

BSL-2 ENHANCED CONTAINMENT PRACTICES FOR HANDLING POTENTIAL SARS-COV-2 SPECIMENS AND SAMPLES

Scope

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 EHS Biosafety when research plans and procedures are reviewed for IBC approval.

Definitions:

COVID-19 specimens/samples: any material that is received for analysis or research use that is known or suspected to be contaminated with SARS-CoV-2 virus or SARS-CoV-2 genetic material.

BSL-2+: BSL-2 containment practices and procedures with enhancements based on institutional and national biosafety recommendations, e.g., BSL-3 practices such as double gloves, rear-closing gowns, and respirators.

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/sample processing wastes can be inactivated prior to disposal in the regular trash. The waste treatment cycle needs to be validated using a biological indicator (BI) prior to use. EHS will provide a biological Indicator with $\geq \log 5$ bacterial spore population 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 UNL 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/samples are secured in a way that these materials will not be distributed without appropriate approvals. Additionally, no materials requiring BSL-2+ containment may be transferred to another lab without prior notification to, and approval by, the IBC via the Biosafety Officer. This notification is necessary in order to assure that permit restrictions are not violated. It is also necessary to assure the receiving lab has the facilities and biosafety support structure in place to work safely with these materials before these materials are in hand.
- 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 the UNL Biosafety Officer for guidance under these circumstances.
- The designated oversight person should communicate with the UNL Biosafety Officer via ibc@unl.edu whenever requests are received to perform COVID-19 specimen/sample processing/handling not previously captured in an IBC protocol 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 UNL IRB.

Biocontainment practices summary:

- Specimens/samples from on-site locations must be packaged and transported to the lab in a secondary container that meets the criteria in the EHS SOP ***Transport of Biohazardous Materials at UNL***; 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/samples, the room must be posted with the BSL-2+ signage provided by EHS and no one aside from the individuals who are authorized for this work can be permitted lab entry while work is occurring with the samples.
- Personal protective equipment includes the following:
 - A disposable, fluid-resistant rear-closing 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
 - Respirators* for aerosol-generating activities conducted outside of primary containment (i.e., biosafety cabinet or sealed rotors or centrifuge safety cups.)



**Respirator use requires enrollment in the UNL Respiratory protection program, medical qualification and annual training. Contact EHS for details.*

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 biohazardous 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/sample procedures will be carried out by one person working in the BSC at a time.
- If processing requires centrifugation of specimens/samples prior to inactivation, 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, respirators must be worn or centrifugation cannot be performed in the lab. Contact the UNL Biosafety Officer 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. Add a small amount of water to the bag prior to closing. Securely close the bag with a rubber band or zip tie and surface disinfect it 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 allowed to sit for the appropriate contact time before removal from the BSC. Disinfect the outside of the tray prior to removal from the BSC. Place pipets in a lined cardboard box for disposal as indicated in the EHS SOP **Sharps Handling and Disposing**.
- 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 should be mostly closed to allow steam to enter the bag. Follow autoclave operation instructions as outlined in the EHS SOP **Autoclave Operation and Use**. Contact EHS Biosafety for assistance with validating the autoclave before future use.

References:

1. Zhang W, Du R-H, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect.* 2020;9(1):386-389. doi:10.1080/22221751.2020.1729071
2. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol.* 2004;203(2):622-630. doi:10.1002/path.1560
3. UNL IBC and EHS. SARS-CoV-2 Biosafety and Biocontainment Research Guide. Published online August 2020.
4. American Biological Safety Association (ABSA) International; Considerations for Handling Potential SARS-CoV-2 Samples. Accessed August 13, 2020. https://absa.org/wp-content/uploads/2020/03/ABSA2020_Covid-19-dr3.pdf

Resource Links:

- 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>
- Institutional Biosafety Committee Email ibc@unl.edu
- Matt Anderson, PhD, RBP(ABSA), CBSP(ABSA), UNL Biosafety Officer manderson11@unl.edu