

Yale Environmental Health & Safety

Biosafety Precautions with Clinical Specimens due to COVID-19

Yale Environmental Health & Safety (EHS) is sending this reminder to laboratories and other groups that are registered to receive or will be receiving unfixed human specimens as part of their research in relation to potential COVID-19 risks. Work with unfixed human tissues requires the use of Universal Precautions and Biosafety Level 2 containment and work practices, because all human materials must be treated as potentially infectious. Given the presence of COVID-19 across the world, this update is provided as a reminder to strictly adhere to established biosafety practices.

Upper and lower respiratory system specimens, such as nasopharyngeal and oropharyngeal swabs, sputum, mucus, and lung tissues are of primary concern. But, stool and blood/serum samples also require the use of universal precautions. Please use the following guidelines to help ensure the safety of your lab or work area.

CDC Guidance Information for Laboratories

For those labs who will handle specimens from COVID-19 patients, the CDC has also published interim laboratory biosafety guidelines that can be accessed from the following website.

<https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html>

(Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19))

Yale EHS Biosafety Level 2 Enhanced Procedures

Yale EHS advocates the use of advanced biosafety precautions for handling higher risk biohazards and unknowns. These practices recommend that all work with biohazards be conducted inside the biosafety cabinet, including the collection of all waste within the biosafety cabinet. Emphasis is also on ensuring that there is zero skin contact by biohazards and that all potentially contaminated items are decontaminated before removal from the biosafety cabinet.

A visual description of Yale EHS's BSL-2 Enhanced work practices can be found at the following website.

<https://ehs.yale.edu/sites/default/files/files/bsl2-work-practices.pdf>

Risk Assessment:

If possible, request that the group providing the tissue to you alert you regarding 1) whether there are known pathogens present in the tissues and 2) potential whether pathogen risks based on symptoms or other information obtained regarding the tissue source. If you determine that the tissues are potentially higher-risk than the level of biocontainment you are using, please notify the EHS Office at 203-785-3550 for assistance.

Packaging and Transport:

This information is for hand-carried transport from one campus location to another by walking. Please refer to EHS eShipGlobal training for any shipments on public roadways in vehicles or other methods of transfer in commerce (trains, planes, etc.): <https://ehs.yale.edu/shipping-biological-materials>.

Remind those sending you human specimens, or staff picking them up that it is important to consistently follow decontamination protocols at the starting point.

- Ensure that the exterior of the primary tube, bag or other container is disinfected after collection.
 - Use disinfectants that contain between 62-90% Ethanol, 1-5% household bleach in water, ≥ 0.5 hydrogen peroxide. Notify EHS if you are utilizing another disinfectant so that a check of the disinfectant's active ingredients can be evaluated.
- After a sufficient contact time (1 to 3 minutes for SARS-CoV-2 with these disinfectants), place the primary container inside a clean secondary container.
- Both primary and secondary containers must be leak-proof.
 - Have paper towels in the bottom of the secondary container to absorb any liquids if there was 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.

Make sure those disinfecting the exterior of the containers are aware not to potentially re-contaminate these containers by touching them with potentially contaminated gloves or placing the containers down on a potentially contaminated surface.

If your staff is responsible for taking the specimens, ensure that they have on full face protection, such as a face-shield, or safety glasses and a surgical mask, as well as gloves and either a lab coat or disposable gown. If you are dealing with samples from known or high-risk COVID-19 patients, a minimum of an N-95 respirator will be required.

Double gloving may be utilized, with disinfectant spraying and removal of the outer gloves (and discarding as biomedical waste) after sealing and decontaminating the primary specimen container.

Those taking and packaging the specimens can disinfect their outer gloves in between steps. They can also place the specimen containers on a clean paper towel or on a disinfectant-wet paper towel prior to packaging.

As a precaution, spray or wipe the exterior of the secondary transport container with an appropriate disinfectant as noted above.

Hands should be washed for 30 seconds with soap and water following the removal of personal protective equipment. Personal protective equipment is not required for the transport of appropriately sealed and decontaminated transport containers.

Storage:

Place your samples inside a freezer or other predetermined storage location that is labeled with the universal biohazard symbol.

Ensure that the samples are coded and 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. Maintain your inventory in a secure manner and keep the freezer or other predetermined storage location with this inventory locked when the laboratory is unoccupied.

Personal Protective Equipment (PPE):

Dedicate PPE within your laboratory for your experiments. Do not wear PPE to other non-lab areas and remove prior to leaving the laboratory. Depending on the number of labs you have and the nature of the work, researchers may need multiple lab coats for each area to avoid potential cross contamination.

Wear a lab coat or solid-front back-fastening gown, preferably with a knit or grip cuff. Coverage of the wrists is 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 as well as the inside of the lab coat cuff.

Ensure that your 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 to your elbow.

Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.

Consider double glove for all work within the biological safety cabinet (biosafety cabinet) if performing higher risk work. With two gloves, researchers can remove the outer pair before exiting the biosafety cabinet and don a new pair each time they reenter the biosafety cabinet.

Face Protection

Each person in the lab should have their own pair of safety glasses. These can be sanitized after each use with 70% Ethanol and allowed to air dry.

Wear a chin-length face shield or safety glasses and a mask if working outside of the biosafety cabinet with biohazards on the bench. This will protect the researcher's facial mucous membranes from exposure in the event a spill outside the biosafety cabinet during transfer of material to and from the incubator.

It will also help to prevent you from touching your eyes, nose and mouth when working within the biosafety cabinet.

Remove PPE before leaving the laboratory. Placing a hook within the lab area will facilitate this requirement.

If double gloving and using a disposable face shield, follow these PPE removal steps:

- Remove your outer gloves first, then your lab coat or gown, followed by the inner gloves. Take your face protection off last. Do not touch your face with gloved hands. Remove gloves and other clothing aseptically, from the inside out, and avoid touching the contaminated outer side of the glove.

Decontaminate reusable PPE as soon as feasible after it has been contaminated. Small areas can be spot treated with a suitable disinfectant, such as 1-10% household bleach. Lab coats can also be autoclaved or sent to a laundry facility equipped to handle biohazardous PPE. Disposable PPE can be placed within a biohazard bag, treated and discarded as biomedical waste.

Wash your hands with soap and water for 30 seconds after removing PPE and before leaving the laboratory.

Initial Processing/Lysing of Specimens from COVID-19 Patients:

If you are obtaining specimens from COVID-19 patients or suspect patients for testing or genetic analysis, please use these guidelines:

- Communicate the importance of the initial disinfection of the exterior of the primary and secondary transport containers as noted in the section above on sample collection.
- Perform all initial steps with these clinical samples inside the biosafety cabinet.
- Make sure that your wrists are not exposed, and are sufficiently covered (e.g. longer gloves over a lab coat with banded cuffs).
 - Wear safety glasses or a full face-shield, a lab coat and exam gloves (consider double gloving).
- Follow the lysing product instructions and allow the required contact time prior to considering the specimens to be “inactivated.”

Note: +ssRNA viruses, like SARS-CoV-2, are considered infectious as full-length RNA, as it could still establish an infection if it can gain entry to a human cell. Continue to treat full-length RNA using Universal Precautions and BSL-2 containment practices.

- Disinfect the exterior of items prior to removal from the biosafety cabinet with the disinfectant you have selected for your research or procedures (62 – 90% Ethanol, 1-5% household bleach in water, and \geq 0.5% Hydrogen Peroxide are suitable active ingredients for inactivation of SARS-CoV-2.
- Continue to utilize PPE for working with RNA from this virus outside of the biosafety cabinet. A lab coat, safety glasses and mask or chin-length face shield, and gloves.
- Follow the PPE removal (doffing) and hand washing guidance as noted in this document.

Engineering Controls:

Perform all work within a biosafety cabinet.

This includes discarding waste within the biosafety cabinet, as moving your hands in and out of the biosafety cabinet will disrupt the protective air curtain at the front access opening.

Place all items required for the experiment within the biosafety cabinet before starting work.

Wipe items down with disinfectant prior to placement within the biosafety cabinet.

Keep the front and rear grilles clear when working within the biosafety cabinet. Avoid blocking the rear grille.

Do not store items on top of the biosafety cabinet. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow.

Depending on the location of the biosafety cabinet within the room, opening and closing the room door can significantly interfere with biosafety cabinet airflow.

Wipe items down with disinfectant prior to removal from the biosafety cabinet.

Wipe down biosafety cabinet with disinfectant after use (work surface, grilles, sides, back and inside front view screen).

Decontaminate liquid waste with household bleach diluted 10% against the volume of the waste. Allow at least a 30-minute contact time for full decontamination.

Collect dry waste (e.g. gloves, pipet wrappers, paper towels) in a small biohazard bag inside the biosafety cabinet. Seal bag prior to removal from biosafety cabinet.

Transport waste to autoclave in a leakproof container.

Work Practices:

- Keep your hands away from your face and avoid touching your eyes, nose or mouth in the work area.
- Never eat, drink, smoke or apply cosmetics in the work area.
- Never mouth pipette. Always use mechanical pipetting devices.
- Avoid the use of sharps where possible and work very carefully with them if they are required.

- Disinfect work surfaces after experiments with an appropriate disinfectant and ensure that you are following the 5 C's of decontamination. These include cleaning, chemical select, concentration of the chemical, contact time and (surface) coverage.
- Wash your hands after removing PPE and before leaving the laboratory.

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. If you are using the flow cytometry core facility, sorts involving biohazards are directed to sorters equipped with biocontainment controls.

If you have your own high-speed cell sorter, please notify Yale EHS at 203-785-3550 to schedule evaluation of your process and for testing the containment of your cell sorter for the containment of biohazards.

Centrifugation

Use sealed rotors or safety buckets as secondary containment for centrifugation. Load and unload the rotor or safety buckets within the biosafety cabinet. Do not overfill primary containers, limit to $< \frac{3}{4}$ 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 allowing to air dry. Wipe the throw line within the centrifuge with disinfectant and remove your biohazard sign. In the event of a spill during centrifugation, follow the spill response procedures outlined below (evacuate the lab and notify Yale EHS at 203-785-3555).

Sharps Elimination or Sharps Precautions:

Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible.

Alternatives to glass Pasteur pipettes include: plastic pipettes, plastic transfer pipettes, plastic gel loading pipette tips and pipette tip extenders, aspirators, and flexible plastic aspiration pipettes.

Some researchers will either score and break the end off a 1 ml or 5 ml plastic pipette or remove the wool plug and use for aspirating cultures.

If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use and discard intact needles and syringes immediately after use. Use a one-handed disposal method (keep a hand behind your back or by your side, and do not place your other hand on or near the opening of the sharps container).

Never recap, bend, break or otherwise manipulate sharps by hand.

If you must remove the needle from the syringe, use forceps, tweezers, a Kelly clamp or small pliers for this purpose.

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 readily inactivated with disinfectants that contain 62 – 90% Ethanol, 1-5% bleach in water solutions, and $\geq 0.5\%$ Hydrogen Peroxide.

Practice the 5 C's of Disinfection and Decontamination at all times.

Cleaning.

Ensure that the area is cleaned prior to initiating the disinfection process where applicable. Some surfaces may be very clean prior to initiating decontamination. Other situations can utilize a “double” disinfection process, where the first application ensures that the surfaces are cleaned and the 2nd application is the formal disinfection procedure.

Chemical.

Ensure that the chemical disinfectant that you select for decontamination has proven efficacy against the biohazards in use or anticipated. Refer to Section 10 of the Yale Biological Safety Manual for more information on disinfectants, and an NIH Chart on the selection of disinfectants appears on Page 10-6.

<https://ehs.yale.edu/sites/default/files/files/biosafety-manual.pdf>

Concentration. The amount of the chemical matters significantly. Follow the manufacturer's recommendations for dilution if purchasing a commercial disinfectant. Household bleach works well in the range of 1 – 10% dilution with water, but higher concentrations may be needed for certain biohazards. Alcohols generally have better efficacy in the range of 70 – 85%. Iodine based disinfectants, unlike bleach, lose effectiveness at higher concentrations as iodine molecules will begin to bind each other, leaving less free iodine available for decontamination.

Contact time.

No disinfectant works immediately. Disinfectants must be left on the surfaces or items to be decontaminated for a specified time and this varies by individual biohazard.

Contact times of 1, 3, 5 or 10 minutes or even longer may be needed. A good rule of thumb is to apply disinfectant to get all surfaces glistening wet with disinfectant and allow it to air dry. If your disinfectant has a higher evaporation rate (e.g. alcohols), and a longer contact time is needed, you may need to apply the disinfectant twice.

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.

Coverage.

Ensure that all surfaces are completely covered with the disinfectant. Merely spraying the disinfectant on a surface, especially if only applied quickly or lightly, can leave spaces in between the disinfectant drops. Also, air bubbles that form from spraying indicate that the disinfectant doesn't ever contact most of the space below the air bubble. In this case, spraying disinfectant and wiping with a disinfectant-wet paper towel would ensure that adequate coverage has been achieved.

If you are working with human specimens, ensure that you are also using a dilute solution of household bleach in water or another EPA registered tuberculocidal disinfectant for conformity with the OSHA Bloodborne Pathogens Standard.

Biomedical waste:

No changes are required to the existing biomedical waste disposal protocols.

- Sharps (needles, syringes with or without needles attached, razor blades, scalpels, etc.) are placed within a needle box or sharps container.
- Items that are not medical sharps, but those that could puncture a bag, are placed within the bag-lined cardboard box. These include all other solid non-sharp items such as serological pipettes, flasks, plates, paper towels, gloves. Glass Pasteur pipettes may also be placed inside these boxes.
- Soft items such as gloves and paper towels, can be placed in a biohazard waste bag, and then moved to a cardboard waste box when full.

Note: high risk wastes, such as those that contain human pathogens or higher risk recombinant DNA research wastes, must be autoclaved prior to disposal inside the cardboard biohazard waste box containers.

Autoclavable solid red buckets are available for this purpose if needed from the EHS Environmental Affairs Office.

- Liquid biohazard waste can either be autoclaved on slow-exhaust or chemically inactivated (usually household bleach is utilized at a final concentration of 10% against the volume of the waste to be treated for a contact time of 30 minutes prior to disposal via the sink drain).

Do not use bleach to inactivate human urine as respiratory irritant chemicals (e.g. Monochloramine) can be formed from the reaction of bleach and urea in urine. **Notify EHS for assistance prior to inactivating human urine.**

Regulations to permit urine and blood to be discarded down the sink drain if connected to the sanitary sewer system. Ensure that those processing liquid waste in this manner are wearing a chin-length face shield or other combinations of full face protection. Disposal of this waste down the drain is followed by flushing disinfectant down the drain after to disinfect the sink trap and running water for 30 seconds to 1-minute following disposal.

Removal of PPE:

In general, the most contaminated item is removed first. Usually, these are the outer pair of gloves if two pairs of gloves are worn.

If only a single pair of gloves are worn, disinfect your gloves with your lab's disinfectant (such as 70% Ethanol or 1-10% bleach) when you have finished your work and allow at least a 30 second contact time. 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. It is recommended that you perform this 30 second disinfection wash before any step in the PPE removal that could potentially have contact with these areas.

Wet some paper towels with your lab's disinfectant and set aside. Disinfectant wipes can also be placed in the PPE removal location for the decontamination of reusable items after removal.

After this 30 second decontamination step, remove your lab coat or gown.

After removing your lab coat or gown, check for any visible contamination on your lab coat if reusable. Spray these areas with your lab's disinfectant (which would have been selected for the biohazard in use) and allow to air dry. If wearing a disposable gown or lab coat, place in the biomedical waste container after removal.

Spray your gloves with your lab's disinfectant and "wash" your gloved hands together for 30 seconds to disinfect them once again.

Remove your face shield or safety glasses and mask if worn. Place disposable items in the biological waste. Use the disinfectant-wet paper towels or disinfectant wipes to wipe down the face shield and safety glasses with disinfectant and allow to air dry.

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.

Hand Washing:

Hands must be washed promptly after the removal of PPE.

Use the nearest hand washing sink and use soap and warm water and wash your hands for 30 seconds. Dry your hands with paper towels. **If you do not have a hands-free sink, allow the water to continue running while you dry your hands and then use those paper towels or other paper towels to shut off the sink faucets.**

If you are not near a hand washing sink, use a hand sanitizer with 60% or greater alcohol and allow contact for 30 seconds. Follow with a full hand washing at the nearest sink after using the hand sanitizer.

CDC Handwashing videos can be accessed at the following web site:

<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.
- Notify your supervisor if they are available.
- Call Yale Health Employee Health (203-432-0123) or Yale Health Acute Care (203-432-0123) for next steps.
 - You need to contact a healthcare provider as soon as possible for a discussion of your incident.
 - Call the Yale EHS Emergency Line to report the incident (203-785-3555).

For splashes or contamination to the eyes, nose or mouth:

- Immediately flush your face in the nearest eye wash for 15 minutes.
- Notify your supervisor if they are available.
- Call Yale Health Employee Health (203-432-0123) or Yale Health Acute Care (203-432-0123) for next steps.
 - You need to contact a healthcare provider as soon as possible for a discussion of your incident.
 - Call the Yale EHS Emergency Line to report the incident (203-785-3555).

Make sure that you are aware of the location of the eye wash before beginning work.

Make sure you have been trained on how to use the eye wash in or outside your lab.

The eye wash in your area must be flushed with water for at least one minute once each week.

Spills:

Spills, or the release of biohazardous materials (e.g. pathogens, toxins, human materials, human cells) outside of primary containment will generate aerosols that have the potential to contaminate those in the room from the splash and splatter immediately, and through exposure to aerosols, within seconds following the spill.

If there is a spill in your laboratory, hold your breath, move everyone out of the spill area, including those who have been contaminated with biological materials. Assist those who are contaminated with their personal clean up. Place any contaminated PPE and personal clothing inside a biohazard bag.

Call the Yale EHS Emergency Line at 203-785-3555 to report the incident.

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 EHS 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 Yale EHS at 203-785-3550 if you have any questions regarding the biosafety precautions for the safe handling of your unfixed human specimens.