WUHAN INSTITUTE OF VIROLOGY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:

Dr. Zhengli Shi, Co-Investigator. Senior Research Scientist at Wuhan Institute of Virology (WIV) Chinese Academy of Sciences, will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to oversee the laboratory implementation at WIV. At a regular basis, Dr. Shi will meet with other Co-PIs to refine study protocols, report back results, and prepare publications. Dr. Shi has been working on the discovery and characterization of novel viruses from bats and other wildlife since 2004. This included the discovery that Chinese horseshoe bats are the natural reservoir of SARSr-CoVs and the likely origin of SARS-CoV. Her lab at WIV isolated SARSr-CoVs from bats sharing high homology with human SARS-CoV and demonstrated their interspecies transmission risk, largely confirming bats as the source of SARs. She will lead her team to carry out then systematic studies on the epidemiology, genetic evolution, interspecies infection mechanism and pathogenesis of a series of bat-borne CoVs on this R01 renewal proposal.

Dr. Peng Zhou, Co-Investigator. Research Scientist at Wuhan Institute of Virology (WIV) Chinese Academy of Sciences, will commit will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to be in charge of the diagnostics, genomics, and virus isolation work at WIV. Dr. Zhou have been working on bat virology since 2004, who will contribute his expertise in next generation diagnostic tool development for monitoring bat virus spillover, bat pathogen discovery, and bat viral immunology to this R01 renewal proposal.

Dr. Ben Hu, Co-Investigator. Research Associate and coordinator of Shi's laboratory at WIV, will commit (b) (4), (b) (6) (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to directly supervisor the lab technicians, and coordinate with our collaborators in this R01 proposal for communication, reporting, and or organizing meetings. Dr. Hu has been working on discovery and characterization of viruses in small mammals in bats and rodents since 2010 at WIV, and had extensive experience managing international collaborative projects.

B. Equipment

No equipment over $5,000 will be purchased.

C. Travel

We are requesting $4,314 per year for all years for Dr. Shi to travel to the United States to meet with EcoHealth Alliance (Daszak, Francesco, Olival, Ross) and University of North Carolina at Chapel Hill (Baric, Sims) collaborators. Travel is calculated at one round trip airfare from Wuhan to New York City ($1,000), nine-night hotel in New York City ($288 per night), and 10 days per diem at $76 per day except for first and last day, which have a reduced per diem of $57.

D. Other Direct Costs

We are requesting support for laboratory experiments and related testing costs with a minimum base of 2,000 samples from 1,000 animals per year.

RNA Extractions

We will be running RNA Extractions for 1,000 bats per year (two samples per bat: rectal and blood) in each year of the project. This will cost $6,214 per year (QIAamp ViralRNA Mini Kit with Axygen Pipette Tips and Filter Tubes at $3.11 per sample).

RT-PCR

Costs for 1-Step RT-PCR assays for Coronavirus conducted on 2,000 samples per year for each year of the project total $6,358 and are detailed as follows: Superscript III one step kit ($2.31 per sample); Platinum Tag DNA Polymerase ($0.25 per sample); nuclease-free water ($0.07 per sample); and Axygen Pipette Tips and Filter Tubes ($0.54 per sample).

DNA Sequencing

In each year of the project, DNA Sequencing will be performed on 1,500 samples at a cost of $4.34 per reaction. We request a total of $6,503 per year in each year.

In vitro Infection Experiment

We are requesting support for *in vitro* infection experiments using pseudoviruses carrying the spike proteins (wild type or mutants) or live viruses in cell lines of different origins, binding affinity assays between the spike proteins (wild type or mutants) and different cellular receptor molecules, and humanized mouse experiments. In each year of the project, we request $1,040 for Lipofectamine3000 transfection reagent; $3,612 for GIBCO Fetal Bovine Serum, $517 for GIBCO antibiotic antimycotic, and 2,601 for GIBCO medium that will be used in the *in vitro* infection experiments that will be used for *in vitro* infection experiment, as well as $6,000 for cell lines, in total of $8,639 per year.

Luciferase Immunoprecipitation System (LIPS) Assay

We are requesting $18,642 to support the in each year of the project to develop LIPS assay for bat CoV antibody detection, with detailed cost as follows: $9,827 for Protein A/G UltraLink Resin; $434 for Monoclonal ANTI-FLAG(R) M2 antibody; $5,636 for Renilla Luciferase Assay System; $2,168 for Merck-Millipore MSBVN1B50 MultiScreen HTS; $578 for Axygen Polypropylene PCR Tube Strips.

The Enzyme-Linked Immunosorbent Assay (ELISA)

We also request $2,312 to support the serological testing of 1,000 bat serum samples per year with ELISA plates, at the cost of $2.31 per sample.

E. Indirect Costs

We are requesting an extremely low indirect cost of 8% on all direct costs.