PennEHRS
Environmental Health & Radiation Safety

Biosafety Precautions for Research Labs Handling COVID-19 Clinical Specimens

The University of Pennsylvania Office of Environmental Health & Radiation Safety (EHRS) is providing this guidance to research labs that will be handling and processing human specimens known or suspected to be infected with SARS-CoV-2. In accordance with CDC guidance, virus isolation in cell culture and initial characterization of viral agents recovered in cultures of COVID-19 specimens must be conducted in a Biosafety Level-3 (BSL-3) laboratory. Processing of patient specimens including blood, urine, stool samples, and nasal swabs requires use of a certified Class II Biosafety Cabinet (BSC) and BSL-2 practices. Site- and activity-specific biosafety risk assessments are on-going to determine additional biosafety precautions that are warranted based on situational needs, such as the likelihood to generate infectious droplets and aerosols.

Please refer to the CDC "Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)" for general biosafety guidelines:
https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

Additional information is also available on the EHRS website:
https://ehrs.upenn.edu/guidance-covid-19-research-projects

Biosafety Level	Type of experiment
BSL-3	• Viral isolation, amplification, and characterization in cell culture • Generation of infectious clones • Manipulation and inactivation of materials with high viral titer, including endotracheal (ET) aspirate, lung tissue, bronchoalveolar lavage (BAL)
BSL-2 enhanced	• Processing and inactivation of known or suspected patient samples, including urine, saliva, blood, NP swabs, OP swabs
BSL-2	• Molecular analyses of extracted nucleic acid • Use of inactivated materials (*inactivated per EHRS-approved SOPs)

*Currently approved methods of inactivation include:

- Heat
- Chemical Fixation
- RNA extraction

Specific SOPs must be followed and will be provided upon request. Additional methods of inactivation will require review by the Biosafety Office. If your experimental needs require use of different extraction or inactivation methods, please contact Dr. Colleen Kovacsics (collk@ehrs.upenn.edu) to discuss.

University of Pennsylvania Biosafety Level 2 (BSL-2) Enhanced Procedures

Enhanced biosafety precautions are required for handling known or suspected COVID-19 patient specimens. These practices include use of a certified Class II Biosafety Cabinet (BSC) for all manipulations of infectious materials, collection of all waste inside the BSC, decontamination of waste prior to removal from the BSC, and use of additional PPE. Specific practices are detailed below. If your work involves manipulations not described in this guidance document, please contact Dr. Colleen Kovacsics.

Packaging and Transport:

This information is for hand-carried transport from one campus location to another by walking.

- Ensure that the exterior of the primary tube, bag or other container is disinfected after collection.
    - Approved disinfectants for solid materials include:
        - 70% ethanol, 1 minute contact time
        - 10% bleach, 1 minute contact time
        - Virkon-S, 10 minute contact time
        - Contact EHRS before using other disinfectants to ensure efficacy against SARS-CoV-2.
- Place the primary container inside a clean secondary container. Both primary and secondary containers must be leak-proof.
    - Place paper towels in the bottom of the secondary container to absorb any liquids if case of a leak in the primary container during transport.
    - Place a biohazard label on the outside of the exterior transport container with the lab's contact information if the container is lost.
- Personal protective equipment is not required for the transport of appropriately sealed and decontaminated transport containers.

Receiving Specimens:

- At destination lab, don appropriate PPE (lab coat, safety glasses, double gloves, universal mask) and open transport container inside the BSC.
- Decontaminate primary and secondary containers with disinfectant (10% bleach, 1 minute contact time, wipe dry; follow with second decontamination step with 70% ethanol for 1 minute contact time.).

Storage:

- Place samples inside a refrigerator or freezer that is labeled with the universal biohazard symbol.
- Ensure that the samples are coded or labeled in a way that identifies their contents to the laboratory.
- Keep an inventory of your biohazards for easy retrieval and to prevent accidental use.

Personal Protective Equipment (PPE):

- Use dedicated PPE for specimen processing. Do not wear PPE to other non-lab areas and remove prior to leaving the processing room.
- Wear a lab coat or solid-front back-fastening gown, preferably with a knit or grip cuff. Coverage of the wrists are very important. Avoid using an open-cuff lab coat inside a biosafety cabinet as aerosols generated inside the cabinet will contaminate your jewelry, wrists and forearms and the inside of the lab coat cuff.
- Wear safety glasses.
- Use double glove technique. Ensure that your outer gloves extend over the sleeve of your lab coat. An opening at the wrist will allow aerosols generated within the biosafety cabinet to contaminate your wrist and forearm, extending handwashing to your elbow.
    - Use different color gloves for inner and outer layer. This will allow you to easily visualize any rips or tears.
    - Remove outer layer of gloves when exiting the BSC. Discard into biohazard collection container inside the BSC. Put on fresh outer layer of gloves when going back into the BSC or handling specimen containers.

- Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.
- Wear a universal mask that is stored in the processing space. Do not wear the same mask in regular lab space or public space.
- Remove PPE before leaving the laboratory.
- Disposable PPE must be discarded into biohazard waste bags.
- Decontaminate safety glasses at completion of work by wiping with paper towel saturated with 70% ethanol. Allow to air dry.
- Lab coats must remain in the tissue culture room. Spot clean with 10% bleach or send for laundering if contaminated.
- Wash your hands with soap and water for 30 seconds after removing PPE and before leaving the laboratory.

Processing Specimens from Known or Suspected COVID-19 Patients:

- A dedicated tissue culture room must be available for processing COVID-19 patient specimens. At a minimum, the tissue culture room must have a door that is kept shut while working.
- Perform all work with potentially infectious material inside the BSC.
- Avoid moving hands in and out of the BSC as this will disrupt airflow and compromise containment. Prepare the BSC with needed materials before initiating work. Keep the front and rear grilles clear.
- Discard all waste inside the BSC.
    - Dry waste (paper towels, gloves, pipette tips, tubes, etc): place 2 small biohazard bag in plastic beaker. When full or at completion of work – close the bag and spray the outside with 70% ethanol or 10% bleach. Allow for one minute of contact time. Remove from BSC and place into biohazard sharps container.
    - Liquid waste: decontaminate with 10% bleach final concentration. Allow 30 minutes of contact time before disposing down the drain with running water.
- Decontaminate materials as you work.
    - Pipette tips, serological pipettes: Prepare a small beaker of 10% bleach. Pipette up and down 3 times in 10% bleach before discarding pipettes into dry waste container.
- Decontaminate reusable materials prior to removal from BSC.
    - Spray with 10% bleach, allow 1 minute contact time, wipe dry. Repeat with second decontamination step using 70% ethanol (1 minute contact time).
- Spray BSC with 10% bleach or Virkon-S (10 minute contact time) after use. Make sure to saturate the work surface, grilles, sides, back and inside front sash). Allow required contact time and then wipe clean. Follow with an additional decontamination step with 70% ethanol (1 minute contact time).

Work Practices:

- Keep your hands away from your face and avoid touching your eyes, nose or mouth in the work area.
- Keep personal belongings, including cell phones, earbuds, and exposed jewelry, out of the processing space to avoid accidental contamination. These items must be placed in a safe location outside the lab where clinical samples are being processed.
- Avoid the use of sharps where possible and work very carefully with them if they are required.
    - Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible.

High-Risk Procedures:

- Flow Cytometry
  - High speed sorting of unfixed human cells can generate a large quantity of aerosols in the event of a clog or deflection.
  - **At this time, flow cytometry and FACS of unfixed materials is not permitted**. Contact an EHRS Biosafety Officer if you have questions.
  - Projects utilizing the UPenn Flow Cytometry and Cell Sorting Facility require prior review and approval by Dr. Jonni Moore. Investigators must present a written justification and description of experiments to Dr. Moore (moorej@pennmedicine.upenn.edu) to receive approval.
- Centrifugation
  - Use of sealed rotors or safety buckets as secondary containment is required for centrifugation.
  - Load and unload the rotor or safety buckets within the BSC.
  - Do not overfill primary containers, limit to < ¾ full.
  - Wipe exterior of all centrifuge tubes with disinfectant before loading.
  - Seal rotors or buckets and wipe down with disinfectant, remove outer gloves inside the biosafety cabinet before transport to the centrifuge.
  - Wait 2-5 minutes after the run to allow aerosols to settle in the event of a spill.
  - Transport sealed rotor or safety bucket to biosafety cabinet to complete your experiment. Don new pair of outer gloves before continuing your work inside the biosafety cabinet.
  - Decontaminate the rotor or safety bucket by spraying with 70% ethanol and allow to air dry.
  - In the event of a spill during centrifugation, follow the spill response procedures outlined below.

Decontamination and Disinfection:

- All surfaces and equipment must be disinfected with an appropriate disinfectant after use. This includes all surfaces within the biosafety cabinet, used research materials, equipment, bench tops and other work surfaces, transport and transfer containers.
- SARS-CoV-2 is inactivated with 70% ethanol or 10% bleach with 1 minute contact time. Contact an EHRS Biosafety Officer if you propose to use alternative disinfectants.
- A good rule of thumb is to apply disinfectant to get all surfaces glistening wet and allow it to air dry. One mistake that is often made is that disinfectants are wiped off immediately after it is applied, leaving a dry surface behind with zero disinfectant left to perform disinfection. This can be amended by wiping the surfaces with disinfectant wet paper towels, so that the surface remains wet with the active disinfectant ingredient to maintain the contact time.

Removal of PPE:

- Be cognizant of any step in the removal of PPE that would require you to have contact with your head, hair, neck, skin, face or personal clothing.
- Remove the most contaminated item first. Usually, this is the outer pair of gloves if two pairs of gloves are worn.
- Next, remove your lab coat. Check for any visible contamination on your lab coat if reusable. Spray these areas with your 10% bleach or set lab coat aside for laundering. If wearing a disposable gown or lab coat, place in the biohazard waste container after removal.
- Spray your inner layer of gloves with 70% ethanol and “wash” your gloved hands together for 30 seconds to disinfect them once again.

- Remove safety glasses and set aside to decontaminate. Wipe down with paper towel saturated in 70% ethanol.
- Spray your inner layer of gloves with 70% ethanol and "wash" your gloved hands together for 30 seconds to disinfect them once again.
- Remove your universal mask and store in paper bag (do not store in Ziploc or sealed bags).
- Remove your inner gloves aseptically, or by avoiding contact with the exterior of each glove and in a manner, that prevents the exterior of either glove from contacting your skin.
- Wash your hands with soap and water for 30 seconds. Close the sink faucet off with paper towels after use. Do not touch the faucet handles with your hands after washing to avoid potential re-contamination of your hands.
    - CDC Handwashing videos can be viewed online: https://www.cdc.gov/handwashing/videos.html

Exposures:

- Immediate response to exposures such as cuts, lacerations or splashes to the eyes, nose or mouth is required.
- For punctures or cuts with contaminated sharp objects:
    - Immediately wash the affected area with soap and water for 15 minutes.
- For splashes or contamination to the eyes, nose or mouth:
    - Immediately flush your face in the nearest eyewash for 15 minutes.
- Notify your supervisor if they are available.
- Report for medical evaluation:
    - Employees: Occupational Medicine
        - HUP RAVDIN 2nd floor, 34th & Spruce Streets
        - Hours: Mon – Fri, 8:00am - 3:30 pm
        - Phone: 215-662-2354
    - Students: Student Health
        - 3535 Market Street, Suite 100
        - Hours: check website (https://shs.wellness.upenn.edu/)
        - Phone: 215-746-3535
    - After hours: HUP Emergency Department
        - Ground floor Silverstein Pavilion, 34th and Spruce Streets
- Call EHRS to report the exposure (215-898-4453).

Spills:

- Spills inside the BSC
    - Cover with absorbent material
    - Saturate with 10% bleach
    - Allow 30 minutes contact time
    - Clean and discard materials into biohazard waste bag
    - Disinfect interior of BSC with 10% bleach followed by 70% ethanol. Ensure all surfaces are disinfected (work surface, side and rear panels, inside sash).
- Spills outside the BSC
    - Alert all users in the area
    - Leave the room and shut the door
    - Call EHRS (215-898-4453)

- Keep the spill area evacuated for at least 30 minutes.
- Depending on the nature of the spill, the lab may be able to perform the decontamination, but EHRS will advise on decontamination protocols after the 30-minute evacuation period to allow aerosols to be removed from the lab by the ventilation system.

Please contact a biosafety officer if you have any questions regarding these biosafety guidelines.

Primary EHRS Biosafety Contact:
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267-315-7934

*This guidance document has been adapted from the Yale EHS document "Biosafety Precautions with Clinical Specimens due to COVID-19"