



Considerations for Handling Potential SARS-CoV-2 Samples

These considerations do not supersede any regulatory or country-specific in your locale.

Laboratory practices, techniques, and administrative controls build upon the previous level. Additional controls are indicated at each level.

BSL2

- Whole blood, serum and urine
- Synthetic messenger RNA-based or recombinant protein-based products
- Rapid respiratory testing performed at the point of care (no nucleic acid isolation)
- Viral vector-based products
- Using automated instruments and analyzers (if aerosol containment is a feature) some devices might be older and not contained
- Staining and microscopic analysis of fixed smears
- Examination of bacterial cultures
- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
 - Inactivation methods should be validated
- 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
 - *CDC Source

Additional procedures

- Cytometry – fixed samples

Laboratory Practices and Technique

- Good (Standard) Microbiological Practices
- Access to the laboratory is restricted when work is being conducted
- All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment
- Use safety cups whenever possible to avoid exposure to aerosols

Primary & Secondary Barriers and PPE

- PPE: gown/lab coat, single gloves, surgical mask, eye protection, face shield
- Work behind plexiglass screen in an isolated designated area (minimum)
- Notify others in the lab
- Work with samples done over a plastic-backed benchtop pad
- Surface decontamination at every step using EPA List N disinfectants and contact times
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility
- BSCs, if available, are properly maintained and certified
- Mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory (recommended)
- A method for decontaminating all laboratory wastes should be available in the facility

Administrative Controls:

- Training and competency verification on donning and doffing required PPE
- Training and competency verification for each procedure performed
- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
- Occupational health
 - mandatory reporting of any symptoms, any laboratory exposure
 - consider baseline blood, baseline questionnaire, emergency wallet card
 - consider review of COVID-19 vaccination status
- Demonstrated competency on working in a BSC (if available)

BSL2 with BSL3 practices

- Aliquoting and/or diluting specimens
- Inoculating bacterial or mycological culture media
- Performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
- Nucleic acid extraction procedures involving potentially infected specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis
 - *CDC Source

Additional procedures

- Respiratory samples and secretions
- Stool***
- Cytometry – non-fixed samples
- Inactivated virus lysate
- Work with ANY sample that may produce an aerosol

Laboratory Practices and Technique

- Good (Standard) Microbiological Practices as indicated for BSL2
- Primary & Secondary Barriers and PPE
- PPE: surgical mask (blood)/N-95 (respiratory secretions), double gloves, impervious gown, eye protection with side shields. The use of PPE should be determined after conducting a risk assessment.
- All samples opened inside the BSC in case of spills/leakage. If BSC is not available, don N95 and face shield and work behind plexiglass screen in an isolated designated area, notify other laboratorians, work with samples done over a plastic-backed benchtop pad.
- Surface decontamination at every step using EPA List N disinfectants and contact times

Administrative Controls:

- Scheduled time for handling SARS-CoV-2 samples (best practice)
- Two-person rule for minimizing withdrawing hands from BSC
- Centrifuging of blood specimens is in safety cups or sealed rotor, loaded and unloaded in a BSC
- Training and competency verification on donning and doffing required PPE
- Specific training on use of N95 respirators, if applicable (includes pulmonary function, medical clearance, and fit testing)
- Occupational health
 - mandatory reporting of any symptoms, any laboratory exposure
 - consider baseline blood, baseline questionnaire, emergency wallet card
 - consider review of COVID-19 vaccination status

BSL3

- Virus isolation/propagation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens
- Infectious clone-derived SARS-CoV-2 virus, infectious cDNA SARS-CoV-2 clones and recombinant derivatives[#]
- Infection of experimental animals with any of the above

Additional procedures

- FACS/High Speed Cell Sorting
- Transfer of inactivated samples outside BSL3
- Inactivation by validated methods with any of the above

Laboratory Practices and Technique

- Good (Standard) Microbiological Practices
 - Follow BSL3 practices and procedures according to CDC BMBL 6th ed.
- Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures

Primary & Secondary Barriers and PPE

- All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices
- Respiratory protection is required (N95 or PAPR/CAPR)
- Autoclave waste before disposal
- HEPA filtration of exhaust air is recommended for certain situations**

Transfer of samples outside BSL3- based on risk assessment by biosafety officer

- Ensure lids are tight
- Decontaminate outside of tubes
- Wrap sample with absorbent material
- Individually place into zip-lock bag
- Seal zip-lock bag and change gloves
- Add all wrapped samples into second bag
- Wrap bagged samples in ample packaging and place into designated carrier, firmly attach lid
- Occupational health
 - mandatory reporting of any symptoms, any laboratory exposure
 - consider baseline blood, baseline questionnaire, emergency wallet card
 - consider review of COVID-19 vaccination status

*CDC Guidance for Laboratory <https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html> [accessed 13 December 2021]

**WHO Laboratory Guidance https://apps.who.int/iris/handle/10665/339056?search-result=true&query=covid+biosafety&scope=&rp=10&sort_by=score&order=desc [Accessed 27Mar2022]

***Recent publications have postulated that stool may not be important to the natural epidemiology and transmission of SARS-CoV-2. However, earlier publications pointed to large numbers of particles present in stool from infected people, and some other recent studies have shown some proportion of those particles are infectious. While acknowledging that fecal/oral or fecal/aerosol transmission of SARS-CoV-2 may not be a major route of spread in the natural environment, it must be acknowledged that the laboratory is a non-natural environment where agents may be concentrated, exposures may be through non-natural routes, and energetic processes (e.g. centrifugation) may lead to an exposure and infection risk for laboratorians from contaminated samples that is not present in the natural environment. A complete risk assessment of laboratory processes must account for the special procedural risks in the laboratory.

[#]SARS-CoV/SARS-CoV-2 Chimeric Viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors have been added to the US HHS list of Select Agents and Toxins per Interim Rule effective 17Nov2021.