

SELECT AGENT RESEARCH/BIOHAZARDS. No select agent research.

Agents: SARS-related bat coronaviruses (SARSr-CoV), like WIV1, WIV16 and SHC014. These bat viruses are distantly related to the epidemic human SARS-CoV which emerged in 2003 and caused 8,000 cases and 800 deaths worldwide. While the epidemic human SARS-CoV is a BSL3 select agent, the SARSr-CoV are BSL3 pathogens in the US and not select agents. The proposal will use a SARSr-CoV molecule clone designated WIV1 during the course of these studies, which is NOT a select agent. This strain has not been shown to cause human disease or be transmissible between humans. All recombinant DNA work will use the bat SARSr-CoV WIV1 molecular clone. At the University of North Carolina (US Government select agent certified laboratory), some virus growth studies will be conducted in primary human airways, comparing wildtype SARS-CoV, WIV1 and various SARSr-CoV WIV1 chimeric virus growth kinetics. Wildtype SARS-CoV strain research will not be conducted at the Wuhan Institute of Virology.

Registration status of all entities where select agent(s) will be used. Wildtype SARS-CoV is a select agent. UNC-Chapel Hill is currently registered with the CDC for select agent use, including SARS-CoV, as required by select agent regulations (42 CFR 73). The UNC SARS select agent laboratories are routinely inspected by the environmental health and safety department at UNC and by the CDC. Workers receive select agent and BSL3 training focused on SARS-CoV safety, procedures and protective clothing/PAPR training each year.

Introduction and Background. SARS-CoV caused outbreaks with significant case fatality rates, and there are no vaccines available for this agent. SARS-CoV is classified as a BSL-3 select agent. Wildtype SARS-CoV is currently thought extinct in the wild. The work proposed in this application will involve two aspects: field work and laboratory work, focusing on distantly SARS-like bat coronaviruses (SARSr-CoV). Fieldwork involves the highest risk of exposure to SARSr-related or other bat CoVs, while working in caves with high bat density overhead and the potential for fecal dust to be inhaled. There is also some risk of exposure to pathogens or physical injury while handling bats, civets, rodents or other animals, their blood samples or their excreta. The Co-PIs and field team have extensive experience and certification working with wildlife species and high-biosecurity pathogens (Nipah virus, ebolavirus, SARS), and great care will be taken in the field to limit the risk of accidental exposure to known or unknown animal pathogens. We have strict procedures for handling bats and working with samples from them as they are secured in the field and transported to the lab. Field team members handling animals will be trained to utilize personal protective equipment (PPE) and practice proper environmental disinfection and biosafety techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator during bat handling and sampling. Fully Tyvek suits and HEPA-filtered Powered Air Purifying and Supplied Air Respirator Systems (PAPRs) will additionally be worn in cave systems where there is a higher risk of contact with aerosolized bat feces. All field clothing and equipment will be disinfected using Virkon disinfectant. All biological waste from field surveys will be disposed of in the appropriate container (sharps box or an autoclave bag) and will be autoclaved at local hospitals or university labs. All personnel will be vaccinated against rabies and have a neutralizing antibody titer, in accordance with WHO and CDC recommendations. Field teams will carry rabies boosters in the field and will receive a booster in the event of a potential rabies exposure.

Field safety protocol: Our procedures to deal with bites, needle-sticks etc. are as follows: The wound is washed thoroughly with soap and water to clean away dirt and debris, then vigorously scrubbed with a sterile gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage for until bleeding has stopped. If the wound continues to bleed, medical attention at the nearest hospital is sought. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed reports to a major hospital within 24 hours to

have wound examined and receive a rabies post-exposure booster vaccines as per WHO/CDC protocols. The laboratory work is lower risk, as samples placed in lysis buffer will be non-infectious. Samples placed in viral transport medium and frozen will be stored at ultra-low temperatures (-86°C) until viral isolation is required. Serum will be heat inactivated at 56°C for 30 minutes prior to testing.

Lab biosafety: The University of North Carolina at Chapel Hill, the Institute for Pathogen Biology, the Wuhan Institute of Virology, and the Wuhan University Center for Animal Experiment BSL-3 laboratories all have respective Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Available Treatments: No approved treatments are related for SARS and the SARSr-related bat coronavirus infections. However, therapeutic antibodies and nucleoside analogues have been successfully used in SARS-infected rodents and primates, which could be approved for compassionate use in humans exposed to the SARSr-CoV.

UNC Facilities where the select agent(s) will be used. SARS-CoV will be manipulated in research activities including establishment of viral replication curves, infection of rodent animal models and performance of plaque assays in laboratory spaces that meet operational and procedural criteria for BSL-3 activities as outlined in the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 5th edition, as well as BSL-3 criteria outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016). In addition, all mouse studies at UNC-Chapel Hill will be performed in an approved and registered BSL-3/ABSL-3 laboratory equipped with Techniplast Sealsafe™ HEPA-filtered animal housing for rodents. All animal protocols will be approved by the UNC-Chapel Hill IACUC.

UNC BSL-3/ABSL-3/select agent laboratories are equipped with biosafety cabinets, incubators, centrifuges with containment features, cold storage units, an autoclave, sink, eyewash and life safety equipment, and mechanical system monitors and alarms to support effective isolation and containment of operations involving SARS-CoV and SARSr-CoV. The anterooms to the BSL-3 laboratories house PAPR charging stations, laboratory and safety supplies, and a changing area. For both the BSL-2 and BSL-3 select agent spaces, access to select agents is restricted by the door between the hallway and anteroom and the door between the anteroom and BSL-3 space, requiring a combination of swipe card and punch code for entry. All select agent materials (SARS-CoV virus and genome length RNA) are stored in locked freezers and incubators.

UNC Procedures for monitoring possession, use and transfer of select agents. All personnel who will have access to select agent-regulated materials have been added to the Select Agent registration following security risk assessments prescribed by the CDC Select Agent Program. Personnel have completed training in all aspects of select agent compliance requirements and have adopted changes to standard operating procedures as applicable to assure that these requirements are met. Personnel will follow all procedures prescribed for accessing and securing the laboratory, documenting laboratory activities and materials used, and responding to incidents that could result in theft, loss, or release of select agent-regulated materials. Transfers of select agent-regulated materials will be coordinated by the laboratory managers and Responsible Official in accordance with standard operating procedures, including obtaining appropriate permits for shipping select agent materials and observing all regulations for shipping, both under dangerous goods and select agent regulations. Transfer of select agent RNA in TRIzol from registered BSL-3 to registered BSL-2 space and cDNA from registered BSL-2 space to non-registered BSL-2 space is conducted according to current select agent rules, regulations, and guidelines, including the new inactivation policies released in 2017.

UNC Biosafety, biocontainment, and security of the select agent(s). The Baric laboratories have been operational with BSL-3 core policies and procedures for ~15 years. Standard operating procedures at BSL-3 have been reviewed and approved by the UNC Chapel Hill Institutional Biosafety Committee and undergo both annual review and approval as well as updates as laboratory processes change or biosafety procedures evolve. The content of these documents has been formatted to conform to select agent regulations for the biosafety, security, and incident response plans. Additionally, lab-specific security risk assessments have been completed and recommendations implemented to ensure that security measures and procedures are sufficient to effectively minimize the possibility of unauthorized access to select agent-regulated materials. The UNC Chapel Hill facilities have undergone multiple CDC inspections and are currently in compliance with CDC requirements relating to SARS-CoV and select agent status. Our three-year renewal inspection occurred in June 2018 and we have been renewed for another three years.

UNC Biocontainment resources. All BSL-3 laboratories are under negative pressure, with redundant systems to ensure that negative pressure is maintained. All BSL-3 facilities have autoclaves to decontaminate waste materials as well as approved protocols for treatment or inactivation of any materials leaving the laboratory. All personnel are extensively trained in basic virology and safety protocols before being approved for select agent work and undergo additional extensive training to work with SARS-CoV and related SARSr-CoV as a BSL-3 pathogen. In both laboratories, annual testing is performed to verify that biosafety cabinets, laboratory supply/exhaust systems (including alarms), and other laboratory equipment are functioning as designed. The laboratories are secured at all times, and only personnel who have successfully completed Select Agent clearance and laboratory specific training requirements are permitted to enter without an escort.

P3CO Research. Recognizing the implementation of new gain of function research guidelines under P3CO, SARS-CoV and MERS-CoV are subject to these guidelines, and as such, reverse genetic studies are subject to review. Our group has considerable expertise in interfacing with the appropriate NIH P3CO institutional review boards to review, revise and finalize research designs that have the potential to modify pathogenesis or transmissibility in mammals. **Importantly, we are not proposing to genetically manipulate SARS-CoV** over the course of this proposal. However, we are proposing to genetically manipulate the full length bat SARSr-CoV WIV1 strain molecular clone during the course of the proposal, which is not a select agent, has not been shown to cause human infections, and has not been shown to be transmissible between humans.