From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 11/6/2019 8:08:42 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: 回复:Re: The PDF offprint of your article [JOBB_26] is attached to this email
Attachments: BSL4 Wuhan_Manuscript-20191107_track.docx

Dear James ,
I really think about to have another article with you about the safety management in the laboratory. Sorry I can not go to WHO meeting this time, and I hope to see you soon, maybe in CAS-NAS meeting.

By the way, I write a small paper on Wuhan P4 lab. My attention is to let outside to know a little bit the laboratory and understand why we need the lab. and how to operate the lab. I hope you could have a look and help me to revise it.

Thanks for you help and I am sure your revision will do me a great favor for the publication of this article.

Regards

Zhiming

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Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
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From: LeDuc, James W.
Date: 2019-11-06 10:20
To: Yuan Zhiming
Subject: Re: The PDF offprint of your article [JOBB_26] is attached to this email
Wonderful! Thanks for the good news. Hopefully we will have another one out soon.

Are you going to the WHO meeting on biocontainment labs next week in Geneva? Perhaps I' ll see you there.

Jim

Sent from my iPhone

On Nov 5, 2019, at 5:44 PM, Yuan Zhiming <yzm@wh.iov.cn> wrote:

Hi, James ,

The article Safety and Security in the Age of Synthetic Biology has been published on line. Thanks for your contribution and hope to meet you soon.

Regards

Zhiming

==========
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Professor of Wuhan Institute of Virology
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Article title: Safety and Security in the Age of Synthetic Biology
Article reference: JOBB26

Journal title: Journal of Biosafety and Biosecurity
Corresponding author: Dr. Zhiming Yuan
First author: Dr. James W. LeDuc
PDF offprint dispatch: 5-11-2019

Dear Dr. Yuan,

We are pleased to inform you that a PDF file of your published article Safety and Security in the Age of Synthetic Biology is attached to this e-mail for you to view and download. Please note that this article is published, therefore content updates are no longer possible at this point.

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China's First Biosafety Level 4 (BSL-4) Laboratory for Fighting Infectious Disease

The epidemic of severe acute respiratory syndrome (SARS-CoV) in 2002–2003, which resulted in 8,069 cases of infection and 775 deaths worldwide (Ref2), brought a great challenge to national and international public health systems. It became a touchstone for public health in China as it responded to emerging infectious diseases, and revealed the weaknesses of existing strategies for the prevention and control of such emerging diseases. Complicating the issue, basic and clinical research in response to the epidemic was impeded due to a lack of high containment facilities. Therefore, in order to reduce the potential impact of deadly infectious diseases, including SARS and other highly dangerous infectious risks to human health, the Chinese authority embarked on the construction of a biosafety laboratory network in China, including the BSL-4 National Biosafety Laboratory, Wuhan, Hubei province in central China. ~~(Wuhan).~~

The Chinese Academy of Sciences began the process that would lead to the construction of the BLS-4 laboratory early in 2003, and broke ground in 2015. In the framework of the Sino-French Cooperation Agreement on the Prevention of Emerging Disease Control, signed in 2004, Chinese and French engineers and scientists agreed to collaborate for one decade to complete an internationally recognized BSL-4 laboratory, providing a safe and secure platform for scientists to study high-hazard viruses.

~~On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).~~

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Milestones of the laboratory construction

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As a critical part of the national high-level biosafety laboratory network system, the construction project of the Wuhan BSL-4 National Biosafety Laboratory (NBL) was officially approved by the National Development and Reform Commission in 2005. Subsequently, Chinese and French engineers and designers studied the operational state of the art high-containment laboratories worldwide, analyzed the geological and environmental conditions of the proposed construction site, confirmed the operational role of the laboratory in China, then jointly designed and constructed the laboratory. The physical completion of the laboratory on January 31, 2015, is not only a great symbol of Sino-French friendship, but also an impressive accomplishment of the national high-containment biosafety laboratory network. After the commissioning, certification, and trial operation, the laboratory was successfully accredited as an Animal Biosafety Level-4 (ABSL-4) laboratory by the China National Accreditation for Conformity Assessment in accordance with CNAS-CL05:2009 and national laboratory standards on January 13, 2017 (Ref3), and acquired the official license of handling risk group-4 (RG-4) pathogens from the National Health and Family Planning Commission on August 17, 2017. The award of the accreditation certificate and the experimental activity license demonstrated that the laboratory has the full capacity and authority to handle high-hazard viruses and to study animal models of infection according to the regulations (Ref4). These events were a landmark achievement for the National High-level Biosafety Laboratory System with recognition by the Chinese national authority (Ref5). In addition to the laboratory, a culture collection and repository center called the "National Center for the Preservation of Pathogenic Microorganisms" was established and authorized, relying on the facility and bio-containment environment (Ref###). With these milestones, the NBL, as China's first BSL-4 laboratory, has been put into operation formally and legally, with full capacity and authority to conduct virus stocking and scientific research on virulent high-hazard viruses. The long-term aim of the institute is to establish the NBL as a comprehensive research and development center for infectious diseases, a national biological center, and a WHO reference laboratory. In addition, this laboratory will become a stepping-stone for Chinese and French scientists in fighting infectious disease and will also serve as a cornerstone in global health security. (Fig.1)

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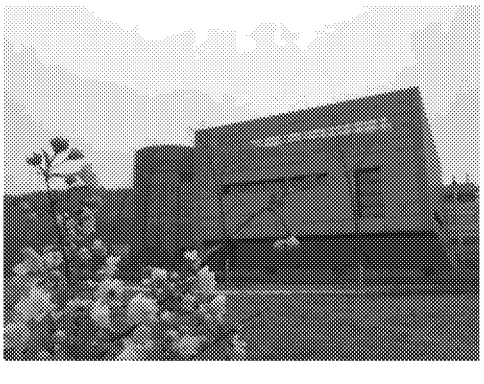

Fig. 1 The BSL-4 facility building

Nature of the laboratory

The laboratory is located in Zhengdian Scientific Park, a few kilometers away from the Yangtze River in the Jiangxia District, Wuhan City, Hubei Province. In addition to the new NBL, one BSL-3, two BSL-2s, molecular diagnosis and cell culture laboratories, and other nearly operational research

facilities and platforms to support virology research and animal rearing are also located in the park, making this research park a modern, comprehensive national and regional virology research and development center.

The BSL-4 laboratory stands as an independent building with a total area of 3266 M^2. It comprises two sections: a square laboratory body structure and a circular auxiliary structure, both inter-linked by a closed corridor. All the equipment and functional units were fitted into the three floors of the square structure. The basement and upper zones are equipped with life maintenance and differential pressure systems (compressed respiratory air and environmental air handling plenums with High Efficiency Particulate Air [HEPA] filters), continuous liquid effluent heat treatment devices and chemical disinfectant tanks, heat exchange systems, water treatment devices, and air conditioning units. All of this equipment is connected to other functional facilities distributed in other zones, within the NBL, through a pipeline network. Thus, all contaminated air, water and solid waste is sterilized/treated before release from the laboratory. (Fig.2)

- **Upper Technical Zone:** Refrigerators, AHU, Exhaust fans, Technical units of shower
- **Equipment interlayer Zone:** Separated from 2nd floor by grilling for maintenance, Air ducts and BIBO.
- **Core laboratory:** Containment with a [illegible] and out on the ceiling.
- **Basement technical zone:** Install the facilities such as electricity, compressed air, wast water treatement, water supply.

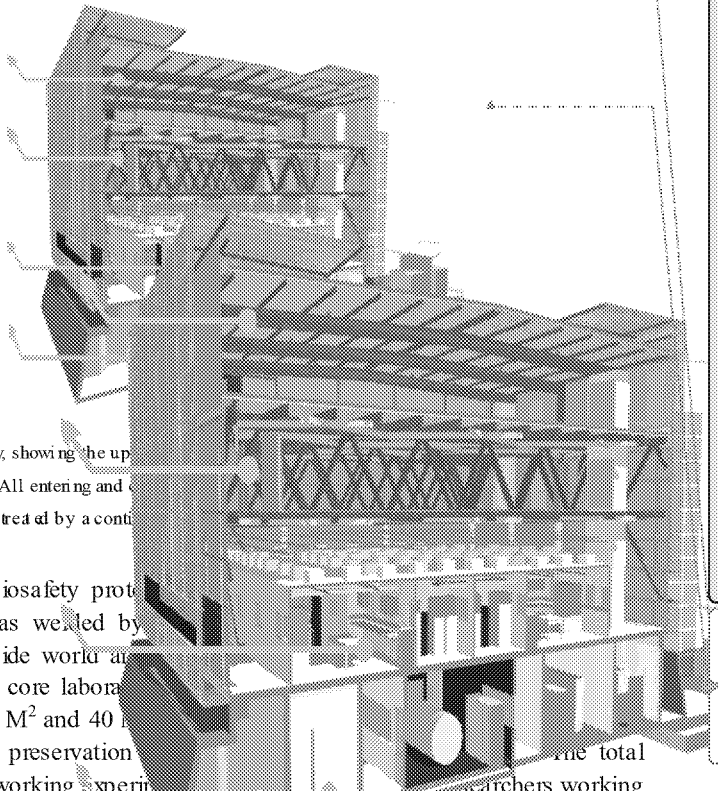

Fig. 2 The Cross-section of the BSL-4 facility, showing the up[illegible] interlayer zone, and the core laboratory zone. All entering and [illegible] solid waste is autoclaved, but liquid waste is treated by a conti[illegible] is transferred from the laboratory.

The BIBO zone is composed of a biosafety prot[illegible] seamlessly welded stainless steel ducts was welded by [illegible] effectively sealed off from the outside world a[illegible] in of the working area is separated into three core labora[illegible] rodents and non-human primates, 28 M^2 and 40 [illegible] room that is 32 M^2 and two virus preservation [illegible] The total containment area is 480 M^2, and its working experi[illegible] researchers working in the lab can see outside to the non-containment ha[illegible] managers can observe the activities occurring inside of it through sealed double-glazed w[illegible] ows installed within the stainless steel wall of each laboratory; this design provides an ambient working environment for researchers in addition to being a practical passage for the purpose of overseeing by the biosafety officer and managers (Fig. 3). The installation of an outer bulletproof glass and outmost porous aluminum plate not only provide

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Comment [A1]: Please change "Technical units of shower" to "technical shower units"
Please change "Exhaust fans" to "exhaust fans"
Please change "Upper Technical Zone" to "Upper technical zone:
Please change "Equipment interlayer Zone" to "Equipment interlayer zone"
Please change "Basement technical Zone" to "Basement technical zone"
Please change "from 2nd" to "from the 2nd"
It's not clear what "by grilling for maintenance" means – maybe "by grills (for maintenance),"?
Please change "Air ducts" to "air ducts"
You might want to spell out BIBO.
Please change "ducts and BIBO." to "ducts, and BIBO"
Please change "get in and out on the ceiling" to "go in and out of the ceiling"

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an extra protective measure, but also bring value in the form of heat insulation and eventual energy savings for the laboratory

The laboratory is composed of 10 technical systems, including the power supply, thermal supply, containment, air treatment system, waste disposal, life maintenance, automatic control system, fire control, security system, and isolation facilities, which guarantees ~~thae~~ stable unidirectional negative pressure gradient air flow and sealed environment in the containment area. It is designed as a suit-type biosafety laboratory, in which the staff inside are completely protected by a whole-body positive-pressure protective suit supplied with conditioned air.

The containment laboratory is fitted with equipment that meets the requirements of biosafety management and high-containment pathogen research, including Labconco biological safety cabinets (BSC), animal breeding and isolators, Tecn independent air transport cages, Tecn animal cages, Ehret monkey cages, a Thermo anatomy table, CO_2 incubators, fluorescence microscopes, quantitative PCR amplifiers, refrigerators, and freezers.

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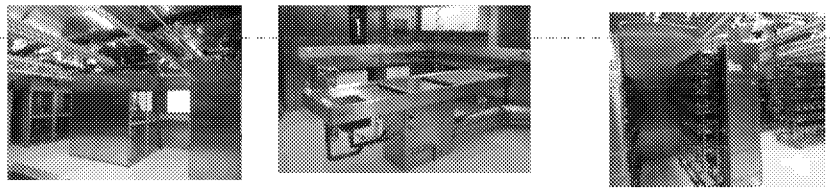
Fig. 3 Two technicians working inside the laboratory



Comment [d2]: Figures need to re-order

Fig. 4: The animal cages for rodent (A) and non-human primate (B) infection, and the autopsy table in three separated room in the BSL-4

Main scientific research priorities

The laboratory is designed and equipped to conduct research on RG-4 pathogens such as the Ebola virus, the Nipah virus, the Crimean-Congo Hemorrhagic Fever (CCHF) virus, the Lassa virus, the Junin virus, the SARS-CoV, the Marburg virus, and so on. According to the lab's biosafety protection level, personnel ability, and management status, the research activities that can be conducted in the laboratory range from low-risk manipulation of cell culture propagation, to rodent infection, and ultimately to the infection of non-human primates. Similarly, pathogen manipulations are gradually conducted from the low-risk CCHF virus to other more virulent pathogens such as the Ebola virus, the Marburg virus, and the Lassa virus. According to the license issued by the National Health Planning Commission and the availability of virus resources, the laboratory has already implemented projects on cell culture models, animal models, pathogenesis studies, and preliminary trials of antiviral drugs as well as vaccine development for the CCHF virus, which used to be called

the Xinjiang hemorrhagic fever virus, causing sporadic animal infection during the last few decades in Northwest China (6).

The laboratory has established short- and long-term collaborative links with counterparts in the USA and France; we are seeking additional beneficial scientific and operational partnerships with other laboratories around the world, with the purpose of sharing specimens, reagents, technology, good practice, and expertise; the eventual goal is for there to be effective collaboration within the international laboratory community to address the threat of emerging and re-emerging infectious diseases locally and internationally (7).

Comment [A3]: "true"? Maybe "beneficial"?

The strategic role and capacity strengthening ~~need re-write the sub-title~~

Comment [A4]: "The strategic plan"

~~On the basis of the~~According to the laboratory's operational orientation and China's national requirements, the laboratory was designed and will operate as the research and development center for infectious disease, as a national biological resource center and as a WHO reference laboratory. As a comprehensive national biosafety research center, it will play an indispensable role in the prevention and control of infectious diseases in China. In order to realize these key goals and functions, we must assure the safe and secure operation of the laboratory, increase its capacity as a core culture collection resource, enlarge its scientific research capacity, support and promote the overall response capacity for public health emergency preparedness, provide expert support to national biosafety strategies, and contribute to the broader laboratory network system. We aim to ensure the safe and efficient operation of the laboratory through the principles of "openness, transparency and sharing," benefiting national security and global health security.

Comment [d5]: Repeat with previous paragraph.

(replaced with the new version of 五种能力建设)

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On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).

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2) WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003. WHO, [HYPERLINK "http://www.who.int/csr/sars/country/table2004_04_21/en/"](2004)
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5) National Development and Reform Commission of China, Planning of high-level biosafety laboratory system construction, 2016. [HYPERLINK "http://www.ndrc.gov.cn/zcfb/zcfbtz/201612/t20161220_830455.html"]

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7) [HYPERLINK "https://www.researchgate.net/scientific-contributions/2148527743_James_W_Le_Duc?_sg=jYwkkXaeO7SEceb3lG8i9a4oToAbdwD8yA2QaJpzASED8nAjJyGxtlp5aKkpsn_aLxiKniLp423eTjyaXHhvjEYZLxqvqhux-cXgiH6GwJRnNilibHccvUC_ezKTwjeZGbRQSIL-NpI3OQvlLQFTbZJmJML4w"] and Zhi-ming Yuan, Network for safe and secure labs., Science, 362:267-267

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[HYPERLINK "http://www.chinadaily.com.cn/china/2015-01/31/content_19457709.htm"] China opens National Biosafety Laboratory in Wuhan

By Cheng Yingqi(chinadaily.com.cn)Updated: 2015-01-31 16:14

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Sent: Sat 3/21/2020 3:05:33 PM (UTC-05:00)
Subject: The Comparison of the Strategies for the Prevention and Control of COVID-19 Eng Version 20200321
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Dear GOARN experts and community ,

I am sure you are all very busy with the response to COVID-19, and in the same time being worrisome and frustrating with the present pandemic situation, just like me, especially after I wrote the letter to you all on 29 February 2020, and the international community doesn't change much of

the control practice in the past three weeks, so that the pandemic evolving so quickly and so fiercely.

But why is that? I am not so sure everyone knows what happens, why it happens? Even after the epidemic evolving so quickly. So I wrote an article on 17 March 2020 and published on China Daily on 19 March 2020.

(https://cn.chinadaily.com.cn/a/202003/19/WS5e731e02a3107bb6b57a7913.html?from=timeline&isappinstalled=0)

As it is in Chinese, so I spend two more days and try my efforts to develop an English version, and would like to share with you here, for the sake of people's health, for those countries with high disease burden, and also for the countries with limited resources and capacity, like Zambia where I am now in.

Do hope you can read it carefully and will find it helpful.

Best regards,

Dr. Ni Daxin

Former GOARN Steering Committee Member

Comparative Study of the Two Kinds of Strategies and Measures for the Prevention and Control of COVID-19

Dr. Ni Daxin
Former Steering Committee Member
of the Global Outbreak Alert and Response Network (GOARN)

On 31 December 2019, an outbreak of pneumonia of unknown pathogen was discovered in Wuhan, China. On 7 January 2020, a new coronavirus was identified as the cause of the pneumonia. Since then, as the epidemic spread to other parts of China and some other countries of the world, it attracted high attention from the international community. On 30 January 2020, the World Health Organization declared the COVID-19 outbreak a public health emergency of international concern. After the outbreak detected, the Chinese government has taken active prevention and control strategies and measures. After more than two months of unremitting efforts, not only has the increase of new cases been effectively controlled, but also the local transmission has been tending to be completely contained by mid-March 2020. But at the same time, the international epidemic went from imported cases and limited local transmission in a few countries in January 2020, to rapid growth in a few countries including the Republic of Korea, Iran and Italy in February, then the global epidemic spread rapidly and accelerated in March. On 11 March 2020, the World Health Organization officially declared the COVID-19 outbreak a global pandemic.

With the spread of the epidemic around the world, different countries have adopted different strategies and measures, and the international academic community has never stopped arguing about how to control the epidemic of COVID-19, the World Health Organization's recommendations have not been well implemented in many countries and areas, especially in countries like the United States of America and Europe, where the World Health Organization's recommendations have almost been ignored. As of 16 March 2020, there were 86,429 confirmed cases and 3,388 deaths in 150 countries except China, including 13,874 new cases and 848 new deaths on March 16th only, and the number of countries with the first confirmed cases report reached seven on the day. However from global perspective, the COVID-19 epidemic is still in the early stage of the pandemic development, widespread community-based transmission has not yet occurred in most countries, and the global pandemic is still accelerating and there is still a long way to reach its peak.

In order to provide support to the prevent and control the global pandemic of COVID-19, this paper tries to analyze the strategies and measures of the prevention and control for the COVID-19 in the world.

1. Classification of the Strategies and Measures for the Prevention and Control of COVID-19

Taking a comprehensive view of the current international strategies and measures for the prevention and control of COVID-19, although there are differences in the specific measures in different countries, but according to the essential characteristics of the strategies and measures adopted, they can be simply classified into the following two categories.

The first category is the strategy and measures adopted by countries such as China, Singapore, Korea and Thailand, etc., which can be called "SARS-like prevention and control strategy and measures" , or Containment strategy, Blocking strategy, and hereafter will be called "SARS-like strategy" in this article .

The second category is the strategy and measures adopted by countries such as the United States of America, Japan, Italy, France and Switzerland, etc., which can be called "pandemic influenza prevention and control strategy and measures" , or mitigation strategy, and hereafter will be called the "Pandemic Flu-like strategy" in the article.

2. Differentiation and Analysis of the Differences between Two Kinds of Strategies and Measures

Through careful analysis and study of different national prevention and control strategies and measures based on their essential differences, the two types of prevention and control

strategies and measures are mainly different in the following areas.

(1) Different Prevention and Control Goals

The prevention and control goals of the two strategies are different. The prevention and control goals of the SARS-like strategy are to control the epidemic, contain the spread completely and eliminate the harm. While the prevention and control goals of Pandemic Flu-like strategy are to control the spread, slow down the epidemic, and reduce the overall harm.

(2) Different Arguments

SASRS-like strategists believe that the main route of transmission of COVID-19 is through close contact and droplet by symptomatic individuals, and active investigation and control through SARS-like prevention and control, the transmission of latent and incubation infections can be interrupted by additional measures, proactive control strategies and measures should be adopted. While Pandemic Flu-like strategists consider that it is almost impossible to completely detect and manage all the source of transmission of the new coronavirus, which infection exists in the latent and incubation period of transmission. The transmission of COVID-19, just like that of an pandemic influenza, can only be slowed down and cannot be completely interrupted. Since it cannot be blocked, all people will sooner or

later become infected, so it is better to adopt strategy and measures to slow its spread, and allow it to spread slowly and in a controlled manner until the population forms an adequate immune barrier or reach herd immunity, and then the intensity of the epidemic will be greatly reduced, making it a seasonal epidemic disease similar to seasonal influenza.

(3) Different Views on Cost-effectiveness

SARS-like strategists believe that, regardless the case-fatality rate of COVID-19 is high or low, since the virus can be effectively controlled and the transmission can be completely blocked by taking proactive prevention and control measures, so hard efforts should be made to minimize the incidence, severe cases and case fatality of the disease, it is worthwhile to pay a greater cost in the short term, and to avoid more significant overall health and socio-economic losses. While Pandemic Flu-like strategists argue that, about 80% of those infected with COVID-19 are mild cases, and the case fatality rate was only a slightly higher than that of the pandemic influenza, which much lower than that of SARS and MERS. At the same time, those infected with the COVID-19 has no specific medicine, and mild cases do not require hospitalization. So under the hypothesis that the transmission could not be interrupted, SARS-like prevention and control strategy and measures will cost too much, and the impact on normal social production and life and the loss for that are extremely heavy, so it is not worth of it, not in line with the

cost-effectiveness principle.

(4) The Key Prevention and Control Measures are Different under the Guidance of the Two Strategies

Because of the different objectives, arguments and cost-effectiveness views, the key prevention and control measures under the guidance of the two strategies are remarkably different.

1. Key Prevention and Control Measures under SARS-like Strategy

Under the SARS-like strategy, in order to stop transmission and reduce health impact, it is necessary to realize "Five Early" , that is, "Early Detection, Early Reporting, Early Investigation, Early Isolation and Early Treatment" , so as to realize the strict management of the source of infection, and to block transmission gradually.

"Early Detection" , that is by improving the awareness and sensitivity of medical staff, early detection of suspected cases, rapid tests and diagnosis should be taken, so as to promote timely and effective management of all patients, which are the most important source of infection for COVID-19.

"Early Reporting" , that is, suspected patients and confirmed

cases need to be reported to the health authority or disease control departments within a specified time, in order to start the investigation and response.

"Early Investigation", that is after receiving reports of confirmed patients, suspected patients or positive tested persons, the department of disease control and prevention needs to send out the epidemiologists to investigate the patients' exposure before the onset of illness and the persons getting contacted with him/her after getting ill, so as to find out the source of infection of the patient and all of the close contacts associated with him/her. Through in-depth "Early Investigation" to identify the transmission chain of each cases, it will be totally possible to achieve an overall identification and management of all possible patient-related infected persons.

"Early Isolation" , that is all confirmed cases should be treated in isolation, all suspected cases should be treated in isolation, and all close contacts should be placed under medical observation and isolation, or be quarantined. "Early Isolation" of confirmed and suspected cases would be effective in preventing transmission of the virus from COVID-19 patients to healthy individuals. "Early Isolation" of close contacts will help to detect new cases of infection including atypical mild cases at an early stage, as well as to ensure strict management of those with latent or incubation period infections, so that they could not spread the virus to other people. Through the single-room

isolation of the suspected patients and close contacts, it can effectively prevent the possible cross-infection between these isolated patients or quarantined people.

"Early Treatment", that is through the effective symptomatic treatment, support treatment and available anti-viral or traditional Chinese medicine treatment, efforts are made to prevent the progression of mild cases to severe or critical, and the severe cases to be given full care, all these efforts are trying to reduce case fatality. At the same time, through "Early Treatment" , it can also achieve the elimination of patients' status as a source of infection.

Through "Five Early" , and to achieve the full hospitalization of all the confirmed cases, suspected cases and positive tested persons, to achieve the full management or quarantine of close contacts, which are called in China "Due Hospitalization, Due Management", it will lead to the effective control of the further spread of the virus, and ultimately interruption of the virus transmission.

2. Key Measures under Pandemic Flu-like Strategy

Under the Pandemic Flu-like strategy, in order to achieve the goal of reducing health impact, the emphasis and the most critical measures are the treatment of severe cases. At the same time in order to avoid medical overload, if necessary,

appropriate measures will be taken to increase social distance. However, no emphasis are placed on the early detection of all cases, the isolation of mild cases, or the tracing and management of close contacts.

Treatment of Severe Cases , that is to give priority to the case of severe, or cases with underlying illnesses for hospital treatment, mainly through active symptomatic, support treatment, to reduce the mortality. At present, there is no specific drug for the treatment of COVID-19 virus, so under the strategy of Pandemic Flu, it is generally to advocate mild cases to be observed at home, if not showing any severe symptoms like short of breath.

Because of the lack of emphasis on early case detection, the tests for new coronavirus is generally not recommended for atypical, mild patients and the close contacts.

When the number of cases increases rapidly or too fast in a region, the number of severe cases exceeds the capacity of the medical institutions, or severe cases crowding out of medical resources, measures may be taken to increase social distance, such as the prohibition or reduction of large-scale gatherings, school suspension, work stoppage, and even declared a state of emergency or a curfew, etc. just like what are happening now rather popularly in the countries with such kind of strategy.

3. Other Common Measures to Prevent and Control

COVID-19

Based on the prevention and control practice of COVID-19 in various countries, other common prevention and control measures are as follows:

(1) Lockdown of cities, villages and roads, as well as closed management for institutions and communities.

(2) Suspension of work, school and production.

(3) Suspension of flights, ships, traffic, etc.

(4) Quarantine at Point of Entries, and traffic health check points.

(5) Mask wearing, hand hygiene, cough etiquette, etc.

(6) Disinfection measures.

All these measures are aimed either at controlling the flow of infectious sources, increasing social distance or protecting vulnerable populations in order to prevent or reduce the spread of the virus and reduce the incidence of the disease. These measures may be more or less adopted to prevent and control the epidemic of COVID-19 in both groups of strategic population, and at the same time, they may be different in each country according to the current epidemic situation, the concept of prevention and control, and cultural customs there. SARS-like strategy may pay more attention to and take more aggressive closure measures, such as in Wuhan, lockdown of the city was used to prevent further spread of new coronavirus infection, other types of closed management in other parts of

China are also taken to prevent infection from spreading out or coming in. while Pandemic Flu-like strategy may place more emphasis on more modest measures to increase social distance, such as fewer gatherings and school suspension. But all of these measures are complementary to the key measures of both types of strategies and can only help or facilitate faster and better outcomes for key interventions.

5. Brief analysis of prevention and control strategies and measures in some countries

(1) China's strategy and measures

China has adopted a SARS-like strategy, focusing on patient discovery and isolation, close contact investigation and strict management. In order to control the spread of infectious sources, the city of Wuhan, with a population of ten millions, was locked down. By adding fixed-point hospitals, building new isolation hospitals and building shelter hospitals, the problem of admission and treatment for huge number of patients in Wuhan was effectively solved, and the patients, the most important source of infection, were effectively under control. At the same time, through general mobilization throughout the country, other provinces and municipalities, while doing well their local prevention and control work, have provided adequate medical personnel, epidemiological investigators, rescue and protection equipment and facilities to Wuhan in the form of counterpart

support, the goal of stopping the spread of the virus has been basically or initially achieved, and the higher case fatality rate in the early stage of the epidemic has been effectively controlled.

The key to the control of epidemic situation in China lies in the effective management of infectious resources, and at the same time, through the lockdown of Wuhan City, the spread of the virus to outside areas has been effectively blocked, which is crucial to the epidemic control of other areas in China, and also contribute to the control of the epidemic in the World at that time period. However, there is almost no widespread community transmission outside of Wuhan, so the various closure measures in these areas should only play an facilitating role in the control of the spread of this disease, meanwhile, the enormous impact of these measures on the social production and life may have caused a large number of unnecessary losses to the national economy and social development.

(2) Singapore's strategy and measures

Although the Singapore government claims that the COVID-19 is just like a large influenza, in the specific prevention and control practice, it has focused on the detection and isolation of patients, and the follow-up investigation and management of close contacts. Therefore, in essence, the goal of prevention and control is to block the spread of the virus, the basic strategy and key measures are still similar to SARS prevention and control

strategy and measures.

(3) Republic of Korea's strategy and measures

The Republic of Korea is also implementing SARS-like prevention and control strategies and measures. After the epidemic rapidly increased and in the case of once-difficult admission of patients, although no drastic containment measures were taken, but the Republic of Korean government resisted huge pressure to increase the detection of suspected patients and close contacts, and their efforts were finally made to achieve the goal of managing all the patients and quarantine all the contacts, so the control results of the epidemic has already seen obviously, daily reported cases have fallen from a peak of more than 1,100 to the dozens now.

(4) Japan's strategy and measures

Japan has adopted a typical pandemic flu-like strategy, with the government making it clear in the early days that it would only encourage hospital treatment for severe cases and home treatment for mild cases, and would not encourage new coronavirus testing for asymptomatic people. But thanks to Japan's self discipline and high level of hygiene, the country has not seen the rapid increase in cases like what seen in European countries, making it one of the very unusual countries using the pandemic flu-like strategy. However, as its prevention and

control strategy is unlikely to stop the spread of the epidemic, the recent COVID-19 in Japan is still in the process of a sustained slow rise.

(5) Iran's strategy and measures

Iran's original intention was to adopt SARS-like prevention and control strategy and measures, to vigorously strengthen the detection of suspected patients, to make efforts to investigate and manage contacts, and to solve the problem of patient admission, but due to long-term economic sanctions, the basic capacity or social economic support is weak, it has not really done the "Due Hospitalization, Due Management", so Iran is not a complete SARS-like strategy country. As a result, although the number of cases has not continued to increase significantly recently, it has been in the peak phase of cases for a longer time, and the effect of future control depends on whether the hospitalization of cases can be effectively solved, and then to further strengthen the close contacts investigation and management.

(6) The United States of America's strategy and measures

The United States of America has a strong Pandemic Flu-like strategy, so it has been strictly limiting the new coronavirus test for suspected cases and encouraging patients with mild illness to stay at home. Earlier outbreaks there rose more slowly than

other countries when aggressive travel restrictions imposed by the United States of America on China, but because cases continue to spread slowly, the source of infection accumulate, new importation from Europe countries introduced, and more testing has recently been done, there has been a rapid increase in the number of cases and deaths. To this end, a number of states, and later on, the United States of America nationwide, have declared a state of emergency, and take a lot of measures to increase social distance.

(7) Italian strategy and measures

In spite of the lockdown imposed in the disease focus areas and even the final nationwide lockdown, and the relatively active testing of new coronavirus, Italy has emphasized the treatment of serious cases, home observation of mild cases was required, both in the focus areas and in other parts of the country. And at one point it was even announced that testing for mild cases would be reduced. So Italy's strategy and measures are closer to those of the Pandemic Flu, which is why Italy has surpassed South Korea and then Iran to become the country with the highest number of cases outside China. And because community transmission has not been effectively controlled, with the increase in the total number of patients, severe cases have emerged to cause medical resources run-off phenomenon, medical staff have to face selective treatment of the "severe cases", that is, priority is given to the treatment of severe cases

with longer "life expectancy" rather than those with advanced age and severe underlying illnesses.

(8) Strategy and measures of the United Kingdom and other European countries

The United Kingdom, as well as most European countries, are strong Pandemic Flu-like strategy, emphasizing that COVID-19 cannot be completely blocked, the treatment of severe cases is the main focus, and patients with mild cases require home observation, limiting new coronavirus testing in patients with mild illness. The United Kingdom, Switzerland, Sweden, and most other European countries, in face of the rapid rise in new cases of COVID-19, directly announced the abandonment of the detection of mild cases, and even said no longer announced the number of confirmed cases. The UK has even gone so far as to say publicly that it wants to natural immunize the population by giving it a 60 per cent or so infection rate, so it has tended to be conservative even in taking measures of increasing social distance, such as not actively enforcing school closures.

6. Analysis of the control effect of two kinds of strategies on COVID-19

(1) SARS-like strategy countries have achieved good results in controlling the epidemic situation

Countries and regions that adopted SARS-like strategies, whether the countries like China and Republic of Korea, where the COVID-19 epidemic was severe in the early stage, or Singapore, Thailand, Vietnam, Hong Kong SAR (China), etc. where the imported cases was the major problem, through actively adopted SARS-like prevention and control strategy and measures, the COVID-19 epidemic situation has been well controlled, even has realized successfully the elimination of local transmission. China, as the first country to find the COVID-19 epidemic this time, has experienced a high intensity and extensive community spread in Wuhan, but has successfully controlled the local spread by actively adopting SARS-like prevention and control strategy and measures, and is about to complete the interruption of local virus transmission. In the case of Republic of Korea, which experienced a sharp increase in the number of cases in the previous period and made it once the number of cases be second only to China, it insisted on the implementation of key measures and quickly reversed the epidemic. At present, the number of new cases has been successfully reduced to double digits, the control and containment of local transmission is within reach.

Countries that have adopted a SARS-like strategy have interrupted the chain of transmission of the virus because of the discovery of key sources of infection and the implementation of management measures. New cases have been effectively contained and the number of severe cases has been brought

under control accordingly, the crude case fatality rate of most other countries or regions except Wuhan, China is relatively low, while the case fatality rate is high in Wuhan due to the medical resources run-off once upon a time.

In addition, the SARS-like strategy countries, exported less cases to other countries. Although imported cases from China were detected in more than 20 countries around the world in the early days of the Wuhan outbreak, with proactive and even aggressive prevention and control measures, other countries has reported very few new cases of COVID-19 imported from China since February 2020.

(2) Pandemic flu-like strategy countries continue increase or even very rapidly

In countries with pandemic flu-like strategy, community transmission continues to occur as a result of a lack of comprehensive and effective management of the source of infection, the level of transmission or the varies of increase rate from country to country affected mainly by differences in the ability to treat cases in different countries, the compliance of the population to treatment at home, the strength and consciousness to increase social distance measures, and the early or late the start of the epidemic. The Italian epidemic continues to rise, the epidemic in vast majority of European countries and the United States of America are soaring in the recent past, and the

epidemic in Japan has been continuing to rise slowly.

With the spread of the disease and the increase in the number of cases in Italy, the number of severe cases has increased accordingly, which has exceeded the capacity of local medical institutions. Medical personnel are facing the dilemma of having to treat patients selectively, and the case fatality rate remains high, to become the world's highest crude case fatality rate country. It remains to be seen whether other countries with pandemic flu-like strategy will experience a similar run on severe cases.

In the case of widespread community transmission and severe epidemic of the virus in Italy, no strict and genuine lockdown was imposed on the severe epidemic area and the country, the epidemic continues to spread inside the focus area, from the focus area to other parts of Italy, and then Italy has continued to spread to Europe and other countries around the world. For this reason, Italy has been the biggest exporter of cases to other countries, and in some ways it has been a major source of the new global pandemic. At the same time, with the number of new cases rapidly rising in most European countries and the United States, these countries have also become an important source of recent cases importation to other countries and act as a booster of the global pandemic.

7. Analysis of the Relationship between the Two Strategies

and Suggestions for the Next Step Control Strategy

At present, the global pandemic is still in its early stage. So if the differences between the two strategies can be quickly resolved, and a global consensus, concerted action and robust joint measures can be reached and taken, among them the most important is that the SARS-like strategy could be promoted by all countries, it is still possible to influence and control the course of the global pandemic, and to halt the global spread of the new coronavirus.

(1) Analysis of the Relationship between the Two Types of Strategies

The fundamental differences between the two strategies are whether the new coronavirus can be completely contained, how to view the cost-effectiveness of prevention and control, and the understanding of containment measures.

A. The possibility of containment or blocking. Whether the new coronavirus can be completely blocked is now entirely possible. First, China, Singapore, Thailand, Vietnam and even the Republic of Korea's control practices have fully explained this point, the China's COVID-19 prevention and control field research report of World Health Organization also gave full recognition. Second, for the "leakage of infectious sources" caused by latent infectious, incubation period infectious and

atypical cases problem which are concerned about by Pandemic flu-strategists, it is entirely possible that these problems can be well solved through the measures of "Five Early" and "Due Hospitalization, Due management", which has also been proved in practice. The incubation infectious of COVID-19 is mainly at the end of the incubation period, and the rate of latent infection is low, it can realize the comprehensive detection and management of all atypical patients, latent infection and incubation period infection, thus effectively prevent the further spread of virus to other healthy people.

B. Cost-effectiveness. As long as people agree that new coronavirus transmission can be stopped, it will become very clear which of the two strategies is more cost-effective. The SARS-like strategy may control the incidence increase in a shorter time, block the spread of the virus, and greatly reduce the overall number of cases, severe cases and deaths, at the same time, it avoids the influence of social distance measures on social production and life for a much longer time. When look it at a global scale, based on a simple analysis of available data and extrapolation, it is much easy to see the cost-effectiveness and health benefits of the two strategies are not even comparable.

C. Understanding of the role and necessity of containment measures. According to the SARS-like strategy, it is necessary to take some measures to control the spread of COVID-19 virus

in the intensive community transmission area, which can reduce the pressure of prevention and control in other areas. As even at the peak of the outbreak in China, and because of domestic management measures for those at risk of infection, limited exportation to the rest of the world were recorded at only earlier stage, and no exportation to Africa. However, after the recent increase of the epidemic in Italy, more European countries and the United Sates of America, due to the adoption of a pandemic-influenza like strategy there, those who may have been infected in the country have not been effectively detected and managed, as a result, more than 20 countries in Africa alone have recently seen imported confirmed cases in a very short time period, and even in some African countries, such as Egypt, Algeria and Senegal, have already led to local transmission, which will be a very worrisome situation. It can be seen that the adoption of SARS-like strategy and measures in areas with widespread community transmission not only has a fundamental impact on the control of the epidemic situation in the country or the region, but also it has a great impact on the world pandemic control, especially on countries with poor capacity and resources, such as Africa, which the epidemic losses there will be incalculable.

It is clear from the comparison of key control measures across the two global strategies, whether in high-prevalence areas where widespread community-based transmission has already occurred, or in low-prevalence areas where only imported cases

or limited local transmission happened there, the key to control and contain the spread of new coronavirus lies in the implementation of "Five Early" and "Due Hospitalization, Due management". And the earlier it is implemented, the easier it is to implement, and the less costly it will be. In contrast, the later it is implemented, the harder it is to implement, and the more costly it will be. The development of the epidemic is mainly affected by the above-mentioned key measures, rather than the lockdown or social distance measures which are considered most difficult to replicate in Western and pandemic flu-like strategy countries. We need to emphasize that these lockdown or social distance measures are necessary only under certain circumstances like massive local transmission. In addition, all these measures only play an facilitating role in controlling the epidemic situation. Even without it, the time for control and containment may be last somehow longer, but if the key control measures be taken timely and effectively, similar containment effect should be able to achieve, just like what we have seen in Singapore and in the Republic of Korea. Therefore, all countries in the world, when take measures to strengthen the treatment of severe cases, they can adopt the SARS-like strategy further, i.e. to implement key control measures to achieve the "Five Early" and "Due hospitalization, Due management", it will effectively control the increase of disease incidence, and easily to reach the target of reduce case fatality rate, and ultimately contain the spread of new coronavirus. With such united efforts, we are still hopefully and able to interrupt the pandemic, to finally contain

or block the new coronavirus transmission, and to make great contribution to the benefit of people in each country, and to the benefit of people around the world.

(2) Recommendations for the control strategy of next step

At present, the COVID-19 global pandemic is still at an early stage, the vast majority of countries have not yet emerged widespread community-based transmission. However, we are now also in a critical period of time for the pandemic, which will be much more difficult to control once the pandemic evolves further and spreads widely in the community in many more countries and regions. Since the outbreak of COVID-19, a large number of disease characteristics and studies have shown that COVID-19 cannot be compared with pandemic influenza in terms of its harm to health and potential harm to social development. At the same time, the transmission of the new coronavirus can be totally contained or blocked, compared with the influenza pandemic, which is almost impossible to do so. Therefore, in the face of this unprecedented challenge to all mankind, as a community of mutual influence on the fate of mankind, countries around the world need to put aside political and ideological differences, and quickly unite to take SARS-like prevention and control strategy and measures, and to act immediately to help without hesitation those countries with highest disease burden now and the countries with the epidemic but without enough resources and capacity, while each country

should do best first to prevent and control the epidemic in their own countries, so that all countries can implement fully and as soon as possible the key measures of "Five Early" and "Due Hospitalization, Due management". Based on these, all countries and regions can take supplementary measures such as lockdown or other social distance measures, in adaptation to the local situation of epidemic and cultural custom etc. In this way, people should have full confidence to make this pandemic the first in the history to be controlled and finally contained.

【Written on 17 March 2020, in Zambia】

From: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Sent: 4/28/2020 5:17:59 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
CC: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Subject: FW: Politico: Trump cuts U.S. research on bat-human virus transmission over China ties https://politi.co/2Sdt9Ke

Assuring you caught this article in Politico. Gray

Health Care

Trump cuts U.S. research on bat-human virus transmission over China ties

The National Institutes of Health on Friday told EcoHealth Alliance, the study's sponsor for the past five years, that all future funding was cut.

The administrative building of the National Institutes of Health. | J. Scott Applewhite/AP Photo

By SARAH OWERMOHLE

04/27/2020 07:02 PM EDT

The Trump administration abruptly cut off funding for a project studying how coronaviruses spread from bats to people after reports linked the work to a lab in Wuhan, China, at the center of conspiracy theories about the Covid-19 pandemic's origins.

The National Institutes of Health on Friday told EcoHealth Alliance, the study's sponsor for the past five years, that all future funding was cut. The agency also demanded that the New York-based research nonprofit stop spending the $369,819 remaining from its 2020 grant, according to emails obtained by POLITICO.

"At this time, NIH does not believe that the current project outcomes align with the program goals and agency priorities," Michael Lauer, the agency's deputy director for extramural research, wrote in a letter to EcoHealth Alliance officials.

The group caught national attention a week ago after reports swirled that millions from its NIH grants had been sent to the Wuhan Institute of Virology, a research facility in the city where the coronavirus pandemic originated. In an email last week to NIH officials, EcoHealth Alliance President Pete Daszak denied giving any money this year to the Wuhan lab, although researchers from the facility have collaborated with EcoHealth Alliance scientists on research supported by an earlier grant.

The Wuhan lab is at the center of conspiracy theories alleging that the coronavirus outbreak began when the virus escaped the facility. U.S. intelligence agencies and scientists have not found any evidence to support the rumors.

Meanwhile, the NIH's strategic plan for studying the novel coronavirus, released Thursday, lays out four key priorities — including understanding its origin and transmission, in line with the EcoHealth alliance's broader investigation of bat coronaviruses. The agency did not respond to a request for comment on its decision to terminate the group's funding.

In a statement, the EcoHealth Alliance said it wanted to know more about the NIH's reasoning. "For the past 20 years our organization has been investigating the sources of emerging diseases such as COVID-19," the group said. "We work in the United States and in over 25 countries with institutions that have been pre-approved by federal funding agencies to do scientific research critical to preventing pandemics. We are planning to talk with NIH to understand the rationale behind their decision."

Suddenly ending a grant early is an unusual move for the NIH, which typically takes such steps only when there is evidence of scientific misconduct or financial improprieties — neither of which it has alleged took place in this case.

The EcoHealth Alliance has received more than $3.7 million since 2015 for its research on the risks of coronavirus spread through bats and the potential for spillover into humans. The effort has produced at least 20 scientific papers, including several published in prominent journals such as Nature.

As recently as April 2018, the NIH issued a press release promoting a study linked to the research project, whose authors included a scientist at the Wuhan lab.

But the project had turned into a political liability for the NIH by the time Lauer emailed Daszak on April 20 asking for a list of all Chinese participants.

A Newsmax reporter asked President Donald Trump about the research project in an April 17 press briefing, suggesting that all $3.7 million had gone to the Wuhan lab.

"We will end that grant very quickly," Trump said. "It was granted quite awhile ago," he added, referencing the Obama administration. "Who was president then, I wonder?"

The NIH awarded the original grant for the project during the Obama administration, but renewed it in July 2019. The funding allotted this year, and cut last week, came from the Trump administration.

Days after Trump's briefing promise, Republican lawmakers wrote to leadership asking that no stimulus funding go to the Wuhan lab, citing State Department cables about safety concerns. The White House did not respond to a request for comment.

By that time, NIH officials had contacted EcoHealth questioning the group about Chinese links to its bat-coronavirus research project.

"We need to know all sites in China that have been in any way linked to this award," Lauer wrote in one email to the researchers. In a separate April 20 message to the group he said "it would be helpful for us to know about all China-based participants in this work since the Type 1 grant started in 2014 — who they were and how much money they received. The sooner you can get us that information, the better."

Daszak told Lauer that EcoHealth would need time to go through its request for information but that "I can categorically state that no fund from [the grant] have been sent to the Wuhan Institute of Virology, nor has any contract been signed."

Within days, NIH told EcoHealth that all future funding was canceled and it would need to stop spending its remaining 2020 grant monies immediately.

EcoHealth Alliance has secured dozens of contracts amounting to millions of dollars from multiple government sources, including health agencies, the Department of Defense and the Department of Homeland Security.

From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 1/18/2020 7:33:47 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]; Shi, Pei yong [peshi@UTMB.EDU]
Subject: 回复: FW: WIRED interview: new coronavirus in Wuhan

Dear Jim,

Thanks for your information and we are working hard now on the related works. Hopefully some results could be relaesed soon.

The lab is under operation during the holiday and I will let you know the situation at the convenient time.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2020-01-14 03:44
To: Shi, Pei yong; Yuan Zhiming
Subject: FW: WIRED interview: new coronavirus in Wuhan

See link below on a story just released this morning in Wired Magazine. I tried to stress the dramatic improvements in PH and technology between 2003 and now—note title.

Too bad she misspelled my name…

Nice work, Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch

Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Molteni, Megan <megan_molteni@wired.com>
Sent: Monday, January 13, 2020 1:07 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: Re: WIRED interview: new coronavirus in Wuhan

Here's a link to the story, which published this morning. Thanks again for sharing your story with me, very cool to see how much has changed in 20 years. If you've ever got any interesting infectious pathogen story tips going forward, don't hesitate to reach out.

Best regards,
Megan

On Fri, Jan 10, 2020 at 8:35 AM Molteni, Megan <megan_molteni@wired.com> wrote:

> Got it. Thanks again, Jim.
>
> On Fri, Jan 10, 2020 at 7:54 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> > Megan, I have not spoken to anyone in China about the techniques they used, but I suspect that they used the same traditional methods to isolate the virus—inoculation of cell cultures. To determine the sequence, I suspect that they did next generation sequencing (see Armstrong, GL et al. Pathogen genomics in public health. NEJM, 26 Dec 2019, 381;26:2569-2580 for an overview of genomics applications in public health. Greg is at CDC).
> >
> > Thanks, Jim
> >
> > **From:** Molteni, Megan <megan_molteni@wired.com>
> > **Sent:** Friday, January 10, 2020 9:27 AM
> > **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> > **Cc:** Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
> > **Subject:** Re: WIRED interview: new coronavirus in Wuhan
> >
> >
> >
> > Jim, thank you so much for this. And Tom, thank you for helping to reconstruct these events. I know it was a long time ago!
> >
> > My only question is based on the news reporting you've seen out of Wuhan (or correspondence you've had with folks over there), is it fair to assume they used similar set of steps to isolate the virus and get a sequence? If not, where do you think they diverged, from a methods standpoint?

Thanks again,
Megan

On Fri, Jan 10, 2020 at 7:03 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:

> Megan, I spoke to Tom Ksiazek who conducted the original SARS isolation. As mentioned below, he was able to isolate the virus from a throat swab taken from Dr Urban on his arrival into Thailand where he was hospitalized and later died. The specimens were immediately sent to CDC and arrived the evening of 13 Mar and were inoculated into cell cultures that same evening. Evidence of virus growth was first seen on day 5 post-inoculation and a sample of the replicating virus was sent that same day to the electron microscopy laboratory where a coronavirus-like particle was visualized by Cynthia Goldsmith. Based on this preliminary information, Dr Dean Erdman and his team designed consensus primers for coronavirus and were able to amplify a product by RT-PCR, which was then sequenced, further supporting that the isolate as a coronavirus. Virus RNA was sent to the DeRisi lab in California where he confirmed coronavirus identity using his then state-of-the-art array.
>
> Tom Ksiazek is copied here and can correct any errors and provide additional details if needed.
>
> Thanks, Jim
>
> **From:** Molteni, Megan <megan_molteni@wired.com>
> **Sent:** Thursday, January 09, 2020 8:57 PM
> **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> **Subject:** Re: WIRED interview: new coronavirus in Wuhan
>
> **WARNING:** This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> Thanks Jim, super helpful. Great to chat with you today. Much appreciated!
>
> On Thu, Jan 9, 2020 at 6:19 PM LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> > Time from receipt of clinical samples to isolation of virus was 5 days; EM identification was done that same day, Inoculated cells on 13 Mar 2003 and had isolate on 18 Mar. More tomorrow.
> >
> > Thanks, Jim
> >
> > **From:** Molteni, Megan <megan_molteni@wired.com>
> > **Sent:** Thursday, January 09, 2020 1:56 PM
> > **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> > **Subject:** Re: WIRED interview: new coronavirus in Wuhan
> >
> > **WARNING:** This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
> >
> > Great, talk soon. Thanks!
> > M

On Thu, Jan 9, 2020 at 11:54 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:

> Perfect—I'll be in my office at 3 CT. Call to 409-266-6516. If by chance I don't/can't answer, the office number is 409-266-6500 and someone should answer.
>
> Thanks, Jim
>
> ---
>
> **From:** Molteni, Megan <megan_molteni@wired.com>
> **Sent:** Thursday, January 09, 2020 1:50 PM
> **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> **Subject:** Re: WIRED interview: new coronavirus in Wuhan
>
> **WARNING:** This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> Hi Jim,
>
> I do appreciate that this kind of science is a massively collaborative effort. But hard to talk to everyone on a daily deadline so I very much appreciate your willingness to describe the work of the team. Can I give you call around 3pm CT today? Which number is better for you?
>
> Many thanks,
> Megan
>
> On Thu, Jan 9, 2020 at 11:32 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> > Hi Megan,
> >
> > I'm happy to chat with you later today or tomorrow. Julie's kind words reflect the combined work of many, many people as I'm sure you appreciate, and all done under her able leadership during a very stressful time. I was coordinating the lab efforts of several talented folks on the front lines actually doing the work.
> >
> > I have a meeting today from 2:00-2:30 CT and again at 4:00-5:00 CT. Tomorrow is generally open from about 10:00 am to 4:00 pm CT. My direct line is 409-266-6516.
> >
> > Thanks, Jim
> >
> > James W. Le Duc, Ph.D.
> > Director
> > Galveston National Laboratory
> > University of Texas Medical Branch
> > Galveston, TX 77555-0610
> > (t) 409-266-6500
> > (f) 409-266-6810
> > (m) 409-789-2012

From: Molteni, Megan <megan_molteni@wired.com>
Sent: Thursday, January 09, 2020 1:13 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: WIRED interview: new coronavirus in Wuhan

Hi Jim,

You probably saw Julie's email a little while ago. Any chance you're free today or tomorrow to reminisce about your work identifying SARS and talk about the current situation in Wuhan?

Many thanks,
Megan

On Thu, Jan 9, 2020 at 11:12 AM Molteni, Megan <megan_molteni@wired.com> wrote:

> Thanks Julie, this is hugely helpful. And I appreciate you providing the context in the midst of a retreat. Will follow up with Jim off-thread.
>
> All the best,
> Megan
>
> On Thu, Jan 9, 2020 at 11:01 AM Gerberding, Julie <julie.gerberding@merck.com> wrote:
>
> > Thanks for tracking this story Megan. I am tied up in a retreat unfortunately. The person who led this work is a brilliant global expert, Dr. James (Jim) LeDuc (copied above) who is now a Professor in charge of emerging pathogens at the University of Texas in Galveston. If you recall, with SARS Canadian scientists initially claimed that the infections were caused by a metapneumovirus but that proved to be a false positive. CDC took a more conservative approach. Though we strongly suspected we had isolated the causative coronavirus very early by observing its corona-like structure under an electron microscope, Dr. LeDuc made sure we first essentially satisfied Koch's postulates of disease causality and proved that we could isolate the virus from infected people, inoculate it into a non-human host and recapitulate the disease, and then isolate it from sites of infection in that host. Our approach involved collaborators in Europe who I believe did the actual animal work at the same time we were doing the genetic sequencing. (One lesson learned from the Canadian experience is that virus are ubiquitous

and that isolating and sequencing one does not necessarily mean it is causing the disease.). Hope this background helps. Dr. LeDuc can correct any lapses in my memory.
Best,
jlg
Sent from my iPhone

On Jan 9, 2020, at 12:30 PM, Molteni, Megan <megan_molteni@wired.com> wrote:

> **EXTERNAL EMAIL** – Use caution with any links or file attachments.
> Dr. Gerberding,
>
> I'm a reporter at WIRED and today I'm writing a quick-turn story about how scientists in China have been able to quickly sequence the infectious agent causing pneumonia-like symptoms in about a dozen patients in Wuhan. Specifically, I'm interested in how those methods compare to the search for the pathogen behind SARS in 2003. I reached out to the CDC to speak to someone on the agency's task force at the time and they recommended I get in touch with you. Do you have any time today for a quick phone interview?
>
> Many thanks,
> Megan
>
> --
> Megan Molteni
> Staff Writer | **WIRED**
> o: 415-276-4924
> c: 332-205-1724
> @MeganMolteni

> > > > > > > delete it from your system.
> > > > > >
> > > > > > --
> > > > > > Megan Molteni
> > > > > > Staff Writer | **WIRED**
> > > > > > o: 415-276-4924
> > > > > > c: 332-205-1724
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> > > > > Staff Writer | **WIRED**
> > > > > o: 415-276-4924
> > > > > c: 332-205-1724
> > > > > @MeganMolteni
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Staff Writer | **WIRED**
o: 415-276-4924
c: 332-205-1724
@MeganMolteni

From: 张晗 [zhanghan@wh.iov.cn]
Sent: 4/4/2019 1:49:34 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: 2019 Call Announcement - Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS
Attachments: 2019 Call Announcement.pdf; Application Form.doc

Dear Prof. LeDuc,

Hope this letter finds you well.

Wuhan National Biosafety (P4) Laboratory of Chinese Academy of Sciences (CAS) has been put into operation from 2018.

Relying on the major science and technology infrastructure, the Advanced Customer Cultivation Project (ACCP) initiated by Wuhan Institute of Virology (WIV), CAS aims to cultivate national high-level biosafety talents, to output significant scientific and technological breakthroughs and achievements, and to promote the scientific and technological support capabilities for biosafety and public health.

Now this project is calling for application in 2019 globally, and the submission deadline is on May 5th. Here we are writing to request your consideration to help to promote this project. If available, could you please review the call announcement and help to forward this notice to the relevant research fellows at your side?

We welcome your potential application and thank you very much for your attention and great support.

Notice website: http://english.whiov.cas.cn/Notice2016/201904/t20190404_207607.html

With best wishes,

--
ZHANG Han
Research Planning Office
Wuhan Institute of Virology
Chinese Academy of Sciences
Xiao Hong Shan 44, Wuchang, Wuhan 430071, Hubei, China

Tel: +86-27-87197115
Email: zhanghan@wh.iov.cn

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Advanced Customer Cultivation Project

2019 Call Announcement

Relying on the major science and technology infrastructure, this project aims to cultivate national high-level biosafety talents, to output significant scientific and technological breakthroughs and achievements, and to promote the scientific and technological support capabilities for biosafety and public health. According to the scientific and technological development programs of China, Chinese Academy of Sciences (CAS) and Wuhan Institute of Virology (WIV), CAS, the 2019 Call Announcement of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS is released. Please apply for the project accordingly. The specific contents are as below:

I. Background

Wuhan National Biosafety Laboratory, CAS (hereinafter referred to as Wuhan P4 laboratory) mainly includes protective facilities and experimental equipment for researches on highly pathogenic pathogens. It is incorporated into the management of national major science and technology infrastructure. The project is guided by the principle of "P4 scientific and technological innovation" which means to conduct scientific experimental activities by utilizing Wuhan P4 laboratory or to conduct scientific researches on the biosafety level 4 (P4) pathogens.

II. Management

Advanced Customer Cultivation Project is funded by CAS. The application, review, management and budget implementation are conducted according to

Measures of Academy-Level Scientific Research Projects of CAS and according to relevant measures of WIV, CAS.

III. Qualification

1. The project leader shall be professor or principal investigator with the doctoral degree.

2. The project leader and team members shall be equipped with high-level research capability, solid research foundation and reliable time commitment. Team application is encouraged.

3. To better cooperate and utilize resources of the sharing research platform, research team at home and abroad is encouraged to jointly apply with the research group from WIV, CAS.

IV. Budget

1. Funding

The project application is globally oriented. The categories of the projects include general project and key project with the budget of RMB 250,000/project/year and RMB 500,000/project/year respectively while dynamic adjustment shall be made according to the total budget appropriated by CAS.

2. Period

(1) The implementation period of the project can be 1 to 3 years while the assessment period is 1 year. The project with excellence in the annual assessment can be further funded preferentially.

(2) The budget will be implemented in WIV, CAS. The budget implementation period is 1 year. During the project execution, the project team shall accept the review and examination on task and budget implementation organized by WIV, CAS, and complies with the relevant measures of project prescribed by our institute.

V. Guideline

1. Application

(1) The applicant's organization should provide the opinions and agreement to the application while providing support for the project implementation.

(2) For research project involving ethics in human tissue and biological samples, the applicant should provide the paper version and the electronic version of approval certificate from ethics committee in his/her organization or superior department in charge. The approval certificates of animal welfare and ethics, experimental animal program and biosafety will be completed by the project implementation organization.

(3) For research project in pathogenic microorganism, the application must be complied with the Regulation on the Biosafety Management of Pathogenic Microbe Labs regulated by the State Council of China and provisions on ethics and biosafety by relevant ministries and departments.

(4) The applicant should be responsible for the truthfulness and legality for the submitted materials, and should not provide the project application involving the confidential information.

2. Approval

(1) Under the guidance of Science and Technology Steering Committee of Wuhan National Biosafety Laboratory, CAS, a review committee consisting of 7 to 9 experts shall be organized for Advanced Customer Project, which will include experts on microbiology, biosafety, ethics, animal experiments and P4 laboratory management. The review committee shall obey the avoidance principle.

(2) The review committee will conduct the proposal selection and after the opinion passed the review by the Science and Technology Steering Committee of Wuhan National Biosafety Laboratory, CAS, results of project approval will be released publicly.

3. Implementation and Assessment

(1) The project leader assumes full responsibility for the Advanced Customer Cultivation Project. The project leader shall fulfill the responsibility as an organizer and shall take charge of the preparation of research plan and implementation scheme for this project and be responsible for the timely accomplishment of the project tasks.

(2) Within 1 month before the end of budget implementation period/project conclusion, the project leader shall submit to WIV, CAS the annual assessment report/summary report for project conclusion and assessment. WIV, CAS will organize the review committee to carry out the project conclusion and assessment, and submit the assessment opinions to the Science and Technology Steering Committee of Wuhan National Biosafety Laboratory, CAS.

(3) The project leader could apply for further funding support while submitting the assessment report. The project with excellence in the annual assessment can be further funded preferentially by the review committee.

(4) The research achievements attained during the project implementation, including theses, monographs, patents, software and database etc. shall be marked with "Funded by Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, Chinese Academy of Sciences". Any achievements unmarked will not be counted in the assessment.

VI. Submission

The applicant shall download the 2019 Application Form of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS, fill in the form according to the instruction, submit the paper version (including signature and organization seal) of the application form, relevant attachments including the approval certificate of human ethics, and the electronic version of the corresponding materials before May 5th, 2019 (subject to the posting time). The paper version of the materials shall be submitted in duplicate. The applicant shall send the electronic version of the materials to chendd@wh.iov.cn.

Address:

Wuhan Institute of Virology, Chinese Academy of Sciences

Room 105, No.3 Building

No.44, Xiaohongshan, Wuchang District, Wuhan, Hubei, China

Postcode: 430071

Contact: CHEN Doudou, +86-27-87197115

Attachment: 2019 Application Form of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS

Wuhan Institute of Virology

Chinese Academy of Sciences

April 4th, 2019

No.	
Grant No.	
Confidentiality Level	

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Advanced Customer Cultivation Project

Application Form

Project name:_____

Project leader (Signature):_____

Organization:_____

Phone number:_____

E-mail:_____

Made by Research Planning Office of Wuhan Institute of Virology, CAS

Filled in on (d/m/y)

Instruction for Form Filling

1. Each item of the application form must be true, complete, accurate and clarified.
2. The "Confidentiality Level" on the cover shall be filled in with "Open".
3. All the application materials shall be submitted in duplicate in A4 book size in print (double page).
4. After the form is filled in completely, the applicant's organization shall review the truthfulness, completeness and effectiveness of the information filled in.
5. The application form shall only be considered effective with the signature of the principal of the applicant's organization.

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Basic Information

<table>
<tr><td colspan="2">Project name</td><td colspan="8"></td></tr>
<tr><td colspan="2">Type of project</td><td colspan="8">☐Frontline of the fundamental ☐Major common key technology ☐Application demonstration research ☐Others</td></tr>
<tr><td colspan="2">Funding Category</td><td colspan="8">☐Key Project ☐General Project</td></tr>
<tr><td colspan="2">Budget</td><td colspan="8">Total estimate: (RMB 10,000 yuan) (Note: please calculate for one year only)</td></tr>
<tr><td colspan="2">Implementation period (one year)</td><td colspan="8">From (d/m/y) to (d/m/y)</td></tr>
<tr><td colspan="2">Assessment period</td><td colspan="8">From (d/m/y) to (d/m/y)</td></tr>
<tr><td rowspan="3">Project leader</td><td>Name</td><td></td><td>Sex</td><td></td><td>Birthday</td><td colspan="3"></td></tr>
<tr><td>Title</td><td></td><td>Duty</td><td></td><td>Highest degree</td><td colspan="3"></td></tr>
<tr><td>Organization</td><td colspan="7"></td></tr>
<tr><td rowspan="2">Research group in WIV, CAS</td><td>Principal Investigator (Signature)</td><td colspan="3"></td><td>Person to contact (Signature)</td><td colspan="3"></td></tr>
<tr><td colspan="8"></td></tr>
<tr><td>Project Implementation</td><td colspan="9">☐Authorization ☐Cooperation ☐Independent Completion</td></tr>
<tr><td rowspan="2">Project team</td><td>Total number</td><td>Senior</td><td>Intermediate</td><td>Junior</td><td>Assistant personnel</td><td>Post-doctor</td><td>Doctor candidate</td><td>Master candidate</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="7">Main participants of the project implementation</td><td>Name</td><td>Age</td><td>Title</td><td>Organization</td><td>Time Commitment（Months）</td><td colspan="2">Task Assignment</td><td>Signature</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td colspan="2"></td><td></td></tr>
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</table>

Text

[= 1 * ROMAN]. Research Background

1. Research purpose

2. Foreign and domestic research background, trend of development

3. References

[= 2 * ROMAN]. Research Contents

1. Research contents

2. Research methods and experimental program

(If the "Project Implementation" is filled in with "Independent Completion", the applicant shall provide the risk assessment report on pathogen experiments.)

3. Expected outcome

4. Key problems and technical difficulties to be solved

5. Innovations of the research proposal

[= 3 * ROMAN]. Research Plan

1. Research schedule

2. Conditions necessary to conduct the research (including lab equipments, instruments and etc.)

IV. Introduction of Leader and Participants

1. Education background

2. Working experience

3. Training experience (including experiments involving biosafety, animal infections and etc.)

[= 7 * ROMAN]. Budget

Unit: RMB 10,000 yuan

Budget Form of Project Expenditure		
Item	Amount	Detailed calculation
1. Equipment		
(1) Equipment purchase		
(2) Trial-manufacture purchase		
(3) Equipment modification and rent		
2. Reagents and consumables		
3. Analysis		
4. Fuel and power		
5. Travel/meeting/international cooperation and exchanges		
6. Publication/literature/information dissemination/intellectual property		
7. Labor costs		
8. Expert consultation		
9. Other expenditure		
Total		

Note: Budget preparation and expenditure execution are conducted according to *Measures of Academy-Level Scientific Research Projects of Chinese Academy of Sciences*.

[= 8 * ROMAN]. Review opinions of applicant's organization

(Note: Please indicate in the opinions that the contents for this project application are non-confidential.)

Organization (official seal) Principal (Signature)

(d/m/y)

[= 9 * ROMAN]. Opinions of the Biosafety Committee in Project Implementation Organization

Chairman of Committee (Signature)

(d/m/y)

[= 10 * ROMAN]. Opinions of Science and Technology Steering Committee of Wuhan National Biosafety Laboratory, CAS

Chairman of Committee (official seal)

(d/m/y)

Attachment

(Only for the "Project Implementation" filled in with "Independent Completion")

I. Risk Assessment Report on Pathogen

1. Pathogenicity and infective dose to human and animal
2. Routes of infection and potential future exposure
3. Epidemiologic data
4. Stability in the environment
5. Prevention and therapeutic program
6. Main contents of the experiments
7. Personal protective measure and accident treatment

II. Qualification Certificates from Applicant or Operating Personnel

(including certificates on biosafety training, animal experiments, and etc.)

From: 侯炜 [houwei@whu.edu.cn]
Sent: 3/31/2019 10:16:59 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: Re: Re: Hi

WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Prof. LeDuc,
Thanks for your reply. Wish to your 552.117 with good healthy. And maybe meet you in China.

> -----原始邮件-----
> 发件人: "LeDuc, James W." <jwleduc@UTMB.EDU>
> 发送时间: 2019-04-01 02:30:23 (星期一)
> 收件人: "侯炜" <houwei@whu.edu.cn>
> 抄送: "Shi, Pei yong" <peshi@UTMB.EDU>
> 主题: Re: Hi
>
> Hi Dr Hou
>
> Thanks for your note. Unfortunately my 552.117 has been seriously ill and I have taken some time off to help her recuperate and will not be in next week. I am copying Dr Shinto see if he might have time to meet with you.
>
> With best regards
>
> Jim
>
> Sent from my iPhone
>
> > On Mar 31, 2019, at 10:39 AM, 侯炜 <houwei@whu.edu.cn> wrote:
> >
> > WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
> >
> >
> > Dear Prof.Leduc,
> >
> > Very long time not to connect with you. Here I 'm in Galveston and will visit Dr. Haitao Hu's Lab next week. We have collaborated on multiple projects that investigate HIV and the associated opportunistic co-infections,and has been published on impactful scientific journals such as Plos Pathogens and Journal of Immunology. So this time I wish to have the opportunities to meet with other colleagues here at UTMB that share common research interests with my research groups in the aera of virology besides Dr. Hu. Could you have time to meet with me before I will leave here next Thursday morning?
> >
> >
> > ------------------------------
> > Wei Hou, M.D.,Ph.D.
> > Professor/Vice Dean
> > State Key Laboratory of Virology/Institute of Medical Virology, School of Basic Medical Sciences
> > Wuhan University, P.R.China
> > Dong-hu Road 185, Wuhan 430071
> > Tel:86-27-68789310(office)
> > E-mail: houwei@whu.edu.cn
> >
> >
> > 侯炜 医学博士
> > 教授/副院长
> > 病毒学国家重点实验室/医学病毒学研究所
> > 武汉大学基础医学院
> > 中国武汉
> > 武汉市东湖路185号，邮编430071

> > 联系电话：86-27-68789310
> > E-mail:houwei@whu.edu.cn
> >
> >
> >
> > 积极思考造就积极人生，消极思考造就消极人生。

Wei Hou, M.D.,Ph.D.
Professor/Vice Dean
State Key Laboratory of Virology/Institute of Medical Virology, School of Basic Medical Sciences
Wuhan University, P.R.China
Dong-hu Road 185, Wuhan 430071
Tel:86-27-68789310(office)
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侯炜　医学博士
教授/副院长
病毒学国家重点实验室/医学病毒学研究所
武汉大学基础医学院
中国武汉
武汉市东湖路185号，邮编430071
联系电话：86-27-68789310
E-mail:houwei@whu.edu.cn

积极思考造就积极人生，消极思考造就消极人生。

From: Boyd, Nancy (NIH/NIAID) [E] [nboyd@niaid.nih.gov]
Sent: 2/29/2020 9:43:57 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: Fwd: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

I imagine you got this but sending anyway…

From: Folkers, Greg (NIH/NIAID) [E] <gfolkers@niaid.nih.gov>
Sent: Saturday, February 29, 2020 10:42 AM
Subject: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

FDA News Release

Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics

For Immediate Release:
February 29, 2020

Today, as part of the U.S. Food and Drug Administration's ongoing and aggressive commitment to address the coronavirus outbreak, the agency issued a new policy for certain laboratories seeking to develop diagnostic tests for coronavirus in order to achieve more rapid testing capacity in the U.S.

"We believe this policy strikes the right balance during this public health emergency," said FDA Commissioner Stephen M. Hahn, M.D. "We will continue to help to ensure sound science prior to clinical testing and follow-up with the critical independent review from the FDA, while quickly expanding testing capabilities in the U.S. We are not changing our standards for issuing Emergency Use Authorizations. This action today reflects our public health commitment to addressing critical public health needs and rapidly responding and adapting to this dynamic and evolving situation."

There is currently an outbreak of respiratory disease caused by a novel coronavirus that was first detected in Wuhan City, Hubei Province, China and which has now been detected in 50 locations internationally, including cases in the United States. The virus has been named "SARS-CoV2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to rapidly spread, leading to significant impact on health care systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the U.S. To effectively respond to the COVID-19 outbreak, rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical. This can best be achieved with wide availability of testing capabilities in health care settings, reference and commercial laboratories, and at the point of care.

The new policy is for certain laboratories that develop and begin to use validated COVID-19 diagnostics before the FDA has completed review of their Emergency Use Authorization (EUA) requests. The FDA can issue an EUA to permit the use, based on scientific data, of certain medical products that may be effective in diagnosing, treating or preventing a disease or condition when there is a determination, by the Secretary of Health and Human Services (HHS), that there is a public health emergency or a significant potential for a public health emergency that has a significant potential to affect national security or the health and security of U.S. citizens, and a declaration that circumstances exist justifying the medical products' emergency use.

On Feb. 4, 2020, the Secretary of HHS determined that there is a public health emergency and that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the COVID-19 outbreak. Rapid detection of COVID-19 cases in the U.S. requires wide availability of diagnostic testing to control the

emergence of a rapidly spreading, severe illness. The FDA has authorized one EUA for COVID-19 that is in use by the U.S. Centers for Disease Control and Prevention (CDC) and some public health labs across the country.

The guidance issued today describes a policy enabling laboratories to immediately use tests they developed and validated in order to achieve more rapid testing capacity in the U.S.

"The global emergence of COVID-19 is concerning, and we appreciate the efforts of the FDA to help bring more testing capability to the U.S.," said Nancy Messonnier, M.D., director of the CDC's Center for the National Center for Immunization and Respiratory Diseases (NCIRD).

The immediately in effect guidance issued today describes the circumstances where the FDA does not intend to object to the use of these tests for clinical testing while the laboratories are pursuing an EUA with the FDA. Importantly, this policy only applies to laboratories that are certified to perform high-complexity testing consistent with requirements under Clinical Laboratory Improvement Amendments.

"We applaud the FDA's approach to speed the path toward emergency use authorization for COVID-19 diagnostics. This step may reduce development costs, speed the process for availability at more testing sites, incentivize private development and, ultimately, help save lives," said Rick Bright, Ph.D., director of the Biomedical Advanced Research and Development Authority (BARDA), part of the HHS Office of the Assistant Secretary for Preparedness and Response. "At BARDA, we are identifying industry partners to develop rapid diagnostics that can be used in commercial and hospital labs or even doctors' offices so that medical professionals and their patients have the information they need to take action."

The FDA guidance provides recommendations for test developers, including information regarding test validation, FDA notification and interim confirmatory clinical testing.

Following the completion of their test validation, laboratories should communicate with the FDA, via email, in order to notify the agency that the test has been validated. Laboratories should submit a completed EUA request within 15 business days of notification.

"Under this policy, we expect certain laboratories who develop validated tests for coronavirus would begin using them right away prior to FDA review," said Jeff Shuren, M.D., J.D., director of the FDA's Center for Devices and Radiological Health. "We believe this action will support laboratories across the country working on this urgent public health situation. We are dedicating all available resources to expediting the review of medical products, including diagnostics, to prevent the spread of this outbreak."

The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

###

Inquiries

Media:
Stephanie Caccomo
301-348-1956
Consumer:
888-INFO-FDA

From: Boyd, Nancy (NIH/NIAID) [E] [nboyd@niaid.nih.gov]
Sent: 2/29/2020 12:51:39 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: Re: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

Oh my! At least it is probably quiet and it will allow you to catch up a bit.

Get Outlook for iOS

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Saturday, February 29, 2020 1:14:25 PM
To: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Subject: RE: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

At the office…

From: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Sent: Saturday, February 29, 2020 11:11 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

You are welcome, Jim. I hope you are having a restful weekend!

Nancy

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Saturday, February 29, 2020 11:17 AM
To: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Subject: Re: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnosticshttp://bit.ly/3adujf6

Thanks for sharing. I saw a draft but hadn't seen the final.

Sent from my iPhone

On Feb 29, 2020, at 9:44 AM, Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov> wrote:

I imagine you got this but sending anyway…

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The immediately in effect guidance issued today describes the circumstances where the FDA does not intend to object to the use of these tests for clinical testing while the laboratories are pursuing an EUA with the FDA. Importantly, this

policy only applies to laboratories that are certified to perform high-complexity testing consistent with requirements underClinical Laboratory Improvement Amendments.

"We applaud the FDA's approach to speed the path toward emergency use authorization for COVID-19 diagnostics. This step may reduce development costs, speed the process for availability at more testing sites, incentivize private development and, ultimately, help save lives," said Rick Bright, Ph.D., director of the Biomedical Advanced Research and Development Authority (BARDA), part of the HHS Office of the Assistant Secretary for Preparedness and Response. "At BARDA, we are identifying industry partners to develop rapid diagnostics that can be used in commercial and hospital labs or even doctors' offices so that medical professionals and their patients have the information they need to take action."

The FDA guidance provides recommendations for test developers, including information regarding test validation, FDA notification and interim confirmatory clinical testing.

Following the completion of their test validation, laboratories should communicate with the FDA, via email, in order to notify the agency that the test has been validated. Laboratories should submit a completed EUA request within 15 business days of notification.

"Under this policy, we expect certain laboratories who develop validated tests for coronavirus would begin using them right away prior to FDA review," said Jeff Shuren, M.D., J.D., director of the FDA's Center for Devices and Radiological Health. "We believe this action will support laboratories across the country working on this urgent public health situation. We are dedicating all available resources to expediting the review of medical products, including diagnostics, to prevent the spread of this outbreak."

The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

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Inquiries

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From: Boyd, Nancy (NIH/NIAID) [E] [nboyd@niaid.nih.gov]
Sent: 1/29/2020 2:26:47 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: RE: Houston Chronicle story

HI Jim. I am sorry to hear that your HI trip is canceled. Better safe than sorry though.

Nice article below! Thanks for sharing.

Just let me know when you are available and want to touch base. Sometime tomorrow?

Nancy

Nancy Boyd
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Office of Biodefense, Research Resources and Translational Research
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National Institutes of Health, DHHS
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From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, January 29, 2020 11:18 AM
To: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Subject: FW: Houston Chronicle story

Fyi. We should chat sometime soon. Unfortunately, I canceled our trip to Hawaii due to the nCoV activities here in the lab.

From: Reyes, Raul <rareyes@UTMB.EDU>
Sent: Wednesday, January 29, 2020 9:31 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>; Holubar, Connie J. <cjholuba@UTMB.EDU>
Cc: Campbell, Stephen <stepcamp@UTMB.EDU>
Subject: Houston Chronicle story

Good morning,

The Chronicle story begins with a quote from a Methodist doctor but then the rest of the story pretty much revolves around the GNL, LeDuc quotes/statements and general background.

Thank you again for speaking to this and other reporters.

Best,

Raul

Why Houston is uniquely situated to be better prepared for the coronavirus threat

JULIE GARCIA

As the number of confirmed coronavirus cases continues to rise globally, Houston-area experts are confident the city is well-equipped to handle a pandemic.

"We're pretty prepared for it," said Dr. Sarfraz Aly, infectious disease physician with Houston Methodist, who pointed out proactive measures being taken, including "screening areas at airports for symptoms. We're ready for this."

Worldwide, more than 6,000 people across 17 countries have been infected with the flu-like virus, and the death toll hit 132 on Tuesday, according to the National Health Commission. Five people have been diagnosed in the U.S. There have been no confirmed cases in Texas; after students at Baylor and Texas A&M each tested negative earlier this month.

"It's kind of a perfect storm. It's a brand new disease, so the general public doesn't have a pre-existing immunity, and it occurred in China when literally everyone goes home to celebrate Lunar New Year," said Dr. James Le Duc, director of the Galveston National Laboratory at the University of Texas Medical Branch at Galveston. "There's a lot of reasons why it's a concern, but the world is doing its best to control it."

On Monday, the George Bush Intercontinental Airport announced it would start screening travelers for the coronavirus as a precaution. The Houston airport is among 15 nationwide chosen by the Centers for Disease Control and Prevention for virus testing.

The largest concentration of patients is in Wuhan, a city in the Hubei province of China, where the virus originated.

It's possible that the virus, which results in severe cold and flu symptoms, was hiding in an animal before spilling over to infect the human population in December. The 2019 novel coronavirus, or 2019-nCoV, is considered a "brand new virus" to the global scientific community, said Le Duc.

"It's called novel because we've never seen it before," he said. This means there's no vaccine for it yet.

On HoustonChronicle.com: Oil prices fall to 3-month low as coronavirus adds to downward pressure

After SARS (severe acute respiratory syndrome) spread to 24 countries in 2003, the U.S. began strategically placing stockpiles of supplies around the country in the event of future pandemics.

"In the past two decades, the U.S. government has invested heavily in preparedness issues," Le Duc said. "Stored across the country are various products — masks, personal protective equipment — that are very appropriate if an outbreak started."

The Strategic National Stockpile is the nation's largest supply of pharmaceuticals and medical supplies "for use in a public health emergency severe enough to cause local supplies to run out," according to the U.S. Department of Health and Human Services.

The goal, Le Duc said, is to be able to deliver supplies to cities like Houston within 12 hours of a federal decision to deploy.

And Houston is uniquely situated to be even better prepared than other cities. The area is home to Galveston's Center for Biodefense and Emerging Infectious Diseases, one of only a handful of high-level containment laboratories in the country. Scientists at the facility have been working on a number of vaccines for different pathogens, including known coronaviruses, since before the outbreak in Wuhan.

Vaccine experiments for novel coronavirus haven't began, Le Duc said. But he does know that scientists will build upon what they learned during similar outbreaks with Ebola, SARS and Middle Eastern Respiratory Syndrome, or MERS.

Chinese health officials quickly published genomic sequences of the coronavirus' isolates, or a culture of microorganisms, but it's not the same as the actual virus that causes infection, Le Duc said. On Tuesday, the Chinese government finally allowed the World Health Organization to send international experts to help with research and containment, a move that had been delayed for weeks.

"If we want to seriously develop therapeutics, antiviral drugs or a vaccine, we need to have access to the real virus that comes from a sick person," Le Duc said. "We're well-positioned to do that work here in the appropriate levels of bio-containment."

According to the Centers for Disease Control and Prevention, the virus is in the same family of coronaviruses as SARS, which originated in civet cats, and MERS, which jumped from camels to people. Similarly, this novel coronavirus likely originated from an animal source, and is now quickly transferring from person to person.

On HoustonChronicle.com: Chinese film 'The Rescue' pulled in Houston due to the coronavirus

Experts agree that common-sense hygienic practices, such as washing your hands multiple times a day and not going to work or school when sick, will help stave off its spread.

The key is monitoring the severity of the symptoms and getting tested early, said Aly of Houston Methodist.

Aly advises that anyone who has traveled to China, or been exposed to someone who has traveled to China within the last two weeks, should go to the emergency room for a diagnostic test if they experience any flu-like symptoms, including fever, cough and respiratory distress.

"If you have been in airports and the symptoms are mild, you're a limited risk, so put on a mask and go to a primary care office," he said. "Once in the hospital, most patients are isolated efficiently. There are masks and gowns readily available, and they would be isolated in a pressurized room."

However, while primary care doctors can test for flu, the diagnostic test for this strain of coronavirus is only available at hospitals.

julie.garcia@chron.com

Twitter.com/reporterjulie

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/2/2020 4:19:35 PM
To: Auchincloss, Hugh (NIH/NIAID) [E] (auchinclossh@niaid.nih.gov) [auchinclossh@niaid.nih.gov]; Erbelding, Emily (NIH/NIAID) [E] [emily.erbelding@nih.gov]; Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Major publication due out Friday
Attachments: Revision SARS2 clone editor suggestions Mar 31 2020.docx; Final Figures Mar 22 2020.pdf

Hugh, Emily and Nancy,

Pasted below is Pei-Yong Shi's note to our communications office about the release tomorrow of our paper on the development of a reverse genetics system and report SARS-CoV-2 virus. This is a major accomplishment and we want to give you a heads up that it will be appearing soon.

Our paper on developing the reverse genetic system and reporter SARS-Cov-2 will be published online tomorrow. This represents one of the most important tools (if not the most important) to study the virus replication, transmission, and pathogenesis.

More importantly, the reporter virus will unleash our limitation to perform serodiagnosis, vaccine evaluation, and therapeutic development. We have already transferred the reagents to New York State Health Department and in the process to share with CDC for serology testing. Our technology has attracted partnership with leading pharmaceutical companies (e.g., Q2 Solutions, Gilead, and others) to jointly fight COVID-19.

Be safe,

Jim

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An infectious cDNA clone of SARS-CoV-2

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Running title: An infectious cDNA clone of SARS-CoV-2

Keywords: Coronavirus, SARS-CoV-2, COVID-19, SARS-CoV, vaccine, antiviral

SUMMARY

The ongoing pandemic of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), underscores the urgency to develop experimental systems for studying this virus and identifying countermeasures. We report a reverse genetic system for SARS-CoV-2. Seven cDNA fragments spanning the SARS-CoV-2 genome were assembled into a full-genome cDNA. RNA transcribed from the full-genome cDNA was highly infectious after electroporation into cells, producing 2.9×10^6 PFU/ml of virus. Compared with a clinical isolate, the infectious clone-derived SARS-CoV-2 (icSARS-CoV-2) exhibited similar plaque morphology, viral RNA profile, and replication kinetics. Additionally, icSARS-CoV-2 retained engineered molecular markers and did not acquire other mutations. A stable mNeonGreen SARS-CoV-2 (icSARS-CoV-2-mNG) was generated by introducing this reporter gene into OFR7 of the viral genome. icSARS-CoV-2-mNG was successfully used to evaluate the antiviral activities of interferon. Collectively, the reverse genetic system and reporter virus provide key reagents to study SARS-CoV-2 and develop countermeasures.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in early 2020 with human cases in Wuhan, China [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It has rapidly rampaged worldwide, causing a pandemic of coronavirus disease (COVID-19) that ranges from fever and breathing difficulty to acute respiratory distress and death [ADDIN EN.CITE ADDIN EN.CITE.DATA]. With over 300,000 people infected in less than 3 months, SARS-CoV-2 causes the most severe disease in older patients and people with comorbidities, including heart disease, diabetes, and other health conditions [ADDIN EN.CITE <EndNote><Cite><Author>Wu</Author><Year>2020</Year><RecNum>7072</RecNum><DisplayText>(Wu and McGoogan, 2020)</DisplayText><record><rec-number>7072</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584734140">7072</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wu, Z.</author><author>McGoogan, J. M.</author></authors></contributors><auth-address>Chinese Center for Disease Control and Prevention, Beijing, China.</auth-address><titles><title>Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention</title><secondary-title>JAMA</secondary-title></titles><periodical><full-title>JAMA</full-title></periodical><dates><year>2020</year><pub-dates><date>Feb 24</date></pub-dates></dates><isbn>1538-3598 (Electronic)
0098-7484 (Linking)</isbn><accession-num>32091533</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/32091533</url></related-urls></urls><electronic-resource-num>10.1001/jama.2020.2648</electronic-resource-num></record></Cite></EndNote>]. Before 2019, six α- and β-coronaviruses were known to cause respiratory diseases of different severity, including four common cold coronaviruses (229E, NL63, OC43, and HKU1) and two highly pathogenic coronaviruses [severe acute

respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), which emerged in 2003 and since 2012, respectively] [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Importantly, with massive hospitalization rates and high mortality, SARS-CoV-2 remains a major threat to humankind and intervention strategies are being rapidly pursued.

A key tool in responding to emergent viruses is the generation of reverse genetic systems to explore and characterize new pathogens. Classically, reverse genetic systems for coronaviruses have been complicated by their large genome size (~30,000 nucleotides) and the existence of bacteriotoxic elements in their genome that make them difficult to propagate [ADDIN EN.CITE <EndNote><Cite><Author>Almazan</Author><Year>2014</Year><RecNum>7158</RecNum><DisplayText>(Almazan et al., 2014)</DisplayText><record><rec-number>7158</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584739078">7158</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Almazan, F.</author><author>Sola, I.</author><author>Zuniga, S.</author><author>Marquez-Jurado, S.</author><author>Morales, L.</author><author>Becares, M.</author><author>Enjuanes, L.</author></authors></contributors><auth-address>Department of Molecular and Cell Biology. Centro Nacional de Biotecnologia (CNB-CSIC), Campus Universidad Autonoma de Madrid, C/ Darwin 3, Cantoblanco, 28049 Madrid, Spain.
Department of Molecular and Cell Biology. Centro Nacional de Biotecnologia (CNB-CSIC), Campus Universidad Autonoma de Madrid, C/ Darwin 3, Cantoblanco, 28049 Madrid, Spain. Electronic address: L.Enjuanes@cnb.csic.es.</auth-address><titles><title>Coronavirus reverse genetic systems: infectious clones and replicons</title><secondary-title>Virus Res</secondary-title></titles><periodical><full-title>Virus Res</full-title></periodical><pages>262-70</pages><volume>189</volume><keywords><keyword>Clone Cells</keyword><keyword>Coronavirus/*genetics</keyword><keyword>Replicon</keyword><k

eyword>Reverse Genetics/*methods</keyword><keyword>Virology/*methods</keyword><keyword>Coronavirus </keyword><keyword>Infectious clones</keyword><keyword>Replicons</keyword><keyword>Reverse genetics</keyword></keywords><dates><year>2014</year><pub-dates><date>Aug 30</date></pub-dates></dates><isbn>1872-7492 (Electronic)
0168-1702 (Linking)</isbn><accession-num>24930446</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/24930446</url></related-urls></urls><custom2>PMC4727449</custom2><electronic-resource-num>10.1016/j.virusres.2014.05.026</electronic-resource-num></record></Cite></EndNote>].

Several approaches have been devised to overcome this barrier, such as multiple plasmid systems to disrupt toxic elements and to reduce deletions/truncations [ADDIN EN.CITE <EndNote><Cite><Author>Yount</Author><Year>2002</Year><RecNum>7014</RecNum><DisplayText>(Yount et al., 2002)</DisplayText><record><rec-number>7014</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584619790">7014</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Yount, B.</author><author>Denison, M. R.</author><author>Weiss, S. R.</author><author>Baric, R. S.</author></authors></contributors><auth-address>Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7435, USA.</auth-address><titles><title>Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>11065-78</pages><volume>76</volume><number>21</number><keywords><keyword>Animals</keyword><keyword>Cell Line</keyword><keyword>Cricetinae</keyword><keyword>DNA, Viral/*analysis</keyword><keyword>Mice</keyword><keyword>Murine hepatitis

virus/*genetics</keyword><keyword>Phenotype</keyword><keyword>RNA Replicase/biosynthesis</keyword><keyword>Tumor Cells, Cultured</keyword><keyword>Viral Proteins/biosynthesis</keyword></keywords><dates><year>2002</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>12368349</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/12368349</url></related-urls></urls><custom2>PMC136593</custom2><electronic-resource-num>10.1128/jvi.76.21.11065-11078.2002</electronic-resource-num></record></Cite></EndNote>]. Using this approach, researchers have developed infectious clones for several coronaviruses, including SARS-CoV, MERS-CoV, and others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Thao *et al.* recently reported a yeast-based synthetic genomics platform for rapid construction of infectious clones for murine hepatitis coronavirus (MHV-CoV), MERS-CoV, and SARS-CoV-2 [ADDIN EN.CITE <EndNote><Cite><Author>Thao</Author><Year>2020</Year><RecNum>7201</RecNum><DisplayText>(Thao et al., 2020)</DisplayText><record><rec-number>7201</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584791671">7201</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Thao, T.T.N.</author><author>Labroussaa, F.</author><author>Ebert, N.</author><author>V'kovski, P.</author><author>Stalder, H.</author><author>Portmann, J.</author><author>Kelly, J.</author><author>Steiner, S.</author><author>Holwerda, M.</author><author>Kratzel, A.</author><author>Gultom, M.</author><author>Laura Laloli,</author><author>Hüsser, L.</author><author>Wider, M.</author><author>Pfaender, S.</author><author>Hirt, D.</author><author>Cippà, V.</author><author>Crespo-Pomar, S.</author><author>Schröder, S.</author><author>Muth, D.</author><author>Niemeyer, D.</author><author>Müller, M.</author><author>Drosten, C.</author><author>Dijkman,

R.</author><author>Jores, J. </author><author>Thiel, V.</author></authors></contributors><titles><title>Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.02.21.959817</electronic-resource-num></record></Cite></EndNote>]. However, the yeast platform-produced SARS-CoV-2 has not been fully characterized for its biological properties (*e.g.*, replication kinetics) in comparison with its original clinical isolate. Such characterization is essential for ensuring the quality of the genetic system to rescue recombinant viruses that recapitulate the biological features of their corresponding clinical isolates. Once validated, the reverse genetic systems allow rapid characterization of novel viruses, development of reporter viruses, and generation of live-attenuated vaccine candidates to respond to emerging infections. Together with animal pathogenesis models, reverse genetic systems offer powerful tools needed to characterize, understand, and respond to emerging virus outbreaks.

In response to the ongoing pandemic of SARS-CoV-2, we have developed a robust reverse genetic system for SARS-CoV-2 and a mNeonGreen reporter virus. Recombinant virus derived from the system recapitulates the replication kinetics of the original clinical isolates. In addition, the mNeonGreen reporter remains stable for at least five passages, allowing its use in long-term studies. Using type-I interferon, we demonstrated that the mNeonGreen virus could be reliably used to study viral replication and pathogenesis as well as to develop vaccine and antiviral drugs.

RESULTS

Design of a SARS-CoV-2 full-length cDNA. An *in vitro* ligation approach, similar to that for constructing the infectious clones of SARS-CoV and MERS [ADDIN EN.CITE ADDIN EN.CITE.DATA], was designed to directionally assemble the full-length cDNA of the SARS-CoV-2 genome (Figure 1A). Our reverse genetic system was based on the virus strain (2019-nCoV/USA_WA1/2020) isolated from the first reported SARS-CoV-2 case in the US [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Viral RNA, extracted from the passage 4 virus from Vero E6 cells, was used as a template for RT-PCR to produce cDNA fragments. Seven contiguous cDNA fragments were constructed to cover the entire viral genome (Figure 1B). Some of the seven cDNA fragments were prepared through RT-PCR, whereas others were generated by chemical synthesis (see Materials and Methods for details). All cDNA fragments were individually cloned into plasmid vectors. For facilitating directional assembly of genome-length cDNA, each cDNA fragment was flanked by a class IIS restriction endonuclease site (BsaI or Esp3I). The class IIS endonucleases recognize asymmetric DNA sequences, cleave outside their recognition sequences, and generate unique cohesive overhangs (Figure 1C). After digestion with BsaI or Esp3I, the seven fragments were directionally ligated to assemble the genome-length cDNA. The unique cohesive ends of each fragment ensured one directional, seamless assembly of the seven fragments with the concomitant loss of the restriction enzyme sites. Figure 1C depicts the details of the seven fragments: F1 (T7 promoter sequence plus nucleotides 1-3,618), F2 (3,619-7,504), F3 (7,505-11,984), F4 (11,985-17,591), F5 (17,592-22,048), F6 (22,049-26,332), and F7 (26,333-29,870 plus a poly$(A)_{29}$ sequence). A T7 promoter and a poly$(A)_{29}$ tail were engineered at the upstream of F1 and the downstream of F7, respectively. *In vitro* transcription of the ligated F1-7 DNA was expected to produce a 5' capped (as cap analog was included in the *in vitro* transcription reaction) and 3' polyadenylated genome-length RNA. To differentiate the infectious clone-derived virus from the parental clinical isolate, we engineered three synonymous nucleotide mutations as markers.

Assembly of a SARS-CoV-2 full-length cDNA. Each of the seven cDNA fragments was cloned into a plasmid and sequenced to ensure no undesired mutations. For assembly of full-length cDNA, the seven cDNA plasmids were digested with BsaI or Esp3I. The resulting cDNA fragments were gel-purified (Fig. 1D), then *in vitro* ligated to assemble the genome-length cDNA in three steps: (i) ligation of F1, F2, F3, and F4 to produce F1-4 cDNA; (ii) ligation of F5, F6, and F7 to produce F5-7 cDNA; and (iii) ligation of F1-4 and F5-7 to produce the full-length F1-7 cDNA. Agarose gel analysis of the ligation (iii) reaction showed a major DNA product representing the size of genome-length cDNA (~29.87 kb, indicated by an arrow in Figure 1E) in addition to several smaller intermediate cDNA products (indicated by circles). *In vitro* transcription using the cDNA template [directly from ligation (iii) without gel purification] generated multiple RNA bands, among which a faint high molecular band may represent the genome-length RNA (indicated by an arrow in Figure 1F) together with several smaller RNA transcripts (indicated by circles).

Recovery of recombinant SARS-CoV-2. To recover recombinant SARS-CoV-2 from the infectious cDNA clone (icSARS-CoV-2), we electroporated *in vitro* transcribed genome-length RNA into Vero E6 cells. The RNA transcription mixture from Figure 1F was directly electroporated into cells without purification. Since N protein was reported to enhance the infectivity of coronavirus RNA transcripts [ADDIN EN.CITE ADDIN EN.CITE.DATA], we co-electroporated an mRNA encoding the SARS-CoV-2 N protein with the full-length RNA. The transfected cells developed cytopathic effects (CPE) on day 4 post-transfection and produced infectious virus [denoted as passage 0 (P0) virus] with a titer of 2.9×10^6 PFU/ml (Figure 2A). It is worth emphasizing that such a high titer of recombinant virus was produced directly from the electroporated cells without additional rounds of cell culture passaging, indicating the robustness of the system and also suggesting a lack of any errors. Next, we compared the replication properties between the recombinant virus and the original clinical isolate. The wild-type icSARS-CoV-2 (icSARS-CoV-2-WT) developed plaques similar to the original clinical

isolate (Figure 2B) and exhibited equivalent replication kinetics on Vero E6 cells (Figure 2C). We did not extend the time points of replication beyond 48 h because CPE was observed at 40-48 h post-infection (p.i.). Northern blot analysis showed that viral mRNA species from the clinical isolate-infected cells and the icSARS-CoV-2-infected cells were identical to the predicted set of genome-length RNA and eight subgenomic RNAs (Figure 2D). Full-genome sequencing showed that the recombinant virus retained the three engineered synonymous mutations with no other sequence changes, demonstrating the rescued virus did not result from contamination by the parental virus isolate (Figure 2E). Furthermore, DNA sequencing chromatogram did not show any partial reversion of the three engineered molecular markers (Figure 2F). Collectively, the results demonstrate that (i) the *in vitro* transcribed full-length RNA is highly infectious upon electroporation into cells and (ii) the recombinant virus recapitulates the replication properties of the original clinical isolate on Vero E6 cells.

Development and characterization of mNeonGreen SARS-CoV-2. Reporter viruses are useful tools to study viral replication and pathogenesis and to develop countermeasure. To establish a reporter SARS-CoV-2 infectious clone, we engineered an mNeonGreen (mNG) gene into the ORF7 of viral genome (Figure 3A), similar to the SARS-CoV reporter [ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>180</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>180</rec-number><foreign-keys><key app="EN" db-id="5txwd0dw9fwdpuesvvjx5pvs90ve5rwttr05" timestamp="1584885311">180</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P. L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome

coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title><abbr-1>Journal of virology</abbr-1></periodical><pages>15511-24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypeptidases/analysis</keyword><keyword>Coronavirus Infections/enzymology/*metabolism</keyword><keyword>Epithelial Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS Virus/*physiology</keyword><keyword>Severe Acute Respiratory Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>16306622</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-urls></urls><custom2>PMC1316022</custom2><electronic-resource-num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-num></record></Cite></EndNote>]. The same *in vitro* ligation and transcription protocols (described above) were used to prepare the mNG full-length RNA. After electroporation, we recovered icSARS-CoV-2-mNG (6.9×10^6 PFU/ml). To examine if the reporter gene attenuates viral replication, we compared the replication properties between the wild-type and reporter viruses on Vero E6 cells. The icSARS-CoV-2-mNG produced plaques similar to those of the icSARS-CoV-WT (compare Figures 3B with 2B). Indistinguishable replication kinetics were observed for the icSARS-CoV-2-mNG and icSARS-CoV-WT (Figure 3C). Infection with icSARS-CoV-2-mNG developed increasing numbers of mNG-positive cells over time (Figure 3D). Concurrently, the fluorescent signals increased from 12 to 48 h p.i. (Figure 3E). At 12-36 h p.i., the level of fluorescent signal correlated with the initial MOIs, whereas a reverse trend was

observed at 48 h p.i., most likely due to earlier CPE caused by the higher MOI. Full-genome sequencing showed that icSARS-CoV-2-mNG retained the three engineered markers with no additional mutations (Figure 3F). These results indicate that icSARS-CoV-2-mNG is initially stable, maintains the wild-type replication, and expresses robust mNG in Vero E6 cells.

Stability of icSARS-CoV-2-mNG. To examine the longer-term stability of icSARS-CoV-2-mNG, we serially passaged the reporter virus on Vero cells for 5 rounds (1 to 2 days per round). Cells infected with equal PFU of passage 1 (P1) or passage 5 (P5) viruses produced comparable numbers of mNG-positive cells (Figure 4A). Next, RT-PCR was performed to verify the retention of mNG in the P1 and P5 viral genomes using two primers targeting the insertion junctions (corresponding to nucleotides 25,068-28,099 of the viral genome). As expected, the RT-PCR products derived from both P1 and P5 mNG viruses were larger than those from the wild-type icSARS-CoV-2 (Figure 4B, lanes 1-3). Digestion of the RT-PCR products with BsrGI (located upstream of the mNG insertion site) and StuI (in the mNG gene) developed distinct cleavage patterns between the reporter and wild-type viruses, whereas P1 and P5 viruses produced an identical digestion pattern (Figure 4B, lanes 4-6). Finally, sequencing the P1 and P5 RT-PCR products confirmed the retention of the mNG gene (data not shown). Altogether, the results demonstrate the stability of icSARS-CoV-2-mNG after five rounds of passaging on Vero E6 cells. Since Vero E6 cells are defective in type-1 interferon production, it remains to be tested if the reporter virus is stable when passaged on interferon-competent cell lines.

Application of icSARS-CoV-2-mNG. To explore the utility of icSARS-CoV-2-mNG, we used the reporter virus to rapidly screen the antiviral activity of a known inhibitor of coronaviruses. We previously showed that pre-treatment of Vero cells with type-I interferon (IFN) inhibits SARS-CoV-2 replication [ADDIN EN.CITE <EndNote><Cite><Author>Lokugamage</Author><Year>2020</Year><RecNum>7202</RecNum><DisplayText>(Lokugamage et al., 2020)</DisplayText><record><rec-number>7202</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a"

timestamp="1584807870">7202</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lokugamage, K.G.</author><author>Hage, A.</author><author>Schindewolf, C.</author><author>Rajsbaum, R.</author><author>Menachery, V.D.</author></authors></contributors><titles><title>SARS-CoV-2 sensitive to type I interferon pretreatment</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.03.07.982264</electronic-resource-num></record></Cite></EndNote>]. Here we explored the dose responsive effect of IFN-a pre-treatment on icSARS-CoV-mNG replication (Figure 4C). No mNG expression was visually observed when the infected cells were pre-treated with the highest dose of IFN-α (1,000 u/ml), whereas a dose-dependent reduction of mNG signal was detected at an intermediate dose (111 u/ml) (Figure 4D). Quantification of the fluorescent readouts estimated an EC_{50} (concentration inhibiting 50% of viral replication) of 101 u/ml, confirming the inhibition of SARS-CoV-2 by IFN-α (Figure 4E). This result is consistent with previous findings that SARS-CoV-2 is sensitive to type-I IFN inhibition. The reporter virus assay required fewer days and less labor when compared with the conventional plaque reduction assay. Collectively, the results indicate that icSARS-CoV-2-mNG could be reliably used to study SARS-CoV-2 replication and to screen antiviral inhibitors.

DISCUSSION

We report the development of a full-length infectious clone and a reporter virus for SARS-CoV-2. One of the key utilities for the reverse genetic system is to facilitate antiviral testing and therapeutic development. The icSARS-CoV-2 mNG reporter virus allows the use of fluorescence as a surrogate readout for viral replication. Compared with a standard plaque assay or $TCID_{50}$ quantification, the fluorescent readout shortens the assay turnaround time by

several days. In addition, the fluorescent readout offers a quantitative measure that is less labor-intensive than the traditional means of viral titer reduction. Furthermore, the mNG virus-based assay could be automated in a high-throughput format to screen compounds against viral replication. As a proof-of-concept, we demonstrated that, after treatments with type-I IFN, the reporter virus reliably revealed efficacy in a rapid and efficient manner. In addition, the stability of the mNG reporter virus allows it to be used for longer-term studies and *in vivo* without fear of losing its fluorescent marker. Thus, this reporter virus offers a huge advantage for research community and pharmaceutical companies to develop therapeutics for COVID-19.

Our reverse genetic system represents a major reagent in the pursuit of understanding SARS-CoV-2 and COVID-19 disease. Compared with the clinical isolate, the recombinant wild-type SARS-CoV-2 has no deficit in terms of viral RNA species produced, plaque morphology, or replication kinetics. Therefore, it may be used as an equivalent to the clinical strain, and mutant viruses can be generated to characterize mutational effect on viral infection. This approach has allowed researchers to identify key viral antagonists of innate immunity for SARS-CoV and MERS-CoV [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Several of these mutant viruses have subsequently been employed as live-attenuated vaccine candidates for SARS-CoV and MERS-CoV [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Using our system, this knowledge may now be applied to the current SARS-CoV-2. Characterizing these mutations may provide insight into SARS-CoV-2 pathogenesis.

Our reverse genetic system also allows exploration of research questions fundamental to understanding the SARS-CoV-2 pandemic. As additional genomic sequences become available, evolutionary mutations can be interrogated for their effect on viral transmission and disease outcome. For example, a 382-nucleotide deletion covering almost the entire ORF8 of SARS-CoV-2 was observed in eight hospitalized patients in Singapore; virus isolation of the deletion strains has not been reported in the study [ADDIN EN.CITE <EndNote><Cite><Author>Su</Author><Year>2020</Year><RecNum>7249</RecNum><Displ

ayText>(Su et al., 2020)</DisplayText><record><rec-number>7249</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584819541">7249</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Su, Y.C.F.</author><author>Anderson, D.E. </author><author>Young, B.E. </author><author>Zhu, F.</author><author>Linster, M. </author><author>Kalimuddin, S. </author><author>Low, J.G.H. </author><author>Yan, Z.</author><author>Jayakumar, J. </author><author>Sun, L.</author><author>Yan, G.Z. </author><author>Mendenhall, I.H.</author><author>Leo, Y.-S.</author><author>Lye, D.C.</author><author>Wang, L.-F.</author><author>Smith, G.J.D.</author></authors></contributors><titles><title>Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.03.11.987222</electronic-resource-num></record></Cite></EndNote>]. A four-amino acid insertion (conferring a possible furin cleavage site) was reported in the spike (S) protein of SARS-CoV-2, but is absent in the S protein of SARS-CoV and other group 2B CoVs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Using the infectious clone, we can now evaluate the impact of these genetic changes by removing the reported sequences from SARS-CoV-2 and examine their effect on virus replication and S protein processing. In addition, mouse models for SARS-CoV-2 have been limited by the absence of virus capable of binding to mouse ACE2 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Point mutations in the receptor binding domain of SARS-CoV-2 S protein may facilitate mouse adaptation and development of a model that recapitulates human diseases in a standard mouse strain. Altogether, the above questions are a few examples of how our infectious clone can be used to advance SARS-CoV-2 research.

In summary, we have developed a robust reverse genetic system for SARS-CoV-2 that can be used to study viral replication and pathogenesis. We have also established an mNG reporter SARS-CoV-2 that is a reliable surrogate for high-throughput drug discovery. The reverse genetic system represents a major tool for the research community and significantly advances opportunities for countermeasure development for COVID-19.

ACKNOWLEDGMENTS

We thank Natalie Thornburg and other colleagues from the Centers for Disease Control and Prevention for providing the clinical virus isolate. We also thank colleagues at UTMB for support and discussions. Research was supported by grants from NIA and NIAID of the NIH (U19AI100625 and R00AG049092 to V.D.M.; R24AI120942 (WRCEVA) to S.C.W.; AI114657 and AI146081 to S.M.; 5UC7AI094660 to J.W.L.). Research was also supported by STARs Award provided by the University of Texas System to V.D.M., trainee funding provided by the McLaughlin Fellowship Fund at UTMB, and IHII Pilot grant to SM. P.-Y.S. was supported by NIH grants AI142759, AI145617, AI[HYPERLINK "https://public.era.nih.gov/grantfolder/piAppDetails/genericStatus.do?encryptedParam=vXeG1axw2cY.YZxjFgAisBblI6OqVj4LxDR99WdVyTI-X4FuJbCLt4g." \t "_blank"], AI136126, and UL1TR001439, and awards from the Kleberg Foundation, John S. Dunn Foundation, Amon G. Carter Foundation, Gilson Longenbaugh Foundation, and Summerfield Robert Foundation. A.M. is supported by a Clinical and Translational Science Award NRSA (TL1) Training Core (TL1TR001440) from NIH.

AUTHOR CONTRIBUTIONS

Conceptualization, X.X., V.D.M., P.-Y.S.; Methodology, X.X., A.M., K.G.L., K.N., X.Z., J.Z., J.L., C.S., N.B., P.A., K.S.P., S.W, S.M., J.W.L., V.D.M, P.-Y.S.; Investigation,

X.X., A.M., K.G.L., K.N., X.Z., J.Z., J.L., C.S., N.B., P.A.; Resources, K.S.P., S.W., C.-T.K.T.; Data Curation, X.X., A.M., K.G.L., K.N., J.L., N.B.; Writing-Original Draft, X.X., K.N., V.D.M., P.-Y.S.; Writing-Review & Editing, X.X., V.D.M., P.-Y.S.; Visualization, X.X., A.M., K.G.L., N.B., and P.-Y.S.; Supervision, X.X., V.D.M., P.-Y.S.; Funding Acquisition, P.A., S.W., S.J., J.W.L., V.D.M., P.-Y.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

MAIN FIGURE TITLES AND LEGENDS

Figure 1 Assembly of a full-length SARS-CoV-2 infection cDNA clone. (A) Genome structure SARS-CoV-2. The open reading frames (ORFs) from the full genome are indicated. (B) Strategy for *in vitro* assembly of an infectious cDNA clone of SARS-CoV-2. The nucleotide sequences and genome locations of the cohesive overhangs are indicated. The wild-type full-length cDNA of SARS-CoV-2 (IC WT) was directionally assembled using *in vitro* ligation. (C) Diagram of the terminal sequences of each cDNA fragment recognized by BsaI and Esp3I. (D) Gel analysis of the seven purified cDNA fragments. Individual fragments (F1 to F7) were digested from corresponding plasmid clones and gel-purified. Seven purified cDNA fragments (50-100 ng) were analyzed on a 0.6% native agarose gel. The 1-kilobase (kb) DNA ladders are indicated. (E) Gel analysis of cDNA ligation products. About 400 ng of purified ligation product was analyzed on a 0.6% native agarose gel. Triangle indicates the full-length (FL) cDNA product. Circles indicate the intermediate cDNA products. (F) Gel analysis of RNA transcripts. About 1 μg of *in vitro* transcribed (IVT) RNAs were analyzed on a 0.6% native agarose gel. DNA ladders are indicated. Since this is a native agarose gel, the DNA size is not directly corelated to the RNA size. Triangle indicates the genome-length RNA transcript. Circles show the shorter RNA transcripts.

Figure 2 Characterization of the wild-type icSARV-CoV-2 (IC WT). (A) Bright-field images of the Vero E6 cells electroporated with RNA transcripts. Cytopathic effects (CPE) appeared in the IC WT RNA-transfected cells on day 4 post-transfection. The titer of the P0 virus (directly from the transfected cells) is shown in plaque-forming units (PFU) per ml. (B) Plaque morphology of the original clinical isolate (WA1=2019-nCoV/USA_WA1/2020) and the recombinant P1 IC WT virus. Plaques were developed in Vero E6 cells on day 2 post-infection. (C) Replication kinetics. Vero E6 cells were infected with the clinical isolate or recombinant P1 IC WT virus at MOI 0.01. Viruses in culture fluids were quantified by plaque assay. Results from triplicate experiments

were presented with error bars indicating standard deviations. (D) Northern blot analysis of full-length and subgenomic RNAs. Numbers indicated the FL (band 1) and eight subgenomic RNAs (bands 2-9). (E) Sequence differences between the original clinical isolate WA1 and the recombinant P1 IC WT. The three silent nucleotide changes were engineered as molecular markers. (F) Chromatograms of Sanger sequencing results. The engineered molecular maker mutations are indicated.

Figure 3 Generation of a mNeonGreen SARS-CoV-2. (A) Assembly of the full-length mNeonGreen (mNG) SARS-CoV-2 cDNA. The mNG gene was placed downstream of the regulatory sequence of ORF7 to replace the ORF7 sequence [ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>7367</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>7367</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584836012">7367</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P. L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>15511-24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypeptidases/analysis</keyword><keyword>Coronavirus Infections/enzymology/*metabolism</keyword><keyword>Epithelial Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><

keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS Virus/*physiology</keyword><keyword>Severe Acute Respiratory Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>16306622</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-urls></urls><custom2>PMC1316022</custom2><electronic-resource-num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-num></record></Cite></EndNote>] in the subclone F7. (B) Plaque morphology of the P1 IC mNG virus. Plaques were developed in Vero E6 cells on day 2 post-infection. (C) Replication kinetics. Vero E6 cells were infected with the wild-type icSARS-CoV-2 (IC WT) or reporter icSARS-CoV-2-mNG (IC mNG) at MOI of 0.01. Viruses in culture medium were quantified by plaque assay. (D) Fluorescence microscopy analysis of P1 mNG virus-infected cells. Vero E6 cells were infected with P1 mNG viruses at MOI of 0.3. Representative mNeonGreen-positive (green) images are shown. (E) Kinetics of fluorescence intensity. Vero E6 cells were infected with MOI of 1.0, 0.3 or 0.1. After background signal subtraction, the fluorescence intensities from 12 to 48 h post-infection are shown. Results from triplicate experiments were presented with bars representing standard deviations. (F) Summary of full-genome sequence of mNG virus (P1 IC mNG). Nucleotides different from the original clinical isolate (WA1) are indicated.

Figure 4 Stability and application of mNeonGreen virus. The stability of mNG virus was analyzed by comparing the fluorescent signals between the cells infected with P1 and P5 reporter viruses. The presence of mNG gene in the P1 and P5 reporter viruses was also verified using RT-PCR. The application of mNG virus was examined by testing the antiviral activity of IFN-α treatment. (A) Fluorescence microscopy analysis of the P1 and P5 mNG virus-infected cells. Vero E6 cells were infected with P1 or P5 virus at an MOI of 0.3. The cells were monitored for mNG-positive signals at 24 h post-infection. Green, mNG; blue, nucleus. (B) Gel analysis of

445 mNG virus stability. Top panel depicts the theoretical results of RT-PCR followed by restriction
446 enzyme digestion. Bottom panel shows the gel analysis of the RT-PCR products before (lanes
447 1-3) and after BsrGI/StuI digestion (lanes 4-6). About 100 ng DNA samples were analyzed on a
448 0.6% agarose gel. The DNA sizes are indicated. (C) Schematic diagram of IFN-α treatment. (D)
449 Representative fluorescence images of reporter virus-infected cells after IFN-α treatment. The
450 doses of IFN-α treatment are indicated. (E) Dose response curve of mNG signal inhibited by
451 IFN-α. The Hillslope and EC_{50} values are indicated. Results from triplicate experiments were
452 presented with bars representing standard deviations.
453

STAR METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
N/A		
Bacterial and Virus Strains		
E. coli strain Top10	ThermoFisher Scientific	Cat# C404006
TransforMax™ EPI300™ Chemically Competent *E. coli*	Lucigen Corporation, Middleton, WI 53562	Cat# C300C105
SARS-CoV strain 2019-nCoV/USA_WA1/2020 (WA1)	World Reference Center of Emerging Viruses and Arboviruses [WRCEVA] at the University of Texas Medical Branch	N/A
Biological Samples		
None		
Chemicals, Peptides, and Recombinant Proteins		
IFN-α A Protein, Recombinant human	Millipore Sigma	Cat# IF007
Critical Commercial Assays		
T7 mMessage mMachine kit	Thermo Fisher Scientific	Cat# AM1344
Ingenio® Electroporation solution	Mirus Bio LLC	Cat# MIR 50117
SuperScript™ IV First-Strand Synthesis System	Thermo Fisher Scientific	Cat# 18091300
Platinum™ SuperFi II DNA Polymerase	Thermo Fisher Scientific	Cat# 12361010
Deposited Data		
N/A		
Experimental Models: Cell Lines		
Vero cells	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Experimental Models: Organisms/Strains		
N/A		
Oligonucleotides		
primer Cov-T7-N-F (TACTGTAATACGA CTCACTATAGGATGTCTGATAATGGA CCCCAAAATC)	Integrated DNA Technologies (Skokie, Illinois)	N/A
primer polyT-N-R (TTTTTTTTTTTTTTTT TTT TTTTTTTTTTTTTTTTTTAGGCCT GAGTTGAGTCAGCAC)	Integrated DNA Technologies (Skokie, Illinois)	N/A
Recombinant DNA		
pUC57-CoV2-F1	This paper	N/A
pCC1-CoV2-F2	This paper	N/A
pCC1-CoV2-F3	This paper	N/A
pUC57-CoV2-F4	This paper	N/A
pUC57-CoV2-F5	This paper	N/A

pUC57-CoV2-F6	This paper	N/A
pCC1-CoV2-F7	This paper	N/A
pCC1-CoV2-F7-mNG	This paper	N/A

Synthesized mNeonGreen gene (sequence-optimized)	This paper and [ADDIN EN.CITE <EndNote><Cite><Author>Shaner</Author><Year>2013</Year><RecNum>177</RecNum><DisplayText>(Shaner et al., 2013)</DisplayText><record><rec-number>177</rec-number><foreign-keys><key app="EN" db-id="txv2awx0sz9dfle59pjpwxxqrzxw25sssvrv" timestamp="1584804789">177</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shaner, Nathan C.</author><author>Lambert, Gerard G.</author><author>Chammas, Andrew</author><author>Ni, Yuhui</author><author>Cranfill, Paula J.</author><author>Baird, Michelle A.</author><author>Sell, Brittney R.</author><author>Allen, John R.</author><author>Day, Richard N.</author><author>Israelsson, Maria</author><author>Davidson, Michael W.</author><author>Wang, Jiwu</author></authors></contributors><titles><title>A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum</title><secondary-title>Nature methods</secondary-title><alt-title>Nat Methods</alt-title></titles><periodical><full-title>Nature Methods</full-title></periodical><pages>	N/A

Software and Algorithms		
ImageJ	NIH	N/A
Prism 8.0 software	GraphPad	N/A
Illustrator CC	Adobe	N/A

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by Lead Contact, Pei-Yong Shi (peshi@utmb.edu)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Virus and Cell Lines

The stock of SARS-CoV-2 strain 2019-nCoV/USA_WA1/2020 was derived from the first patient diagnosed in the US. The virus isolate was originally provided by Dr. Natalie Thornburg from the Centers for Disease Control and Prevention in Atlanta, GA as described previosuly [ADDIN EN.CITE ADDIN EN.CITE.DATA], and amplified on Vero E6 cells at the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch at Galveston (UTMB). The P5 passage was used in this study.

African green monkey kidney epithelial cells (Vero E6; CRL-1586) were purchased from the American Type Culture Collection (ATCC, Bethesda, MD) and maintained in a high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 1% penicillin/streptomycin (P/S). Cells were grown at 37°C with 5% CO_2. All culture medium and antibiotics were purchased from ThermoFisher Scientific (Waltham, MA). All cell lines were tested negative for mycoplasma.

METHOD DETAILS

Cloning the SARS-CoV-2 cDNAs

Two approaches were taken to rapidly obtain stable cDNAs of SARS-CoV-2. Firstly, the cDNAs of fragments F1, F4, F5, and F6 were successfully synthesized from the GenScript company

(Piscataway, NJ) and cloned into a high-copy plasmid pUC57. The F1 contains a T7 promoter sequence at the upstream of the 5′ end of the SARS-CoV-2 sequence. Other cDNA fragments were also synthesized but found unstable after cloning into plasmid pUC57. For overcoming this hurdle, the cDNAs of fragments F2, F3, and F7 were obtained by reverse transcription and PCR (RT-PCR). RT was performed by using the SuperScript™ IV First-Strand Synthesis System (ThermoFisher Scientific) with random hexamer primers and extracellular viral RNA (extracted from the supernatants of SARS-CoV-2-infected Vero E6 cells). The cDNA was used as a template to amplify the fragments F2, F3, and F7 by high fidelity PCR with the Platinum™ SuperFi II DNA Polymerase (ThermoFisher Scientific) according to the manufacturer's instructions. A poly$(T)_{29}$ sequence was introduced by PCR to the 3′ end of the untranslated region of viral genome. The amplicons were cloned into a single-copy vector pCC1BAC (Epicentre) to increase the stability of the cDNA plasmids when propagated in *E. coli*. To ensure a seamless assembly of the full-length cDNA, we introduced two cleavage sites of class IIS restriction enzymes (BsaI and Esp3I) at both ends of each sibling cDNAs during PCR or gene synthesis. To differentiate the infectious clone-derived virus from the parental clinical isolate 2019-nCoV/USA_WA1/2020, we engineered three silent mutations at nucleotide positions 7,486 (A-to-T change), 7,489 (T-to-A change), and 18,058 (T-to-C change). For construct the pCC1-F7-mNG, the gene of mNeonGreen (sequence-optimized) was synthesized and inserted at the downstream of the regulatory sequence of ORF7a to replace the entire ORF7a, according to the study as described previously[ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>6988</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>6988</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1581278772">6988</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P.

L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>15511-24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypeptidases/analysis</keyword><keyword>Coronavirus Infections/enzymology/*metabolism</keyword><keyword>Epithelial Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS Virus/*physiology</keyword><keyword>Severe Acute Respiratory Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>16306622</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-urls></urls><custom2>PMC1316022</custom2><electronic-resource-num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-num></record></Cite></EndNote>]. All subclones were finally validated by Sanger sequencing.

Assembly of a Full-length SARS-CoV-2 cDNA

To assemble the full-length cDNA, we digested individual cDNA plasmids and purified each cDNA fragments. Specifically, F1, F2, F3 and F4 cDNA fragments were obtained by digesting the corresponding plasmids with enzyme BsaI. F5 and F6 fragments were obtained by digesting the plasmids with enzymes Esp3I and PvuI. F7 and F7-mNG cDNA fragments were obtained by

529 digesting the corresponding plasmids by Esp3I and SnaBI. PvuI and SnaBI was included in the
530 digestion to eliminate undesired DNA bands that co-migrated with the targeting fragments on
531 agarose gels. All fragments after restriction enzyme digestion were separated on 0.6% agarose
532 gels, visualized under a darkreader lightbox (Clare Chemical Research, Dolores, CO), excised,
533 and purified using the QIAquick Gel Extraction Kit (Qiagen, Germantown, MD). To assemble the
534 full-length cDNA, we ligated the seven cDNA fragments in a three-step manner. Firstly, equal
535 molar ratio of F1 (0.61 μg), F2 (0.65 μg), F3 (0.75 μg), and F4 (0.94 μg) were ligated in a PCR
536 tube using T4 DNA ligase in a 40 μl-reaction at 4°C for 18 h, resulting in F1-4 DNA. Secondly,
537 equal molar ratio of fragments F5 (0.75 μg), F6 (0.72 μg), and F7 (0.60 μg) were ligated in a
538 separate PCR tube to produce F5-7 DNA using the same ligation condition. Thirdly, without any
539 DNA purification, the two reactions (containing F1-4 and F5-7) were combined (total 80 μl) and
540 topped with additional T4 ligase (2 μl), buffer (2 μl) and nuclease-free water (16 μl) to a 100-μl
541 reaction. The final reaction was incubated at 4°C for 18 h to produce the full-length F1-7 DNA.
542 Afterwards, the full-length cDNA was phenol/chloroform extracted, isopropanol precipitated, and
543 resuspended in 10 μl nuclease-free water.

544 **RNA transcription, Electroporation, Virus production and Quantification**

545 RNA transcript was *in vitro* synthesized by the mMESSAGE mMACHINE™ T7 Transcription Kit
546 (ThermoFisher Scientific) according to the manufacturer's instruction with some modifications. A
547 50-μl reaction was set up by adding 1 μg DNA template and 7.5 μl GTP (cap analog-to-GTP
548 ratio of 1:1). The reaction was incubated at 32°C for 5 h. After removing the template DNA by
549 nuclease per manufacturer's protocol, the RNA was phenol/chloroform extracted and
550 isopropanol precipitated. A SARS-CoV-2 N gene transcript was *in vitro* transcribed from a DNA
551 template using the mMESSAGE mMACHINE™ T7 Transcription Kit with a 2:1 ratio of cap
552 analog to GTP. The N gene DNA template was prepared by PCR using primer Cov-T7-N-F
553 (tactgTAATACGACTCACTATAGGatgtctgataatggaccccaaaatc; the uppercase sequence

represents T7 promoter; the underlined sequence represents the 5' end of N gene) and primer polyT-N-R [$(t)_{37}$aggcctgagttgagtcagcac].

RNA transcripts were electroporated into Vero E6 cells using a protocol as previously described [ADDIN EN.CITE ADDIN EN.CITE.DATA] with some modifications. Twenty micrograms of total RNA transcripts (containing both full-length RNA and short RNAs) and 20 μg N gene transcript were mixed and added to a 4-mm cuvette containing 0.8 ml of Vero E6 cells (8×10^6) in Ingenio® Electroporation Solution (Mirus). Single electrical pulse was given with a GenePulser apparatus (Bio-Rad) with setting of 270V at 950 μF. After 5 min recovery at room temperature, the electroporated cells were seeded into a T-75 flask and incubated at 37°C with 5% CO_2. On the next day, the culture fluid was replaced with 2% FBS DMEM medium. The cells were monitored daily for virus-mediated cytopathic effect (CPE). One milliliter of the P0 virus was inoculated to a T-175 flask containing 80% confluence Vero E6 cells. The infected cells were incubated at 37°C with 5% CO_2 for 2-3 days. Culture supernatants (P1) were harvested when CPE occurred. The amount of infectious virus was determined by a standard plaque assay on Vero E6 cells. All virus cultures were performed in a biosafety level 3 (BSL-3) laboratory with redundant fans in the biosafety cabinets. All personnel wore powered air purifying respirators (Breathe Easy, 3M) with Tyvek suits, aprons, booties and double gloves.

Interferon Treatment

Vero E6 cells were plated as 1.5×10^4 cells/well in a black 96-well plate (Greiner). For interferon treatment, at 6 h post-seeding, cells were treated with various doses of IFN-α (Millipore Sigma). After 14 h of treatment, the culture fluids were replaced with 2% FBS medium, and P1 IC mNG viruses were added to the cells at MOI 0.3 with additional corresponding concentration of IFN-α. At 24 h post-infection, Hoechst 33342 (ThermoFisher Scientific) was added to a final concentration of 0.1% to counterstain the nucleus. The green fluorescence signals were

578 detected by Cytation 5 (BioTek) and the infection rate was calculated according to the
579 manufacturer's instructions.

580 **RNA Extraction, RT-PCR and Sanger Sequencing**

581 250 µl of culture fluids were mixed with three volume of TRIzol™ LS Reagent (Thermo Fisher
582 Scientific). Viral RNAs were extracted per manufacturer's instructions. The final RNAs were
583 dissolved in 30 µl nuclease-free water. 11 µl RNA samples were used for reverse transcription
584 by using the SuperScript™ IV First-Strand Synthesis System (ThermoFisher Scientific) with
585 random hexamer primers. Nine DNA fragments covering the entire viral genome were amplified
586 by PCR with specific primers. The resulting DNAs were cleaned up by the QIAquick PCR
587 Purification Kit and Sanger sequencing was performed at the GENEWIZ facilities (South
588 Plainfield, NJ).

589 **Northern Blot**

590 Vero E6 cells were infected with clinical isolate WA1 or the infectious clone-derived SARS-CoV-
591 2 (IC WT) at MOI 0.01. At 48 h post-infection, total intracellular RNAs were isolated using TRIzol
592 reagent (Invitrogen). Northern blot analysis was performed using total intracellular RNAs as
593 described previously [ADDIN EN.CITE
594 <EndNote><Cite><Author>Narayanan</Author><Year>2008</Year><RecNum>7021</RecNum
595 ><DisplayText>(Narayanan et al., 2008)</DisplayText><record><rec-number>7021</rec-
596 number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a"
597 timestamp="1584637550">7021</key></foreign-keys><ref-type name="Journal
598 Article">17</ref-type><contributors><authors><author>Narayanan, K.</author><author>Huang,
599 C.</author><author>Lokugamage, K.</author><author>Kamitani,
600 W.</author><author>Ikegami, T.</author><author>Tseng, C. T.</author><author>Makino,
601 S.</author></authors></contributors><auth-address>Department of Microbiology and
602 Immunology, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1019,

603 USA. shmakino@utmb.edu</auth-address><titles><title>Severe acute respiratory syndrome
604 coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in
605 infected cells</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J
606 Virol</full-title></periodical><pages>4471-
607 9</pages><volume>82</volume><number>9</number><keywords><keyword>Cell
608 Line</keyword><keyword>*Gene Expression
609 Regulation</keyword><keyword>Humans</keyword><keyword>Interferon Type
610 I/*genetics</keyword><keyword>Mutation</keyword><keyword>Protein
611 Biosynthesis</keyword><keyword>RNA Replicase/*physiology</keyword><keyword>RNA
612 Stability</keyword><keyword>RNA, Messenger/metabolism</keyword><keyword>SARS
613 Virus/pathogenicity/*physiology</keyword><keyword>Viral Nonstructural
614 Proteins/*physiology</keyword></keywords><dates><year>2008</year><pub-
615 dates><date>May</date></pub-dates></dates><isbn>1098-5514 (Electronic)
0022-538X
616 (Linking)</isbn><accession-num>18305050</accession-num><urls><related-
617 urls><url>https://www.ncbi.nlm.nih.gov/pubmed/18305050</url></related-
618 urls></urls><custom2>PMC2293030</custom2><electronic-resource-num>10.1128/JVI.02472-
619 07</electronic-resource-num></record></Cite></EndNote>]. A digoxigenin (DIG)-labeled
620 random-primed probe, corresponding to nucleotides 28,999 to 29,573 of the SARS-CoV-2
621 genome, was used to detect SARS-CoV-2 mRNAs and visualized by DIG luminescent detection
622 kit (Roche, Indianapolis, IN) according to the manufacturer's protocol.

623 **QUANTIFICATION AND STATISTICAL ANALYSIS**

624 All numerical data are presented as the mean±SD (standard deviations). Group comparisons of
625 viral growth kinetics in Figures 2 and 3 were performed using multiple t-test with Bonferroni-
626 Dunn correction in software Prism 8.0 (GraphPad). *p<0.05, significant; **p<0.01, significant;
627 p>0.05, ns (not significant). The 50% effective concentration (EC_{50}) in Figure 4 were estimated

by using a four-parameter logistic regression model from the GraphPad Prism 8 software (GraphPad Software Inc., San Diego CA). Minimal adjustment was made in the software ImageJ to enhance the contrast for bright-field images in Figures 1-3. Blue- and green-fluorescence images were merged in ImageJ. Figures were finally assembled using the software Adobe illustrator CC.

DATA AND SOFTWARE AVAILABILITY

All data are present in this study.

635 [ADDIN EN.REFLIST]

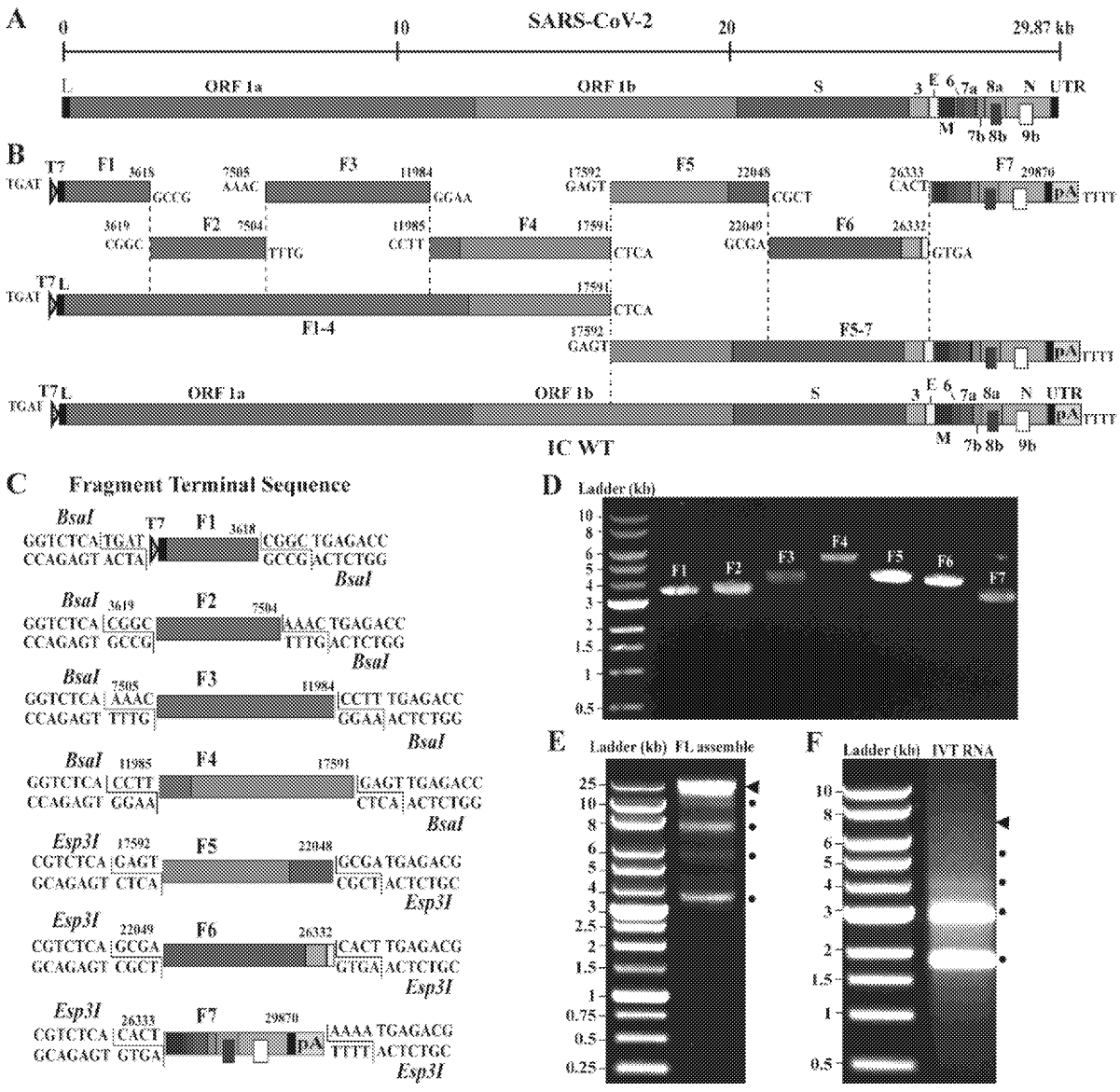


Figure 1

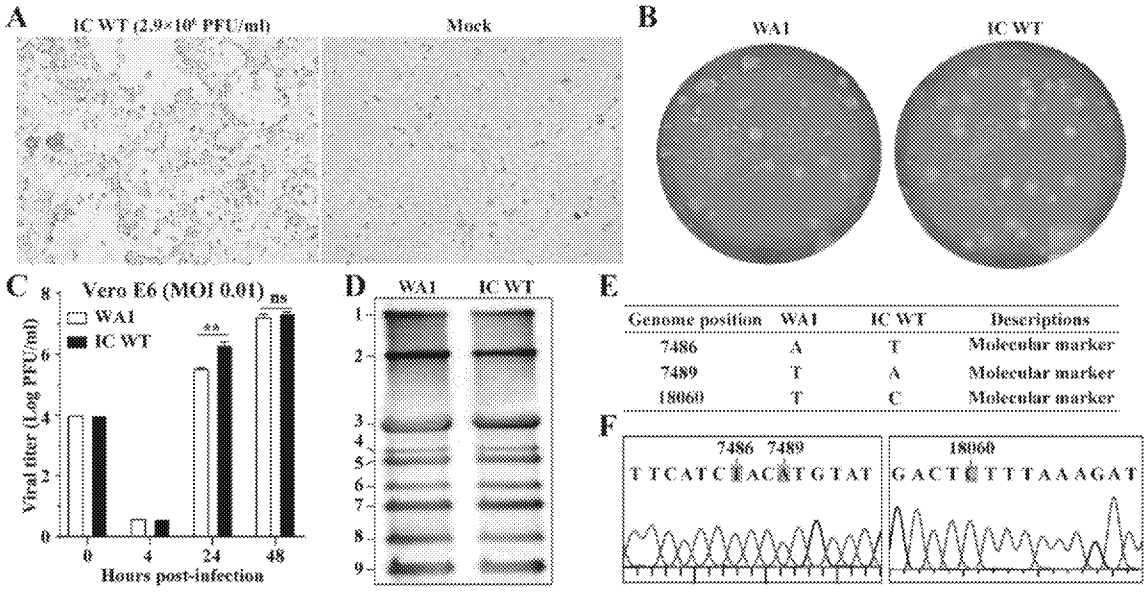
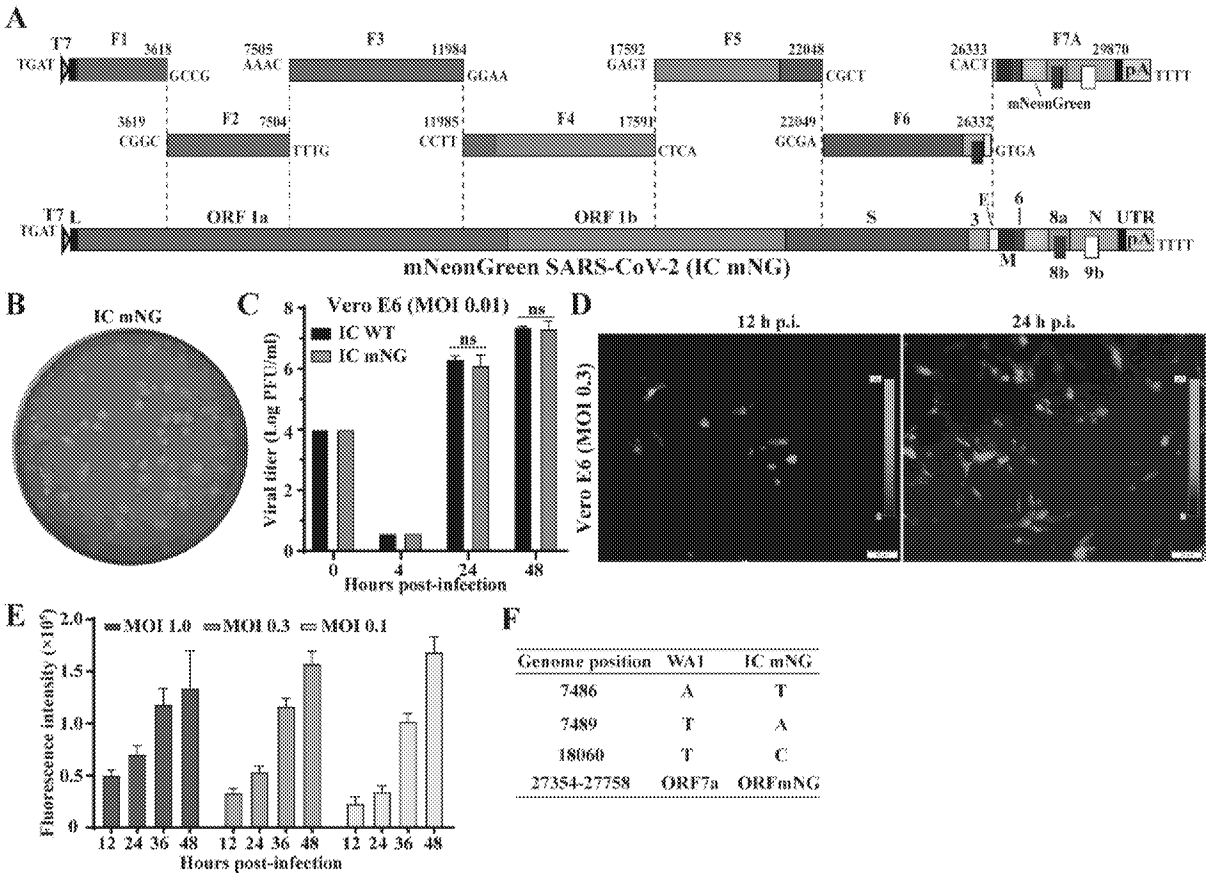


Figure 2



Genome position	WA1	IC mNG
7486	A	T
7489	T	A
18060	T	C
27354-27758	ORF7a	ORFmNG

Figure 3

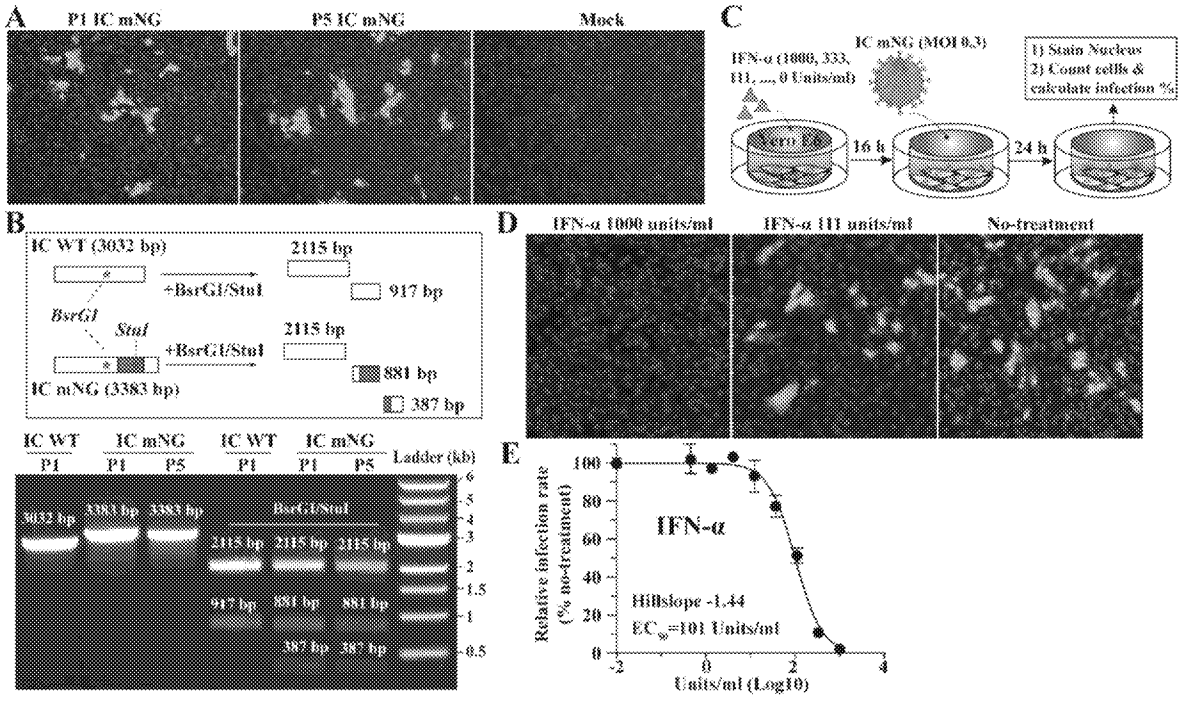


Figure 4

From: LeDuc, James W.
Sent: Thursday, April 16, 2020 10:07 PM
To: zengli Shi
Subject: Fwd: Rubio

Hi Zheng-Li. I hope you are well as surviving all the COVID19 drama. I wonder if you would have time for a phone call sometime soon. Let me know a good number and time and I'll call. The email below is relevant.

I will certainly understand if you are not available but Pei-Yong keeps encouraging me to call.

With all good wishes.

Jim.

My office line is 1 409 266 6516 or cell is 1 409 789 2012 if it's easier for you to call me.

Sent from my iPhone

Begin forwarded message:

> **From:** David Franz <davidrfranz@gmail.com>
> **Date:** April 16, 2020 at 8:04:55 PM CDT
> **To:** "LeDuc, James W." <jwleduc@UTMB.EDU>
> **Subject: Rubio**
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"
>
> Sent from my iPhone

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 2/29/2020 12:14:25 PM
To: Boyd, Nancy (NIH/NIAID) [E] [nboyd@niaid.nih.gov]
Subject: RE: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

At the office…

From: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Sent: Saturday, February 29, 2020 11:11 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

You are welcome, Jim. I hope you are having a restful weekend!

Nancy

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Saturday, February 29, 2020 11:17 AM
To: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Subject: Re: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

Thanks for sharing. I saw a draft but hadn't seen the final.

Sent from my iPhone

On Feb 29, 2020, at 9:44 AM, Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov> wrote:

I imagine you got this but sending anyway…

From: Folkers, Greg (NIH/NIAID) [E] <gfolkers@niaid.nih.gov>
Sent: Saturday, February 29, 2020 10:42 AM
Subject: Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics http://bit.ly/3adujf6

FDA News Release

Coronavirus (COVID-19) Update: FDA Issues New Policy to Help Expedite Availability of Diagnostics

For Immediate Release:
February 29, 2020

Today, as part of the U.S. Food and Drug Administration's ongoing and aggressive commitment to address the coronavirus outbreak, the agency issued a new policy for certain laboratories seeking to develop diagnostic tests for coronavirus in order to achieve more rapid testing capacity in the U.S.

"We believe this policy strikes the right balance during this public health emergency," said FDA Commissioner Stephen M. Hahn, M.D. "We will continue to help to ensure sound science prior to clinical testing and follow-up with the critical independent review from the FDA, while quickly expanding testing capabilities in the U.S. We are not changing our standards for issuing Emergency Use Authorizations. This action today reflects our public health commitment to addressing critical public health needs and rapidly responding and adapting to this dynamic and evolving situation."

There is currently an outbreak of respiratory disease caused by a novel coronavirus that was first detected in Wuhan City, Hubei Province, China and which has now been detected in 50 locations internationally, including cases in the United States. The virus has been named "SARS-CoV2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to rapidly spread, leading to significant impact on health care systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the U.S. To effectively respond to the COVID-19 outbreak, rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical. This can best be achieved with wide availability of testing capabilities in health care settings, reference and commercial laboratories, and at the point of care.

The new policy is for certain laboratories that develop and begin to use validated COVID-19 diagnostics before the FDA has completed review of their Emergency Use Authorization (EUA) requests. The FDA can issue an EUA to permit the use, based on scientific data, of certain medical products that may be effective in diagnosing, treating or preventing a disease or condition when there is a determination, by the Secretary of Health and Human Services (HHS), that there is a public health emergency or a significant potential for a public health emergency that has a significant potential to affect national security or the health and security of U.S. citizens, and a declaration that circumstances exist justifying the medical products' emergency use.

On Feb. 4, 2020, the Secretary of HHS determined that there is a public health emergency and that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the COVID-19 outbreak. Rapid detection of COVID-19 cases in the U.S. requires wide availability of diagnostic testing to control the emergence of a rapidly spreading, severe illness. The FDA has authorized one EUA for COVID-19 that is in use by the U.S. Centers for Disease Control and Prevention (CDC) and some public health labs across the country.

The guidance issued today describes a policy enabling laboratories to immediately use tests they developed and validated in order to achieve more rapid testing capacity in the U.S.

"The global emergence of COVID-19 is concerning, and we appreciate the efforts of the FDA to help bring more testing capability to the U.S.," said Nancy Messonnier, M.D., director of the CDC's Center for the National Center for Immunization and Respiratory Diseases (NCIRD).

The immediately in effect guidance issued today describes the circumstances where the FDA does not intend to object to the use of these tests for clinical testing while the laboratories are pursuing an EUA with the FDA. Importantly, this policy only applies to laboratories that are certified to perform high-complexity testing consistent with requirements under Clinical Laboratory Improvement Amendments.

"We applaud the FDA's approach to speed the path toward emergency use authorization for COVID-19 diagnostics. This step may reduce development costs, speed the process for availability at more testing sites, incentivize private development and, ultimately, help save lives," said Rick Bright, Ph.D., director of the Biomedical Advanced Research and Development Authority (BARDA), part of the HHS Office of the Assistant Secretary for Preparedness and Response. "At BARDA, we are identifying industry partners to develop rapid diagnostics that can be used in commercial and hospital labs or even doctors' offices so that medical professionals and their patients have the information they need to take action."

The FDA guidance provides recommendations for test developers, including information regarding test validation, FDA notification and interim confirmatory clinical testing.

Following the completion of their test validation, laboratories should communicate with the FDA, via email, in order to notify the agency that the test has been validated. Laboratories should submit a completed EUA request within 15 business days of notification.

"Under this policy, we expect certain laboratories who develop validated tests for coronavirus would begin using them right away prior to FDA review," said Jeff Shuren, M.D., J.D., director of the FDA's Center for Devices and Radiological Health. "We believe this action will support laboratories across the country working on this urgent public health situation. We are dedicating all available resources to expediting the review of medical products, including diagnostics, to prevent the spread of this outbreak."

The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

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Inquiries

Media:
Stephanie Caccomo
301-348-1956
Consumer:
888-INFO-FDA

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/24/2020 10:29:26 AM
To: Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
CC: Gregory Sempowski [greg.sempowski@duke.edu]; Gary Zackowitz (zackowig@niaid.nih.gov) [zackowig@niaid.nih.gov]
BCC: Auchincloss, Hugh (NIH/NIAID) [E] (auchinclossh@niaid.nih.gov) [auchinclossh@niaid.nih.gov]
Subject: FW: NBL/RBL testing network
Attachments: 2020.04.16.20067835v1.full.pdf

Hi Nancy,

I spoke to Greg and Tom Denny yesterday at Duke about using the RBL network to assist with testing. There is general interest and clearly a willingness to assist, but we are not clear as to how best to contribute. I wonder if you and Gary could forward to the network the note below from Emily, focusing on the highlighted area and see what kind of response we get from everyone. I am assuming that her comment about link to patient identifiers means either being CLIA certified or having a work around where the lab is screening and forwarding positives to a partner CLIA approved lab for confirmation. I copied you on an earlier note to Emily regarding the CLIA and EUA challenges but have not had a reply.

Also note the attached paper suggesting saliva rather than NP swabs as a specimen for testing.

Let me know if you have heard anything more from Emily or her team on this.

Thanks, Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Erbelding, Emily (NIH/NIAID) [E] <emily.erbelding@nih.gov>
Sent: Monday, April 20, 2020 6:41 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>; Auchincloss, Hugh (NIH/NIAID) [E] <auchinclossh@niaid.nih.gov>
Subject: RE: NBL/RBL testing network

Jim,
There is a forceful movement toward expansion of testing, since most experts believe that reopening businesses and society will not be successful unless public health authorities can immediate detect cases (and onward transmission) in their jurisdictions.

So please engage in the extent to which you can. At the current moment, diagnostic testing supported by NIH would somehow come out of funds appropriated for research. That is probably OK. But the next supplemental appropriation might be different and focused on NIH expanding lab testing however they can.
We have had a lot of internal discussions on this so Carl hasn't dropped it.
Feel free to sketch out for us exactly what the RBL/NBLs could do on diagnostic testing (with link to patient identifiers).
Thanks.
Emily

I am cc'ing Hugh on this for his awareness.

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Monday, April 20, 2020 7:24 PM
To: Erbelding, Emily (NIH/NIAID) [E] <emily.erbelding@nih.gov>
Cc: Boyd, Nancy (NIH/NIAID) [E] <nboyd@niaid.nih.gov>
Subject: NBL/RBL testing network

Hi Emily

I spoke to Carl Dieffenbach last Wednesday, 15 April when he called about a plan to expand COVID testing across the country and Hugh had suggested he speak to me about including the NBL/RBL network. He was going to follow up with you and Nancy Boyd and get back to me. Have you heard anything on this initiative? It sounded urgent when we spoke, so I'm surprised to not have heard anything more. Happy to chat by phone if you like — my direct office line is 409-266-6516.

Thanks, Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
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(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs

Anne L. Wyllie[1]*, John Fournier[2], Arnau Casanovas-Massana[1], Melissa Campbell[2], Maria Tokuyama[3], Pavithra Vijayakumar[4], Bertie Geng[4], M. Catherine Muenker[1], Adam J. Moore[1], Chantal B.F. Vogels[1], Mary E. Petrone[1], Isabel M. Ott[5], Peiwen Lu[3], Arvind Venkataraman[3], Alice Lu-Culligan[3], Jonathan Klein[3], Rebecca Earnest[1], Michael Simonov[6], Rupak Datta[2], Ryan Handoko[2], Nida Naushad[2], Lorenzo R. Sewanan[2], Jordan Valdez[2], Elizabeth B. White[1], Sarah Lapidus[1], Chaney C. Kalinich[1], Xiaodong Jiang[3], Daniel J. Kim[3], Eriko Kudo[3], Melissa Linehan[3], Tianyang Mao[3], Miyu Moriyama[3], Ji Eun Oh[3], Annsea Park[3], Julio Silva[3], Eric Song[3], Takehiro Takahashi[3], Manabu Taura[3], Orr-El Weizman[3], Patrick Wong[3], Yexin Yang[3], Santos Bermejo[7], Camila Odio[8], Saad B. Omer[1,2,9,10], Charles S. Dela Cruz[7], Shelli Farhadian[2], Richard A. Martinello[2,7,11], Akiko Iwasaki[3,12], Nathan D. Grubaugh[1#*], Albert I. Ko[1#*]

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Key words: SARS-CoV-2, COVID-19, saliva, diagnostics

Abstract

Rapid and accurate SARS-CoV-2 diagnostic testing is essential for controlling the ongoing COVID-19 pandemic. The current gold standard for COVID-19 diagnosis is real-time RT-PCR detection of SARS-CoV-2 from nasopharyngeal swabs. Low sensitivity, exposure risks to healthcare workers, and global shortages of swabs and personal protective equipment, however, necessitate the validation of new diagnostic approaches. Saliva is a promising candidate for SARS-CoV-2 diagnostics because (1) collection is minimally invasive and can reliably be self-administered and (2) saliva has exhibited comparable sensitivity to nasopharyngeal swabs in detection of other respiratory pathogens, including endemic human coronaviruses, in previous studies. To validate the use of saliva for SARS-CoV-2 detection, we tested nasopharyngeal and saliva samples from confirmed COVID-19 patients and self-collected samples from healthcare workers on COVID-19 wards. When we compared SARS-CoV-2 detection from patient-matched nasopharyngeal and saliva samples, we found that saliva yielded greater detection sensitivity and

consistency throughout the course of infection. Furthermore, we report less variability in self-sample collection of saliva. Taken together, our findings demonstrate that saliva is a viable and more sensitive alternative to nasopharyngeal swabs and could enable at-home self-administered sample collection for accurate large-scale SARS-CoV-2 testing.

Introduction

Efforts to control SARS-CoV-2, the novel coronavirus causing COVID-19 pandemic, depend on accurate and rapid diagnostic testing. These tests must be (***1***) sensitive to mild and asymptomatic infections to promote effective self isolation and reduce transmission within high risk groups[1]; (***2***) consistent to reliably monitor disease progression and aid clinical decisions[2]; and (***3***) scalable to inform local and national public health policies, such as when social distancing measures can be safely relaxed. However, current SARS-CoV-2 testing strategies often fail to meet these criteria, in part because of their reliance on nasopharyngeal swabs as the widely recommended sample type for real-time RT-PCR. Although nasopharyngeal swabs are commonly used in respiratory virus diagnostics, they show relatively poor sensitivity for SARS-CoV-2 detection in early infection and are inconsistent during serial testing[2–6]. Moreover, collecting nasopharyngeal swabs causes discomfort to patients due to the procedure's invasiveness, limiting compliance for repeat testing, and presents a considerable risk to healthcare workers, because it can induce patients to sneeze or cough, expelling virus particles[7]. The procedure is also not conducive to large-scale testing, because there are widespread shortages of swabs and personal protective equipment for healthcare workers[8], and self-collection of nasopharyngeal swabs is difficult and less sensitive for virus detection[9]. These challenges will be further exacerbated as the COVID-19 pandemic intensifies in low income countries. Given the limitations, a more reliable and less resource-intensive sample collection method, ideally one that accommodates self-collection in the home, is urgently needed.

Saliva sampling is an appealing alternative to nasopharyngeal swab, since collecting saliva is non-invasive and easy to self-administer. An analysis of nasopharyngeal and saliva concordance for RT-PCR detection of respiratory pathogens, including two seasonal human coronaviruses, suggests comparable diagnostic sensitivity between the two sample types[10,11]. Preliminary findings indicate that (***1***) SARS-CoV-2 can be detected from the saliva of COVID-19 patients[12] and (***2***) self-collected saliva samples have comparable SARS-CoV-2 detection sensitivity to nasopharyngeal swabs collected by healthcare workers from mild and subclinical COVID-19 cases[13]. Critically, however, no rigorous evaluation of the sensitivity of SARS-CoV-2 detection in saliva with respect to nasopharyngeal swabs has been conducted from inpatients during the course of COVID-19 infection.

In this study, we evaluated SARS-CoV-2 detection in paired nasopharyngeal swabs and saliva samples collected from COVID-19 inpatients and asymptomatic healthcare workers at moderate-to-high risk of COVID-19 exposure. Our results indicate that using saliva for SARS-CoV-2 detection is more sensitive and consistent than using nasopharyngeal swabs. Overall, we demonstrate that saliva should be considered as a reliable sample type to alleviate COVID-19 testing demands.

Results

Higher SARS-CoV-2 titers detected from saliva than nasopharyngeal swabs from inpatients

To determine if saliva performs as well as the U.S. CDC recommendation of using nasopharyngeal swabs for SARS-CoV-2 diagnostics, we collected clinical samples from 44 COVID-19 inpatient study participants (**Table 1**). This cohort represents a range of COVID-19 patients with severe disease, with 19 (43%) requiring intensive care, 10 (23%) requiring mechanical ventilation, and 2 (5%) deceased as of April 5th, 2020. Using the U.S. CDC SARS-CoV-2 RT-PCR assay, we tested 121 self-collected saliva or healthcare worker-administered nasopharyngeal swabs from this cohort. We found strong concordance between the U.S. CDC "N1" and "N2" primer-probe sets (**Extended Data Fig. 1**), and thus calculated virus titers (virus copies/mL) using only the "N1" set. From all positive samples tested (n = 46 nasopharyngeal, 37 saliva), we found that the geometric mean virus titers from saliva were about 5× higher than nasopharyngeal swabs ($p < 0.05$, Mann-Whitney test; **Fig. 1a**). When limiting our analysis to only patient-matched nasopharyngeal and saliva samples (n = 38 for each sample type), we found that SARS-CoV-2 titers from saliva were significantly higher than nasopharyngeal swabs (p = 0.0001, Wilcoxon test; **Fig. 1b**). Moreover, we detected SARS-CoV-2 from the saliva but not the nasopharyngeal swabs from eight matching samples (21%), while we only detected SARS-CoV-2 from nasopharyngeal swabs and not saliva from three matched samples (8%; **Fig. 1c**). Overall, we found higher SARS-CoV-2 titers from saliva than nasopharyngeal swabs from hospital inpatients.

Table 1. COVID-19 inpatient cohort characteristics

	All study participants (n = 44)	Study participants with paired nasopharyngeal and saliva samples (n = 29)
Gender, male	23 (52%)	16 (55%)
Age range, years	23-92 (mean = 61)	23-91 (mean = 59)
ICU on admission, *n*	6 (14%)	4 (14%)
ICU during hospital stay, *n*	19 (43%)	12 (41%)
Mechanical ventilation, *n*	10 (23%)	6 (21%)
Deceased (April 5th), *n*	2 (5%)	1 (4%)
Total samples collected, *n*	121	76

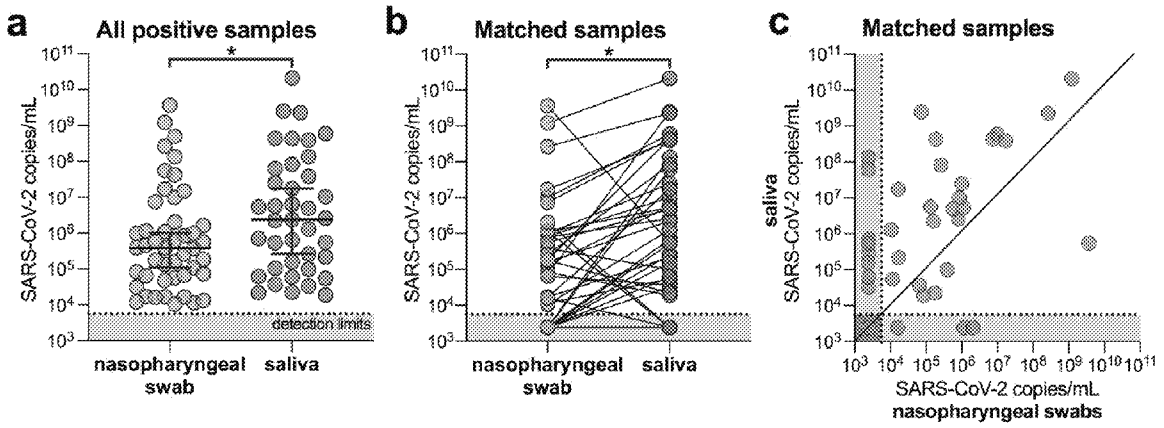

Figure 1. SARS-CoV-2 titers are higher in the saliva than nasopharyngeal swabs from hospital inpatients. (**a**) All positive nasopharyngeal swabs (n = 46) and saliva samples (n = 39) were compared by a Mann-Whitney test ($p < 0.05$). Bars represent the median and 95% CI. Our assay detection limits for SARS-CoV-2 using the US CDC "N1" assay is at cycle threshold 38, which corresponds to 5,610 virus copies/mL of sample (shown as dotted line and grey area). (**b**) Patient matched samples (n = 38), represented by the connecting lines, were compared by a Wilcoxon test test ($p < 0.05$). (**c**) Patient matched samples (n = 38) are also represented on a scatter plot. All of the data used to generate this figure, including the raw cycle thresholds, can be found in **Supplementary Data 1**. **Extended Data Fig. 1** shows the correlation between US CDC assay "N1" and "N2" results.

Less temporal SARS-CoV-2 variability when testing saliva from inpatients

As temporal SARS-CoV-2 diagnostic testing from nasopharyngeal swabs is reported to be variable[2,3], we tested longitudinal nasopharyngeal and saliva samples from inpatients to determine which sample type provided more consistent results. From 22 participants with multiple nasopharyngeal swabs and 12 participants with multiple saliva samples, we found that SARS-CoV-2 titers generally decreased in both sample types following the reported date of symptom onset (**Fig. 2a**). Our nasopharyngeal swab results are consistent with previous reports of variable SARS-CoV-2 titers and results[2,3]: we found 5 instances where a participant's nasopharyngeal swab was negative for SARS-CoV-2 followed by a positive result during the next collection (5/33 repeats, 33%; **Fig. 2b**). In longitudinal saliva collections from 12 patients, however, there were no instances in which a sample tested negative and was later followed by a positive result. As true negative test results are important for clinicians to track patient improvements and for decisions regarding discharges, our data suggests that saliva is a more consistent sample type than nasopharyngeal swabs for monitoring temporal changes in SARS-CoV-2 titers.

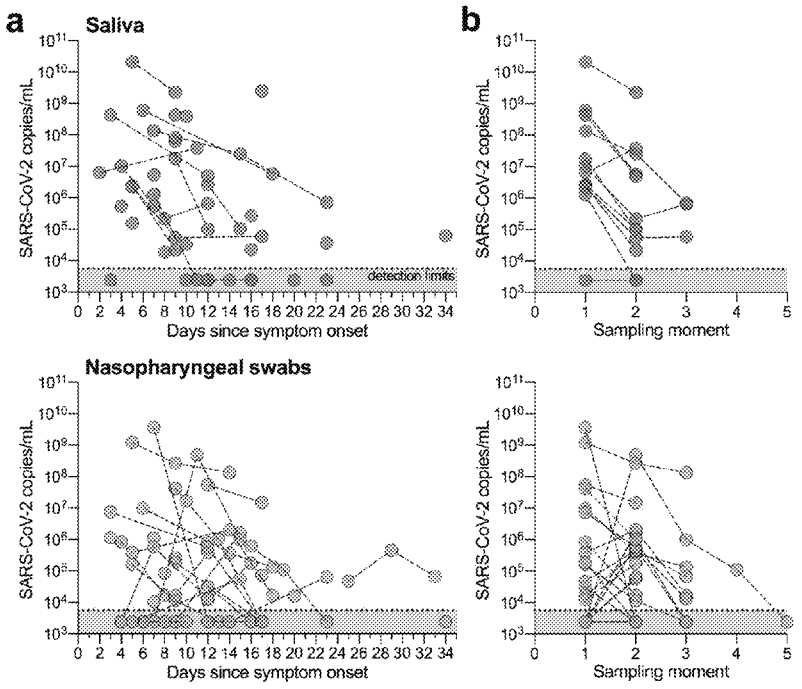

Figure 2: SARS-CoV-2 detection is less variable between repeat sample collections with saliva. (a) Longitudinal SARS-CoV-2 titers from saliva or nasopharyngeal swabs are shown as days since symptom onset. Each circle represents a separate sample, which are connected to additional samples from the same patient by a dashed line. Our assay detection limits for SARS-CoV-2 using the US CDC "N1" assay is at cycle threshold 38, which corresponds to 5,610 virus copies/mL of sample (shown as dotted line and grey area). (**b**) The data are also shown by sampling moment (sequential collection time) to highlight the differences in virus titers between collection points. All of the data used to generate this figure, including the raw cycle thresholds, can be found in **Supplementary Data 1**.

More consistent self-sampling from healthcare workers using saliva

Validating saliva for the detection of subclinical SARS-CoV-2 infections could prove transformative for both remote patient diagnostics and healthcare worker surveillance. To investigate this, we enrolled 98 asymptomatic healthcare workers into our study and collected saliva and/or nasopharyngeal swabs on average every 2.9 days (range = 1-8 days, **Table 2**). To date, we have detected SARS-CoV-2 in saliva from two healthcare workers who were negative by nasopharyngeal swabs using both the US CDC "N1" and "N2" tests and did not report any symptoms. The saliva from one of these individuals again tested positive alongside a matching negative nasopharyngeal swab upon repeat testing 2 days later. Virus titers from asymptomatic healthcare workers' saliva are lower than what we typically detect from symptomatic inpatients (**Fig. 3a**), which likely supports the lack of symptoms.

Our limited data supports that saliva may be more sensitive for detecting asymptomatic or pre-symptomatic infections; however, a larger sample size is needed to confirm. As nasopharyngeal swab sampling inconsistency may be one of the potential issues for false negatives (**Fig. 2**), monitoring an internal control for proper sample collection, human RNase P, may provide an alternative evaluation technique. While human RNase P detection was

better from saliva in both the inpatient and healthcare worker cohorts (**Fig. 3b**), this alone may not indicate better virus detection. More importantly, we found that human RNase P detection was more variable from nasopharyngeal swabs collected from inpatients (p = 0.0001, F test for variances) and self-collected from healthcare workers (p = 0.0002; **Fig. 3b**). Our results suggest that saliva may also be an appropriate, and perhaps more sensitive, alternative to nasopharyngeal swabs for screening asymptomatic or pre-symptomatic SARS-CoV-2 infections.

Table 2. Healthcare worker cohort

	All study participants (n=98)	Participants with matching samples (n=33)
Gender, male	16 (16%)	5 (15%)
Age range, years (average)	22-67 (36)	22-61 (36)
Total samples collected, n	244	64

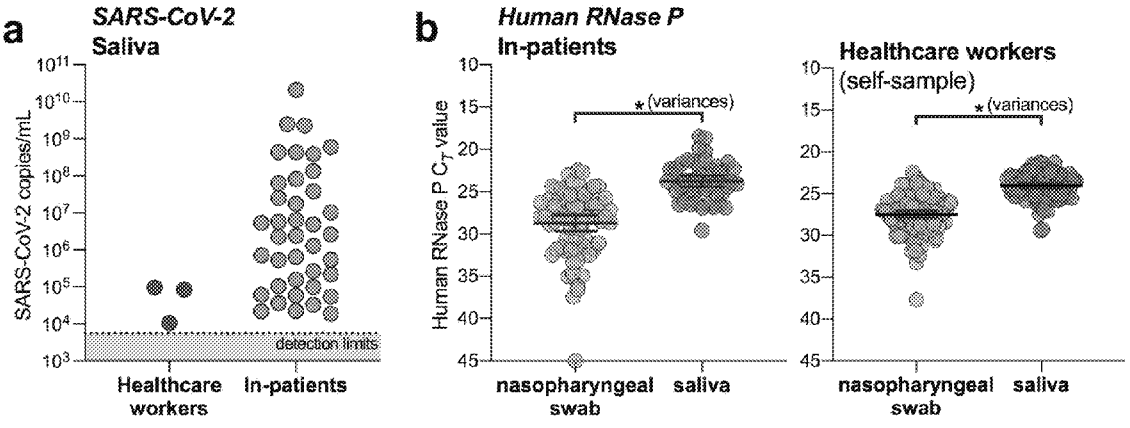

Figure 3. Saliva is an alternative for SARS-CoV-2 screening from healthcare workers and asymptomatic cases. (**a**) SARS-CoV-2 titers measured from the saliva of healthcare workers and inpatients. Our assay detection limits for SARS-CoV-2 using the US CDC "N1" assay is at cycle threshold 38, which corresponds to 5,610 virus copies/mL of sample (shown as dotted line and grey area). (**b**) RT-PCR cycle thresholds (Ct) values for human RNase P, and internal control for sample collection, from either inpatients (left panel) or health care workers (right panel) were compared by variances using the F test (p = 0.0001 for inpatients; p = 0.0002 for healthcare workers). All of the data used to generate this figure, including the raw cycle thresholds, can be found in **Supplementary Data 1**.

Discussion

Our study demonstrates that saliva is a viable and preferable alternative to nasopharyngeal swabs for SARS-CoV-2 detection. We found that the sensitivity of SARS-CoV-2 detection

from saliva is comparable, if not superior to nasopharyngeal swabs in early hospitalization and is more consistent during extended hospitalization and recovery. Moreover, the detection of SARS-CoV-2 from the saliva of two asymptomatic healthcare workers despite negative matched nasopharyngeal swabs suggests that saliva may also be a viable alternative for identifying mild or subclinical infections. With further validation, widespread implementation of saliva sampling could be transformative for public health efforts: saliva self-collection negates the need for direct healthcare worker-patient interaction, a source of several major testing bottlenecks and overall nosocomial infection risk[14–16], and alleviates supply demands on swabs and personal protective equipment.

As SARS-CoV-2 viral loads differ between mild and severe cases[17], a limitation of our study is the primary focus on COVID-19 inpatients, many with severe disease. While more data are required to more rigorously compare the efficacy of saliva in the hospital setting to earlier in the course of infection, findings from two recent studies support its potential for detecting SARS-CoV-2 from both asymptomatic individuals and outpatients[13,18]. As infectious virus has been detected from the saliva of COVID-19 patients[12], ascertaining the relationship between virus genome copies and infectious virus particles in the saliva of pre-symptomatic individuals[19] will play a key role in understanding the dynamics of asymptomatic transmission[1,20].

Stemming from the promising results for SARS-CoV-2 detection in asymptomatic individuals[13], a saliva SARS-CoV-2 detection assay has already gained approval through the U.S. Food and Drug Administration emergency use authorization[18]. To meet the growing testing demands, however, our findings support the need for immediate validation and implementation of saliva for SARS-CoV-2 diagnostics in certified clinical laboratories.

Methods

Ethics

All study participants were enrolled and sampled in accordance to the Yale University HIC-approved protocol #2000027690. Demographics, clinical data and samples were only collected after the study participant had acknowledged that they had understood the study protocol and signed the informed consent. All participant information and samples were collected in association with study identifiers.

Participant enrollment

Inpatients

Patients admitted to Yale New Haven Hospital (a 1541-bed tertiary care medical center in New Haven, CT, USA), who tested positive for SARS-CoV-2 by nasopharyngeal and/or oropharyngeal swab (CDC approved assay) were invited to enroll in the research study. Exclusion criteria were age under 18 years, non-English speaking and clinical, radiological or laboratory evidence for a non-infectious cause of fever or respiratory symptoms or a microbiologically-confirmed infectious source (e.g. gastrointestinal, urinary, cardiovascular) other than respiratory tract for symptoms and no suspicion for COVID-19 infection.

Healthcare workers

Asymptomatic healthcare workers (e.g., without fever or respiratory symptoms) with occupational exposure to patients with COVID-19 were invited to enroll in the study. Study participation enabled active surveillance to ensure early detection following exposure and to further protect other healthcare workers and patients.

Sample collection

Inpatients

Nasopharyngeal and saliva samples were obtained every three days throughout their clinical course. Nasopharyngeal samples were taken by registered nurses using the BD universal viral transport (UVT) system. The flexible, mini-tip swab was passed through the patient's nostril until the posterior nasopharynx was reached, left in place for several seconds to absorb secretions then slowly removed while rotating. The swab was placed in the sterile viral transport media (total volume 3 mL) and sealed securely. Saliva samples were self-collected by the patient. Upon waking, patients were asked to avoid food, water and brushing of teeth until the sample was collected. Patients were asked to repeatedly spit into a sterile urine cup until roughly a third full of liquid (excluding bubbles), before securely closing it. All samples were stored at room temperature and transported to the research lab at the Yale School of Public Health within 5 hours of sample collection.

Healthcare workers

Healthcare workers were asked to collect a self-administered nasopharyngeal swab and a saliva sample every three days for a period of 2 weeks. Samples were stored at +4°C until being transported to the research lab.

SARS-CoV-2 detection

On arrival at the research lab, total nucleic acid was extracted from 300 µl of viral transport media from the nasopharyngeal swab or 300 µl of whole saliva using the MagMAX Viral/Pathogen Nucleic Acid Isolation kit (ThermoFisher Scientific) following the manufacturer's protocol and eluted into 75 µl of elution buffer. For SARS-CoV-2 RNA detection, 5 µl of RNA template was tested as previously described[21,22], using the US CDC real-time RT-PCR primer/probe sets for 2019-nCoV_N1 and 2019-nCoV_N2 and the human RNase P (RP) as an extraction control. Samples were classified as positive for SARS-CoV-2 when both N1 and N2 primer-probe sets were detected <38 C_T. Virus copies were quantified using a 10-fold dilution standard curve of RNA transcripts that we previously generated[21]. As results from N1 and N2 were comparable (**Extended Data Fig. 1**), all virus copies are shown as calculated using the N1 primer-probe set.

Statistical analysis

Statistical analyses were conducted in GraphPad Prism 8.0.0 as described in the Results.

Acknowledgments

We gratefully acknowledge the study participants for their time and commitment to the study. We thank all members of the clinical team at Yale-New Haven Hospital for their

dedication and work which made this study possible. We also thank S. Taylor and P. Jack for technical discussions.

Funding

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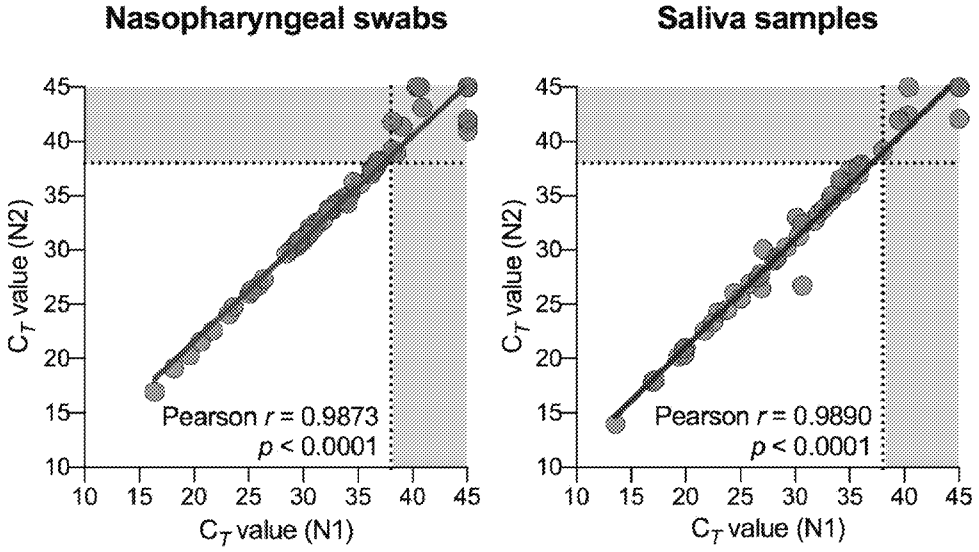
Extended data



Extended Data Fig. 1. Concordance between SARS-CoV-2 detection using US CDC "N1" and "N2" primer and probe sets. Ct = RT-PCR cycle threshold. Dotted line and grey areas indicate the limits of detection.

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Sent: 1/17/2020 11:45:22 AM
To: Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
CC: Holubar, Connie J. [cjholuba@UTMB.EDU]
Subject: select agent inspection notes

Hi Nancy,

We just completed the exit briefing for the BSL4 select agents inspection. The CDC team was very complementary of our staff and PIs both about the status of the facility and the records they reviewed. They had no major issues, but noted findings on: lack of records documentation and follow-up action for the Shope lab (resolved during discussions during the briefing); minor comments on our failure to explicitly mention security in our documentation; ongoing discussion regarding the significance of a glove tear that occurred in the Shope; recommendation to not store select agents in the same box as non-select agents; questions on the BAS upgrade certification (ongoing as systems are upgraded); and documentation in greater detail as the proposed use of material removed from the long-term storage. These are all very minor observations and I do not expect any significant issues going forward.

They were very complementary on our maintenance records of suit wear and repairs that Tom K and Miguel have implemented. Also specifically mentioned the good work of Miguel and his team in overall building operations and safety, and Johnny Peterson and his team in aerobiology.

I mentioned to them that we are attempting to obtain an isolate of the new Coronavirus from Wuhan, China, and asked about biocontainment level and that we assumed that it would not be considered as a select agent. If someone at NIAID is coordinating work on the nCoV, please let me know as we have some resources that will be useful for further analysis and countermeasure development.

Overall, a good report.

I will be in DC Thursday and Friday of next week for an NSABB meeting being held at Hyatt Regency. I let Hugh know that I would be in town and he suggested that we meet for coffee Thursday morning and today followed up with a note saying that Tony would like to meet then also. I'll let you know if anything significant is discussed.

Enjoy the long weekend!

Jim

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Sent: 4/3/2019 8:43:23 PM
To: 侯炜 [houwei@whu.edu.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Re: very nice to visit UTMB

Dear Dr Hou

I'm so happy that you enjoyed your visit and had an opportunity to meet my colleague Dr Shi. I hope that we can find a way to work together in the future.

I hope the remainder of your trip is enjoyable.

With best wishes

Jim

Sent from my iPhone

> On Apr 3, 2019, at 6:12 PM, 侯炜 <houwei@whu.edu.cn> wrote:
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
>
> Hi Prof.Le Duc,
> Yesterday afternoon I visited Prof. Shi to discuss my recently work, and to introduce you and other American collaborators' work with us in the 1980s at the Institute of Medical virology. And Dr. Hu also showed me around the campus of UTMB. Beautiful campus, I took a lot of photos.
> Your old friend,Prof. Zhan-qiu Yang retired at the end of last year. Now the faculties of the Institute are 11 persons, including 4 professors. And the institute needs young people, and it needs more international cooperation from you like before.
> Tomorrow morning I will be back. I wish your 552.117 a speedy recovery,and hopefully meet you soon in China, even Wuhan city.
>
> ---
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> 武汉大学基础医学院
> 中国武汉
> 武汉市东湖路185号，邮编430071
> 联系电话：86-27-68789310
> E-mail:houwei@whu.edu.cn
>
>
>
> 积极思考造就积极人生，消极思考造就消极人生。

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/3/2019 8:43:23 PM
To: 侯炜 [houwei@whu.edu.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Re: very nice to visit UTMB

Dear Dr Hou

I'm so happy that you enjoyed your visit and had an opportunity to meet my colleague Dr Shi. I hope that we can find a way to work together in the future.

I hope the remainder of your trip is enjoyable.

With best wishes

Jim

Sent from my iPhone

> On Apr 3, 2019, at 6:12 PM, 侯炜 <houwei@whu.edu.cn> wrote:
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
>
> Hi Prof.Le Duc,
> Yesterday afternoon I visited Prof. Shi to discuss my recently work, and to introduce you and other American collaborators' work with us in the 1980s at the Institute of Medical virology. And Dr. Hu also showed me around the campus of UTMB. Beautiful campus, I took a lot of photos.
> Your old friend,Prof. Zhan-qiu Yang retired at the end of last year. Now the faculties of the Institute are 11 persons, including 4 professors. And the institute needs young people, and it needs more international cooperation from you like before.
> Tomorrow morning I will be back. I wish your 552.117 a speedy recovery,and hopefully meet you soon in China, even Wuhan city.
>
> ---
> Wei Hou, M.D.,Ph.D.
> Professor/Vice Dean
> State Key Laboratory of Virology/Institute of Medical Virology, School of Basic Medical Sciences
> Wuhan University, P.R.China
> Dong-hu Road 185, Wuhan 430071
> Tel:86-27-68789310(office)
> E-mail: houwei@whu.edu.cn
>
>
> 侯炜 医学博士
> 教授/副院长
> 病毒学国家重点实验室/医学病毒学研究所
> 武汉大学基础医学院
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Sent: 3/31/2019 1:30:23 PM
To: 侯炜 [houwei@whu.edu.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Re: Hi

Hi Dr Hou

Thanks for your note. Unfortunately my 552.117 has been seriously ill and I have taken some time off to help her recuperate and will not be in next week. I am copying Dr Shinto see if he might have time to meet with you.

With best regards

Jim

Sent from my iPhone

> On Mar 31, 2019, at 10:39 AM, 侯炜 <houwei@whu.edu.cn> wrote:
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
>
> Dear Prof.Leduc,
>
> Very long time not to connect with you. Here I 'm in Galveston and will visit Dr. Haitao Hu's Lab next week. We have collaborated on multiple projects that investigate HIV and the associated opportunistic co-infections,and has been published on impactful scientific journals such as Plos Pathogens and Journal of Immunology. So this time I wish to have the opportunities to meet with other colleagues here at UTMB that share common research interests with my research groups in the aera of virology besides Dr. Hu. Could you have time to meet with me before I will leave here next Thursday morning?
>
>
> ---
> Wei Hou, M.D.,Ph.D.
> Professor/Vice Dean
> State Key Laboratory of Virology/Institute of Medical Virology, School of Basic Medical Sciences
> Wuhan University, P.R.China
> Dong-hu Road 185, Wuhan 430071
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> 侯炜 医学博士
> 教授/副院长
> 病毒学国家重点实验室/医学病毒学研究所
> 武汉大学基础医学院
> 中国武汉
> 武汉市东湖路185号，邮编430071
> 联系电话：86-27-68789310
> E-mail:houwei@whu.edu.cn
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From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/28/2020 3:52:29 PM
To: Handley, Gray (handleygr@niaid.nih.gov) [handleygr@niaid.nih.gov]
Subject: FW: Regarding GNL and Programs with China

From: LeDuc, James W.
Sent: Tuesday, April 28, 2020 3:52 PM
To: Ravishankar, Sid <Sid.Ravishankar@mail.house.gov>
Cc: Matthews, Douglas W. <dmatthew@UTMB.EDU>; Sheer, Lauren E. <lesheer@utmb.edu>; Crosby, Katy <Katy.Crosby@mail.house.gov>; Carey, Laura <Laura.Carey@mail.house.gov>; White, Jennifer Hendrixson <Jennifer.Hendrixson.White@mail.house.gov>; Keck, Zachary <Zachary.Keck@mail.house.gov>; Bair, James <James.Bair@mail.house.gov>; Russell, Chris <Chris.Russell@mail.house.gov>
Subject: RE: Regarding GNL and Programs with China

Hi Sid,

To the best of my knowledge, there were no restrictions put in place in 2014 or later that would have limited NIH's ability to make grants to the lab in Wuhan or any others. That said, I would refer you to NIH since this is clearly out of my lane.

Best regards,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Ravishankar, Sid <Sid.Ravishankar@mail.house.gov>
Sent: Tuesday, April 28, 2020 2:14 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Matthews, Douglas W. <dmatthew@UTMB.EDU>; Sheer, Lauren E. <lesheer@utmb.edu>; Crosby, Katy <Katy.Crosby@mail.house.gov>; Carey, Laura <Laura.Carey@mail.house.gov>; White, Jennifer Hendrixson <Jennifer.Hendrixson.White@mail.house.gov>; Keck, Zachary <Zachary.Keck@mail.house.gov>; Bair, James <James.Bair@mail.house.gov>; Russell, Chris <Chris.Russell@mail.house.gov>
Subject: Regarding GNL and Programs with China

Jim,

Thank you again for the very helpful conversation last week, and for providing the contacts at NASEM, who we will follow up with.

We were hoping that you might be able to clarify one point that has been cropping up lately and on which we'd like some ground truth. To your knowledge, were there any restrictions put in place in 2014 or thereafter that would have limited the ability of NIH or others to make grants to labs like the one in Wuhan? Any specific information you can provide would be much appreciated.

I have CCed on this email the other folks from the Foreign Affairs Committee majority staff who joined us on the call earlier as well, to ensure we all have each other's contact information.

Thank you,
Sid

____
Sid Ravishankar
Staff Director
House Foreign Affairs Committee
Subcommittee on Oversight & Investigations
Subcommittee Chairman Joaquin Castro (TX-20)
U.S. House of Representatives
O&I Subcommittee: (202) 226-6434
Castro Office: (202) 225-3236
Cell: (202) 322-2610

From: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Sent: 4/29/2020 8:06:55 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: quick question

Jim: Embassy Beijing is asking what was the official name of your DoD supported training program and some other background information. Can you send me that?

Also, can you assure these responses to their questions are accurate? I provided the text in black earlier and the red text is my response to their follow-up questions – all based on our conversations.

1) Did this training take place in the U.S., China, or in both countries?

Since 2013, the Galveston National Laboratory (GNL) of the University of Texas Medical Branch (UTMB), part of the NIH Biodefense Laboratory Network, provided laboratory safety and security training for high-level biocontainment facilities in China, including the Wuhan Institute of Virology

In the U.S. at UTMB facility.

2) Is this relationship still ongoing?

This relationship has been facilitated since 2015 through an ongoing dialogue and regular collaboration meetings co-sponsored by the Chinese Academies of Science and the U.S. National Academies of Science, Engineering and Medicine with cooperation from the Chinese CDC and others.

The training ended in 2016. The collaboration meetings convened by the CAS and the U.S. NAS, and highly regarded by the participating scientists, continued to be convened nearly each year since 2015. We understand there will not likely be a meeting this year.

3) Why was DOD funding discontinued?

This UTMB training engagement ended in 2016 when DoD funding was exhausted and not replenished from 2017 onwards.

This funding was expended on the training of scientists and facility operators to assure biosafety and biosecurity at high-containment laboratories around the world. The funding was provided by DoD, following an Congressional earmark in its appropriation, through two awards of five years each. In year seven, the awarded funding had been fully utilized. Despite requests from UTMB, DoD and other USG Agencies approached for support chose not to provide additional funding. UTMB understood this decision had to do with the overall USG position on relations with China but only DoD could say what factors were actually determinative.

4) Was there a formal name for this program? If so, please include.

According to GNL leadership, the relationship with the Wuhan Institute of Virology included the provision of training to scientists and biosafety and engineering professionals from Wuhan, as well as from other biocontainment labs in China and the China CDC. This [name of program] included operations training as the Wuhan Institute of Virology prepared to open their BSL-4 facility as part of essential global research collaboration which is necessary to develop countermeasures against the world's most dangerous public health threats.

Many thanks,

Gray

F. Gray Handley
Associate Director for International Research Affairs
National Institute of Allergy and Infectious Diseases
National Institute of Health
U.S. Department of Health and Human Services

Tel: 301 594 6128
Fax: 301 480 2954
handleygr@niaid.nih.gov

5601 Fishers Lane, Room 1E50
Bethesda, MD 20892-9802

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/29/2020 12:08:11 PM
To: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Subject: RE: quick question

The Kunming lab is a BSL4 and I believe that it has just started operations. The lab is associated with the Chinese Academy of Medical Sciences and is part of the Institute of Medical Biology that is headquartered in Kunming. Kunming is a major city in the south of China in Yunnan Province.

We have an MOU with them, although I'm not sure if it was ever finalized. Only covers scientific collaborations and no exchange of funds.

Thanks, Jim

From: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Sent: Wednesday, April 29, 2020 12:00 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: quick question

Jim, What level is the lab in Kunming and where is it? I will not volunteer that information but expect to be asked. Gray

---

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 11:39 AM
To: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Cc: Holubar, Connie J. <cjholuba@UTMB.EDU>
Subject: FW: quick question

Gray, my colleague Connie Holubar raises some concern that are valid as noted below. The 10K encounters includes training for our own UTMB staff, which was at least half to three quarters of all those trained. Further, we had very few trainees from China. We trained one building engineer from Wuhan and the two post-docs mentioned below. We also trained four individuals from Kunming (where another BSL4 is located) on building operations, and we sent a team to Kunming to offer on-site training at their facility. So we had relatively little engagement with China throughout the training center history.

Thanks, Jim

From: Holubar, Connie J. <cjholuba@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:57 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: quick question

552.111

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:14 AM
To: Holubar, Connie J. <cjholuba@UTMB.EDU>
Subject: FW: quick question

Just FYI.

From: LeDuc, James W.
Sent: Wednesday, April 29, 2020 9:12 AM
To: 'Handley, Gray (NIH/NIAID) [E]' <handleygr@niaid.nih.gov>
Subject: RE: quick question

Hi Gray,

The title for both projects was National Biocontainment Training Center. Final reports for both are attached for your information.

The Center was supported by two separate awards: W81XWH-09-2-0053 covering the period 22-05-2009 to 21-12-2014 and W81XWH-11-2-0148 covering the period 07-2011 to 07-2016. See below for specific answers. Let me know if you need additional information. Thank you for addressing these issues. I continue to believe that this is a success story and we are proud of our contributions.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
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Galveston, TX 77555-0610
(t) 409-266-6500
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(m) 409-789-2012

From: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Sent: Wednesday, April 29, 2020 8:07 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: quick question

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In the U.S. at UTMB facility. Training was provided to partners from about 70 different countries with over 10,000 training encounters offered over the life of the training center, including a few from China. Training was provided both on site using a purpose made training center on the UTMB campus and augmented with training in the Galveston National Laboratory active biocontainment suites and mechanical spaces and at host nation facilities. Training was offered to two categories of learners, those laboratory scientists who would be working in biocontainment and those building engineers who were responsible for the safe and secure operations of the laboratory infrastructure. Training included long-term training for post-doctoral fellows working in biocontainment and specific to China, we hosted Han Xia, PhD during her post-doctoral training working on Crimean-Congo hemorrhagic fever virus in the GNL BSL4 laboratories. A second post-doctoral fellow, Chan Shao, PhD, was supported using other funds after the training center award expired. Both Dr Xia and Dr Shao have now returned to the Wuhan Institute of Virology where they are working on the current coronavirus pandemic.

2) Is this relationship still ongoing?

This relationship has been facilitated since 2015 through an ongoing dialogue and regular collaboration meetings co-sponsored by the Chinese Academies of Science and the U.S. National Academies of Science, Engineering and Medicine with cooperation from the Chinese CDC and others.

The training ended in 2016. The collaboration meetings convened by the CAS and the U.S. NAS, and highly regarded by the participating scientists, continued to be convened nearly each year since 2015. We understand there will not likely be a meeting this year. We just learned today that a joint virtual meeting will be held as early as May, 2020, again jointly sponsored by the CAS and NAS. Details are just being developed.
We continue to have scientist-to-scientist dialogue and collaborations with colleagues in China and elsewhere around the world.

3) Why was DOD funding discontinued?

This UTMB training engagement ended in 2016 when DoD funding was exhausted and not replenished from 2017 onwards.

This funding was expended on the training of scientists and facility operators to assure biosafety and biosecurity at high-containment laboratories around the world. The funding was provided by DoD, following an Congressional earmark in its appropriation, through two awards of five years each. In year seven, the awarded funding had been fully utilized. Despite requests from UTMB, DoD and other USG Agencies approached for support chose not to provide additional funding. UTMB understood this decision had to do with the overall USG position on relations with China but only DoD could say what factors were actually determinative. OK. We continue to seek funding for the training center as it clearly addresses an urgent global need with the continuing proliferation of biocontainment labs around the world.

4) Was there a formal name for this program? If so, please include.

According to GNL leadership, the relationship with the Wuhan Institute of Virology included the provision of training to scientists and biosafety and engineering professionals from Wuhan, as well as from other biocontainment labs in China and the China CDC. This [name of program] included operations training as the Wuhan Institute of Virology prepared to open their BSL-4 facility as part of essential global research collaboration which is necessary to develop countermeasures against the world's most dangerous public health threats.
National Biocontainment Training Center

Many thanks,

Gray

F. Gray Handley
Associate Director for International Research Affairs
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Tel: 301 594 6128
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5601 Fishers Lane, Room 1E50
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From: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Sent: 4/29/2020 10:43:23 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
CC: Holubar, Connie J. [cjholuba@UTMB.EDU]
Subject: RE: quick question

Very helpful and just in time. Thanks to you both. Gray

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 11:39 AM
To: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
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(t) 409-266-6500
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National Biocontainment Training Center

Many thanks,

Gray

F. Gray Handley
Associate Director for International Research Affairs
National Institute of Allergy and Infectious Diseases
National Institute of Health
U.S. Department of Health and Human Services

Tel: 301 594 6128 5601 Fishers Lane, Room 1E50
Fax: 301 480 2954 Bethesda, MD 20892-9802
handleygr@niaid.nih.gov

From: 石正丽 [zlshi@wh.iov.cn]
Sent: 4/19/2020 6:13:32 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
CC: Yuan Zhiming [yzm@wh.iov.cn]; Shi, Pei yong [peshi@UTMB.EDU]
Subject: Re: RE: 回复: RE: Fwd: Rubio
Attachments: a nCoV and WIV-drf2-zl.docx

Sorry, I forgot the reviewed document.

-----原始邮件-----
发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>
发送时间:2020-04-20 01:55:34 (星期一)
收件人: "石正丽" <zlshi@wh.iov.cn>, "Yuan Zhiming" <yzm@wh.iov.cn>
抄送: "Shi, Pei yong" <peshi@UTMB.EDU>
主题: RE: 回复: RE: Fwd: Rubio

Thank you Zhengli and Zhiming for your comments and the reference publications. I did not receive the document I sent for your review so if you made comments on that, please resend. I'm afraid that this discussion will continue for some time regarding where early coronavirus work was being done, the role, if any, of the Wuhan CDC in research on bat-associated coronaviruses, and exactly when scientists at WIV first became aware of the new coronavirus and had possession of specimens in the WIV and where was that work done (level of biocontainment). Next week will be busy…

Best wishes, Jim

From: 石正丽 <zlshi@wh.iov.cn>
Sent: Saturday, April 18, 2020 11:30 PM
To: Yuan Zhiming <yzm@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; Shi, Pei yong <peshi@UTMB.EDU>
Subject: Re: 回复: RE: Fwd: Rubio

Dear James,

Thank you for your clarifying.

I've added some detailed information for your reference. I'm also sending you some papers published for your reference.

Best regards,

Zhengli,

-----原始邮件-----

发件人:"Yuan Zhiming" <yzm@wh.iov.cn>

发送时间:2020-04-19 10:55:33 (星期日)

收件人: "James LeDuc" <jwleduc@UTMB.EDU>, zlshi <zlshi@wh.iov.cn>

抄送: "Shi Peiyong" <peshi@UTMB.EDU>

主题: 回复: RE: Fwd: Rubio

Dear Jim,

Thanks for your informaiton. I really appreciate your help and your action, we need to let some people understand well the the mission of high-level biosafey lab. what we do, and how we do inside. We all know that the labs were built not for causing epidmic, but for proventing the epidemic, and the labs are managed according to interantional guildline and national accquirement, Wuhan's lab is among the others.

I will contact Zhengli to see what she can do for your report.

Regards

Zhiming

==========

Yuan Zhiming, Ph. D.

Professor of Wuhan Institute of Virology

President of Wuhan Branch

Chinese Academy of Sciences

Wuhan 430071, China

Tel: 86-27-87198195(O)

86-27-87197242(L)

Fax: 86-27-87199480

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From: LeDuc, James W.

Date: 2020-04-19 00:44

To: 石正丽

CC: Shi, Pei yong; Yuan Zhiming

Subject: RE: Fwd: Rubio

Dear Zhengli,

Thank you for your response. I understand completely and I certainly do not wish to compromise you personally or your research activities. Given our long history of collaborations between the GNL and the WIV, I have been approached repeatedly for details on our work. Attached is a draft summary that I will be providing to the leadership of our University of Texas system and likely to Congressional committees that are being formed now. Please review carefully and make any changes that you would like. I want this to be as accurate as possible and I certainly do not want to misrepresent any of your valuable contributions. I need to submit this on Monday, 20 April, so your prompt reply would be very much appreciated. I have copied Zhiming for his comments as well.

With best wishes,

Jim

From: 石正丽 <zlshi@wh.iov.cn>
Sent: Saturday, April 18, 2020 9:20 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Fwd: Rubio

Dear James,

Thank you for your email and consideration our communication.

Due to the complicated situation, I don't think it's a right time to communicate by the call.

What I can tell you is that this virus is not a leaky from our lab or any other labs.It's a shame to make this scientific question so complicated.

I hope to talk with you whenever the COVID-19 is over and world is calme and believe in the science.

Best regards,
Zhengli,

> -----原始邮件-----
> **发件人:**"LeDuc, James W." <jwleduc@UTMB.EDU>

发送时间:2020-04-17 11:06:38 (星期五)
收件人: "zengli Shi" <zlshi@wh.iov.cn>
抄送:
主题: Fwd: Rubio

Hi Zheng-Li. I hope you are well as surviving all the COVID19 drama. I wonder if you would have time for a phone call sometime soon. Let me know a good number and time and I'll call. The email below is relevant.

I will certainly understand if you are not available but Pei-Yong keeps encouraging me to call.

With all good wishes.

Jim.

My office line is 1 409 266 6516 or cell is 1 409 789 2012 if it's easier for you to call me.

Sent from my iPhone

Begin forwarded message:

> **From:** David Franz <davidrfranz@gmail.com>
> **Date:** April 16, 2020 at 8:04:55 PM CDT
> **To:** "LeDuc, James W." <jwleduc@UTMB.EDU>
> **Subject: Rubio**
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"
>
> Sent from my iPhone

From: Handley, Gray (NIH/NIAID) [E] [handleygr@niaid.nih.gov]
Sent: 4/29/2020 12:09:20 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: RE: quick question

Thanks!

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 1:08 PM
To: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Subject: RE: quick question

The Kunming lab is a BSL4 and I believe that it has just started operations. The lab is associated with the Chinese Academy of Medical Sciences and is part of the Institute of Medical Biology that is headquartered in Kunming. Kunming is a major city in the south of China in Yunnan Province.

We have an MOU with them, although I'm not sure if it was ever finalized. Only covers scientific collaborations and no exchange of funds.

Thanks, Jim

From: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Sent: Wednesday, April 29, 2020 12:00 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: quick question

Jim, What level is the lab in Kunming and where is it? I will not volunteer that information but expect to be asked. Gray

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 11:39 AM
To: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Cc: Holubar, Connie J. <cjholuba@UTMB.EDU>
Subject: FW: quick question

Gray, my colleague Connie Holubar raises some concern that are valid as noted below. The 10K encounters includes training for our own UTMB staff, which was at least half to three quarters of all those trained. Further, we had very few trainees from China. We trained one building engineer from Wuhan and the two post-docs mentioned below. We also trained four individuals from Kunming (where another BSL4 is located) on building operations, and we sent a team to Kunming to offer on-site training at their facility. So we had relatively little engagement with China throughout the training center history.

Thanks, Jim

From: Holubar, Connie J. <cjholuba@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:57 AM

To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: quick question

552.111

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:14 AM
To: Holubar, Connie J. <cjholuba@UTMB.EDU>
Subject: FW: quick question

Just FYI.

From: LeDuc, James W.
Sent: Wednesday, April 29, 2020 9:12 AM
To: 'Handley, Gray (NIH/NIAID) [E]' <handleygr@niaid.nih.gov>
Subject: RE: quick question

Hi Gray,

The title for both projects was National Biocontainment Training Center. Final reports for both are attached for your information.

The Center was supported by two separate awards: W81XWH-09-2-0053 covering the period 22-05-2009 to 21-12-2014 and W81XWH-11-2-0148 covering the period 07-2011 to 07-2016. See below for specific answers. Let me know if you need additional information. Thank you for addressing these issues. I continue to believe that this is a success story and we are proud of our contributions.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Sent: Wednesday, April 29, 2020 8:07 AM

To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: quick question

Jim: Embassy Beijing is asking what was the official name of your DoD supported training program and some other background information. Can you send me that?

Also, can you assure these responses to their questions are accurate? I provided the text in black earlier and the red text is my response to their follow-up questions – all based on our conversations.

1) Did this training take place in the U.S., China, or in both countries?

Since 2013, the Galveston National Laboratory (GNL) of the University of Texas Medical Branch (UTMB), part of the NIH Biodefense Laboratory Network, provided laboratory safety and security training for high-level biocontainment facilities in China, including the Wuhan Institute of Virology

In the U.S. at UTMB facility. Training was provided to partners from about 70 different countries with over 10,000 training encounters offered over the life of the training center, including a few from China. Training was provided both on site using a purpose made training center on the UTMB campus and augmented with training in the Galveston National Laboratory active biocontainment suites and mechanical spaces and at host nation facilities. Training was offered to two categories of learners, those laboratory scientists who would be working in biocontainment and those building engineers who were responsible for the safe and secure operations of the laboratory infrastructure. Training included long-term training for post-doctoral fellows working in biocontainment and specific to China, we hosted Han Xia, PhD during her post-doctoral training working on Crimean-Congo hemorrhagic fever virus in the GNL BSL4 laboratories. A second post-doctoral fellow, Chan Shao, PhD, was supported using other funds after the training center award expired. Both Dr Xia and Dr Shao have now returned to the Wuhan Institute of Virology where they are working on the current coronavirus pandemic.

2) Is this relationship still ongoing?

This relationship has been facilitated since 2015 through an ongoing dialogue and regular collaboration meetings co-sponsored by the Chinese Academies of Science and the U.S. National Academies of Science, Engineering and Medicine with cooperation from the Chinese CDC and others.

The training ended in 2016. The collaboration meetings convened by the CAS and the U.S. NAS, and highly regarded by the participating scientists, continued to be convened nearly each year since 2015. We understand there will not likely be a meeting this year. We just learned today that a joint virtual meeting will be held as early as May, 2020, again jointly sponsored by the CAS and NAS. Details are just being developed.
We continue to have scientist-to-scientist dialogue and collaborations with colleagues in China and elsewhere around the world.

3) Why was DOD funding discontinued?

This UTMB training engagement ended in 2016 when DoD funding was exhausted and not replenished from 2017 onwards.

This funding was expended on the training of scientists and facility operators to assure biosafety and biosecurity at high-containment laboratories around the world. The funding was provided by DoD, following an Congressional earmark in its appropriation, through two awards of five years each. In year seven, the awarded funding had been fully utilized. Despite requests from UTMB, DoD and other USG Agencies approached for support chose not to provide additional funding. UTMB understood this decision had to do with the overall USG position on relations with China but

only DoD could say what factors were actually determinative. OK. We continue to seek funding for the training center as it clearly addresses an urgent global need with the continuing proliferation of biocontainment labs around the world.

4) Was there a formal name for this program? If so, please include.

According to GNL leadership, the relationship with the Wuhan Institute of Virology included the provision of training to scientists and biosafety and engineering professionals from Wuhan, as well as from other biocontainment labs in China and the China CDC. This [name of program] included operations training as the Wuhan Institute of Virology prepared to open their BSL-4 facility as part of essential global research collaboration which is necessary to develop countermeasures against the world's most dangerous public health threats.
National Biocontainment Training Center

Many thanks,

Gray

F. Gray Handley
Associate Director for International Research Affairs
National Institute of Allergy and Infectious Diseases
National Institute of Health
U.S. Department of Health and Human Services

Tel: 301 594 6128 5601 Fishers Lane, Room 1E50
Fax: 301 480 2954 Bethesda, MD 20892-9802
handleygr@niaid.nih.gov

From: Zheng郑大胜 [d.zheng@wh.iov.cn]
Sent: 11/11/2019 10:01:49 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: Re:RE: Re:RE: Re:RE: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Prof. LeDuc,

Just now, actually five minutes ago, Prof. Yuan Zhiming tells me that he is not suitable for strongly recoomend me to your laboratory and that I could choose somewhere else to visit. The tone "not suitable for" reminds me of the reply to my transferring proposal of international collaboration between GNL and WNBL in June and July of 2016.

So far I have to look for other opportunities to USA. Thank you very much for the sincere assistance to me all the time.

Best Regards,

Dasheng
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ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969
==========

At 2019-10-17 00:49:34, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dasheng,

As I tried to explain in my earlier message of 2 Oct 2019, I need additional information on the objective of your training and how it will contribute to your position in Wuhan and our ongoing collaborations. I cannot commit to a one year visit without knowing what the expectations will be for your fellowship. Pei-Yong Shi will be in Wuhan soon and I suggest that you meet with him and Zhiming Yuan to discuss how your fellowship will build on our ongoing work together.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Zheng郑大胜 [d.zheng@wh.iov.cn]
Sent: 11/11/2019 10:01:49 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: Re:RE: Re:RE: Re:RE: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Prof. LeDuc,

Just now, actually five minutes ago, Prof. Yuan Zhiming tells me that he is not suitable for strongly recoomend me to your laboratory and that I could choose somewhere else to visit. The tone "not suitable for" reminds me of the reply to my transferring proposal of international collaboration between GNL and WNBL in June and July of 2016.

So far I have to look for other opportunities to USA. Thank you very much for the sincere assistance to me all the time.

Best Regards,

Dasheng
--
ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969
==========

At 2019-10-17 00:49:34, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dasheng,

As I tried to explain in my earlier message of 2 Oct 2019, I need additional information on the objective of your training and how it will contribute to your position in Wuhan and our ongoing collaborations. I cannot commit to a one year visit without knowing what the expectations will be for your fellowship. Pei-Yong Shi will be in Wuhan soon and I suggest that you meet with him and Zhiming Yuan to discuss how your fellowship will build on our ongoing work together.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 1/30/2020 5:13:05 PM
To: Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
Subject: FW: UTMB/GNL Coronavirus Activity
Attachments: GNL Coronavirus Update for Congressman Weber (002).docx

Here is the document we sent to Representative Weber's office earlier today. It gives you a good overview of the campus-wide response activities.

From: Holubar, Connie J. <cjholuba@UTMB.EDU>
Sent: Thursday, January 30, 2020 2:18 PM
To: Harvey, Tom <Tom.Harvey@mail.house.gov>
Cc: Matthews, Douglas W. <dmatthew@UTMB.EDU>; Sheer, Lauren E. <lesheer@utmb.edu>; Lidstone, Sheila <shlidsto@UTMB.EDU>; LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: UTMB/GNL Coronavirus Activity

Mr. Harvey,

On behalf of Dr. Jim Le Duc, I've attached a document that summarizes the initiatives that are underway at UTMB with regard to the new Coronavirus.

Let us know if you have any questions or need further updates.

Kind regards,

Connie Jean Holubar, MS, MBA
Director of Operations
Galveston National Laboratory

The University of Texas Medical Branch
301 University Blvd., Galveston, TX 77555-0128
P 409.266.6518 E cjholuba@utmb.edu

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Monday, January 27, 2020 11:10 AM
To: Harvey, Tom <Tom.Harvey@mail.house.gov>
Cc: Holubar, Connie J. <cjholuba@UTMB.EDU>; Matthews, Douglas W. <dmatthew@UTMB.EDU>; Lidstone, Sheila <shlidsto@UTMB.EDU>
Subject: RE: GNL Coronavirus Activity

Hi Tom,

I'll pull a summary together and send later today. As you might imagine, we're busy on several fronts.

Best regards,

Jim

James W. Le Duc, Ph.D.

Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Harvey, Tom <Tom.Harvey@mail.house.gov>
Sent: Monday, January 27, 2020 11:00 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: GNL Coronavirus Activity

Hello Jim,

I wanted to reach out to see whether the GNL had materials you could share regarding your efforts to respond to the Wuhan Coronavirus.

I saw the information y'all have placed on the front page of your website, but I wanted to see if there was anything else I could share with the Congressman to keep him informed of how Texas 14 and UTMB is at the forefront of responding to this health scare. We would love to highlight GNL's efforts as appropriate.

Thank you,
Tom

Tom Harvey
Sr. Legislative Assistant
Congressman Randy K. Weber (TX-14)
107 Cannon House Office Building
(202) 225-2831

utmb Health
Galveston National Laboratory

GNL/UTMB Efforts Against the new Coronavirus

January 29, 2020

Healthcare Preparedness and Diagnostics

UTMB is following CDC guidance regarding diagnostics for suspect cases and has volunteered to assist state and local health departments by providing surge capacity for diagnostic testing, should the need arise.

The Galveston National Laboratory is prepared to perform virus isolation attempts under appropriate biocontainment conditions, should a patient be encountered.

UTMB announced today that we will begin screening all patients for recent travel to China and for symptoms associated with this virus, such as fever, cough or sore throat.

UTMB students, staff and faculty returning from China (or other high risk areas) are being screened and evaluated individually to determine if home quarantine is warranted.

Our Biocontainment Care Unit, built to care for patients with highly contagious diseases, is prepared to open if needed. Additional Personal Protective Equipment is being acquired to have on hand.

UTMB's infectious disease task force and emergency response groups have convened and are meeting regularly to monitor the situation and take action as needed.

Countermeasure Development and Science

UTMB has several existing funded research projects on Coronaviruses, including nationally respected translational programs for vaccine and therapy development against both SARS and MERS. Senior investigators at UTMB will focus on **two separate strategies for vaccine development** for the nCoV. One is a continuation of the work Dr. Kent Tseng has pioneered with collaborators at Baylor College of Medicine focused on SARS and MERS coronaviruses and adapting that strategy to the development of a vaccine for the new virus. The second is the vaccine platform that Dr. Pei-Yong Shi and his team developed for Zika virus that is being adapted to target nCoV.

In addition, Dr. Tseng has developed a very viable mouse model with an adapted immune system that allows the mouse to be infected with human Coronavirus diseases. This will be critically important in early demonstration of vaccine efficacy (as well as for testing of antiviral drug candidates).

It's important to note that gaining access to the live virus is essential for these experiments. To date we still have not received an isolate. We have requested this from both our colleagues in Wuhan and also from CDC. We are continuing, however, to develop the vaccine candidates using the published sequence information that was provided by Chinese officials. **Assistance in access to the live virus would be very much appreciated.**

The Galveston National Lab stands ready to accommodate additional priority research on this new virus as requested by NIH or other national or state officials.

Collaborations with China

We have been working for many years with our U.S. National Academy of Sciences in partnership with the Chinese Academy of Sciences, the Chinese CDC and the Wuhan Institute of Virology to develop strong relationships with the Chinese biocontainment research labs, particularly the BSL4 laboratory in Wuhan. Through these collaborations, we have established close personal friendships with many Chinese leaders and have been in frequent communications with experts in Wuhan as the coronavirus outbreak has unfolded. **This kind of personal relationship is extremely valuable and it is important to sustain these collaborations.**

We have assisted the Wuhan Institute of Virology in developing their high containment training facilities and have had staff, researchers and students from Wuhan train with us in the Galveston National Laboratory on both facilities operations and maintenance best practices and on the conduct of research in biocontainment. Former PhD Fellows trained here are currently working in the Wuhan high containment labs on the new coronoavirus.

Public Information

UTMB experts are serving as resources for regional and national media regarding Coronaviruses, countermeasure development strategies and outbreak response.

Our infectious disease experts and Galveston National Laboratory leadership are participating with national and international public health organizations in their global efforts and sharing information with state and county health department officials.

We continue to participate on the Governor's Task Force on Infectious Diseases and collaborate with state health department officials. Our next meeting will be Feb. 4.

Our researchers are closely monitoring this fast-developing story, reviewing newly released papers and contributing to the scientific discussions.

Our goal is to assist with the public information needs, which continue to be significant within the framework of this quickly developing situation. Our communications efforts are, for the most part, responsive in nature at this time, with several television and news outlet interviews being conducted each day. Our focus has been on minimizing panic through education and the sharing of scientific information about these types of viruses, as well as providing information about the role of the Galveston National Lab in these situations.

Beyond the responsive press, Dr. Jim Le Duc wrote an editorial that ran in the Houston Chronicle on Jan. 22 about the improved relationships between the US and Chinese research and public health communities. It is linked here: [HYPERLINK "https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.houstonchronicle.com%2Fopinion%2Foutlook%2Farticle%2FU-S-China-relationship-good-news-with-the-bad-14992714.php&data=02%7C01%7Ccjholuba%40utmb.edu%7C3277d7240e3c4fb5f82708d79f5aedd9%7C7bef256d85db4526a72d31aea2546852%7C0%7C0%7C637153085199564138&sdata=us7JJuPQecki51DqCE8mzZ1UjsKUlBTNZqiT5TZnMz8%3D&reserved=0"]

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 1/24/2020 7:01:19 AM
To: Robert Kadlec (OS/ASPR/IO) [Robert.Kadlec@hhs.gov]; Hugh Auchincloss [E] [auchinclossh@niaid.nih.gov]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Fwd: No Snakes?

See text at bottom for more on sequence analysis.

Also I just learned that Wuhan leadership is requesting we raise our request for the isolates to higher political level. Can we get our ambassador involved? Scientists are eager to share. This is a political decision now.

Thanks. Jim

Sent from my iPhone

Begin forwarded message:

From: "Ksiazek, Thomas G." <tgksiaze@UTMB.EDU>
Date: January 24, 2020 at 2:33:14 AM EST
To: "LeDuc, James W." <jwleduc@UTMB.EDU>, "Tseng, Chien-Te K." <sktseng@UTMB.EDU>, "Menachery, Vineet" <vimenach@UTMB.EDU>, Tesh Robert <rbtesh22@gmail.com>, "Holubar, Connie J." <cjholuba@UTMB.EDU>
Subject: Fwd: No Snakes?

Tom Ksiazek

Sent from a portable device

Begin forwarded message:

From: Dean Erdman <derdman05@gmail.com>
Date: January 23, 2020 at 22:29:42 CST
To: "Ksiazek, Thomas G." <tgksiaze@UTMB.EDU>
Subject: No Snakes?

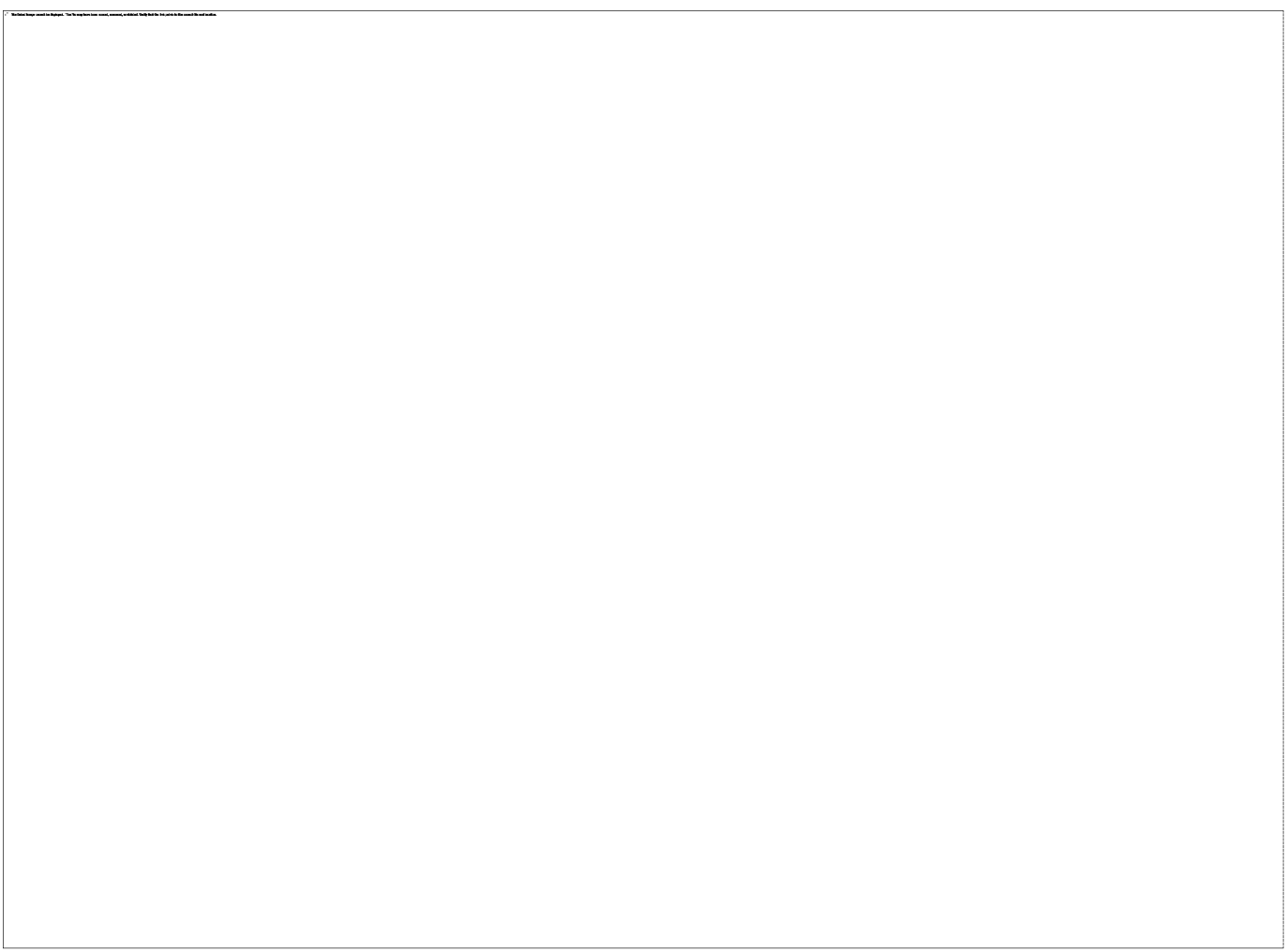
nCoV's relationship to bat coronaviruses & recombination signals (no snakes)

Novel 2019 coronavirus

david.l.robertson

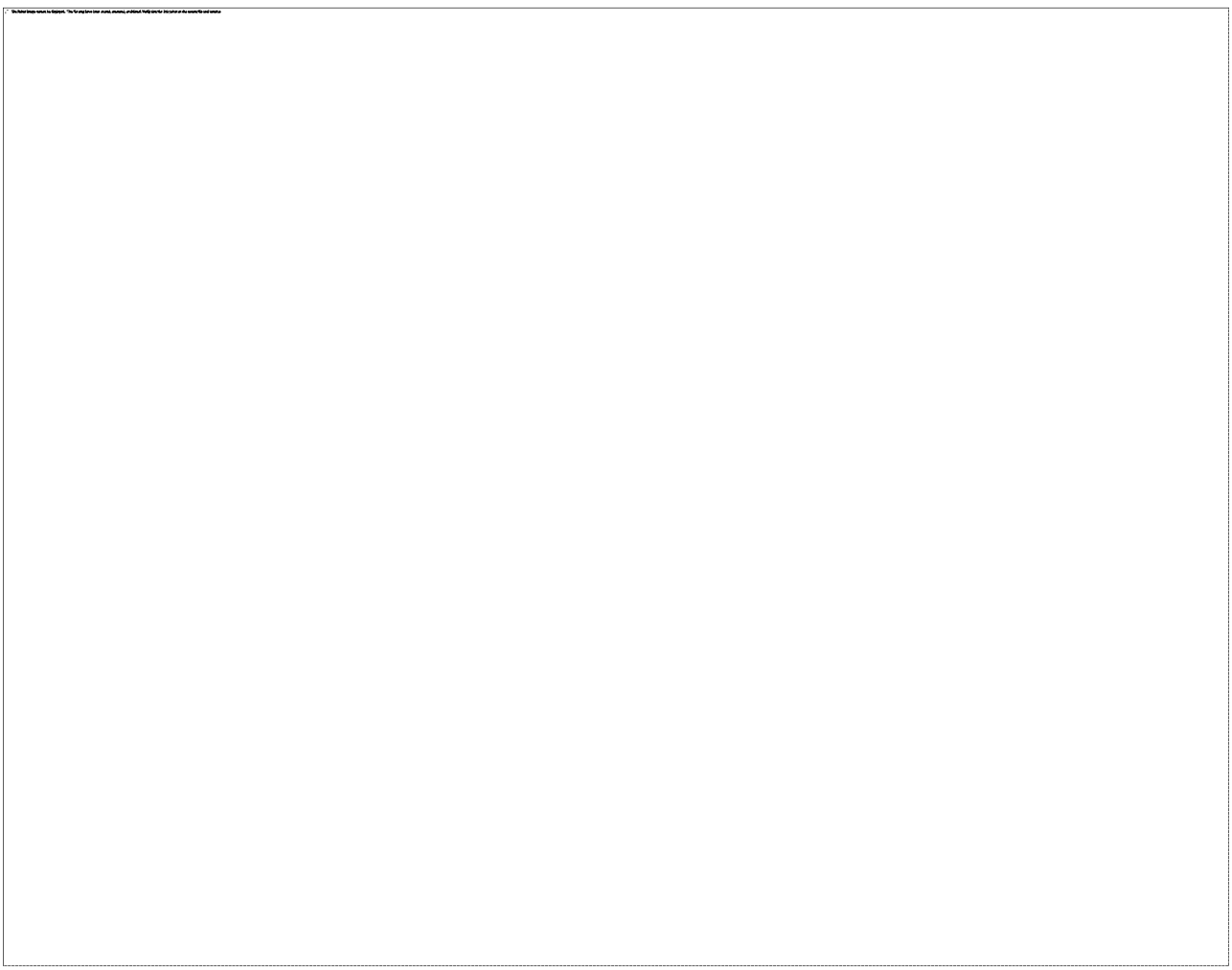
2
1d

With Xiaowei Jiang at XJTLU we've carried out a preliminary evolutionary analysis to characterise the evolutionary origins of the Wuhan virus, nCoV. Focus of our analysis is on the Wuhan-Hu-1 virus (accession no. MN908947, released on GenBank by Shanghai Public Health Clinical Center and School of Public Health, Fudan University, Shanghai, China) as all nCoV cluster together so will share the same evolutionary ancestry. It's clear from phylogenetic analysis the new human virus is most closely related to bat coronaviruses in the Betacoronaviruses genera. While this is apparent from both the previously reported BLAST and full-genome phylogenetic analysis the closest related bat viruses (CoVZC45 and CoVZXC21) are in fact recombinants with shared breakpoints either side of ORF1b:



nCoV-recombination-analysis-v22416×1768 748 KB

The phylogenetic clustering of the Wuhan-Hu-1 virus is consistently as a sister group to the SARS-related bat coronaviruses. Interestingly, a third bat coronavirus (Longquan_140) is a recombinant involving the Wuhan virus lineage in part of ORF1a.



1680-3014692×540 50.6 KB

This analysis has detected three bat coronavirus recombinants (two with shared breakpoints) involving the nCoV lineage indicating greater diversity in the Chinese Sarbecovirus group than previously appreciated. The clustering of the related Sarbecovirus viruses from Kenya and Europe suggest the Wuhan virus is still part of the Sabecovirus sub-genre, and these recombination events probably occurred in bats. Although. given the propensity of coronaviruses to switch hosts, involvement of another species cannot be discounted. There is also a very good chance that a non-bat intermediate species is responsible for the beginning of the current outbreak in Wuhan. Given the tight clustering of the nCoV viruses in phylogenetic trees it seems most likely one event has occurred.

Several of these bat coronaviruses have been previously detected to be recombinant under-scoring the importance of doing appropriate analysis when analysing these viruses using phylogenetic methods. Recombination, in this case between divergent coronaviruses circulating in bats, violates our assumption of a single evolutionary tree and so needs to be considered carefully when inferring coronavirus evolution from complete genome alignments. We're looking into the patterns of breakpoints to see if there's any clues to the significance (or not) of these recombination events.

We'd like to thank the researchers and health professionals for making the nCoV data available. Credit also needs to be given to the surveillance projects for generating the data that is now available for comparison and to the software developers for making the tools we've used freely available: FigTree, available at: http://tree.bio.ed.ac.uk/software/figtree/; 27 GARD, available at http://www.hyphy.org 15; MAFFT, available at https://mafft.cbrc.jp/alignment/software/; 7 PhyML, available at http://www.atgc-montpellier.fr/phyml/; 9 and RDP4, available at http://www.atgc-montpellier.fr/phyml/ 17.

- **created**

1d

- **last reply**

9h

- 13 **replies**
- 8.5k **views**
- 9 **users**
- 5 **likes**
- 8 **links**

-  4

 3

david.l.robertson
1d

Just to be clear Spike is at positions 21717 to 25693 in our diversity plot and recombination analysis so to the right of the recombination breakpoint in the bat viruses CoVZC45 and CoVZXC21. In a Spike phylogeny nCoV clusters with these bat viruses. There is no evidence of snakes being involved as incorrectly reported here https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25682 189!

m.koopmans
22h

Hi David
Thanks for sharing this. Interesting dive into the hidden world of these viruses in their reservoir (presumably). I guess there will be insufficient sampling of bat viruses do dabble at when this may have occurred?

Would also like to hear your opinion on the "snake"paper. I see it criticised but am not familiar enough with the specific analyses to make a real assessment.

Marion

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Commentary

- Zoonotic origins of human coronavirus 2019 (HCoV-19 / SARS-CoV-2): why is this work important?

Gary Wong, Yu-Hai Bi, Qi-Hui Wang, Xin-Wen Chen, Zhi-Gang Zhang, Yong-Gang Yao

Fulltext HTML PDF

Abstract: The ongoing pandemic of coronavirus disease 2019 (COVID-19), caused by infection with human coronavirus 2019 (HCoV-19 / SARS-CoV-2 / 2019-nCoV), is a global threat to the human population. Here, we briefly summarize the available data for the zoonotic origins of HCoV-19, with reference to the other two epidemics of highly virulent coronaviruses, SARS-CoV and MERS-CoV, which cause severe pneumonia in humans. We propose to intensify future efforts for tracing the origins of HCoV-19, which is a very important scientific question for the control and prevention of the pandemic.

2019年人类冠状病毒(HCoV-19)引起人畜共患病溯源研究的重要性

摘要: 2019年人类冠状病毒（HCoV-19/SARS-CoV-2/2019-nCoV）感染引起2019年肺炎爆发，造成对人类全球性威胁。作者参考两种引起人类严重肺炎的新型冠状病毒SARS-CoV和MERS-CoV的流行病学数据，总结了HCoV-19人畜共患病起源的现有数据。作者建议今后应加紧HCoV-19的溯源研究，为控制和预防该病提供重要的科学依据。

Cite this article: Gary Wong, Yu-Hai Bi, Qi-Hui Wang, Xin-Wen Chen, Zhi-Gang Zhang, Yong-Gang Yao. 2020: Zoonotic origins of human coronavirus 2019 (HCoV-19 / SARS-CoV-2): why is this work important?. *Zoological Research*, 41(3): 213-219. doi: 10.24272/j.issn.2095-8137.2020.031

Reviews

- The pathological role of ferroptosis in ischemia/reperfusion-related injury

Hong-Fa Yan, Qing-Zhang Tuo, Qiao-Zhi Yin, Peng Lei

Fulltext HTML PDF

Abstract: Ischemia/reperfusion (I/R) is a pathological process that occurs in numerous organs throughout the human body, and it is frequently associated with severe cellular damage and death. Recently it has emerged that ferroptosis, a new form of regulated cell death that is caused by iron-dependent lipid peroxidation, plays a significantly detrimental role in many I/R models. In this review, we aim to revise the pathological process of I/R and then explore the molecular pathogenesis of ferroptosis. Furthermore, we aim to evaluate the role that ferroptosis plays in I/R, providing evidence to support the targeting of

ferroptosis in the I/R pathway may present as a therapeutic intervention to alleviate ischemia/reperfusion injury (IRI) associated cell damage and death.

铁死亡在缺血再灌注相关损伤中的病理作用

摘要: 缺血再灌注(I/R)是一种发生在人体多个器官的病理过程，常会导致严重的细胞损伤，甚至死亡。参与I/R导致的细胞死亡的分子机制一直是研究热点。以铁依赖的脂质过氧化作为特征的铁死亡（Ferroptosis）近来被发现了在多种I/R相关疾病中都扮演重要角色。本综述总结了I/R的病理过程，并探讨了其分子机制。这些实验证据支持铁死亡在I/R通路中的靶向角色，因此干预铁死亡可能减轻I/R相关细胞损伤和死亡。

Cite this article: Hong-Fa Yan, Qing-Zhang Tuo, Qiao-Zhi Yin, Peng Lei. 2020: The pathological role of ferroptosis in ischemia/reperfusion-related injury. *Zoological Research*, 41(3): 220-230. doi: 10.24272/j.issn.2095-8137.2020.042

- **Interfacial phenomena of water striders on water surfaces: a review from biology to biomechanics**

Jing-Ze Ma, Hong-Yu Lu, Xiao-Song Li, Yu Tian

Fulltext HTML PDF

Abstract: Water striders have intrigued researchers for centuries from the viewpoints of biology to biomechanics. In this review, we introduce the basic theories and techniques of physics and force measurement for biomechanical research into water striders. Morphological and behavioral traits of water striders are summarized and discussed from biomechanical perspectives, along with comparative study. This integrated review also highlights potential directions for studies on water-walking arthropods, which might inspire future biological and biomechanical research.

水黾在水面上的界面现象：从生物学到生物力学

摘要: 水黾在生物学和生物力学领域引起了广泛关注。该文介绍了水黾生物力学研究中的物理理论和力值测量方法。从生物力学角度，该文总结探讨了水黾的形态特征和行为特征，并着重讨论了有关水黾的比较研究趋势。此综述也为未来对水上行走生物的可能研究方向提出了展望。

Cite this article: Jing-Ze Ma, Hong-Yu Lu, Xiao-Song Li, Yu Tian. 2020: Interfacial phenomena of water striders on water surfaces: a review from biology to biomechanics. *Zoological Research*, 41(3): 231-246. doi: 10.24272/j.issn.2095-8137.2020.029

Articles

- **Decoding the evolution and transmissions of the novel pneumonia coronavirus (SARS-CoV-2 / HCoV-19) using whole genomic data**

Wen-Bin Yu, Guang-Da Tang, Li Zhang, Richard T. Corlett

Fulltext HTML PDF

Abstract: The outbreak of COVID-19 started in mid-December 2019 in Wuhan, China. Up to 29 February 2020, SARS-CoV-2 (HCoV-19 / 2019-nCoV) had infected more than 85 000 people in the world. In this study, we used 93 complete genomes of SARS-CoV-2 from the GISAID EpiFlu™ database to investigate the evolution and human-to-human transmissions of SARS-CoV-2 in the first two months of the outbreak. We constructed haplotypes of the SARS-CoV-2 genomes, performed phylogenomic analyses and estimated the potential population size changes of the virus. The date of population expansion was calculated based on the expansion parameter tau (τ) using the formula $t=\tau/2u$. A total of 120 substitution sites with 119 codons, including 79 non-synonymous and 40 synonymous substitutions, were found in eight coding-regions in the SARS-CoV-2 genomes. Forty non-synonymous substitutions are potentially associated with virus adaptation. No combinations were detected. The 58 haplotypes (31 found in samples from China and 31 from outside China) were identified in 93 viral genomes under study and could be classified into five groups. By applying the reported bat coronavirus genome (bat-RaTG13-CoV) as the outgroup, we found that haplotypes H13 and H38 might be considered as ancestral haplotypes, and later H1 was derived from the intermediate haplotype H3. The population size of the SARS-CoV-2 was estimated to have undergone a recent expansion on 06 January 2020, and an early expansion on 08 December 2019. Furthermore, phyloepidemiologic approaches have recovered specific directions of human-to-human transmissions and the potential sources for international infected cases.

基于全基因组数据解析新型冠状病毒演化和传播

摘要: 2019年12月中旬新型肺炎疫情(COVID-19)在湖北武汉爆发，截至2020年2月29日，新冠病毒SARS-CoV-2（2019-nCoV/HCoV-19）已感染全球85 000多人。在本研究中，我们使用了GISAID EpiFluTM数据库中的93个完整新冠病毒基因组来解析新冠病毒在疫情爆发前两个月的演化，以及人际传播情况。我们采用系统发育网状分析方法，构建了SARS-CoV-2基因组的单倍型的演化关系，并进一步对病毒的潜在种群大小变化进行估算。利用公式t =τ/ 2u，基于扩展参数tau（τ）推算了病毒可能发生扩张（即大量人传人）日期。在新冠病毒基因组的八个编码区中共发现120个的变异位点，关联119个密码子有79个非同义和40个同义替换。我们猜测40个改变氨基酸属性的非同义替换可能与病毒适应性有关。另外，我们分析中没有检测到病毒重组事件。从93个病毒基因组中鉴定出58种单倍型，将其划分为五组用于传播和扩散分析。通过蝙蝠冠状病毒基因组（bat-RaTG13-CoV）作为外群，我们发现单倍型H13和H38可能是古老的单倍型，单倍型H1是从单倍型H3演化而来。种群扩张时间估算发现在2020年1月6日和2019年12月8日可能发生大量人传人。此外，我们利用谱系流行病学方法回溯了一些人际传播事件。同时，这种方法可以用于寻找感染病例的病毒感染源。

Cite this article: Wen-Bin Yu, Guang-Da Tang, Li Zhang, Richard T. Corlett. 2020: Decoding the evolution and transmissions of the novel pneumonia coronavirus (SARS-CoV-2 / HCoV-19) using whole genomic data. *Zoological Research*, 41(3): 247-257. doi: 10.24272/j.issn.2095-8137.2020.022

- **Social avoidance behavior in male tree shrews and prosocial behavior in male mice toward unfamiliar conspecifics in the laboratory**

Rong-Jun Ni, Yang Tian, Xin-Ye Dai, Lian-Sheng Zhao, Jin-Xue Wei, Jiang-Ning Zhou, Xiao-Hong Ma, Tao Li

Fulltext HTML PDF

Abstract: Adult male tree shrews vigorously defend against intruding male conspecifics. However, the characteristics of social behavior have not been entirely explored in these males. In this study, male wild-type tree shrews (*Tupaia belangeri chinensis*) and C57BL/6J mice were first allowed to familiarize themselves with an open-field apparatus. The tree shrews exhibited a short

duration of movement (moving) in the novel environment, whereas the mice exhibited a long duration of movement. In the 30 min social preference-avoidance test, target animals significantly decreased the time spent by the experimental tree shrews in the social interaction (SI) zone, whereas experimental male mice exhibited the opposite. In addition, experimental tree shrews displayed a significantly longer latency to enter the SI zone in the second 15 min session (target-present) than in the first 15 min session (target-absent), which was different from that found in mice. Distinct behavioral patterns in response to a conspecific male were also observed in male tree shrews and mice in the first, second, and third 5 min periods. Thus, social behaviors in tree shrews and mice appeared to be time dependent. In summary, our study provides results of a modified social preference-avoidance test designed for the assessment of social behavior in tree shrews. Our findings demonstrate the existence of social avoidance behavior in male tree shrews and prosocial behavior in male mice toward unfamiliar conspecifics. The tree shrew may be a new animal model, which differs from mice, for the study of social avoidance and prosocial behaviors.

实验室中雄性树鼩对陌生同类表现出社交回避行为而雄性小鼠则表现出亲社会行为

摘要: 成年雄性树鼩对入侵的同类防御剧烈。但是，这些雄性树鼩的社交行为特征尚未得到全面探究。本研究中，首先让雄性野生的中国树鼩和C57BL/6J小鼠熟悉测试用的旷场箱子，树鼩在新颖的旷场环境中运动的时间较短，而小鼠则表现较长的运动时间。在30分钟的社会偏好与回避实验中，目标树鼩（入侵者）显著减少了实验树鼩（居住者）在社会交互区域（SI）中的停留时间，而实验小鼠表现出相反的结果。此外，实验树鼩表现出在第二个15分钟测试（存在目标树鼩）中进入SI中的潜伏期比在第一个15分钟测试（无目标树鼩）中更长，这与在小鼠中发现的结果不同。在第一个、第二个和第三个5分钟时间内，还观察到了雄性树鼩和小鼠对于同类入侵者表现出不同的行为范式，因此，树鼩和小鼠的社交行为似乎表现出时间依赖性。总而言之，我们的研究提供了一种改良的社会偏好与回避实验方法用于评估树鼩的社交行为。我们的发现表明，雄性树鼩对陌生同类表现出社交回避行为，而雄性小鼠对陌生同类表现出亲社会行为。树鼩可能是一种新的用于研究社会回避和亲社会行为的动物模型，这不同于小鼠社交模型。

Cite this article: Rong-Jun Ni, Yang Tian, Xin-Ye Dai, Lian-Sheng Zhao, Jin-Xue Wei, Jiang-Ning Zhou, Xiao-Hong Ma, Tao Li. 2020: Social avoidance behavior in male tree shrews and prosocial behavior in male mice toward unfamiliar conspecifics in the laboratory. *Zoological Research*, 41(3): 258-272. doi: 10.24272/j.issn.2095-8137.2020.034

- **Impact of sympatric carnivores on den selection of wild giant pandas**

Xin-Lei Lai, Wen-Liang Zhou, Hua-Lei Gao, Meng Wang, Kai Gao, Bao-Wei Zhang, Fu-Wen Wei, Yong-Gang Nie

Fulltext HTML PDF

Abstract: Interspecific killing is a primary reason for the low survival rates of some animal species. The giant panda (*Ailuropoda melanoleuca*) is an altricial eutherian mammal and thus, in comparison to other infants, panda cubs are highly vulnerable, which may significantly influence the selection of breeding sites by females. Here, we used infrared camera traps to monitor giant panda dens for 5.5 years in Foping National Nature Reserve (FNNR) to determine how interspecific factors affect den selection by wild female pandas. Results indicated that Asian black bears (*Ursus thibetanus*), yellow-throated martens (*Martes flavigula*), leopard cats (*Prionailurus bengalensis*), and masked palm civets (*Paguma larvata*) visited the dens frequently, and the presence of these species negatively influenced den selection by female pandas. Interestingly, the presence of rodents and terrestrial birds appeared to indicate den safety, and female giant pandas were not averse and even preferred dens with a high abundance index of rodents and terrestrial birds. The den suitability index (DSI) was a reliable tool for evaluating whether dens were suitable for female giant pandas to give birth to and rear cubs, with preference for dens with high DSI

values. This study increases our understanding of the den selection criteria of female giant pandas and the main threats to the survival of their cubs, thus providing important guidance for the conservation and management of this species.

同域分布食肉动物对野生大熊猫巢穴选择的影响

摘要: 种间捕杀是导致某些动物物种存活率低的主要原因。大熊猫(*Ailuropoda melanoleuca*)幼仔一般被认为是晚成性，与其它动物相比，其幼仔出生时非常脆弱，这直接影响雌性个体对繁殖巢穴的选择。我们利用红外相机技术对佛坪国家自然保护区的大熊猫潜在巢穴进行了长达五年多的监测，以确定同域物种如何影响野生雌性大熊猫的巢穴选择。结果表明，亚洲黑熊(*Ursus thibetanus*)、黄喉貂(*Martes flavigula*)、豹猫(*Prionailurus bengalensis*)和果子狸(*Paguma larvata*)等食肉动物频繁出现在大熊猫洞穴，而这些食肉动物的到访对雌性大熊猫产仔洞穴的选择产生了负面影响。有趣的是，雌性大熊猫对啮齿类和鸟类的出现并不敏感，甚至更偏好选择啮齿类和鸟类丰度指数高的巢穴，这些物种的出现似乎在一定程度表明了巢穴的安全性。我们引入巢穴适宜性指数(DSI) 来评估巢穴是否适合雌性大熊猫产仔和育幼，结果显示DSI是一个评估巢穴适宜性的可靠工具，雌性大熊猫优先选择具有高DSI值的巢穴。本研究提高了我们对雌性大熊猫繁殖巢穴选择标准和幼仔生存所受主要威胁的认识，为该物种的保护和管理提供了重要的指导作用。

Cite this article: Xin-Lei Lai, Wen-Liang Zhou, Hua-Lei Gao, Meng Wang, Kai Gao, Bao-Wei Zhang, Fu-Wen Wei, Yong-Gang Nie. 2020: Impact of sympatric carnivores on den selection of wild giant pandas. *Zoological Research*, 41(3): 273-280. doi: 10.24272/j.issn.2095-8137.2020.027

- **Targeting lentiviral vectors to primordial germ cells (PGCs): An efficient strategy for generating transgenic chickens**

Zi-Qin Jiang, Han-Yu Wu, Jing Tian, Ning Li, Xiao-Xiang Hu

Fulltext HTML PDF

Abstract: Recent advances in avian transgenic studies highlight the possibility of utilizing lentiviral vectors as tools to generate transgenic chickens. However, low rates of gonadal chimerism and germ line transmission efficiency still limit the broad usage of this method in creating transgenic chickens. In this study, we implemented a simple strategy using modified lentiviral vectors targeted to chicken primordial germ cells (PGCs) to generate transgenic chickens. The lentiviral vectors were pseudotyped with a modified Sindbis virus envelope protein (termed M168) and conjugated with an antibody specific to PGC membrane proteins. We demonstrated that these optimized M168-pseudotyped lentiviral vectors conjugated with SSEA4 antibodies successfully targeted transduction of PGCs *in vitro* and *in vivo*. Compared with the control, 50.0%–66.7% of chicken embryos expressed green fluorescent protein (GFP) in gonads transduced by the M168-pseudotyped lentivirus. This improved the targeted transduction efficiency by 30.0%–46.7%. Efficient chimerism of exogenous genes was also observed. This targeting technology could improve the efficiency of germ line transmission and provide greater opportunities for transgenic poultry studies.

慢病毒靶向感染PGCs制备转基因鸡的方法研究

摘要: 慢病毒作为制备转基因鸡的经典工具, 通常利用水泡型口炎病毒包膜蛋白 (Vesicular stomatitis virus G, VSVG) 进行病毒包装, 经包膜后的病毒具有广泛的细胞感染性。然而这种非特异感染细胞的性质导致利用慢病毒制备转基因鸡性腺嵌合率较低，进而引起转基因后代筛选效率偏低。为解决这一科学问题，本研究利用改装后的Sindbis病毒包膜蛋白 (M168) 包装慢病毒, 在特异抗体的介导下靶向感染鸡的原始生殖细胞 (Primordial germ cells, PGCs) 制备转基因鸡, 以期提高转基因鸡制备及筛选效率, 加速转基因禽类的研究进展。本研究首先建立了以第二代慢病毒包装系统为主体的

病毒包装体系，并优化了靶向感染系统中抗体浓度与M168假型化慢病毒（M168-LVs）之间最佳的结合比例；随后通过体外分离培养PGCs，并对其进行病毒靶向感染研究。结果显示SSEA4抗体介导的M168-LVs与VSVG-LVs相比，能靶向感染PGCs而不感染饲养层细胞和鸡成纤维细胞（DF-1）；最后结合三期换壳法，利用SSEA4抗体介导的M168-LVs显微注射到鸡胚盘下腔进行转基因鸡制备研究。结果显示SSEA4抗体介导的M168-LVs转染的鸡胚中有50.0%–66.7%的性腺表达GFP, 转染效率与对照组VSVG-LVs相比提高了30.0%–36.7%，说明SSEA4抗体介导的M168-LVs在体内不仅能够靶向感染PGCs, 还提高了性腺的嵌合比率, 为进一步进行转基因鸡的制备研究提供了理论依据，并为加速转基因禽类的研究进展提供方法基础。

Cite this article: Zi-Qin Jiang, Han-Yu Wu, Jing Tian, Ning Li, Xiao-Xiang Hu. 2020: Targeting lentiviral vectors to primordial germ cells (PGCs): An efficient strategy for generating transgenic chickens. *Zoological Research*, 41(3): 281-291. doi: 10.24272/j.issn.2095-8137.2020.032

- **How little is known about "the little brown frogs": description of three new species of the genus *Leptobrachella* (Anura: Megophryidae) from Yunnan Province, China**

Jin-Min Chen, Kai Xu, Nikolay A. Poyarkov, Kai Wang, Zhi-Yong Yuan, Mian Hou, Chatmongkon Suwannapoom, Jian Wang, Jing Che

Fulltext HTML PDF

Abstract: Asian leaf-litter toads of the genus *Leptobrachella* represent a great anuran diversification in Asia. Previous studies have suggested that the diversity of this genus is still underestimated. During herpetological surveys from 2013 to 2018, a series of *Leptobrachella* specimens were collected from the international border areas in the southern and western parts of Yunnan Province, China. Subsequent analyses based on morphological and molecular data revealed three distinct and previously unknown lineages, which we formally describe as three new species herein. Among them, we describe a new species that occurs at the highest known elevation for *Leptobrachella* in China. Four species of *Leptobrachella*, including two new species, are found in the same reserve. Furthermore, our results suggest that the population from Longchuan County, Yunnan, may represent an additional new species of *Leptobrachella*, although we tentatively assigned it to *Leptobrachella* cf. *yingjiangensis* due to the small sample size examined. Lastly, we provide the first description of females of *L. yingjiangensis*. Our results further highlight that both micro-endemism and sympatric distributions of species are common patterns in *Leptobrachella*, that contribute to taxonomic and conservation challenges in these frogs. We provide an identification key for *Leptobrachella* known to occur in Yunnan. Given the lack of knowledge on species diversity of *Leptobrachella* along international border areas, we recommend that future studies include trans-boundary collaborative surveys.

我们对"小棕蛙"的多样性知之甚少：描述中国云南省掌突蟾属（无尾目：角蟾科）三个新种

摘要: 掌突蟾属（两栖类：角蟾科）物种具有极其丰富的多样性。先前的研究表明，该属的物种数目仍然被大大低估。基于2013年至2018年的野外调查，我们从中国云南省南部和西部的国界地区收集了一系列掌突蟾属标本。随后，结合形态学和分子数据进行的联合分析揭示了3个掌突蟾属新物种，并在本文中对它们进行了正式描述。其中，我们发现了在中国分布海拔最高的掌突蟾属物种；在同一保护区内发现了4种掌突蟾属物种，包括2种新种。此外，我们的结果表明，来自云南省陇川县的掌突蟾属种群可能代表了另一个新物种，由于我们获得的样品有限，暂将其命名为疑似盈江掌突蟾。我们也首次提供了盈江掌突蟾雌性的描述。我们的结果进一步表明，地方特有种和同域物种分布是掌突蟾属物种的常见分化模式，这对这类青蛙的分类和保护提出了挑战。文章同时提供了云南省掌突蟾属已知种的检索表。鉴于国界线区域被低估的多样性，我们建议未来的研究应包括跨国合作调查。

Cite this article: Jin-Min Chen, Kai Xu, Nikolay A. Poyarkov, Kai Wang, Zhi-Yong Yuan, Mian Hou, Chatmongkon Suwannapoom, Jian Wang, Jing Che. 2020: How little is known about "the little brown frogs": description of three new species of the genus *Leptobrachella* (Anura: Megophryidae) from Yunnan Province, China. *Zoological Research*, 41(3): 292-313. doi: 10.24272/j.issn.2095-8137.2020.036

- **Novel insights into host-pathogen interactions of large yellow croakers (*Larimichthys crocea*) and pathogenic bacterium *Pseudomonas plecoglossicida* using time-resolved dual RNA-seq of infected spleens**

Yi Tang, Ge Xin, Ling-Min Zhao, Li-Xing Huang, Ying-Xue Qin, Yong-Quan Su, Wei-Qiang Zheng, Bin Wu, Nan Lin, Qing-Pi Yan

Fulltext HTML PDF

Abstract: Host-pathogen interactions are highly complex, involving large dynamic changes in gene expression during infection. These interactions are fundamental to understanding anti-infection immunity of hosts, as well as the pathogenesis of pathogens. For bacterial pathogens interacting with animal hosts, time-resolved dual RNA-seq of infected tissue is difficult to perform due to low pathogen load in infected tissue. In this study, an acute infection model of *Larimichthys crocea* infected by *Pseudomonas plecoglossicida* was established. The spleens of infected fish exhibited typical symptoms, with a maximum bacterial load at two days post-injection (dpi). Time-resolved dual RNA-seq of infected spleens was successfully applied to study host-pathogen interactions between *L. crocea* and *P. plecoglossicida*. The spleens of infected *L. crocea* were subjected to dual RNA-seq, and transcriptome data were compared with those of noninfected spleens or *in vitro* cultured bacteria. Results showed that pathogen-host interactions were highly dynamically regulated, with corresponding fluctuations in host and pathogen transcriptomes during infection. The expression levels of many immunogenes involved in cytokine-cytokine receptor, Toll-like receptor signaling, and other immune-related pathways were significantly up-regulated during the infection period. Furthermore, metabolic processes and the use of oxygen in *L. crocea* were strongly affected by *P. plecoglossicida* infection. The WGCNA results showed that the metabolic process was strongly related to the entire immune process. For *P. plecoglossicida*, the expression levels of motility-related genes and flagellum assembly-related genes were significantly up-regulated. The results of this study may help to elucidate the interactions between *L. crocea* and *P. plecoglossicida*.

应用基于时间序列的双物种转录组学研究大黄鱼与变形假单胞菌之间的宿主-病原互作

摘要: 宿主与病原之间的相互作用非常复杂，在感染过程中涉及基因表达的大量动态变化。这些相互作用对于理解宿主的抗感染能力以及病原体的致病机理是至关重要的。由于病原在感染组织中的载菌量很低，限制了双物种转录组测序在细菌性病原与动物宿主的互作研究的应用。本研究建立了变形假单胞菌（*Pseudomonas plecoglossicida*）-大黄鱼（*Larimichthys crocea*）的急性感染模型，病原在脾脏中有较高的载菌量。受感染鱼的脾脏表现出典型症状，并且在感染后2天（dpi）时细菌载量达到最大。对受感染大黄鱼的脾脏进行病原与宿主双物种转录组测序，并与PBS注射的大黄鱼脾脏和体外培养的细菌的转录组数据进行比较。结果表明，病原体与宿主之间的相互作用是高度动态调控的，在感染过程中，大黄鱼和变形假单胞菌转录组会发生相对应的变化。在感染期间，大黄鱼涉及细胞因子-细胞因子受体，Toll样受体信号传导和其他免疫相关途径的许多基因的表达水平显着上调。此外，变形假单胞菌感染严重影响了大黄鱼的代谢过程和氧气的使用。对于变形假单胞菌，运动相关基因和鞭毛组装相关基因的表达水平显着上调。应用WGCNA进行时序分，析结果表明，代谢过程与整个免疫过程密切相关。这项研究的结果有助于阐明在感染期间大黄鱼和变形假单胞菌之间的相互作用。

Cite this article: Yi Tang, Ge Xin, Ling-Min Zhao, Li-Xing Huang, Ying-Xue Qin, Yong-Quan Su, Wei-Qiang Zheng, Bin Wu, Nan Lin, Qing-Pi Yan. 2020: Novel insights into host-pathogen interactions of large yellow croakers (*Larimichthys crocea*) and pathogenic bacterium *Pseudomonas plecoglossicida* using time-resolved dual RNA-seq of infected spleens. *Zoological Research*, 41(3): 314-327. doi: 10.24272/j.issn.2095-8137.2020.035

- **Whole-genome sequencing of leopard coral grouper (*Plectropomus leopardus*) and exploration of regulation mechanism of skin color and adaptive evolution**

Yang Yang, Li-Na Wu, Jing-Fang Chen, Xi Wu, Jun-Hong Xia, Zi-Ning Meng, Xiao-Chun Liu, Hao-Ran Lin

Fulltext HTML PDF

Abstract: Leopard coral groupers belong to the *Plectropomus* genus of the Epinephelidae family and are important fish for coral reef ecosystems and the marine aquaculture industry. To promote future research of this species, a high-quality chromosome-level genome was assembled using PacBio sequencing and Hi-C technology. A 787.06 Mb genome was assembled, with 99.7% (784.57 Mb) of bases anchored to 24 chromosomes. The leopard coral grouper genome size was smaller than that of other groupers, which may be related to its ancient status among grouper species. A total of 22 317 protein-coding genes were predicted. This high-quality genome of the leopard coral grouper is the first genomic resource for *Plectropomus* and should provide a pivotal genetic foundation for further research. Phylogenetic analysis of the leopard coral grouper and 12 other fish species showed that this fish is closely related to the brown-marbled grouper. Expanded genes in the leopard coral grouper genome were mainly associated with immune response and movement ability, which may be related to the adaptive evolution of this species to its habitat. In addition, we also identified differentially expressed genes (DEGs) associated with carotenoid metabolism between red and brown-colored leopard coral groupers. These genes may play roles in skin color decision by regulating carotenoid content in these groupers.

豹纹鳃棘鲈(*Plectropomus leopardus*)全基因组测序以及皮肤颜色调控机制与适应性进化探索

摘要: 豹纹鳃棘鲈俗称东星斑，属于鲈形目，石斑鱼科，鳃棘鲈属，是一种重要的珊瑚礁鱼类，也是目前海水养殖石斑鱼类中市场价格昂贵的种类。我们使用PacBio三代测序和Hi-C技术对其基因组进行了测序与组装。豹纹鳃棘鲈染色体全长787.06 Mb，其中99.7% (784.57 Mb)的碱基可以锚定在24条染色体上，共检测到22 317个蛋白编码基因。豹纹鳃棘鲈的基因组小于其他石斑鱼的基因组，这可能与其在石斑鱼类中的原始地位有关。基于基因组数据的系统发育分析表明，13种鱼类中，棕点石斑鱼与豹纹鳃棘鲈具有较近的亲缘关系。豹纹鳃棘鲈基因组中的扩张基因主要与免疫反应和运动能力有关，这可能与该物种的适应性进化有关。此外，在对不同体色（红色和棕色）豹纹鳃棘鲈的转录组分析中，我们还发现了一些与类胡萝卜素代谢相关基因的差异表达。这些基因可能通过调节豹纹鳃棘鲈体内类胡萝卜素的含量，从而在皮肤颜色调节中发挥作用。

Cite this article: Yang Yang, Li-Na Wu, Jing-Fang Chen, Xi Wu, Jun-Hong Xia, Zi-Ning Meng, Xiao-Chun Liu, Hao-Ran Lin. 2020: Whole-genome sequencing of leopard coral grouper (*Plectropomus leopardus*) and exploration of regulation mechanism of skin color and adaptive evolution. *Zoological Research*, 41(3): 328-340. doi: 10.24272/j.issn.2095-8137.2020.038

Letters to the editor

- **Discovery of first active breeding den of Chinese mountain cat (*Felis bieti*)**

Xue-Song Han, Huai-Qing Chen, Zheng-Yi Dong, Ling-Yun Xiao, Xiang Zhao, Zhi Lu

Fulltext HTML PDF

Abstract: In mid-September 2018, during a field survey in Chiat'ung, Sanjiangyuan (Three-River-Source) Region, Tibetan Plateau, China, we discovered the first active breeding den of the Chinese mountain cat (*Felis bieti*), inhabited by one adult female and two kittens. Based on fieldwork over the following months, five breeding dens were discovered, and 33 sightings were recorded. In addition, at least five individuals were confirmed to inhabit this overlooked region, and much previously unknown information concerning this cat species and its ecology was revealed for the first time.

首个荒漠猫（*Felis bieti*）活跃繁殖洞穴的记录

摘要: 荒漠猫（Felis bieti）主要分布在青藏高原东缘，是中国特有的两种食肉动物之一。作为猫科动物中最不为人知的物种之一，荒漠猫自其1892年科学发现后仍缺乏足够的野外生态学研究工作。2018年9月中旬，在位于三江源地区青海省玉树州称多县嘉塘草原进行野外调查时，发现了一个由一只荒漠猫成年雌性及其两只幼崽栖息的繁殖洞穴。基于在随后五个月中的红外相机监测、样线调查以及社区访谈工作，累计在嘉塘草原内发现荒漠猫繁殖洞穴五处（2017年2处属1个家庭，2018年3处属1个家庭），以及33笔巢外个体目击记录。此外，通过荒漠猫尾部花纹的差异，确定嘉塘草原及周边区域至少存在5只荒漠猫个体生存。

Cite this article: Xue-Song Han, Huai-Qing Chen, Zheng-Yi Dong, Ling-Yun Xiao, Xiang Zhao, Zhi Lu. 2020: Discovery of first active breeding den of Chinese mountain cat (*Felis bieti*). *Zoological Research*, 41(3): 341-344. doi: 10.24272/j.issn.2095-8137.2020.039

- **Responses of cuckoo hosts to alarm signals of different nest intruders in non-nesting areas**

Jiao-Jiao Wang, Lai-Kun Ma, Wei Liang, Can-Chao Yang

Fulltext HTML PDF

Abstract: The "call for help" hypothesis proposes that alarm calls produced by a bird can transmit warning information to both conspecific and interspecific neighbors. Neighbors who are attracted by social transmission might benefit from knowing about the presence of danger or by gaining information about the presence of predators or brood parasites nearby. Brood parasite hosts can distinguish threats from different intruders and exhibit varied responses correspondingly. However, most previous studies have conducted sound playback at host nest sites and focused on conspecific individuals attracted by the alarm calls. In this study, we used random location playback to investigate the responses of different host species to alarm signals of oriental reed warblers (*Acrocephalus orientalis*) toward different intruders (brood parasite, predator, and harmless control) in order to reveal how hosts evaluate different threats from different intruders using vocal information in non-nesting areas during the breeding season. We found that the alarm calls given in response to different intruders incurred similar numbers of approaching species for both conspecific and interspecific birds. However, the number of attracted individuals differed significantly among the various species, with conspecifics and vinous-throated parrotbills (*Paradoxornis webbianus*) dominating, both of which are major hosts of common cuckoos (*Cuculus canorus*). Nevertheless, interspecific birds did not present any aggressive behavior according to the alarm calls, which implied that visual information may be needed for further confirmation of threats. In addition, determining whether alarm call structure promoted an evolutionary convergence phenomenon still needs further verification.

杜鹃宿主在非巢区对不同巢入侵者报警信号的反应

摘要: “呼救”假说认为鸟类发出的报警声可以向同种和异种邻居传递相关的警戒信息，邻居可以通过这些社会信息，获取附近捕食者或巢寄生者的情况而从中受益。巢寄生宿主可以区分不同入侵者，并根据不同的威胁做出反应。然而，以往大多数研究都是针对宿主巢进行的声音回放，并且焦点集中在被报警声吸引的同种个体上。本研究使用东方大苇莺（*Acrocephalus orientalis*）对不同入侵者（巢寄生者、捕食者和无害对照）的报警声作为回放刺激，在繁殖季节非巢区采用随机位点回放的方法研究了同种和异种宿主对报警声的反应，以揭示宿主如何使用声音信息评估来自不同入侵者的威胁。结果发现，不同报警声之间所招引的个体数量，无论对于同种还是异种均为相似。但被招引个体的数量在不同物种之间存在显著差异，以报警声同种（即东方大苇莺）和棕头鸦雀（*Paradoxornis webbianus*）为主，它们都是大杜鹃（*Cuculus canorus*）的主要宿主。然而，回放中异种宿主并没有表现出任何攻击性行为，这表明进一步确认威胁可能需要视觉信息的参与。此外，报警声的结构是否促进了趋同进化现象还有待进一步验证。

Cite this article: Jiao-Jiao Wang, Lai-Kun Ma, Wei Liang, Can-Chao Yang. 2020: Responses of cuckoo hosts to alarm signals of different nest intruders in non-nesting areas. *Zoological Research*, 41(3): 345-350. doi: 10.24272/j.issn.2095-8137.2020.030

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From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Thursday, January 23, 2020 8:03 PM
To: Shi, Pei yong;LeDuc, James W.
Cc: brusek;David Franz;George F GAO;mifangl
Subject: 回复: RE: Op Ed in Houston Chronicle

Dear all,
Thanks for your suggestion and I do agree with you. I will try my best to promote the sharing of strain and experience betweet us and let you know later.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: Shi, Pei yong
Date: 2020-01-22 23:56
To: LeDuc, James W.; 袁志明
CC: Benjamin Rusek (BRusek@nas.edu); Dave Franz (davidrfranz@gmail.com); George F GAO; Mifang Liang
Subject: RE: Op Ed in Houston Chronicle
I totally agree with Jim. The timing is critical here.

- Pei-Yong

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, January 22, 2020 9:29 AM
To: 袁志明 <yzm@wh.iov.cn>
Cc: Benjamin Rusek (BRusek@nas.edu) <BRusek@nas.edu>; Dave Franz (davidrfranz@gmail.com) <davidrfranz@gmail.com>; George F GAO <gaof@im.ac.cn>; Mifang Liang <mifangl@hotmail.com>; Shi,

Pei yong <peshi@UTMB.EDU>
Subject: Re: Op Ed in Houston Chronicle

Thanks Zhiming. You are in a very challenging position and doing a great job. I would however recommend that you organize and quickly implement a way to share reference isolates. With cases occurring outside China, others will soon have their own isolates and China will have lost the opportunity for leadership. And if scientific publications start appearing from Chinese investigators without the world having independent access to a strain, China will likely be heavily criticized.

Keep up the good work

Jim
Sent from my iPhone

On Jan 22, 2020, at 6:30 AM, 袁志明 <yzm@wh.iov.cn> wrote:

Dear Jim,
Thanks for your information and your positive attitute to Chinese public health response system and the practice. We are still work hard the on the novel coronovirus and hope to get the help from your team.

Regards

Zhiming

-----原始邮件-----
发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>
发送时间:2020-01-22 06:33:54 (星期三)
收件人: "Benjamin Rusek (BRusek@nas.edu)" <BRusek@nas.edu>, "Dave Franz (davidrfranz@gmail.com)" <davidrfranz@gmail.com>, "Yuan Zhiming" <yzm@wh.iov.cn>, "George F GAO" <gaof@im.ac.cn>, "Mifang Liang" <mifangl@hotmail.com>, "Shi, Pei yong" <peshi@UTMB.EDU>
抄送:
主题: Op Ed in Houston Chronicle
Ben, Dave, Zhiming, George, Mifang and Pei-Yong

The attached, slightly modified to include mention of the new case in Washington State, is scheduled to appear in Wednesday 22 Jan's Houston Chronicle. Note mention of the NASEM/CAS collaborations.

Just FYI,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 6/30/2019 7:12:52 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]; 步志高 [buzhigao@caas.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]; David Franz [davidrffranz@gmail.com]; brusek [BRusek@nas.edu]
Subject: 回复:Synthetic biology commentary

Hi, Jim,

You have done a great preparation of this manuscript and I hope I could contributed my effeort. As you indicated, we should show our understanding and principle for the mangement of the biosafety laboratory, with a aim to promote the global cooperation in the related field. I will return you back this week.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2019-06-22 03:58
To: Yuan Zhiming; Zhigao Bu (buzhigao@caas.cn)
CC: Shi, Pei yong; Dave Franz (davidrfranz@gmail.com); Benjamin Rusek (BRusek@nas.edu)
Subject: Synthetic biology commentary

Dear Zhiming and Zhigao,

I hope this note finds you well on this first day of summer. I write to propose a joint commentary to be submitted for publication in Zhiming's of *Journal of Biosafety and Biosecurity* on the topic of biosafety and biosecurity in the age of synthetic biology. This is a relevant topic and our shared publication would offer an excellent example of the benefits of our joint China-USA dialogue. Having such a co-authored publication would be tangible evidence of the importance we all place on working together to solve challenges of global importance. I have taken the liberty of preparing a first draft of such a manuscript and I invite you both to be co-authors. Dave, Ben and Pei-Yong have reviewed and I have incorporated their comments. Your additions, deletions and modifications will certainly further improve the quality of the piece and make it most relevant to the issues we all face daily in managing a large biocontainment facility. (Please use track change as you edit the piece.)

As you will see, I tried to address four separate areas that impact current and future work in synthetic biology, starting from the position that many of the relevant safeguards needed are already in place through our existing programs in biosafety and biosecurity. I then talk about the importance of leadership at all organizational levels, as Dave Franz has so eloquently spoken about in the past. The last area is the importance of *Institutional* leadership, and here I would especially value your input. At the GNL and elsewhere in the USA, we rely heavily on the Institutional Biosafety Committee (IBC) for final review and approval for studies involving recombinant DNA, and more broadly to studies involving pathogens in general. I don't know if a similar committee exists in China or in other countries around the world. Your thoughts and input particularly on this point would certainly improve the manuscript and make it more relevant to a broader community of scientists.

Attached please find a first draft for your review and consideration. I hope that you will agree to work with me on this important project. I look forward to hearing from you soon.

With best wishes,

Jim

James W. Le Duc, Ph.D.
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(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 11/27/2019 5:56:04 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: 回复:draft manuscript

Dear James ,

Thanks for your comments and your revision and I will revise the manuscript according to your suggestion. Hopefully this paper will be published soon and let the outside understand our lab and our mission.
Thanks

Regards
Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
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Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2019-11-26 05:42
To: Yuan Zhiming
CC: Shi, Peiyong
Subject: draft manuscript
Hi Zhiming,

Sorry for the delay in responding to your request for comments on your draft manuscript. I finally had a chance to review it and my comments are attached. I think the paper is nicely written and will be of interest to readers following the development of biocontainment labs in China. You have done a good job in recording capabilities, and you may wish to expand a bit more by mentioning the maximum number of small or large (non-human primates) you are able to manage at a single time in the facility. We are frequently asked these questions, and most product developers want sufficiently large single studies to have statistical significance, so many of our larger studies involve about 20 NHP. There may be good reasons not to quantify your capabilities as well, which I fully understand.

You rightly credit the collaborations with the French in building the laboratory; however, if your goal is to have a truly international impact, you may wish to broaden comments on potential collaboration/collaborators as mentioned in one comment.

If I understand you correctly, you will be publishing the paper in your biosafety journal. If so, you may wish to expand your comments on your training efforts to prepare your staff to safely and securely work in the new

facility. You may also wish to mention something about your security profile. As I recall, the entire campus has limited access with guards at entrances. You may wish to comment on other mechanisms in place to limit access to high-risk pathogens—card-key access to labs, security personnel, etc. You will not want to go into too much detail, but it might be appropriate, especially given the focus of your journal, to let readers know that security is an important aspect of your program.

Very nicely done! Thank you for the opportunity to review the draft.

With best wishes,

Jim

James W. Le Duc, Ph.D.
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(f) 409-266-6810
(m) 409-789-2012

From: 石正丽 <zlshi@wh.iov.cn>
Sent: Friday, March 6, 2020 8:05 AM
To: LeDuc, James W.
Cc: Yuan Zhiming;Shi, Pei yong
Subject: Re: Vox article

Dear Jim,

Thank you for your information.

It's a difficult time for us. We will be fine.

The rumors always ran faster than the reality. That's problem of internet world.

Best regards,
Zhengli,

-----原始邮件-----
发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>
发送时间:2020-03-05 22:50:33 (星期四)
收件人: "Yuan Zhiming" <yzm@wh.iov.cn>, zlshi <zlshi@wh.iov.cn>
抄送: "Shi, Pei yong" <peshi@UTMB.EDU>
主题: Vox article

Dear Zhiming and Zhengli,

I hope you are both well during this very difficult time.

The link below is to an article just published that may be of interest to you.

With all good wishes for your personal health and safety as we all work together to control the new virus.

Jim

James W. Le Duc, Ph.D.
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University of Texas Medical Branch
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(m) 409-789-2012

From: Eliza Barclay <eliza.barclay@vox.com>
Sent: Wednesday, March 04, 2020 8:49 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Wuhan

Hi Jim,
The story went up today. Thanks so much for your help with it, and let me know if you see any inaccuracies to fix or updates I should make.
Very best, and hope to stay in touch,
Eliza
https://www.vox.com/2020/3/4/21156607/how-did-the-coronavirus-get-started-china-wuhan-lab

On Fri, Feb 28, 2020 at 3:06 PM Eliza Barclay <eliza.barclay@vox.com> wrote:

> Sure, will do.
>
> On Feb 28, 2020, at 12:16 PM, LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> Better to call after about 4 pm CT. We're kinda busy…
>
> Thanks, Jim
>
> **From:** Eliza Barclay <eliza.barclay@vox.com>
> **Sent:** Friday, February 28, 2020 1:07 PM
> **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> **Cc:** Keusch, Gerald T <keusch@bu.edu>
> **Subject:** Re: Wuhan
>
>
>
> Thanks for the connection, Jerry.

And thanks so much for the quick response, Jim. I will give you a call in about an hour.

Best,

Eliza

On Fri, Feb 28, 2020 at 10:50 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:

> Hi Jerry,
>
> Thanks for the introduction and happy to meet you, Eliza. I'm happy to chat about this issue at your convenience. My direct office line is 409-266-6516.
>
> Thanks, Jim
>
> James W. Le Duc, Ph.D.
>
> Director
>
> Galveston National Laboratory
>
> University of Texas Medical Branch
>
> Galveston, TX 77555-0610
>
> (t) 409-266-6500
>
> (f) 409-266-6810
>
> (m) 409-789-2012
>
> **From:** Keusch, Gerald T <keusch@bu.edu>
> **Sent:** Friday, February 28, 2020 11:48 AM
> **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> **Cc:** Eliza Barclay <eliza.barclay@vox.com>
> **Subject:** Wuhan

Hi Jim,

I was talking to Eliza Barclay from Vox (copied above) who was referred to me by our friend Peter Daszak. Eliza is working on a story to address the various conspiracy theories being bandied about on the origin of the Covid19 virus. One of the issues, of course, was the Wuhan laboratory as a source – whether accidental or deliberate – and the questions being raised about it biosecurity and biosafety protocols. I said that I was absolutely confident that they had proper protocols and trained people in place, in part because I am was aware that GNL had connections with that lab, had trained many of their staff, and that you have been there.

Eliza will follow up and if you have the time she would like to talk with you. She is trying to gather the scientific argument and be able to translate it for a general audience to be able to distinguish between evidence and conspiracy.

Hope all is well.

Jerry

Gerald T. Keusch, M.D.

Professor of Medicine and International Health

Boston University School of Medicine

Associate Director, National Emerging Infectious Diseases Laboratories

620 Albany Street

Boston, MA 02118

--

Eliza Barclay • Science Editor

<~WRD363.jpg>

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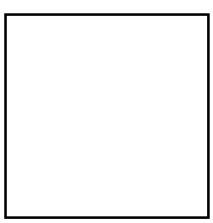
Eliza Barclay • Science Editor



Follow Vox on Twitter • Instagram • Facebook
—

From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Saturday, April 18, 2020 9:56 PM
To: LeDuc, James W.;zlshi
Cc: Shi, Pei yong
Subject: 回复: RE: Fwd: Rubio

Dear Jim，

Thanks for your informaiton. I really appreciate your help and your action, we need to let some people understand well the the mission of high-level biosafey lab. what we do, and how we do inside. We all know that the labs were built not for causing epidmic, but for proventing the epidemic, and the labs are managed according to interantional guildline and national accquirement, Wuhan's lab is among the others.

I will contact Zhengli to see what she can do for your report.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2020-04-19 00:44
To: 石正丽
CC: Shi, Pei yong; Yuan Zhiming
Subject: RE: Fwd: Rubio
Dear Zhengli,

Thank you for your response. I understand completely and I certainly do not wish to compromise you personally or your research activities. Given our long history of collaborations between the GNL and the

WIV, I have been approached repeatedly for details on our work. Attached is a draft summary that I will be providing to the leadership of our University of Texas system and likely to Congressional committees that are being formed now. Please review carefully and make any changes that you would like. I want this to be as accurate as possible and I certainly do not want to misrepresent any of your valuable contributions. I need to submit this on Monday, 20 April, so your prompt reply would be very much appreciated. I have copied Zhiming for his comments as well.

With best wishes,

Jim

From: 石正丽 <zlshi@wh.iov.cn>
Sent: Saturday, April 18, 2020 9:20 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Fwd: Rubio

Dear James,

Thank you for your email and consideration our communication.

Due to the complicated situation, I don't think it's a right time to communicate by the call.

What I can tell you is that this virus is not a leaky from our lab or any other labs.It's a shame to make this scientific question so complicated.

I hope to talk with you whenever the COVID-19 is over and world is calme and believe in the science.

Best regards,
Zhengli,

-----原始邮件-----
发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>
发送时间:2020-04-17 11:06:38 (星期五)
收件人: "zengli Shi" <zlshi@wh.iov.cn>
抄送:
主题: Fwd: Rubio

Hi Zheng-Li. I hope you are well as surviving all the COVID19 drama. I wonder if you would have time

for a phone call sometime soon. Let me know a good number and time and I'll call. The email below is relevant.

I will certainly understand if you are not available but Pei-Yong keeps encouraging me to call.

With all good wishes.

Jim.

My office line is 1 409 266 6516 or cell is 1 409 789 2012 if it's easier for you to call me.

Sent from my iPhone

Begin forwarded message:

From: David Franz <davidrfranz@gmail.com>
Date: April 16, 2020 at 8:04:55 PM CDT
To: "LeDuc, James W." <jwleduc@UTMB.EDU>
Subject: Rubio
WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.

I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"

Sent from my iPhone

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 2/12/2020 8:41:52 AM
To: Shi, Pei yong [peshi@UTMB.EDU]; df@wh.iov.cn; zlshi [zlshi@wh.iov.cn]
CC: yzm [yzm@wh.iov.cn]; wangyy [wangyy@wh.iov.cn]; Ksiazek, Thomas G. [tgksiaze@UTMB.EDU]
Subject: RE: RE: sharing of isolates of 2019nCoV

I strongly agree. We need to show international scientific collaborations at this time of potentially global crisis.

Thank you Fei for your continued efforts.

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Shi, Pei yong <peshi@UTMB.EDU>
Sent: Wednesday, February 12, 2020 7:10 AM
To: df@wh.iov.cn; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: RE: RE: sharing of isolates of 2019nCoV

Thanks, Fei
Although US CDC has already shared the virus isolate with a number of US institutions (including UTMB) last week, it is still important to successfully transfer and share the isolate(s) from China.
Best,
- Pei-Yong

From: df@wh.iov.cn <df@wh.iov.cn>
Sent: Wednesday, February 12, 2020 3:34 AM
To: Shi, Pei yong <peshi@UTMB.EDU>; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: Re: RE: sharing of isolates of 2019nCoV

No prompt reply from the Custom until today. President Bai is trying to push it in Beijing. Please wait for a while.

With best

Dr. Fei Deng
Virus Resource and Bioinformation Center,
Wuhan Institute of Virology, Chinese Academy of Sciences.
Tel/Fax:0086-27-87198465
Email: df@wh.iov.cn

From: Shi, Pei yong
Date: 2020-02-05 20:41
To: df@wh.iov.cn; zlshi
CC: LeDuc, James W.; yzm; wangyy; Ksiazek, Thomas G.
Subject: RE: FW: sharing of isolates of 2019nCoV

Hi Fei,

Thanks for the update. We look forward to further progress.

Best,

- Pei-Yong

From: df@wh.iov.cn <df@wh.iov.cn>
Sent: Wednesday, February 5, 2020 5:57 AM
To: Shi, Pei yong <peshi@UTMB.EDU>; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: Re: FW: sharing of isolates of 2019nCoV

Thanks for your information.

We are trying to discuss this with the General Administration of Customs in Beijing directly.

I will keep on contacting with you.

Best wishes,

Fei

Dr. Fei Deng
Virus Resource and Bioinformation Center,
Wuhan Institute of Virology, Chinese Academy of Sciences.
Tel/Fax:0086-27-87198465
Email: df@wh.iov.cn

From: Shi, Pei yong
Date: 2020-02-04 22:52
To: df@wh.iov.cn; zlshi
CC: LeDuc, James W.; Yuan Zhiming; wangyy@wh.iov.cn; Ksiazek, Thomas G.
Subject: FW: sharing of isolates of 2019nCoV

Dear Fei and Zhengli,

Please see the response from President Bai. Zhiming and Yanyi were copied on the original email. Let us know anything we could help to facilitate the isolate transfer.

Best regards,

- Pei-Yong

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Tuesday, February 4, 2020 8:39 AM
To: Shi, Pei yong <peshi@UTMB.EDU>
Subject: FW: sharing of isolates of 2019nCoV

From: "president-office@cas.cn" <president-office@cas.cn>
Date: February 3, 2020 at 11:20:56 PM EST
To: dgriffi6 <dgriffi6@jhmi.edu>, MHamburg <MHamburg@nas.edu>
Cc: mlowenth <mlowenth@nas.edu>, Peggy Hamburg <peggy@hbfam.net>, jwleduc <jwleduc@nas.edu>, jhilderbr <jhilderbr@arizona.edu>, BRsek <BRsek@nas.edu>, jboright <jboright@nas.edu>, clbai <clbai@cas.cn>, zhangyp <zhangyp@cashq.ac.cn>, gaof <gaof@im.ac.cn>, jh-cao <jh-cao@cashq.ac.cn>, liyin <liyin@cashq.ac.cn>, sunhui <sunhui@cashq.ac.cn>, wangyy <wangyy@wh.iov.cn>, yzm <yzm@wh.iov.cn>
Subject: sharing of isolates of 2019nCoV

Diane E. Griffin, Vice President, NAS,
Margaret Hamburg, Foreign Secretary, NAM

Dear Prof. Griffin and Prof. Hamburg,

Thank you for your concerns on the recent outbreak of the 2019 novo-coronavirus epidemic. Upon receiving your letter dated January 28, my colleagues have discussed with Dr. George Fu Gao and other experts and we are willing to share isolates of the 2019 nCoV with the international community. We believe this is critical to engaging joint international efforts to contain the spread of the virus.

The National Biosafety Laboratory Wuhan of the Chinese Academy of Sciences is prepared and willing to work with The University of Texas Medical Branch and other international research institutions on the specifics for the sharing and distribution of the isolates. We are in the process of getting it ready.

I look forward to hearing your further advice on this matter.

With best regards,

Chunli Bai
President
Chinese Academy of Sciences
The Alliance of International Science Organizations (ANSO)

From: LeDuc, James W.
Sent: Thursday, May 7, 2020 3:28 PM
To: Jessica Tucker (jessica.tucker@nih.gov)
Subject: FW: News query from Nature

Hi Jessica,

I just tried to call but missed you. Please see inquiry below and questions to be asked. Let's chat before I respond to her. I don't want to get in troubl

From: Nidhi Subbaraman <nidhi.subbaraman@us.nature.com>
Sent: Thursday, May 07, 2020 1:58 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: News query from Nature

Dr Le Duc, I am reporting on the coronavirus for the news team at Nature, based in Washington DC.

I am looking into a story about gain of function and dual use research, and writing to ask if you'd be free for a brief call about this. We're wondering if the pandemic will re-ignite the debate about this research area – what should be allowed, what should be published, how the work should be communicated to the public. I'm also curious how you see the rumors about Shi Zhengli's lab in Wuhan influencing this.

I'm reaching out to reserachers who work in this area, or have participated in policy discussions in the past to ask how they see the pandmic tinting that discussion. (I covered the NSABB meeting in Jan where a version of this discussion came up https://www.nature.com/articles/d41586-020-00210-5)

Would value your thoughts on this. Please let me know if you're available for a phone call Friday or Monday.

Thank you, Nidhi

--
Nidhi Subbaraman (she/her)
Senior reporter, *Nature*
https://www.nature.com/news
O: +1 202 626 2523 | C: +1 857 228 8502 | @nidhisubs

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 11/8/2019 4:56:47 PM
To: Yuan Zhiming [yzm@wh.iov.cn]
Subject: RE: 回复: Re: The PDF offprint of your article [JOBB_26] is attached to this email

Thanks Zhiming. I will try to look at your manuscript while in Geneva next week.

Best wishes,

Jim

From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Wednesday, November 06, 2019 8:09 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: 回复: Re: The PDF offprint of your article [JOBB_26] is attached to this email

Dear James ,
I really think about to have another article with you about the safety management in the laboratory. Sorry I can not go to WHO meeting this time, and I hope to see you soon, maybe in CAS-NAS meeting.

By the way, I write a small paper on Wuhan P4 lab. My attention is to let outside to know a little bit the laboratory and understand why we need the lab. and how to operate the lab. I hope you could have a look and help me to revise it.

Thanks for you help and I am sure your revision will do me a great favor for the publication of this article.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2019-11-06 10:20
To: Yuan Zhiming
Subject: Re: The PDF offprint of your article [JOBB_26] is attached to this email

Wonderful! Thanks for the good news. Hopefully we will have another one out soon.

Are you going to the WHO meeting on biocontainment labs next week in Geneva? Perhaps I' ll see you there.

Jim

Sent from my iPhone

On Nov 5, 2019, at 5:44 PM, Yuan Zhiming <yzm@wh.iov.cn> wrote:

Hi, James ,

The article Safety and Security in the Age of Synthetic Biology has been published on line. Thanks for your contribution and hope to meet you soon.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: Elsevier - PDF Offprint
Date: 2019-11-06 04:50
To: yzm
Subject: The PDF offprint of your article [JOBB_26] is attached to this email

---

Article title: Safety and Security in the Age of Synthetic Biology
Article reference: JOBB26
Journal title: Journal of Biosafety and Biosecurity
Corresponding author: Dr. Zhiming Yuan
First author: Dr. James W. LeDuc
PDF offprint dispatch: 5-11-2019

Dear Dr. Yuan,

We are pleased to inform you that a PDF file of your published article Safety and Security in the Age of Synthetic Biology is attached to this e-mail for you to view and download. Please note that this article is published, therefore content updates are no longer possible at this point.

If you wish to order paper offprints, please go to
https://authors.elsevier.com/authorforms/JOBB26/88b5eb54513e722e9a6a67bd55c2f754

You can also order a copy of the issue on the Elsevier Webshop: http://webshop.elsevier.com .
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Get started at > http://service.elsevier.com

[T-13a-20151509]

<JOBB26.pdf>

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 1/23/2020 9:20:12 PM
To: Yuan Zhiming [yzm@wh.iov.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]; brusek [BRusek@nas.edu]; David Franz [davidrfranz@gmail.com]; George F GAO [gaof@im.ac.cn]; mifangl [mifangl@hotmail.com]
Subject: Re: Op Ed in Houston Chronicle

Wonderful news. This is the right decision at the right time. Let me know if we can help.

Keep up the great work! Jim

Sent from my iPhone

On Jan 23, 2020, at 9:03 PM, Yuan Zhiming <yzm@wh.iov.cn> wrote:

Dear all,
Thanks for your suggestion and I do agree with you. I will try my best to promote the sharing of strain and experience betweet us and let you know later.

Regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: Shi, Pei yong
Date: 2020-01-22 23:56
To: LeDuc, James W.; 袁志明
CC: Benjamin Rusek (BRusek@nas.edu); Dave Franz (davidrfranz@gmail.com); George F GAO; Mifang Liang
Subject: RE: Op Ed in Houston Chronicle
I totally agree with Jim. The timing is critical here.

- Pei-Yong

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, January 22, 2020 9:29 AM
To: 袁志明 <yzm@wh.iov.cn>
Cc: Benjamin Rusek (BRusek@nas.edu) <BRusek@nas.edu>; Dave Franz (davidrfranz@gmail.com) <davidrfranz@gmail.com>; George F GAO <gaof@im.ac.cn>; Mifang Liang <mifangl@hotmail.com>; Shi, Pei yong <peshi@UTMB.EDU>
Subject: Re: Op Ed in Houston Chronicle

Thanks Zhiming. You are in a very challenging position and doing a great job. I would however recommend that you organize and quickly implement a way to share reference isolates. With cases occurring outside China, others will soon have their own isolates and China will have lost the opportunity for leadership. And if scientific publications start appearing from Chinese investigators without the world having independent access to a strain, China will likely be heavily criticized.

Keep up the good work

Jim
Sent from my iPhone

On Jan 22, 2020, at 6:30 AM, 袁志明 <yzm@wh.iov.cn> wrote:

Dear Jim,
Thanks for your information and your positive attitute to Chinese public health response system and the practice. We are still work hard the on the novel coronovirus and hope to get the help from your team.

Regards

Zhiming

-----原始邮件-----
发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>
发送时间:2020-01-22 06:33:54 (星期三)
收件人: "Benjamin Rusek (BRusek@nas.edu)" <BRusek@nas.edu>, "Dave Franz (davidrfranz@gmail.com)" <davidrfranz@gmail.com>, "Yuan Zhiming" <yzm@wh.iov.cn>, "George F GAO" <gaof@im.ac.cn>, "Mifang Liang" <mifangl@hotmail.com>, "Shi, Pei yong" <peshi@UTMB.EDU>
抄送:
主题: Op Ed in Houston Chronicle

Ben, Dave, Zhiming, George, Mifang and Pei-Yong

The attached, slightly modified to include mention of the new case in Washington State, is scheduled to appear in Wednesday 22 Jan's Houston Chronicle. Note mention of the NASEM/CAS collaborations.

Just FYI,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: LeDuc, James W.
Sent: Wednesday, May 20, 2020 10:34 AM
To: Auchincloss, Hugh (NIH/NIAID) [E] (auchinclossh@niaid.nih.gov)
Subject: call
Attachments: U.S. Probes University of Texas Links to Chinese Lab Scrutinized Over Coronavirus - WSJ.pdf

Hi Hugh,

We should chat about the status of the probe by DoEd mentioned in the attached article. Nothing urgent, I just want to keep you informed. Let me know when you would be available for a brief 15 min call later today or this week.

Thanks, Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: LeDuc, James W.
Sent: Saturday, April 18, 2020 11:44 AM
To: 石正丽
Cc: Shi, Pei yong;Yuan Zhiming
Subject: RE: Fwd: Rubio
Attachments: a nCoV and WIV-drf2.docx

Dear Zhengli,

Thank you for your response. I understand completely and I certainly do not wish to compromise you personally or your research activities. Given our long history of collaborations between the GNL and the WIV, I have been approached repeatedly for details on our work. Attached is a draft summary that I will be providing to the leadership of our University of Texas system and likely to Congressional committees that are being formed now. Please review carefully and make any changes that you would like. I want this to be as accurate as possible and I certainly do not want to misrepresent any of your valuable contributions. I need to submit this on Monday, 20 April, so your prompt reply would be very much appreciated. I have copied Zhiming for his comments as well.

With best wishes,

Jim

From: 石正丽 <zlshi@wh.iov.cn>
Sent: Saturday, April 18, 2020 9:20 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Fwd: Rubio

Dear James,

Thank you for your email and consideration our communication.

Due to the complicated situation, I don't think it's a right time to communicate by the call.

What I can tell you is that this virus is not a leaky from our lab or any other labs.It's a shame to make this scientific question so complicated.

I hope to talk with you whenever the COVID-19 is over and world is calme and believe in the science.

Best regards,

Zhengli,

-----原始邮件-----

发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>

发送时间:2020-04-17 11:06:38 (星期五)

收件人: "zengli Shi" <zlshi@wh.iov.cn>

抄送:

主题: Fwd: Rubio

Hi Zheng-Li. I hope you are well as surviving all the COVID19 drama. I wonder if you would have time for a phone call sometime soon. Let me know a good number and time and I'll call. The email below is relevant.

I will certainly understand if you are not available but Pei-Yong keeps encouraging me to call.

With all good wishes.

Jim.

My office line is 1 409 266 6516 or cell is 1 409 789 2012 if it's easier for you to call me.

Sent from my iPhone

Begin forwarded message:

> **From:** David Franz <davidrfranz@gmail.com>
> **Date:** April 16, 2020 at 8:04:55 PM CDT
> **To:** "LeDuc, James W." <jwleduc@UTMB.EDU>
> **Subject: Rubio**
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"
>
> Sent from my iPhone

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/29/2020 10:38:48 AM
To: Handley, Gray (handleygr@niaid.nih.gov) [handleygr@niaid.nih.gov]
CC: Holubar, Connie J. [cjholuba@UTMB.EDU]
Subject: FW: quick question

Gray, my colleague Connie Holubar raises some concern that are valid as noted below. The 10K encounters includes training for our own UTMB staff, which was at least half to three quarters of all those trained. Further, we had very few trainees from China. We trained one building engineer from Wuhan and the two post-docs mentioned below. We also trained four individuals from Kunming (where another BSL4 is located) on building operations, and we sent a team to Kunming to offer on-site training at their facility. So we had relatively little engagement with China throughout the training center history.

Thanks, Jim

From: Holubar, Connie J. <cjholuba@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:57 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: RE: quick question

552.111

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Wednesday, April 29, 2020 9:14 AM
To: Holubar, Connie J. <cjholuba@UTMB.EDU>
Subject: FW: quick question

Just FYI.

From: LeDuc, James W.
Sent: Wednesday, April 29, 2020 9:12 AM
To: 'Handley, Gray (NIH/NIAID) [E]' <handleygr@niaid.nih.gov>
Subject: RE: quick question

Hi Gray,

The title for both projects was National Biocontainment Training Center. Final reports for both are attached for your information.

The Center was supported by two separate awards: W81XWH-09-2-0053 covering the period 22-05-2009 to 21-12-2014 and W81XWH-11-2-0148 covering the period 07-2011 to 07-2016. See below for specific answers. Let me know if you need additional information. Thank you for addressing these issues. I continue to believe that this is a success story and we are proud of our contributions.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Handley, Gray (NIH/NIAID) [E] <handleygr@niaid.nih.gov>
Sent: Wednesday, April 29, 2020 8:07 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: quick question

Jim: Embassy Beijing is asking what was the official name of your DoD supported training program and some other background information. Can you send me that?

Also, can you assure these responses to their questions are accurate? I provided the text in black earlier and the red text is my response to their follow-up questions – all based on our conversations.

1) Did this training take place in the U.S., China, or in both countries?

Since 2013, the Galveston National Laboratory (GNL) of the University of Texas Medical Branch (UTMB), part of the NIH Biodefense Laboratory Network, provided laboratory safety and security training for high-level biocontainment facilities in China, including the Wuhan Institute of Virology

In the U.S. at UTMB facility. Training was provided to partners from about 70 different countries with over 10,000 training encounters offered over the life of the training center, including a few from China. Training was provided both on site using a purpose made training center on the UTMB campus and augmented with training in the Galveston National Laboratory active biocontainment suites and mechanical spaces and at host nation facilities. Training was offered to two categories of learners, those laboratory scientists who would be working in biocontainment and those building engineers who were responsible for the safe and secure operations of the laboratory infrastructure. Training included long-term training for post-doctoral fellows working in biocontainment and specific to China, we hosted Han Xia, PhD during her post-doctoral training working on Crimean-Congo hemorrhagic fever virus in the GNL BSL4 laboratories. A second post-doctoral fellow, Chan Shao, PhD, was supported using other funds after the training center award expired. Both Dr Xia and Dr Shao have now returned to the Wuhan Institute of Virology where they are working on the current coronavirus pandemic.

2) Is this relationship still ongoing?

This relationship has been facilitated since 2015 through an ongoing dialogue and regular collaboration meetings co-sponsored by the Chinese Academies of Science and the U.S. National Academies of Science, Engineering and Medicine with cooperation from the Chinese CDC and others.

The training ended in 2016. The collaboration meetings convened by the CAS and the U.S. NAS, and highly regarded by the participating scientists, continued to be convened nearly each year since 2015. We understand there will not likely be a meeting this year. We just learned today that a joint virtual meeting will be held as early as May, 2020, again jointly sponsored by the CAS and NAS. Details are just being developed.
We continue to have scientist-to-scientist dialogue and collaborations with colleagues in China and elsewhere around the world.

3) Why was DOD funding discontinued?

This UTMB training engagement ended in 2016 when DoD funding was exhausted and not replenished from 2017 onwards.

This funding was expended on the training of scientists and facility operators to assure biosafety and biosecurity at high-containment laboratories around the world. The funding was provided by DoD, following an Congressional earmark in its appropriation, through two awards of five years each. In year seven, the awarded funding had been fully utilized. Despite requests from UTMB, DoD and other USG Agencies approached for support chose not to provide additional funding. UTMB understood this decision had to do with the overall USG position on relations with China but only DoD could say what factors were actually determinative. OK. We continue to seek funding for the training center as it clearly addresses an urgent global need with the continuing proliferation of biocontainment labs around the world.

4) Was there a formal name for this program? If so, please include.

According to GNL leadership, the relationship with the Wuhan Institute of Virology included the provision of training to scientists and biosafety and engineering professionals from Wuhan, as well as from other biocontain ment labs in China and the China CDC. This [name of program] included operations training as the Wuhan Institute of Virology prepared to open their BSL-4 facility as part of essential global research collaboration which is necessary to develop countermeasures against the world's most dangerous public health threats.
National Biocontainment Training Center

Many thanks,

Gray

F. Gray Handley
Associate Director for International Research Affairs
National Institute of Allergy and Infectious Diseases
National Institute of Health
U.S. Department of Health and Human Services

Tel: 301 594 6128
Fax: 301 480 2954
handleygr@niaid.nih.gov

5601 Fishers Lane, Room 1E50
Bethesda, MD 20892-9802

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 5/3/2019 1:56:10 PM
To: Shan, Chao [chshan@UTMB.EDU]; Shi, Pei yong [peshi@UTMB.EDU]; Bente, Dennis A. [dabente@UTMB.EDU]; yzm@wh.iov.cn; hanxia@wh.iov.cn
Subject: RE: Wuhan CCHFV application
Attachments: Application Form Wuhan-UTMB May 3 2019-jwl.doc

Please see attached with some minor edits and questions in track change. Very nicely done! Good luck to us all!

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Shan, Chao <chshan@UTMB.EDU>
Sent: Friday, May 03, 2019 11:05 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>; Shi, Pei yong <peshi@UTMB.EDU>; Bente, Dennis A. <dabente@UTMB.EDU>; yzm@wh.iov.cn; hanxia@wh.iov.cn
Subject: Wuhan CCHFV application

Dear All,

Here is the application I wrote with help from Han for Wuhan collaboration. Please take a look and let me know if anything needs to be changed.

Thanks very much for all the supports from you.

Best,
Chao

No.	
Grant No.	
Confidentiality Level	Open

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Advanced Customer Cultivation Project

Application Form

Project name: Vaccine Development and Polyclonal Antiserum for Crimean-Congo Hemorrhagic Fever Virus

Project leader (Signature):

Organization: University of Texas Medical Branch, Texas, USA

Phone number: +1(409) 266-6500

E-mail: jwleduc@utmb.edu

Made by Research Planning Office of Wuhan Institute of Virology, CAS

Filled in on 30/4/2019

Instruction for Form Filling

1. Each item of the application form must be true, complete, accurate and clarified.
2. The "Confidentiality Level" on the cover shall be filled in with "Open".
3. All the application materials shall be submitted in duplicate in A4 book size in print (double page).
4. After the form is filled in completely, the applicant's organization shall review the truthfulness, completeness and effectiveness of the information filled in.
5. The application form shall only be considered effective with the signature of the principal of the applicant's organization.

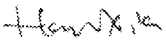
.

Basic Information

Project name	Vaccine Development and Polyclonal Antiserum for Crimean-Congo Hemorrhagic Fever Virus					
Type of project	☐Frontline of the fundamental ☐Major common key technology √Application demonstration research ☐Others					
Funding Category	√Key Project ☐General Project					
Budget	Total estimate: 50 (RMB 10,000 yuan) (Note: please calculate for one year only)					
Implementation period (one year)	From (01/07/2019) to (31/12/2019)					
Assessment period	From (01/07/2019) to (31/12/2021)					
Project leader	Name	James Le Duc	Sex	M	Birthday	11/23/1945
	Title	Professor	Duty	Director	Highest degree	Ph.D.
	Organization	University of Texas Medical Branch, Texas, USA				
Research group in WIV, CAS	Principal Investigator (Signature)	Zhiming Yuan			Person to contact (Signature)	Chao Shan
Project Implementation	☐Authorization √Cooperation ☐Independent Completion					

Project team	Total number	Senior	Intermediate	Junior	Assistant personnel	Post-doctor	Doctor candidate	Master candidate
	6	6	0	0	0	0	0	0

Main participants of the project implementation	Name	Age	Title	Organization	Time Commitment (Months)	Task Assignment	Signature
	James Le Duc	73	Professor	UTMB	3	Project leader	
	Pei-Yong Shi	53	Professor	UTMB	3	Co-Project leader	
	Dennis Bente	43	Associate professor	UTMB	3	Co-Project leader	

	Zhiming Yuan	56	Professor	WHIOV	3	Project Leader in WHIOV	
	Chao Shan	34	Professor	WHIOV	6	Vaccine development and efficacy test	
	Han Xia	36	Associate professor	WHIOV	6	Anti-CCHFV polyclonal antibody generation	

Text

[= 1 * ROMAN]. Research Background

1. Research purpose

The purposes of the project (1) Develop a replicon-based DNA vaccine for Crimean-Congo hemorrhagic fever virus (CCHFV). (2) Generation horse source polyclonal antibody for anti-CCHFV therapy.

2. Foreign and domestic research background, trend of development

Crimean-Congo hemorrhagic fever (also known as Xinjiang hemorrhagic fever) is caused by CCHFV in humans. CCHFV is a tick-borne virus with a wide geographical distribution, including Africa, the Balkans, the Middle East, Russia, western Asia and eastern Asia and needs high containment laboratory to conduct research. There are currently no licensed vaccines to prevent CCHFV-associated disease. CCHFV causes severe disease in human beings with a reported mortality rate of 3%–30% [ADDIN EN.CITE <EndNote><Cite><Author>Bente</Author><Year>2013</Year><RecNum>110</RecNum><DisplayText>(1)</DisplayText><record><rec-number>110</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556812014" guid="a4688135-8d5c-4363-a3ef-afb1028964bb">110</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bente, D. A.</author><author>Forrester, N. L.</author><author>Watts, D. M.</author><author>McAuley, A. J.</author><author>Whitehouse, C. A.</author><author>Bray, M.</author></authors></contributors><titles><title>Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity</title><secondary-title>Antiviral Res</secondary-title></titles><periodical><full-title>Antiviral Res</full-title></periodical><pages>159-89</pages><volume>100</volume><number>1</number><edition>2013/07/29</edition><keywords><keyword>Animals</keyword><keyword>Genetic Variation</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Hemorrhagic Fever, Crimean</keyword><keyword>History, 20th Century</keyword><keyword>History, 21st Century</keyword><keyword>Humans</keyword><keyword>Phylogeny</keyword><keyword>Arbovirus</keyword><keyword>Bunyavirus</keyword><keyword>Crimean–Congo hemorrhagic fever virus</keyword><keyword>Nairovirus</keyword><keyword>Tick-borne virus</keyword><keyword>Viral hemorrhagic fever</keyword></keywords><dates><year>2013</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1872-9096</isbn><accession-num>23906741</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/23906741</url></related-urls></urls><electronic-resource-num>1

0.1016/j.antiviral.2013.07.006</electronic-resource-num><language>eng</language></record></Cite></EndNote>].

Currently, there are mainly two forms of the vaccine of CCFHV. Inactivated CCHFV vaccine: the vaccine is made on suckling mouse brain and used only in Bulgaria and is not approved for use in other countries [ADDIN EN.CITE <EndNote><Cite><Author>Mousavi-Jazi</Author><Year>2012</Year><RecNum>106</RecNum><DisplayText>(2)</DisplayText><record><rec-number>106</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556812014" guid="9eda4adb-313b-4322-9da6-36fce16d1a95">106</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mousavi-Jazi, M.</author><author>Karlberg, H.</author><author>Papa, A.</author><author>Christova, I.</author><author>Mirazimi, A.</author></authors></contributors><titles><title>Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine</title><secondary-title>Vaccine</secondary-title></titles><periodical><full-title>Vaccine</full-title></periodical><pages>6225-9</pages><volume>30</volume><number>44</number><edition>2012/08/14</edition><keywords><keyword>Adult</keyword><keyword>Antibodies, Neutralizing</keyword><keyword>Antibodies, Viral</keyword><keyword>Enzyme-Linked Immunospot Assay</keyword><keyword>Female</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Humans</keyword><keyword>Interferon-gamma</keyword><keyword>Male</keyword><keyword>Middle Aged</keyword><keyword>Neutralization Tests</keyword><keyword>T-Lymphocytes</keyword><keyword>Vaccines, Inactivated</keyword><keyword>Viral Vaccines</keyword></keywords><dates><year>2012</year><pub-dates><date>Sep</date></pub-dates></dates><isbn>1873-2518</isbn><accession-num>22902680</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/22902680</url></related-urls></urls><electronic-resource-num>10.1016/j.vaccine.2012.08.003</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. A formalin-inactivated preparation of CCHFV was also found to be protective against CCHFV infection in mice[ADDIN EN.CITE <EndNote><Cite><Author>Canakoglu</Author><Year>2015</Year><RecNum>121</RecNum><DisplayText>(3)</DisplayText><record><rec-number>121</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556812016" guid="2fe103ce-96a5-46c6-9f76-59e484a881cd">121</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Canakoglu, N.</author><author>Berber, E.</author><author>Tonbak, S.</author><author>Ertek, M.</author><author>Sozdutmaz, I.</author><author>Aktas, M.</author><author>Kalkan, A.</author><author>Ozdarendeli, A.</author></authors></contributors><titles><title>Immunization of knock-out α/β interferon receptor mice against high lethal dose of Crimean-Congo hemorrhagic fever virus with a cell culture based

vaccine</title><secondary-title>PLoS Negl Trop Dis</secondary-title></titles><periodical><full-title>PLoS Negl Trop Dis</full-title></periodical><pages>e0003579</pages><volume>9</volume><number>3</number><edition>2015/03/11</edition><keywords><keyword>Animals</keyword><keyword>Cell Culture Techniques</keyword><keyword>Female</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Humans</keyword><keyword>Immunization</keyword><keyword>Mice</keyword><keyword>Mice, Knockout</keyword><keyword>Receptor, Interferon alpha-beta</keyword><keyword>Viral Vaccines</keyword></keywords><dates><year>2015</year><pub-dates><date>Mar</date></pub-dates></dates><isbn>1935-2735</isbn><accession-num>25760444</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/25760444</url></related-urls></urls><custom2>PMC4356576</custom2><electronic-resource-num>10.1371/journal.pntd.0003579</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. Subunit CCHFV vaccine. Since CCHFV glycoproteins are displayed on the surface of the virion, it was used as the target to develop vaccine. Using vaccinia virus as vehicle to deliver the glycoproteins of CCHFV was shown to provide 100% protection to lethally challenged mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]. DNA vaccine expressing nucleoprotein (NP) and ubiquitin-linked versions of GPC-derived Gn and Gc or virus-like particle vaccination has been shown to confer protection against lethal CCHFV challenge [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mice fed transgenic plants expressing the CCHFV glycoproteins and immunized with purified glycoprotein from *Drosophila* insect cell developed antibodies against the glycoproteins, while the latter did not confer the protection[ADDIN EN.CITE ADDIN EN.CITE.DATA].

Polyclonal antibody was widely used for antiviral treatment. The FDA has approved the production of anti-rabies virus polyclonal antibodies for commercial use. The polyclonal anti-Ebola antibody from horse can effectively protect mice from lethal Ebola virus infection. During the 2014 Ebola outbreak, the monoclonal anti-Ebola antibody was used to treat Ebola virus infection and rescued patient life. Those cases indicate that antibody therapy may plays an important role in prevention and control outbreaks of emerging diseases[ADDIN EN.CITE ADDIN EN.CITE.DATA].

Comment [LJW1]: this suggests that the horse anti-Ebola antibody was successfully used to treat humans during the West Africa Ebola outbreak. We need to be clear that it was monoclonal antibody used for treatment as referenced. See suggested changes

3. References

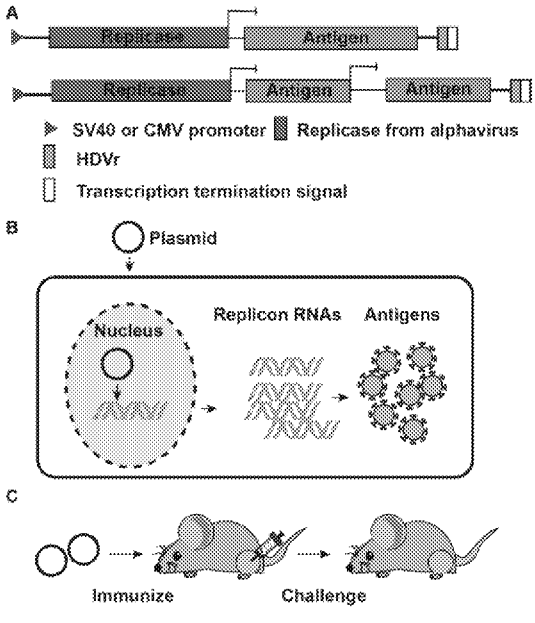
[ADDIN EN.REFLIST]

[= 2 * ROMAN]. Research Contents

1. Research contents

Aim 1 Develop a replicon-based DNA vaccine for Crimean-Congo hemorrhagic fever virus (CCHFV). We will first develop the DNA-launched replicon of alphavirus due to its rapid replication capacity in mammalian cells to deliver foreign genes (Figure 1A). The replicon will be launched by eukaryotic cytomegalovirus (CMV) promoter or SV40 promoter. After the replicon is built, we will engineer the CCHFV glycoproteins G_N and G_C (e.g., GPC open-reading frame) into the replicon DNA plasmids and characterize the immunogenicity and vaccine efficacy in a mouse model (Figure 1 B&C). We choose CCHFV as a target vaccine because (i) CCHFV represents the second most widespread of all medically important arboviruses (after dengue virus) and (ii) WHO and NIAID have classified it as an R&D Blueprint priority disease and Category A priority pathogen, respectively.

Aim 2 Generation horse ~~source~~ polyclonal antibody for anti-CCHFV therapy. VSV-CCHFV-GPC recombinant virus will be generated and used to immunized the horse. Multiple booster doses may be used to ~~boost horse and facilitate~~enhance the horse immune response. The polyclonal antibody from the horse will be analyzed for specificity and titer. Once positive antibody ~~wa~~is confirmed, the horse antibody will be purified ~~from horse~~ and assayed for protective and therapeutic efficacy in ~~used for~~ mice. ~~efficacy study.~~



2. Research methods and experimental program

Figure 1 Experimental scheme. (A) A CHIKV (alphavirus) replicon-based

antigen deliver system. CHIKV replicon RNA containing one or two antigen-expressing subgenomes be ~~is~~ launched through DNA plasmid using eukaryotic promoter (red triangle) to transcribe CHIKV replicon RNA inside mammalian cells. An HDVr (hepatitis delta ribozyme; orange box) and a transcription termination signal sequence (white box) will be ~~are~~ engineered at the 3' end of viral RNA. (B) DNA plasmid will be~~is~~ delivered into cells to launch CHIKV replicon. The replication of replicon RNAs results in robust expression of antigens. (C) Immunization of mice with CHIKV replicon DNA. The plasmid DNA will be~~is~~ delivered to animals. The vaccinated animals will be~~are~~ analyzed for immunogenicity and challenged for efficacy testing.

Aim 1 Develop a replicon-based DNA vaccine for Crimean-Congo hemorrhagic fever virus (CCHFV).

(1) **Construct alphavirus replicon as delivery system.** Alphavirus replicons are genomes with one or more of the structural protein genes deleted, but with all nonstructural genes and cis-acting sequences retained such that they replicate once introduced or produced in the cytoplasm (Fig. 1A). However, because the compatible alphavirus structural proteins are missing, infectious virus cannot be produced. The alphavirus replicon subgenomic promoter can be left intact such that subgenomic RNA is produced, which for alphaviruses is in high molar excess compared to the genomic RNA. Thus, if a foreign gene is used to replace the alphavirus structural polyprotein encoded by the subgenomic RNA, large amounts are expressed but viral spread cannot occur. Here we choose chikungunya virus (CHIKV) replicon among other alphaviruses in this proposal.

Comment [LJW2]: not sure what is meant in this sentence. "We choose chik virus recplion as our delivery system in this proposal."?

(2) **Antigen selection of CCHFV vaccine.** Like other Bunyaviruses, CCHFV contains a tri-segmented, negative sense RNA genome: small (S), medium (M), and large (L) segments. Among the six genetically distinct clades of CCHFV, there are 20, 31, and 22% sequence divergence for S, M, and L segment, respectively [ADDIN EN.CITE <EndNote><Cite><Author>Bente</Author><Year>2013</Year><RecNum>110</RecNum><DisplayText>(1)</DisplayText><record><rec-number>110</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556812014" guid="a4688135-8d5c-4363-a3ef-afb1028964bb">110</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bente, D. A.</author><author>Forrester, N. L.</author><author>Watts, D. M.</author><author>McAuley, A.

J.</author><author>Whitehouse, C. A.</author><author>Bray, M.</author></authors></contributors><titles><title>Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity</title><secondary-title>Antiviral Res</secondary-title></titles><periodical><full-title>Antiviral Res</full-title></periodical><pages>159-89</pages><volume>100</volume><number>1</number><edition>2013/07/29</edition><keywords><keyword>Animals</keyword><keyword>Genetic Variation</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Hemorrhagic Fever, Crimean</keyword><keyword>History, 20th Century</keyword><keyword>History, 21st Century</keyword><keyword>Humans</keyword><keyword>Phylogeny</keyword><keyword>Arbovirus</keyword><keyword>Bunyavirus</keyword><keyword>Crimean–Congo hemorrhagic fever virus</keyword><keyword>Nairovirus</keyword><keyword>Tick-borne virus</keyword><keyword>Viral hemorrhagic fever</keyword></keywords><dates><year>2013</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1872-9096</isbn><accession-num>23906741</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/23906741</url></related-urls></urls><electronic-resource-num>10.1016/j.antiviral.2013.07.006</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. The M segment encodes the glycoprotein precursor (GPC) that is processed to two structural glycoproteins G_N and G_C, along with several non-structural G_N and G_C are the major antigenic proteins that elicit protective immune response in humans, as observed with other Bunyaviruses [ADDIN EN.CITE <EndNote><Cite><Author>Faburay</Author><Year>2016</Year><RecNum>131</RecNum><DisplayText>(12)</DisplayText><record><rec-number>131</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556820677" guid="3638de14-24e8-4407-8efe-5a76314e8107">131</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Faburay, B.</author><author>Wilson, W. C.</author><author>Gaudreault, N. N.</author><author>Davis, A. S.</author><author>Shivanna, V.</author><author>Bawa, B.</author><author>Sunwoo, S. Y.</author><author>Ma, W.</author><author>Drolet, B. S.</author><author>Morozov,

I.</author><author>McVey, D. S.</author><author>Richt, J. A.</author></authors></contributors><titles><title>A Recombinant Rift Valley Fever Virus Glycoprotein Subunit Vaccine Confers Full Protection against Rift Valley Fever Challenge in Sheep</title><secondary-title>Sci Rep</secondary-title></titles><periodical><full-title>Sci Rep</full-title></periodical><pages>27719</pages><volume>6</volume><edition>2016/06/14</edition><keywords><keyword>Animals</keyword><keyword>Antibodies, Neutralizing</keyword><keyword>Antibody Formation</keyword><keyword>Glycoproteins</keyword><keyword>Immunoglobulin G</keyword><keyword>Liver</keyword><keyword>Lymph Nodes</keyword><keyword>Recombinant Proteins</keyword><keyword>Rift Valley Fever</keyword><keyword>Rift Valley fever virus</keyword><keyword>Sheep</keyword><keyword>Sheep Diseases</keyword><keyword>Temperature</keyword><keyword>Vaccines, Subunit</keyword><keyword>Viremia</keyword><keyword>Virulence</keyword></keywords><dates><year>2016</year><pub-dates><date>06</date></pub-dates></dates><isbn>2045-2322</isbn><accession-num>27296136</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/27296136</url></related-urls></urls><custom2>PMC4906348</custom2><electronic-resource-num>10.1038/srep27719</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. Monoclonal antibodies against CCHFV G_N and G_C potently neutralize diverse CCHFV strains *in vitro* [ADDIN EN.CITE <EndNote><Cite><Author>Zivcec</Author><Year>2017</Year><RecNum>132</RecNum><DisplayText>(13)</DisplayText><record><rec-number>132</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556820738" guid="1092b9f5-fd56-4d1e-ab08-64c4c801ea82">132</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zivcec, M.</author><author>Guerrero, L. I. W.</author><author>Albariño, C. G.</author><author>Bergeron, É</author><author>Nichol, S. T.</author><author>Spiropoulou, C. F.</author></authors></contributors><titles><title>Identification of broadly neutralizing monoclonal antibodies against Crimean-Congo hemorrhagic fever virus</title><secondary-title>Antiviral

Res</secondary-title></titles><periodical><full-title>Antiviral Res</full-title></periodical><pages>112-120</pages><volume>146</volume><edition>2017/08/24</edition><keywords><keyword>Animals</keyword><keyword>Antibodies, Monoclonal</keyword><keyword>Antibodies, Neutralizing</keyword><keyword>Antibodies, Viral</keyword><keyword>Epitopes</keyword><keyword>Glycoproteins</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Hemorrhagic Fever, Crimean</keyword><keyword>Humans</keyword><keyword>Mutation</keyword><keyword>Neutralization Tests</keyword><keyword>Phylogeny</keyword><keyword>Sequence Analysis, DNA</keyword><keyword>Crimean-Congo hemorrhagic fever virus</keyword><keyword>Monoclonal antibodies</keyword><keyword>Neutralization assay</keyword><keyword>Virus-like particles</keyword></keywords><dates><year>2017</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1872-9096</isbn><accession-num>28842265</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/28842265</url></related-urls></urls><electronic-esource-num>10.1016/j.antiviral.2017.08.014</electronic-resource-num><language>eng</language></record></Cite></EndNote>], and adoptive transfer of these antibodies confer *in vivo* protection in suckling mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These results support the possibility of cross-protection against all strains from the six CCHFV clades. The results also justify expression of the complete GPC polyprotein for vaccine development.

(3) **Construction of CCHFV vaccine candidates.** We will engineer the full open-reading frame of CCHFV GPC into the CHIKV replicon plasmid. The complete GPC will be used~~is selected~~ to ensure the correct processing and conformation of the individual G_N and G_C proteins. The GPC sequence from clinical CCHFV strain FK16116 (China) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and Turkey200406546 (UTMB), rather than laboratory strain IbAr10200, will be used for human codon optimization and inserted into the replicon plasmids. The resulting GPC-replicon plasmids will be evaluated in cell culture for the expression and processing of GN and GC proteins using Western blot. Once the protein expression has been confirmed, we will test the GPC-replicon plasmid in a CCHFV mouse model.

(4) **Testing CCHFV vaccine candidates *in vivo*.** We will test the GPC-replicon plasmids for immunogenicity, safety, and efficacy in a mouse model. Over the last seven decades, attempts have been made to establish an animal model for CCHF in adult mice, rats, guinea pigs, hamsters, rabbits, and other laboratory animals. They met with very limited success showing little or no signs of infection or disease when infected with CCHFV. Until 2010, the only animal that manifested disease after CCHFV infection was the newborn suckling mouse. In recent years, small animal models have been developed using STAT-1$^{-/-}$ [ADDIN EN.CITE <EndNote><Cite><Author>Bente</Author><Year>2010</Year><RecNum>115</RecNum><DisplayText>(16)</DisplayText><record><rec-number>115</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556812015" guid="3f1f6230-804e-46e3-8483-dac0c54cf730">115</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bente, D. A.</author><author>Alimonti, J. B.</author><author>Shieh, W. J.</author><author>Camus, G.</author><author>Ströher, U.</author><author>Zaki, S.</author><author>Jones, S. M.</author></authors></contributors><titles><title>Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>11089-100</pages><volume>84</volume><number>21</number><edition>2010/08/25</edition><keywords><keyword>Animals</keyword><keyword>Disease Models, Animal</keyword><keyword>Disease Susceptibility</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Hemorrhagic Fever, Crimean</keyword><keyword>Humans</keyword><keyword>Mice</keyword><keyword>Mice, Knockout</keyword><keyword>STAT1 Transcription Factor</keyword></keywords><dates><year>2010</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>1098-5514</isbn><accession-num>20739514</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/20739514</url></related-urls></urls><custom2>PMC2953203</custom2><electronic-resource-num>10.1128/JVI.01383-10</electronic-resource-num><language>eng</language></record></Cite></EndNote>] or IFNAR$^{-/-}$ (type-I interferon receptor) knockout mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Since these mice succumb to CCHFV infection, we will test our vaccine candidates in the IFNAR$^{-/-}$ mice. More recently, transient

depletion of interferon receptors through intraperitoneal injection with a monoclonal antibody (Mab MAR1-5A3, Leinco Technologies) immediately prior to challenge has been shown to allow efficient viral replication [ADDIN EN.CITE ADDIN EN.CITE.DATA]. We will also use the wild-type C57BL/6 mice to evaluate the vaccine efficacy since this model allows a full immune response to immunization while allowing the virus to replicate after challenge. The mouse efficacy experiments with CCHFV will be performed by Dr. Chao Shan and Dr. Han Xia at the BSL-4 facility at Wuhan Institute of Virology, Chinese Academy of Sciences.

Using the IFNAR$^{-/-}$ mice, we will intramuscularly immunize different doses of replicon-GPC plasmid (1, 5, 10, and 20 μg; n=12 per group, male and female) or PBS (as sham control) using the TriGrid Delivery System. On day 28 post-immunization, the immunized mice will be bled and measured for antibody titers using CCHFV VLP ELISA [ADDIN EN.CITE <EndNote><Cite><Author>Zivcec</Author><Year>2015</Year><RecNum>136</RecNum><DisplayText>(20)</DisplayText><record><rec-number>136</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556821443" guid="951bec17-5bed-4e75-80a1-9f1b854ce673">136</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zivcec, M.</author><author>Metcalfe, M. G.</author><author>Albariño, C. G.</author><author>Guerrero, L. W.</author><author>Pegan, S. D.</author><author>Spiropoulou, C. F.</author><author>Bergeron, É</author></authors></contributors><titles><title>Assessment of Inhibitors of Pathogenic Crimean-Congo Hemorrhagic Fever Virus Strains Using Virus-Like Particles</title><secondary-title>PLoS Negl Trop Dis</secondary-title></titles><periodical><full-title>PLoS Negl Trop Dis</full-title></periodical><pages>e0004259</pages><volume>9</volume><number>12</number><edition>2015/12/01</edition><keywords><keyword>Antibodies, Monoclonal</keyword><keyword>Antibodies, Neutralizing</keyword><keyword>Antibodies, Viral</keyword><keyword>Drug Evaluation, Preclinical</keyword><keyword>Genes, Reporter</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Molecular Sequence Data</keyword><keyword>Sequence Analysis, DNA</keyword><keyword>Transcription,

Genetic</keyword><keyword>Virion</keyword><keyword>Virus Internalization</keyword><keyword>Virus Replication</keyword></keywords><dates><year>2015</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>1935-2735</isbn><accession-num>26625182</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/26625182</url></related-urls></urls><custom2>PMC4666410</custom2><electronic-resource-num>10.1371/journal.pntd.0004259</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. On the same day, the animals will be challenged with 100 PFU of clinical CCHFV strain FK16116 (China). The challenged animals will be measured for the following parameters for efficacy: (i) Viremia and viral loads in different organs using qRT-PCR and plaque assays, (ii) weight loss, (iii) survival (Deaths are expected on days 2-6 post-challenge in the sham group), (iv) antibody titers on 28 days after challenge, and (v) T cell activation. Comparison of the antibody titers before and after challenge will indicate if the vaccine elicits sterilizing immunity (i.e., no detectable viremia and no increase in antibody titers after challenge). Using the wild-type mice, we will immunize C57BL/6 mice with the GPC-replicon plasmid and measure the five parameters described above. The immunized mice will be challenged with 100 PFU of clinical CCHFV strain FK16116 (China) by the intraperitoneal route; however, to facilitate viral infection and replication, the animals will be pretreated with 2 mg of interferon receptor-blocking antibodies one day before the CCHFV challenge, and further treated with 0.5 mg of antibody at 24 h after challenge [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These *in vivo* studies will reveal the immunogenicity, safety, and efficacy of the vaccine candidates. Comparison of the results from different dosage groups (1, 5, 10, and 20 μg DNA) will also allow us to estimate the correlates of protections against CCHFV infection in the mouse model.

Aim 2 Generation horse ~~source~~ polyclonal antibody for anti-CCHFV therapy.

(1) Production of VSV-CCHFV-GPC recombinant virus.

(2) Immunization of the horse for polyclonal antibody production. VSV-CCHFV-GPC recombinant virus (1×10^6 PFU) will be used to immunized a horse by intramuscularly (multi-point injection) route. On day 28 post-immunization, the immunized ~~horse~~mice will be bled and measured for antibody titers using CCHFV VLP ELISA [ADDIN EN.CITE <EndNote><Cite><Author>Zivcec</Author><Year>2015</Year><RecNum>136</RecNum><

Comment [LW3]: confirm that this is what you mean.

DisplayText>(20)</DisplayText><record><rec-number>136</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556821443" guid="951bec17-5bed-4e75-80a1-9f1b854ce673">136</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zivcec, M.</author><author>Metcalfe, M. G.</author><author>Albariño, C. G.</author><author>Guerrero, L. W.</author><author>Pegan, S. D.</author><author>Spiropoulou, C. F.</author><author>Bergeron, É</author></authors></contributors><titles><title>Assessment of Inhibitors of Pathogenic Crimean-Congo Hemorrhagic Fever Virus Strains Using Virus-Like Particles</title><secondary-title>PLoS Negl Trop Dis</secondary-title></titles><periodical><full-title>PLoS Negl Trop Dis</full-title></periodical><pages>e0004259</pages><volume>9</volume><number>12</number><edition>2015/12/01</edition><keywords><keyword>Antibodies, Monoclonal</keyword><keyword>Antibodies, Neutralizing</keyword><keyword>Antibodies, Viral</keyword><keyword>Drug Evaluation, Preclinical</keyword><keyword>Genes, Reporter</keyword><keyword>Hemorrhagic Fever Virus, Crimean-Congo</keyword><keyword>Molecular Sequence Data</keyword><keyword>Sequence Analysis, DNA</keyword><keyword>Transcription, Genetic</keyword><keyword>Virion</keyword><keyword>Virus Internalization</keyword><keyword>Virus Replication</keyword></keywords><dates><year>2015</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>1935-2735</isbn><accession-num>26625182</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/26625182</url></related-urls></urls><custom2>PMC4666410</custom2><electronic-resource-num>10.1371/journal.pntd.0004259</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. If the antibody titer reaches desired point, the horse~~blood~~ will be bled for antibody purification. If not, ~~a~~the booster innoculation will be given~~happened~~ to facilitate the immune response and antibody titer will be assayed at ~~wait another~~ 28 days.

Comment [LW4]: what's the target titer?

(3) Polyclonal antibody purification from horse blood. Once desired anti-CCHFV antibody titer is reached, the horse ~~blood~~ will be bled. Protein G Agarose will be used to purify the horse polyclonal antibody based on the manufacture manual. The eluted polyclonal antibody will be ~~dialyzed~~dialyzed in PBS and aliquoted. Purified polyclonal antibody will be measured by CCHFV VLP ELISA to confirm antibody titer.

Comment [LJW5]: identify titer desired someplace in the proposal

(4) Mice efficacy study.

Using the IFNAR$^{-/-}$ mice, we will intraperitoneally inject different doses of purified antibody (or PBS (as sham control) one day before CCHFV FK16116 infection. On the day of infection, the blood will be collected and 100 PFU CCHFV will be injected subcutaneously. The challenged animals will be measured for the following parameters for efficacy: (i) Viremia and viral loads in different organs using qRT-PCR and plaque assays, (ii) weight loss, (iii) survival (Deaths are expected on days 2-6 post-challenge in the sham group).

3. Expected outcome

The proposed experiments will generate a CCHFV vaccine candidate built upon the FK16116 and Turkey200406546 strain. The cross-protection against different stains will be evaluated after challenge. Comparison of the immunogenicity and efficacy from the mouse experiments will allow us to down select the final candidate. In an unlikely situation that a single-shot immunization of the replicon-GPC plasmid is not sufficient to elicit protective antibody titers or full protection against CCHFV infection, we will boost the animals with a second dose of replicon-GPC plasmid. The mouse efficacy results will allow us to strategize further regarding preclinical development, including efficacy test in a non-human primate model. In addition, by producing~~selecting~~ the horse polyclonal antibody, ~~from horse~~ we will ~~allow us~~ have ~~the~~ potential candidate for anti-CCHFV treatment.

Comment [LJW6]: please confirm that this is your intent

4. Key problems and technical difficulties to be solved

Vaccine development. The proposed study will establish a DNA replicon platform for CHIKV. ~~And~~ tThise platform will ~~be~~ served as the vehicle to deliver the CCHFV-GPC for our vaccine candidate. The cell culture and mouse study will define the expression pattern. This information will be critical to guide vaccine and therapeutics development. We will also explore the cross-protection by

Comment [LJW7]: should these activities be listed as problems to be resolved over the course of the study?

using ~~when use~~ two different antigens to immunize mice. The polyclonal antibody will provide potential treatment option for CCHFV infection.

5. Innovations of the research proposal

The innovation of this project derives from the research plan that integrates three powerful components into a translational product: (i) the robust self-amplifying ability of alphavirus replicon, (ii) the ease of DNA plasmid as a vector to launch the replicative RNAs (using the mammalian transcription machinery), and (iii) the cutting-edge device for DNA delivery in clinical use. Although alphavirus replicon has been used for protein expression and vaccine development for almost three decades, previous efforts have mainly focused on the virus-like particle-RNA packaging (VLP) approach [ADDIN EN.CITE <EndNote><Cite><Author>Lundstrom</Author><Year>2017</Year><RecNum>137</RecNum><DisplayText>(21)</DisplayText><record><rec-number>137</rec-number><foreign-keys><key app="EN" db-id="fw5a0favmxv0w2ex9wqvwxwnz9pat9z0szft" timestamp="1556823011" guid="45f9f958-084b-4a4f-a13b-67585a1d3c8d">137</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lundstrom, K.</author></authors></contributors><titles><title>Oncolytic Alphaviruses in Cancer Immunotherapy</title><secondary-title>Vaccines (Basel)</secondary-title></titles><periodical><full-title>Vaccines (Basel)</full-title></periodical><volume>5</volume><number>2</number><edition>2017/04/12</edition><keywords><keyword>cancer immunotherapy</keyword><keyword>oncolytic alphaviruses</keyword><keyword>tumor eradication</keyword></keywords><dates><year>2017</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>2076-393X</isbn><accession-num>28417936</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/28417936</url></related-urls></urls><custom2>PMC5492006</custom2><electronic-resource-num>10.3390/vaccines5020009</electronic-resource-num><language>eng</language></record></Cite></EndNote>]. For the VLP approach, viral structural proteins are supplied in trans to package the viral replicon RNA (carrying an antigen of interest) into VLPs; such VLPs are used to immunize animals. The drawbacks of the VLP approach include the requirement of eukaryotic cell culture electroporation for VLP production, moderate

yield of VLP manufacture (when compared with the doses required for vaccination and treatment), pre-immune inhibition when multiple rounds of VLP infection are needed, and the "cold chain" transportation from manufactures to clinics. All the above drawbacks of the VLP approach could be mitigated by the proposed DNA-launched replicon in this project.

Despite the potential concern of integration of exogenous DNA into the cellular chromosome, the DNA plasmid approach has been actively pursued for vaccine development and cancer therapy. For example, the current frontrunner ZIKV vaccine in the phase II clinical trial is built upon a DNA plasmid (expressing two viral structure proteins prM and E). This type of traditional DNA vaccine requires multiple high doses to achieve protective immunity. For the ZIKV DNA vaccines, each human volunteer requires three shots, with 1 to 5 mg of DNA per shot, to achieve short-term neutralizing antibody titers [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It should be noted that the adverse potential of DNA integration into the cellular genome remains to be determined in clinics. Compared with the traditional non-amplifying DNA vaccine, our DNA-launched replicon is self-replicative, and requires a much lower dose to achieve efficacy. The significantly reduced dose will minimize the risk of potential DNA integration. In addition, due to the self-amplifying nature of the proposed replicon DNA platform, protective immunity and efficacy could potentially be achieved with a single dose to control explosive outbreaks, which is particularly important when responding to public health emergency. Therefore, the replicon DNA platform has the potential to overcome the most critical weakness of the traditional DNA vaccine, and could be developed into a transformative new DNA delivery technology.

Collectively, we hypothesize that, in combination with the cutting-edge device for delivery, the DNA-launched replicon platform will transform into robust translational products for vaccine and therapeutics development. Since the goal of the Advanced Customer Cultivation Project is to develop solutions to prevent and control human diseases, the combination of the well-proven alphaviral replicon system with the ease of DNA engineering and the state-of-the-art DNA delivery represents a practical innovation for vaccine platform development.

Passive immunotherapy with sera of animal origin has been used for over 120 years to treat bacterial and viral infections, envenomations and drug intoxications. The lower manufacturing costs of hyperimmune equine antisera therefore represents an attractive alternate avenue of treatment,

especially to developing and third-world countries, compared to the more costly production process of viral specific mAbs. However, currently the study of anti-CCHFV antisera via the immunization of horses and the safety and efficiency has not been reported. Since the highly replication efficiency and the safety of the VSV vector, in this study the VSV-CCHFV-GP will be used~~chose~~ as the antigen for horse.

[= 3 * ROMAN]. Research Plan

1. Research schedule

Aim	Tasks and milestones	2019											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Clone CHIKV replicon and SEAP reporter							X	X	X	X	X	X
	Characterize SEAP replicons in cell culture												
	Characterize SEAP replicon in mice												
	Clone GPC CHIKV replicon												
	Test GPC replicon in cell culture												
	Test efficacy in mouse model												
2	Generation VSV-CCHFV-GP recombinant virus							X	X	X	X	X	X
	Immunization horse and characterization antibody response												
	Purify antibody from horse												
	Test efficacy in mouse model												

Aim	Tasks and milestones	2020											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Clone CHIKV replicon and SEAP reporter												
	Characterize SEAP replicons in cell culture	X	X	X									
	Characterize SEAP replicon in mice				X	X	X	X					
	Clone GPC CHIKV replicon								X	X	X	X	
	Test GPC replicon in cell culture												X
	Test efficacy in mouse model												
2	Generation VSV-CCHFV-GP recombinant virus												
	Immunization horse and characterization antibody response	X	X	X	X	X	X	X	X				
	Purify antibody from horse									X	X	X	X
	Test efficacy in mouse model												

Aim	Tasks and milestones	2021											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Clone CHIKV replicon and SEAP reporter												
	Characterize SEAP replicons in cell culture												
	Characterize SEAP replicon in mice												
	Clone GPC CHIKV replicon												
	Test GPC replicon in cell culture	X	X	X									
	Test efficacy in mouse model				X	X	X	X	X	X	X	X	
2	Generation VSV-CCHFV-GP recombinant virus												
	Immunization horse and characterization antibody response												
	Purify antibody from horse												
	Test efficacy in mouse model				X	X	X	X	X	X	X	X	

2. Conditions necessary to conduct the research (including lab equipments, instruments and etc.)

Biosafety cabinet

PCR thermal cycler

Real-Time PCR thermal cycler

Table top centrifuge

Water bath

DNA gel electrophoresis system

Protein gel electrophoresis system

TriGrid Delivery System (Ichor, for DNA vaccine delivery)

ChemiDoc System

Pipettes

CO2 incubators

Microscopy

Refrigerator

-80 freezer

-30 freezer

Liquid nitrogen tank

IV. Introduction of Leader and Participants

James Le Duc, PhD, is the director of the Galveston National Laboratory, one of the largest active biocontainment facilities on a U.S. academic campus. Dr. Le Duc joined UTMB in late 2006 from the Centers for Disease Control and Prevention in Atlanta, where he was the influenza coordinator and director of the Division of Viral and Rickettsial Diseases. With more than four decades of experience working in the fields of biodefense and public health, his work has taken him around the world, from West Africa, where he began his professional career as a field biologist working for the Smithsonian Institution, to Brazil and Panama during a 23-year career as a U.S. Army officer in the medical research and development command.

Pei-Yong Shi, PhD, is I.H. Kempner Professor of Human Genetics, University of Texas Medical Branch, Galveston Texas, USA. He is an elected Fellow of American Academy of Microbiology, adjunct Professor of Emerging Infectious Diseases at the Duke-NUS Graduate Medical School in Singapore, and Honorary Professor at the Wuhan Institute of Virology, Chinese Academy of Sciences. He received his Ph.D. in virology in 1996 from Georgia State University. After postdoctoral training at Yale University, he joined Bristol-Myers Squibb as a Principal Scientist to develop HIV and HCV therapeutics from 1998 to 2000. He then moved to the Wadsworth Center, New York State Department of Health, to study West Nile virus. From 2008 to 2015, he served as Dengue Unit Head and Executive Director to lead drug discovery at Novartis Institute for Tropical Diseases. His group developed the first infectious clones of the epidemic strain of West Nile virus and Zika virus, discovered two RNA cap methylation activities of flavivirus NS5 protein, identified essential RNA elements for flavivirus replication, established various platforms for flavivirus vaccine and drug discovery, and pioneered therapeutics development for dengue virus. He is internationally recognized for his scholar and administrative accomplishments at leading research institution, public health sector, and pharmaceutical industry. Dr. Shi has more than 20 years experiences in handling BSL2 and ABSL2 agent.

Dennis A. Bente, DVM, Ph.D., is an associate professor from University of Texas Medical Branch, Galveston Texas, USA. Dr. Bente received his DVM in 2000 and Ph.D. in 2003 from University of Veterinary Medicine at Germany. The goal of Dr. Bente's research is to better understand the

transmission and pathogenesis of tick - borne hemorrhagic fever viruses and to develop countermeasures to combat the disease. The intersection between arbovirology and hemorrhagic fever research requires an interdisciplinary approach, involving virology (classical techniques as well as molecular techniques such as reverse genetics), immunology (human and animal models), and tick physiology. Dr. Bente's is the first laboratory in the world to establish a tick - host transmission model in a BSL-4 setting. A number of collaborations have been established with other virologists at UTMB, including Drs. Alan Barrett, Thomas Ksiazek, David Beasley, Alexander Freiberg and Thomas Geisbert, that include studies on Crimean - Congo hemorrhagic fever virus, Kyasanur forest disease virus, Alkhurma hemorrhagic fever virus, and West - Nile virus. Dr. Bente has more than 10 years experiences in handling BSL2, BSL3, BSL4, ABSL2, ABSL3 and ABSL4 agent.

Zhiming Yuan, Ph.D., professor from Wuhan Institute of Virology, the director of the Wuhan National biosafety laboratory (BSL-4), and the President of Wuhan Branch, Chinese Academy of Sciences. His research interest including: (1) Diagnosis, evolution, and pathogenesis of aborviruses, (2) Tropical Disease vector control with microbial agents, and (3) Laboratory biorisk management and applied biosafety research.

Chao Shan, Ph.D., professor from Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China. Dr. Shan Received his Ph.D. in Biochemistry and Molecular Biology in Wuhan Institute of Virology, Chinese Academy of Sciences in China in 2014. And he joined Novartis Institute for Tropical Diseases (NITD) from 2013 to 2015. He served as postdoctoral fellow in University of Texas Medical Branch from 2015 to 2019. During his training at Wuhan Institute of Virology and NITD, he worked with Dengue virus, Japanese encephalitis virus and EV71 virus *in vitro*. After joining in UTMB in December 2015, Dr. Shan has completed BSL3, BSL4, ABSL2, ABSL3 and ABSL4 training in University of Texas Medical Branch. He built the first reverse genetic system for Zika virus and developed the first live-attenuated Zika vaccine in the world.

Han Xia, Ph.D., is associate professor from Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China. Dr. Xia received his Ph.D. in Biochemistry and Molecular Biology in Wuhan Institute of Virology, Chinese Academy of Sciences in China in 2014. And she served as postdoctoral fellow and the

complete the BSL-4 training in University of Texas Medical Branch from 2013 to 2016, working with reverse genetic system developing of CCHFV and CCHFV-vector-host interaction through NGS strategy. Currently, her research interest is the epidemiology, diagnoses, and evolution of arbovirus.

[= 7 * ROMAN].Budget

Unit: RMB 10,000 yuan

Budget Form of Project Expenditure		
Item	Amount	Detailed calculation
1. Equipment		
(1) Equipment purchase	0	
(2) Trial-manufacture purchase	0	
(3) Equipment modification and rent	0	
2. Reagents and consumables	15	Cloning and related reagent, kit, sequencing, cell culture reagent and consumables
3. Analysis	15	Horse purchase, immunization, antibody purification, DNA synthesis
4. Fuel and power	3	Transport
5. Travel/meeting/international cooperation and exchanges	5	Project meeting for WHIOV and UTMB, hotel etc.
6. Publication/literature/information dissemination/intellectual property	3	Publication and patent
7. Labor costs	5	Subsidy
8. Expert consultation	1	Project consultation
9. Other expenditure	3	Shipping fee about reagent and material
Total	50	

Note: Budget preparation and expenditure execution are conducted according to *Measures of Academy-Level Scientific Research Projects of Chinese Academy of Sciences*.

[= 8 * ROMAN]. Review opinions of applicant's organization

Our joint proposal, "Vaccine Development and Polyclonal Antiserum for Crimean-Congo Hemorrhagic Fever Virus" represents the culmination of many years of collaboration between the Galveston National Laboratory, the U.S. National Academy of Sciences and the Wuhan Institute of Virology, Wuhan National Biosafety Laboratory of the Chinese Academy of Sciences. We are very excited about the possibility of collaborating in the implementation of this important study and making use of the unique resources of both our biocontainment laboratories.

The study is non-confidential and we anticipate that our findings will be published in the peer reviewed scientific literature under shared co-authorship.

Organization (official seal) Principal (Signature)

03/05/2019

[= 9 * ROMAN]. Opinions of the Biosafety Committee in Project Implementation Organization

Chairman of Committee (Signature)

(d/m/y)

[= 10 * ROMAN]. Opinions of Science and Technology Steering Committee of Wuhan National Biosafety Laboratory, CAS

Chairman of Committee (official seal)

(d/m/y)

[ADDIN EN.REFLIST]

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 6/10/2019 4:07:48 PM
To: Zheng郑大胜 [d.zheng@wh.iov.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]; Grimaldo, Miguel A. [magrimal@UTMB.EDU]
Subject: RE: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Dasheng,

It's nice to hear from you again and to learn of your continued interest in working with us here at the GNL. I am happy to prepare a letter of invitation for your proposed visit, but it would be useful to understand a bit more as to the purpose of your stay here. Do you envision conducting a research study, and if so, what is the topic? If you are only seeking additional training in biosafety, biosecurity and building operations, that would be easier to accomplish, although the support we enjoyed previously that allowed us to provide biosafety training at no cost to users is no longer available and we now have a fee for the training. It would also be useful to learn the most convenient dates from your perspective for a visit.

I look forward to hearing back from you will additional details.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Zheng郑大胜 <d.zheng@wh.iov.cn>
Sent: Wednesday, June 05, 2019 8:43 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Mendoza, Imelda <imendoza@UTMB.EDU>
Subject: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Prof LeDuc,

May I ask for a favor from you to write an invitation letter with the same purpose as previous one so that I could seek another funding for longer stay at your academia?

You have always been appreciated greatly to provide opportunities for academic exchanges. Hopefully I could do something in return.

Best Wishes,

Dasheng
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ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969
==========

At 2015-04-01 21:43:13, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

> Dear Dr Zheng,
>
> Thank you for the update, and best of luck as you continue to seek funding for your scholarship.
>
> With best regards,
>
> Jim
>
> James W. Le Duc, Ph.D.
> Director
> Galveston National Laboratory
> University of Texas Medical Branch
> Galveston, TX 77555-0610
> (t) 409-266-6500
> (f) 409-266-6810
> jwleduc@utmb.edu
>
> **From:** dsn.zheng@163.com [mailto:dsn.zheng@163.com] **On Behalf Of** d.zheng@wh.iov.cn
> **Sent:** Tuesday, March 31, 2015 9:36 PM
> **To:** LeDuc, James W.
> **Cc:** Mendoza, Imelda; Bente, Dennis A.; Xia, Han
> **Subject:** Re:Chinese Scholarship to Visit UTMB
>
> Dear Dr. James LeDuc,
>
> Thank you for inviting me to the GNL in writing the invitation letter which provides opportunity of visit and study at your honored laboratory. Unfortunately I havenot gotten any acceptance news from the Chinese Scholarship Committee after the scheduled admission deadline. I am afraid I have to look for other funding resources.

Best Wishes,

Dasheng

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Zheng, Dasheng PhD
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Wuhan National Biosafety Laboratory
Chinese Academy of Sciences
Wuhan, P.R.China.

Tel: 86-27-5186 1004

Mobile: 86-135 1729 0969

Email: d.zheng@wh.iov.cn
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At 2014-11-26 10:44:53, "Bente, Dennis A." <dabente@UTMB.EDU> wrote:
- 隐藏引用文字 -

Dear Dasheng,

Han translated the requirements for me and we are happy to write an invitation letter for you. I talked to Dr. Le Duc, director of the Galveston National Laboratory, and he agreed to write a letter for you. I copied him on this email. Dr. Le Duc will also involve our building engineer, Miguel Grimaldo, in the process of planning your visit.

Best wishes,

Dennis

From: dsn.zheng@163.com [mailto:dsn.zheng@163.com] **On Behalf Of** d.zheng@wh.iov.cn
Sent: Saturday, November 22, 2014 1:46 AM
To: Bente, Dennis A.
Cc: Xia, Han
Subject: Re:RE: Nice to meet you at Wuhan

Hi Den,

At this moment I have a chance to apply for some fund to support my idea to UTMB from the China Scholarship Committee. May I ask for your help in writing an invitation letter as a prerequisite for this fund? The webpage (in Chinese only) of this fund is as follow:

http://www.csc.edu.cn/Chuguo/43988dd354584badbeb2faf380d99859.shtml

Could Han do a little interpretation so as to make sure what we need to do? According to the Item 14 of the fund bidding approach shown in the webpage, the applicant should have an invitation from abroad in advance.

In my proposal of visit to your lab, I shall accept trainings in high BSL laboratories at first; then, conduct experiments for some time, which is the solid work of this visit; and, last but the most important part, have lessons in biosafety management of GNL, playing as one reason for the fund. You might have better plans. Anyway I will follow your steps since I'm a trainee.

Your assistance would be appreciated greatly.

Best Regards,

Dasheng

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Zheng, Dasheng
==========
Wuhan Institute of Virology
Chinese Academy of Sciences
Mid 44, Wuchang Xiaohongshan

Wuhan 430071, P.R.China.

Mobile: 86-13517290969

Email: dsn.zheng@163.com

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From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 11/25/2019 3:42:54 PM
To: Yuan Zhiming [yzm@wh.iov.cn]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: draft manuscript
Attachments: BSL4 Wuhan_Manuscript-20191107_track-jwl comments Nov19.docx

Hi Zhiming,

Sorry for the delay in responding to your request for comments on your draft manuscript. I finally had a chance to review it and my comments are attached. I think the paper is nicely written and will be of interest to readers following the development of biocontainment labs in China. You have done a good job in recording capabilities, and you may wish to expand a bit more by mentioning the maximum number of small or large (non-human primates) you are able to manage at a single time in the facility. We are frequently asked these questions, and most product developers want sufficiently large single studies to have statistical significance, so many of our larger studies involve about 20 NHP. There may be good reasons not to quantify your capabilities as well, which I fully understand.

You rightly credit the collaborations with the French in building the laboratory; however, if your goal is to have a truly international impact, you may wish to broaden comments on potential collaboration/collaborators as mentioned in one comment.

If I understand you correctly, you will be publishing the paper in your biosafety journal. If so, you may wish to expand your comments on your training efforts to prepare your staff to safely and securely work in the new facility. You may also wish to mention something about your security profile. As I recall, the entire campus has limited access with guards at entrances. You may wish to comment on other mechanisms in place to limit access to high-risk pathogens—card-key access to labs, security personnel, etc. You will not want to go into too much detail, but it might be appropriate, especially given the focus of your journal, to let readers know that security is an important aspect of your program.

Very nicely done! Thank you for the opportunity to review the draft.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

China's First Biosafety Level 4 (BSL-4) Laboratory for Fighting Infectious Disease

The epidemic of severe acute respiratory syndrome (SARS-CoV) in 2002–2003, which resulted in 8,069 cases of infection and 775 deaths worldwide (Ref2), brought a great challenge to national and international public health systems. It became a touchstone for public health in China as it responded to emerging infectious diseases, and revealed the weaknesses of existing strategies for the prevention and control of such emerging diseases. Complicating the issue, basic and clinical research in response to the epidemic was impeded due to a lack of high containment facilities. Therefore, in order to reduce the potential impact of deadly infectious diseases, including SARS and other highly dangerous infectious risks to human health, the Chinese authority embarked on the construction of a biosafety laboratory network in China, including the BSL-4 National Biosafety Laboratory, Wuhan, Hubei province in central China. ~~(Wuhan).~~

Comment [LJW1]: check numbers; I had 8437 total with 813 deaths from WHO. You may wish to just say over 8,000 cases and nearly 10% mortality or something similar to avoid specific numbers.

The Chinese Academy of Sciences began the process that would lead to the construction of the BLS-4 laboratory early in 2003, and broke ground in 2015. In the framework of the Sino-French Cooperation Agreement on the Prevention of Emerging Disease Control, signed in 2004, Chinese and French engineers and scientists agreed to collaborate for one decade to complete an internationally recognized BSL-4 laboratory, providing a safe and secure platform for scientists to study high-hazard viruses.

~~On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).~~

~~The Chinese Academy of Sciences began the process that would lead to the construction of the BLS-4 laboratory early in 2003, and broke ground in 2015. In the framework of the Sino-French Cooperation Agreement on the Prevention of Emerging Disease Control, signed in 2004, Chinese and French engineers and scientists agreed to collaborate for one decade to complete an internationally recognized BSL-4 laboratory, providing a safe and secure platform for scientists to study high-hazard viruses.~~

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Milestones of the laboratory construction

As a critical part of the national high-level biosafety laboratory network system, the construction project of the Wuhan BSL-4 National Biosafety Laboratory (NBL) was officially approved by the National Development and Reform Commission in 2005. Subsequently, Chinese and French engineers and designers studied the operational state of the art high-containment laboratories worldwide, analyzed the geological and environmental conditions of the proposed construction site, confirmed the operational role of the laboratory in China, then jointly designed and constructed the laboratory. The physical completion of the laboratory on January 31, 2015, is not only a great symbol of Sino-French friendship, but also an impressive accomplishment of the national high-containment biosafety laboratory network. After the commissioning, certification, and trial operation, the laboratory was successfully accredited as an Animal Biosafety Level-4 (ABSL-4) laboratory by the China National Accreditation for Conformity Assessment in accordance with CNAS-CL05:2009 and national laboratory standards on January 13, 2017 (Ref3), and acquired the official license of handling risk group-4 (RG-4) pathogens from the National Health and Family Planning Commission on August 17, 2017. The award of the accreditation certificate and the experimental activity license demonstrated that the laboratory has the full capacity and authority to handle high-hazard viruses and to study animal models of infection according to the regulations (Ref4). These events were a landmark achievement for the National High-level Biosafety Laboratory System with recognition by the Chinese national authority (Ref5). In addition to the laboratory, a culture collection and repository center called the "National Center for the Preservation of Pathogenic Microorganisms" was established and authorized, relying on the facility and bio-containment environment (Ref###). With these milestones, the NBL, as China's first BSL-4 laboratory, has been put into operation formally and legally, with full capacity and authority to conduct virus stocking and scientific research on virulent high-hazard viruses. The long-term aim- of the institute is to establish the NBL as a comprehensive research and development center for infectious diseases, a national biological center, and a WHO reference laboratory. In addition, this laboratory will become a stepping-stone for Chinese and French scientists in fighting infectious disease and will also serve as a cornerstone in global health security. (Fig.1)

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Comment [LJW2]: Do you want to limit this to French scientists? Perhaps "..a stepping stone for Chinese, French and other international partners in fighting..."

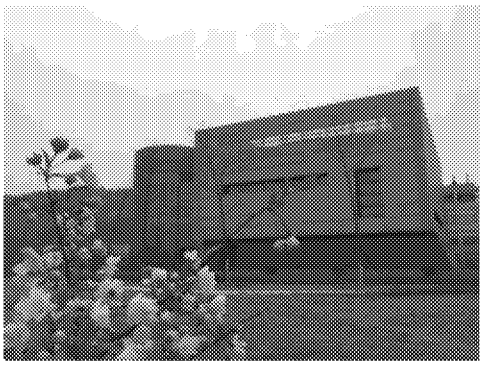

Fig. 1 The BSL-4 facility building

Nature of the laboratory

The laboratory is located in Zhengdian Scientific Park, a few kilometers away from the Yangtze River in the Jiangxia District, Wuhan City, Hubei Province. In addition to the new NBL, one BSL-3, two BSL-2s, molecular diagnosis and cell culture laboratories, and other nearly operational research

facilities and platforms to support virology research and animal rearing are also located in the park, making this research park a modern, comprehensive national and regional virology research and development center.

The BSL-4 laboratory stands as an independent building with a total area of 3266 M^2. It comprises two sections: a square laboratory body structure and a circular auxiliary structure, both inter-linked by a closed corridor. All the equipment and functional units were fitted into the three floors of the square structure. The basement and upper zones are equipped with life maintenance and differential pressure systems (compressed respiratory air and environmental air handling plenums with High Efficiency Particulate Air [HEPA] filters), continuous liquid effluent heat treatment devices and chemical disinfectant tanks, heat exchange systems, water treatment devices, and air conditioning units. All of this equipment is connected to other functional facilities distributed in other zones, within the NBL, through a pipeline network. Thus, all contaminated air, water and solid waste is sterilized/treated before release from the laboratory. (Fig.2)

- **Upper Technical Zone:** Refrigerators, AHU, Exhaust fans, Technical units of shower
- **Equipment interlayer Zone:** Separated from 2nd floor by grilling for maintenance, Air ducts and BIBO.
- **Core laboratory:** Containment with a [illegible] and out on the ceiling.
- **Basement technical zone:** Install the facilities such as electricity, compressed air, wast water treatement, water supply.

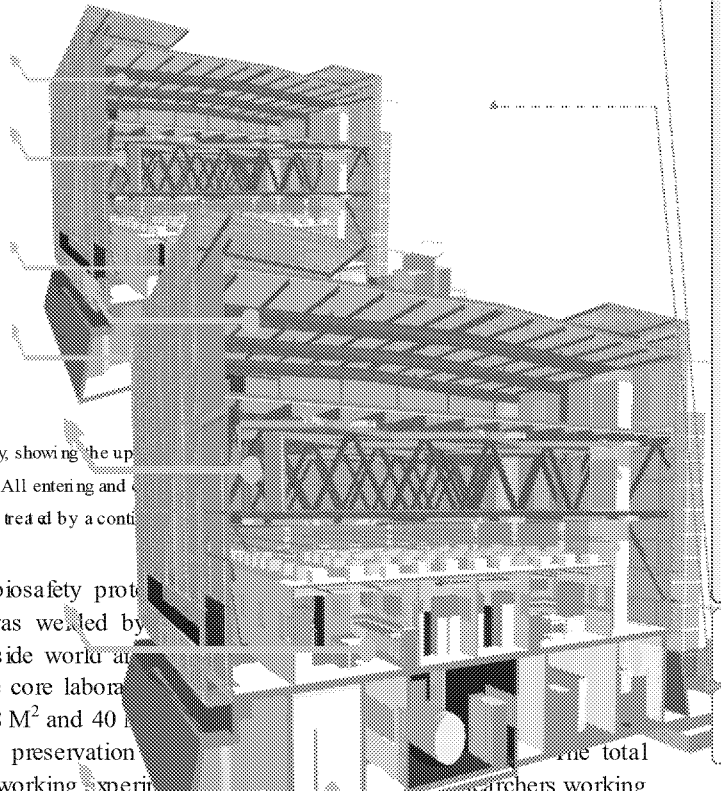

Fig. 2 The Cross-section of the BSL-4 facility, showing the up[illegible] interlayer zone, and the core laboratory zone. All entering and [illegible] solid waste is autoclaved, but liquid waste is treated by a conti[illegible] is transferred from the laboratory.

The BSL-4 zone is composed of a biosafety prot[illegible] is surrounded by a ring corridor, pipes and ducts was welded by [illegible] effectively sealed off from the outside world a[illegible] in of the working area is separated into three core labora[illegible] rodents and non-human primates, 28 M^2 and 40 [illegible] room that is 32 M^2 and two virus preservation [illegible] The total containment area is 480 M^2, and its working experi[illegible] researchers working in the lab can see outside to the non-containment ha[illegible] managers can observe the activities occurring inside of it through sealed double-glazed w[illegible] ows installed within the stainless steel wall of each laboratory; this design provides an ambient working environment for researchers in addition to being a practical passage for the purpose of overseeing by the biosafety officer and managers (Fig. 3). The installation of an outer bulletproof glass and outmost porous aluminum plate not only provide

[PAGE * MERGEFORMAT]

Comment [A3]: Please change "Technical units of shower" to "technical shower units"
Please change "Exhaust fans" to "exhaust fans"
Please change "Upper Technical Zone" to "Upper technical zone:
Please change "Equipment interlayer Zone" to "Equipment interlayer zone"
Please change "Basement technical Zone" to "Basement technical zone"
Please change "from 2nd" to "from the 2nd"
It's not clear what "by grilling for maintenance" means – maybe "by grills (for maintenance),"?
Please change "Air ducts" to "air ducts"
You might want to spell out BIBO.
Please change "ducts and BIBO." to "ducts, and BIBO"
Please change "get in and out on the ceiling" to "go in and out of the ceiling"

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Comment [LJW4]: Not sure what this means. You may wish to define in greater detail.

an extra protective measure, but also bring value in the form of heat insulation and eventual energy savings for the laboratory

The laboratory is composed of 10 technical systems, including the power supply, thermal supply, containment, air treatment system, waste disposal, life maintenance, automatic control system, fire control, security system, and isolation facilities, which guarantees the stable unidirectional negative pressure gradient air flow and sealed environment in the containment area. It is designed as a suit-type biosafety laboratory, in which the staff inside are completely protected by a whole-body positive-pressure protective suit supplied with conditioned air.

Comment [LJW5]: Would you like include mention of the maximum number of personnel that can be supported by the BSL4 air supply system at any given time?

The containment laboratory is fitted with equipment that meets the requirements of biosafety management and high-containment pathogen research, including Labconco biological safety cabinets (BSC), animal breeding and isolators, Tecn independent air transport cages, Tecn animal cages, Ehret monkey cages, a Thermo anatomy table, CO_2 incubators, fluorescence microscopes, quantitative PCR amplifiers, refrigerators, and freezers.

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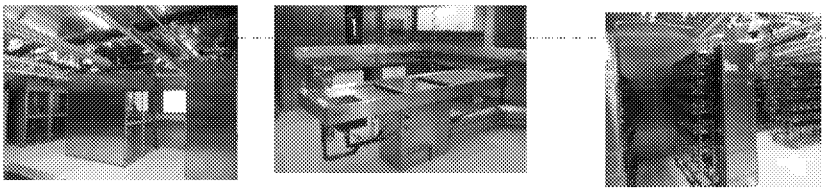
Fig. 3 Two technicians working inside the laboratory



Comment [d6]: Figures need to re-order

Fig. 4: The animal cages for rodent (A) and non-human primate (B) infection, and the autopsy table in three separated room in the BSL-4

Main scientific research priorities

The laboratory is designed and equipped to conduct research on RG-4 pathogens such as the Ebola virus, the Nipah virus, the Crimean-Congo Hemorrhagic Fever (CCHF) virus, the Lassa virus, the Junin virus, the SARS-CoV, the Marburg virus, and so on. According to the lab's biosafety protection level, personnel ability, and management status, the research activities that can be conducted in the laboratory range from low-risk manipulation of cell culture propagation, to rodent infection, and ultimately to the infection of non-human primates. Similarly, pathogen manipulations are gradually conducted from the low-risk CCHF virus to other more virulent pathogens such as the Ebola virus, the Marburg virus, and the Lassa virus. According to the license issued by the National Health Planning Commission and the availability of virus resources, the laboratory has already implemented projects on cell culture models, animal models, pathogenesis studies, and preliminary trials of antiviral drugs as well as vaccine development for the CCHF virus, which used to be called

Comment [LJW7]: Is SARS-Cov considered a RG-4 pathogen in China? I think we handle it at BSL3. This will be important if you want to share strains.

the Xinjiang hemorrhagic fever virus, causing sporadic animal infection during the last few decades in Northwest China (6).

The laboratory has established short- and long-term collaborative links with counterparts in the USA and France; we are seeking additional beneficial scientific and operational partnerships with other laboratories around the world, with the purpose of sharing specimens, reagents, technology, good practice, and expertise; the eventual goal is for there to be effective collaboration within the international laboratory community to address the threat of emerging and re-emerging infectious diseases locally and internationally (7).

Comment [A8]: "true"? Maybe "beneficial"?

The strategic role and capacity strengthening ~~**(need re-write the sub-title)**~~

Comment [A9]: "The strategic plan"

~~On the basis of the~~According to the laboratory's operational orientation and China's national requirements, the laboratory was designed and will operate as the research and development center for infectious disease, as a national biological resource center and as a WHO reference laboratory. As a comprehensive national biosafety research center, it will play an indispensable role in the prevention and control of infectious diseases in China. In order to realize these key goals and functions, we must assure the safe and secure operation of the laboratory, increase its capacity as a core culture collection resource, enlarge its scientific research capacity, support and promote the overall response capacity for public health emergency preparedness, provide expert support to national biosafety strategies, and contribute to the broader laboratory network system. We aim to ensure the safe and efficient operation of the laboratory through the principles of "openness, transparency and sharing", benefiting national security and global health security.

Comment [d10]: Repeat with previous paragraph.

Comment [LJW11]: Would you like to mention your training program to prepare staff for work in biocontainment?

(replaced with the new version of 五种能力建设)

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On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).

References

1) David Cyranoski, Inside the Chinese lab poised to study world's most dangerous pathogens, Nature, 2017, 542: 399-400,
2) WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003. WHO, [HYPERLINK "http://www.who.int/csr/sars/country/table2004_04_21/en/"](2004)
3) State Council of the People's Republic of China, Regulation on administration of biosafety in pathogenic microorganism laboratories, 2018, [HYPERLINK "http://jiuban.moa.gov.cn/fwllm/zxbs/xzxk/spyj/201706/t20170606_5662359.htm"]
4) China National Standards Committee, Laboratory-general requirements for biosafety (GB19489-2008), 2008, [HYPERLINK "http://c.gb688.cn/bzgk/gb/showGb?type=online&hcno=EB3B94B543F6E4CD18C044DE6AB64CEC"].
5) National Development and Reform Commission of China, Planning of high-level biosafety laboratory system construction, 2016. [HYPERLINK "http://www.ndrc.gov.cn/zcfb/zcfbtz/201612/t20161220_830455.html"]

Comment [d12]: Page not found

Comment [d13]: Not the right link

6) [HYPERLINK
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term=Moming%20A%5BAuthor%5D&cauthor=tr
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cauthor_uid=29524182"],[HYPERLINK
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term=Shen%20S%5BAuthor%5D&cauthor=true
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cauthor_uid=29524182"],[HYPERLINK
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e&cauthor_uid=29524182"],[HYPERLINK
"https://www.ncbi.nlm.nih.gov/pubmed/?
term=Guo%20R%5BAuthor%5D&cauthor=true&

cauthor_uid=29524182"].[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Hu%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=29524182"].[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=29524182"].[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Deng%20F%5BAuthor%5D&cauthor=true&cauthor_uid=29524182"].[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/?term=Sun%20S%5BAuthor%5D&cauthor=true&cauthor_uid=29524182"], Prevalence and Phylogenetic Analysis of Crimean-Congo Hemorrhagic Fever Virus in Ticks from Different Ecosystems in Xinjiang, China, [HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/29524182" \o "Virologica Sinica."] 2018, 33(1):67-73.

7) [HYPERLINK "https://www.researchgate.net/scientific-contributions/2148527743_James_W_Le_Duc?_sg=jYwkkXaeO7SEceb3lG8i9a4oToAbdwD8yA2QaJpzASED8nAjJyGxtlp5aKkpsn_aLxiKniLp423eTjyaXHhvjEYZLxqvqhux-cXgiH6GwJRnNilibHccvUC_ezKTwjeZGbRQSIL-NpI3OQvlLQFTbZJmJML4w"] and Zhi-ming Yuan, Network for safe and secure labs., Science, 362:267-267

Related news and reports

[HYPERLINK "http://www.chinadaily.com.cn/china/2015-01/31/content_19457709.htm"] China opens National Biosafety Laboratory in Wuhan

By Cheng Yingqi(chinadaily.com.cn)Updated: 2015-01-31 16:14

[PAGE * MERGEFORMAT]

From: LeDuc, James W.
Sent: Tuesday, January 21, 2020 4:34 PM
To: Benjamin Rusek (BRusek@nas.edu);Dave Franz (davidrfranz@gmail.com);Yuan Zhiming;George F GAO;Mifang Liang;Shi, Pei yong
Subject: Op Ed in Houston Chronicle
Attachments: Chinese Response to New Virus_Le Duc 21Jan revised.docx

Ben, Dave, Zhiming, George, Mifang and Pei-Yong

The attached, slightly modified to include mention of the new case in Washington State, is scheduled to appear in Wednesday 22 Jan's Houston Chronicle. Note mention of the NASEM/CAS collaborations.

Just FYI,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
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(m) 409-789-2012

Chinese Response to New Virus: Good News/Bad News

By James W. Le Duc

Fast action and open communications by China is helping the world prepare for another potentially devastating infectious disease outbreak. While the situation is rapidly evolving, there is good news that may not make the headlines. Many will recall the dark days in the spring of 2003 when Asia and the world were threatened by the appearance of a new virus disease, Severe Acute Respiratory Syndrome, or SARS, which first appeared in southern China and quickly spread to other countries around the world, ultimately causing over 8000 cases with nearly 10% of those ending in death. SARS was caused by a novel coronavirus then unknown to medical science. There was no known cure, no diagnostic tests and little understanding of where it came from or how it was spread, although person-to-person transmission was obvious as health care workers treating the first cases were themselves among the early victims. Initially, China was reluctant to share information or alert the international community of the magnitude of the epidemic, leading to international criticism and a dangerous global health situation. Fortunately, China reversed its position, opened to collaborations with the WHO, U.S. and others, and the epidemic was eventually controlled.

Today, with another novel coronavirus discovered in China, the start is very different. In quick measure, Chinese health officials recognized that a new disease had emerged, quickly isolated patients, and instituted an impressive set of interventions in attempts to limit disease spread and characterize the new pathogen. Importantly, they have been transparent in sharing their findings with the world, thus allowing other nations to take precautions and be on the lookout for the new disease. Already, the genome of the new virus was sequenced and posted for easy access by international experts, allowing rapid exploration of possible treatments, development of diagnostics and epidemiological investigations.

China's ability to respond quickly and efficiently to this new threat is the result of nearly two decades of investments and collaborations to improve public health in China. The Chinese Centers for Disease Control incorporates many of the strengths of our own CDC, but is designed to meet the needs of a 1.4 billion plus population. In addition, China has invested in building a robust scientific capacity and partnered with containment laboratories such as ours to incorporate best practices when studying dangerous pathogens.

The current outbreak demonstrates a welcome openness to health information sharing with the global community. To diagnose an outbreak early requires astute healthcare providers able to recognize when something new or unusual is occurring; however, clinical recognition alone is meaningless if there is no capacity to investigate cases or characterize the disease-causing agent.

For the last few years, our National Academy of Science, Engineering and Medicine has worked with the Chinese Academy of Sciences to build relationships and share information on emerging diseases and advancements in vaccines and treatments. In Galveston, we welcomed leading Chinese health officials to collaborate on biocontainment facility design, biosafety training and laboratory operations. This dialogue, along with U.S.-based educational opportunities for Chinese students, benefit us all.

China's response to the new coronavirus demonstrates their investments in physical laboratories and scientific collaborations over the past decade are paying dividends, not only to China, but the entire world. Control of a new disease efficiently transmitted person-to-person is nearly impossible as we witnessed during the 2009 novel influenza pandemic and much must still be done together during this quickly evolving situation.

The outbreak is still in the early stages, but it is now clear that the new virus may be transmitted person-to-person, although the efficiency of such transmission remains in question. A few hundred patients have been identified, deaths occurred and the disease has spread from the epicenter in Wuhan to major cities in China and other Asian countries. Our CDC is now screening travelers arriving from Wuhan at U.S. airports, and the WHO is set to consider a global emergency response. With millions about to travel for the Chinese New Year, avoiding a global catastrophe must be the current goal.

The good news is that, at a time when US-China relations are being tested on many fronts, relations within the public health and scientific research arenas remain open and positive, which lays a solid foundation for curtailing this latest threat.

James Le Duc, PhD, is the Director of the Galveston National Laboratory at the University of Texas Medical Branch and a professor in UTMB's Department of Microbiology and Immunology.

705 words in body

From: LeDuc, James W.
Sent: Friday, March 20, 2020 2:40 PM
To: Yuan Zhiming
Cc: Shi, Pei yong
Subject: RE: 回复: Vox article

Dear Zhiming,

Thank you for your kinds words and your heroic efforts to control the epidemic in Wuhan. Your success is an inspiration to all of us as we work to halt transmission here in the USA and in other countries. We are just at the start of the explosion of cases in our region and we expect that over the next few weeks we will see very high numbers of cases requiring hospitalization and ICU support. We are preparing as best we can, but as you know well the challenges will be substantial.

Pei Yong and his team continue to make remarkable progress on different aspects of study and it would be excellent if we could identify areas for collaborations.

With all good wishes,

Jim

James W. Le Duc, Ph.D.
Director
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(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Friday, March 20, 2020 1:33 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: 回复: Vox article

Dear Jim，
I am sincerely hope everything goes well with you and your family!
The 2019 novel coronavirus (SARS-CoV-2) outbreak is a major challenge for global public health security. Infection with SARS-CoV-2 has been associated with serious acute respiratory distress syndrome with large number of patients' hospitalization and relatively high mortality. We had a very hard time in combating the

infection in Wuhan, the epicenter of the COVID-19 in China, and now we can see the situation goes in good direction, with no reported confirmed case, no reported suspected case in last two days here.
My colleagues and I, have been working on characterization of pathogens, antiviral screen, vaccine development, animal modeling since the early January this year, and some progresses have been made. I hope our understanding of the virus and the technology could be valuable in the global fighting to the virus.
As I can see from the media, the virus is spreading in your country, and more people are infected during the last days, and the situation worries me a lot. I am confident that we could finally curb the spreading of the virus with our joint effort, and our life will return back to the normal soon. I do not know what I can do in the special moment and I hope you could protect you and your family.
Best regards

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2020-03-05 22:50
To: Yuan Zhiming; zlshi
CC: Shi, Pei yong
Subject: Vox article

Dear Zhiming and Zhengli,

I hope you are both well during this very difficult time.

The link below is to an article just published that may be of interest to you.

With all good wishes for your personal health and safety as we all work together to control the new virus.

Jim

James W. Le Duc, Ph.D.
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From: Eliza Barclay <eliza.barclay@vox.com>
Sent: Wednesday, March 04, 2020 8:49 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Wuhan

Hi Jim,
The story went up today. Thanks so much for your help with it, and let me know if you see any inaccuracies to fix or updates I should make.
Very best, and hope to stay in touch,
Eliza
https://www.vox.com/2020/3/4/21156607/how-did-the-coronavirus-get-started-china-wuhan-lab

On Fri, Feb 28, 2020 at 3:06 PM Eliza Barclay <eliza.barclay@vox.com> wrote:

> Sure, will do.
>
> On Feb 28, 2020, at 12:16 PM, LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> Better to call after about 4 pm CT. We're kinda busy...
>
> Thanks, Jim
>
> **From:** Eliza Barclay <eliza.barclay@vox.com>
> **Sent:** Friday, February 28, 2020 1:07 PM
> **To:** LeDuc, James W. <jwleduc@UTMB.EDU>
> **Cc:** Keusch, Gerald T <keusch@bu.edu>
> **Subject:** Re: Wuhan
>
>
>
> Thanks for the connection, Jerry.
> And thanks so much for the quick response, Jim. I will give you a call in about an hour.
> Best,
> Eliza
>
> On Fri, Feb 28, 2020 at 10:50 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:
>
> > Hi Jerry,
> >
> > Thanks for the introduction and happy to meet you, Eliza. I'm happy to chat about this issue at your convenience. My direct office line is 409-266-6516.
> >
> > Thanks, Jim

James W. Le Duc, Ph.D.
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(m) 409-789-2012

From: Keusch, Gerald T <keusch@bu.edu>
Sent: Friday, February 28, 2020 11:48 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Eliza Barclay <eliza.barclay@vox.com>
Subject: Wuhan

Hi Jim,

I was talking to Eliza Barclay from Vox (copied above) who was referred to me by our friend Peter Daszak. Eliza is working on a story to address the various conspiracy theories being bandied about on the origin of the Covid19 virus. One of the issues, of course, was the Wuhan laboratory as a source – whether accidental or deliberate – and the questions being raised about it biosecurity and biosafety protocols. I said that I was absolutely confident that they had proper protocols and trained people in place, in part because I am was aware that GNL had connections with that lab, had trained many of their staff, and that you have been there.

Eliza will follow up and if you have the time she would like to talk with you. She is trying to gather the scientific argument and be able to translate it for a general audience to be able to distinguish between evidence and conspiracy.

Hope all is well.

Jerry

Gerald T. Keusch, M.D.
Professor of Medicine and International Health
Boston University School of Medicine
Associate Director, National Emerging Infectious Diseases Laboratories
620 Albany Street
Boston, MA 02118

--
Eliza Barclay • Science Editor

<~WRD363.jpg>

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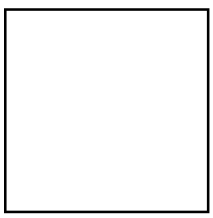
Eliza Barclay • Science Editor



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From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/2/2020 4:19:35 PM
To: Auchincloss, Hugh (NIH/NIAID) [E] (auchinclossh@niaid.nih.gov) [auchinclossh@niaid.nih.gov]; Erbelding, Emily (NIH/NIAID) [E] [emily.erbelding@nih.gov]; Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: Major publication due out Friday
Attachments: Revision SARS2 clone editor suggestions Mar 31 2020.docx; Final Figures Mar 22 2020.pdf

Hugh, Emily and Nancy,

Pasted below is Pei-Yong Shi's note to our communications office about the release tomorrow of our paper on the development of a reverse genetics system and report SARS-CoV-2 virus. This is a major accomplishment and we want to give you a heads up that it will be appearing soon.

Our paper on developing the reverse genetic system and reporter SARS-Cov-2 will be published online tomorrow. This represents one of the most important tools (if not the most important) to study the virus replication, transmission, and pathogenesis.

More importantly, the reporter virus will unleash our limitation to perform serodiagnosis, vaccine evaluation, and therapeutic development. We have already transferred the reagents to New York State Health Department and in the process to share with CDC for serology testing. Our technology has attracted partnership with leading pharmaceutical companies (e.g., Q2 Solutions, Gilead, and others) to jointly fight COVID-19.

Be safe,

Jim

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An infectious cDNA clone of SARS-CoV-2

Xuping Xie[1*], Antonio Muruato[1,2], Kumari G. Lokugamage[2], Krishna Narayanan[2], Xianwen Zhang[1], Jing Zou[1], Jianying Liu[2], Craig Schindewolf[2], Nathen E. Bopp[3], Patricia V. Aguilar[3], Kenneth S. Plante[2,4], Scott C. Weaver[2,4,5,6,7,8], Shinji Makino[2], James W. LeDuc[2,9], Vineet D. Menachery[2,5,7*], Pei-Yong Shi[1,5,8,10,11,12*]

[1]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston TX, USA

[2]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston TX, USA

[3]Department of Pathology, University of Texas Medical Branch, Galveston TX, USA

[4]World Reference Center for Emerging Viruses and Arboviruses, University of Texas Medical Branch, Galveston TX, USA

[5]Institute for Human Infection and Immunity, University of Texas Medical Branch, Galveston TX, USA

[6]Institute for Translational Sciences, University of Texas Medical Branch, Galveston, TX, USA

[7]Department of Pathology and Center for Biodefense & Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX, USA

[8]Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX, USA

[9]Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, USA

[10]Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA

[11]Department of Pharmacology & Toxicology, University of Texas Medical Branch, Galveston, TX, USA

[12]Lead Contact

*Correspondence: X.X. (xuxie@UTMB.edu), V.D.M. (vimenach@UTMB.edu), or P.-Y.S. (peshi@UTMB.edu)

Running title: An infectious cDNA clone of SARS-CoV-2

Keywords: Coronavirus, SARS-CoV-2, COVID-19, SARS-CoV, vaccine, antiviral

SUMMARY

The ongoing pandemic of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), underscores the urgency to develop experimental systems for studying this virus and identifying countermeasures. We report a reverse genetic system for SARS-CoV-2. Seven cDNA fragments spanning the SARS-CoV-2 genome were assembled into a full-genome cDNA. RNA transcribed from the full-genome cDNA was highly infectious after electroporation into cells, producing 2.9×10^6 PFU/ml of virus. Compared with a clinical isolate, the infectious clone-derived SARS-CoV-2 (icSARS-CoV-2) exhibited similar plaque morphology, viral RNA profile, and replication kinetics. Additionally, icSARS-CoV-2 retained engineered molecular markers and did not acquire other mutations. A stable mNeonGreen SARS-CoV-2 (icSARS-CoV-2-mNG) was generated by introducing this reporter gene into OFR7 of the viral genome. icSARS-CoV-2-mNG was successfully used to evaluate the antiviral activities of interferon. Collectively, the reverse genetic system and reporter virus provide key reagents to study SARS-CoV-2 and develop countermeasures.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in early 2020 with human cases in Wuhan, China [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It has rapidly rampaged worldwide, causing a pandemic of coronavirus disease (COVID-19) that ranges from fever and breathing difficulty to acute respiratory distress and death [ADDIN EN.CITE ADDIN EN.CITE.DATA]. With over 300,000 people infected in less than 3 months, SARS-CoV-2 causes the most severe disease in older patients and people with comorbidities, including heart disease, diabetes, and other health conditions [ADDIN EN.CITE <EndNote><Cite><Author>Wu</Author><Year>2020</Year><RecNum>7072</RecNum><DisplayText>(Wu and McGoogan, 2020)</DisplayText><record><rec-number>7072</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584734140">7072</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wu, Z.</author><author>McGoogan, J. M.</author></authors></contributors><auth-address>Chinese Center for Disease Control and Prevention, Beijing, China.</auth-address><titles><title>Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention</title><secondary-title>JAMA</secondary-title></titles><periodical><full-title>JAMA</full-title></periodical><dates><year>2020</year><pub-dates><date>Feb 24</date></pub-dates></dates><isbn>1538-3598 (Electronic)
0098-7484 (Linking)</isbn><accession-num>32091533</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/32091533</url></related-urls></urls><electronic-resource-num>10.1001/jama.2020.2648</electronic-resource-num></record></Cite></EndNote>]. Before 2019, six α- and β-coronaviruses were known to cause respiratory diseases of different severity, including four common cold coronaviruses (229E, NL63, OC43, and HKU1) and two highly pathogenic coronaviruses [severe acute

respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), which emerged in 2003 and since 2012, respectively] [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Importantly, with massive hospitalization rates and high mortality, SARS-CoV-2 remains a major threat to humankind and intervention strategies are being rapidly pursued.

A key tool in responding to emergent viruses is the generation of reverse genetic systems to explore and characterize new pathogens. Classically, reverse genetic systems for coronaviruses have been complicated by their large genome size (~30,000 nucleotides) and the existence of bacteriotoxic elements in their genome that make them difficult to propagate [ADDIN EN.CITE <EndNote><Cite><Author>Almazan</Author><Year>2014</Year><RecNum>7158</RecNum><DisplayText>(Almazan et al., 2014)</DisplayText><record><rec-number>7158</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584739078">7158</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Almazan, F.</author><author>Sola, I.</author><author>Zuniga, S.</author><author>Marquez-Jurado, S.</author><author>Morales, L.</author><author>Becares, M.</author><author>Enjuanes, L.</author></authors></contributors><auth-address>Department of Molecular and Cell Biology. Centro Nacional de Biotecnologia (CNB-CSIC), Campus Universidad Autonoma de Madrid, C/ Darwin 3, Cantoblanco, 28049 Madrid, Spain.
Department of Molecular and Cell Biology. Centro Nacional de Biotecnologia (CNB-CSIC), Campus Universidad Autonoma de Madrid, C/ Darwin 3, Cantoblanco, 28049 Madrid, Spain. Electronic address: L.Enjuanes@cnb.csic.es.</auth-address><titles><title>Coronavirus reverse genetic systems: infectious clones and replicons</title><secondary-title>Virus Res</secondary-title></titles><periodical><full-title>Virus Res</full-title></periodical><pages>262-70</pages><volume>189</volume><keywords><keyword>Clone Cells</keyword><keyword>Coronavirus/*genetics</keyword><keyword>Replicon</keyword><k

eyword>Reverse Genetics/*methods</keyword><keyword>Virology/*methods</keyword><keyword>Coronavirus </keyword><keyword>Infectious clones</keyword><keyword>Replicons</keyword><keyword>Reverse genetics</keyword></keywords><dates><year>2014</year><pub-dates><date>Aug 30</date></pub-dates></dates><isbn>1872-7492 (Electronic)
0168-1702 (Linking)</isbn><accession-num>24930446</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/24930446</url></related-urls></urls><custom2>PMC4727449</custom2><electronic-resource-num>10.1016/j.virusres.2014.05.026</electronic-resource-num></record></Cite></EndNote>].

Several approaches have been devised to overcome this barrier, such as multiple plasmid systems to disrupt toxic elements and to reduce deletions/truncations [ADDIN EN.CITE <EndNote><Cite><Author>Yount</Author><Year>2002</Year><RecNum>7014</RecNum><DisplayText>(Yount et al., 2002)</DisplayText><record><rec-number>7014</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584619790">7014</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Yount, B.</author><author>Denison, M. R.</author><author>Weiss, S. R.</author><author>Baric, R. S.</author></authors></contributors><auth-address>Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7435, USA.</auth-address><titles><title>Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>11065-78</pages><volume>76</volume><number>21</number><keywords><keyword>Animals</keyword><keyword>Cell Line</keyword><keyword>Cricetinae</keyword><keyword>DNA, Viral/*analysis</keyword><keyword>Mice</keyword><keyword>Murine hepatitis

virus/*genetics</keyword><keyword>Phenotype</keyword><keyword>RNA Replicase/biosynthesis</keyword><keyword>Tumor Cells, Cultured</keyword><keyword>Viral Proteins/biosynthesis</keyword></keywords><dates><year>2002</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>12368349</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/12368349</url></related-urls></urls><custom2>PMC136593</custom2><electronic-resource-num>10.1128/jvi.76.21.11065-11078.2002</electronic-resource-num></record></Cite></EndNote>]. Using this approach, researchers have developed infectious clones for several coronaviruses, including SARS-CoV, MERS-CoV, and others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Thao *et al.* recently reported a yeast-based synthetic genomics platform for rapid construction of infectious clones for murine hepatitis coronavirus (MHV-CoV), MERS-CoV, and SARS-CoV-2 [ADDIN EN.CITE <EndNote><Cite><Author>Thao</Author><Year>2020</Year><RecNum>7201</RecNum><DisplayText>(Thao et al., 2020)</DisplayText><record><rec-number>7201</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584791671">7201</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Thao, T.T.N.</author><author>Labroussaa, F.</author><author>Ebert, N.</author><author>V'kovski, P.</author><author>Stalder, H.</author><author>Portmann, J.</author><author>Kelly, J.</author><author>Steiner, S.</author><author>Holwerda, M.</author><author>Kratzel, A.</author><author>Gultom, M.</author><author>Laura Laloli,</author><author>Hüsser, L.</author><author>Wider, M.</author><author>Pfaender, S.</author><author>Hirt, D.</author><author>Cippà, V.</author><author>Crespo-Pomar, S.</author><author>Schröder, S.</author><author>Muth, D.</author><author>Niemeyer, D.</author><author>Müller, M.</author><author>Drosten, C.</author><author>Dijkman,

R.</author><author>Jores, J. </author><author>Thiel, V.</author></authors></contributors><titles><title>Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.02.21.959817</electronic-resource-num></record></Cite></EndNote>]. However, the yeast platform-produced SARS-CoV-2 has not been fully characterized for its biological properties (*e.g.*, replication kinetics) in comparison with its original clinical isolate. Such characterization is essential for ensuring the quality of the genetic system to rescue recombinant viruses that recapitulate the biological features of their corresponding clinical isolates. Once validated, the reverse genetic systems allow rapid characterization of novel viruses, development of reporter viruses, and generation of live-attenuated vaccine candidates to respond to emerging infections. Together with animal pathogenesis models, reverse genetic systems offer powerful tools needed to characterize, understand, and respond to emerging virus outbreaks.

In response to the ongoing pandemic of SARS-CoV-2, we have developed a robust reverse genetic system for SARS-CoV-2 and a mNeonGreen reporter virus. Recombinant virus derived from the system recapitulates the replication kinetics of the original clinical isolates. In addition, the mNeonGreen reporter remains stable for at least five passages, allowing its use in long-term studies. Using type-I interferon, we demonstrated that the mNeonGreen virus could be reliably used to study viral replication and pathogenesis as well as to develop vaccine and antiviral drugs.

RESULTS

Design of a SARS-CoV-2 full-length cDNA. An *in vitro* ligation approach, similar to that for constructing the infectious clones of SARS-CoV and MERS [ADDIN EN.CITE ADDIN EN.CITE.DATA], was designed to directionally assemble the full-length cDNA of the SARS-CoV-2 genome (Figure 1A). Our reverse genetic system was based on the virus strain (2019-nCoV/USA_WA1/2020) isolated from the first reported SARS-CoV-2 case in the US [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Viral RNA, extracted from the passage 4 virus from Vero E6 cells, was used as a template for RT-PCR to produce cDNA fragments. Seven contiguous cDNA fragments were constructed to cover the entire viral genome (Figure 1B). Some of the seven cDNA fragments were prepared through RT-PCR, whereas others were generated by chemical synthesis (see Materials and Methods for details). All cDNA fragments were individually cloned into plasmid vectors. For facilitating directional assembly of genome-length cDNA, each cDNA fragment was flanked by a class IIS restriction endonuclease site (BsaI or Esp3I). The class IIS endonucleases recognize asymmetric DNA sequences, cleave outside their recognition sequences, and generate unique cohesive overhangs (Figure 1C). After digestion with BsaI or Esp3I, the seven fragments were directionally ligated to assemble the genome-length cDNA. The unique cohesive ends of each fragment ensured one directional, seamless assembly of the seven fragments with the concomitant loss of the restriction enzyme sites. Figure 1C depicts the details of the seven fragments: F1 (T7 promoter sequence plus nucleotides 1-3,618), F2 (3,619-7,504), F3 (7,505-11,984), F4 (11,985-17,591), F5 (17,592-22,048), F6 (22,049-26,332), and F7 (26,333-29,870 plus a poly$(A)_{29}$ sequence). A T7 promoter and a poly$(A)_{29}$ tail were engineered at the upstream of F1 and the downstream of F7, respectively. *In vitro* transcription of the ligated F1-7 DNA was expected to produce a 5' capped (as cap analog was included in the *in vitro* transcription reaction) and 3' polyadenylated genome-length RNA. To differentiate the infectious clone-derived virus from the parental clinical isolate, we engineered three synonymous nucleotide mutations as markers.

Assembly of a SARS-CoV-2 full-length cDNA. Each of the seven cDNA fragments was cloned into a plasmid and sequenced to ensure no undesired mutations. For assembly of full-length cDNA, the seven cDNA plasmids were digested with BsaI or Esp3I. The resulting cDNA fragments were gel-purified (Fig. 1D), then *in vitro* ligated to assemble the genome-length cDNA in three steps: (i) ligation of F1, F2, F3, and F4 to produce F1-4 cDNA; (ii) ligation of F5, F6, and F7 to produce F5-7 cDNA; and (iii) ligation of F1-4 and F5-7 to produce the full-length F1-7 cDNA. Agarose gel analysis of the ligation (iii) reaction showed a major DNA product representing the size of genome-length cDNA (~29.87 kb, indicated by an arrow in Figure 1E) in addition to several smaller intermediate cDNA products (indicated by circles). *In vitro* transcription using the cDNA template [directly from ligation (iii) without gel purification] generated multiple RNA bands, among which a faint high molecular band may represent the genome-length RNA (indicated by an arrow in Figure 1F) together with several smaller RNA transcripts (indicated by circles).

Recovery of recombinant SARS-CoV-2. To recover recombinant SARS-CoV-2 from the infectious cDNA clone (icSARS-CoV-2), we electroporated *in vitro* transcribed genome-length RNA into Vero E6 cells. The RNA transcription mixture from Figure 1F was directly electroporated into cells without purification. Since N protein was reported to enhance the infectivity of coronavirus RNA transcripts [ADDIN EN.CITE ADDIN EN.CITE.DATA], we co-electroporated an mRNA encoding the SARS-CoV-2 N protein with the full-length RNA. The transfected cells developed cytopathic effects (CPE) on day 4 post-transfection and produced infectious virus [denoted as passage 0 (P0) virus] with a titer of 2.9×10^6 PFU/ml (Figure 2A). It is worth emphasizing that such a high titer of recombinant virus was produced directly from the electroporated cells without additional rounds of cell culture passaging, indicating the robustness of the system and also suggesting a lack of any errors. Next, we compared the replication properties between the recombinant virus and the original clinical isolate. The wild-type icSARS-CoV-2 (icSARS-CoV-2-WT) developed plaques similar to the original clinical

isolate (Figure 2B) and exhibited equivalent replication kinetics on Vero E6 cells (Figure 2C). We did not extend the time points of replication beyond 48 h because CPE was observed at 40-48 h post-infection (p.i.). Northern blot analysis showed that viral mRNA species from the clinical isolate-infected cells and the icSARS-CoV-2-infected cells were identical to the predicted set of genome-length RNA and eight subgenomic RNAs (Figure 2D). Full-genome sequencing showed that the recombinant virus retained the three engineered synonymous mutations with no other sequence changes, demonstrating the rescued virus did not result from contamination by the parental virus isolate (Figure 2E). Furthermore, DNA sequencing chromatogram did not show any partial reversion of the three engineered molecular markers (Figure 2F). Collectively, the results demonstrate that (i) the *in vitro* transcribed full-length RNA is highly infectious upon electroporation into cells and (ii) the recombinant virus recapitulates the replication properties of the original clinical isolate on Vero E6 cells.

Development and characterization of mNeonGreen SARS-CoV-2. Reporter viruses are useful tools to study viral replication and pathogenesis and to develop countermeasure. To establish a reporter SARS-CoV-2 infectious clone, we engineered an mNeonGreen (mNG) gene into the ORF7 of viral genome (Figure 3A), similar to the SARS-CoV reporter [ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>180</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>180</rec-number><foreign-keys><key app="EN" db-id="5txwd0dw9fwdpuesvvjx5pvs90ve5rwttr05" timestamp="1584885311">180</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P. L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome

206 coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the
207 conducting airways of the lungs</title><secondary-title>J Virol</secondary-
208 title></titles><periodical><full-title>J Virol</full-title><abbr-1>Journal of virology</abbr-
209 1></periodical><pages>15511-
210 24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypepti
211 dases/analysis</keyword><keyword>Coronavirus
212 Infections/enzymology/*metabolism</keyword><keyword>Epithelial
213 Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><
214 keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS
215 Virus/*physiology</keyword><keyword>Severe Acute Respiratory
216 Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-
217 dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
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218 (Linking)</isbn><accession-num>16306622</accession-num><urls><related-
219 urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-
220 urls></urls><custom2>PMC1316022</custom2><electronic-resource-
221 num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-
222 num></record></Cite></EndNote>]. The same *in vitro* ligation and transcription protocols
223 (described above) were used to prepare the mNG full-length RNA. After electroporation, we
224 recovered icSARS-CoV-2-mNG (6.9×10^6 PFU/ml). To examine if the reporter gene attenuates
225 viral replication, we compared the replication properties between the wild-type and reporter
226 viruses on Vero E6 cells. The icSARS-CoV-2-mNG produced plaques similar to those of the
227 icSARS-CoV-WT (compare Figures 3B with 2B). Indistinguishable replication kinetics were
228 observed for the icSARS-CoV-2-mNG and icSARS-CoV-WT (Figure 3C). Infection with icSARS-
229 CoV-2-mNG developed increasing numbers of mNG-positive cells over time (Figure 3D).
230 Concurrently, the fluorescent signals increased from 12 to 48 h p.i. (Figure 3E). At 12-36 h p.i.,
231 the level of fluorescent signal correlated with the initial MOIs, whereas a reverse trend was

observed at 48 h p.i., most likely due to earlier CPE caused by the higher MOI. Full-genome sequencing showed that icSARS-CoV-2-mNG retained the three engineered markers with no additional mutations (Figure 3F). These results indicate that icSARS-CoV-2-mNG is initially stable, maintains the wild-type replication, and expresses robust mNG in Vero E6 cells.

Stability of icSARS-CoV-2-mNG. To examine the longer-term stability of icSARS-CoV-2-mNG, we serially passaged the reporter virus on Vero cells for 5 rounds (1 to 2 days per round). Cells infected with equal PFU of passage 1 (P1) or passage 5 (P5) viruses produced comparable numbers of mNG-positive cells (Figure 4A). Next, RT-PCR was performed to verify the retention of mNG in the P1 and P5 viral genomes using two primers targeting the insertion junctions (corresponding to nucleotides 25,068-28,099 of the viral genome). As expected, the RT-PCR products derived from both P1 and P5 mNG viruses were larger than those from the wild-type icSARS-CoV-2 (Figure 4B, lanes 1-3). Digestion of the RT-PCR products with BsrGI (located upstream of the mNG insertion site) and StuI (in the mNG gene) developed distinct cleavage patterns between the reporter and wild-type viruses, whereas P1 and P5 viruses produced an identical digestion pattern (Figure 4B, lanes 4-6). Finally, sequencing the P1 and P5 RT-PCR products confirmed the retention of the mNG gene (data not shown). Altogether, the results demonstrate the stability of icSARS-CoV-2-mNG after five rounds of passaging on Vero E6 cells. Since Vero E6 cells are defective in type-1 interferon production, it remains to be tested if the reporter virus is stable when passaged on interferon-competent cell lines.

Application of icSARS-CoV-2-mNG. To explore the utility of icSARS-CoV-2-mNG, we used the reporter virus to rapidly screen the antiviral activity of a known inhibitor of coronaviruses. We previously showed that pre-treatment of Vero cells with type-I interferon (IFN) inhibits SARS-CoV-2 replication [ADDIN EN.CITE <EndNote><Cite><Author>Lokugamage</Author><Year>2020</Year><RecNum>7202</RecNum><DisplayText>(Lokugamage et al., 2020)</DisplayText><record><rec-number>7202</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a"

timestamp="1584807870">7202</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lokugamage, K.G.</author><author>Hage, A.</author><author>Schindewolf, C.</author><author>Rajsbaum, R.</author><author>Menachery, V.D.</author></authors></contributors><titles><title>SARS-CoV-2 sensitive to type I interferon pretreatment</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.03.07.982264</electronic-resource-num></record></Cite></EndNote>]. Here we explored the dose responsive effect of IFN-a pre-treatment on icSARS-CoV-mNG replication (Figure 4C). No mNG expression was visually observed when the infected cells were pre-treated with the highest dose of IFN-α (1,000 u/ml), whereas a dose-dependent reduction of mNG signal was detected at an intermediate dose (111 u/ml) (Figure 4D). Quantification of the fluorescent readouts estimated an EC_{50} (concentration inhibiting 50% of viral replication) of 101 u/ml, confirming the inhibition of SARS-CoV-2 by IFN-α (Figure 4E). This result is consistent with previous findings that SARS-CoV-2 is sensitive to type-I IFN inhibition. The reporter virus assay required fewer days and less labor when compared with the conventional plaque reduction assay. Collectively, the results indicate that icSARS-CoV-2-mNG could be reliably used to study SARS-CoV-2 replication and to screen antiviral inhibitors.

DISCUSSION

We report the development of a full-length infectious clone and a reporter virus for SARS-CoV-2. One of the key utilities for the reverse genetic system is to facilitate antiviral testing and therapeutic development. The icSARS-CoV-2 mNG reporter virus allows the use of fluorescence as a surrogate readout for viral replication. Compared with a standard plaque assay or $TCID_{50}$ quantification, the fluorescent readout shortens the assay turnaround time by

several days. In addition, the fluorescent readout offers a quantitative measure that is less labor-intensive than the traditional means of viral titer reduction. Furthermore, the mNG virus-based assay could be automated in a high-throughput format to screen compounds against viral replication. As a proof-of-concept, we demonstrated that, after treatments with type-I IFN, the reporter virus reliably revealed efficacy in a rapid and efficient manner. In addition, the stability of the mNG reporter virus allows it to be used for longer-term studies and *in vivo* without fear of losing its fluorescent marker. Thus, this reporter virus offers a huge advantage for research community and pharmaceutical companies to develop therapeutics for COVID-19.

Our reverse genetic system represents a major reagent in the pursuit of understanding SARS-CoV-2 and COVID-19 disease. Compared with the clinical isolate, the recombinant wild-type SARS-CoV-2 has no deficit in terms of viral RNA species produced, plaque morphology, or replication kinetics. Therefore, it may be used as an equivalent to the clinical strain, and mutant viruses can be generated to characterize mutational effect on viral infection. This approach has allowed researchers to identify key viral antagonists of innate immunity for SARS-CoV and MERS-CoV [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Several of these mutant viruses have subsequently been employed as live-attenuated vaccine candidates for SARS-CoV and MERS-CoV [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Using our system, this knowledge may now be applied to the current SARS-CoV-2. Characterizing these mutations may provide insight into SARS-CoV-2 pathogenesis.

Our reverse genetic system also allows exploration of research questions fundamental to understanding the SARS-CoV-2 pandemic. As additional genomic sequences become available, evolutionary mutations can be interrogated for their effect on viral transmission and disease outcome. For example, a 382-nucleotide deletion covering almost the entire ORF8 of SARS-CoV-2 was observed in eight hospitalized patients in Singapore; virus isolation of the deletion strains has not been reported in the study [ADDIN EN.CITE <EndNote><Cite><Author>Su</Author><Year>2020</Year><RecNum>7249</RecNum><Displ

ayText>(Su et al., 2020)</DisplayText><record><rec-number>7249</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584819541">7249</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Su, Y.C.F.</author><author>Anderson, D.E. </author><author>Young, B.E. </author><author>Zhu, F.</author><author>Linster, M. </author><author>Kalimuddin, S. </author><author>Low, J.G.H. </author><author>Yan, Z.</author><author>Jayakumar, J. </author><author>Sun, L.</author><author>Yan, G.Z. </author><author>Mendenhall, I.H.</author><author>Leo, Y.-S.</author><author>Lye, D.C.</author><author>Wang, L.-F.</author><author>Smith, G.J.D.</author></authors></contributors><titles><title>Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2</title><secondary-title>bioRxiv</secondary-title></titles><periodical><full-title>bioRxiv</full-title></periodical><dates><year>2020</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1101/2020.03.11.987222</electronic-resource-num></record></Cite></EndNote>]. A four-amino acid insertion (conferring a possible furin cleavage site) was reported in the spike (S) protein of SARS-CoV-2, but is absent in the S protein of SARS-CoV and other group 2B CoVs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Using the infectious clone, we can now evaluate the impact of these genetic changes by removing the reported sequences from SARS-CoV-2 and examine their effect on virus replication and S protein processing. In addition, mouse models for SARS-CoV-2 have been limited by the absence of virus capable of binding to mouse ACE2 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Point mutations in the receptor binding domain of SARS-CoV-2 S protein may facilitate mouse adaptation and development of a model that recapitulates human diseases in a standard mouse strain. Altogether, the above questions are a few examples of how our infectious clone can be used to advance SARS-CoV-2 research.

In summary, we have developed a robust reverse genetic system for SARS-CoV-2 that can be used to study viral replication and pathogenesis. We have also established an mNG reporter SARS-CoV-2 that is a reliable surrogate for high-throughput drug discovery. The reverse genetic system represents a major tool for the research community and significantly advances opportunities for countermeasure development for COVID-19.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Conceptualization, X.X., V.D.M., P.-Y.S.; Methodology, X.X., A.M., K.G.L., K.N., X.Z., J.Z., J.L., C.S., N.B., P.A., K.S.P., S.W, S.M., J.W.L., V.D.M, P.-Y.S.; Investigation,

X.X., A.M., K.G.L., K.N., X.Z., J.Z., J.L., C.S., N.B., P.A.; Resources, K.S.P., S.W., C.-T.K.T.; Data Curation, X.X., A.M., K.G.L., K.N., J.L., N.B.; Writing-Original Draft, X.X., K.N., V.D.M., P.-Y.S.; Writing-Review & Editing, X.X., V.D.M., P.-Y.S.; Visualization, X.X., A.M., K.G.L., N.B., and P.-Y.S.; Supervision, X.X., V.D.M., P.-Y.S.; Funding Acquisition, P.A., S.W., S.J., J.W.L., V.D.M., P.-Y.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

MAIN FIGURE TITLES AND LEGENDS

Figure 1 Assembly of a full-length SARS-CoV-2 infection cDNA clone. (A) Genome structure SARS-CoV-2. The open reading frames (ORFs) from the full genome are indicated. (B) Strategy for *in vitro* assembly of an infectious cDNA clone of SARS-CoV-2. The nucleotide sequences and genome locations of the cohesive overhangs are indicated. The wild-type full-length cDNA of SARS-CoV-2 (IC WT) was directionally assembled using *in vitro* ligation. (C) Diagram of the terminal sequences of each cDNA fragment recognized by BsaI and Esp3I. (D) Gel analysis of the seven purified cDNA fragments. Individual fragments (F1 to F7) were digested from corresponding plasmid clones and gel-purified. Seven purified cDNA fragments (50-100 ng) were analyzed on a 0.6% native agarose gel. The 1-kilobase (kb) DNA ladders are indicated. (E) Gel analysis of cDNA ligation products. About 400 ng of purified ligation product was analyzed on a 0.6% native agarose gel. Triangle indicates the full-length (FL) cDNA product. Circles indicate the intermediate cDNA products. (F) Gel analysis of RNA transcripts. About 1 µg of *in vitro* transcribed (IVT) RNAs were analyzed on a 0.6% native agarose gel. DNA ladders are indicated. Since this is a native agarose gel, the DNA size is not directly corelated to the RNA size. Triangle indicates the genome-length RNA transcript. Circles show the shorter RNA transcripts.

Figure 2 Characterization of the wild-type icSARV-CoV-2 (IC WT). (A) Bright-field images of the Vero E6 cells electroporated with RNA transcripts. Cytopathic effects (CPE) appeared in the IC WT RNA-transfected cells on day 4 post-transfection. The titer of the P0 virus (directly from the transfected cells) is shown in plaque-forming units (PFU) per ml. (B) Plaque morphology of the original clinical isolate (WA1=2019-nCoV/USA_WA1/2020) and the recombinant P1 IC WT virus. Plaques were developed in Vero E6 cells on day 2 post-infection. (C) Replication kinetics. Vero E6 cells were infected with the clinical isolate or recombinant P1 IC WT virus at MOI 0.01. Viruses in culture fluids were quantified by plaque assay. Results from triplicate experiments

were presented with error bars indicating standard deviations. (D) Northern blot analysis of full-length and subgenomic RNAs. Numbers indicated the FL (band 1) and eight subgenomic RNAs (bands 2-9). (E) Sequence differences between the original clinical isolate WA1 and the recombinant P1 IC WT. The three silent nucleotide changes were engineered as molecular markers. (F) Chromatograms of Sanger sequencing results. The engineered molecular maker mutations are indicated.

Figure 3 Generation of a mNeonGreen SARS-CoV-2. (A) Assembly of the full-length mNeonGreen (mNG) SARS-CoV-2 cDNA. The mNG gene was placed downstream of the regulatory sequence of ORF7 to replace the ORF7 sequence [ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>7367</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>7367</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584836012">7367</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P. L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>15511-24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypeptidases/analysis</keyword><keyword>Coronavirus Infections/enzymology/*metabolism</keyword><keyword>Epithelial Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><

keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS Virus/*physiology</keyword><keyword>Severe Acute Respiratory Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>16306622</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-urls></urls><custom2>PMC1316022</custom2><electronic-resource-num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-num></record></Cite></EndNote>] in the subclone F7. (B) Plaque morphology of the P1 IC mNG virus. Plaques were developed in Vero E6 cells on day 2 post-infection. (C) Replication kinetics. Vero E6 cells were infected with the wild-type icSARS-CoV-2 (IC WT) or reporter icSARS-CoV-2-mNG (IC mNG) at MOI of 0.01. Viruses in culture medium were quantified by plaque assay. (D) Fluorescence microscopy analysis of P1 mNG virus-infected cells. Vero E6 cells were infected with P1 mNG viruses at MOI of 0.3. Representative mNeonGreen-positive (green) images are shown. (E) Kinetics of fluorescence intensity. Vero E6 cells were infected with MOI of 1.0, 0.3 or 0.1. After background signal subtraction, the fluorescence intensities from 12 to 48 h post-infection are shown. Results from triplicate experiments were presented with bars representing standard deviations. (F) Summary of full-genome sequence of mNG virus (P1 IC mNG). Nucleotides different from the original clinical isolate (WA1) are indicated.

Figure 4 Stability and application of mNeonGreen virus. The stability of mNG virus was analyzed by comparing the fluorescent signals between the cells infected with P1 and P5 reporter viruses. The presence of mNG gene in the P1 and P5 reporter viruses was also verified using RT-PCR. The application of mNG virus was examined by testing the antiviral activity of IFN-α treatment. (A) Fluorescence microscopy analysis of the P1 and P5 mNG virus-infected cells. Vero E6 cells were infected with P1 or P5 virus at an MOI of 0.3. The cells were monitored for mNG-positive signals at 24 h post-infection. Green, mNG; blue, nucleus. (B) Gel analysis of

445 mNG virus stability. Top panel depicts the theoretical results of RT-PCR followed by restriction
446 enzyme digestion. Bottom panel shows the gel analysis of the RT-PCR products before (lanes
447 1-3) and after BsrGI/StuI digestion (lanes 4-6). About 100 ng DNA samples were analyzed on a
448 0.6% agarose gel. The DNA sizes are indicated. (C) Schematic diagram of IFN-α treatment. (D)
449 Representative fluorescence images of reporter virus-infected cells after IFN-α treatment. The
450 doses of IFN-α treatment are indicated. (E) Dose response curve of mNG signal inhibited by
451 IFN-α. The Hillslope and EC_{50} values are indicated. Results from triplicate experiments were
452 presented with bars representing standard deviations.
453

454 **STAR METHODS**

455 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
N/A		
Bacterial and Virus Strains		
E. coli strain Top10	ThermoFisher Scientific	Cat# C404006
TransforMax™ EPI300™ Chemically Competent *E. coli*	Lucigen Corporation, Middleton, WI 53562	Cat# C300C105
SARS-CoV strain 2019-nCoV/USA_WA1/2020 (WA1)	World Reference Center of Emerging Viruses and Arboviruses [WRCEVA] at the University of Texas Medical Branch	N/A
Biological Samples		
None		
Chemicals, Peptides, and Recombinant Proteins		
IFN-α A Protein, Recombinant human	Millipore Sigma	Cat# IF007
Critical Commercial Assays		
T7 mMessage mMachine kit	Thermo Fisher Scientific	Cat# AM1344
Ingenio® Electroporation solution	Mirus Bio LLC	Cat# MIR 50117
SuperScript™ IV First-Strand Synthesis System	Thermo Fisher Scientific	Cat# 18091300
Platinum™ SuperFi II DNA Polymerase	Thermo Fisher Scientific	Cat# 12361010
Deposited Data		
N/A		
Experimental Models: Cell Lines		
Vero cells	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Experimental Models: Organisms/Strains		
N/A		
Oligonucleotides		
primer Cov-T7-N-F (TACTGTAATACGA CTCACTATAGGATGTCTGATAATGGA CCCCAAAATC)	Integrated DNA Technologies (Skokie, Illinois)	N/A
primer polyT-N-R (TTTTTTTTTTTTTTTT TTT TTTTTTTTTTTTTTTTTTAGGCCT GAGTTGAGTCAGCAC)	Integrated DNA Technologies (Skokie, Illinois)	N/A
Recombinant DNA		
pUC57-CoV2-F1	This paper	N/A
pCC1-CoV2-F2	This paper	N/A
pCC1-CoV2-F3	This paper	N/A
pUC57-CoV2-F4	This paper	N/A
pUC57-CoV2-F5	This paper	N/A

pUC57-CoV2-F6	This paper	N/A
pCC1-CoV2-F7	This paper	N/A
pCC1-CoV2-F7-mNG	This paper	N/A

Synthesized mNeonGreen gene (sequence-optimized)	This paper and [ADDIN EN.CITE <EndNote><Cite><Author>Shaner</Author><Year>2013</Year><RecNum>177</RecNum><DisplayText>(Shaner et al., 2013)</DisplayText><record><rec-number>177</rec-number><foreign-keys><key app="EN" db-id="txv2awx0sz9dfle59pjpwxxqrzxw25sssvrv" timestamp="1584804789">177</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shaner, Nathan C.</author><author>Lambert, Gerard G.</author><author>Chammas, Andrew</author><author>Ni, Yuhui</author><author>Cranfill, Paula J.</author><author>Baird, Michelle A.</author><author>Sell, Brittney R.</author><author>Allen, John R.</author><author>Day, Richard N.</author><author>Israelsson, Maria</author><author>Davidson, Michael W.</author><author>Wang, Jiwu</author></authors></contributors><titles><title>A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum</title><secondary-title>Nature methods</secondary-title><alt-title>Nat Methods</alt-title></titles><periodical><full-title>Nature Methods</full-title></periodical><pages>	N/A

Software and Algorithms		
ImageJ	NIH	N/A
Prism 8.0 software	GraphPad	N/A
Illustrator CC	Adobe	N/A

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by Lead Contact, Pei-Yong Shi (peshi@utmb.edu)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Virus and Cell Lines

The stock of SARS-CoV-2 strain 2019-nCoV/USA_WA1/2020 was derived from the first patient diagnosed in the US. The virus isolate was originally provided by Dr. Natalie Thornburg from the Centers for Disease Control and Prevention in Atlanta, GA as described previosuly [ADDIN EN.CITE ADDIN EN.CITE.DATA], and amplified on Vero E6 cells at the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch at Galveston (UTMB). The P5 passage was used in this study.

African green monkey kidney epithelial cells (Vero E6; CRL-1586) were purchased from the American Type Culture Collection (ATCC, Bethesda, MD) and maintained in a high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 1% penicillin/streptomycin (P/S). Cells were grown at 37°C with 5% CO_2. All culture medium and antibiotics were purchased from ThermoFisher Scientific (Waltham, MA). All cell lines were tested negative for mycoplasma.

METHOD DETAILS

Cloning the SARS-CoV-2 cDNAs

Two approaches were taken to rapidly obtain stable cDNAs of SARS-CoV-2. Firstly, the cDNAs of fragments F1, F4, F5, and F6 were successfully synthesized from the GenScript company

(Piscataway, NJ) and cloned into a high-copy plasmid pUC57. The F1 contains a T7 promoter sequence at the upstream of the 5′ end of the SARS-CoV-2 sequence. Other cDNA fragments were also synthesized but found unstable after cloning into plasmid pUC57. For overcoming this hurdle, the cDNAs of fragments F2, F3, and F7 were obtained by reverse transcription and PCR (RT-PCR). RT was performed by using the SuperScript™ IV First-Strand Synthesis System (ThermoFisher Scientific) with random hexamer primers and extracellular viral RNA (extracted from the supernatants of SARS-CoV-2-infected Vero E6 cells). The cDNA was used as a template to amplify the fragments F2, F3, and F7 by high fidelity PCR with the Platinum™ SuperFi II DNA Polymerase (ThermoFisher Scientific) according to the manufacturer's instructions. A poly$(T)_{29}$ sequence was introduced by PCR to the 3′ end of the untranslated region of viral genome. The amplicons were cloned into a single-copy vector pCC1BAC (Epicentre) to increase the stability of the cDNA plasmids when propagated in *E. coli*. To ensure a seamless assembly of the full-length cDNA, we introduced two cleavage sites of class IIS restriction enzymes (BsaI and Esp3I) at both ends of each sibling cDNAs during PCR or gene synthesis. To differentiate the infectious clone-derived virus from the parental clinical isolate 2019-nCoV/USA_WA1/2020, we engineered three silent mutations at nucleotide positions 7,486 (A-to-T change), 7,489 (T-to-A change), and 18,058 (T-to-C change). For construct the pCC1-F7-mNG, the gene of mNeonGreen (sequence-optimized) was synthesized and inserted at the downstream of the regulatory sequence of ORF7a to replace the entire ORF7a, according to the study as described previously[ADDIN EN.CITE <EndNote><Cite><Author>Sims</Author><Year>2005</Year><RecNum>6988</RecNum><DisplayText>(Sims et al., 2005)</DisplayText><record><rec-number>6988</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1581278772">6988</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sims, A. C.</author><author>Baric, R. S.</author><author>Yount, B.</author><author>Burkett, S. E.</author><author>Collins, P.

L.</author><author>Pickles, R. J.</author></authors></contributors><auth-address>Department of Epidemiology, University of North Carolina at Chapel Hill, 2107 McGavran-Greenberg Hall, CB 7435, Chapel Hill, NC 27599-7435, USA. sims0018@email.unc.edu</auth-address><titles><title>Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J Virol</full-title></periodical><pages>15511-24</pages><volume>79</volume><number>24</number><keywords><keyword>Carboxypeptidases/analysis</keyword><keyword>Coronavirus Infections/enzymology/*metabolism</keyword><keyword>Epithelial Cells/*virology</keyword><keyword>Humans</keyword><keyword>Lung/*virology</keyword><keyword>Peptidyl-Dipeptidase A</keyword><keyword>SARS Virus/*physiology</keyword><keyword>Severe Acute Respiratory Syndrome/*pathology/virology</keyword></keywords><dates><year>2005</year><pub-dates><date>Dec</date></pub-dates></dates><isbn>0022-538X (Print)
0022-538X (Linking)</isbn><accession-num>16306622</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16306622</url></related-urls></urls><custom2>PMC1316022</custom2><electronic-resource-num>10.1128/JVI.79.24.15511-15524.2005</electronic-resource-num></record></Cite></EndNote>]. All subclones were finally validated by Sanger sequencing.

Assembly of a Full-length SARS-CoV-2 cDNA

To assemble the full-length cDNA, we digested individual cDNA plasmids and purified each cDNA fragments. Specifically, F1, F2, F3 and F4 cDNA fragments were obtained by digesting the corresponding plasmids with enzyme BsaI. F5 and F6 fragments were obtained by digesting the plasmids with enzymes Esp3I and PvuI. F7 and F7-mNG cDNA fragments were obtained by

529 digesting the corresponding plasmids by Esp3I and SnaBI. PvuI and SnaBI was included in the
530 digestion to eliminate undesired DNA bands that co-migrated with the targeting fragments on
531 agarose gels. All fragments after restriction enzyme digestion were separated on 0.6% agarose
532 gels, visualized under a darkreader lightbox (Clare Chemical Research, Dolores, CO), excised,
533 and purified using the QIAquick Gel Extraction Kit (Qiagen, Germantown, MD). To assemble the
534 full-length cDNA, we ligated the seven cDNA fragments in a three-step manner. Firstly, equal
535 molar ratio of F1 (0.61 μg), F2 (0.65 μg), F3 (0.75 μg), and F4 (0.94 μg) were ligated in a PCR
536 tube using T4 DNA ligase in a 40 μl-reaction at 4°C for 18 h, resulting in F1-4 DNA. Secondly,
537 equal molar ratio of fragments F5 (0.75 μg), F6 (0.72 μg), and F7 (0.60 μg) were ligated in a
538 separate PCR tube to produce F5-7 DNA using the same ligation condition. Thirdly, without any
539 DNA purification, the two reactions (containing F1-4 and F5-7) were combined (total 80 μl) and
540 topped with additional T4 ligase (2 μl), buffer (2 μl) and nuclease-free water (16 μl) to a 100-μl
541 reaction. The final reaction was incubated at 4°C for 18 h to produce the full-length F1-7 DNA.
542 Afterwards, the full-length cDNA was phenol/chloroform extracted, isopropanol precipitated, and
543 resuspended in 10 μl nuclease-free water.

544 **RNA transcription, Electroporation, Virus production and Quantification**

545 RNA transcript was *in vitro* synthesized by the mMESSAGE mMACHINE™ T7 Transcription Kit
546 (ThermoFisher Scientific) according to the manufacturer's instruction with some modifications. A
547 50-μl reaction was set up by adding 1 μg DNA template and 7.5 μl GTP (cap analog-to-GTP
548 ratio of 1:1). The reaction was incubated at 32°C for 5 h. After removing the template DNA by
549 nuclease per manufacturer's protocol, the RNA was phenol/chloroform extracted and
550 isopropanol precipitated. A SARS-CoV-2 N gene transcript was *in vitro* transcribed from a DNA
551 template using the mMESSAGE mMACHINE™ T7 Transcription Kit with a 2:1 ratio of cap
552 analog to GTP. The N gene DNA template was prepared by PCR using primer Cov-T7-N-F
553 (tactgTAATACGACTCACTATAGGatgtctgataatggaccccaaaatc; the uppercase sequence

554 represents T7 promoter; the underlined sequence represents the 5' end of N gene) and primer
555 polyT-N-R [$(t)_{37}$aggcctgagttgagtcagcac].
556 RNA transcripts were electroporated into Vero E6 cells using a protocol as previously described
557 [ADDIN EN.CITE ADDIN EN.CITE.DATA] with some modifications. Twenty micrograms of
558 total RNA transcripts (containing both full-length RNA and short RNAs) and 20 μg N gene
559 transcript were mixed and added to a 4-mm cuvette containing 0.8 ml of Vero E6 cells (8×10^6) in
560 Ingenio® Electroporation Solution (Mirus). Single electrical pulse was given with a GenePulser
561 apparatus (Bio-Rad) with setting of 270V at 950 μF. After 5 min recovery at room temperature,
562 the electroporated cells were seeded into a T-75 flask and incubated at 37°C with 5% CO_2. On
563 the next day, the culture fluid was replaced with 2% FBS DMEM medium. The cells were
564 monitored daily for virus-mediated cytopathic effect (CPE). One milliliter of the P0 virus was
565 inoculated to a T-175 flask containing 80% confluence Vero E6 cells. The infected cells were
566 incubated at 37°C with 5% CO_2 for 2-3 days. Culture supernatants (P1) were harvested when
567 CPE occurred. The amount of infectious virus was determined by a standard plaque assay on
568 Vero E6 cells. All virus cultures were performed in a biosafety level 3 (BSL-3) laboratory with
569 redundant fans in the biosafety cabinets. All personnel wore powered air purifying respirators
570 (Breathe Easy, 3M) with Tyvek suits, aprons, booties and double gloves.

571 **Interferon Treatment**

572 Vero E6 cells were plated as 1.5×10^4 cells/well in a black 96-well plate (Greiner). For interferon
573 treatment, at 6 h post-seeding, cells were treated with various doses of IFN-α (Millipore Sigma).
574 After 14 h of treatment, the culture fluids were replaced with 2% FBS medium, and P1 IC mNG
575 viruses were added to the cells at MOI 0.3 with additional corresponding concentration of IFN-α.
576 At 24 h post-infection, Hoechst 33342 (ThermoFisher Scientific) was added to a final
577 concentration of 0.1% to counterstain the nucleus. The green fluorescence signals were

detected by Cytation 5 (BioTek) and the infection rate was calculated according to the manufacturer's instructions.

RNA Extraction, RT-PCR and Sanger Sequencing

250 µl of culture fluids were mixed with three volume of TRIzol™ LS Reagent (Thermo Fisher Scientific). Viral RNAs were extracted per manufacturer's instructions. The final RNAs were dissolved in 30 µl nuclease-free water. 11 µl RNA samples were used for reverse transcription by using the SuperScript™ IV First-Strand Synthesis System (ThermoFisher Scientific) with random hexamer primers. Nine DNA fragments covering the entire viral genome were amplified by PCR with specific primers. The resulting DNAs were cleaned up by the QIAquick PCR Purification Kit and Sanger sequencing was performed at the GENEWIZ facilities (South Plainfield, NJ).

Northern Blot

Vero E6 cells were infected with clinical isolate WA1 or the infectious clone-derived SARS-CoV-2 (IC WT) at MOI 0.01. At 48 h post-infection, total intracellular RNAs were isolated using TRIzol reagent (Invitrogen). Northern blot analysis was performed using total intracellular RNAs as described previously [ADDIN EN.CITE <EndNote><Cite><Author>Narayanan</Author><Year>2008</Year><RecNum>7021</RecNum><DisplayText>(Narayanan et al., 2008)</DisplayText><record><rec-number>7021</rec-number><foreign-keys><key app="EN" db-id="59ztpes0ededwse2sr7xwv21zz02f5zw5w5a" timestamp="1584637550">7021</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Narayanan, K.</author><author>Huang, C.</author><author>Lokugamage, K.</author><author>Kamitani, W.</author><author>Ikegami, T.</author><author>Tseng, C. T.</author><author>Makino, S.</author></authors></contributors><auth-address>Department of Microbiology and Immunology, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1019,

603 USA. shmakino@utmb.edu</auth-address><titles><title>Severe acute respiratory syndrome
604 coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in
605 infected cells</title><secondary-title>J Virol</secondary-title></titles><periodical><full-title>J
606 Virol</full-title></periodical><pages>4471-
607 9</pages><volume>82</volume><number>9</number><keywords><keyword>Cell
608 Line</keyword><keyword>*Gene Expression
609 Regulation</keyword><keyword>Humans</keyword><keyword>Interferon Type
610 I/*genetics</keyword><keyword>Mutation</keyword><keyword>Protein
611 Biosynthesis</keyword><keyword>RNA Replicase/*physiology</keyword><keyword>RNA
612 Stability</keyword><keyword>RNA, Messenger/metabolism</keyword><keyword>SARS
613 Virus/pathogenicity/*physiology</keyword><keyword>Viral Nonstructural
614 Proteins/*physiology</keyword></keywords><dates><year>2008</year><pub-
615 dates><date>May</date></pub-dates></dates><isbn>1098-5514 (Electronic)
0022-538X
616 (Linking)</isbn><accession-num>18305050</accession-num><urls><related-
617 urls><url>https://www.ncbi.nlm.nih.gov/pubmed/18305050</url></related-
618 urls></urls><custom2>PMC2293030</custom2><electronic-resource-num>10.1128/JVI.02472-
619 07</electronic-resource-num></record></Cite></EndNote>]. A digoxigenin (DIG)-labeled
620 random-primed probe, corresponding to nucleotides 28,999 to 29,573 of the SARS-CoV-2
621 genome, was used to detect SARS-CoV-2 mRNAs and visualized by DIG luminescent detection
622 kit (Roche, Indianapolis, IN) according to the manufacturer's protocol.

623 **QUANTIFICATION AND STATISTICAL ANALYSIS**

624 All numerical data are presented as the mean±SD (standard deviations). Group comparisons of
625 viral growth kinetics in Figures 2 and 3 were performed using multiple t-test with Bonferroni-
626 Dunn correction in software Prism 8.0 (GraphPad). *p<0.05, significant; **p<0.01, significant;
627 p>0.05, ns (not significant). The 50% effective concentration (EC_{50}) in Figure 4 were estimated

by using a four-parameter logistic regression model from the GraphPad Prism 8 software (GraphPad Software Inc., San Diego CA). Minimal adjustment was made in the software ImageJ to enhance the contrast for bright-field images in Figures 1-3. Blue- and green-fluorescence images were merged in ImageJ. Figures were finally assembled using the software Adobe illustrator CC.

DATA AND SOFTWARE AVAILABILITY

All data are present in this study.

635 [ADDIN EN.REFLIST]

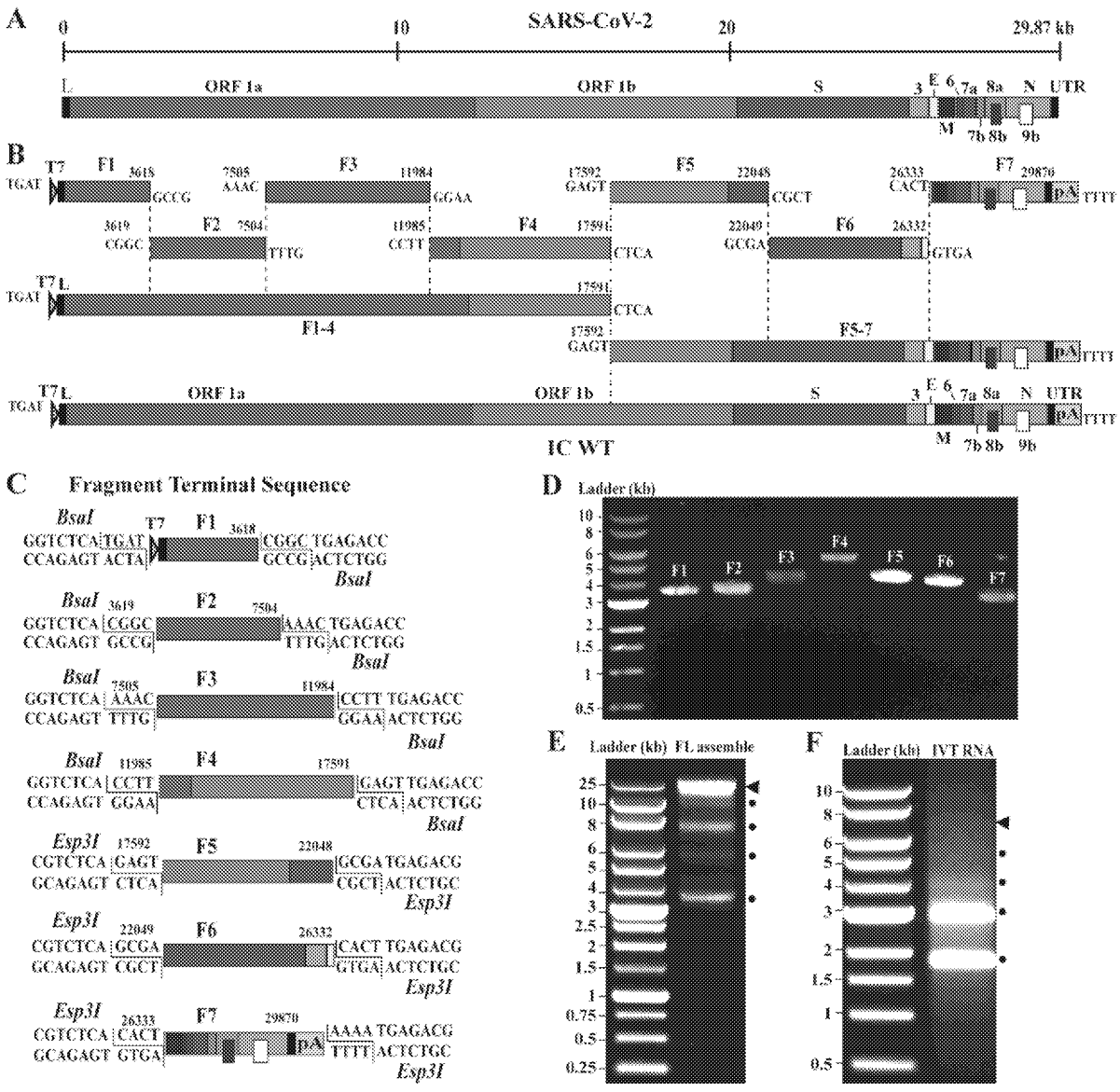


Figure 1

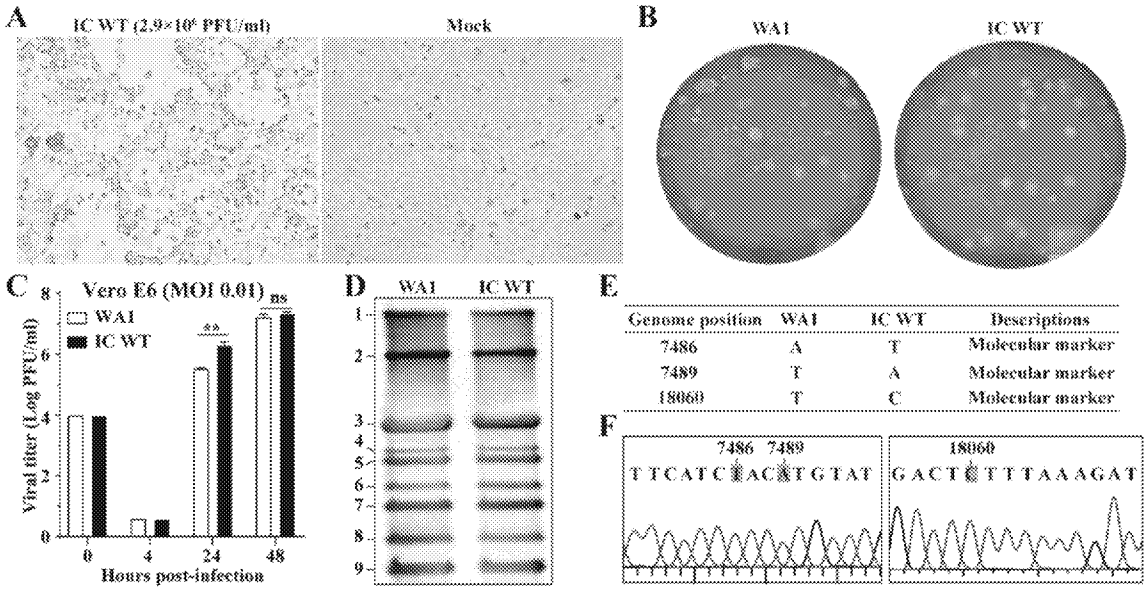
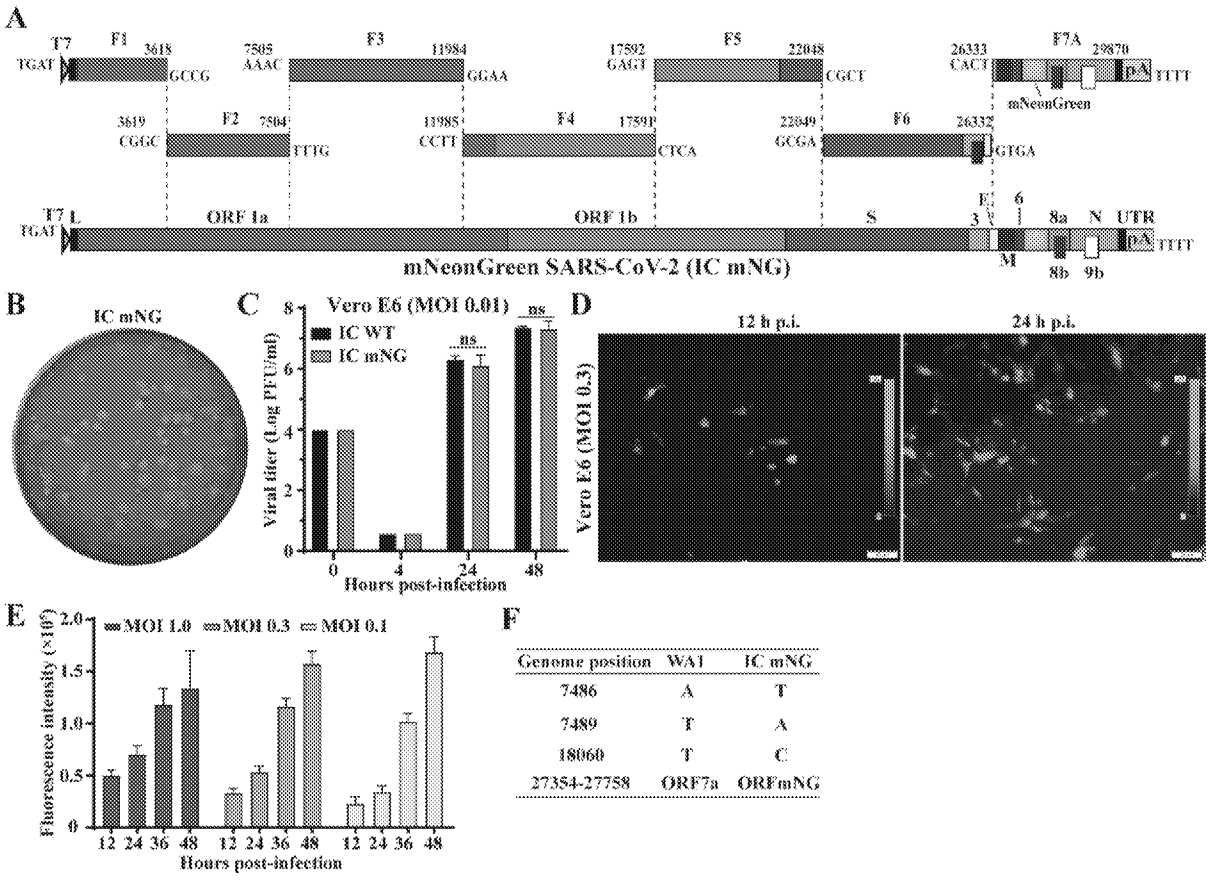


Figure 2



Genome position	WA1	IC mNG
7486	A	T
7489	T	A
18060	T	C
27354-27758	ORF7a	ORFmNG

Figure 3

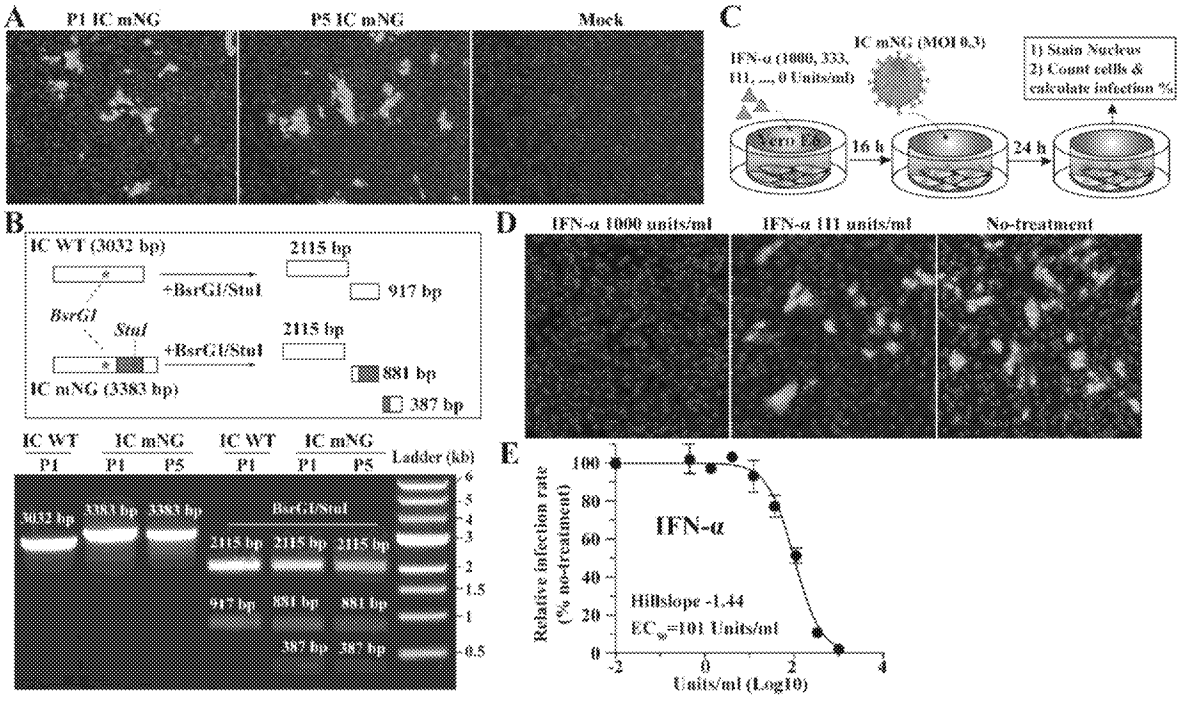


Figure 4

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 1/17/2020 11:45:22 AM
To: Nancy (NIH/NIAID) Boyd (NBoyd@niaid.nih.gov) [NBoyd@niaid.nih.gov]
CC: Holubar, Connie J. [cjholuba@UTMB.EDU]
Subject: select agent inspection notes

Hi Nancy,

We just completed the exit briefing for the BSL4 select agents inspection. The CDC team was very complementary of our staff and PIs both about the status of the facility and the records they reviewed. They had no major issues, but noted findings on: lack of records documentation and follow-up action for the Shope lab (resolved during discussions during the briefing); minor comments on our failure to explicitly mention security in our documentation; ongoing discussion regarding the significance of a glove tear that occurred in the Shope; recommendation to not store select agents in the same box as non-select agents; questions on the BAS upgrade certification (ongoing as systems are upgraded); and documentation in greater detail as the proposed use of material removed from the long-term storage. These are all very minor observations and I do not expect any significant issues going forward.

They were very complementary on our maintenance records of suit wear and repairs that Tom K and Miguel have implemented. Also specifically mentioned the good work of Miguel and his team in overall building operations and safety, and Johnny Peterson and his team in aerobiology.

I mentioned to them that we are attempting to obtain an isolate of the new Coronavirus from Wuhan, China, and asked about biocontainment level and that we assumed that it would not be considered as a select agent. If someone at NIAID is coordinating work on the nCoV, please let me know as we have some resources that will be useful for further analysis and countermeasure development.

Overall, a good report.

I will be in DC Thursday and Friday of next week for an NSABB meeting being held at Hyatt Regency. I let Hugh know that I would be in town and he suggested that we meet for coffee Thursday morning and today followed up with a note saying that Tony would like to meet then also. I'll let you know if anything significant is discussed.

Enjoy the long weekend!

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: LeDuc, James W.
Sent: Sunday, February 9, 2020 4:15 PM
To: Yuan Zhiming
Cc: Shi, Pei yong
Subject: Suggestions
Attachments: Questions on nCoV in Wuhan lab.docx

Dear Zhiming,

I am devastated to see the evolving nCoV epidemic unfolding in Wuhan and I just hope that you, your family and the larger Institute colleagues are well and surviving this very difficult time.

I want to suggest that you conduct a thorough review of the laboratory activities associated with research on coronaviruses so that you are fully prepared to answer questions dealing with the origin of the virus. I'm sure that you have considered this already, but attached are some areas where you may wish to investigate and be prepared to address. You might even consider preparing a manuscript that addresses these topics in an effort to be transparent and proactive. I would be pleased to work with you on such a paper if you think that would be helpful.

I raise these issues since I am receiving questions along these lines more and more frequently. Initially they came from social media and other "alternate information sources" but in the last few days I have been approached by senior officials and major reputable newspapers. Most link the opening of the new BSL4 lab as a possible source of the virus. Clearly addressing this will be essential, with any kind of documentation you might have available used to back up your comments. (It's not clear to me where the coronavirus work was/is actually being conducted.)

I have the utmost respect and admiration for Dr Shi and I am in no way casting doubt on her or her colleagues. I just think that we need to aggressively address these rumors and presumably false accusations quickly and provide definitive, honest information to counter misinformation. If there are weaknesses in your program, now is the time to admit them and get them corrected.

I trust that you will take my suggestions in the spirit of one friend trying to help another during a very difficult time.

Jim

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(f) 409-266-6810
(m) 409-789-2012

Investigation into the possibility that the nCoV was the result of a release from the Wuhan Institute of Virology (main campus or new BSL3/BSL4 facilities).

The questions below deal with the period 1 October 2019 to the present:

Where is coronavirus research conducted?

What level of biocontainment?

How many different laboratories actually handle live virus?

Where are coronavirus stocks stored?

Is there an inventory record of each isolate of each coronavirus kept? If so, are there any discrepancies between the record and actual current inventory number (i.e., is there evidence to suggest that virus stocks may have been stolen or used without proper record?)

Physical Security

Is there controlled access to the laboratories and freezers where coronavirus stocks are held? (Locked doors; card-key access; biometric readers; others?) Were any breaches in security noted/access to the facility by unauthorized individuals?

Is the laboratory constantly monitored by security personnel (24/7)? If not, you could briefly summarize your physical security program during the period in question.

Is there any evidence to suggest a mechanical failure in biocontainment during the time in question?

-were biological safety cabinets used and appropriately certified?

-Exhaust air filtration systems working correctly?

-Autoclaves and waste stream disinfection systems working properly?

-Animal husbandry and management?

-Waste stream integrity (think of FMD leak in UK a few years ago)

Virus Stocks (You may wish to explain that many novel coronaviruses are known only from their sequence and are not able to replicate in culture)

When was the nCoV first handled in your laboratory?

What was the source of that virus? (patient specimen or field collected animal or other?)

During the period in question, what other coronaviruses (that replicate in culture) are stored/handled in your laboratories?

What are the coronaviruses in your possession that are most closely related to nCoV based on genetic sequences and are able to replicate in culture?

Is anyone on your team conducting gain of function studies, recombination studies or any other studies that may have resulted in the creation of the nCoV?

Personnel

How many people have access to the coronavirus stocks and laboratory?

Senior investigators? Junior investigators? Technical support staff? Post-docs? Students? Animal handlers? Janitors and other cleaning staff? Building support personnel? Others?

Is there any evidence to suggest that a disgruntled employee may have had access to the coronavirus stocks? (Recently fired or reprimanded individual(s), for example.)

Does the Institute have an occupational health clinic where employees and students can go to seek medical care? If so, was there any indication of unusual illness similar to that seen for nCoV among Institute staff? If yes, when were cases first seen relative to the nCoV outbreak.

Does a serum bank exist for staff and students working on infectious agents? If yes, could a current serum and the most recent banked sera be serologically tested for antibody to nCoV in an effort to document seroconversion? (If positive, this would not be able to differentiate between community acquired and occupational acquired infection, but absence of evidence of infection would be helpful in ruling out the lab as a source of infection.)

Have any of individuals working at the Wuhan Institute of Virology (main campus or BSL4 campus) been infected with the nCoV? Family members of employees?

If yes, when was the date of onset of illness for the first case recognized? (How does this date compare with the progress of the epidemic; that is, was this among the first cases recognized or later after the outbreak was well underway? How does this compare to the first recognition of nCoV in the greater Wuhan community?)

Where the individuals infected involved in coronavirus research?

Geography

(These questions deal with any association between the physical location of the lab(s) and the districts in Wuhan where the illness was first seen. The assumption is that someone suffering an occupational exposure would go to their local hospital for treatment.)

Where and when were the first Wuhan (or Hubei Province) residents infected with the nCoV first identified (hospital or clinic name/date of earliest cases)?

Do staff members of the Institute reside in the district serviced by this (these) hospital/clinic (s)?

Were any Institute staff members seen for clinical illness at this/these hospital (s) during the time in question?

Do staff members of the Wuhan Institute of Virology frequent the sea food/live market first associated with the nCoV outbreak?

Did any staff member visit the market in the weeks prior to it being closed?

If so, how many staff frequent the market? How often would they visit the market during the period of interest? Have any become infected with nCoV?

From: jwleduc@UTMB.EDU
Sent: Thursday, April 16, 2020 10:07 PM
To: zengli Shi
Subject: Fwd: Rubio

Hi Zheng-Li. I hope you are well as surviving all the COVID19 drama. I wonder if you would have time for a phone call sometime soon. Let me know a good number and time and I'll call. The email below is relevant.

I will certainly understand if you are not available but Pei-Yong keeps encouraging me to call.

With all good wishes.

Jim.

My office line is 1 409 266 6516 or cell is 1 409 789 2012 if it's easier for you to call me.

Sent from my iPhone

Begin forwarded message:

> **From:** David Franz <davidrfranz@gmail.com>
> **Date:** April 16, 2020 at 8:04:55 PM CDT
> **To:** "LeDuc, James W." <jwleduc@UTMB.EDU>
> **Subject: Rubio**
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"
>
> Sent from my iPhone

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> **Date:** April 16, 2020 at 8:04:55 PM CDT
> **To:** "LeDuc, James W." <jwleduc@UTMB.EDU>
> **Subject: Rubio**
>
> WARNING: This email originated from outside of UTMB's email system. Do not click links or open attachments unless you recognize the sender and know the content is safe.
>
> I heard from someone in government this evening that Senator Rubio is starting to push for AN investigation regarding Wuhan lab. Just found it on the web at Forbes by Kenneth Repoza. Title of article is "eight senators call for investigation into coronavirus origins"
>
> Sent from my iPhone

From: LeDuc, James W.
Sent: Wednesday, February 12, 2020 8:42 AM
To: Shi, Pei yong; df@wh.iov.cn; zlshi
Cc: yzm; wangyy; Ksiazek, Thomas G.
Subject: RE: RE: sharing of isolates of 2019nCoV

I strongly agree. We need to show international scientific collaborations at this time of potentially global crisis.

Thank you Fei for your continued efforts.

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Shi, Pei yong <peshi@UTMB.EDU>
Sent: Wednesday, February 12, 2020 7:10 AM
To: df@wh.iov.cn; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: RE: RE: sharing of isolates of 2019nCoV

Thanks, Fei
Although US CDC has already shared the virus isolate with a number of US institutions (including UTMB) last week, it is still important to successfully transfer and share the isolate(s) from China.
Best,

- Pei-Yong

From: df@wh.iov.cn <df@wh.iov.cn>
Sent: Wednesday, February 12, 2020 3:34 AM
To: Shi, Pei yong <peshi@UTMB.EDU>; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: Re: RE: sharing of isolates of 2019nCoV

No prompt reply from the Custom until today. President Bai is trying to push it in Beijing. Please wait for a while.

With best

Dr. Fei Deng
Virus Resource and Bioinformation Center,
Wuhan Institute of Virology, Chinese Academy of Sciences.
Tel/Fax:0086-27-87198465
Email: df@wh.iov.cn

From: Shi, Pei yong
Date: 2020-02-05 20:41
To: df@wh.iov.cn; zlshi
CC: LeDuc, James W.; yzm; wangyy; Ksiazek, Thomas G.
Subject: RE: FW: sharing of isolates of 2019nCoV

Hi Fei,
Thanks for the update. We look forward to further progress.
Best,

- Pei-Yong

From: df@wh.iov.cn <df@wh.iov.cn>
Sent: Wednesday, February 5, 2020 5:57 AM
To: Shi, Pei yong <peshi@UTMB.EDU>; zlshi <zlshi@wh.iov.cn>
Cc: LeDuc, James W. <jwleduc@UTMB.EDU>; yzm <yzm@wh.iov.cn>; wangyy <wangyy@wh.iov.cn>; Ksiazek, Thomas G. <tgksiaze@UTMB.EDU>
Subject: Re: FW: sharing of isolates of 2019nCoV

Thanks for your information.
We are trying to discuss this with the General Administration of Customs in Beijing directly.
I will keep on contacting with you.

Best wishes,
Fei

Dr. Fei Deng
Virus Resource and Bioinformation Center,
Wuhan Institute of Virology, Chinese Academy of Sciences.
Tel/Fax:0086-27-87198465
Email: df@wh.iov.cn

From: Shi, Pei yong

Date: 2020-02-04 22:52
To: df@wh.iov.cn; zlshi
CC: LeDuc, James W.; Yuan Zhiming; wangyy@wh.iov.cn; Ksiazek, Thomas G.
Subject: FW: sharing of isolates of 2019nCoV

Dear Fei and Zhengli,

Please see the response from President Bai. Zhiming and Yanyi were copied on the original email. Let us know anything we could help to facilitate the isolate transfer.

Best regards,

- Pei-Yong

From: LeDuc, James W. <jwleduc@UTMB.EDU>
Sent: Tuesday, February 4, 2020 8:39 AM
To: Shi, Pei yong <peshi@UTMB.EDU>
Subject: FW: sharing of isolates of 2019nCoV

From: "president-office@cas.cn" <president-office@cas.cn>
Date: February 3, 2020 at 11:20:56 PM EST
To: dgriffi6 <dgriffi6@jhmi.edu>, MHamburg <MHamburg@nas.edu>
Cc: mlowenth <mlowenth@nas.edu>, Peggy Hamburg <peggy@hbfam.net>, jwleduc <jwleduc@nas.edu>, jhilderbr <jhilderbr@arizona.edu>, BRsek <BRsek@nas.edu>, jboright <jboright@nas.edu>, clbai <clbai@cas.cn>, zhangyp <zhangyp@cashq.ac.cn>, gaof <gaof@im.ac.cn>, jh-cao <jh-cao@cashq.ac.cn>, liyin <liyin@cashq.ac.cn>, sunhui <sunhui@cashq.ac.cn>, wangyy <wangyy@wh.iov.cn>, yzm <yzm@wh.iov.cn>
Subject: sharing of isolates of 2019nCoV

Diane E. Griffin, Vice President, NAS,
Margaret Hamburg, Foreign Secretary, NAM

Dear Prof. Griffin and Prof. Hamburg,

Thank you for your concerns on the recent outbreak of the 2019 novo-coronavirus epidemic. Upon receiving your letter dated January 28, my colleagues have discussed with Dr. George Fu Gao and other experts and we are willing to share isolates of the 2019 nCoV with the international community. We believe this is critical to engaging joint international efforts to contain the spread of the virus.

The National Biosafety Laboratory Wuhan of the Chinese Academy of Sciences is prepared and willing to work with The University of Texas Medical Branch and

other international research institutions on the specifics for the sharing and distribution of the isolates. We are in the process of getting it ready.

I look forward to hearing your further advice on this matter.

With best regards,

Chunli Bai

Chunli Bai
President
Chinese Academy of Sciences
The Alliance of International Science Organizations (ANSO)

From: 郑大胜Zheng [d.zheng@wh.iov.cn]
Sent: 11/24/2020 9:04:27 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
CC: Grimaldo, Miguel A. [magrimal@UTMB.EDU]
Subject: Happy Thanksgiving!

Dear James,
Dear Miguel,

Thanks for giving me the opportunities of staying in Galveston and being trained at GNL. I am so grateful to you all and your kind assistance.

Wish you happier and healthier than ever!

Kind Regards,

Dasheng
--
ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969
==========

在 2018-09-05 03:41:45, "LeDuc, James W." <jwleduc@UTMB.EDU> 写道：

Dear Dasheng,

I am sorry to learn that you will not be continuing at the Wuhan laboratory and I certainly wish you well as you seek another position. Unfortunately, we have no vacancies here at the GNL, but I am copying Miguel on this message in case he is aware of other jobs elsewhere.

I would be pleased to offer a letter of recommendation limited to your training here at the GNL.

With all good wishes for your future success.

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch

Galveston, TX 77555-0610

(t) 409-266-6500

(f) 409-266-6810

(m) 409-789-2012

From: 郑大胜Zheng <d.zheng@wh.iov.cn>
Sent: Friday, August 31, 2018 3:24 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re:RE: Re:Re: Happy New Year!

Dear Prof LeDuc,

I am deeply impressed by you and your laboratory so that may I ask for work opportunity directly with you at your laboratory.

Before this June the director of the Wuhan P4 lab loses his word to promote me as a quality manager, although Prof. Rene Courcol proposes, who is the French quality inspector to the Wuhan P4 lab. I have to look for new "la vie" by 2019 New Year, the end of current work contract.

Your consideration or recommendation would be appreciated greatly as I am confident in fruitful collaborations with you and your laboratory on biosafety and other relevant topics.

Best Wishes,

Dasheng

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ZHENG Dasheng, PhD

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At 2018-01-10 00:56:48, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

> Dear Dasheng,

It is good to hear from you and to learn that you remain interested in collaborations on biosafety and other relevant topics. We look forward to working with you, Yuan Zhiming and your other colleagues as you bring your beautiful new facility to full operational status. Please let us know if we can be of help to you during these exciting times.

With all good wishes,

Jim

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(f) 409-266-6810
(m) 409-789-2012

From: 郑大胜Zheng [mailto:d.zheng@wh.iov.cn]
Sent: Tuesday, January 09, 2018 1:26 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re:Re: Happy New Year!

Dear James,

Surely I am eager at stronger collaborations with GNL while proposing cooperations to the Wuhan P4 director, Prof. Yuan Zhiming, as soon as my back to Wuhan from Galveston. However, the leader has special arrangements on me although am not suitable for. Update now I am ready for indulging myself into biosafety other than notion of disguise.

Please let me know if any chance I am of help in collaborations.

Dasheng

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ZHENG Dasheng, PhD

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Tel: +86-27-5186-1004 Fax: +86-27-5186-1006

Mob: +86-135 1729 0969

==========

在 2017-12-22 22:17:02，"LeDuc, James W." <jwleduc@UTMB.EDU> 写道：

Thank you very much for your kind note and good holiday wishes. I am very pleased that you were able to spend time with us and that the experiences at UTMB were valuable. I look forward to many years of fruitful collaborations.

With all good wishes for a Merry Christmas and a healthy and prosperous New Year

Jim

Sent from my iPhone

On Dec 22, 2017, at 1:55 AM, 郑大胜Zheng <d.zheng@wh.iov.cn> wrote:

Dear Prof. LeDuc,

Hope this email give you more health and happiness. I really appreciate your instruction and supervision in my stay at GNL, which is my ideal model of biocontainment as you and your colleagues taught me what and how the biocontainments provide protection to people working on pathogenic microorganisms. Your invitation plays important role in my life. I shall look for more opportunities for fruitful cooperation with GNL.

Wish you a merry Christmas and Happy New Year!

Best Regards,

Dasheng

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Zheng, Dasheng PhD

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At 2017-01-27 22:31:18, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dasheng,

I wish you and your team good health and lasting prosperity as you begin the Chinese New Year. May the coming year bring you much success and fruitful collaborations.

With all good wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
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(t) 409-266-6500
(f) 409-266-6810
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From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 11/24/2020 9:29:49 PM
To: 郑大胜Zheng [d.zheng@wh.iov.cn]
CC: Grimaldo, Miguel A. [magrimal@UTMB.EDU]
Subject: Re: Happy Thanksgiving!

Thank you Dasheng. I hope you are well.

Best wishes, Jim

Sent from my iPhone

On Nov 24, 2020, at 9:05 PM, 郑大胜Zheng <d.zheng@wh.iov.cn> wrote:

Dear James,
Dear Miguel,

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Sent from my iPhone

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Dasheng

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Zheng, Dasheng PhD

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Director
Galveston National Laboratory
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Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 9/15/2020 10:17:07 PM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: 回复:Dr. Shi named to prestigious new distinguished chair

Contratulation for Peiyong's excellent works and great recognition.
Best wishes.

Zhiming

==========
Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2020-09-15 03:34
To: Yuan Zhiming
Subject: Dr. Shi named to prestigious new distinguished chair
Great recognition of Pei-Yong's work.

Best wishes,

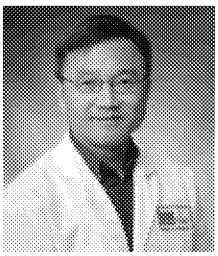
Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: UTMB Broadcast Account <UTMBbroad@UTMB.EDU>
Sent: Monday, September 14, 2020 2:14 PM
Subject: From the President ad interim and the Executive Vice President and Provost: Dr. Shi named to prestigious new distinguished chair

Dr. Pei-Yong Shi named inaugural holder of the John Sealy Distinguished Chair in Innovations in Molecular Biology

We are very pleased to announce that Dr. Pei-Yong Shi, professor in Biochemistry and Molecular Biology and vice chair for Innovation and Commercialization, has been named the inaugural holder of the John Sealy Distinguished Chair in Innovations in Molecular Biology at UTMB.



This new endowed position, made possible through a $1 million contribution from The Sealy & Smith Foundation, will promote and advance UTMB's outstanding and innovative programs in infectious diseases research. We are deeply grateful to the foundation and its Board of Directors for investing in the future health and well-being of Galveston, our region and beyond through this generous gift.

Dr. Shi joined UTMB in 2015 and is internationally recognized for his research accomplishments in virology, drug discovery, vaccine development, pathogen diagnosis and cancer therapy.

When the Zika virus spread across the globe in 2015 and 2016, Dr. Shi and his lab were on the cutting edge of research related to the virus. His lab immediately pushed our knowledge of the virus forward by developing the first genetically engineered clone of it early in that epidemic. This month, he published new work detailing a mutation in the virus that likely led to its sudden spread and its serious consequences for babies born to mothers infected with Zika.

Now, in response to the COVID-19 pandemic, Dr. Shi and his team have once again worked quickly to adapt their research techniques and collaborate to meet this new global challenge. They were the first to engineer a reverse genetic system of the novel coronavirus that causes COVID-19, allowing scientists to safely make the virus in the lab and manipulate it in a petri dish.

Shi and his team also have developed tools to streamline the COVID-19 vaccine development process as research teams around the world work to create life-saving preventives. His team just recently made headlines for using an enzyme produced by fireflies, or fluorescent tag, to develop better tests for COVID-19 and to better understand this new virus.

With this latest support from The Sealy & Smith Foundation, we are confident Dr. Shi and his lab will continue to make groundbreaking discoveries that will make our world a healthier place.

Please join us in congratulating Dr. Shi on this tremendous honor and in thanking The Sealy & Smith Foundation for its ongoing, visionary support of UTMB's mission.

Thank you.

Ben G. Raimer, MD, MA, FAAP
President *ad interim*

Charles P. Mouton, MD, MS, MBA
Executive Vice President and Provost, and
Dean, School of Medicine

utmb Health

Working together to work wonders™

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 7/15/2019 3:55:00 PM
To: 'Yuan Zhiming' [yzm@wh.iov.cn]
CC: Holubar, Connie J. [cjholuba@UTMB.EDU]; Shi, Pei yong [peshi@UTMB.EDU]; Benjamin Rusek (BRusek@nas.edu) [BRusek@nas.edu]; Dave Franz (davidrfranz@gmail.com) [davidrfranz@gmail.com]
Subject: RE: 回复: Synthetic biology commentary
Attachments: Biosecurity in the age to synthetic biology 15 Jul2019-drt final.docx

Hi Zhiming,

Attached please find a draft of our commentary that includes your suggested edits as well as a draft abstract, an author line and our contact information, draft keywords and a conflict of interest statement. I also inserted a couple of website references where appropriate. Please review and modify as you see fit. If you are happy with the text, please feel free to submit directly to your Journal of Biosafety and Biosecurity. Let me know if you think it needs additional work. On review of the first issue of your journal, I note a paper with a similar title, but on reading it, their focus is a bit diffe rent from what we discuss and it does not appear to me to have much duplication.

Have you received your visa for the meeting later this month in DC?

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
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(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Monday, July 15, 2019 1:22 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: 回复: Synthetic biology commentary

Dear Jim ,

I am afraid you did not recevied my last mail and send you again.

Zhiming

Dear Jim,

Sorry for my delayed feedback. As you explained, the developemnt of synthetic biology bring us some challenge on biosafety and biosecurity management, and we could show our understanding this challenge and your concern. You have well organized the draft, and I am sure the readers could benefit for it.

I have insert some words in the text for your consideration.

If there is anything needed my attention, please let me know.

Regards

Zhiming

Dear Zhiming and Zhigao,

I hope this note finds you well on this first day of summer. I write to propose a joint commentary to be submitted for publication in Zhiming's of *Journal of Biosafety and Biosecurity* on the topic of biosafety and biosecurity in the age of synthetic biology. This is a relevant topic and our shared publication would offer an excellent example of the benefits of our joint China-USA dialogue. Having such a co-authored publication would be tangible evidence of the importance we all place on working together to solve challenges of global importance. I have taken the liberty of preparing a first draft of such a manuscript and I invite you both to be co-authors. Dave, Ben and Pei-Yong have reviewed and I have incorporated their comments. Your additions, deletions and modifications will certainly further improve the quality of the piece and make it most relevant to the issues we all face daily in managing a large biocontainment facility. (Please use track change as you edit the piece.)

As you will see, I tried to address four separate areas that impact current and future work in synthetic biology, starting from the position that many of the relevant safeguards needed are already in place through our existing programs in biosafety and biosecurity. I then talk about the importance of leadership at all organizational levels, as Dave Franz has so eloquently spoken about in the past. The last area is the importance of *Institutional* leadership, and here I would especially value your input. At the GNL and elsewhere in the USA, we rely heavily on the Institutional Biosafety Committee (IBC) for final review and approval for studies involving recombinant DNA, and more broadly to studies involving pathogens in general. I don't know if a similar committee exists in China or in other countries around the world. Your thoughts and input particularly on this point would certainly improve the manuscript and make it more relevant to a broader community of scientists.

Attached please find a first draft for your review and consideration. I hope that you will agree to work with me on this important project. I look forward to hearing from you soon.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610

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Safety and Security in the Age of Synthetic Biology

James W. Le Duc* and Zhiming Yuan**

Abstract:

Synthetic biology offers great potential for benefit to society, human health and agriculture; however, it also raises new concerns about safety and security. Application of established tools of biosafety and biosecurity along with strong individual and institutional leadership will help alleviate risks, but these may require refinement to address successfully the potential challenges of synthetic biology. Institutional biosafety committees (IBC) are key to providing institutional oversight and as technology evolves, especially in the case of synthetic biology where many different specialized fields may play a role, membership of the IBC needs to adapt to ensure that sufficient knowledge and experience is available to evaluate projects and recognize potentially dangerous experiments. Recommendations regarding avian influenza gain of function studies may offer a valuable framework of points to consider relevant future studies involving synthetic biology.

The rise of synthetic biology, employing novel techniques like gene editing, can create new biological pathways and even microbes not known to exist in nature. As we consider the safety and security challenges that may be associated with this rapidly advancing field, we should not ignore the proven tools that have kept scientists and society safe and secure for decades. These tools are biosafety and biosecurity, a set of biocontainment precautions that make it safe to manipulate dangerous biological agents in an enclosed laboratory facility, along with the physical security controls that secure the research environment. In addition, equally

critical are the fundamentals of *individual* leadership and *institutional* oversight. Together, the pragmatic use of these tools and measures ensures that research on microbes, including the modifications of known microbes, or even the recreation of extinct pathogens or the *de novo* construction of a completely novel microbe remains safe and secure while allowing researchers the freedom necessary to advance life science for the benefit of society. These tools have evolved and improved to meet the changing demands of researchers, and they remain essential resources to control the dynamic landscape heralded by synthetic biology. Any attempt to mitigate against new risks associated with microbiological research will rely on this foundation; however, these tools may need additional refinement to successfully address all the potential challenges associated with synthetic biology.

Biosafety: Ensuring that those working in microbiology are well prepared to work safely with microbes is essential. Such training is not glamorous and it may be easily overlooked when preparing budgets or scheduling projects, yet the risks of human error are arguably the greatest threat to the investigators, their laboratory and the community at large. Besides the physical biocontainment, a laboratory specific biosafety procedures manual and the associated training ensures that the entire workforce, from the individual investigators and staff to the animal care staff and building maintenance and engineers understands and appreciates the need for mitigating risk through safe techniques for handling potentially dangerous microbes and associated equipment. In labs that work with dangerous pathogens, such individuals may receive formal training through academic coursework. This is often augmented by facility-specific safety orientation and perhaps followed by a period of mentorship provide by an experienced individual working side-by-side with the trainee. Unfortunately, such training may be a luxury in many

programs, and novel approaches are needed to adapt and make available core biosafety training to everyone involved in synthetic biology.

Biosecurity: Similarly, ensuring that the research enterprise operates with access controls and physical security and that a trusted workforce is implementing the studies as designed is essential. In recent years, there has been a global proliferation of biocontainment laboratories. In most cases, these biocontainment facilities are well designed and constructed to meet the modern demands of biosecurty. To ensure research and product development conducted on dangerous pathogens occurs in a safe and secure environment, these facilities must have controlled access that limits admittance to only vetted persons. This ensures that only known individuals enter the facility and limits access to pathogens to only those who are essential and properly trained, thereby mitigating the risks of nefarious use of technology or pathogens. Some programs may also attempt to inventory quantities of pathogens; however, given that by their nature they replicate easily, this measure has limited value in terms of security. The biosecurity in synthetic biology is largely dependent on the trusted workforce in the laboratory, and therefore a great deal of attention must be paid to a culture of safety, as well as careful personnel recruitment, background screenings, and adherence to strict policies and procedures regarding laboratory access.

Leadership: Enhancing physical security is relatively easy; but assuring that researchers and workers are reliable is more challenging. This requires that leaders manage the laboratory in full compliance with all regulations and safe management practices, know their team members well and ensure that an appropriate safety and security culture pervades their institution. Leadership is a shared responsibility that involves engaging anyone with supervisory responsibility, be it a team leader, a principal investigator or the institution's director.

Leadership extends beyond the laboratory itself and ideally includes a second level, engagement by the biotech industry and service providers. Commercial partners provide technical support and products such as oligonucleotides that may encode potentially pathogenic attributes or be used to modify or construct a dangerous or extinct pathogen. Many commercial providers are part of the International Gene Synthesis Consortium ([HYPERLINK "https://genesynthesisconsortium.org/"]) a group of gene synthesis companies that routinely screen requests and vet customers to ensure that requests for potentially dangerous sequences are carefully reviewed. They make it a practice to know their customers and their research needs. As this commercial sector expands globally, competition for market share and a desire to reduce costs may lead to less rigorous screening of requests, possibly resulting in greater availability of potentially dangerous sequences and a decrease in the industry's ability to maintain a familiarity between the gene synthesis providers and their customers. Going forward, it will be important to sustain these best practices established by the gene synthesis industries to mitigate against the misuse of synthetic biology.

Institutional Oversight: As part of their leadership responsibilities, laboratory directors, principal investigators and independent scientists provide direct oversight to their immediate staff. Many organizations have an additional formal review process that provides institutional oversight of research activities. This may come from an Institutional Biosafety Committee (IBC) or another committee with equivalent responsibilities. IBCs are a requirement for institutions receiving U.S. National Institutes of Health funding and they provide local review and oversight of research involving recombinant DNA studies (.https://osp.od.nih.gov/biotechnology/institutional-biosafety-committees/). In China, the IBCs play an increasing role in the oversight and biorisk assessment of novel techniques and

experiments concerning the manipulation of pathogens and recombinant DNA, and only projects that pass a rigorous review can be implemented in the laboratory. These committees ensure that work conducted within a facility is done safely, securely, and in a responsible manner. Historically, this model has been instrumental in providing local institutional oversight of research activities and in establishing a national framework for consistent conduct of nearly all forms of research involving recombinant studies or synthetic biology.

However, globally, all institutions may not have an IBC, and those that do may face challenges in ensuring that their committee members represent a sufficiently broad array of technical skills necessary to provide effective oversight and perform an adequate risk assessment that is needed for many synthetic biology projects. As technology evolves, especially in the case of synthetic biology where many different specialized fields may play a role, membership of the IBC needs to adapt to ensure that sufficient knowledge and experience is available to evaluate projects and recognize potentially dangerous experiments.

Challenge and Perspectives: One area of research that received considerable attention recently is *gain of function* studies, especially those investigations attempting to identify key molecular changes that might lead to efficient person-to-person transmission of avian influenza viruses. In the U.S.A., the National Science Advisory Board for Biosecurity (NSABB) considered the risks associated with gain of function studies, focusing especially on those studies that may hold potential to increase the virulence or transmissibility of a pathogen. After more than a year of careful consideration, the NSABB proposed the following points to be considered when reviewing the risk of planned gain-of-function experiments. (https://osp.od.nih.gov/wp-content/uploads/2016/06/NSABB_Final_Report_Recommendations_Evaluation_Oversight_Proposed_Gain_of_Function-Research.pdf).

Such research would be of special concern and warrant additional review if:

- The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.
- The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

In addition, the NSABB recommends that the following principles be considered prior to initiating the study:

- The proposed research has been reviewed and is scientifically meritorious
- The pathogen generated is likely able to arise naturally
- The potential risks as compared to the potential benefits to society are justified
- There are no feasible, equally efficacious alternative methods to address the same scientific question.
- The investigator and institution have demonstrated capacity and commitment to conduct the study safely and securely.
- The results will be broadly shared to realize full potential benefits.
- Management of risks and ongoing oversight will be in place throughout the course of the study.
- The propose research is ethically justified.

These NSABB principles may serve as a guide as IBCs evolve to meet the challenges of synthetic biology. Expertise that may be required includes a full understanding of biohazardous agents; biological containment structure and operations; care and use of laboratory animals (or plants, if appropriate) in containment; the conduct of a comprehensive risk-benefit assessment;

ethics; legal concerns as reflected in local, state and national laws; ecological considerations; and the potential public health impact of proposed investigations. Establishing an IBC that is competent in these many fields represents a significant challenge to program leadership.

Many countries are relying on regulations targeting Genetically Modified Organisms to regulate synthetic biology (see: [HYPERLINK "https://www.loc.gov/law/help/restrictions-on-gmos/usa.php"] for relevant laws in the U.S.A.). As synthetic biology advances, these regulations may be insufficient to meet future oversight needs, given their focus only on known organisms.

A path forward for countries and institutions engaged in research involving synthetic biology but lacking a framework for oversight might include ensuring that a solid foundation for training in biosafety is available, coupled with appropriate laboratory facilities where appropriate biosecurity precautions are in place. Leaders at all levels should be expected to ensure that best safety and security practices are used in a culture of open and honest communication, and commercial suppliers of synthetic genes should screen requests and know their customers and their research. These attributes are already in place in many organizations working with highly-hazardous pathogens and may be easily refined to address the unique challenges of synthetic biology. The greatest unmet need of many research organizations may be in having an adequately experienced institutional oversight capability such as the IBC. If absent, one should be established. If present, it may be appropriate to review the committee's mandate and composition so that the diversity of technical skills and experience is available to help institutions ensure that their research in synthetic biology is done in a safe and secure manner that will ultimately benefit society.

*James W. Le Duc, Ph.D. Director, Galveston National Laboratory, Professor, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, USA, 77555-0610

(email: [HYPERLINK "mailto:jwleduc@utmb.edu"])

** Zhiming Yuan, Ph. D. Professor of Wuhan Institute of Virology, President of Wuhan Branch Chinese Academy of Sciences, Wuhan 430071, China

(email: [HYPERLINK "mailto:yzm@wh.iov.cn"])

Conflict of interest

We claim no conflicts of interest associated with our commentary "Safety and security in the age of synthetic biology."

Keywords

Synthetic biology; biosafety; biosecurity; Institutional Biosafety Committees; Leadership in science.

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 7/31/2019 10:44:13 AM
To: 袁志明 [yzm@wh.iov.cn]
Subject: RE: FW: PPT slides from July 26
Attachments: Biosecurity in the age to synthetic biology 31 Jul 2019-final.docx

Hi Zhiming,

Thanks for your note. A slight revision of our commentary is attached. Please review and edit as you like, then go ahead and submit it to Dr. Xu Jainguo.

Please feel free to use the slides I prepared that draw from the points of our commentary. Please add your name to the first slide credits.

I hope you enjoy the BWC discussion and give my best regards to Weiwen. He and his colleagues did a nice job at the conference in DC.

Best wishes,

Jim

From: 袁志明 <yzm@wh.iov.cn>
Sent: Tuesday, July 30, 2019 11:12 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: FW: PPT slides from July 26

Dear Jim

I was regretted to have not attend the US-China synthetic biology conference in US. Zhang Weiwen is my good friend, and he told me the success of this meeting and your contribution for the conference. We all agree to work together to have this forum as an another platform for discussing the main chanllenge between US-China scientist besides our CAS-NAS channel.

I am in Geneva for BWC expert meeting, I could brief our main idea on biosafety and biosecurity aroused in synthetic biology in the meeting if you have no objection.

As to the manuscript, I have not do the submittion, I could submit this manuscript to Dr. Xu Jianguo directedly. Would you please send me this final version of this manuscirpt?

Regards

Zhiming

-----原始邮件-----

发件人:"LeDuc, James W." <jwleduc@UTMB.EDU>

发送时间:2019-07-31 01:38:41 (星期三)

收件人:"Yuan Zhiming" <yzm@wh.iov.cn>

抄送:

主题:FW: PPT slides from July 26

Zhiming,

Attached are the slides I used for the presentation at the recenthat you unfortunately could not attend. As you will see, the slides are basically drawn from our recent commentary and I mentioned you by name and our collaborations on this when I gave the talk. As you will see from the note below and my separate response, some participants asked for copies of the slides which I have given to them.

Weiwen Zhang from Tianjin University was the lead person from the Chinese delegation.

Did you submit our commentary to your journal? Is there anything I need to do?

I hope you are enjoying summer in Wuhan.

Thanks, Jim

From: Andrea Lapp <alapp1@jhu.edu>
Sent: Tuesday, July 30, 2019 11:10 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: PPT slides from July 26

Hi Jim,
Our colleagues from China have asked if you would mind sharing your slides with them? This is completely optional and we will not share without your permission. However, please let me know if it is ok to share your PPT with them.
Thanks,
Andrea

Andrea R. Lapp
Events Director
Johns Hopkins Center for Health Security
621 E. Pratt Street, Suite 210
Baltimore, MD 21202
443-286-9494
alapp1@jhu.edu
www.centerforhealthsecurity.org

Safety and Security in the Age of Synthetic Biology

James W. Le Duc* and Zhiming Yuan**

Abstract:

Synthetic biology offers great potential for benefit to society, human health and agriculture; however, it also raises new concerns about safety and security. Application of established tools of biosafety and biosecurity along with strong individual and institutional leadership will help alleviate risks, but these may require refinement to address successfully the potential challenges of synthetic biology. Institutional biosafety committees (IBC) are key to providing institutional oversight and as technology evolves, especially in the case of synthetic biology where many different specialized fields may play a role. Membership of the IBC needs to adapt to ensure that sufficient knowledge and experience is available to evaluate projects and recognize potentially dangerous experiments. Recommendations regarding avian influenza gain of function studies may offer a valuable framework of points to consider relevant future studies involving synthetic biology.

The rise of synthetic biology, employing novel techniques like gene editing, can create new biological pathways and even microbes not known to exist in nature. As we consider the safety and security challenges that may be associated with this rapidly advancing field, we should not ignore the proven tools that have kept scientists and society safe and secure for decades. These tools are biosafety and biosecurity, a set of biocontainment precautions that make it safe to manipulate dangerous biological agents in an enclosed laboratory facility, along with the physical security controls that protect the research environment. In addition, equally critical are the fundamentals of *individual* leadership and *institutional* oversight. Together, the pragmatic use of these tools and measures ensures that research on microbes, including the modifications of known microbes, or even the recreation of extinct pathogens or the *de novo* construction of a completely novel microbe remains safe and secure while allowing researchers the freedom necessary to advance life science for the benefit of society. These tools have evolved and improved to meet the changing demands of researchers, and they remain essential resources to control the dynamic landscape heralded by synthetic biology. Any attempt to mitigate against new risks associated with microbiological research will rely on this foundation; however, these tools may need additional refinement to address successfully all the potential challenges associated with synthetic biology.

Biosafety: Ensuring that those working in microbiology are well prepared to work safely with microbes is essential. Such training is not glamorous and it may be easily overlooked when preparing budgets or scheduling projects, yet the risks of human error are arguably the greatest threat to the investigators, their laboratory and the community at large. Besides the physical biocontainment, a laboratory specific biosafety procedures manual and the associated training ensures that the entire workforce, from the individual investigators and staff to the animal care

personnel and building maintenance and engineers understands and appreciates the need for mitigating risk through safe techniques for handling potentially dangerous microbes and associated equipment. In labs that work with dangerous pathogens, such individuals may receive formal training through academic coursework. This is often augmented by facility-specific safety orientation and perhaps followed by a period of mentorship provide by an experienced individual working side-by-side with the trainee. Unfortunately, such training may be a luxury in many programs, and novel approaches are needed to adapt and make available core biosafety training to everyone involved in synthetic biology.

Biosecurity: Similarly, it is essential to ensure that the research enterprise operates with access controls and physical security and that a trusted workforce is implementing the studies as designed. In recent years, there has been a global proliferation of biocontainment laboratories. In most cases, these biocontainment facilities are well-designed and constructed to meet the modern demands of biosecurty. To ensure research and product development conducted on dangerous pathogens occurs in a safe and secure environment, these facilities must have controlled access that limits admittance to only vetted persons. This ensures that only known individuals enter the facility and limits access to pathogens to only those who are essential and properly trained, thereby mitigating the risks of nefarious use of technology or pathogens. Some programs may also attempt to inventory quantities of pathogens; however, given that by their nature they replicate easily, this measure has limited value in terms of security. Biosecurity in synthetic biology is largely dependent on the trusted workforce in the laboratory, and therefore a great deal of attention must be paid to a culture of safety, as well as careful personnel recruitment, background screenings, and adherence to strict policies and procedures regarding laboratory access.

Leadership: Enhancing physical security is relatively easy; however, assuring that researchers and workers are reliable is more challenging. This requires that leaders manage the laboratory in full compliance with all regulations and safe management practices, know their team members well and ensure that an appropriate safety and security culture pervades their institution. Leadership is a shared responsibility that involves engaging anyone with supervisory responsibility, be it a team leader, a principal investigator or the institution's director.

Leadership in synthetic biology extends beyond the laboratory itself and ideally includes a second level, engagement by the biotech industry and service providers. Commercial partners provide technical support and products such as oligonucleotides that may encode potentially pathogenic attributes or may be used to modify or construct a dangerous or extinct pathogen. Many commercial providers are part of the International Gene Synthesis Consortium ([HYPERLINK"https://genesynthesisconsortium.org/"]) a group of gene synthesis companies that routinely screen requests and vet customers to ensure that requests for potentially dangerous sequences are carefully reviewed. They make it a practice to know their customers and their research needs. As this commercial sector expands globally, competition for market share and a desire to reduce costs may lead to less rigorous screening of requests, possibly resulting in greater availability of potentially dangerous sequences and a decrease in the industry's ability to maintain a familiarity between the gene synthesis providers and their customers. As synthetic biology activities expand, it will be important to sustain these best practices established by the gene synthesis industries to mitigate against possible misuse.

Institutional Oversight: As part of their leadership responsibilities, laboratory directors, principal investigators and independent scientists provide direct oversight to their immediate staff. Many organizations have an additional formal review process that provides institutional

oversight of research activities. This may come from an Institutional Biosafety Committee (IBC) or another committee with equivalent responsibilities. IBCs are a requirement for institutions receiving U.S. National Institutes of Health funding and they provide local review and oversight of research involving recombinant DNA studies (.https://osp.od.nih.gov/biotechnology/institutional-biosafety-committees/). In China, the IBCs play an increasing role in the oversight and biorisk assessment of novel techniques and experiments concerning the manipulation of pathogens and recombinant DNA, and only projects that pass a rigorous review can be implemented in the laboratory. These committees ensure that work conducted within a facility is done safely, securely, and in a responsible manner. Historically, this model has been instrumental in providing local institutional oversight of research activities and in establishing a national framework for consistent conduct of nearly all forms of research involving recombinant studies or synthetic biology.

However, globally, all institutions may not have an IBC, and those that do may face challenges in ensuring that their committee members represent a sufficiently broad array of technical skills necessary to provide effective oversight and perform an adequate risk assessment that is needed for many synthetic biology projects. As technology evolves, especially in the case of synthetic biology where many different specialized fields may play a role, membership of the IBC needs to adapt to ensure that sufficient knowledge and experience is available to evaluate projects and recognize potentially dangerous experiments.

Challenge and Perspectives: One area of research that received considerable attention recently is *gain of function* studies, especially those investigations attempting to identify key molecular changes that might lead to efficient person-to-person transmission of avian influenza viruses. In the U.S.A., the National Science Advisory Board for Biosecurity (NSABB) considered the risks

associated with gain of function studies, focusing especially on those studies that may hold potential to increase the virulence or transmissibility of a pathogen. After more than a year of careful consideration, the NSABB proposed the following points to be considered when reviewing the risk of planned gain-of-function experiments. (https://osp.od.nih.gov/wp-content/uploads/2016/06/NSABB_Final_Report_Recommendations_Evaluation_Oversight_Proposed_Gain_of_Function-Research.pdf).

Such research would be of special concern and warrant additional review if:

- The pathogen generated is likely highly transmissible and likely capable of wide and uncontrollable spread in human populations.
- The pathogen generated is likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

In addition, the NSABB recommends that the following principles be considered prior to initiating the study:

- The proposed research has been reviewed and is scientifically meritorious
- The pathogen generated is likely able to arise naturally
- The potential risks as compared to the potential benefits to society are justified
- There are no feasible, equally efficacious alternative methods to address the same scientific question.
- The investigator and institution have demonstrated capacity and commitment to conduct the study safely and securely.
- The results will be broadly shared to realize full potential benefits.

- Management of risks and ongoing oversight will be in place throughout the course of the study.
- The proposed research is ethically justified.

These NSABB principles may serve as a guide as IBCs evolve to meet the challenges of synthetic biology. Expertise that may be required includes a full understanding of biohazardous agents; biological containment structure and operations; care and use of laboratory animals (or plants, if appropriate) in containment; the conduct of a comprehensive risk-benefit assessment; ethics; legal concerns as reflected in local, state and national laws; ecological considerations; and the potential public health impact of proposed investigations. Establishing an IBC that is competent in these many fields represents a significant challenge to program leadership.

Many countries are relying on regulations targeting Genetically Modified Organisms to regulate synthetic biology (see: [HYPERLINK "https://www.loc.gov/law/help/restrictions-on-gmos/usa.php"] for relevant laws in the U.S.A.). As synthetic biology advances, these regulations may be insufficient to meet future oversight needs, given their focus only on known organisms.

A path forward for countries and institutions engaged in research involving synthetic biology but lacking a framework for oversight might include ensuring that a solid foundation for training in biosafety is available, coupled with appropriate laboratory facilities where adequate biosecurity precautions are in place. Leaders at all levels should be expected to ensure that best safety and security practices are used in a culture of open and honest communication, and commercial suppliers of synthetic genes should screen requests and know their customers and their research. These attributes are already in place in many organizations working with highly-

hazardous pathogens and may be easily refined to address the unique challenges of synthetic biology. The greatest unmet need of many research organizations may be in having an adequately experienced institutional oversight capability such as the IBC. If absent, one should be established. If present, it may be appropriate to review the committee's mandate and composition so that the diversity of technical skills and experience is available to help institutions ensure that their research in synthetic biology is done in a safe and secure manner that will ultimately benefit society.

*James W. Le Duc, Ph.D. Director, Galveston National Laboratory, Professor, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, USA, 77555-0610

(email: [HYPERLINK "mailto:jwleduc@utmb.edu"])

** Zhiming Yuan, Ph. D. Professor of Wuhan Institute of Virology, President of Wuhan Branch Chinese Academy of Sciences, Wuhan 430071, China

(email: [HYPERLINK "mailto:yzm@wh.iov.cn"])

Conflict of interest

We claim no conflicts of interest associated with our commentary "Safety and security in the age of synthetic biology."

Keywords

Synthetic biology; biosafety; biosecurity; Institutional Biosafety Committees; Leadership in science.

From: Yuan Zhiming [yzm@wh.iov.cn]
Sent: 2/20/2021 1:16:08 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: 回复:Happy New Year

Dear Jim,

Nice to have heard you again. During the last year, we all experienced the hardest time, fighting against the virus, fighting against the rumors. The lab. operated smoothly he efficiently, providing a crucial platform for pathogen identifiication, animal modeling, antiviral drug screening and vaccine development, and we are very proud of the role and achievements of the laboratory. Here, I would like to express my sincere thanks to you and your colleagues for your assitance for the safety and secure operation of the lab.
I really hope you could come back here after the epidemic and we could share our understanding on lab management and infectious disease control.

Best regards and looking forward to seeing you in near future.

Zhiming

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Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
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From: LeDuc, James W.
Date: 2021-02-12 10:05
To: Yuan Zhiming
Subject: Happy New Year
Dear Zhiming,

I hope that your are well and healthy! Maryellen and I take this opportunity to wish you and your family a very happy new year, filled with good health and happiness. We are all happy to see the past year come to an end and are looking forward to a COVID-19 free new year, filled with good friends and great joy.

With my very best wishes,

Jim

James W. Le Duc
(m) 409-789-2012

From: Ting YUAN 袁婷 [yuanting@westlake.edu.cn]
Sent: 4/29/2021 12:33:43 AM
To: LeDuc, James W. [jwleduc@UTMB.EDU]
Subject: 回复 : Invitation for Biosafety Advisory Committee of Westlake University
Attachments: appointment_letter.jpeg

Dear Professor LeDuc,

Thank you for accepting our invitation, please find attached the appointment letter signed by Professor Yigong Shi. We would like to ask your opinion on the design of our facility and our project proposal once we have them in maybe early next month.

Best,
Ting

袁婷 Ting Yuan
主任助理 Director Assistant
应急医学研究中心 Center for Infectious Disease Research, CIDR
Tel: +86 571 87089772
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18 Shilongshan Rd, Cloud Town, Xihu District, Hangzhou, Zhejiang, China
西湖大學
WESTLAKE UNIVERSITY

发件人: LeDuc, James W.
发送时间: 2021年4月26日 22:47
收件人: Ting YUAN 袁婷
主题: RE: Invitation for Biosafety Advisory Committee of Westlake University

Dear Ting Yuan,

Thank you for the kind offer to join the Biosafety Advisory Committee of Westlake University in Hangzhou, China. It would be my pleasure to join Professor Yigong Shi and my colleagues Professor Pei-Yong Shi and Professor Zhiming Yuan in this very important activity. I look forward to learning more about the new laboratory and how I might best contribute to your success.

With best wishes,

Jim

James W. Le Duc

University of Texas Medical Branch
Cell 409-789-2012

From: Ting YUAN 袁婷 <yuanting@westlake.edu.cn>
Sent: Monday, April 26, 2021 4:40 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Invitation for Biosafety Advisory Committee of Westlake University

Dear Professor LeDuc,

This is Ting Yuan, the Director Assistant of Center for Infectious Disease Research (CIDR), Westlake University (Hangzhou, China). We are writing to you because we wish that we have the honor to invite you as a member of Biosafety Advisory Committee of Westlake University.

Westlake University is a new type of private non-profit university led by Professor Yigong Shi, who is an outstanding structural biologist. CIDR was established by Westlake University in response to the emerging global infectious disease COVID-19. It aims to make transformative scientific advances that lead to an understanding of a broad spectrum of infectious diseases. Thus, BSL-3 laboratories will be the most needed facility right now.

You are an extraordinary microbiologist excel on infectious disease and biosafety so that we can get invaluable suggestions from you while we are building our BSL-3 labs at CIDR. The Westlake University Biosafety Advisory Committee will recruit experts domestically and abroad like Professor Peiyong Shi from UTMB, Professor Zhiming Yuan from Wuhan Institute of Virology, etc. It will guide our Biosafety Committee in terms of lab design and biosafety management during the first few years of lab operation. You are highly recommended by Professor Shi when we asked him for advice on the member list.

We would greatly appreciate that if you take the invitation. The invitation letter will be sent to you later. Thank you for your time.

Best!
Ting Yuan

袁婷 Ting Yuan
主任助理 Director Assistant
应急医学研究中心 Center for Infectious Disease Research, CIDR
Tel: +86 571 87089772
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18 Shilongshan Rd, Cloud Town, Xihu District, Hangzhou, Zhejiang, China

西湖大學
WESTLAKE UNIVERSITY

西湖大學
WESTLAKE UNIVERSITY

APPOINTMENT LETTER

Dear Professor Jamse LeDuc

We are pleased to appoint you as member of Biosafety Advisory Committee of Westlake University.

2021.4.27

日期 Date

签名 Signature

From: jwleduc@utmb.edu [jwleduc@utmb.edu]
Sent: 11/24/2020 9:29:48 PM
To: 郑大胜Zheng [d.zheng@wh.iov.cn]
CC: Grimaldo, Miguel A. [magrimal@UTMB.EDU]
Subject: Re: Happy Thanksgiving!

Thank you Dasheng. I hope you are well.

Best wishes, Jim

Sent from my iPhone

On Nov 24, 2020, at 9:05 PM, 郑大胜Zheng <d.zheng@wh.iov.cn> wrote:

Dear James,
Dear Miguel,

Thanks for giving me the opportunities of staying in Galveston and being trained at GNL. I am so grateful to you all and your kind assistance.

Wish you happier and healthier than ever!

Kind Regards,

Dasheng
--
ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969
==========

在 2018-09-05 03:41:45, "LeDuc, James W." <jwleduc@UTMB.EDU> 写道：

Dear Dasheng,

I am sorry to learn that you will not be continuing at the Wuhan laboratory and I certainly wish you well as you seek another position. Unfortunately, we have no vacancies here at the GNL, but I am copying Miguel on this message in case he is aware of other jobs elsewhere.

I would be pleased to offer a letter of recommendation limited to your training here at the GNL.

With all good wishes for your future success.

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: 郑大胜Zheng <d.zheng@wh.iov.cn>
Sent: Friday, August 31, 2018 3:24 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re:RE: Re:Re: Happy New Year!

Dear Prof LeDuc,

I am deeply impressed by you and your laboratory so that may I ask for work opportunity directly with you at your laboratory.

Before this June the director of the Wuhan P4 lab loses his word to promote me as a quality manager, although Prof. Rene Courcol proposes, who is the French quality inspector to the Wuhan P4 lab. I have to look for new "la vie" by 2019 New Year, the end of current work contract.

Your consideration or recommendation would be appreciated greatly as I am confident in fruitful collaborations with you and your laboratory on biosafety and other relevant topics.

Best Wishes,

Dasheng

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ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology

Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006
Mob: +86-135 1729 0969

==========

At 2018-01-10 00:56:48, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dasheng,

It is good to hear from you and to learn that you remain interested in collaborations on biosafety and other relevant topics. We look forward to working with you, Yuan Zhiming and your other colleagues as you bring your beautiful new facility to full operational status. Please let us know if we can be of help to you during these exciting times.

With all good wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: 郑大胜Zheng [mailto:d.zheng@wh.iov.cn]
Sent: Tuesday, January 09, 2018 1:26 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re:Re: Happy New Year!

Dear James,

Surely I am eager at stronger collaborations with GNL while proposing cooperations to the Wuhan P4 director, Prof. Yuan Zhiming, as soon as my back to Wuhan from Galveston. However, the leader has special arrangements on me although am not suitable for. Update now I am ready for indulging myself into biosafety other than notion of disguise.

Please let me know if any chance I am of help in collaborations.

Dasheng

--

ZHENG Dasheng, PhD

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National Biosafety Laboratory
Institute of Virology

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Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006

Mob: +86-135 1729 0969

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在 2017-12-22 22:17:02, "LeDuc, James W." <jwleduc@UTMB.EDU> 写道：

Thank you very much for your kind note and good holiday wishes. I am very pleased that you were able to spend time with us and that the experiences at UTMB were valuable. I look forward to many years of fruitful collaborations.

With all good wishes for a Merry Christmas and a healthy and prosperous New Year

Jim

Sent from my iPhone

On Dec 22, 2017, at 1:55 AM, 郑大胜Zheng <d.zheng@wh.iov.cn> wrote:

Dear Prof. LeDuc,

Hope this email give you more health and happiness. I really appreciate your instruction and supervision in my stay at GNL, which is my ideal model of biocontainment as you and your colleagues taught me what and how the biocontainments provide protection to people working on pathogenic microorganisms. Your invitation plays important role in my life. I shall look for more opportunities for fruitful cooperation with GNL.

Wish you a merry Christmas and Happy New Year!

Best Regards,

Dasheng

--

Zheng, Dasheng PhD

==========

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==========

At 2017-01-27 22:31:18, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dasheng,

I wish you and your team good health and lasting prosperity as you begin the Chinese New Year. May the coming year bring you much success and fruitful collaborations.

With all good wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 4/26/2021 2:47:12 PM
To: Ting YUAN 袁婷 [yuanting@westlake.edu.cn]
BCC: Shi, Pei yong [peshi@UTMB.EDU]; Yuan Zhiming [yzm@wh.iov.cn]
Subject: RE: Invitation for Biosafety Advisory Committee of Westlake University

Dear Ting Yuan,

Thank you for the kind offer to join the Biosafety Advisory Committee of Westlake University in Hangzhou, China. It would be my pleasure to join Professor Yigong Shi and my colleagues Professor Pei-Yong Shi and Professor Zhiming Yuan in this very important activity. I look forward to learning more about the new laboratory and how I might best contribute to your success.

With best wishes,

Jim

James W. Le Duc
University of Texas Medical Branch
Cell 409-789-2012

From: Ting YUAN 袁婷 <yuanting@westlake.edu.cn>
Sent: Monday, April 26, 2021 4:40 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Invitation for Biosafety Advisory Committee of Westlake University

Dear Professor LeDuc,

This is Ting Yuan, the Director Assistant of Center for Infectious Disease Research (CIDR), Westlake University (Hangzhou, China). We are writing to you because we wish that we have the honor to invite you as a member of Biosafety Advisory Committee of Westlake University.

Westlake University is a new type of private non-profit university led by Professor Yigong Shi, who is an outstanding structural biologist. CIDR was established by Westlake University in response to the emerging global infectious disease COVID-19. It aims to make transformative scientific advances that lead to an understanding of a broad spectrum of infectious diseases. Thus, BSL-3 laboratories will be the most needed facility right now.

You are an extraordinary microbiologist excel on infectious disease and biosafety so that we can get invaluable suggestions from you while we are building our BSL-3 labs at CIDR. The Westlake University Biosafety Advisory Committee will recruit experts domestically and abroad like Professor Peiyong Shi from UTMB, Professor Zhiming Yuan from Wuhan Institute of Virology, etc. It will guide our Biosafety Committee in terms of lab design and biosafety management during the first few years of lab operation. You are highly recommended by Professor Shi when we asked him for advice on the member list.

We would greatly appreciate that if you take the invitation. The invitation letter will be sent to you later. Thank you for your time.

Best!
Ting Yuan

袁婷 Ting Yuan
主任助理 Director Assistant
应急医学研究中心 Center for Infectious Disease Research, CIDR
Tel: +86 571 87089772
Cell: 15927246429
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地址：中国浙江省杭州市西湖区云栖小镇石龙山街18号
18 Shilongshan Rd, Cloud Town, Xihu District, Hangzhou, Zhejiang, China
西湖大學
WESTLAKE UNIVERSITY

To: Zheng郑大胜[d.zheng@wh.iov.cn]
Cc: Shi, Pei yong[peshi@UTMB.EDU]; Grimaldo, Miguel A.[magrimal@UTMB.EDU]
From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Mon 6/10/2019 4:07:49 PM (UTC-05:00)
Subject: RE: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Dasheng,

It's nice to hear from you again and to learn of your continued interest in working with us here at the GNL. I am happy to prepare a letter of invitation for your proposed visit, but it would be useful to understand a bit more as to the purpose of your stay here. Do you envision conducting a research study, and if so, what is the topic? If you are only seeking additional training in biosafety, biosecurity and building operations, that would be easier to accomplish, although the support we enjoyed previously that allowed us to provide biosafety training at no cost to users is no longer available and we now have a fee for the training. It would also be useful to learn the most convenient dates from your perspective for a visit.

I look forward to hearing back from you will additional details.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
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(f) 409-266-6810
(m) 409-789-2012

From: Zheng郑大胜 <d.zheng@wh.iov.cn>
Sent: Wednesday, June 05, 2019 8:43 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Mendoza, Imelda <imendoza@UTMB.EDU>
Subject: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Prof LeDuc,

May I ask for a favor from you to write an invitation letter with the same purpose as previous one so that I could seek another funding for longer stay at your academia?

You have always been appreciated greatly to provide opportunities for academic exchanges. Hopefully I could do something in return.

Best Wishes,

Dasheng
--
ZHENG Dasheng, PhD
==========
National Biosafety Laboratory
Institute of Virology
Wuhan, Chinese Academy of Sciences
Hubei 430071, P.R.China.
Tel: +86-27-5186-1004 Fax: +86-27-5186-1006

Mob: +86-135 1729 0969
==========

At 2015-04-01 21:43:13, "LeDuc, James W." <jwleduc@UTMB.EDU> wrote:

Dear Dr Zheng,

Thank you for the update, and best of luck as you continue to seek funding for your scholarship.

With best regards,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
jwleduc@utmb.edu

From: dsn.zheng@163.com [mailto:dsn.zheng@163.com] **On Behalf Of** d.zheng@wh.iov.cn
Sent: Tuesday, March 31, 2015 9:36 PM
To: LeDuc, James W.
Cc: Mendoza, Imelda; Bente, Dennis A.; Xia, Han
Subject: Re:Chinese Scholarship to Visit UTMB

Dear Dr. James LeDuc,

Thank you for inviting me to the GNL in writing the invitation letter which provides opportunity of visit and study at your honored laboratory. Unfortunately I havenot gotten any acceptance news from the Chinese Scholarship Committee after the scheduled admission deadline. I am afraid I have to look for other funding resources.

Best Wishes,

Dasheng

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Zheng, Dasheng PhD
==========
Wuhan National Biosafety Laboratory
Chinese Academy of Sciences
Wuhan, P.R.China.

Tel: 86-27-5186 1004
Mobile: 86-135 1729 0969
Email: d.zheng@wh.iov.cn
==========

At 2014-11-26 10:44:53, "Bente, Dennis A." <dabente@UTMB.EDU> wrote:
- 隐藏引用文字 -
Dear Dasheng,

Han translated the requirements for me and we are happy to write an invitation letter for you. I talked to Dr. Le Duc, director of the Galveston National Laboratory, and he agreed to write a letter for you. I copied him on this email. Dr. Le Duc will also involve our building engineer, Miguel Grimaldo, in the process of planning your visit.

Best wishes,

Dennis

From: dsn.zheng@163.com [mailto:dsn.zheng@163.com] **On Behalf Of** d.zheng@wh.iov.cn
Sent: Saturday, November 22, 2014 1:46 AM
To: Bente, Dennis A.
Cc: Xia, Han
Subject: Re:RE: Nice to meet you at Wuhan

Hi Den,

At this moment I have a chance to apply for some fund to support my idea to UTMB from the China Scholarship Committee. May I ask for your help in writing an invitation letter as a prerequisite for this fund? The webpage (in Chinese only) of this fund is as follow:

http://www.csc.edu.cn/Chuguo/43988dd354584badbeb2faf380d99859.shtml

Could Han do a little interpretation so as to make sure what we need to do? According to the Item 14 of the fund bidding approach shown in the webpage, the applicant should have an invitation from abroad in advance.

In my proposal of visit to your lab, I shall accept trainings in high BSL laboratories at first; then, conduct experiments for some time, which is the solid work of this visit; and, last but the most important part, have lessons in biosafety management of GNL, playing as one reason for the fund. You might have better plans. Anyway I will follow your steps since I'm a trainee.

Your assistance would be appreciated greatly.

Best Regards,

Dasheng

--
Zheng, Dasheng
==========

Wuhan Institute of Virology
Chinese Academy of Sciences
Mid 44, Wuchang Xiaohongshan
Wuhan 430071, P.R.China.

Mobile: 86-13517290969
Email: dsn.zheng@163.com
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To: Zheng郑大胜[d.zheng@wh.iov.cn]
Cc: Yuan Zhiming[yzm@wh.iov.cn]
Bcc: Shi, Pei yong[peshi@UTMB.EDU]
From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Wed 10/2/2019 10:26:25 AM (UTC-05:00)
Subject: RE: Re:RE: Re:RE: Re:Chinese Scholarship to Visit UTMB

Dear Dasheng,

Thank you for your recent note. At present, we have very limited opportunities for training of international partners due to budget restrictions. If you can give me some additional information regarding the training you are requesting and how that will contribute to your position at the National Biosafety Laboratory in Wuhan, perhaps we can find a way forward. It will be important for your training to be seen as part of the overall collaborations we have in place between the GNL and the National Biosafety Laboratory, so the endorsement of your proposed training by Zhiming Yuan will be essential.

I look forward to hearing from you soon.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

To: Yuan Zhiming[yzm@wh.iov.cn]
Cc: Shi, Pei yong[peshi@UTMB.EDU]

From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Fri 3/20/2020 2:40:25 PM (UTC-05:00)
Subject: RE: 回复: Vox article

Dear Zhiming,

Thank you for your kinds words and your heroic efforts to control the epidemic in Wuhan. Your success is an inspiration to all of us as we work to halt transmission here in the USA and in other countries. We are just at the start of the explosion of cases in our region and we expect that over the next few weeks we will see very high numbers of cases requiring hospitalization and ICU support. We are preparing as best we can, but as you know well the challenges will be substantial.

Pei Yong and his team continue to make remarkable progress on different aspects of study and it would be excellent if we could identify areas for collaborations.

With all good wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Yuan Zhiming <yzm@wh.iov.cn>
Sent: Friday, March 20, 2020 1:33 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: 回复: Vox article

Dear Jim ,
I am sincerely hope everything goes well with you and your family!
The 2019 novel coronavirus (SARS-CoV-2) outbreak is a major challenge for global public health security. Infection with SARS-CoV-2 has been associated with serious acute respiratory distress syndrome with large number of patients' hospitalization and relatively high mortality. We had a very hard time in combating the infection in Wuhan, the epicenter of the COVID-19 in China, and now we can see the situation goes in good direction, with no reported confirmed case, no reported suspected case in last two days here.
My colleagues and I, have been working on characterization of pathogens, antiviral screen, vaccine development, animal modeling since the early January this year, and some progresses have been made. I hope our understanding of the virus and the technology could be valuable in the global fighting to the virus.
As I can see from the media, the virus is spreading in your country, and more people are infected during the last days, and the situation worries me a lot. I am confident that we could finally curb the spreading of the virus with our joint effort, and our life will return back to the normal soon. I do not know what I can do in the special moment and I hope you could protect you and your family.
Best regards

Zhiming

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Yuan Zhiming, Ph. D.
Professor of Wuhan Institute of Virology
President of Wuhan Branch
Chinese Academy of Sciences
Wuhan 430071, China
Tel: 86-27-87198195(O)
86-27-87197242(L)
Fax: 86-27-87199480
==========

From: LeDuc, James W.
Date: 2020-03-05 22:50
To: Yuan Zhiming; zlshi
CC: Shi, Pei yong
Subject: Vox article

Dear Zhiming and Zhengli,

I hope you are both well during this very difficult time.

The link below is to an article just published that may be of interest to you.

With all good wishes for your personal health and safety as we all work together to control the new virus.

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
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(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

From: Eliza Barclay <eliza.barclay@vox.com>
Sent: Wednesday, March 04, 2020 8:49 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Re: Wuhan

Hi Jim,
The story went up today. Thanks so much for your help with it, and let me know if you see any inaccuracies to fix or updates I should make.
Very best, and hope to stay in touch,
Eliza
https://www.vox.com/2020/3/4/21156607/how-did-the-coronavirus-get-started-china-wuhan-lab

On Fri, Feb 28, 2020 at 3:06 PM Eliza Barclay <eliza.barclay@vox.com> wrote:

> Sure, will do.

> On Feb 28, 2020, at 12:16 PM, LeDuc, James W. <jwleduc@utmb.edu> wrote:

Better to call after about 4 pm CT. We're kinda busy...

Thanks, Jim

From: Eliza Barclay <eliza.barclay@vox.com>
Sent: Friday, February 28, 2020 1:07 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Keusch, Gerald T <keusch@bu.edu>
Subject: Re: Wuhan

Thanks for the connection, Jerry.
And thanks so much for the quick response, Jim. I will give you a call in about an hour.
Best,
Eliza

On Fri, Feb 28, 2020 at 10:50 AM LeDuc, James W. <jwleduc@utmb.edu> wrote:

> Hi Jerry,
>
> Thanks for the introduction and happy to meet you, Eliza. I'm happy to chat about this issue at your convenience. My direct office line is 409-266-6516.
>
> Thanks, Jim
>
> James W. Le Duc, Ph.D.
> Director
> Galveston National Laboratory
> University of Texas Medical Branch
> Galveston, TX 77555-0610
> (t) 409-266-6500
> (f) 409-266-6810
> (m) 409-789-2012

From: Keusch, Gerald T <keusch@bu.edu>
Sent: Friday, February 28, 2020 11:48 AM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Cc: Eliza Barclay <eliza.barclay@vox.com>
Subject: Wuhan

Hi Jim,

I was talking to Eliza Barclay from Vox (copied above) who was referred to me by our friend Peter Daszak. Eliza is working on a story to address the various conspiracy theories being bandied about on the origin of the Covid19 virus. One of the issues, of course, was the Wuhan laboratory as a source – whether accidental or deliberate – and the questions being raised about it biosecurity and biosafety protocols. I said that I was absolutely confident that they had proper protocols and trained people in place, in part because I am was aware that GNL had connections with that lab, had trained many of their staff, and that you have been there.

Eliza will follow up and if you have the time she would like to talk with you. She is trying to gather the scientific argument and be able to translate it for a general audience to be able to distinguish between evidence and conspiracy.

Hope all is well.

Jerry

Gerald T. Keusch, M.D.
Professor of Medicine and International Health
Boston University School of Medicine
Associate Director, National Emerging Infectious Diseases Laboratories
620 Albany Street
Boston, MA 02118

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Eliza Barclay • Science Editor

<~WRD363.jpg>

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Eliza Barclay • Science Editor

Follow Vox on Twitter • Instagram • Facebook
—

To: Yuan Zhiming[yzm@wh.iov.cn]
Cc: Shi, Pei yong[peshi@UTMB.EDU]

From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Mon 11/25/2019 3:42:54 PM (UTC-06:00)
Subject: draft manuscript
BSL4 Wuhan_Manuscript-20191107_track-jwl comments Nov19.docx

Hi Zhiming,

Sorry for the delay in responding to your request for comments on your draft manuscript. I finally had a chance to review it and my comments are attached. I think the paper is nicely written and will be of interest to readers following the development of biocontainment labs in China. You have done a good job in recording capabilities, and you may wish to expand a bit more by mentioning the maximum number of small or large (non-human primates) you are able to manage at a single time in the facility. We are frequently asked these questions, and most product developers want sufficiently large single studies to have statistical significance, so many of our larger studies involve about 20 NHP. There may be good reasons not to quantify your capabilities as well, which I fully understand.

You rightly credit the collaborations with the French in building the laboratory; however, if your goal is to have a truly international impact, you may wish to broaden comments on potential collaboration/collaborators as mentioned in one comment.

If I understand you correctly, you will be publishing the paper in your biosafety journal. If so, you may wish to expand your comments on your training efforts to prepare your staff to safely and securely work in the new facility. You may also wish to mention something about your security profile. As I recall, the entire campus has limited access with guards at entrances. You may wish to comment on other mechanisms in place to limit access to high-risk pathogens—card-key access to labs, security personnel, etc. You will not want to go into too much detail, but it might be appropriate, especially given the focus of your journal, to let readers know that security is an important aspect of your program.

Very nicely done! Thank you for the opportunity to review the draft.

With best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
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China's First Biosafety Level 4 (BSL-4) Laboratory for Fighting Infectious Disease

The epidemic of severe acute respiratory syndrome (SARS-CoV) in 2002–2003, which resulted in 8,069 cases of infection and 775 deaths worldwide (Ref2), brought a great challenge to national and international public health systems. It became a touchstone for public health in China as it responded to emerging infectious diseases, and revealed the weaknesses of existing strategies for the prevention and control of such emerging diseases. Complicating the issue, basic and clinical research in response to the epidemic was impeded due to a lack of high containment facilities. Therefore, in order to reduce the potential impact of deadly infectious diseases, including SARS and other highly dangerous infectious risks to human health, the Chinese authority embarked on the construction of a biosafety laboratory network in China, including the BSL-4 National Biosafety Laboratory, Wuhan, Hubei province in central China. ~~(Wuhan).~~

Comment [LJW1]: check numbers; I had 8437 total with 813 deaths from WHO. You may wish to just say over 8,000 cases and nearly 10% mortality or something similar to avoid specific numbers.

The Chinese Academy of Sciences began the process that would lead to the construction of the BLS-4 laboratory early in 2003, and broke ground in 2015. In the framework of the Sino-French Cooperation Agreement on the Prevention of Emerging Disease Control, signed in 2004, Chinese and French engineers and scientists agreed to collaborate for one decade to complete an internationally recognized BSL-4 laboratory, providing a safe and secure platform for scientists to study high-hazard viruses.

~~On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).~~

~~The Chinese Academy of Sciences began the process that would lead to the construction of the BLS-4 laboratory early in 2003, and broke ground in 2015. In the framework of the Sino-French Cooperation Agreement on the Prevention of Emerging Disease Control, signed in 2004, Chinese and French engineers and scientists agreed to collaborate for one decade to complete an internationally recognized BSL-4 laboratory, providing a safe and secure platform for scientists to study high-hazard viruses.~~

~~The epidemic of severe acute respiratory syndrome (SARS-CoV) in 2002–2003, which resulted in 8,069 cases of infection and 775 deaths worldwide (Ref2), brought a great challenge to national and international public health systems. It became a touchstone for public health in China as it responded to emerging infectious diseases, and revealed the weaknesses of existing strategies for the prevention and control of such emerging diseases. Complicating the issue, basic and clinical research in response to the epidemic was impeded due to a lack of high containment facilities. Therefore, in order to reduce the potential impact of deadly infectious diseases, including SARS and other highly dangerous infectious risks to human health, the Chinese authority embarked on the construction of a biosafety laboratory network in China, including the BSL-4 National Biosafety Laboratory (Wuhan).~~

Milestones of the laboratory construction

[PAGE * MERGEFORMAT]

As a critical part of the national high-level biosafety laboratory network system, the construction project of the Wuhan BSL-4 National Biosafety Laboratory (NBL) was officially approved by the National Development and Reform Commission in 2005. Subsequently, Chinese and French engineers and designers studied the operational state of the art high-containment laboratories worldwide, analyzed the geological and environmental conditions of the proposed construction site, confirmed the operational role of the laboratory in China, then jointly designed and constructed the laboratory. The physical completion of the laboratory on January 31, 2015, is not only a great symbol of Sino-French friendship, but also an impressive accomplishment of the national high-containment biosafety laboratory network. After the commissioning, certification, and trial operation, the laboratory was successfully accredited as an Animal Biosafety Level-4 (ABSL-4) laboratory by the China National Accreditation for Conformity Assessment in accordance with CNAS-CL05:2009 and national laboratory standards on January 13, 2017 (Ref3), and acquired the official license of handling risk group-4 (RG-4) pathogens from the National Health and Family Planning Commission on August 17, 2017. The award of the accreditation certificate and the experimental activity license demonstrated that the laboratory has the full capacity and authority to handle high-hazard viruses and to study animal models of infection according to the regulations (Ref4). These events were a landmark achievement for the National High-level Biosafety Laboratory System with recognition by the Chinese national authority (Ref5). In addition to the laboratory, a culture collection and repository center called the "National Center for the Preservation of Pathogenic Microorganisms" was established and authorized, relying on the facility and bio-containment environment (Ref###). With these milestones, the NBL, as China's first BSL-4 laboratory, has been put into operation formally and legally, with full capacity and authority to conduct virus stocking and scientific research on virulent high-hazard viruses. The long-term aim- of the institute is to establish the NBL as a comprehensive research and development center for infectious diseases, a national biological center, and a WHO reference laboratory. In addition, this laboratory will become a stepping-stone for Chinese and French scientists in fighting infectious disease and will also serve as a cornerstone in global health security. (Fig.1)

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Comment [LJW2]: Do you want to limit this to French scientists? Perhaps "..a stepping stone for Chinese, French and other international partners in fighting..."

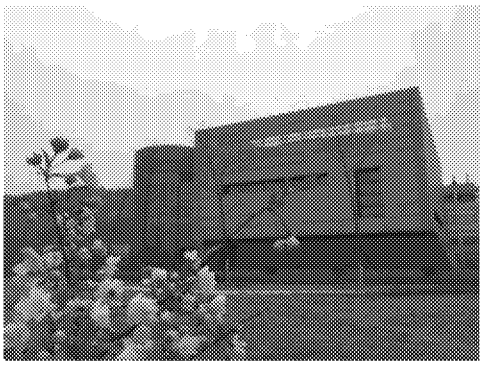

Fig. 1 The BSL-4 facility building

Nature of the laboratory

The laboratory is located in Zhengdian Scientific Park, a few kilometers away from the Yangtze River in the Jiangxia District, Wuhan City, Hubei Province. In addition to the new NBL, one BSL-3, two BSL-2s, molecular diagnosis and cell culture laboratories, and other nearly operational research

facilities and platforms to support virology research and animal rearing are also located in the park, making this research park a modern, comprehensive national and regional virology research and development center.

The BSL-4 laboratory stands as an independent building with a total area of 3266 M^2. It comprises two sections: a square laboratory body structure and a circular auxiliary structure, both inter-linked by a closed corridor. All the equipment and functional units were fitted into the three floors of the square structure. The basement and upper zones are equipped with life maintenance and differential pressure systems (compressed respiratory air and environmental air handling plenums with High Efficiency Particulate Air [HEPA] filters), continuous liquid effluent heat treatment devices and chemical disinfectant tanks, heat exchange systems, water treatment devices, and air conditioning units. All of this equipment is connected to other functional facilities distributed in other zones, within the NBL, through a pipeline network. Thus, all contaminated air, water and solid waste is sterilized/treated before release from the laboratory. (Fig.2)

- **Upper Technical Zone:** Refrigerators, AHU, Exhaust fans, Technical units of shower
- **Equipment interlayer Zone:** Separated from 2nd floor by grilling for maintenance, Air ducts and BIBO.
- **Core laboratory:** Containment with a [illegible] and out on the ceiling.
- **Basement technical Zone:** [illegible] such as electricity, compressed air, [illegible] water treatement, water supply.

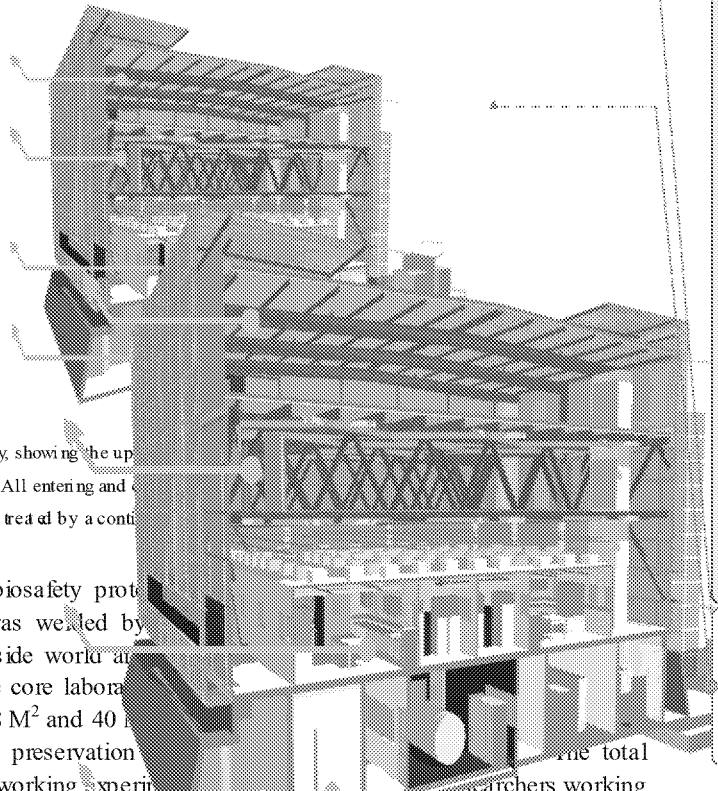

Fig. 2 The Cross-section of the BSL-4 facility, showing the up[illegible] interlayer zone, and the core laboratory zone. All entering and [illegible] solid waste is autoclaved, but liquid waste is treated by a conti[illegible] is transferred from the laboratory.

The core zone is composed of a biosafety prot[illegible] seamlessly welded stainless steel ducts was welded by [illegible] effectively sealed off from the outside world a[illegible] in of the working area is separated into three core labora[illegible] rodents and non-human primates, 28 M^2 and 40 [illegible] room that is 32 M^2 and two virus preservation [illegible] The total containment area is 480 M^2, and its working experi[illegible] searchers working in the lab can see outside to the non-containment ha[illegible] agers can observe the activities occurring inside of it through sealed double-glazed w[illegible] ows installed within the stainless steel wall of each laboratory; this design provides an ambient working environment for researchers in addition to being a practical passage for the purpose of overseeing by the biosafety officer and managers (Fig. 3). The installation of an outer bulletproof glass and [illegible] utmost porous aluminum plate not only provide

[PAGE * MERGEFORMAT]

Comment [A3]: Please change "Technical units of shower" to "technical shower units"
Please change "Exhaust fans" to "exhaust fans"
Please change "Upper Technical Zone" to "Upper technical zone:
Please change "Equipment interlayer Zone" to "Equipment interlayer zone"
Please change "Basement technical Zone" to "Basement technical zone"
Please change "from 2nd" to "from the 2nd"
It's not clear what "by grilling for maintenance" means – maybe "by grills (for maintenance),"?
Please change "Air ducts" to "air ducts"
You might want to spell out BIBO.
Please change "ducts and BIBO." to "ducts, and BIBO"
Please change "get in and out on the ceiling" to "go in and out of the ceiling"

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Comment [LJW4]: Not sure what this means. You may wish to define in greater detail.

an extra protective measure, but also bring value in the form of heat insulation and eventual energy savings for the laboratory

The laboratory is composed of 10 technical systems, including the power supply, thermal supply, containment, air treatment system, waste disposal, life maintenance, automatic control system, fire control, security system, and isolation facilities, which guarantees the stable unidirectional negative pressure gradient air flow and sealed environment in the containment area. It is designed as a suit-type biosafety laboratory, in which the staff inside are completely protected by a whole-body positive-pressure protective suit supplied with conditioned air.

Comment [LJW5]: Would you like include mention of the maximum number of personnel that can be supported by the BSL4 air supply system at any given time?

The containment laboratory is fitted with equipment that meets the requirements of biosafety management and high-containment pathogen research, including Labconco biological safety cabinets (BSC), animal breeding and isolators, Tecn independent air transport cages, Tecn animal cages, Ehret monkey cages, a Thermo anatomy table, CO_2 incubators, fluorescence microscopes, quantitative PCR amplifiers, refrigerators, and freezers.

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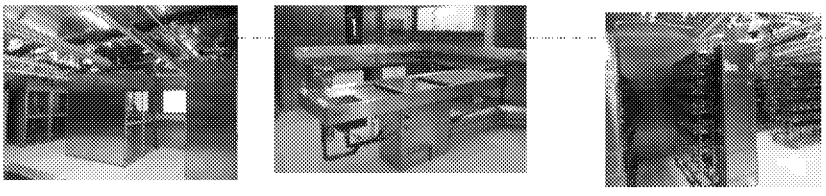
Fig. 3 Two technicians working inside the laboratory



Comment [d6]: Figures need to re-order

Fig. 4: The animal cages for rodent (A) and non-human primate (B) infection, and the autopsy table in three separated room in the BSL-4

Main scientific research priorities

The laboratory is designed and equipped to conduct research on RG-4 pathogens such as the Ebola virus, the Nipah virus, the Crimean-Congo Hemorrhagic Fever (CCHF) virus, the Lassa virus, the Junin virus, the SARS-CoV, the Marburg virus, and so on. According to the lab's biosafety protection level, personnel ability, and management status, the research activities that can be conducted in the laboratory range from low-risk manipulation of cell culture propagation, to rodent infection, and ultimately to the infection of non-human primates. Similarly, pathogen manipulations are gradually conducted from the low-risk CCHF virus to other more virulent pathogens such as the Ebola virus, the Marburg virus, and the Lassa virus. According to the license issued by the National Health Planning Commission and the availability of virus resources, the laboratory has already implemented projects on cell culture models, animal models, pathogenesis studies, and preliminary trials of antiviral drugs as well as vaccine development for the CCHF virus, which used to be called

Comment [LJW7]: Is SARS-Cov considered a RG-4 pathogen in China? I think we handle it at BSL3. This will be important if you want to share strains.

the Xinjiang hemorrhagic fever virus, causing sporadic animal infection during the last few decades in Northwest China (6).

The laboratory has established short- and long-term collaborative links with counterparts in the USA and France; we are seeking additional beneficial scientific and operational partnerships with other laboratories around the world, with the purpose of sharing specimens, reagents, technology, good practice, and expertise; the eventual goal is for there to be effective collaboration within the international laboratory community to address the threat of emerging and re-emerging infectious diseases locally and internationally (7).

Comment [A8]: "true"? Maybe "beneficial"?

The strategic role and capacity strengthening ~~**(need re-write the sub-title)**~~

Comment [A9]: "The strategic plan"

~~On the base of the~~According to the laboratory's operational orientation and China's national requirements, the laboratory was designed and will operate as the research and development center for infectious disease, as a national biological resource center and as a WHO reference laboratory. As a comprehensive national biosafety research center, it will play an indispensable role in the prevention and control of infectious diseases in China. In order to realize these key goals and functions, we must assure the safe and secure operation of the laboratory, increase its capacity as a core culture collection resource, enlarge its scientific research capacity, support and promote the overall response capacity for public health emergency preparedness, provide expert support to national biosafety strategies, and contribute to the broader laboratory network (system). We aim to ensure the safe and efficient operation of the laboratory through the principles of "openness, transparency and sharing", benefiting national security and global health security.

Comment [d10]: Repeat with previous paragraph.

Comment [LJW11]: Would you like to mention your training program to prepare staff for work in biocontainment?

(replaced with the new version of 五种能力建设)

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On February 22, 2017, an article entitled "Inside the Chinese lab poised to study world's most dangerous pathogens," by David Cyranoski, elicited a range of opinions in the form of discussions among scientists, both in China and abroad. Some scientists regard China's first Biosafety Level 4 (BSL-4) laboratory as a "big status symbol in biology" that will usefully contribute to and benefit global health security, whereas others express considerable concern regarding the potential biosafety and biosecurity risk posed by the new laboratory (Ref1).

References

1) David Cyranoski, Inside the Chinese lab poised to study world's most dangerous pathogens, Nature, 2017, 542: 399-400,
2) WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003. WHO, [HYPERLINK "http://www.who.int/csr/sars/country/table2004_04_21/en/"](2004)
3) State Council of the People's Republic of China, Regulation on administration of biosafety in pathogenic microorganism laboratories, 2018, [HYPERLINK "http://jiuban.moa.gov.cn/fwllm/zxbs/xzxk/spyj/201706/t20170606_5662359.htm"]
4) China National Standards Committee, Laboratory-general requirements for biosafety (GB19489-2008), 2008, [HYPERLINK "http://c.gb688.cn/bzgk/gb/showGb?type=online&hcno=EB3B94B543F6E4CD18C044DE6AB64CEC"].
5) National Development and Reform Commission of China, Planning of high-level biosafety laboratory system construction, 2016. [HYPERLINK "http://www.ndrc.gov.cn/zcfb/zcfbtz/201612/t20161220_830455.html"]

Comment [d12]: Page not found

Comment [d13]: Not the right link

6) [HYPERLINK
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"https://www.ncbi.nlm.nih.gov/pubmed/?
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7) [HYPERLINK "https://www.researchgate.net/scientific-contributions/2148527743_James_W_Le_Duc?_sg=jYwkkXaeO7SEceb3lG8i9a4oToAbdwD8yA2QaJpzASED8nAjJyGxtlp5aKkpsn_aLxiKniLp423eTjyaXHhvjEYZLxqvqhux-cXgiH6GwJRnNilibHccvUC_ezKTwjeZGbRQSIL-NpI3OQvlLQFTbZJmJML4w"] and Zhi-ming Yuan, Network for safe and secure labs., Science, 362:267-267

Related news and reports

[HYPERLINK "http://www.chinadaily.com.cn/china/2015-01/31/content_19457709.htm"] China opens National Biosafety Laboratory in Wuhan

By Cheng Yingqi(chinadaily.com.cn)Updated: 2015-01-31 16:14

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From: LeDuc, James W. [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: 5/6/2019 4:16:05 PM
To: Gail Bernabe (gbernabe@niaid.nih.gov) [gbernabe@niaid.nih.gov]
CC: Shi, Pei yong [peshi@UTMB.EDU]
Subject: FW: Call Announcement - Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, Chinese Academy of Sciences
Attachments: Call Announcement.pdf.pdf; Application Form.doc

Gail, thanks for taking my call this morning and for your suggestions as to possible funding sources for collaborative work with China. Attached are the original announcement and the application form for the program we discussed. We have already submitted a proposal and if successful, we would begin work in Wuhan later this year. My goal is to identify a similar funding mechanism that would allow the US side partners to receive similar support for these collaborations. Our vision is that the work will be conducted in true collaboration with some undertaken in the US and some in Wuhan by investigators that are in frequent contact and visiting each other frequently.

Best wishes,

Jim

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
Galveston, TX 77555-0610
(t) 409-266-6500
(f) 409-266-6810
(m) 409-789-2012

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences Advanced Customer Cultivation Project

From: 张晗 <zhanghan@wh.iov.cn>
Sent: Thursday, May 24, 2018 9:40 PM
To: LeDuc, James W. <jwleduc@UTMB.EDU>
Subject: Call Announcement - Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Dear Prof. LeDuc,

Wuhan National Biosafety （P4） Laboratory of Chinese Academy of Sciences （CAS） has been put into operation recently. Relying on the major science and technology infrastructure, the Advanced Customer Cultivation Project initiated by Wuhan Institute of Virology, CAS aims to cultivate national high-level biosafety talents, to output significant scientific and technological breakthroughs and achievements, and to promote the scientific and technological support capabilities for biosafety and public health.

Now this project is open for application globally. Here we are writing to request your consideration to help to promote this project. If available, could you please review the call announcement and help to forward this notice to the relevant research fellows in your side?

We welcome your potential application and thank you very much for your great support.

project website : http://english.whiov.cas.cn/Notice2016/201805/t20180518_192593.html

With best wishes,

ZHANG Han

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Advanced Customer Cultivation Project

Call Announcement

Relying on the major science and technology infrastructure, this project aims to cultivate national high-level biosafety talents, to output significant scientific and technological breakthroughs and achievements, and to promote the scientific and technological support capabilities for biosafety and public health. According to the scientific and technological development programs of China, Chinese Academy of Sciences (CAS) and Wuhan Institute of Virology (WIV), CAS, the Call Announcement of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS is released. Please apply for the project accordingly. The specific contents are as below:

I. Background

Wuhan National Biosafety Laboratory, CAS (hereinafter referred to as Wuhan P4 laboratory) mainly includes protective facilities and experimental equipments for researches on highly pathogenic pathogens. It is incorporated into the management of national major science and technology infrastructure. The project is guided by the principle of "P4 scientific and technological innovation" which means to conduct scientific experimental activities by utilizing Wuhan P4 laboratory or to conduct scientific researches on the biosafety level 4 (P4) pathogens.

II. Management

Advanced Customer Cultivation Project is funded by CAS. The application, review, management and budget implementation are conducted according to

Measures of Academy-Level Scientific Research Projects of CAS and according to relevant measures of WIV, CAS.

III. Qualification

1. The project leader shall be professor or principal investigator with the doctoral degree.

2. The project leader and team members shall be equipped with high-level research capability, solid research foundation and reliable time commitment. Team application is encouraged.

3. To better cooperate and utilize resources of the sharing research platform, research team at home and abroad is encouraged to jointly apply with the research group from WIV, CAS.

IV. Budget

1. Funding

The project application is globally oriented. The categories of the projects include general project and key project with the budget of RMB 250,000/project/year and RMB 500,000/project/year respectively while dynamic adjustment shall be made according to the total budget appropriated by CAS.

2. Period

(1) The implementation period of the project can be 1 to 3 years while the assessment period is 1 year. The project with excellence in the annual assessment can be further funded preferentially.

(2) The budget will be implemented in WIV, CAS. The budget implementation period is 1 year. During the project execution, the project team shall accept the review and examination on task and budget implementation organized by WIV, CAS, and complies with the relevant measures of project prescribed by our institute.

V. Guideline

1. Application

(1) The applicant's organization should provide the opinions and agreement to the application while providing support for the project implementation.

(2) The experimental activities on high-level pathogenic microorganism must be complied with relevant Chinese requirements on biosafety laboratory. At the meanwhile, the project members shall be experienced and the participating organization shall provide guarantee conditions.

2. Approval

(1) Under the guidance of Academic Committee of WIV, CAS, a review committee consisting of 7 to 9 experts shall be organized for Advanced Customer Project, which will include experts on microbiology, biosafety, ethics, animal experiments and P4 laboratory management. The review committee shall obey the avoidance principle.

(2) The committee will conduct the proposal selection and after the opinion passed the review by WIV, CAS, results of project approval will be released publicly.

3. Implementation and Assessment

(1) The project leader assumes full responsibility for the Advanced Customer Cultivation Project. The project leader shall fulfill the responsibility as an organizer and shall take charge of the preparation of research plan and implementation scheme for this project and be responsible for the timely accomplishment of the project tasks.

(2) Within 2 months before the end of budget implementation period/project conclusion, the project leader shall submit to WIV, CAS the annual assessment report/summary report for project conclusion and assessment. The review committee will organize the project conclusion and assessment, and submit the assessment opinions to WIV, CAS. A general report will be submitted to CAS.

(3) The research achievements attained during the project implementation, including theses, monographs, patents, software and database etc. shall be marked with "Funded by Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, Chinese Academy of Sciences". Any achievements unmarked will not be counted in the assessment.

V. Submission

The applicant shall download the Application Form of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS, fill in the form according to the instruction, submit the paper version (including signature and organization seal) of the application form, the approval certificate of animal welfare and ethics, the guarantee certificate of research condition for high-level pathogens and the electronic version of the corresponding materials before June 18th, 2018 (subject to the posting time). The paper version of the materials shall be submitted in duplicate. The foreign applicant shall send the electronic version of the materials to zhanghan@wh.iov.cn, while the Chinese applicant shall sent the electronic materials to chendd@wh.iov.cn.

Address:

Wuhan Institute of Virology, Chinese Academy of Sciences

Room 105, No.3 Building

No.44, Xiaohongshan, Wuchang District, Wuhan, Hubei, China

Postcode: 430071

Contact: +86-27-87197115

Attachment: Application Form of Advanced Customer Cultivation Project of Wuhan National Biosafety Laboratory, CAS

Wuhan Institute of Virology

Chinese Academy of Sciences

May 18th, 2018

Confidentiality level	

Wuhan National Biosafety Laboratory, Chinese Academy of Sciences

Advanced Customer Cultivation Project

Application Form

Project name:_____

Project leader (Signature):_____

Organization:_____

Phone number:_____

E-mail:_____

Made by Scientific Research Office of Wuhan Institute of Virology, CAS

Filled in on (d/m/y)

Instruction for Form Filling

[= 1 * ROMAN]. Instruction for form filling

1. The main body of the project application form consists of seven parts: “research background”, “research contents”, “technical route”, “expected outcomes”, “basic information of organization”, “introduction of the leader and participants” and “budget”.
2. The content of application form will be taken as important basis for project review and the signing of assignment, therefore, each item of the application form must be true, complete, accurate and clarified.
3. The application content of this project must obey the application requirements of the Advanced Customer Cultivation Project of Wuhan Institute of Virology, CAS.
4. The text of the application form shall be filled in by the “Times New Roma” typeface in 12pt fonts. The text (includes the title) shall be written with a 1.5 times spacing. For all the items without any content to be filled in, please fill in “none”.
5. For name of organization, please fill in the full name which shall be consistent with that on the official seal of the organization. The paper print of application form shall be consistent with the electronic version filled in online. The paper print shall be signed by the project leader and the date of form filling shall be truthfully indicated.
6. After the form is filled in completely, the applicant’s organization shall review the truthfulness, completeness and effectiveness of the information filled in.

[= 2 * ROMAN]. Instruction for application

1. The applicant shall be responsible for the truthfulness and completeness of the application materials.
2. All the application materials shall be submitted in duplicate in A4 book size in print (double page) or in regular script.

Basic Information

<table>
<tr><td colspan="2">Project name</td><td colspan="9"></td></tr>
<tr><td colspan="2">Type of project</td><td colspan="9">☐Frontline of the fundamental ☐Major common key technology ☐Application demonstration research ☐Others</td></tr>
<tr><td colspan="2">Budget</td><td colspan="9">Total estimate: (RMB 10,000 yuan)</td></tr>
<tr><td colspan="2">Implementation period</td><td colspan="9">From (d/m/y) to (d/m/y)</td></tr>
<tr><td colspan="2">Assessment period</td><td colspan="9">From (d/m/y) to (d/m/y)</td></tr>
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<tr><td rowspan="4">Project leader</td><td>Name</td><td></td><td>Sex</td><td></td><td>Birthday</td><td colspan="4"></td></tr>
<tr><td>Type of certificate</td><td></td><td>Certificate No.</td><td colspan="6"></td></tr>
<tr><td>Highest degree</td><td colspan="8"></td></tr>
<tr><td>Title</td><td colspan="3"></td><td>Duty</td><td colspan="4"></td></tr>
<tr><td>Research group in WIV, CAS</td><td>Principal Investigator (Signature)</td><td colspan="3"></td><td>Person to contact (Signature)</td><td colspan="4"></td></tr>
<tr><td rowspan="2">Project team</td><td>Total number</td><td>Senior</td><td>Intermediate</td><td>Junior</td><td>Assistant personnel</td><td>Post-doctor</td><td>Doctor candidate</td><td>Master candidate</td></tr>
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<tr><td rowspan="7">Leader and participants of the project</td><td>Name</td><td>Age</td><td>Title</td><td>Degree of education</td><td>Time Commitment（Months）</td><td colspan="2">Task Assignment</td><td>Signature</td></tr>
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Text

[= 1 * ROMAN]. Research Background

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[= 2 * ROMAN]. Research Contents

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[= 3 * ROMAN]. Technical Route

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[= 4 * ROMAN]. Expected Outcomes

(within 200 characters)

[= 5 * ROMAN]. Basic Information of the Organization

(within 300 characters)

[= 6 * ROMAN]. Introduction of Leader and Participants

(within 300 characters)

[= 7 * ROMAN]. Budget

Unit: RMB 10,000 yuan

Budget Form of Project Expenditure		
Item	Amount	Detailed calculation
1. Equipment		
(1) Equipment purchase		
(2) Trial-manufacture purchase		
(3) Equipment modification and rent		
2. Reagents and consumables		
3. Analysis		
4. Fuel and power		
5. Travel/meeting/international cooperation and exchanges		
6. Publication/literature/information dissemination/intellectual property		
7. Labor costs		
8. Expert consultation		
9. Other expenditure		
Total		

Note: Budget preparation and expenditure execution are conducted according to *Measures of Academy-Level Scientific Research Projects of Chinese Academy of Sciences*.

[= 8 * ROMAN]. Review opinions of applicant's organization Organization (official seal) Principal (Signature) (d/m/y)
[= 9 * ROMAN]. Opinions of the Review Committee Chairman of Committee (Signature) (d/m/y)
[= 10 * ROMAN]. Opinions of Wuhan Institute of Virology, CAS Director General (official seal) (d/m/y)

To: George F GAO[gaof@im.ac.cn]
Cc: Shi, Pei yong[peshi@UTMB.EDU]; Tseng, Chien-Te K.[sktseng@UTMB.EDU]
From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Thur 1/16/2020 1:55:36 PM (UTC-06:00)
Subject: new Coronavirus
Chinese Response to New Virus Shows Promise_Le Duc-14Jan20.docx
Lethal and nonlethal ACE2 transgenic mouse lineages_2009.pdf

Hi George,

Congratulations on China's response to the emergence of another new coronavirus. Under your strong leadership, you and your colleagues have prepared China well for this new threat and I think that it is important that your efforts are recognized. The link below is to an article published earlier this week in WIRED magazine where I am quoted on the stark differences in response between SARS and now. In addition, I just submitted the attached essay to the Houston Chronicle as an Op Ed. I haven't heard yet if it has been accepted, but if they don't take it, I'll try elsewhere. This is a good story at a time when we need one.

As you might expect, we are following the evolving story on nCoV from Wuhan very closely and we are eager to get an isolate for antiviral testing. Dr Tseng's lab here in the GNL has developed a transgenic mouse model for SARS that is very useful and we are anxious to see if it can be used for the nCoV as well. Any suggestions on how we might obtain an isolate would be most appreciated, and if you would like to send an investigator here to the GNL to work with Dr Tseng on the antiviral screening and further development of the animal model, we would welcome the collaborations. A copy of his publication is attached.

With best wishes,

Jim

Here's a link to the story, which published this morning.

James W. Le Duc, Ph.D.
Director
Galveston National Laboratory
University of Texas Medical Branch
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Chinese Response to New Virus Shows Promise

Fast action and open communications by China may be saving the world from another devastating infectious disease outbreak. Many will recall the dark days in the spring of 2003 when Asia and the world were threatened by the appearance of a new virus disease, Severe Acute Respiratory Syndrome, or SARS, which first appeared in southern China and quickly spread to other countries around the world, ultimately causing over 8000 cases with nearly 10% of those ending in death. SARS was caused by a novel coronavirus then unknown to medical science. There was no known cure, no diagnostic tests and little understanding of where it came from or how it was spread, although person-to-person transmission was obvious as health care workers treating the first cases were themselves among the early victims. Initially, China was reluctant to share information or alert the international community of the magnitude of the epidemic, leading to international criticism and a dangerous global health situation. Fortunately, under pressure, China reversed its position, opened its borders to collaborations with the WHO, U.S. and others, and the epidemic was eventually controlled.

Today, another novel coronavirus has been discovered, again in China; however, this time, less than two decades later, things are very different. Chinese health officials recognized that a new disease had emerged, quickly isolated the patients, and instituted an impressive set of interventions to limit the spread of disease and characterize the new pathogen. Importantly, they have been very transparent in sharing their findings with the world, thus allowing other nations to be on the lookout for the new disease. The outbreak is still in the early stages and fortunately, preliminary results suggest that the new virus is not easily transmitted from person-to-person. While only about 40 patients have been identified, there has been at least one death, and a patient is now hospitalized in Thailand, having traveled from the outbreak site in Wuhan, China. The genome of the new virus was completely sequenced and posted for easy access by experts around the world, allowing rapid exploration of possible treatments, development of diagnostics and epidemiological investigations.

China's ability to respond quickly and efficiently to this new threat is the result of nearly two decades of investments and collaborations to improve public health in China. The Chinese Centers for Disease Control incorporates many of the strengths of our own CDC, but is designed to meet the needs of a 1.4 billion plus population. In addition, China has invested in building a robust scientific capacity and partnered with containment laboratories such as ours to incorporate best practices when studying dangerous pathogens.

The current outbreak clearly demonstrates a new openness to health information sharing with the global community. To diagnose an outbreak early requires astute healthcare providers able to recognize when something new or unusual is occurring; however, clinical recognition alone is meaningless if there is no capacity to investigate the cases or characterize the disease-causing agent.

For the last few years, the D.C.-based National Academy of Science, Engineering and Medicine has worked with the Chinese Academy of Sciences to build relationships and share information on emerging diseases and advancements in the development of vaccines and treatments. In Galveston, we have welcomed leading Chinese health officials to learn about biocontainment facility design and construction, biosafety training and laboratory operations. These collaborations, along with U.S.-based educational opportunities for Chinese students, benefit us all.

China's response to the new coronavirus clearly demonstrates that their investments both in physical laboratories and scientific diplomacy over the past decade are paying dividends, not only to China, but the entire world. Since infectious diseases do not recognize international borders, much must still be done with this current and quickly evolving situation, including the sharing of clinical material, information on containment and treatment options. The international community can assist with studies to determine the original source of infection, assumed to be zoonotic.

At a time when US-China relations are being tested, it is important to note that relations within the public health and scientific research arenas remain positive, which is a success story worth sharing.

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Differential Virological and Immunological Outcome of Severe Acute Respiratory Syndrome Coronavirus Infection in Susceptible and Resistant Transgenic Mice Expressing Human Angiotensin-Converting Enzyme 2[▽]

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We previously reported that transgenic (Tg) mice expressing human angiotensin-converting enzyme 2 (hACE2), the receptor for severe acute respiratory syndrome coronavirus (SARS-CoV), were highly susceptible to SARS-CoV infection, which resulted in the development of disease of various severity and even death in some lineages. In this study, we further characterized and compared the pathogeneses of SARS-CoV infection in two of the most stable Tg lineages, AC70 and AC22, representing those susceptible and resistant to the lethal SARS-CoV infection, respectively. The kinetics of virus replication and the inflammatory responses within the lungs and brains, as well as the clinical and pathological outcomes, were assessed in each lineage. In addition, we generated information on lymphocyte subsets and mitogen-mediated proliferation of splenocytes. We found that while both lineages were permissive to SARS-CoV infection, causing elevated secretion of many inflammatory mediators within the lungs and brains, viral infection appeared to be more intense in AC70 than in AC22 mice, especially in the brain. Moreover, such infection was accompanied by a more profound immune suppression in the former, as evidenced by the extensive loss of T cells, compromised responses to concanavalin A stimulation, and absence of inflammatory infiltrates within the brain. We also found that $CD8^+$ T cells were partially effective in attenuating the pathogenesis of SARS-CoV infection in lethality-resistant AC22 mice. Collectively, our data revealed a more intense viral infection and immunosuppression in AC70 mice than in AC22 mice, thereby providing us with an immunopathogenic basis for the fatal outcome of SARS-CoV infection in the AC70 mice.

Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged as a public health threat in November 2002, and by July 2003 this previously unknown virus had spread to 29 countries in five continents. This outbreak resulted in more than 8,000 cases and 774 deaths and was accompanied by a devastating social, economic, and medical impact worldwide (15). It is generally believed that the reservoirs of SARS-CoV are the Chinese horseshoe bat (*Rhinolophus sinicus*), palm civet cat, and other exotic animal species. These species are sold in markets as sources of food for human consumption and are believed to be responsible for the first cases in southern China (16, 20). Currently, it is a matter of debate as to whether SARS-CoV will make the transition from animals to humans or if such a transition will again result in a global pandemic. However, SARS-CoV and the conditions that fostered the first outbreak continue to exist in southern China, posing a threat for its reemergence. Thus, effective prophylactic or therapeutic strategies against SARS beyond supportive care are needed should reemergence of the virus occur in the future.

The exact mechanism of SARS pathogenesis remains unknown. Evidence has shown that SARS-CoV is transmitted by large droplets, likely via aerosol or fecal-oral routes, with the lungs being the main pathological target. SARS patients exhibited a wide-ranging clinical course, characterized mainly by fever, dry cough, dyspnea, lymphopenia, various degrees of pancytopenia, arterial hypoxemia, and rapidly progressing changes in chest radiography (15). Studies with postmortem lung tissues revealed diffuse alveolar damages, with prominent hyperplasia of pneumocytes, and an increased accumulation of activated macrophages. Strikingly, these pulmonary manifestations usually occurred after the clearance of viremia and in the absence of infections by other opportunistic agents. The pulmonary damage in SARS patients could be caused directly by viral destruction of permissive alveolar and bronchial epithelial cells. Such a delay in revealing reactive hemophagocytosis and other pathological manifestations within the lungs of patients severely affected by SARS strongly suggested that overly intense host inflammatory responses to the infection may play a major role in the pathogenesis of SARS. The likelihood of SARS being an immune-mediated disease was further supported by the highly elevated expression of various innate inflammatory cytokines in the circulation of SARS patients, a state commonly referred to as a "cytokine storm" (1, 3, 24, 30). However, in the absence of recurring SARS epidemics, an

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animal model that mimics human disease is critical for defining the exact cellular and molecular basis of SARS pathogenesis, in order to develop effective preventive and therapeutic strategies against SARS.

The animal species permissive for SARS-CoV infection include mice (young and aged) and some of their derivatives, e.g., "knockout" and transgenic (Tg) mice, hamsters, ferrets, and various nonhuman primates. Unfortunately, infection in these animal models does not result in clinical diseases resembling those reported for human SARS cases (26, 29), and in the case of primates, the costs of studying them are quite high. We have focused our studies on characterizing the pathogenesis of SARS-CoV infection in Tg mice expressing human angiotensin-converting enzyme 2 (hACE2), the functional receptor of SARS-CoV (19), established in our laboratories. Our initial characterization from two different lineages of hACE2 Tg mice (AC70 and AC63) clearly demonstrated that the Tg expression of hACE2 makes the otherwise resistant mice highly susceptible to SARS-CoV infection, resulting in an overwhelming infection, especially in the lungs and brains of both lineages, accompanied by a clinical illness of varying severity (32). Specifically, mice of the AC70 lineage developed an acute wasting syndrome that resulted in 100% mortality within a week following the infection, whereas AC63 mice eventually recovered from the diseases without suffering any mortality, despite progressive weight loss and other signs of illness. Although SARS likely stems from an unregulated and often excessive inflammatory response, the exact nature of the host responses and their correlation with the severity of the diseases associated with SARS-CoV infection are not entirely clear. The exhibition of such a strikingly different final outcome to SARS-CoV infection, i.e., lethal versus nonlethal, among lineages of hACE2 Tg mice makes it useful for establishing the correlates between host responses and SARS pathogenesis. The small litter size and the inconsistent hACE2 expression in AC63 mice led us choose the other lethality-resistant hACE2 Tg lineage, AC22, for the subsequent characterization of host responses to SARS-CoV infection.

In this study, we infected hACE2 Tg AC70 and AC22 mice with an equal amount of SARS-CoV (i.e., 10^6 50% tissue culture infective doses [$TCID_{50}$]) to compare the correlates between various aspects of host immune responses (e.g., proinflammatory cytokines, modulation of lymphocyte subsets, and mitogen-induced proliferation of lymphocytes) and the pathogenesis of SARS-CoV infection. The data presented in this study extend our previously reported observations concerning the differential pathogenesis of SARS-CoV infection in hACE2 Tg mouse lineages that are either highly susceptible or resistant to lethality following SARS-CoV infection. We believe that our results provide insight into the cellular and molecular basis of host immune responses relevant to the final outcome of murine SARS-CoV infection.

MATERIALS AND METHODS

Mice. Tg mice expressing human ACE2 were generated as previously described (32). Among the five established Tg lineages, three (i.e., AC12, AC50, and AC70) and two (i.e., AC22 and AC63) were susceptible and resistant to lethality in response to SARS-CoV, respectively (Table 1). The tissue expression profiles of hACE2 in AC22 and AC70 mice were developed following semiquantitative reverse transcription-PCR (RT-PCR) by using hACE2-specific primers (forward, 5′-AGGATGTGCGAGTGGCTA-3′; reverse, 5′-AGGGCCATCAG GATGTCC-3′), as we previously described (35). For this study, we chose the AC70 and AC22 lineages, which are two of the most stable lines with regard to hACE2 expression, breeding efficiency, and litter size.

TABLE 1. Differential outcome of hACE2 Tg mouse lineage to SARS-CoV infection

Transgenic lineage	$TCID_{50}$ (log_{10}) of SARS-CoV (Urbani strain)	Morbidity (weight loss, etc.)	Mortality (%)	Mean survival time (days p.i.)
AC70	3	+	100	6.2
AC50	3	+	100	6.9
AC12	3	+	100	4.5
AC22	6	+	0	—[a]
AC63	6	+	0	—

[a] —, not applicable.

In some experiments, $CD8^+$ T-cell-depleted Tg AC22 mice were used for assessing the role of this T-cell subset in the host responses against SARS-CoV infection. To deplete $CD8^+$ T cells, we injected (intraperitoneally [i.p.]) two doses (50 μg/per dose, 3 days apart) of anti-mouse CD8 monoclonal antibody (clone 2.43) or an isotype-matched rat immunoglobulin G (IgG) antibody (clone SFR8) as controls. The extent of depletion was assessed at day 2 after the last antibody treatment by obtaining splenocytes and analyzing them for the presence of $CD3^+$ $CD8^+$ T cells by flow cytometry. To ensure a persistent state of cell depletion during the course of SARS-CoV infection, we treated Tg AC22 mice with either anti-CD8 antibody or control antibody at days −4, −1, +2, +5, and +8, where day 0 was defined as the time of SARS-CoV challenge.

SARS-CoV and cells. The Urbani strain of SARS-CoV, kindly provided to us by T. G. Ksiazek, Centers for Disease Control and Prevention (Atlanta, GA), was used throughout this study. Vero E6 cells (ATCC) were used for virus infectivity assays. The original stock of SARS-CoV, designated passage 1, received two additional passages in Vero E6 cells. The titer of this last passage 3 was determined and expressed as $TCID_{50}$/ml, and the virus was stored at −80°C, and used throughout this study. All experiments involving infectious virus were conducted at the University of Texas Medical Branch, Galveston, TX, under an animal use and care protocol approved by the University of Texas Medical Branch IACUC in AALAC-accredited animal biosafety level 3 and biosafety level 3 laboratories.

Infection of mice, body weight, illness score, and mortality. Anesthetized Tg mice, their non-Tg littermates, and $CD8^+$ T-cell-depleted Tg mice, ranging from 8 to 20 weeks of age, were infected intranasally (i.n.) with 60 μl of SARS-CoV in phosphate-buffered saline (PBS) that contained the indicated doses of infectious virus. Control mice were inoculated with the same volume of PBS. Infected mice were weighed daily to allow us to monitor disease progression. In addition, the severity of illness in infected mice was scored independently by two investigators who used a previously described (9), standardized 1-to-5 grading system as follows: 0, healthy; 1, barely ruffled fur; 2, ruffled fur but active; 3, ruffled fur and inactive; 4, ruffled fur, inactive, and hunched; and 5, dead. In some experiments, infected mice were sacrificed at indicated time intervals to obtain lungs and brains for determining viral infectivity titers, staining for viral antigen by immunohistochemistry (IHC), profiling the inflammatory responses, and analyzing the histopathology. We also harvested the spleens of uninfected and SARS-CoV-infected Tg mice at days 2 and 4 in separate experiments to determine CD4 T-cell, CD8 T-cell, B-cell, and non-T- non-B-cell subsets and their response to concanavalin A (ConA) stimulation as described below.

Virus titers in the lungs and brains of infected mice. The lungs and brain specimens obtained from mice at the indicated time points after infection were weighed and homogenized in a PBS–10% fetal calf serum solution using the TissueLyser-Qiagen (Retsch, Haan, Germany) to yield 10% tissue suspensions. After clarification by centrifugation, serial 10-fold dilutions of the tissue suspensions were prepared and assayed in Vero E6 cells to determine viral titers (32). The titers of individual samples were expressed as $TCID_{50}$ per gram of tissues.

IHC and histopathology. Lung and brain tissues, obtained as described above, were fixed in 10% neutral buffered formalin, embedded in paraffin, and processed for the subsequent IHC and histopathology studies, as described previously (32). Briefly, 5-μm sections were used to detect the expression of SARS-CoV nucleocapsid (N) protein by using standard IHC by sequential incubation with rabbit-specific anti-SARS-CoV N protein antibody, phosphatase-conjugated secondary antibodies, and naphthol-fast red (as a substrate). Slides were coun-

terstained with hematoxylin, and antigen expression was examined under different magnifications. The hematoxylin-eosin-stained paraffin sections were used for routine histopathological studies.

Cytokine and chemokine profiling. Gamma-irradiated lung and brain homogenates were subjected to inflammatory profiling by using the 23-plex Cytometric Bead Array (Bio-Rad, Hercules, CA), as described previously (32).

Flow cytometry and ConA-induced proliferation of splenocytes. Splenocytes were stained for fluorescein isothiocyanate- or phycoerythrin-conjugated anti-CD3, -CD4, -CD8, -B220, and -CD14 and their corresponding isotype-matched control antibodies (all from CalTag Laboratories). These samples were then analyzed with FACScan and CelQuest software (BD Biosciences), as described previously (33). For determining the capacity of splenocytes to proliferate in response to mitogen stimulation, we cultured 2×10^5 cells/200 μl in triplicate in 96-well, U-bottomed microtiter plates in the presence or absence of ConA (2.5 μg/ml; Sigma-Aldrich) for 3 days. The cultures were pulsed with 1 μCi/well [^{3}H]thymidine (New England Nuclear) for the last 12 to 16 h in the culture. The total incorporation of [^{3}H]thymidine was determined by liquid scintillation counting and expressed as counts per minute (cpm) or stimulation index, which was calculated as total cpm of ConA-stimulated cells/total cpm of unstimulated cells.

Statistical analysis. Statistical analyses were performed by using a two-tailed, unpaired Student *t* test. Unless otherwise indicated, means ± standard errors of the means are shown.

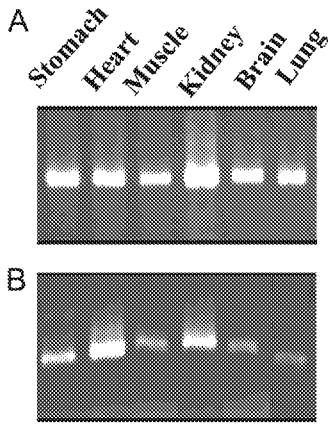

FIG. 1. Tissue expression profile of hACE2 in the Tg mouse lineages AC70 (A) and AC22 (B). DNA-free RNAs extracted from different organs of Tg mice at 6 to 8 weeks of age were subjected to RT-PCR analysis to evaluate the expression of hACE2 mRNA. The RT-PCR products were analyzed on 2% agarose gels. The data shown are representative of two independently conducted experiments.

RESULTS

Differential susceptibility of hACE2 Tg mice of different lineages to SARS-CoV infection. Our observations on the pathogenesis of SARS-CoV infection in two different lineages of hACE2 Tg mice, i.e., the AC70 and AC63 lineages, were reported previously (32). We continued to characterize the remaining three lineages with regard to their susceptibility, clinical manifestations (i.e., ruffled fur, lethargy, rapid and shallow breathing, and weight loss), and mortality, if any. As summarized in Table 1, all Tg lineages established were highly susceptible to infection compared to their non-Tg littermates. Like that of AC70 mice, infection of AC50 and AC12 mice with 10^3 $TCID_{50}$ of SARS-CoV resulted in the mice developing an acute wasting syndrome and eventually succumbing to the infection with a 100% mortality rate, with a mean survival time of less than 1 week postinfection (p.i.). While the 50% lethal dose of SARS-CoV for AC70 mice was approximately 1.7 log units, the prospective 50% lethal doses for AC50 and AC12 mice were not determined, largely due to the scarcity of the available animals. In contrast to the lethality-susceptible lineages, AC22 mice, following infection with up to 10^6 $TCID_{50}$ of SARS-CoV, survived despite exhibiting weight loss and other signs of clinical illness. Importantly, the transcriptional levels of hACE2 expression in various tissues of AC22 mice, especially those of the lungs and brain, were lower than those in Tg AC70 mice (Fig. 1). This observation was consistent with our earlier observation that Tg mice for which SARS-CoV infection was lethal (e.g., AC70 mice) had much higher levels of hACE2 expression than Tg mice for which infection was not lethal (e.g., AC63 mice) (32).

Differential SARS-CoV-induced morbidity and mortality between AC70 and AC22 mice. To further establish the possible correlates between host responses and such a strikingly different outcome of the infection, groups of 21 and 31 age-matched AC70 and AC22 mice, respectively, were inoculated (i.n.) with the same dose of SARS-CoV, i.e., 10^6 $TCID_{50}$/60 μl of SARS-CoV. Infected mice were monitored daily for morbidity and cumulative mortality, if any. In addition, three mice of each lineage were sacrificed at daily intervals until day 5 and three AC22 mice were also sacrificed at days 6, 8, 10, and 12, thereby allowing us to assess the infectivity titers of SARS-CoV in the lungs and brains, two of the most prominent tissues shown to support viral replication in our hACE2 Tg mice (32). As shown in Fig. 2, infected AC70 mice started to manifest various signs of illness, including ruffled fur, lethargy, rapid and shallow breathing, trembling, and immobility, accompanied by a relentless weight loss, starting at day 3 p.i. The weight loss of infected AC70 mice at day 3 was approximately 15%, and it reached up to 20% of the animals' total body weight before death by day 6 p.i. Despite their susceptibility to the infection, as evidenced by the progressive weight loss, which could reach an average of about 30%, infected AC22 mice gradually regained the lost weight, starting at day 9 p.i. In addition, infected AC22 mice exhibited a much milder disease than the infected AC70 mice and eventually recovered without suffering any mortality, which suggested to us that this lineage was indeed resistant to lethal infection by SARS-CoV.

Kinetics of viral replication in the lungs and brain. Based on the striking differences in the clinical symptoms as well as the mortality after SARS-CoV infection in AC70 and AC22 mice, we compared the kinetics and distribution of SARS-CoV replication in the lungs and brains between these two lineages. Viral replication in the lungs reached a maximum at day 1 p.i., in which averages of $10^{8.5}$ and $10^{8.7}$ $TCID_{50}$ SARS-CoV/gram were recovered from AC70 and AC22 mice, respectively, and gradually declined thereafter (Fig. 3). However, at day 5 p.i. a significantly higher level of viral replication was sustained in the lungs of a single surviving AC70 mouse than in any AC22 mice. A low-grade viral replication in the lungs of some infected AC22 mice continued until day 8 p.i. In contrast to the subtle dissimilarity of the viral replication in the lungs, the magnitudes and kinetics of viral infection within the brains of these two lineages were remarkably different. Specifically, a low level of infectious virus ($\sim 10^{2.7}$) was first detected in the brains of infected AC70 mice at day 2. Viral replication within this tissue proceeded rapidly thereafter, reached a maximum of $\sim 10^8$ $TCID_{50}$/g at day 3, and remained prominent through day

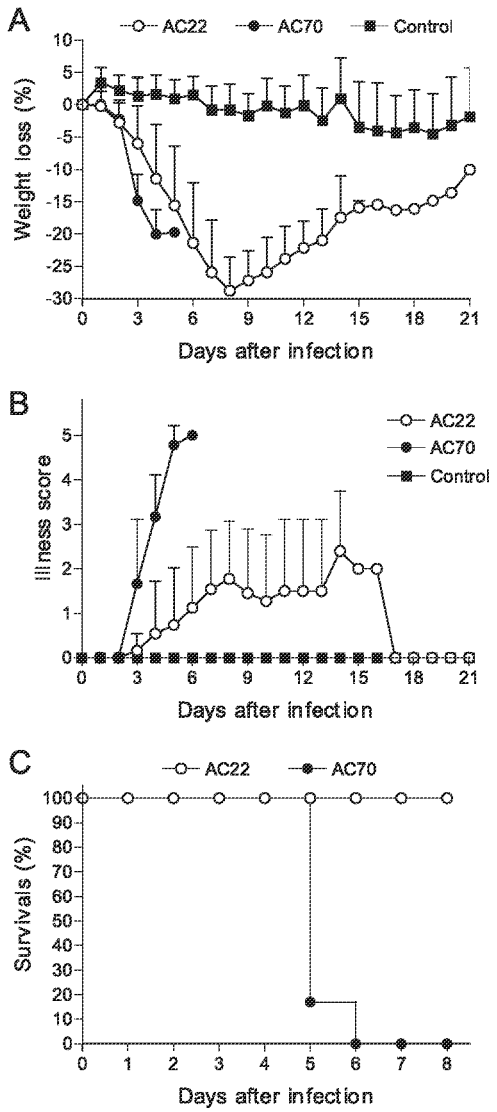

FIG. 2. Differential outcomes of SARS-CoV infection in AC70 and AC22 Tg mice. Groups of hACE2 Tg and age-matched non-Tg mice (control) (n = 14 to 31 mice/group) were infected intranasally with 10^6 $TCID_{50}$ of SARS-CoV (Urbani strain). The severity of clinical illness, i.e., weight loss (A), average illness score (B), and cumulative mortality (C), of infected mice was recorded daily as described in Materials and Methods. Error bars indicate standard deviations.

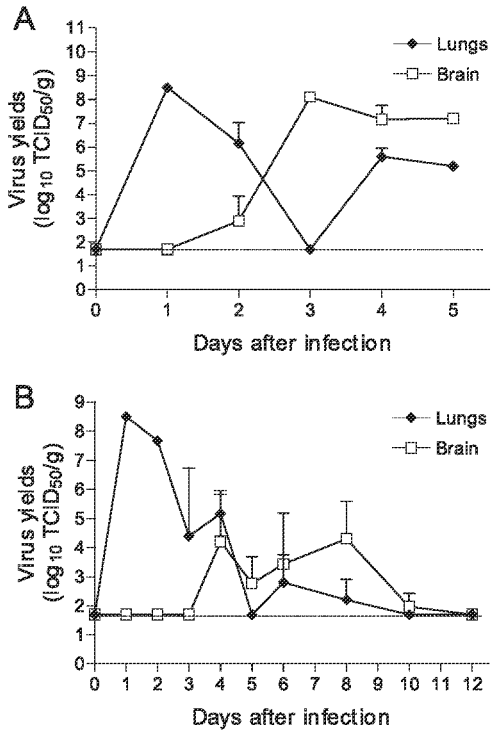

FIG. 3. Kinetics of viral replication in the lungs and brains of SARS-CoV-infected AC70 and AC22 mice. AC70 (A) and AC22 (B) mice were infected with SARS-CoV as described for Fig. 1. Three mice from each group were sacrificed at the indicated days after infection for determining infectious virus titers in the lungs and brains by the standard infectivity assay in Vero E6 cells. The viral titers were expressed as $\log_{10}$ $TCID_{50}$ virus per gram of tissue. Data are shown as means ± standard deviations for three animals at each time point, except for AC70 mice at day 5, where only one mouse survived the infection.

5, at which time a titer of ~10^7 $TCID_{50}$/g was routinely recovered. In contrast, SARS-CoV replication in the brains of infected AC22 mice was relatively benign, in that a modest level of infectious virus (~10^4) was initially demonstrated on day 4 and gradually declined to a barely detectable level at both days 8 and 10 p.i.

IHC and histopathology. The differential kinetics of viral replication and the final outcomes of SARS-CoV infection exhibited by AC70 and AC22 mice prompted us to investigate the temporal and spatial patterns of viral distribution and the pathological changes within the lungs and brains of infected animals. IHC staining for the SARS-CoV N protein clearly indicated that bronchiolar and alveolar epithelial cells and the neuronal cells were the primary targets of SARS-CoV infection in both Tg lineages. As shown in Fig. 4, an intense expression of viral antigen was first detected in the cytoplasm of bronchial epithelial cells, and occasionally in alveolar epithelial cells, at day 1. This viral antigen subsequently spread to the alveolar epithelial cells at day 2 but was undetectable within the lungs at day 3 and day 4 p.i. for the AC70 and AC22 mice, respectively. In contrast to its early expression within the lungs, SARS-CoV N protein in the brain was not detected until day 3 and day 4 in infected AC70 and AC22 mice, respectively (Fig. 5). Sustained expression of viral antigen was demonstrated until days 5 and 10 in the brains of AC70 and AC22 mice, respectively. This temporal expression of viral protein in the brain, as detected by IHC, was largely consistent with that revealed by the viral infectivity titers (Fig. 3).

Histopathological studies of the lungs did not reveal any obvious difference in the pulmonary pathologies between these two lineages, even though the infection-associated pathological process was faster in the AC70 than in the AC22 mice. Pathological changes in the lungs of both lineages started on day 1 and were characterized by a minimal-to-mild perivascular and peribronchiolar inflammatory infiltration, accompanied by the swelling and blebbing of epithelial cells lining bronchi and bronchioles (Fig. 6). Such a pathological process was followed by the accumulation of cell debris, necrotic epithelial cells, and

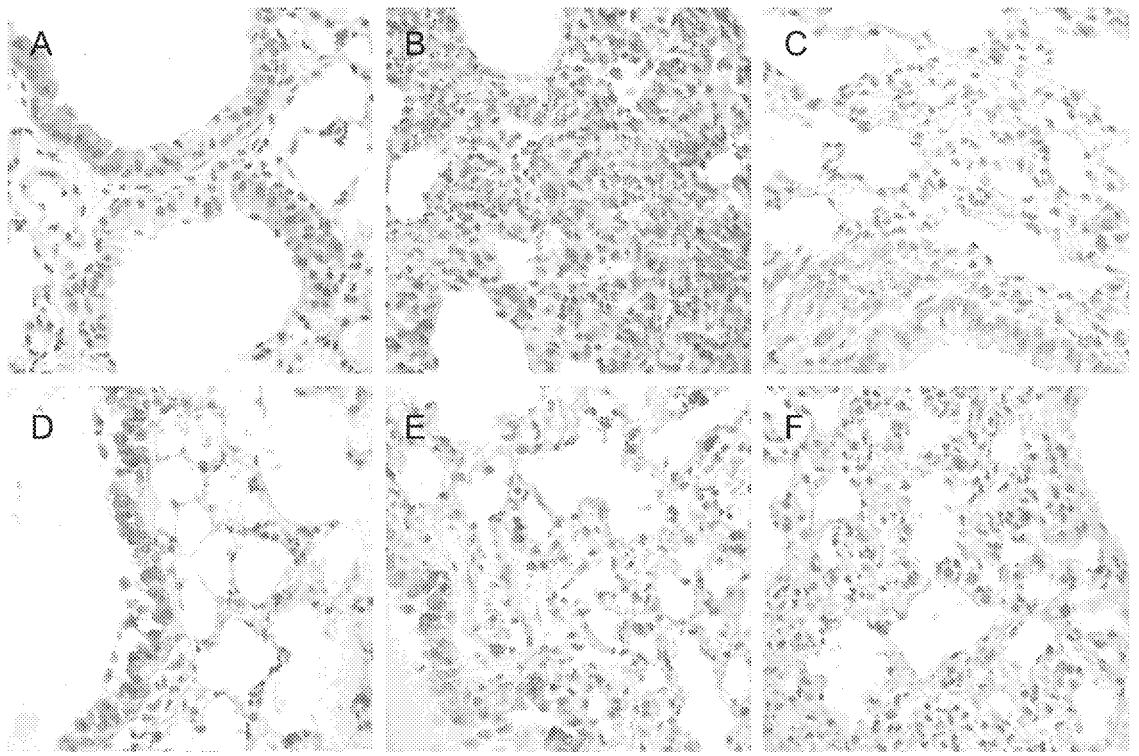

FIG. 4. SARS-CoV antigen expression in the lungs of AC70 and AC22 mice. Paraffin-embedded lung sections of SARS-CoV-infected AC70 mice (A to C) and AC22 mice (D to F) were analyzed for the expression of SARS-CoV nucleocapsid protein by IHC, as described in Materials and Methods. Profound viral infection, as indicated by the intense staining of viral antigen (red), was first detected in the cytoplasm of bronchial epithelial cells (A and D) at day 1, subsequently spread to the alveolar epithelial cells at day 2 (B and E), and subsided to either an undetectable level (C) or a lower level (F) in AC70 and AC22 mice at day 3, respectively. Original magnifications, ×40.

inflammatory cells within the bronchiolar lumen, along with interstitial thickening on day 2 p.i. As a consequence of reduced cellular infiltration, the interstitial thickening gradually subsided on day 3 in infected AC70 mice. In contrast, interstitial thickening intensified in infected AC22 mice until day 4 p.i. and was accompanied by infiltrating macrophages, pyknotic cells, and necrotic cells within the alveolar spaces. Mild inflammatory responses remained detectable in some areas of the lungs until day 4 and day 6 p.i for AC70 and AC22 mice, respectively.

Compared to the relatively indistinguishable pulmonary pathologies, a substantial difference in the pathologies of the brain was apparent between these two Tg lineages. Specifically, perivascular cuffing in the meninge and brain in the absence of other signs of inflammation was demonstrated infrequently in some AC70 mice at day 3 or day 4 p.i. (Fig. 7). In contrast, prominent perivascular lymphocytic cuffing in the meninge was consistently observed in AC22 mice on day 4 and spread to the brain parenchyma by day 5, where it was accompanied by a time-dependent infiltration of mononuclear cells within the central nervous system (CNS) until day 21 p.i., at which point the experiment was terminated. These results suggested that AC22 mice were superior to AC70 mice in mounting a full spectrum of inflammatory responses upon challenge by SARS-CoV.

Cytokine profiling of SARS-CoV-infected AC70 versus AC22 mice. SARS pathogenesis likely stems from exuberant acute inflammatory responses within the lungs (23). Our results that revealed the differential clinical and pathological outcomes between AC70 and AC22 mice in response to SARS-CoV infection led us to profile SARS-CoV-induced cytokine responses in these two Tg lineages by using BioPlex analysis. The results showed that AC70 mice were capable of secreting elevated levels of interleukin-12 p40 (IL-12p40), KC, RANTES, and monocyte chemoattractant protein 1 (MCP-1) in the lungs at at least one time point during the course of a 5-day infection, However, AC22 mice appeared to be more immunologically competent in mounting inflammatory responses, resulting in the production not only of the aforementioned cytokines at higher levels but also of three additional cytokines (IL-1α, IL-1β, and IL-6) that were not detected in infected AC70 mice (Fig. 8). Other cytokines, including IL-2, IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, gamma interferon, and tumor necrosis factor alpha, were not detected.

Cytokine responses were also measured in the brains of infected animals. The production of a total of 13 out of 23 cytokines that can be measured simultaneously, including IL-1α, IL-1β, IL-6, IL-8 (KC), IL-9, IL-10, IL-12p40, MIP-1α, MIP-1β, MCP-1, eotaxin, granulocyte colony-stimulating factor, and RANTES, was significantly induced in the brains of both Tg lineages at at least one time point during the entire course of infection (Fig. 9). Additionally, the kinetics and the magnitudes of the cytokine responses within each lineage appeared to positively correlate with the extent of virus replication (Fig. 3). However, there was no direct

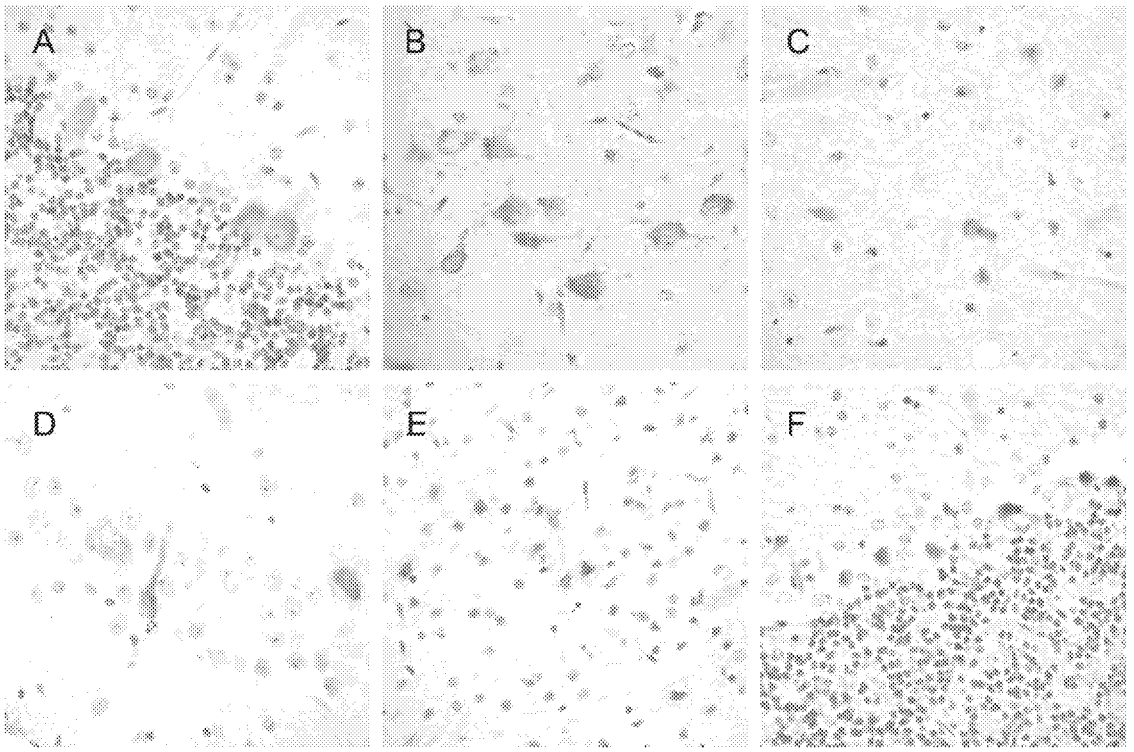

FIG. 5. SARS-CoV antigen expression in the brains of AC70 and AC22 mice. The brains of infected AC70 (A to C) and AC22 (D to F) mice were fixed, sectioned, and processed for the staining of SARS-CoV N protein as for Fig. 3. Viral antigen could be consistently detected in many neuronal cells of AC70 mice from day 3 (A) and remained readily detectable at days 4 and 5 (B and C). The earliest time for detecting viral antigen in the neuronal cells of infected AC22 mice was day 4 (D), and it remained detectable at days 6 and 10 after infection (E and F). Original magnifications, ×40.

correlation between the extent of viral replication and the magnitude of inflammatory responses when these two Tg lineages were compared. Specifically, despite much higher viral titers (~4 log units) detected in the brains of AC70 mice than in those of AC22 mice, such an overwhelming viral infection in AC70 mice failed to induce inflammatory cell infiltrates, a finding which was readily demonstrable in AC22 mice, in this organ (Fig. 7).

Alteration of the lymphocyte subsets and ConA-induced proliferation of splenocytes of infected mice. Many viruses are capable of immunoevasion to establish their infection. The compromised ability of infected AC70 mice to elicit a full-blown inflammatory response in the brain led us to question whether SARS-CoV could induce a generalized immunosuppression in the infected hosts. To evaluate the impact of SARS-CoV infection on the host immune response, both uninfected and infected AC22 and AC70 mice were sacrificed at days 2 and 4 p.i. for assessing the lymphocyte subsets and ConA-induced proliferation of splenocytes. While SARS-CoV infection did not result in any noticeable change in the total number of cells recovered and their constituents of the lymphocyte subsets at day 2 p.i. (data not shown), it caused a significant reduction in the total number of splenocytes, especially those of selected lymphocyte subsets, in these two lineages of Tg mice at day 4 p.i. compared to equivalent findings in uninfected mice (Table 2). Specifically, while SARS-CoV infection significantly reduced the number of recovered CD4 T cells ($P = 0.005$) in AC22 mice, it spared any profound impact on the total numbers of splenocytes, CD8 T cells, B cells, and non-T non-B cells. In contrast, SARS-CoV infection exerted a more profound impact in AC70 mice beyond significantly reducing the number of CD4 T cells in the spleen ($P = 0.025$). In fact, it also caused significant reductions in the total splenocytes ($P = 0.032$) and the CD8 T cells ($P = 0.001$). This marked reduction in the total numbers of T cells, especially the $CD8^+$ subset, in infected AC70 mice was further underlined by the significantly increased CD4/CD8 ratio ($P < 0.018$) compared to that in uninfected mice. Interestingly, similar to the case for AC22 mice, the populations of B cells and non-T non-B cells in the spleens of AC70 mice were not significantly altered upon challenge by SARS-CoV.

In addition to causing a striking reduction in the numbers of selected T-cell subsets, SARS-CoV infection significantly impaired ConA-mediated proliferation of splenocytes of both AC22 and AC70 mice ($P < 0.01$) compared to that in uninfected mice (Table 3; Fig. 10). Importantly, such a compromised ConA-mediated proliferative response observed in infected mice appeared to be more severe in AC70 than in AC22 mice ($P < 0.01$). Because ConA is a T-cell-specific mitogen, the more intense defect of infected AC70 than AC22 mice in responding to this mitogen was consistent with the more profound loss of splenic T cells in infected AC70 than in AC22 mice, i.e., 53% and 29%, respectively, compared to their uninfected controls.

Protective role of $CD8^+$ T cells against SARS-CoV infection in AC22 mice. While SARS-CoV infection drastically reduced

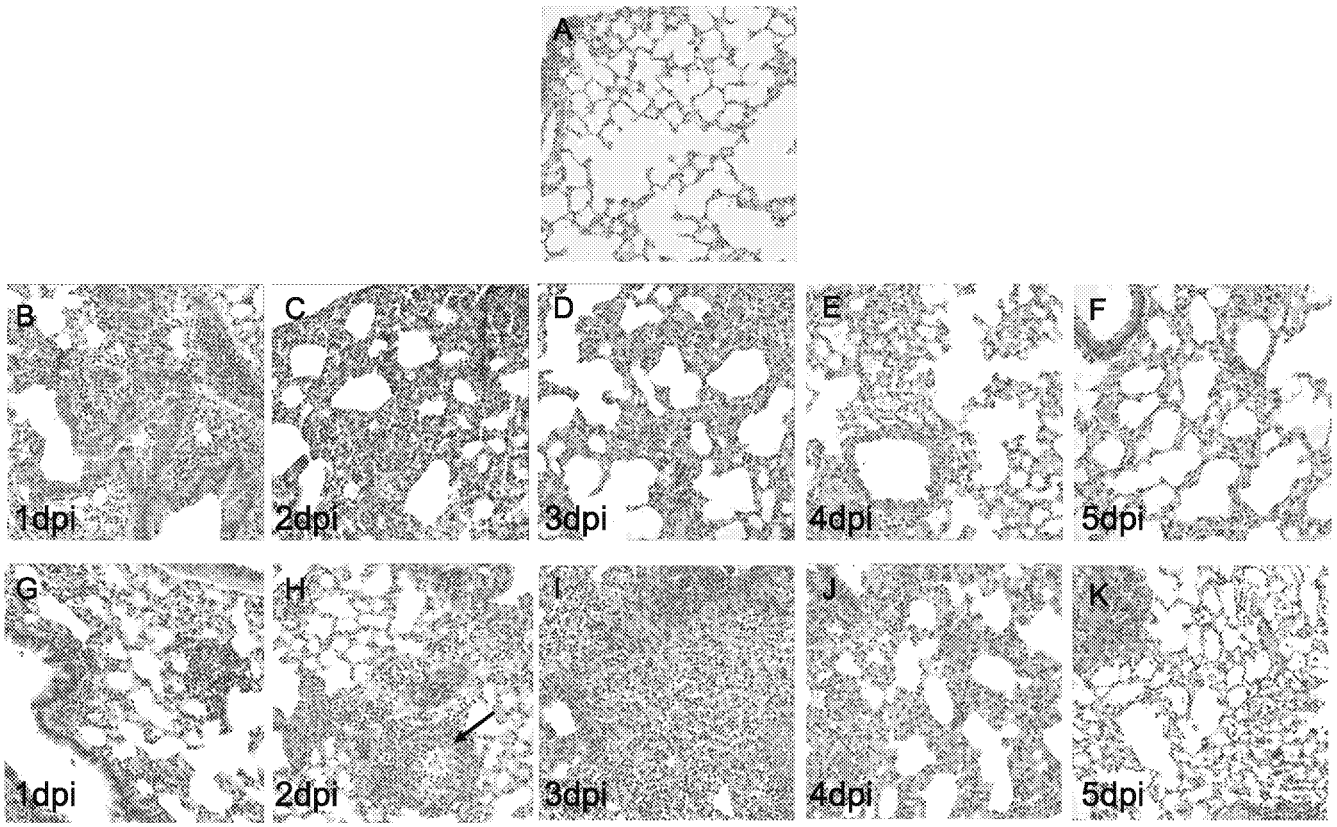

FIG. 6. Lung pathology of SARS-CoV-infected AC70 and AC22 mice. We examined paraffin-embedded, hematoxylin- and eosin-stained lung sections obtained from mock-infected (A) and SARS-CoV-infected AC70 (B to F) and AC22 (G to K) mice at the indicated time points after infection. Lung pathology in both lineages started at 1 day p.i. (dpi) (with mild mononuclear cell infiltration around blood vessels and bronchioles, accompanied by swelling and blebbing of epithelial cells of bronchi and bronchioles (B and G). Accumulation of cell debris within the lumen (arrow), interstitial thickening, and inflammatory cellular infiltrates were observed at day 2 (C and H). Peribronchial inflammation continued, as the damaged pneumocytes and disrupted epithelial lining were readily detectable through days 3 and 4 (B, I, E, and J) and gradually subsided thereafter, with a minimal-to-mild cellular infiltration observed at day 5 (F and K). Original magnifications, ×20.

the total numbers of $CD4^+$ and $CD8^+$ T-cell subsets in lethality-susceptible Tg AC70 mice, it significantly reduced only $CD4^+$, and not $CD8^+$, T cells in Tg AC22 mice (Table 2). To determine whether this largely intact $CD8^+$ T-cell subset might contribute to the relatively benign clinical and/or pathological phenotypes of infected AC22 mice, we infected CD8-depleted AC22 mice with SARS-CoV and monitored weight loss and the titers of infectious virus and pathology in the lungs and brain. As shown in Fig. 11A, treating Tg AC22 mice with only two doses of anti-mouse CD8 antibody, as described in Materials and Methods, was sufficient to deplete the great majority of CD8 T cells, compared to the numbers of these cells in control antibody-treated mice. Interestingly, depletion of CD8 T cells appeared to exacerbate pulmonary infection, as evidenced by the ~2-log-unit increase in the yields of infectious SARS-CoV within the lungs, but not the brain, at both days 2 and 4 p.i. (Fig. 11B), accompanied by the more prominent weight loss (Fig. 11C) and profound lung pathology (Fig. 11D) than those elicited in control antibody-treated mice. Taken together, these results suggest that this $CD8^+$ subset of T cells play a positive role in the host defense against SARS-CoV infection.

DISCUSSION

The data presented in this study greatly extend in at least seven ways our previous report concerning the differential pathogenesis of SARS-CoV infection in hACE2 Tg mouse lineages established in our laboratories (32). First, we demonstrated that among five lineages of hACE2 Tg mice, all of which exhibited clinical manifestations of various severity following SARS-CoV infection, mice three lineages (AC70, AC50, and AC12) inevitably died within a week after infection, whereas mice of the other two (AC63 and AC22) eventually recovered from the illness without suffering any mortality (Table 1). Such a strikingly different disease outcome elicited in these Tg mice in response to SARS-CoV infection provides an ample opportunity for studying the likely impact of the complex virus-host interactions on the pathogenesis of SARS-CoV in an animal model. Second, by using hACE2 Tg AC70 and AC22 mice as the lethal and nonlethal models of SARS-CoV infection, respectively, we established the kinetics of the disease course (i.e., illness score and weigh loss) and/or the rate of mortality (Fig. 2). These results clearly indicated that despite the similar onset and progression of clinical manifestations,

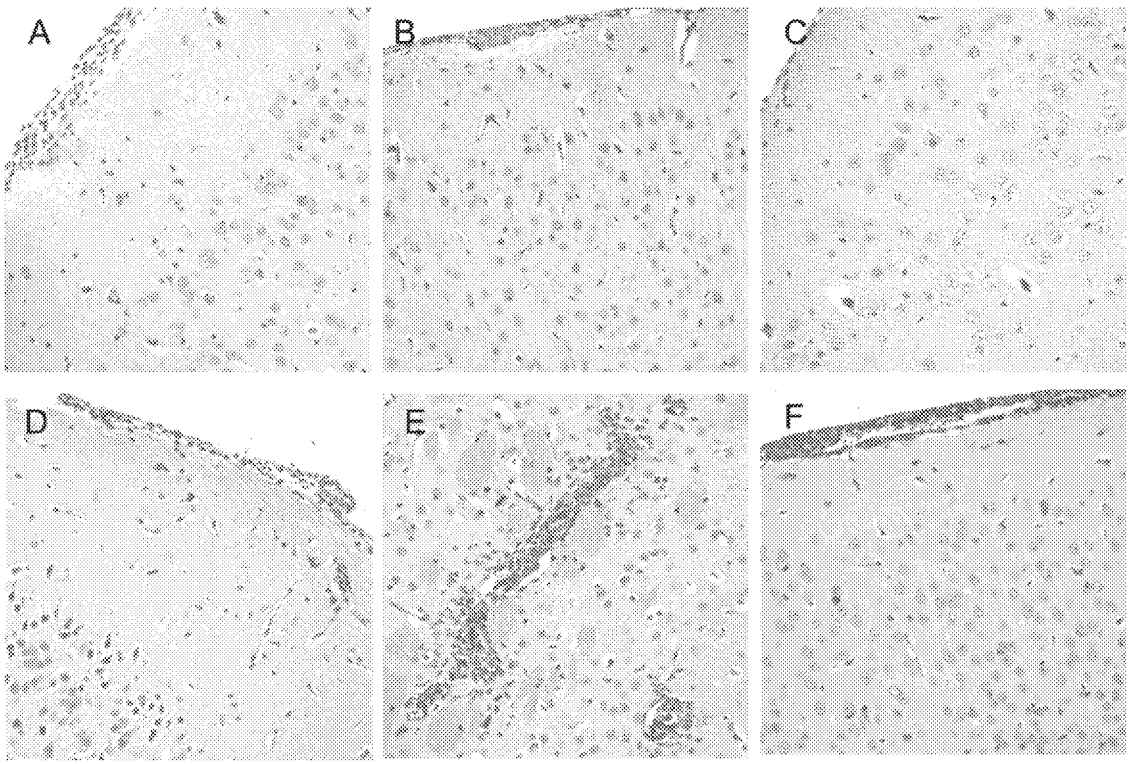

FIG. 7. Brain pathology of SARS-CoV-infected AC70 and AC22 mice. Brains harvested from infected mice daily from day 1 to 5 for both hACE2 lineages and every 3 to 4 days thereafter were paraffin embedded, sectioned, and stained with hematoxylin and eosin. No obvious brain pathology was observed prior to days 3 and 4 in infected AC70 and AC22 mice, respectively. Perivascular cuffing in the meninge was observed only in a single infected AC70 mouse at day 3 (A). Very little pathology, if any, could be detected in the brains of infected AC70 mice thereafter (B [day 4] and C [day 5]). In contrast, perivacular cuffing was consistently detected in all infected AC22 mice, starting at day 4 (D). A time-dependent and prominent inflammatory infiltration was observed at day 6 (E). Perivascular cuffing persisted through day 21 (F), when the study was terminated. Original magnifications, ×20.

infected AC22 mice exhibited much milder disease than did infected AC70 mice and completely recovered around day 17 p.i. Third, we showed the kinetics of replication and the cellular distribution of SARS-CoV within the lungs and brains, two of the most severely affected tissues, and identified bronchial epithelial cells, alveolar epithelial cells, and neuronal cells as the main target cells of SARS-CoV infection in both lineages (Fig. 3 to 5). Additionally, the significant delay in detecting brain infection in both lineages, compared to the early and intense viral replication within the lungs, clearly indicated that SARS-CoV infection was first established in the respiratory system before spreading to the CNS, an observation consistent with our previous finding and an earlier report by McCray and colleagues on hACE2 Tg mice (21, 32). However, after the same group presented a more thorough examination, they concluded that SARS-CoV enters the brains of K18-hACE2 Tg mice primarily via the olfactory bulb, resulting in extensive brain infection (22); whether this olfactory route of SARS-CoV entry could also be responsible for the subsequent intense brain infection in our Tg mice requires additional studies. Fourth, while the overall lung pathologies presented the two lineages were largely indistinguishable (Fig. 6), the pathological features within the brains were markedly different with regard to the cellular responses, in that infected AC22 but not AC70 mice consistently exhibited a time-dependent infiltration of inflammatory cells in the brains, as revealed by the prominent accumulation of mononuclear cells and activation of microglial cells within the CNS (Fig. 7). Fifth, despite the minimal cellular responses to SARS-CoV infection within the brains, AC70 mice were as capable as AC22 mice in producing elevated levels of proinflammatory cytokines and chemokines there in response to SARS-CoV infection (Fig. 9). However, AC22 mice appeared to be superior to AC70 mice in inducing inflammatory responses within the lungs, where there was a more intense secretion of an array of soluble inflammatory mediators, some of which were not detected in infected AC70 mice (Fig. 8). Sixth, our results clearly demonstrated that SARS-CoV infection in hACE2 Tg mice can cause a generalized suppression of host immunity, at least in part through the depletion of T cells (Tables 2 and 3; Fig. 10). Interestingly, the extent of T-cell loss, especially loss of the CD8 subset, appeared to positively correlate with the susceptibility of mice to the lethal SARS-CoV infection. Specifically, in contrast to the drastically reduced number of CD8 T cells in the lethality-sensitive AC70 mice, the number of this CD8 T-cell subset was basically not affected in the resistant AC22 mice. Finally, our study also identifies the positive role of CD8 T cells in protecting AC22 mice against excessive respiratory infection and pathology and the onset of illness (e.g., weight loss) (Fig. 11).

SARS has been generally recognized as an acute viral pneumonia, with the lungs as its main pathological target. However, we found that SARS-CoV, like other animal and human coro-

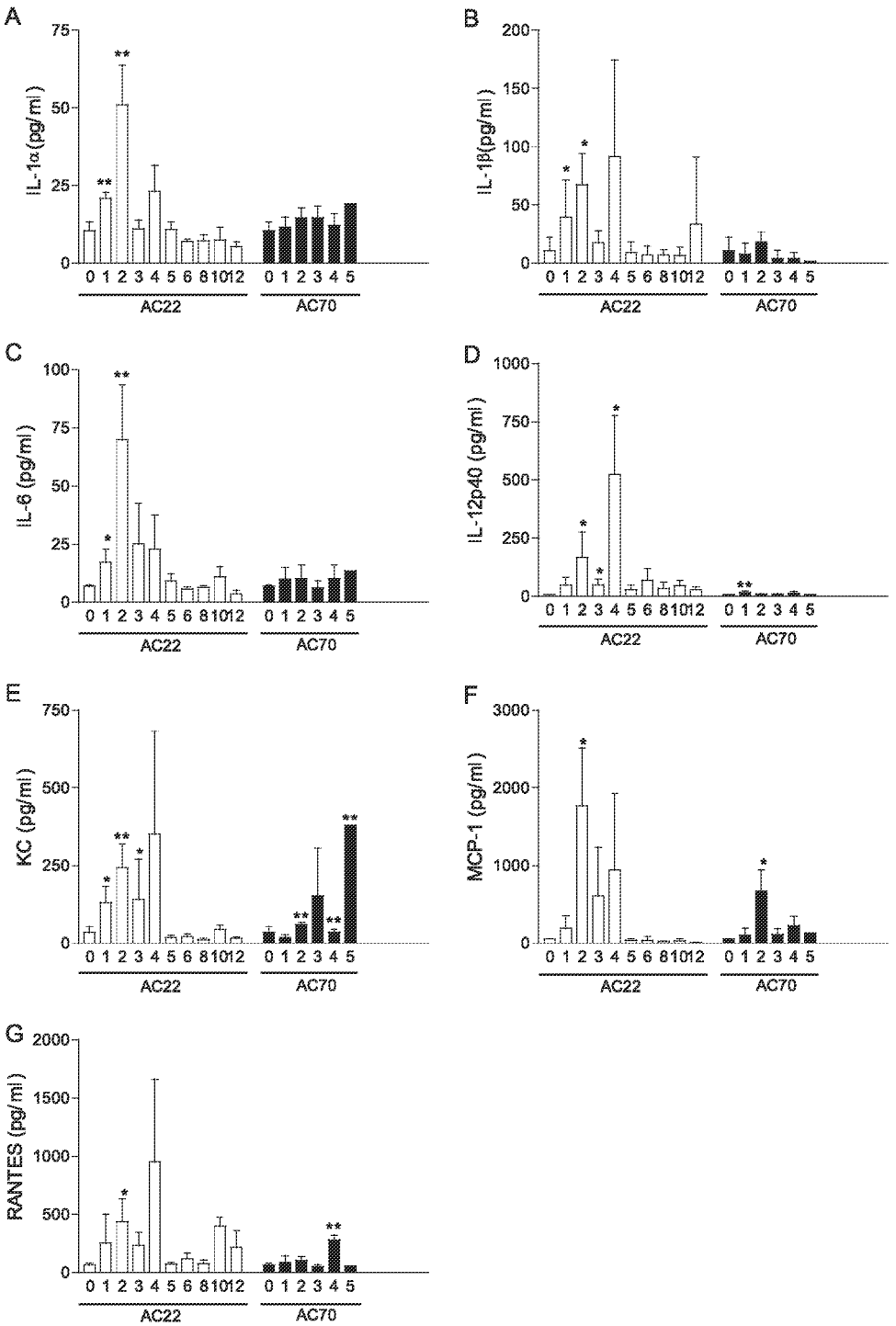

FIG. 8. Kinetics of the cytokine responses in the lungs of SARS-CoV-infected AC70 and AC22 mice. Lung homogenates derived from AC70 and AC22 mice at the indicated time points after infection were used to assess the levels of chemokines and cytokines by Bio-Plex analysis. Duplicate samples of individual specimens were assayed. Results are shown as means ± standard deviations for three animals at the indicated time points, except for day 5, at which only two AC70 mice that survived the infection were used. *, $P < 0.05$; **, $P < 0.01$ (Student's t test, compared to mock-infected mice).

naviruses, could infect the CNS in our Tg models. In fact, studies of brain sections obtained from SARS patients who died as a result of this disease have clearly demonstrated, by IHC, real-time PCR, in situ hybridization, and electron microscopy, the expression of SARS-CoV exclusively within the neuronal cells (5, 10, 40). The susceptibility of neuronal cells to SARS-CoV infection has been underscored by recent studies using both wild-type and hACE2 Tg mice infected with either

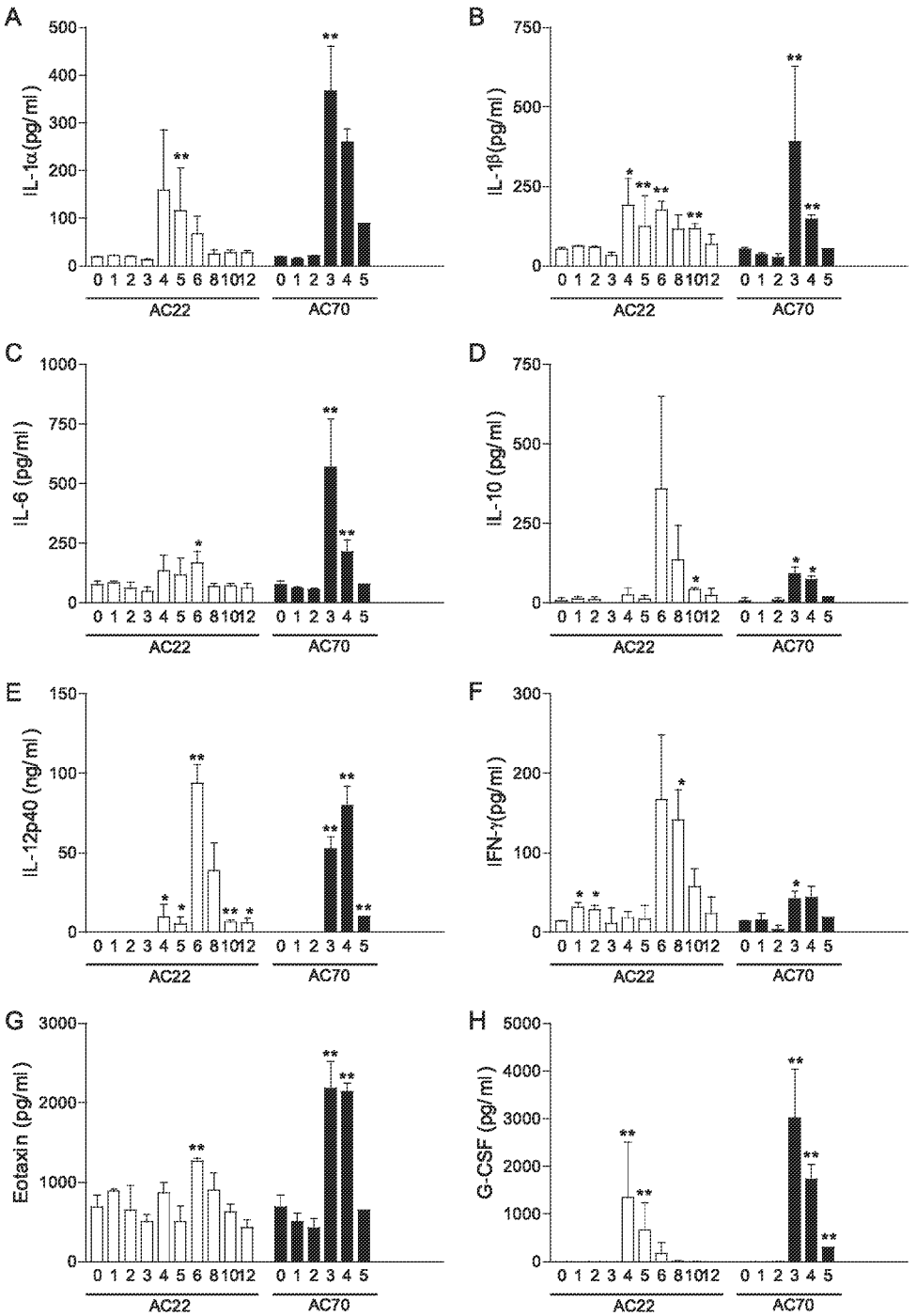

FIG. 9. Kinetics of the cytokine responses in the brains of SARS-CoV-infected AC70 and AC22 mice. Homogenates of the brains harvested from AC70 and AC22 mice at the indicated time points after SARS-CoV infection were used to measure the expression of various cytokines and chemokines by Bio-Plex analysis. Duplicate samples of individual specimens were assayed. Results are shown as means ± standard deviations for three animals at the indicated time points, except for day 5, at which only two AC70 mice that survived the infection were used. *, $P < 0.05$; **, $P < 0.01$ (Student's t test, compared to mock-infected mice).

the clinical isolates or mouse-adapted SARS-CoV (11, 26, 29, 35; C.-T. K. Tseng et al., unpublished data). In addition, two of the four human glioma cell lines tested in our laboratories appeared to be permissive to productive SARS-CoV infection (Tseng et al., unpublished data). Thus, identification of the neuronal cells as the major target of SARS-CoV infection in the brains of both lineages of Tg mice further confirmed their permissiveness to this CoV infection.

Early pathological studies with lung specimens obtained from the patients who died of SARS and in whom the disease progressed slowly identified type I and II alveolar pneumocytes and, possibly, pulmonary macrophages as the primary targets

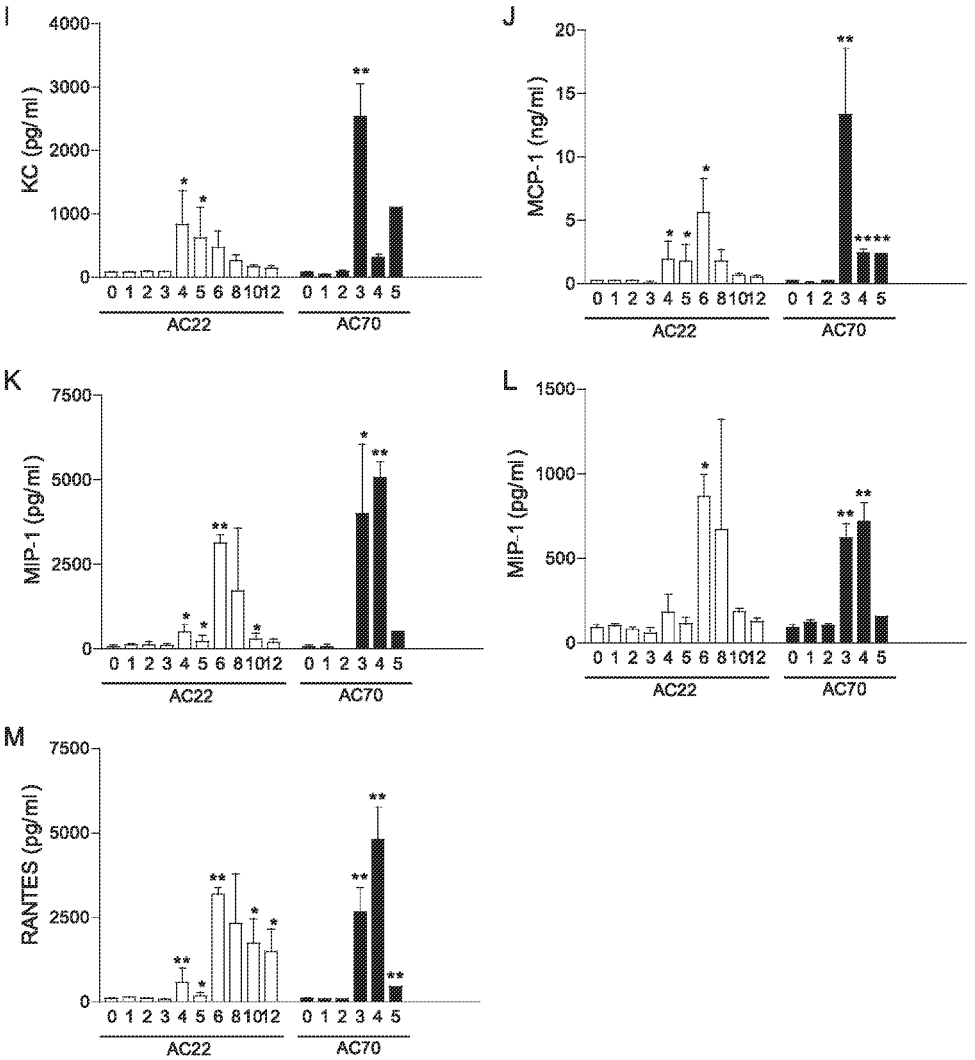

FIG. 9—*Continued.*

of SARS-CoV infection (2, 24, 31). However, the possibility that the pathogenesis might initiate within the respiratory bronchioles came about due to the revelation of prominent bronchitis with a marked necrosis of epithelial cells, loss of cilia, squamous metaplasia, and fibrin deposition within the bronchi in the lungs of patients who died following a more rapid clinical course of SARS (7, 24). Furthermore, human primary bronchial and other ciliated airway epithelial cells

TABLE 2. Total cell counts and lymphocyte subsets in the spleens of SARS-CoA-infected AC70 and AC22 mice and mock-infected control mice

Cells	AC22 mice					AC70 mice				
	No. of cells (10^6) or ratio[a]				P[b]	No. of cells (10^6) or ratio				P
	Mock infection		SARS infection			Mock infection		SARS infection		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Splenocytes	130.0	10.0	130.0	10.0	0.698	166.7	11.5	100.0	28.3	0.032
CD4 cells	41.4	2.5	25.4	2.7	0.005	52.3	3.9	32.1	7.5	0.025
CD8 cells	19.9	0.7	18.1	4.2	0.652	31.0	1.9	7.0	0.1	0.001
CD4/CD8 ratio	2.1	0.0	1.4	0.3	0.095	1.7	0.2	4.6	1.2	0.018
B cells	49.4	2.9	58.7	9.4	0.278	58.1	1.0	43.5	9.6	0.060
Non-T non-B cells	24.3	1.0	27.9	7.5	0.599	25.2	8.7	17.4	11.4	0.414

[a] Means and standard deviations are for three or four mice.
[b] Student's *t* test for mock-infected versus infected mice.

TABLE 3. ConA-stimulated proliferation of splenic T cells in uninfected and SARS-CoV-infected AC70 and AC22 mice

Mice	Mock infection						SARS-CoV infection					
	[^{3}H]thymidine uptake (cpm)				SI[a]		[^{3}H]thymidine uptake (cpm)				SI	
	Stimulated cultures		Unstimulated cultures				Stimulated cultures		Unstimulated cultures			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AC70	4,370	1,790	86	39	54	27	552	246	138	138	6.0	3
AC22	4,700	725	68	14	70	15	1,845	841	83	19	24	13

[a] SI, stimulation index (cpm of ConA-stimulated cultures/cpm of unstimulated cultures).

have also been demonstrated, in vitro, to be permissive to productive SARS-CoV infection (14, 27, 34). Thus, our IHC study results, which revealed epithelial cells lining the respiratory tract, especially the bronchi and bronchioles, and alveolar epithelial cells as the prime cells harboring SARS-CoV, led us to suggest that SARS-CoV infection in hACE2 Tg mice may induce a faster course of clinical illness, perhaps similar to that in SARS patients having a rapidly progressing form of the disease.

SARS has been proposed to stem from exuberant innate inflammatory responses with diffuse alveolar damages as the most characteristic pathological feature (6, 7, 24). Specifically, SARS-CoV infection has been reported to minimally induce the expression of antiviral cytokines (e.g., interferons and IL-12p40), moderately upregulate the expression of proinflammatory cytokines (e.g., tumor necrosis factor alpha and IL-6), and significantly promote the production of proinflammatory chemokines (e.g., MIP-1a, IP-10, RANTES, and MCP-1) in patients (17, 28). Elevated and prolonged expressions of chemokines (i.e., MIP-1, IP-10, CXCL8, and CXCL9) have been

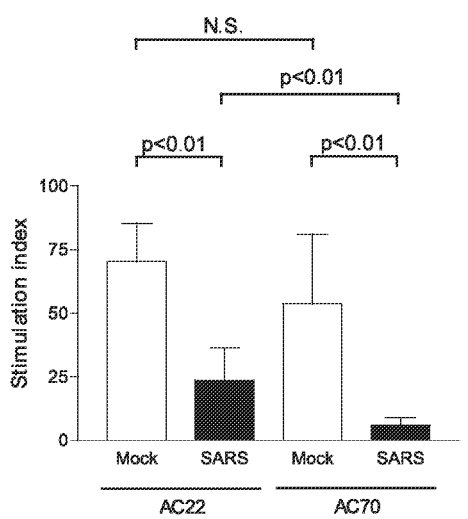

FIG. 10. SARS-CoV significantly inhibits ConA-mediated proliferation of T cells in infected AC70 and AC22 mice. AC70 and AC22 mice were either uninfected or infected (i.n.) with 10^6 $TCID_{50}$ SARS-CoV. Splenocytes were prepared from individual mice and tested for their proliferation in response to ConA (2.5 μg/ml) stimulation, as described in Materials and Methods. Student's *t* test was used to determine the *P* values between the indicated groups for statistical significance. N.S., not significant. Error bars indicate standard deviations.

detected not only in SARS patients but also in experimentally infected wild-type and lethality-sensitive hACE2 Tg mice (8, 10, 13, 32, 35, 36, 38). In this study, we extended this observation to hACE2 Tg mice that were resistant to the lethal SARS-CoV infection. Interestingly, while the kinetics and magnitude of the cytokine responses within each lineage appeared to positively correlate with the extent of viral replication in each tissue, no such correlation in the brain could be observed when findings for these two Tg lineages were compared.

The cellular sources and the overall impact of these virally induced inflammatory mediators (Fig. 8 and 9) on the pathogenesis and/or clearance of SARS-CoV remain to be determined. Neuronal cells have recently been shown to release abundant IL-6 in SARS-CoV-infected K18-hACE2 mice that rapidly succumbed to infection with minimal cellular infiltration within the brain (22). Thus, the overwhelming viral infection in the absence of readily detectable cellular infiltrates within the brains of infected AC70 mice makes neuronal cells and, possibly, other brain cells the likely producers of these inflammatory mediators within the brain. Despite the less profound brain infection, SARS-CoV-infected AC22 mice consistently showed a time-dependent infiltration of inflammatory cells. Thus, it is likely that infiltrating cells might effectively make up the shortfall of cytokine responses elicited by moderately infected brain cells in this Tg lineage. The ability to elicit an optimal acute inflammatory response is essential, not only to limit early microbial infections but also to ensure the onset of adaptive responses to effectively resolve the infections. However, an excess inflammatory response often leads to immune-mediated pathology and diseases. Thus, it is tempting to hypothesize that the highly elevated levels of inflammatory mediators detected in our study might contribute to exacerbated clinical and pathological outcomes of SARS-CoV-infected Tg mice. While it is highly desirable to determine which cytokine(s), alone or in combination, is likely to be responsible for the onset of clinical illness and even death in infected Tg mice, choosing which cytokine(s) from minimums of 7 and 13 potential candidates within the lungs and brain, respectively, is a major undertaking and is beyond the scope of this study.

It has been shown that SARS-CoV infection in clinical patients was accompanied by a transient, but extensive, lymphopenia with a preferential reduction in the number of CD4 and CD8 T cells (4, 12, 18, 39). Importantly, the severity of T-cell loss has been positively correlated with an adverse outcome in SARS patients. Thus, a much more pronounced T-cell loss in the lethality-susceptible AC70 mice than in the lethality-resistant AC22 mice extends this correlation to the mouse

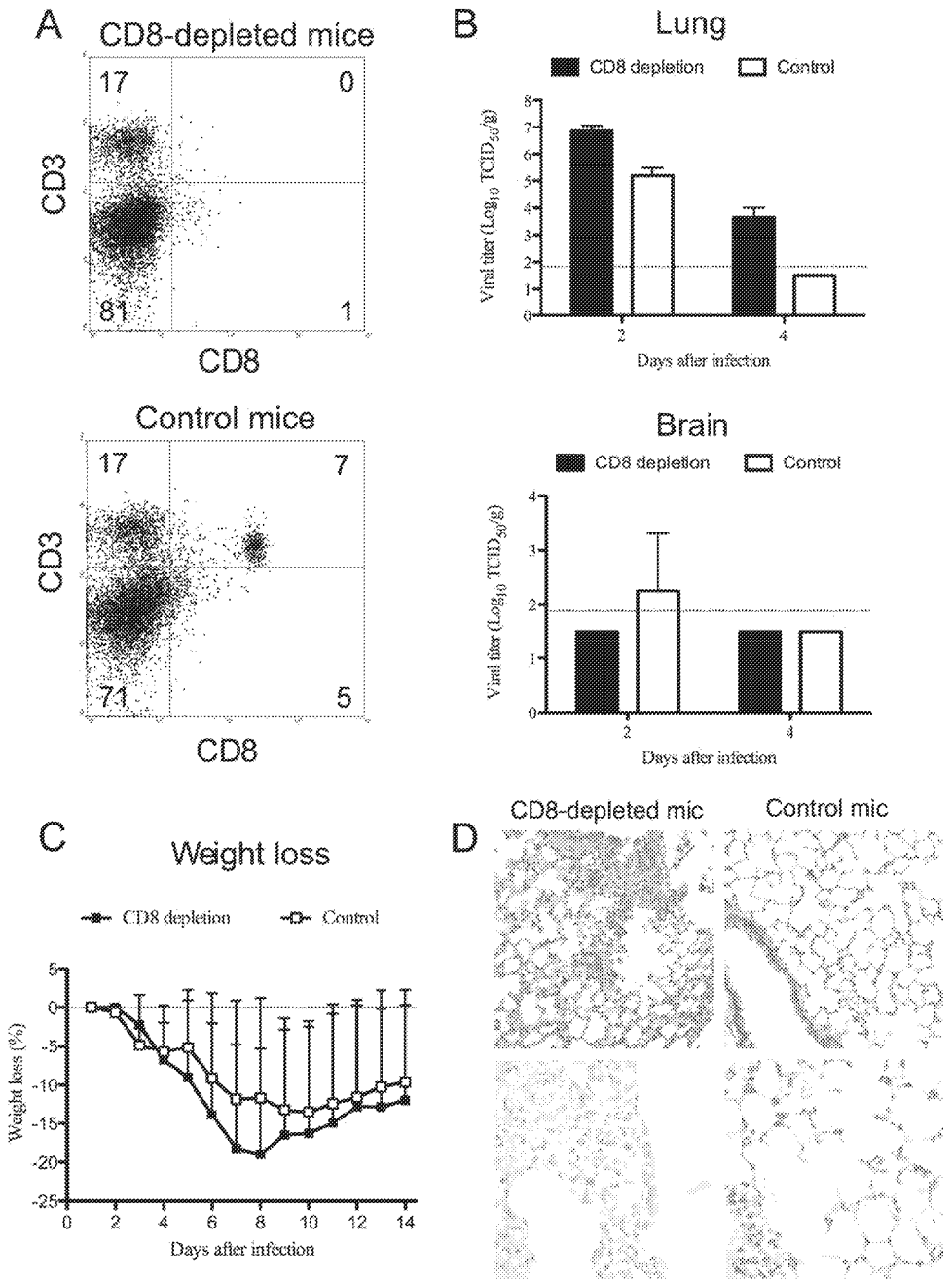

FIG. 11. Exacerbated pathogenesis of SARS-CoV infection in CD8-depleted Tg AC22 mice. Two groups of Tg AC22 mice (12 mice per group) were subjected to multiple doses (i.p.) of rat anti-mouse CD8 monoclonal antibody and an isotype-matched irrelevant rat monoclonal antibody (as control), respectively, as described in Materials and Methods. They were then infected i.n. with 10^6 $TCID_{50}$ SARS-CoV. Two mice/group were sacrificed after two doses of antibody treatment for assessing by flow cytometry the efficacy of antibody-mediated CD8 depletion in the spleens, whereas the effect of CD8 depletion on the pathogenesis of SARS-CoV infection was evaluated by virologic, clinical, and pathological parameters, as described in the text. Briefly, two additional mice were sacrificed at days 2 and 4 p.i. to allow assessment of virus infectivity and pathology in the lungs and brains, and the remaining mice were monitored for the onset of illness (i.e., weight loss). It appeared that a two-dose specific-antibody treatment regimen effectively depleted most of the $CD8^+$ T cells from the spleens (A). SARS-CoV infection of CD8-depleted mice resulted in increased infection in the lungs, but not in the brains, at both days 2 and 4 p.i. (B). This was accompanied by an increased weight loss (C), as well as more pronounced histopathology and the retention of viral NC antigen at day 4 p.i., as revealed by hematoxylin and eosin staining and IHC, respectively (D).

model for SARS-CoV infection. Although the underlying mechanism of SARS-CoV-associated lymphopenia in patients, as well as in the Tg mice described in this study, remains unclear, the absence of ACE2 expression in lymphocytes (11) makes the direct lysis of lymphocytes by this virus unlikely. Sequestration of lymphocytes in affected tissues also seems unlikely, at least in our AC70 mice, in which SARS-CoV infection failed to elicit a persistent infiltration of mononuclear

cells within the brain. Rather, cytokine-mediated apoptosis of uninfected lymphocytes may be the cause of acute lymphopenia, as suggested by others (12, 37). In this regard, further investigation is needed to discern whether some of the cytokines that were produced by SARS-CoV-infected Tg mice could cause apoptosis of T cells.

While a profound T-cell loss was readily detectable in our Tg mouse lineages, especially the lethality-susceptible AC70 mice, neither B cells nor non-T non-B lymphocytes were noticeably affected (Table 2), which led to the possibility that T cells were the preferred targets for manipulation by SARS-CoV in our Tg mouse model. More strikingly, in contrast to the grossly diminished number of CD8 T cells in AC70 mice which rapidly succumbed to lethal infection, this T-cell subset was largely unaffected by SARS-CoV in the lethality-resistant AC22 mice, a finding which implied that this CD8 subset of T cells might have a protective role in AC22 mice against SARS-CoV infection. We employed a depletion technique using rat anti-mouse CD8 antibody to investigate the role that $CD8^+$ T cells might have.

Elimination of most of the CD8 T cells in the spleens of AC22 mice resulted in an increased respiratory infection, accompanied by more intense lung pathology, compared to that in control mice (Fig. 11B and D). Although $CD8^+$ T cells were also effective in attenuating weight loss (Fig. 11C), we noted that the extent of weigh loss in infected AC22 mice treated with control rat IgG antibody was much less than that in untreated mice (10 to 15% versus 35% weight loss). High-dose intravenous IgG has been widely used as a potent immune modulator for the treatment of autoimmune diseases and many infectious diseases. This modulation occurs most likely, in part, through the Fc portion of the IgG molecule (25). Thus, multiple i.p. injections with 50 μg/injection of irrelevant rat IgG into AC22 mice might provide a yet-to-be identified immune regulatory mechanism in protecting against excess infection and weight loss. While $CD8^+$ T cells have a protective role against SARS-CoV infection, the exact mechanisms underlying this CD8-mediated protection in AC22 mice remain undefined. Because the clearance of many viral infections requires antigen-specific T cells, it is tempting to hypothesize that these protective $CD8^+$ T cells were likely SARS-CoV specific. The development of primary T-cell responses in an immunocompetent host usually takes about 4 days after an initial encounter with invading pathogens. Thus, the observation that a noticeable difference in the virally induced weight loss between CD8-depleted and control AC22 mice could not be detected until day 5 and continued through day 8 p.i. (Fig. 11C) might argue for the SARS-CoV-specific nature of these protective $CD8^+$ T cells. While $CD8^+$ T cells could attenuate the pathogenesis of SARS-CoV, other cellular elements of the immune system, especially CD4 T cells, are likely needed to provide more complete protection against SARS-CoV in our Tg mouse model. Additional studies are warranted to identify epitope-specific $CD8^+$ T cells and determine the contribution of $CD4^+$ T cells in the host defense against SARS-CoV in our Tg mouse model.

In summary, our studies have provided cellular and molecular insights into the differential regulation of host immune responses against SARS-CoV infection in two lineages of hACE2 Tg mice that were either susceptible or resistant to lethal SARS-CoV infection. Importantly, the less severe loss of T cells, accompanied by the ability to recover from SARS-CoV-associated acute clinical illness, makes our lethality-resistant lineage, i.e., AC22, particularly useful for dissecting both the innate and adaptive arms of the host immunity against the SARS-CoV infection. In addition, the fatal outcome of the disease in AC70 mice make this lineage attractive as a stringent model for adoptive transfer studies aimed at evaluation of the molecular and cellular bases responsible for the protection against SARS-CoV infection.

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From: LeDuc, James W.[/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=937DF08E29C4439E88A04BABFFB162AD-JWLEDUC]
Sent: Tue 1/21/2020 4:33:54 PM (UTC-06:00)
Subject: Op Ed in Houston Chronicle
Chinese Response to New Virus_Le Duc 21Jan revised.docx

Ben, Dave, Zhiming, George, Mifang and Pei-Yong

The attached, slightly modified to include mention of the new case in Washington State, is scheduled to appear in Wednesday 22 Jan's Houston Chronicle. Note mention of the NASEM/CAS collaborations.

Just FYI,

Jim

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Chinese Response to New Virus: Good News/Bad News

By James W. Le Duc

Fast action and open communications by China is helping the world prepare for another potentially devastating infectious disease outbreak. While the situation is rapidly evolving, there is good news that may not make the headlines. Many will recall the dark days in the spring of 2003 when Asia and the world were threatened by the appearance of a new virus disease, Severe Acute Respiratory Syndrome, or SARS, which first appeared in southern China and quickly spread to other countries around the world, ultimately causing over 8000 cases with nearly 10% of those ending in death. SARS was caused by a novel coronavirus then unknown to medical science. There was no known cure, no diagnostic tests and little understanding of where it came from or how it was spread, although person-to-person transmission was obvious as health care workers treating the first cases were themselves among the early victims. Initially, China was reluctant to share information or alert the international community of the magnitude of the epidemic, leading to international criticism and a dangerous global health situation. Fortunately, China reversed its position, opened to collaborations with the WHO, U.S. and others, and the epidemic was eventually controlled.

Today, with another novel coronavirus discovered in China, the start is very different. In quick measure, Chinese health officials recognized that a new disease had emerged, quickly isolated patients, and instituted an impressive set of interventions in attempts to limit disease spread and characterize the new pathogen. Importantly, they have been transparent in sharing their findings with the world, thus allowing other nations to take precautions and be on the lookout for the new disease. Already, the genome of the new virus was sequenced and posted for easy access by international experts, allowing rapid exploration of possible treatments, development of diagnostics and epidemiological investigations.

China's ability to respond quickly and efficiently to this new threat is the result of nearly two decades of investments and collaborations to improve public health in China. The Chinese Centers for Disease Control incorporates many of the strengths of our own CDC, but is designed to meet the needs of a 1.4 billion plus population. In addition, China has invested in building a robust scientific capacity and partnered with containment laboratories such as ours to incorporate best practices when studying dangerous pathogens.

The current outbreak demonstrates a welcome openness to health information sharing with the global community. To diagnose an outbreak early requires astute healthcare providers able to recognize when something new or unusual is occurring; however, clinical recognition alone is meaningless if there is no capacity to investigate cases or characterize the disease-causing agent.

For the last few years, our National Academy of Science, Engineering and Medicine has worked with the Chinese Academy of Sciences to build relationships and share information on emerging diseases and advancements in vaccines and treatments. In Galveston, we welcomed leading Chinese health officials to collaborate on biocontainment facility design, biosafety training and laboratory operations. This dialogue, along with U.S.-based educational opportunities for Chinese students, benefit us all.

China's response to the new coronavirus demonstrates their investments in physical laboratories and scientific collaborations over the past decade are paying dividends, not only to China, but the entire world. Control of a new disease efficiently transmitted person-to-person is nearly impossible as we witnessed during the 2009 novel influenza pandemic and much must still be done together during this quickly evolving situation.

The outbreak is still in the early stages, but it is now clear that the new virus may be transmitted person-to-person, although the efficiency of such transmission remains in question. A few hundred patients have been identified, deaths occurred and the disease has spread from the epicenter in Wuhan to major cities in China and other Asian countries. Our CDC is now screening travelers arriving from Wuhan at U.S. airports, and the WHO is set to consider a global emergency response. With millions about to travel for the Chinese New Year, avoiding a global catastrophe must be the current goal.

The good news is that, at a time when US-China relations are being tested on many fronts, relations within the public health and scientific research arenas remain open and positive, which lays a solid foundation for curtailing this latest threat.

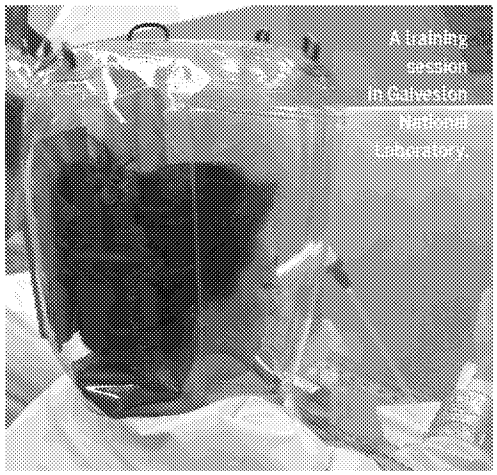
James Le Duc, PhD, is the Director of the Galveston National Laboratory at the University of Texas Medical Branch and a professor in UTMB's Department of Microbiology and Immunology.

705 words in body

EDITORIAL

Network for safe and secure labs

The current outbreak of Ebola virus in the Democratic Republic of the Congo is a reminder that dangerous diseases exist in many corners of the world and that they can cause substantial human suffering and financial devastation locally and internationally. In response, institutions and nations are constructing maximum biocontainment laboratories (MCLs) to address these threats. MCLs operate at the highest level of biological containment to diagnose, perform research on, and validate cures for life-threatening diseases like Ebola. There are more than 50 MCLs that are operational, under construction, or in advanced planning around the world. The global proliferation of these facilities raises questions about how to ensure their safe and secure operations while enhancing their contributions to science and global health. One solution is to establish an MCL network that enables the sharing of best practices, collaboration, transparency, and exchange of specimens and technology.



A training session in Galveston National Laboratory

"These labs handle the world's most dangerous pathogens..."

A multitude of challenges are associated with MCLs. Even at the idea stage, a serious issue is the objection of local communities to the construction of an MCL in their neighborhood. Several MCL operations were delayed or never realized because of public concern. Gaining community trust and support is therefore vital to planning and operating MCLs, so a network of such labs would be valuable for sharing experiences and providing guidance in these situations.

Besides the millions of dollars that it costs to build a modern MCL, there are annual operations—maintenance, utility, and security—that can amount to 5 to 10% of the construction costs. Moreover, there is a need for experienced guidance and qualified oversight to ensure that an MCL is built and operated safely and securely. Yet, few such resources exist, and available training opportunities are inconsistent and often costly. An MCL network could fill the personnel pipeline more efficiently by connecting experienced personnel and professional societies to develop standards for globally accepted training and create mentoring opportunities.

Importantly, MCLs must share a culture of responsibility. These labs handle the world's most dangerous pathogens known, and there must be safeguards to prevent theft or misuse. At the same time, security must be balanced against mechanisms that support collaboration, including specimen sharing. Again, by working together through an MCL network to develop standards and guidelines, a culture of responsibility could be fortified.

We direct a newly constructed MCL in Wuhan, China (Z.Y.), and an established MCL in the United States (J.W.L.), in Galveston, Texas. In preparation for the opening of the new China MCL, we engaged in short- and long-term personnel exchanges focused on biosafety training, building operations and maintenance, and collaborative scientific investigations in biocontainment. We succeeded in transferring proven best practices to the new Wuhan facility. Both labs recently signed formal cooperative agreements that will streamline future scientific and operational collaborations on dangerous pathogens, although funding for research and the logistics of exchanging specimens are challenges that we have yet to solve. Ours is a promising first step in MCL partnerships; however, wider national, regional, and international cooperation is needed. We benefited from meetings jointly sponsored by the U.S. National Academy of Sciences and the Chinese National Academy of Sciences, and from World Health Organization initiatives, but stakeholders are not limited to human and animal health. Our partnership still requires input from foundations and governmental agencies that are involved in security, commerce, and transportation, as well as from the commercial sector.

Not every country requires an MCL, but every country can benefit from the collaborative operation of these labs. We encourage existing MCLs to convene a forum that brings together all stakeholders to conceive of an MCL network so that these critical labs can tackle urgent global health needs safely, securely, and productively.

–James W. Le Duc and Zhiming Yuan

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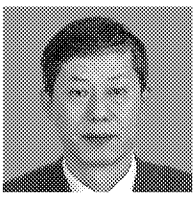

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10.1126/science.aav7120

Response to Your Texas Public Information Act Request (Nelson PIA #1)

Taylor, Matthew H. <mhtaylor@UTMB.EDU>

Fri 3/4/2022 3:19 PM

To: Chris Nelson <cnelson@judicialwatch.org>

Cc: Henze, Kaelan <kahenze@UTMB.EDU>

Good Afternoon:

The link below contains UTMB's response to your Public Information Act request (Nelson PIA #1) received by the University of Texas Medical Branch ("UTMB") for certain emails of a named former UTMB employee. The remainder of the information has been submitted to the Attorney General of Texas for a ruling.

Documents to release

Please note that this link will expire in three (3) business days, and I suggest that you copy the information to another source so you will have continued access. Once this link expires, any requests for this same information will be considered a redundant request under Texas Government Code 552.232.

Best Regards

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utmb Health

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