

**Office of Research
Compliance & Ethics**

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Memorandum

TO: UND Faculty
FROM: Matthew Nilles, Chair, Institutional Biosafety Committee (IBC)
John Mihelich, Interim Vice President for Research and Economic Development
RE: Research on COVID-19/SARS-CoV-19 and samples that could contain SARS-CoV-19
DATE: April 30, 2020

In recent months the COVID-19 pandemic has caused significant changes to work and life. UND researchers are responding to this newly emerged disease by initiating research to understand the disease, COVID-19, and the virus, SARS-CoV-2. UND's Institutional Biosafety Committee is charged with reviewing and approving all biological research conducted at UND for safety and security. To support this research and help ensure it is conducted safely and in a manner that helps contain the disease and protect researchers, UND's Institutional Biosafety Committee has developed regulations to guide researchers to safely work on COVID-19/SARS-CoV-2. These regulations are required for work with any samples that contain or may contain SARS-CoV-2, including research samples from clinical sources not related to COVID-19 research. Note: These regulations do not apply to standard clinical samples used for diagnostic purposes.

If you have questions or concerns with these new regulations please send them to Matthew Nilles, Chair of the Institutional Biosafety Committee at matthew.nilles@UND.edu.

UND LABORATORY REQUIREMENTS FOR SARS-CoV-2 (COVID-19) RESEARCH

The following Institutional Biosafety Committee (IBC) requirements and laboratory biosafety guidelines must be followed regarding any work at UND or by UND investigators with materials containing or derived from the SARS-CoV-2 virus.

Investigators working with SARS-CoV-2 should regularly check for any revisions to this document, changes may occur as knowledge of the virus progresses. PIs are responsible for keeping current on biosafety procedures.

The IBC will not conduct an expedited review for COVID-19 related work or approve such work as a protocol change. However, if you are planning to propose COVID-19 research, the IBC is committed to helping the review process move quickly and to conducting safe research.

The IBC is requesting that all PIs planning to conduct any research related to COVID-19/SARS-CoV-2 provide the following information prior to submitting any IBC application:

1. A description of the materials needed, where the materials will be acquired, and how the materials will be transported to UND.
2. The experimental procedures to be performed and how any samples will be manipulated or handled.
3. A completed UND risk assessment form (a template is available on the IBC website).
4. A listing of everyone in the laboratory who will be working on the COVID-19 project, including:
 - a. Names
 - b. Academic degrees
 - c. Project responsibilities
 - d. Prior experience and/or training directly related to project responsibilities

The Biological Safety Officer or the IBC chair must approve any orders or requests to obtain any COVID-19/SARS-CoV-2 related materials, and an IBC approval must be completed before a material transfer agreement (MTA) will be signed.

Laboratory Biosafety Requirements

The current available literature regarding the viral load of SARS-CoV-2 in blood, plasma, and urine suggests these patient samples have low titers and may safely be manipulated with strict BSL2 containment procedures and practices by investigators experienced in handling human pathogens. Note that there is no guarantee of a sample having low or no viral titer so care still must be used in handling these samples. Biosafety levels and their required practices are described in Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition <https://www.cdc.gov/labs/BMBL.html>

Nasal and pharyngeal swabs have been shown to contain high loads of SARS-CoV-2 virus. **Currently these materials need BSL2 containment with enhanced precautions.** Materials post-extraction can be handled at BSL2 containment with BSL2 practices.

Investigators registering to work with nasal swabs, pharyngeal swabs, and other known high viral titer samples need BSL2 containment with enhanced precautions (BSL-2 enhanced) including : ALL work must be performed in the biosafety cabinet, closed rotor centrifuges or centrifuge "cups", centrifuge rotors loaded and opened in the biosafety cabinet, hazardous wastes decontaminated or bagged within the biosafety cabinet...no tossing materials into a nearby red-bag-lined container. Closed lab coat, double gloves use of a N95 respirator or at minimum surgical mask, and eye protection are mandatory. Daily self-monitoring for fever, cough, or fatigue mandatory. Signage should be posted to restrict access to the biosafety cabinet area during manipulations of SARS-CoV-2.

Be advised that current data in the literature suggest a prolonged release of viral material in feces extending past patient symptoms. These are potential high titer samples which require additional precautionary measures.

Research samples containing suspected high titers of SARS-CoV-2 that have not been inactivated and validated by an accepted method must be manipulated in BSL2 containment with enhancements as specified above and summarized at the end of the document.

Research involving culturing of ALL primary cells derived from humans must be screened for SARS-CoV-2 prior to culture to ensure propagation of the virus does not occur. The IBC recommends the use of rtPCR. Documentation that samples are SARS-CoV-2 negative from the clinical source is acceptable. A log must be kept of the receipt, screening method, and results. Any samples that test positive must be destroyed with 10% freshly made bleach (30 min exposure time) or autoclaving.

Note that work with patient materials or other human-derived clinical or control samples may require Institutional Review Board (IRB) approval.

NATURE OF THE MATERIAL

The SARS-CoV-2 virus is a positive strand, or sense strand, RNA virus. This means that the RNA extracted from a virus-containing sample is still officially considered "infectious" because it can be directly read by the existing translation machinery in your cells and generate viral proteins resulting in the generation of more SARS-CoV-2. Thus, work with SARS-CoV-2 must be done with great attention to safety standards and avoidance of aerosols and sharps.

SURVIVAL OF MATERIAL OUTSIDE HOST

At present, and with very preliminary data, the virus is suggested to survive on surfaces for a number of days and may persist as an airborne threat for a number of hours. Given these qualities, the current pandemic, and the lack of a vaccine or other effective treatment, a great deal of care needs to be given to any project involving the SARS-CoV-2 virus both in terms of planning of experiments and in the manipulations involved.

MATERIAL REGISTRATION

All research laboratories working with SARS-CoV-2 virus, nucleic acids extracted from virus-containing samples, or any natural or synthetic nucleic acid molecules capable of coding for the virus are required to be approved by the IBC in advance of obtaining or working with material.

CONTAINMENT REQUIREMENTS

The IBC considers the current CDC guidance regarding containment procedures for working with the SARS-CoV-2 virus as a MINIMUM standard. In some instances, the IBC is requiring a more stringent containment plan as noted below.

Procedures/Lab Work Requiring BSL3 Containment

As per the CDC, virus isolation in cell culture and initial characterization or propagation of viral agents recovered from cultures of SARS-CoV-2 specimens are only allowed in Biosafety Level 3 (BSL3) labs operating with BSL3 practices. **The IBC has extended the requirement for BSL3 containment to include ANY work with the SARS-CoV-2 virus that involves culture or propagation of the virus OR application of the virus or viral genome, RNA or cDNA, to cultured cells or animals. The viral genome, RNA or cDNA are considered infectious by the CDC. These procedures are strictly prohibited on the UND campus as there is currently no approved BSL3 laboratory available.**

Procedures/Lab Work Requiring BSL2 Containment

As per the CDC, routine diagnostic testing of specimens, such as (but not limited to) the activities listed below, can be handled in a BSL-2 containment laboratory using BSL-2 practices. The IBC is requiring that laboratories handling samples containing SARS-CoV-2, work in a biosafety cabinet that is isolated from the general lab space, such as in a separate room, and that the area be capable of restricted access during manipulation of these potentially high titer samples. Higher stringency procedures are also required as noted above.

ALL research manipulations of SARS-CoV-2 material need to occur in a biological safety cabinet. Laboratories working with extracted RNA or other SARS-CoV-2 genetic material must also work in a biosafety cabinet at BSL2 containment. **Important Note: cell culture and animal research is restricted as stated under BSL3 containment above.** Materials must be autoclaved or chemically inactivated prior to disposal. Chemically inactivated solids must be placed in a red bag and sealed prior to disposal in a red bag-lined biohazard container. Bleach-inactivated liquids (10% final volume of bleach) may be disposed down the drain.

Examples of routine specimen testing requiring BSL-2 containment:

- Using automated instruments and analyzers - the IBC requires that all automated instruments/analyzers used for research involving SARS-CoV-2 be equipped with some form of aerosol containment system that prevents release of aerosols during processing of samples potentially containing live virus. In addition, experiments with such equipment that may involve potentially live virus must have SOPs in place regarding the loading and unloading of such equipment in a manner that prevents spills and potential exposures. Fixed cells do not require containment for sorting or other manipulations.
- Staining and microscopic analysis of fixed smears
- Examination of (patient-derived) bacterial cultures
- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues - the IBC requires that the inactivation method has previously been shown to be 100% effective. Experimental inactivation methods such as microwaving or irradiation will need to be an accepted procedure published in the literature or proven at the bench for efficacy.
- Molecular analysis of extracted nucleic acid preparations
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container - Note that

shipping SARS-CoV-2 containing samples falls under DOT regulations and requires that the individual packaging the materials has taken biological material shipping training within the past 2 years.

- Using inactivated specimens, such as specimens in nucleic acid extraction buffer
- Performing electron microscopic studies with glutaraldehyde-fixed grids

TECHNICAL EXPERTISE/PERSONNEL REQUIREMENTS

At this point in time the IBC requires that only trained scientists be allowed to work with materials containing potentially active SARS-CoV-2 or genetic material extracted thereof. No undergraduate students should work with the material at this time.

! Individuals who are immunosuppressed or have medical conditions that might contribute to negative outcomes if infected by SARS-CoV-2 are strongly discouraged from working with the material !

Individuals working with SARS-CoV-2 virus must monitor their health and immediately report any potential illness, especially fever, cough, shortness of breath, or flu-like symptoms, to their PI/Lab Director and the Office of Safety. Any individual experiencing symptoms noted above must self-quarantine for 14 days.

Personal Protective Equipment (PPE)

BSL2 containment requires gloves, a labcoat, and eye protection for anticipated splashes or sprays when handling materials outside the biosafety cabinet. The IBC is requiring eye protection for all manipulations involving potentially live SARS-CoV-2 virus including when one is working in a biosafety cabinet. The labcoat must be worn buttoned, snapped or zipped closed, or be of a design that has a solid front and ties in the back. Work with samples that potentially have high viral titers requires strict adherence to BSL-2 procedures and requires additional PPE (BSL-2 enhanced). A summary of PPE requirements is listed in a Table at the end of this document.

TRANSPORTATION

Materials containing SARS-CoV-2 must be triple packaged for transportation within UND. This means the sample, in its original tube or container, must be packaged within two leak-proof outer containers. One way to accomplish this is to place the original tube or container in a ziplock bag, seal, decontaminate the outside of this bag, and then place this bagged material in a Tupperware-type container. **Transport of materials must occur by the most direct route possible that avoids transit through common areas such as eateries or high traffic areas.**

DO NOT SEAL DRY ICE OR LIQUID NITROGEN IN A CONTAINMENT VESSEL!

If you want to keep materials cool, place the secondary container (box or ziplock bag) on top of a layer of coolant. If you put a coolant such as dry ice or liquid nitrogen inside a container and seal it, the container has a good chance of exploding.

Containers that hold SARS-CoV-2 material must only be opened in a biosafety cabinet.

DISINFECTION

There are a number of options available per EPA list N (<https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>). Any disinfectant used **must have an EPA registration number, including bleach** (typically labeled germicidal bleach, check the bottle for the EPA number). The IBC prefers the use of 10% bleach due to its ease of use and speed of kill. You should have your 10% bleach solution pre-made the same day prior to working with SARS-CoV-2. Time is of the essence if there is a spill. You must have disinfection material ready to use. A container of 10% bleach should be placed in the biosafety cabinet prior to beginning work with SARS-CoV-2 so that arms and hands do not leave the biosafety cabinet to initiate decontamination. For quick and effective decontamination, the volume of 10% bleach used should at least equal the amount of material spilled.

DISPOSAL

The IBC requires that all human pathogens/infectious agents be autoclaved or thoroughly chemically inactivated prior to disposal in red bag-lined biohazard box. Solid materials that have been chemically inactivated should be placed in a red bag and sealed prior to disposal in a red bag-lined biohazard container. Liquids decontaminated with a final concentration of 10% bleach may be disposed down the drain after a **minimum 30 minute kill time**.

SPILLS

Any spill of SARS-CoV-2 material should have occurred in a biosafety cabinet. Otherwise, you have already violated these requirements.

If material is spilled in the biosafety cabinet, immediately apply 10% bleach solution or paper towels soaked in 10% bleach. The volume of 10% bleach should be similar to the volume of material spilled. Wait 10 minutes for a complete kill. Wipe up, dispose in a biohazard box, re-wipe the area with 10% bleach, then wipe with water or 70% ethanol to remove residual to prevent corrosion of stainless steel surfaces.

A spill outside of a biosafety cabinet is unacceptable and presents a number of problems. First, all staff members in the room where the spill has occurred are now considered potentially exposed and, thus, must self-quarantine for 14 days. Second, the spill must be immediately decontaminated to prevent potential spread of infectious material. In the event of a spill outside the biosafety cabinet, immediately cover the spill with 10% bleach or with paper towels soaked in 10% bleach. The volume of 10% bleach should be similar to the volume of material spilled. Wait 10 minutes for a complete kill. The survival of SARS-CoV-2 on surfaces is expected to be a number of hours so shoes, socks, pants, etc. are all suspect in the event of a spill outside the biosafety cabinet and must be removed carefully to minimize the chance of generating inhalable aerosols and bagged at the site for sterilization by autoclaving.

SAFETY INSPECTIONS

Laboratories conducting COVID related research will be subject to frequent safety inspections within the initial six months of the project to ensure compliance set forth in this document. Any concerns will be immediately addressed at the time of inspection with a written report to follow. A six month and one year formal review will be initiated to address any concerns or procedural alterations. Safety inspections will continue twice yearly for the duration of the project.

EXPOSURES

All exposures MUST be reported to the PI/Lab Director, UND IBC, and the Office of Safety

immediately. Exposed individuals will be required to self-quarantine for 14 days and monitor for symptoms.

NON-COMPLIANCE

Failure to comply with these requirements will result in an investigation by the IBC and the Office of Safety. No further work can proceed until the issue is resolved. The IBC can revoke any prior approval of the research where non-compliance has occurred.

	BSL-1	BSL-2	BSL-2 with Enhanced Precautions	BSL-3
PPE	Lab Coat, gloves, eye protection, mask and face shield working outside a biosafety cabinet	BSL-1	BSL-2 Plus: N95 mask or at minimum surgical mask, double gloves, impervious gown	No work allowed at UND
Laboratory	Notification of work provided to lab members	BSL-1 Plus: Personnel training and competency verification in handling infectious agents, Exposure monitoring	BSL-2 Plus: Daily self-monitoring reports	
Decontamination	Surface decon at every step using EPA List N disinfectants with required contact times	BSL-1 Plus: Autoclave or incinerate all solid waste, Autoclave liquid waste or treat with 10% EPA grade bleach	Same as BSL-2	
Primary Barriers	Plastic-backed benchtop pad, Samples stored/transported in leak proof containers	BSL-1 Plus: All aerosol generating procedures performed in biosafety cabinet, use centrifuge safety cups, Waste containers sealed prior to removing from biosafety cabinet	BSL-2 Plus: All work performed inside a biosafety cabinet, Rotors loaded/unloaded in biosafety cabinet, Minimize withdrawing hands from biosafety cabinet – use 2 person rule	