

Research Activities with Known or Likely Infected Specimens from Humans or Animal Models	Assigned Biosafety Level	Contact for Guidance
<ul style="list-style-type: none"> • Handling of material with high concentrations of live virus (e.g. viral cultures, virus propagation, virus isolation or neutralization assays) or large volumes of infectious materials. This includes virus isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens. • Inoculation of animals for potential recovery of SARS-CoV-2 • Research studies/protocols involving animal inoculation for confirmation and/or characterization of presumed SARS-CoV-2 agents 	BSL-3 / ABSL-3	<p>Randy A. Albrecht, Ph.D. Director, Emerging Pathogens Facility Global Health and Emerging Pathogens Institute (212) 241-8255 randy.albrecht@mssm.edu https://icahn.mssm.edu/research/global-health/emerging-pathogens</p>
<ul style="list-style-type: none"> • Processing blood and other human biospecimens including tissue from COVID-19 patients • Performing research experiments using human cells or bodily fluids that do not involve activities with the potential to propagate SARS-CoV-2 • Aliquoting and/or diluting biospecimens • Pre-lysis nucleic acid extraction procedures involving potentially SARS-CoV-2 infected specimens • Preparation and chemical- or heat-fixing of smears for microscopic analysis 	BSL-2+ (BSL-2 Enhanced)	<p>Landau Buissereth, MS Associate Director, Environmental Health & Safety / Acting Biosafety Officer Environmental Health & Safety (212) 241-3761 landau.buissereth@mssm.edu http://intranet1.mountsinai.org/compliance/env_health_safety.asp</p>
<ul style="list-style-type: none"> • Using automated instruments and analyzers (if aerosol containment is a feature) • Staining and microscopic analysis of fixed smears • Examination of bacterial cultures • Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues (<i>Inactivation methods should be validated</i>) • Post-lysis nucleic acid extraction procedures involving potentially infected specimens • 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) • Using inactivated specimens (such as specimens in nucleic acid extraction buffer) • Performing electron microscopic studies with glutaraldehyde- fixed grids 	BSL-2	<p>Landau Buissereth, MS Associate Director, Environmental Health & Safety / Acting Biosafety Officer Environmental Health & Safety (212) 241-3761 landau.buissereth@mssm.edu http://intranet1.mountsinai.org/compliance/env_health_safety.asp</p>

Please note that all proposed research with SARS-CoV-2 (COVID-19) requires:

1. Review by Environmental Health & Safety (Biological Safety Officer)
2. Review and approval by the Institutional Biosafety Committee (IBC): <https://icahn.mssm.edu/research/ibc>
3. An approved Standard Operating Procedure (SOP), submitted as part of the IBC approval process.

Updated Biosafety Guidelines for SARS-CoV-2 (COVID-19): Research Laboratories

BSL-2+ (BSL-2 Enhanced) work with COVID-19 patient specimens requires the following enhancements to standard BSL-2:

- Any procedure with the potential to generate aerosols or droplets (e.g. vortexing, cell sorting, ELISA plate washing, pipetting, cap removal) will be performed in a certified Class II Biological Safety Cabinet (BSC). BSC must be decontaminated after use with an EPA approved disinfectant for SARS-CoV-2 ([List N](#)).
- Required PPE for personnel (*at minimum*):
 - disposable solid-front tie-back isolation gown
 - eye protection (face shield or goggles)
 - double pair of gloves
 - surgical / procedure mask
- Centrifugation of specimens must be performed using sealed centrifuge rotors / safety cups. Samples must be loaded and unloaded within BSC.
- If specific manipulations **cannot** be performed within a biosafety cabinet **and** have the potential to generate aerosols or droplets, N95* respirators or other appropriate respiratory protection must be utilized (in place of surgical /procedure masks), in addition to the minimum required PPE for BSL-2+. Additional PPE (e.g. eye protection) and/or engineering controls may also be required per risk assessment.
*Guidance regarding respirators, including information about respirator fit testing and medical clearance, is available on the following webpage (see EH&S section): <http://researchroadmap.mssm.edu/reference/covid-19-guidance/>
- The use of sharps should be eliminated wherever possible.
- The laboratory must have a door that can be closed at all times and restrict access when experiments are in progress. Ideally, a tissue culture room within a laboratory suite should be utilized. Rooms that open directly to a common (non-laboratory) corridor should be avoided if possible.
- Dedicated equipment should be utilized for storage and use of specimens (e.g. incubator, section of freezer). Storage equipment should be labeled to indicate the contents and also secured if possible.

Additional PPE Considerations: Please note that standard PPE requirements apply for BSL-2 work (laboratory coat and gloves; eye and face protection, as needed. Refer to EH&S safety manuals and policies; hyperlinks available on [SECTOR](#)). Access and use of a BSL-3 facility requires specific PPE procedures and practices. Use surgical masks at all times on campus in accordance with current institutional policy for COVID-19 pandemic response.

References:

- **Mount Sinai COVID-19 Guidelines:**
<https://www.mountsinai.org/about/covid19>
- **COVID-19 Research Guidance for MSHS**
<http://researchroadmap.mssm.edu/reference/covid-19-guidance/>
- **Duke University - SARS-CoV-2 (COVID-19) Research Laboratory Biosafety Guidelines:**
[https://www.safety.duke.edu/sites/default/files/SARS-CoV-2%20\(COVID-19\)%20Biosafety%20Guidelines.pdf](https://www.safety.duke.edu/sites/default/files/SARS-CoV-2%20(COVID-19)%20Biosafety%20Guidelines.pdf)
- **ABSA International - Considerations for Handling Potential SARS-CoV-2 Samples:**
https://absa.org/wp-content/uploads/2020/03/ABSA2020_Covid-19-dr3.pdf
- **Centers for Disease Control and Prevention (CDC) - Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19):**
<https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html>
- **Centers for Disease Control and Prevention (CDC) - Laboratory Biosafety and COVID-19: Questions and Answers:**
<https://www.cdc.gov/coronavirus/2019-ncov/lab/biosafety-faqs.html>
- **World Health Organization (WHO) - Laboratory biosafety guidance related to coronavirus disease (COVID-19) - Interim guidance 19 March 2020:**
<https://apps.who.int/iris/handle/10665/331500>
- **United States Environmental Protection Agency (EPA) - Pesticide Registration - List N: Disinfectants for Use Against SARS-CoV-2:**
<https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>
- **Occupational Safety and Health Administration - Safety and Health Topics - Control and Prevention:**
<https://www.osha.gov/SLTC/covid-19/controlprevention.html>
- **List N: Disinfectants for Use Against SARS-CoV-2:**
<https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>

Frequently Asked Questions (FAQs)
Biosafety Guidelines for SARS-CoV-2 (COVID-19): Research Laboratories

- 1. I am using nasal swabs collected from patients with acute COVID19 for host nucleic acid extraction. Can these be handled at BSL-2+ with applicable personal protective equipment (PPE), or is BSL-3 containment required?**

Biospecimens from patients with acute COVID-19 can be handled at BSL-2+ containment (i.e., a BSL-2 laboratory with some BSL-3 practices/procedures) for non-propagative procedures. Please refer to the guidelines for additional information.

- 2. I am working with fresh surgically removed tissue from an autopsy of a patient with COVID-19. It is being used for nucleic acid extraction and/or other downstream applications, such as single-cell RNA-seq (scRNA-seq) technology, commonly known as 10X. Is BSL-3 containment required?**

- BSL-2+ would apply for nucleic acid extraction procedures involving known or potentially infected patient specimen until the sample is lysed and can be transferred to a BSL-2 laboratory to continue the nucleic acid evaluation.
- BSL-2 would apply for molecular analysis of extracted nucleic acid preparations.

- 3. I am working with fresh surgically removed tissue from an autopsy of a patient with COVID-19. Single cell microfluidics will be utilized to process the samples. No cell sorting process (e.g. FACS) will be performed. What biosafety level would be applicable?**

Aliquoting and/or diluting of patient specimens, and performing diagnostic tests that do not involve activities with the potential to propagate virus, can be performed at BSL-2+.

- 4. I would like to disaggregate fresh tissue from a patient with COVID-19 into a cellular suspension. What is the applicable biosafety level? Can the suspension then be distributed for processes such as direct single cell molecular methods, or similar procedures, at a lower or higher containment level?**

Non-propagative procedures with COVID-19 patient specimens can be performed at BSL-2+. Any procedure with the potential to propagate virus (SARS-CoV-2) must be performed in a BSL-3 facility. Procedures with the potential to generate aerosols should be performed in a biosafety cabinet with the operator wearing appropriate PPE. In general, procedures involving the use of potentially infectious patient specimens should be done at BSL-2+. Work with inactivated material and extracted nucleic acid preparations would be applicable to BSL-2.

5. What is the applicable biosafety level for extraction and molecular methods on OCT, frozen blocks, blood, and serum? Is BSL-2+ applicable?

- BSL-2+ would apply for nucleic acid extraction procedures up to the lysis step. Lysed biospecimen can be transferred to a BSL-2 laboratory to continue the nucleic acid extraction.
- BSL-2 would apply for molecular analysis of extracted nucleic acid preparations.

6. I am collecting ambient air samples from treatment areas of COVID-19 patients. What is the applicable biosafety level?

Generally, initial processing and other non-propagative procedures utilizing material from air sampling can be performed at BSL-2+ (provided there will be no amplification or concentration of the pathogen prior to use). Projects utilizing air sampling processes will be reviewed on a case-by-case basis per risk assessment. Any procedure with the potential to propagate virus (SARS-CoV-2) must be performed in a BSL-3 facility.

7. Animals will be inoculated with SARS-CoV-2 in the ABSL-3 facility. As part of our studies, we intend to perform routine laboratory procedures on isolated tissue such as lung, spleen, and isolated blood. These include RNA extraction, assessment of markers of inflammation such as cytokines, and histology work. Can these procedures be conducted in a BSL-2+ laboratory setting, or is a BSL-3 containment necessary?

Work with experimentally infected cell/tissues (including materials from animals infected with SARS-CoV-2) would need to be done at BSL-3 / ABSL-3. Once the isolated tissue from animals has been inactivated using a validated method within a BSL-3 / ABSL-3 facility, it can be used at a lower containment level. Please refer to the guidelines; and the BSL-3 / ABSL-3 Facility Manual and specific SOP(s); for additional information.

8. We are starting a study that only involves healthy human subjects (no symptoms and no known history of COVID-19). Would it be adequate to process specimens from this study using BSL-2 practices (instead of BSL2+)?

If the subjects present as healthy (no symptoms and no history of COVID-19), and are not known or likely to have COVID-19 per professional judgment, BSL-2 should be appropriate. Please perform all manipulations with the specimens in a biosafety cabinet.

Please note that, as COVID-19 is assumed to be widespread within the community, it is best practice to wear BSL-2+ recommended PPE when working with patient samples, and other enhancements to BSL-2 as detailed in the guidelines.