

BSL-2 with Enhanced Containment Practices for Activities Involving Potential COVID-19/SARS-CoV-2 Specimens

SARS-CoV-2, a newly identified coronavirus currently circulating in the United States as well in other countries worldwide, is the causative agent of COVID-19. Early studies have demonstrated that the virus can be detected in a range of clinical specimens collected from infected individuals including blood, oral swabs and anal swabs.¹ Based on studies involving SARS-CoV, it is likely that virus can also be detected in some organs and tissues including the respiratory and gastrointestinal tracts.²

There are still many unknowns about SARS-CoV-2 transmissibility. The range of disease outcomes can range from asymptomatic to life-threatening. There is no approved, validated vaccine or anti-viral available at this time, and this is unlikely to change for some time. For these reasons, viable clinical specimens must be handled in a way that:

1. The end user is not exposed to any form of aerosol during the specimen manipulation process. Examples of aerosol-generating procedures include (but are not limited to): uncapping specimen containers, vortexing/mixing, grinding, sectioning, pipetting, pouring/decanting liquids and centrifugation.
2. Exposure risk associated with specimen handling and untreated wastes generated during the processing/analyses should be limited to the fewest number of personnel as possible.

To achieve this, viable/unfixed clinical specimens from known or strongly suspected COVID-19+ subjects should be handled/processed/analyzed under the following laboratory BSL-2 with enhanced containment practices (BSL-2+) conditions per institutional³ and national biosafety⁴ recommendations.

NOTE: This document is not intended to be exhaustive of all scenarios and is intended for guidance only. Applicable practices and alternatives will need to be determined in conjunction with VEHS Biosafety when research plans and procedures are reviewed for IBC approval.

Lab facility requirements:

- The lab must be equipped with at least one currently certified biosafety cabinet (BSC), a sink, a functional eyewash, and a door that can be closed to restrict access.
- Aerosol-generating equipment must be either placed inside the BSC for processing steps, or the equipment must have containment features that will capture aerosols from the process (i.e., safety cups or sealed rotor).
- The lab should not be shared with those not operating at BSL-2+ containment conditions.
- The lab should have access to an autoclave for on-site treatment of solid wastes, such that COVID-19 specimen processing wastes can be inactivated prior to hand-off to Environmental Services to protect this group from accidental exposure. The waste treatment cycle needs to be validated using a biological indicator prior to use. VEHS Biosafety will provide a [3M Attest Pack](#) and instructions for this process.

Lab oversight requirements: The lab needs to have a designated oversight person who is experienced and fully qualified to perform and train individuals in the technical procedures requiring BSL-2+ containment. This person must be current with institutional biosafety requirements for personnel who work with infectious materials.

- This person is responsible for ensuring that all personnel identified for BSL-2+ activities have completed the necessary biosafety training and have demonstrated proficiency in carrying out the specified technical procedures successfully under the prescribed biosafety practice conditions. These qualifications should be documented at the lab level.
- The designated oversight person is also responsible for ensuring that inventory documentation is maintained and that viable/unfixed COVID-19 specimens are secured in a way that these materials will not be distributed without appropriate approvals. Per institutional policy⁵, materials may not be transferred without notification of and approval by the IBC.
- The designated oversight person should ensure that no personnel other than those identified for the BSL-2+ work enter the space unescorted while COVID-19 specimen handling is underway. If equipment service, facility repair, etc. is required, please contact [Robin Trundy](#) for guidance under these circumstances.

- The designated oversight person should communicate with [Robin Trundy](#) or [VEHS Biosafety](#) whenever requests are received to perform COVID-19 specimen processing/handling not previously captured in an IBC amendment. An additional COVID-19 specimen processing record will be needed for projects/studies that are not part of the original approval for cross-referencing purposes with VHRPP.

Biocontainment practices summary:

- Specimens from on-site locations must be packaged and transported to the lab in a secondary container that meets the criteria in [Transporting Biological Materials on Campus](#) document; secondary containers must be opened and primary containers removed from packaging inside a BSC to ensure that no leaking or damaged primary containers are handled outside of containment.
- Before work is initiated with specimens, the room must be posted with the COVID-19 signage provided by VEHS and no one aside from the individuals who are authorized for this work can be permitted lab entry.
- Personal protective equipment includes the following:
 - A disposable, fluid-resistant wraparound gown
 - 2 pairs of fluid-resistant disposable gloves: 1 pair under the gown sleeve, 1 over the gown sleeve
 - Safety glasses or a face shield

NOTE: Personnel need to wear clothing that completely covers any exposed skin on the lower extremities. Long hair should be tied back.
- Before initiating work, personnel need to verify that:
 - The biosafety cabinet is functioning properly and staged appropriately for work
 - The sink and eyewash are accessible and functional
 - A biomaterials spill kit is available
 - A secondary container for biowaste bags is staged near the BSC
- Glass vessels and sharps should be eliminated from lab procedures whenever possible.
- Specimens may only be opened and handled in the currently certified Class II BSC identified in the IBC registration by the personnel specified in the IBC registration covering this activity.
- Specimen procedures will be carried out by one person working in the BSC at a time.
- If processing requires centrifugation of specimens prior to inactivation, specimen containers must be loaded and unloaded into centrifuge safety cups or a sealed rotor inside the BSC. Alternately, if safety cups or a sealed rotor will not be used, stage the centrifuge inside the BSC for this step. If neither configuration is feasible, centrifugation cannot be performed in the lab. Contact [Robin Trundy](#) for guidance.
- An EPA-rated tuberculocidal disinfectant or an [EPA-rated disinfectant effective for destruction of SARS-CoV-2](#) must be used to disinfect any surfaces impacted during specimen handling.
- Solid process wastes must be collected in a biohazard bag inside the BSC. The bag should be secured closed with a rubber band or zip tie and surface disinfected before removal from the BSC and placement in a dedicated biowaste container.
- Disposable pipets must be treated with disinfectant inside the BSC. These should be transferred as a bundle into a biohazard bag to be closed with a rubber band or zip tie. This bag should be treated and transferred as outlined in the previous bullet.
- Gloved hands must be surface disinfected before removal from the BSC, or the outer gloves will be removed before removing hands from the BSC.
- Gloves and disposable body coverings removed at conclusion of BSC cleaning and disinfection must be collected in a biohazard bag that gets processed with the other COVID-19 associated wastes.
- Biohazardous waste must be autoclaved using a validated cycle at the end of the session unless a dedicated, clearly marked waste receptacle with lid is available in the room. Do not accumulate more than the capacity of the one load for the autoclave.

- When autoclaving waste, bags must not be opened. Tape a [chemical integrator strip](#) on the lower part of a bag and position this bag in the center of the autoclave tray. Run the autoclave using the cycle parameters that were validated using the [3M Attest Pack](#) (to be provided by VEHS Biosafety). If the integrator results indicate that minimal sterilization conditions were NOT achieved, rerun the cycle with an additional integrator. Contact [VEHS Biosafety](#) for assistance with validating the autoclave before future use.

References:

1. Wei Zhang, Rong-Hui Du, Bei Li, Xiao-Shuang Zheng, et al. (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, *Emerging Microbes & Infections*, 9:1, 386-389.
2. Yanqing Ding, Li He, Qingling Zhang, Zhonxi Huang, Xiaoyan Che, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways, *Journal of Pathology*, 2004:203, 622-630.
3. Vanderbilt Institutional Biosafety Committees/VEHS Biosafety; Biosafety & Biocontainment Action Grid: Research Activities Involving COVID-19 Specimens & SARS-CoV-2 Materials.
4. American Biological Safety Association (ABSA) International; Considerations for Handling Potential SARS-CoV-2 Samples.
5. Vanderbilt Institutional Biosafety Committees Policy; Biosafety Level 2 with Enhanced Practices (BSL-2+) for Basic Research Applications.

Resource Links:

- 3M Attest Pack Biological Indicator Instructions
<https://multimedia.3m.com/mws/media/4977430/attest-bi-test-pack-for-steam-1276-package-insert-english.pdf>
- Proper OK Chemical Integrator Product Information
<https://www.proppermfg.com/product/ok-chemical-indicator-strips/>
- EPA List of Disinfectants Rated for Destruction of SARS-CoV-2
<https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>
- Transporting Biological Research Materials on Campus
https://www.vumc.org/safety/sites/vumc.org.safety/files/public_files/bio/transport-bio-on-campus.pdf
- Responding to Personnel Exposures & Spills Involving Biological Materials
https://www.vumc.org/safety/sites/vumc.org.safety/files/public_files/bio/responding-to-bio-exposures-and-spills.pdf
- VEHS Biosafety Email
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- Robin Trundy, Biosafety Officer
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