

JHU IBC SARS-CoV-2 (COVID-19) LABORATORY REQUIREMENTS

(Current as of March 26, 2020. Any subsequent updates supersede this document)

2020-03-26 Update...

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.

Nasal and pharyngeal swabs have been shown to contain high loads of SARS-CoV-2 virus. Currently JHH Clinical Pathology located in the Meyer Building has chosen to work with these materials according to BSL3 containment practices and, in most instances, in a BSL3 containment laboratory. Materials post-extraction are being handled at BSL2 containment with BSL2 practices. The JHU IBC acknowledges the technical expertise of this group and recognizes that they will adjust practices to accommodate sample loads and requirements.

Our information from Clinical Pathology is that they are currently NOT off-loading sample processing of materials such as nasal swabs and pharyngeal swabs. Investigators claiming they are to receive such materials need to specify in their registration materials where these materials are coming from so we can keep track of sample traffic origin.

Investigators registering to work with nasal swabs, pharyngeal swabs, and other known high viral titer samples at BSL2 containment will need to have access to a certified biosafety cabinet that is isolated from their general laboratory space and has the ability to have restricted access during times of work with SARS-CoV-2. BSL3 containment strategies should be used with these samples including ALL work 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 box. Closed lab coat, double gloves, eye protection 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 that deserve your respect.

IMPORTANT NOTE: Allgas Corporation is requiring that ALL investigators working with SARS-CoV-2 must decontaminate their gas cylinders with 70% ethanol prior to returning to Allgas.

The Johns Hopkins Institutional Biosafety Committee (JHU IBC) is requiring the following rules be followed regarding any work at JHU or by JHU investigators with materials containing or derived from the SARS-CoV-2 virus.

As noted below, all research involving SARS-CoV-2 virus, including the use of nucleic acids coding for SARS-CoV-2, require registration with the Biosafety Office and IBC approval prior to obtaining or working with the material.

Research samples containing suspected high titers of SARS-CoV-2 that have not been inactivated by an accepted method must be manipulated in strict BSL2 containment with enhanced requirements specified above in the 2020-03-26 update.

Due to the nature of the virus, extracted or otherwise isolated genetic material of SARS-CoV-2 virus will only be permitted in facilities that strictly adhere to BSL2 containment requirements.

Note that work with patient materials or other human-derived clinical or control samples may require 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 register with the Biosafety Office and be approved by the JHU IBC in advance of obtaining or working with material. The Infectious Agent/Pathogen form is here: <https://www.hopkinsmedicine.org/hse/forms/PathogenToxinRegistration.pdf>

CONTAINMENT REQUIREMENTS

The JHU 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 JHU 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 of viral agents recovered from cultures of SARS-CoV-2 specimens are only allowed in Biosafety Level 3 (BSL3) labs operating with BSL3 practices. **Important note:** The JHU 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. BSL2 containment is appropriate once a sample has been extracted or otherwise inactivated.

Please note that BSL3 containment laboratories are unique lab spaces with a specified set of requirements including, but not limited to, sealed cleanable surfaces, ceilings, walls and floors (no drop ceilings), monitored negative air flow, a dedicated exhaust system equipped with HEPA exhaust filters and redundant fan systems, an anteroom with door interlocks to the main lab space, high stringency PPE and training, and are tested, and must pass, failure scenarios including power or exhaust fan loss. All BSL3 laboratories at JHU are registered with the Biosafety Office and are annually certified for proper function and containment. **No other laboratories at JHU are acceptable for BSL3 containment work unless approval is granted in writing by the JHU Biosafety Office.**

Procedures/Lab Work Requiring BSL2 Containment

As per CDC, routine diagnostic testing of specimens, such as the activities listed below, can be handled in a BSL-2 containment laboratory using BSL-2 practices. **Important note:** The JHU 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 in the 2020-03-26 update above.

Laboratories working with extracted RNA or other SARS-CoV-2 genetic material must also work in a biosafety cabinet at BSL2 containment, but are not required to incorporate the enhanced stringency requirements and procedures noted in the 2020-03-26 update. ALL research manipulations of SARS-CoV-2 material still need to occur in a biological safety cabinet, however, and materials must be autoclaved or chemically inactivated prior to disposal. Chemically inactivated solids must be placed into a red bag and sealed prior to disposal in a red bag-lined biohazard box. Bleach-inactivated liquids (10% final volume of bleach) may be disposed down the drain.

Formalin or otherwise-fixed material does not require a biosafety cabinet post fixation.

- Using automated instruments and analyzers - Note that the JHU 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 - Note that the JHU 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 the hazardous goods shipping class offered by HSE or another entity within the past 2 years. Check with HSE shipping specialists regarding compliance before shipping SARS-CoV-2 containing materials
- 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 JHU 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 or graduate 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 Biosafety Office (biosafety@jhu.edu). Any individual experiencing symptoms noted above should self-quarantine.

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 JHU 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 should be worn buttoned, snapped or zipped closed, or be of a design that has a solid front and ties in the back.

TRANSPORTATION

Materials containing SARS-CoV-2 must be triple packaged for transportation within Johns Hopkins. 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.

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.

Transport of materials must occur by the most direct route possible that avoids transit through common areas such as eateries or high traffic areas. Maximize transit through JHU buildings and avoid patient care areas by using tunnels and service elevators whenever possible.

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

DISINFECTION

A 10% solution of household bleach in water is ample killing power for SARS-CoV-2. Although there are a number of options available per EPA, the JHU IBC prefers the use of bleach due to its ease of use and speed of kill. You should have your 10% bleach solution pre-made 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 BSC prior to beginning work with SARS-CoV-2 so that arms and hands do not leave the BSC 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 JHU IBC requires that all human pathogens/infectious agents be autoclaved or thoroughly chemically inactivated prior to disposal in red bag-lined biohazard box. **DO NOT PLACE SARS-CoV-2 MATERIALS THAT HAVE NOT BEEN PREVIOUSLY DECONTAMINATED DIRECTLY INTO A 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 box. 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 BSC. Otherwise you have already violated these requirements.

If material is spilled in the BSC, 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 5 minutes for a complete kill. Wipe up, dispose in a biohazard box, rewipe the area with 10% bleach, then wipe with water or 70% ethanol to remove residual bleach which can be quite corrosive for the stainless surfaces of a BSC.

A spill outside of a BSC 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 be considered for a 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 5 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 BSC and must be removed carefully and bagged to minimize the chance of generating inhalable aerosols.

EXPOSURES

All exposures **MUST** be reported to the PI/Lab Director and the Biosafety Office (biosafety@jhu.edu) immediately. Exposed individuals will be required to self-quarantine for 14 days and monitor for symptoms if required by Occupational Health.