

Biosafety Precautions with Clinical Specimens from COVID-19 Patients

Executive Summary

Columbia University Environmental Health & Safety (EH&S) in conjunction with the Institutional Biological Safety Committee (IBC) has prepared this guidance for 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 human specimens requires the use of [standard AKA universal precautions](#) and Enhanced biosafety Level 2 (BSL-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 (See Appendix 1 Biocontainment levels and training required for research materials).

CDC/ABSA 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](#) (Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)). The IBC considers the current CDC guidance regarding containment procedures for working with the SARS-CoV-2 virus as a minimum standard. See also Appendix 2; ABSA chart.

1. Procedures/Lab Work Requiring BSL-2 Containment

Specimens that have been inactivated by an established method can be worked on with BSL-2 practices. At BSL-2, aerosol-generating procedures are also performed in a biosafety cabinet.

2. Procedures/Lab Work Requiring *Enhanced* BSL-2 Containment

Nasal and pharyngeal swabs have been shown to contain high loads of SARS-CoV-2 virus and must be worked on at enhanced BSL-2 containment. Be advised that current data in the literature suggest a prolonged release of viral material in feces extending past patient symptoms. These are potential high titer samples. The current available literature regarding the viral load of SARS-CoV-2 in blood, plasma, urine and organs (except lung) suggests these patient samples have lower viral titer. Note that there is no guarantee of a sample having low or no viral titer so care must still be used in handling these specimens. Enhanced BSL-2 containment is performed in typical laboratory tissue culture room and employs practices borrowed from specialized BSL-3 containment labs. Investigators planning to work with nasal swabs, pharyngeal swabs, BAL, tracheal washes, lung tissue and other known high viral titer samples at enhanced BSL-2 containment will need to have access to a certified biosafety cabinet that is isolated from their general laboratory space and has

the ability to have restricted access during times of work with specimens. Signage should be posted to ensure restricted access during manipulation of these potentially high titer specimens. Enhanced containment practices (**detailed below**) should be used with these samples including ALL work in the biosafety cabinet, closed rotor centrifuges or centrifuge "cups", centrifuge rotors loaded and opened in the biosafety cabinet, biohazardous wastes decontaminated or bagged within the biosafety cabinet. Closed lab coat, double gloves, eye protection mandatory. Self-monitoring for fever, cough, or fatigue mandatory.

3. Procedures/Lab Work Requiring BSL3 Containment

As per the CDC, cell culture of clinical specimens, virus isolation in cell culture and initial characterization of viral agents recovered from cultures of SARS-CoV-2 specimens are only allowed in Biosafety Level 3 (BSL-3) labs operating with BSL-3 practices. This requirement for BSL-3 containment is extended to include ANY work with the SARS-CoV-2 virus that involves culture or propagation of the virus OR introduction of the full length virus or viral genome, RNA or cDNA, to cultured cells or animals. Please note that BSL-3 containment laboratories are unique lab spaces with a specified set of requirements including, but not limited to, sealed cleanable surfaces, ceilings, walls and floors (no drop ceilings), monitored negative air flow, a dedicated exhaust system equipped with HEPA exhaust filters and redundant fan systems, an anteroom with door interlocks to the main lab space, high stringency PPE and training, and are tested, and must pass, failure scenarios including power or exhaust fan loss. All BSL-3 laboratories at Columbia University are registered with the Biosafety Office, the NYC-DOHMH and are evaluated annually for proper function and containment.

4. Procedures/Lab Work with full length viral genomes

The SARS-CoV-2 virus is a positive strand, or sense strand, RNA virus. This means that the RNA extracted from a virus-containing sample is still officially considered "infectious" because it can be directly read by the existing translation machinery in cells and generate viral proteins resulting in the generation of more SARS-CoV-2.

Getting Started

Research with infectious material may require review by the Rapid Research Review Team (RRRT) and/or the IBC and research with recombinant DNA must require review by the IBC, both through submission of a Rascal Appendix A. When compiling a Rascal Appendix A, choose "BSL-2" for "Enhanced BSL-2". Research with patient materials or other human-derived clinical or control samples may require IRB approval. Training requirements are met by completion of applicable Rascal Courses. See Appendix 1.

Risk Assessment:

If your lab cannot meet any of the expectations for work at enhanced BSL-2 or suspect that the specimens you receive are potentially higher-risk than the level of biocontainment you are using, please notify biosafety@columbia.edu. Regardless of the source of the sample, *Universal aka Standard precautions* must be observed when handling all specimens.

Biosafety Level 2 Enhanced Procedures

Technical Expertise/Personnel Requirements

At this time the IBC requires that only trained scientists be allowed to work with materials containing potentially active SARS-CoV-2. A visual description of BSL-2 Enhanced work practices can be found at the following website

<https://research.columbia.edu/sites/default/files/content/EHS/COVID-19/EnhancedBSL-2-SpecimenProcessingandCellCulture.pdf>. A Rascal course on enhanced BSL-2 procedures (TC5500) with a post test and attestation satisfies training requirements but ultimately, training is provided by lab members with the most experience in handling pathogens through hands-on work and directly observed competency assessment.

Laboratory Locations

As well as having a certified biosafety cabinet, labs that work with infectious material from COVID-19 patients should ideally be small tissue culture rooms. These are low traffic areas with no concurrent unrelated work taking place and less potential for cross-contamination. Rooms should have a handwashing sink and the centrifuge that will be used to process the specimens. Biosafety Officers (biosafety@columbia.edu) can determine whether your room meets these criteria. Signage should be posted to restrict access to the tissue culture room during manipulations of infectious specimens.

Sample Collection

Samples will be collected by clinical staff using standard CDC-recommended protocols specific to COVID-19 by staff wearing recommended personal protective equipment (PPE) that consists of a gown, gloves, masks and eye protection as indicated by clinical guidelines. Samples will be placed into durable, sealable plastic transport containers. Tupperware also adequate.

Packaging and Transport:

Disinfection of Sample and Transport Containers

Sample and transport containers will be disinfected/wiped down using a standard 10% bleach solution followed by a wipe-down with alcohol solution with at least 70% alcohol.

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, 10% household bleach in water, $\geq 0.5\%$ hydrogen peroxide. Notify biosafety@columbia.edu 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.

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.

Sample Packaging for Transport

This information is for hand-carried transport from one campus location to another by walking. Samples will be packaged for transport using a standard triple-packaging scheme to withstand the normal shocks, temperature and pressure changes common during regular transport without leaking to the outside of the container. This will include placement of samples into a plastic screw-

top container (primary receptacle) which will then be cushioned. Samples will then be placed into a leak-proof Ziploc specimen bag (secondary receptacle), after which the specimen bag will be placed into a standard, dedicated hard-plastic transport box (with one outer surface with minimum dimensions of 100 mm x 100 mm). This transport box is labeled with standard hazard labels, all of which are durable and self-adhesive and do not overlap. UN3373 packaging standards are also acceptable (see Guidance document pg. 7:

<https://research.columbia.edu/system/files/EHS/Policies/BiologicalMaterialsShippingManual.pdf>

Please refer the EH&S shipping manual for transport in vehicles or other methods of transfer in commerce (trains, planes, etc.):

<https://research.columbia.edu/system/files/EH&S/Policies/BiologicalMaterialsShippingManual.pdf>.

Logistics for Sample Delivery

If your staff is responsible for accepting the specimens delivered from a courier, ensure all steps are followed under the Chain of Custody and Storage sections, below.

Logistics for Sample Pick-up

Laboratory personnel will receive the sealed transport container from the nursing station or outside the patient room. Personnel must observe all applicable PPE requirements in patient treatment areas. Following arrival in the lab the sealed transport container will be disinfected and wiped again prior to opening the container within a certified biological safety cabinet.

Chain of Custody

Upon delivery to the laboratory, samples must be registered in the lab by a research coordinator for sample tracking purposes.

Specimen identification:

Ensure that the samples are coded and labeled in a way that identifies their contents to the laboratory. Most likely exposure to infectious materials is due to human error; mistakenly removing infectious material from the laboratory. The lab must implement an easy to understand tracking system to discriminate infectious from inactive material. This could be different colored tubes, colored labels (“cryodots”), colored sharpies etc. Infectious materials should not be transferred to laboratories that lack the appropriate controls to handle them safely.

Security

Frozen materials that contain SARS-CoV-2 should be maintained plastic boxes (not cardboard) that are labeled to identify the hazard as “SARS-CoV-2”. Keep an inventory of your infectious

biohazards for easy retrieval and to prevent accidental use. Maintain your frozen materials and inventory in a secure manner. Freezers should be kept locked, and with a biohazard symbol on the freezer door (self-adhesive labels available from biosafety@columbia.edu). Freezers should be located inside a biobank facility or the principal investigators laboratory where the specimen work is authorized; not in common areas.

Personal Protective Equipment (PPE):

General Requirements

Proper laboratory attire consisting of long pants or the equivalent, closed shoes and a shirt with sleeves is required at all times. 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.

Body and Hand Protection

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 can easily 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 gloves for all work within the 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 or other equipment.

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

PPE Care and Disposal

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 and PPE aseptically, from the inside out, and avoid touching the potentially 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 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 regulated medical waste.

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

Buddy system/two person rule

Working with a partner can make your work flow much smoother. The buddy can assist the person working at the biosafety cabinet so that they can keep their hands inside the cabinet as much as possible. Buddies assist by reading the protocol to the operator, and checking their microbiological technique. Buddies transport centrifuge safety cups and partners check each other's PPE is worn correctly

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

Inactivation procedures that are not established methods must be validated.

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 certified 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 any inactivation procedures and allow the required contact time prior to considering the specimens to be “inactivated.”

Note: positive strand RNA viruses, like SARS-CoV-2, are considered infectious as full-length RNA, since given the right experimental conditions, an infectious virus can be produced. 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 (70% Ethanol, 10% 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 certified 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. There is no tossing materials from the biosafety cabinet into a nearby red-bag bin or sharps container.

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

Set up the biosafety cabinet for a clean-to-dirty linear workflow, and avoid cross-contamination of work zones within the cabinet.

When performing tissue culture, 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 inside or on top of the biosafety cabinet. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow. Consider posting signage, where feasible.

Depending on the location of the biosafety cabinet within the room, opening and closing the room door can significantly interfere with biosafety cabinet airflow. An air conditioner can also disrupt the 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 10-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.

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.
- Waste generated within the biosafety cabinet is collected inside the biosafety cabinet and decontaminated with bleach whenever possible.
- 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 selection, 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 including FACS 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. Please refer to [ISAC guidelines](#) for further info.

If you have your own high-speed cell sorter, please notify biosafety@columbia.edu to schedule evaluation of your process 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 < 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.

Transport sealed rotor or safety bucket to biosafety cabinet to complete your experiment. Don a new pair of outer gloves before returning to continue your work inside the biosafety cabinet.

If you are working with a buddy, this task can be completed by the buddy so you can keep your hands inside the biosafety cabinet.

Infectious liquid residue from the threads of tubes can still potentially accumulate in the wells of the centrifuge tube holders. Wipe exterior of all centrifuge tubes with disinfectant when unloading

Wait 20 minutes after the run to allow aerosols to settle in the event of a spill. Wipe centrifuge bowl and the throw line within the centrifuge with disinfectant.

Decontaminate the rotor or safety bucket by spraying with 70% ethanol and allowing to air dry. In the event of a spill during centrifugation, follow the spill response procedures outlined below

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 of 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 70% Ethanol, 10% 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. For a list of EPA-approved disinfectants appropriate for use against SARS CoV-2, please visit the link here - <https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2>

Note, chemicals not listed on “List N” can also be used for disinfection of SARS-CoV 2, provided they are an EPA-registered product with a label claim against human coronaviruses.

Concentration. The amount of the chemical matters significantly. Follow the manufacturer's recommendations for dilution if purchasing a commercial disinfectant. Household bleach works well at 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 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 more than once.

One mistake that is often made is that disinfectants are wiped off immediately after they are applied, leaving a dry surface behind with zero disinfectant left to perform disinfection. This can be amended by wiping the surfaces with paper towels wetted with disinfectant, 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.

Repurposing of Room.

A room that has been used for specimen handling must be given a terminal cleaning before the room is used for any other type of work, for example regular tissue culture. Terminal cleaning is using a disinfectant to wipe down anything that may have been contacted by the hands or by materials used for viral work. This would include interior of biosafety cabinets, countertops, centrifuge rotors and centrifuge bowls, centrifuge control buttons, freezer and incubator handles.

A sign on the door should indicate that Facilities workers should not enter the space. For Facilities to clean the room an EH&S clearance should be solicited and the room receive terminal cleaning.

Biomedical Waste:

All waste goes into red bags or sharps containers. There are no regular trash cans in the room. No changes are required to existing biomedical waste disposal protocols.

- Sharps (needles, syringes with or without needles attached, razor blades, scalpels, pipette tips and Pasteur pipettes, serological pipettes etc.) are treated with bleach whenever possible and placed within a sharps container. Never overfill sharps containers. Properly close containers before removal from the laboratory to the hallway following normal procedures.
- Soft items such as gloves and paper towels can be placed in a red biohazard waste bag. In the Superblock and Hammer buildings, red bags must be tied tightly and moved, within the container they are housed in, to the grey TB02 dumpster in the hallway. In the ICRC building red bags are housed in cardboard boxes. Red bags must be tied tightly, within the cardboard box they are housed in, and placed in the hallway.
- Liquid biohazard waste can be chemically inactivated. Household bleach is utilized at a final concentration of 10% against the volume of the waste to be treated for a contact time of 10 minutes prior to disposal via the sink drain.

Irritant chemicals can be formed when bleach is used to treat human urine (e.g. Monochloramine) which can be formed from the reaction of bleach and urea in urine. Human urine has not been shown to contain SARS-CoV-2 and can be directly disposed into the drain and flushed with plenty of water.

Removal of PPE:

In general, the most potentially 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, when you have finished your work, disinfect your gloves by spraying them with your lab's disinfectant (such as 70% Ethanol or 10% bleach) and "wash" your gloved hands together for 30 seconds to disinfect them. 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. Gloves that are knowingly contaminated should be immediately disinfected and replaced at their point of use.

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.

Once again disinfect your gloves by spraying them with your lab's disinfectant and "wash" your gloved hands together for 30 seconds to disinfect them

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.

- Immediately wash the affected area:
 - Punctures or cuts with contaminated sharp objects, splashes wash with soap and water for 15 minutes.
 - Contamination to the eyes, nose or mouth wash with water for 15 minutes
- Notify your supervisor if they are available.

Call Workforce Health and Safety (212-305-7590; Harkness Pavilion, 1st Floor, 180 Fort Washington Ave.) for next steps. You need to contact a healthcare provider as soon as possible for a discussion of your incident. An accident report must be completed and submitted to biosafety@columbia.edu either by the individual or the medical provider.

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.

Medical Surveillance

Individuals who are immunosuppressed or have medical conditions that might contribute to negative outcomes if infected by SARS-CoV-2 are strongly discouraged from working with the material.

EH&S is tasked with investigating COVID illness that may be related to a lab exposure (laboratory acquired) versus community-acquired. Because of asymptomatic spread EH&S is concerned about development of clusters of infection in a group of people who work together. Lab workers should have an acute awareness of symptoms and report any COVID-19 illness to their supervisor and biosafety@columbia.edu within 24h. Any individual experiencing symptoms noted above should self-quarantine. The University's occupational health provider, Workforce Health and Safety (212-305-7590) can advise on additional steps for medical care.

Spills:

Spills, or the release of biohazardous materials (e.g. pathogens, toxins, human materials, and 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, 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.

Depending on the nature of the spill, the lab may be able to perform the Decontamination. More information in the EH&S spill policy

<https://research.columbia.edu/system/files/EHS/Policies/BiologicalSpills.pdf>

Please contact biosafety@columbia.edu if you have any questions regarding the biosafety precautions for the safe handling of your unfixed human specimens.

Appendices

Appendix 1. Grid of biocontainment level and specimen type.

Appendix 2. ABSA Considerations for Handling Potential SARS-CoV-2 Samples.

https://absa.org/wp-content/uploads/2020/03/ABSA2020_Covid-19-dr3.pdf

References

WHO Standard Precautions in Healthcare. https://www.who.int/docs/default-source/documents/health-topics/standard-precautions-in-health-care.pdf?sfvrsn=7c453df0_2

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>

SARS-CoV-2 FACS sorting protocols. <https://isac-net.org/news/news.asp?id=497501>

Content kindly provided by Yale University EH&S and John's Hopkins Biological Safety Program.

Appendix 1. Biocontainment levels and training required for research materials

	Universal / Standard precautions	BSL-2	Enhanced BSL-2	BSL-3	ABSL-2	ABSL-3
Rascal Training	PI attests	TC0509 TC3550 (lab work). TC0025 (specimen collection)	TC0509 TC3550 TC5500	TC0509 TC3550 TC5500	TC0509	TC0509
Other training	WHO Standard Precautions (References)	N/A	Hands-on provided by PI	Contact EH&S		Contact EH&S/CM
Viral culture from a repository Viral isolation from specimens Culture of cells from clinical specimens	X			X		
Respiratory specimens (swab, BAL, endotracheal wash, lung tissue). Other specimens (blood, urine, fecal)	X		X			
Cadaverous tissue	X	X	X (lung)			
Cutting frozen sections	X	X				
Unfixed cells for Fluorescence-Activated Cell Sorting (FACS)	X		X			
Material subjected to viral inactivation in the University's Biobank		X				
Material subjected to viral inactivation in a Columbia University research laboratory		X				
Material not inactivated, processed for shipping	X		X			
Material subjected to viral inactivation before receiving at Columbia University (non-infectious)		X				
Full length viral RNA		X				
Pseudotyped viral vector (lentiviral, VSV)		X				
Viral genes in plasmids		X				
Introducing SAR-CoV-2 into animals	X					X
Introducing Pseudotyped viral vector into animals					X	

