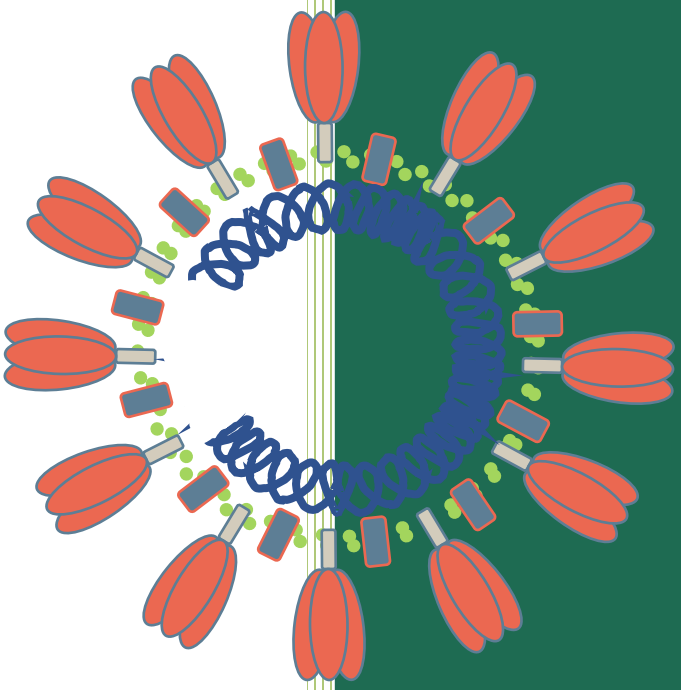
2020

UAB COVID-19 Containment Guidance for Researchers

This lab-specific manual applies to the following laboratory:

PI:
Building:
Room:



UAB Biosafety Program
Justin Roth 03/23/2020

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Biosafety Level-2 *Enhanced* Laboratories (BSL2+):

Biosafety Level 2+ (BSL2+) is not a containment level officially recognized by the NIH or CDC, but the term is often used to describe BSL-2 laboratory facilities that operate using BSL-3 practices and procedures. This enhanced containment level is used for biological agents that pose a higher risk than standard agents found in BSL2 laboratories, but the greater associated risks can be adequately addressed through more stringent practices and procedures. There is no standardized list of microorganisms, procedures, or research projects that should be conducted at BSL2+. This level of containment will be determined by the Biosafety Officer and/or the Institutional Biosafety Committee (IBC) based on a risk assessment of the agent(s) in use, the facilities and PPE available, and the procedures being conducted.

The PI is ultimately responsible for providing training and ensuring staff and students are proficient in the practices and procedures required of them. This manual is provided to assist Principal Investigators (PIs) in the development of a laboratory-specific biosafety manual with instructions to safely handle and manipulate COVID-19+ patient samples under BSL2+ laboratory conditions. If other agents are to be used in this research space, an Agent-Specific Safety Sheet should be appended to provide basic background information of each agent. Standard operating procedures for experiments where safety is a concern (see "Safety SOPs" on page 15) should also be included. Training dates should be maintained in the provided table, and incident response plans easily located. Additions/changes to this template that will render the final manual more useful for the laboratory's safety needs are encouraged. If any laboratory determines the need to deviate from the minimal work practices discussed in this manual, then these alterations along with a written explanation must be submitted to the Biosafety Officer for approval.

***NOTICE: SARS-CoV2 research**: At this time, the CDC or the NIH guidelines clearly **define research with the actual virus as BSL3**, which means all research isolating and propagating the virus, including culturing of permissive cells obtained from (or exposed to) COVID-19^{+} patient samples, must be performed in SEBLAB.

Diagnostic testing: CDC and NIH guidelines have designated BSL2 containment for diagnostic testing. Clinical diagnostic testing includes processing **limited amounts of blood (5-10 ml)**, which may require the use of centrifuges. Centrifuges must be equipped with centrifuge safety cups or sealed centrifuge rotors to reduce the risk of exposure to laboratory personnel.

Research with COVID-19 patient blood/cell isolation: At this time, there are no definitive guidelines from the NIH or CDC regarding the BSL classification of COVID-19 sample-based research.

Research purpose is defined as the use of more than one blood vial or >10 ml of blood, and shall be conducted according to the BSL2+ requirements outlined in this document:

- Processing will ***require the use of centrifuges equipped with centrifuge safety cups or sealed centrifuge rotors*** to reduce the risk of exposure to laboratory personnel.
- Processing should not be done in large, open floorplan laboratories, but rather in smaller laboratories with directional airflow.
- Samples subjected to published methods for inactivating coronaviruses may be exempt from these requirements. Contact Biosafety@uab.edu for guidance.

Additional Resources: In addition to this manual, the National Institutes of Health (NIH) and the UAB Institutional Biosafety Committee (IBC) require the lab to follow biosafety procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (http://www.cdc.gov/biosafety/publications/bmbl5/).

For research involving recombinant DNA, the lab must also follow the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm).

Overview of Biosafety Stipulations for BSL2+ Work:

1. **Laboratory:** Since BSL2+ builds on BSL2 requirements, the laboratory must meet all BSL2 facility requirements
   - Of particular importance is the routine assurance that airflow is directional into the laboratory, preferably with both visual and audible readouts, and the availability of a handwashing sink
   - See ***Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition***
   - Or, ask for the "UAB Lab-Specific Biosafety Plan Template for BSL-2 Laboratories"
2. **Administrative Controls and Standard Operating Procedures**
   - Enrollment in Occupational Medicine
   - Completion of all appropriate on-line training (HS200, Bio303, Bio301L, Bio500…)
   - SOPs for guidance of the specific procedures conducted, with demonstrated proficiency
   - Agent-specific Awareness and Exposure Response Plans
   - Project Registration and IBC Approval must be granted before the start of any non-exempt work or work with SARS-CoV-2 material other than unmodified patient samples
3. **Safety Equipment:**
   - All work with viable material must be conducted in an annually-certified biosafety cabinet, or other containment device
   - Centrifugations: must be conducted within gasketed safety buckets or gasketed rotors that are loaded and unloaded in the BSC
4. **PPE: Based on Agent-specific Risk Assessment and Procedures (see detailed section below)**
   - Additions to the minimum PPE may be required by the IBC on a case-by-case basis and will be specified in your approval letter and/or during an in-lab biosafety visit
5. **Waste**: All waste is inactivated, either chemically or by autoclave, before being offered to Stericycle as medical waste
6. **Sharps**: must be limited. If needles must be used, then safety needles are required. Alternatives to glass must be used wherever possible

Detailed COVID-19^{+} Biosafety Requirements and Procedures:

1. **Physical Containment:** All infectious disease work designated for BSL2 containment must be performed in a BSL2 laboratory that is compliant with the annual UAB Research Safety Review Program. Contact a biosafety representative at 934-2487, or biosafety@uab.edu for consultations prior to your lab review.

   ***SARS-CoV2 research**: At this time, the CDC and the NIH guidelines clearly **define research with the actual virus as BSL3**, which means all research isolating and propagating the virus will have to be performed in the UAB SEBLAB**. This includes any culture involving cells isolated from, or exposed, to COVID-19^{+} patient tissues that are permissive to virus replication.**

   a. **Directional Airflow:** All BSL2+ laboratories must have inward directional airflow from the public corridor. Visual or electronic monitoring devices are strongly recommended to ensure airflow directionality and pressure differentials are maintained.

   b. **Security:** All doors to BSL2+ areas must remain closed and locked to restrict entry of unapproved visitors. Doors may not be propped open, including doors to interior suites requiring directional airflow.

c. **Tissue Culture:** Tissue culture work must not be conducted within the main BSL2 laboratory, but isolated within a separate suite, with directional airflow inward, compared to the rest of the laboratory.

d. **Signage:**
- A biohazard sign must be posted at the entrance to the lab. This sign must include the biosafety level (BSL2+), a biohazard symbol, "Restricted Access Only," and "COVID-19+ Patient Samples" (see Appendix VII, page 23).
- The name and phone number of the PI or facility supervisor, and lab entry procedures (including required personal protective equipment) must also be posted for entry. Contact biosafety@uab.edu if you need help with appropriate signage.
- All incubators and freezers used with infectious materials must bear a biohazard label. Doors to the laboratory should be lockable and self-closing. Negative airflow into the BSL2+ suite from the main laboratory or anteroom/vestibule should be visually confirmed on the pressure monitors before entry.

2. Safety Equipment.

a. **Biosafety Cabinet (BSC):** An annually certified Class II BSC must be present for all work with COVID-19^{+} patient samples (see below for more information on BSCs). If work with viable material is proposed to occur outside of an approved primary containment device, then a risk assessment must be performed by the Biosafety Officer prior to beginning such experiments.
- We strongly encourage COVID-19+ work to be done in a TC room with directional airflow inward from the rest of the laboratory, with doors that remain closed.
- BSCs must be positioned in the lab such that fluctuations of the room air supply and exhaust do not disrupt the proper airflow within the BSC.
- The best placement of a BSC is a location with minimal walking paths and away from doors and windows. Note that HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory, but only if the BSC is certified annually.
- If the blower on the BSC is not left on continuously, it should be turned on and run for 5 minutes to allow several complete exchanges of air before work begins. At the beginning of the work session, plastic-backed absorbent paper can be placed on the work surface (optional) but must not obstruct air flow. The work area should be arranged clean-to-dirty to reduce the chance of contamination. Discarded material should be added to a small, red biohazard bag within the cabinet.
- Work with all materials must be done 4-6 inches inside the sash. Keep containers of liquids capped when not in use.
- Double-gloving is a PPE requirement at BSL2+. Remove (or disinfect, if appropriate) the outer pair of gloves and deposit in a solid waste bag if they are visibly contaminated or compromised. Remove the outer pair of gloves and deposit in a solid waste bag before removing hands from the BSC. At the end of the work session, all items to be removed from the BSC must be decontaminated. The surface of the BSC must be wiped down with 70% EtOH, and the sash lowered.

b. **Vacuum lines:** Vacuum lines to be used for aspiration must be equipped with an in-line filter and a vacuum flask (two flasks connected in series are recommended, but not required), containing

an appropriate disinfectant in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is full. At the end of the work session, aspirate a small volume of concentrated disinfectant through the vacuum tubing, into the vacuum flask.

c. **Centrifuges:** Swinging-bucket centrifuge buckets must be equipped with aerosol-tight safety covers. Microcentrifuges must have aerosol-tight rotors capable of being removed while sealed so that the rotor can be unloaded in the BSC. See section 5e below for more information on centrifugation procedures.

3. **Personal Protective Equipment (PPE).** The following minimal PPE must be worn when working with COVID-19+ patient samples:
   a. Protective gowns:
      - Disposable barrier gown, or
      - Blue wrap around, closed-front gown (disinfected after each, stays in containment), or
      - Approved aprons/smocks (disinfected after each, stays in containment), in combination with disposable sleeves, and
      - Dedicated shoes or disposable shoe covers
   b. Double gloves:
      - Inner gloves should be inside the gown sleeve and outer gloves should cover the gown sleeves
      - The outer gloves are to be (i) disinfected or (ii) removed and replaced every time prior to exiting the BSC
      - Outer gloves should be replaced if their integrity is suspect or if they are visibly contaminated
      - Inner gloves are to be disinfected and removed prior to washing hands thoroughly and exiting the laboratory
   c. Recommended:
      - Eye protection (glasses or faceshield), if only to prevent face-touching
      - N95 respirators (requires fit-testing through Occupational Medicine) if there is a reasonable anticipation that aerosols will be released inside the laboratory

   *Certain procedures may require additional PPE. Contact a Biosafety Officer as appropriate.

4. **Spill Kit.** The BSL2+ lab must have a spill kit, or the components of such, readily accessible in the event of a spill. The spill kit should have:
   a. an easy-to-read outline of the spill response SOP (both in and outside the BSC)
   b. gloves
   c. surgical masks
   d. safety glasses or goggles
   e. clean PPE, disposable gown or clean scrubs, and plastic shoe covers, in case clothing not covered by lab coat becomes contaminated
   f. paper towels to absorb contaminated liquids
   g. disinfectant (do not prepare final dilution until needed)
   h. tongs or forceps to pick up broken glass
   i. a biohazard waste container large enough to handle wet, contaminated paper towels.

5. **General Procedures for working in a BSL2+.** Specific BSL3 work practices (outlined below) should be employed within a BSL2+ lab, conforming to the BMBL and UAB Biosafety Manual (see link below). If the IBC has determined that your lab requires additional controls, please add them to the outline below. UAB Biosafety Manual link: http://www.uab.edu/ohs/images/docs/bio/biosafetymanual.pdf

   Additional practices include the following recommendations:

   a. Whenever possible, BSL2+ work should be conducted during normal working hours to enable adequate response to a severe adverse incident.

   b. Appropriate PPE (listed above) must be donned at all times in the BSL2+ area.

   c. **Sharps**: should be avoided whenever possible in a BSL2+ laboratory. Plastic aspirating pipettes (e.g., Corning cat. # 4975; Falcon cat # 3575; Fisher Cat # 13-675-123) should be substituted for glass Pasteur pipettes. Needles with safety devices are recommended wherever possible. If conventional needles are required, they must never be re-capped, and must be disposed of in a rigid, red sharps waste container. Never reach into a sharps container to retrieve discarded items. Do not allow a sharps container to become more than ¾ full. Reminder: syringes without needles must never be discarded in regular trash.

   d. **There are two distinct SARS-CoV-2 waste categories, as pertains to the Department of Transportation Regulations:**
      1. Category A waste: This is anything contaminated with SARS-CoV-2 virus cultures or purified virus preparations**. All of this waste must be chemically disinfected or autoclaved before it can be offered as medical waste to Stericycle.**
      2. Category B waste: All material potentially contaminated with COVID-19^{+} patient tissues (i.e., nothing that has been cultured) **can be** offered to Stericycle directly as medical waste. If your lab has both categories of waste we recommend treating all waste as Category A. If you have questions, contact UAB Biosafety at biosafety@uab.edu

      UAB Biosafety guidelines for handling waste:

      - **Solid Waste:** All potentially contaminated solutions and vessels must be decontaminated before being removed from the biosafety cabinet. This includes the outer pair of gloves. Solid waste must be collected in a small biohazard bag inside the biosafety cabinet. Pipette tips can alternatively be collected in a disposable plastic bottle (e.g., an empty 500 mL media bottle), containing 10% bleach. Once full (and after all material has been allowed to soak for >30 minutes) the exterior of the bottle can be disinfected and the liquid poured off in the sink. The remaining solids are disposed of in the biohazard waste. Upon completing all work, the biohazard bag in the BSC must be closed, sprayed with appropriate disinfectant, and deposited into a larger biohazard waste container near the biosafety cabinet. Ideally, the larger biohazard waste container to collect BSL2+ waste will have a foot-operated lid. If this is unavailable, then another biohazard bin with a lid is highly recommended. It is the laboratory's responsibility to ensure medical waste is properly packaged and treated (if necessary-see Category A waste description, above) before offering it to Stericycle.

- **Liquid Waste:** should be aspirated into a vacuum flask containing an appropriate disinfectant in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is full. Be mindful of the discarded liquid level before and after aspirating liquid waste to prevent overfilling. The vacuum flask must sit for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with disinfectant at the recommended final concentration, allowing a minimum time of 30 minutes to inactivate the agent. A simple 500 mL bottle containing disinfectant may be suitable to collect non-aspirated liquid waste.

  *A note on bleach: Household bleach is effective and inexpensive, but it is also volatile and corrosive. Bleach-soaked paper towels should not be autoclaved because autoclaving 1) releases chlorine, a chemical hazard, and 2) will corrode the autoclave over time. All 10% (0.5% final concentration sodium hypochlorite) household bleach solutions should be prepared fresh prior to each work session, when needed. If 10% bleach is used to decontaminate a spill within the BSC, residual bleach should be removed using a wipe down with 70% ethanol (EtOH).

e. **Centrifugation:** All centrifuging rotors/buckets should be gasketed and designed to eliminate aerosol exposures and allow samples to be loaded and unloaded in the BSC. All centrifugation procedures (apart from swinging bucket TC centrifugation) should be described to biosafety before work begins (including the location of the centrifuge, and the procedure followed (see below).
   i. Centrifuge tubes must be prepared and sealed in the biosafety cabinet. This includes methods to ensure tubes are properly balanced. Fixed angle rotors should be loaded in the BSC as well, and the entire rotor sprayed with 70% EtOH before removal of the rotor from the BSC. For ultracentrifugation with swinging bucket rotors (e.g., SW28), individual safety buckets must be prepared in the BSC, securely closed, wiped down with 70% EtOH, and then transported to the centrifuge in the respective rack for those buckets. Aerosol-tight safety cups for low-speed centrifugation must be loaded, closed, and wiped down with 70% EtOH prior to removal from the BSC; they must also be unloaded in the BSC. After centrifugation, the centrifuge lid must be opened cautiously and the rotor quickly inspected for a failure, which could have generated aerosols in the centrifuge chamber. The rotor and chamber must be misted with 70% EtOH, and the rotor (or swinging buckets/safety cups) transported into the BSC for further work. At the end of the procedure, rotors and/or buckets must be decontaminated.

f. **Vortexing:** must be done inside the BSC.

g. **Secondary Containment is highly recommended:** A rigid container with a sealable lid that can hold the total capacity of potentially spilled biological liquids should be used when transferring COVID-19^{+} samples from one area of the laboratory to another, if there is a likelihood of exposure (e.g., patient blood, compared to isolated lymphocytes). A Tupperware-type container is sufficient, and the lid of the Tupperware container can be removed or left ajar once the container is in the incubator, to enable gas exchange. To remove the tissue culture dishes from the

incubator, close the Tupperware container with the lid before taking the dishes out of the incubator.

h. **Storage and Inventory:** Samples obtained from COVID-19^{+} patients must be stored in leak-proof secondary containers in a -80C freezer clearly marked with a warning label to indicate that biohazards are present. Detailed inventories are strongly encouraged, as SARS-CoV-2 is likely to be listed as a Select Agent in the future.

6. Accidents and spills

a. Spills

i. ***Small spills (<10 mL) inside the BSC:***

- First, lower the sash for 5 minutes to allow the blower to move aerosols through the HEPA filter.
- During this time, check to see if the spill is fully contained within the BSC, if any PPE has become contaminated, or if any breach of containment has occurred (e.g., a splash where droplets have escaped the BSC and fallen on the floor). If there has been a breach of containment, response should be as for a spill outside the BSC.
- Small spills (<10 ml) can be decontaminated by layering paper towels soaked in appropriate disinfectant on top of the spill, allowing 20 min. for the disinfectant to inactivate the agent, then depositing the paper towels in the biohazard waste bag in the BSC.
- If using bleach, residual bleach can be wiped off with paper towels sprayed with 70% EtOH, and the towels deposited in the biohazard waste bag.
- Small spills inside the BSC that do not involve an exposure do not require notification of the IBC or Biosafety Officer, but do require notification of the PI, who will direct further training (e.g. retraining on pipetting techniques, or organization of materials and instruments in the BSC) to minimize the risk of recurrence. Note: a spill of media or buffer not containing the agent does not represent a biohazard, but paper towels used to wipe it up should be deposited in the biohazard bag in the BSC.

ii. ***Large spills (>10 ml) inside the BSC, will likely splatter droplets outside the BSC):***

Large spills should be treated with caution.

- Leave the BSC running.
- Disinfect outer gloves and sleeves.
- Remove outer gloves and sleeves (if applicable) before exiting the BSC.
- Disinfect/dispose of gowns according to the doffing procedure.
- Close the door to the room as you leave, remove any additional PPE and any contaminated clothing (check the sleeves of your lab coat) and place in a biohazard bag for disposal. Everyone in the room at the time of the spill should thoroughly wash their hands, using disinfectant soap.
- If there is an aerosol risk, promptly exit the laboratory and post spill-cleanup signage.
- Notify the PI. If you are absolutely sure that there has been no exposure and no breach of containment, proceed as for a small spill inside the BSC.

- If there has been overt exposure (e.g., actual contact of bare skin), wash skin with soap and water for 15 minutes, contact the campus Biosafety Officer, and initiate the exposure response plan.
- In the absence of an exposure allow 30 min. for any potential aerosols to settle. Don clean PPE, re-enter the room, cover the spill with paper towels, soak with appropriate diluted disinfectant, starting at the perimeter and working inward toward the center. Allow the appropriate contact time to inactivate the agent. Deposit soaked towels in biohazard waste. The interior of the BSC should be decontaminated by wiping down the walls, sash, and equipment with disinfectant.
- If the spill has entered the BSC drain pan, more extensive decontamination must be performed. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. The drain pan should be decontaminated, flushed with water and the drain tube removed. After decontamination with corrosive disinfectants (e.g., bleach), remember to wipe down the BSC with 70% EtOH to remove residual chemicals. If no overt exposure has occurred, and the spill was completely contained within the BSC, the Biosafety Officer/Specialist does not need to be informed. The PI should review the incident to revise procedures to minimize the risk of recurrence.

iii. ***Small spills of COVID-19+ patient samples (<10 mL) outside the BSC.*** A small spill, in this circumstance, is defined as a spill with low potential to aerosolize, presents no inhalational hazard, and no endangerment to people or the environment. These steps should be clarified by the PI, based on the sample material being worked with and the relative risk of infectious material in the sample. **The following is an example that may be appropriate:**

- First, ascertain the extent of the spill. Simply dropping a 150 mm dish contained inside a closed secondary container does not constitute a spill outside the BSC, since there is no breach of containment—as long as the secondary container stays closed.
- If other personnel are present, alert them immediately. Keep in mind that spills can generate aerosols. If aerosols are likely:
  - quickly check to ascertain the extent of the spill: Is PPE contaminated? (Gloves, lab coat, pants cuffs, shoes?) Is bare skin is exposed? Has liquid splashed over a large area? If shoes are visibly contaminated, decontaminate them with appropriate disinfectant, then evacuate the room, doffing and disinfecting PPE before exiting the door. Make sure to remove gloves (or outer gloves, if double-gloved) before touching the door knob.
  - Post a sign on the door warning personnel not to enter.
  - Remove any additional PPE and place it in a biohazard bag, wash hands and face thoroughly.
  - Allow 30 min. for aerosols to settle. During this time, notify the PI.
  - After 30 min., don fresh PPE, re-enter the room, cover the spill with paper towels, then soak them with disinfectant starting at the periphery and moving inward toward the center. Be sure to check for and decontaminate small splashes beyond the main affected area.

- Leave the soaked towels in place for the contact time required to inactivate the agent. Leave the room during this time.
- After the appropriate inactivation time, transfer soaked paper towels to biohazard waste. Wipe up the residual spill with more paper towels. Give the area a final wipe-down with paper towels using the appropriate disinfectant.

iv. ***Large spills (>10 mL) outside the BSC.*** A large spill, in this circumstance, is defined as a spill that spreads rapidly, presents an inhalational hazard, endangers people or the environment, and/or involves personal injury or rescue and should be handled as an emergency. In practical terms, this might be a spill of more than 10 ml splattering over a large area, thus presenting the possibility of aerosolization and widespread contamination. **Specific procedures should be developed based on the nature of your work and the relative risk of infectious material. The following is an example that may be appropriate:**

- If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols. Ascertain the extent of the spill: possible overt exposure, splash on shoes or soles of shoes, contamination of PPE.
- If shoes are contaminated, disinfect them before evacuating the room (if shoes are extensively contaminated, you should remove them as you leave the room). After removing gloves (or outer gloves, if double-gloved), evacuate the room, closing the door as you leave.
- Remove additional PPE. Wash hands and face thoroughly. Post a sign on the door warning personnel not to enter. Allow 30 min. for aerosols to settle. During this time, notify the PI and the Biosafety Officer. If the spill is too difficult to manage alone, seek help from the Biosafety Officer (934-2487) or UAB PD (934-3535) after business hours.
- If spill cleanup is feasible, don fresh PPE (including eye and respiratory protection), and re-enter the room after the 30 minutes has transpired. Cover the spill with paper towels, and soak the towels with appropriate disinfectant, working from the outside toward the center. Allow the appropriate contact time for inactivation. If there is any broken glass associated with the spill, pick it up with tongs or forceps, and transfer it to a biohazardous broken glass container. Pick up soaked paper towels, and transfer to a biohazard bag. Give the area a final wipe-down with paper towels using the appropriate disinfectant.

All Spills outside of the BSC that involve breach of containment, regardless of exposure, must be reported to a Biosafety Officer.

b. Accidents

Accidents include the release of COVID-19^{+} material due to equipment failure (e.g. tube failure in the centrifuge), needle-sticks, or other injuries concomitant with a breach of containment.

i. ***Centrifugation.*** If tube failure is suspected (sudden clunking or automatic shut-down due to imbalance), leave the centrifuge lid closed for 30 min. to allow aerosols to settle. During this time, notify the PI. Open the lid cautiously to check the integrity of the rotor/tubes. If the rotor looks intact, spray the rotor with 70% EtOH, and transport it into the BSC before unloading centrifuge tubes. If a tube has cracked or collapsed within a swinging bucket, decontaminate the tube and bucket inside the BSC. If there appears to be a leak or spill inside

the centrifuge, decontaminate the centrifuge chamber by cautiously opening the centrifuge, adding paper towels to soak up any contaminated liquids, then liberally spraying disinfectant onto the walls and inside the lid of the centrifuge, so that disinfectant pools at the bottom of the chamber. (e.g., about 0.5-1 liter). Close the centrifuge for the appropriate contact time. Clean up the soaked paper towels as for a major spill outside the BSC. In the event of a catastrophic failure in the centrifuge (e.g., swinging bucket coming off the rotor at 22,000 rpm, damaging the centrifuge, and releasing sample into the centrifuge chamber), keep the centrifuge lid closed for 30 min. During this time, notify the PI. If the contamination is too extensive to manage alone, ask the Biosafety Officer for assistance (934-2487). Decontamination is similar to a major spill outside the BSC. Lay paper towels inside the centrifuge chamber, and soak with 10% bleach (or other approved disinfectant). Spray the inside of the centrifuge jacket with 70% EtOH. Close the lid for the appropriate contact time. Clean up as for a major spill outside the BSC.

ii. ***Sharps*** should be avoided whenever possible for work with COVID-19^{+} materials. However, if there is a needle-stick, briefly bleed the wound (squeeze it to produce a couple of drops of blood), then wash thoroughly with soap and water for 15 min. Report the incident to the PI and the Biosafety Officer and initiate the SARS-CoV-2 exposure response plan.

iii. ***Other accidents*** might include slips, falls, or collisions with other personnel, leading to spills of COVID-19^{+} material or injuries. Additional help may be required in the event of personal injury, in which case assisting personnel must be made aware of the presence of uncontained agent so that they can respond appropriately. In the event of a major spill involving serious personal injury or requiring rescue, call UAB Police at 911 from a campus phone, or 934-3535 from a mobile device.

iv. ***Follow-up of exposures or injuries involving COVID-19+ material:*** Follow the SARS-CoV-2 Exposure Symptom Reporting Plan (below)

Appendix I. Assurance Statement

All lab personnel must read the contents of this manual and sign & date below. By signing this page, lab personnel agree to abide by the safety precautions and procedures discussed herein.

I have read, understand, and agree to adhere to the biosafety procedures contained within

Principal Investigators:

Name	Title	Signature	Date
	Principal Investigator		
	Principal Investigator		
	Principal Investigator		
	Principal Investigator		
	Principal Investigator		
	Principal Investigator		

Laboratory Staff (Undergraduates and minors are not allowed to enter COVID-19^{+} research areas):

Student/staff Name (BlazerID)	PI or Supervisor	Signature	Date

Appendix II. Building/Room Access Checklist:

To be completed for each investigator utilizing this BSL2+ Facility

Principal Investigator:	
Lab Location:	
Office Phone:	
24/7 contact (cell phone/pager):	
IBC Protocol #(s):	

For unescorted access to this facility you must successfully complete the following:

	Online Training Completed[1]					Other Requirements			
Staff/student Name:	**HS200**	**Bio301L**	**Bio303**	**Bio304**	**Bio500**	**Shadowing & Mentoring[2]**	**Occ. Med. Enrollment[3]**	**Consult with Biosafety[4]**	**Access granted**

1. **Online courses:** are available through the UAB Learning System (https://uab.edu/learningsystem/)
   - Hazard Communication
   - Medical Waste Management for Labs
   - Basic Biosafety
   - Biosafety Cabinets and Fume Hoods
   - Bloodborne Pathogens
2. **Shadowing/mentoring:** Laboratory personnel are not allowed to work with agents until they have been trained by the PI who supervises their work, or a designated technical expert. The worker should demonstrate good microbiological skills and an understanding of this SOP prior to being permitted to work with the agent.
3. **Enrollment with Occupational Medicine:** http://www.uab.edu/ehs/occupational-medicine
4. **Biosafety Consults:** Safety discussion with Biosafety to verify **u**nderstanding, and acceptance of the information contained and sign Assurance Statement (Appendix I).
5. **IBC Approval:** must be granted before the start of any non-exempt work or work with SARS-CoV-2 material other than unmodified COVID-19^{+} patient samples.

Appendix III. COVID-19+ Agent-Specific Safety Plan

BIOLOGICAL AGENT(S): SARS-COV-2 INFECTED (COVID-19+) PATIENT SAMPLES

PHYSICAL PROPERTIES:	
MORPHOLOGY (PARTICLE/GENOME)	***Coronaviridae* family, *Betacoronavirus* genus,** **Positive-sensed, single-stranded mRNAgenome, enveloped with pleomorphic morphology of approximately 80-140 nm in diameter.**
STRAINS/VARIANTS (DESCRIBE)	**NA**

AGENT RISK FACTORS:					
RISK GROUP LEVEL		**3 (virus and virus cultures)**	**2 (patient samples)**		
HOST/VECTOR RANGE		**Humans**			
INFECTIOUS DOSE		**Unknown**			
MEDICAL OPTIONS		**Prophylaxis** NA	**Vaccines** Experimental trials ongoing	**Treatments** NA	**Surveillance** NA
SEVERITY OF DISEASE	UNTREATED:	**Mild ☑**	**Moderate☑**	**Severe ☑**	**Lethal ☑**
	TREATED:	NA	NA	NA	NA
NATURAL MODES OF TRANSMISSION		**Droplet/aerosol, mucous membrane exposures (Direct and indirect)**			
POTENTIAL LABORATORY EXPOSURE ROUTES: SOURCE:		**mucosal membranes** **spills, splashes, contaminated gloves**	**parenteral inoculation** -unknown, but likely **needlesticks, exposures to open wounds**	**Ingestion** -unknown, but likely	**Inhalation (droplet/aerosol)** **improper use of containment devices (BSC, centrifuge safety cups).**
ENVIRONMENTAL STABILITY		**Hours ☑**	**days ☑**	**weeks**	**months**
GENETIC MODIFICATIONS (DOES THE MODIFICATION ALTER ANY RISK FACTORS?)		**NA**			
REGIONAL PREVALENCE		**Indigenous**	**Emerging ☑**	**exotic**	

PROCEDURAL RISK FACTORS:			
ANIMAL MODELS?	AEROSOL-PRODUCING PROCEDURES?	SHARPS USED?	VOLUME/CONCENTRATIONS USED?
CULTURE/PROPAGATION	Projects involving the culture of SARS-Cov-2 requires **BSL3 containment in SEBLAB**. This includes the culture of COVID-19+ patient samples permissive to virus replication		

DESCRIBE OTHER RISK FACTORS	**Other Bloodborne pathogens present in human tissues**

CONTAINMENT REQUIREMENTS:		
	BIOSAFETY LEVEL A/BSL 1-3	**ADDITIONAL CONSIDERATIONS** (SPECIAL PRACTICES, SAFETY EQUIPMENT, AND FACILITY SAFEGUARDS NEEDED)
LAB	**2 +**	**BSL3 PRACTICES AND PROCEDURES** (AS SPECIFIED ABOVE)
ANIMAL FACILITIES	**NA**	
POSTED SIGNAGE	**"BIOHAZARD (BSL2+), RESTRICTED ACCESS, COVID-19+ PATIENT SAMPLES"**	
PPE REQUIRED		
DISINFECTANTS & INACTIVATION	**Disinfectants:** • Agents with $TCID_{50}$ of ≥4 (log_{10}) at 30 seconds contact time: ○ **Based on murine hepatitis virus, see PMID 19692148** ○ Household bleach (1:25-fold dilution to 0.21% NaClO) ○ Lysol Disinfectant Spray (0.100% Alkyl dimethyl benzyl ammonium saccharinate/79.0% ethanol) ○	**Inactivation:**
REQUIRED SAFETY TRAINING	**EH&S Courses:** • Hazard Communication • Medical Waste Management for Labs • Basic Biosafety • Biosafety Cabinets and Fume Hoods • Bloodborne Pathogens * Training Matrix and Decision Tree: **http://www.uab.edu/ohs/training** ***Classes are on** The UAB Learning System: **http://www.uab.edu/learningsystem**	**Investigator or Lab Provided Training:** **Agent-specific training (this document) and supervisor attestation of proficiency**

EXPOSURE AND INCIDENT RESPONSE PROCEDURES:	
MUCOSAL MEMBRANES	**Flush eyes, mouth or nose at eyewash station for 15 minutes**
DERMAL	**wash area with soap and water for 15 minutes**

SYMPTOMS

Symptom	Percentage
Fever	87.9%
Dry cough	67.7%
Fatigue	38.1%
Sputum production	33.4%
Shortness of breath	18.6%
Sore throat	13.9%
Headache	13.6%
Myalgia/arthralgia	14.8%
Chills	11.4%
Nausea/vomiting	5.0%
Nasal congestion	4.8%
Diarrhea	3.7%
Mild to moderate disease	80%
Severe disease	13.8%
Critical disease	6.1%

INCUBATION PERIOD

VARIABLE: 1-14 DAYS (AVG 5.5)

MEDICAL RESPONSE

SEE: SARS-COV-2 EXPOSURE & SYMPTOM REPORTING PLAN (APPENDIX VI)

LIFE THREATENING INJURIES

- Campus phone: dial 911
- Outside line: 934-3535

TO SEEK MEDICAL ATTENTION AFTER HOURS

- Report to the UAB Emergency Department

REPORTING

1. **Whether or not you're seeking medical attention, ALL incidents are reported to the lab supervisor (Supervisor's Emergency contact number:_____ _____)**
2. **Supervisors report ALL incidents to UAB EH&S at 934-2487**
3. **Supervisors should also report all injuries/exposures requiring medical treatment to HR**

PLEASE SEE INSTRUCTIONS AND FORMS FOR ON-THE-JOB-INJURY

FOR MEDICAL CLAIM COVERAGE, YOU MUST FILL OUT:
1) An OJI Application for Benefits form, 2) A RELEASE OF INFORMATION FORM, 3.) The Trend tracker Incident Report
***An incident/accident must be reported verbally by the employee to the employee's supervisor as soon as possible but no later than two calendar days following the incident/accident or following the onset of the illness or disease. Your failure to report an incident within two working days may jeopardize your On-the-Job Injury Program benefits.

ADDITIONAL REFERENCES:

BMBL 5TH EDITION	http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf
CANADIAN MSDS	http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds50e-eng.php
CDC	http://www.cdc.gov
NEJM 2020, MAR 17, PMID 32182409	Aerosol and Surface Stability of **SARS-CoV-2** as Compared with SARS-CoV-1.
ABSA	https://my.absa.org/tiki-index.php?page=Riskgroups

Appendix IV

Persistence of coronaviruses on different types of inanimate surfaces

Type of surface	Virus	Strain / isolate	Inoculum (viral titer)	Temperature	Persistence	Reference
Steel	MERS-CoV	Isolate HCoV-EMC/2012	10^5	20°C	48 h	[21]
				30°C	8–24 h	
	TGEV	Unknown	10^6	4°C	≥ 28 d	[22]
				20°C	3–28 d	
				40°C	4–96 h	
	MHV	Unknown	10^6	4°C	≥ 28 d	[22]
				20°C	4–28 d	
				40°C	4–96 h	
	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Aluminium	HCoV	Strains 229E and OC43	5×10^3	21°C	2–8 h	[24]
Metal	SARS-CoV	Strain P9	10^5	RT	5 d	[25]
Wood	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
Paper	SARS-CoV	Strain P9	10^5	RT	4–5 d	[25]
	SARS-CoV	Strain GVU6109	10^6	RT	24 h	[26]
			10^5		3 h	
			10^4		< 5 min	
Glass	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Plastic	SARS-CoV	Strain HKU39849	10^5	22°-25°C	≤ 5 d	[27]
	MERS-CoV	Isolate HCoV-EMC/2012	10^5	20°C	48 h	[21]
				30°C	8–24 h	
	SARS-CoV	Strain P9	10^5	RT	4 d	[25]
	SARS-CoV	Strain FFM1	10^7	RT	6–9 d	[28]
	HCoV	Strain 229E	10^7	RT	2–6 d	[28]
PVC	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Silicon rubber	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Surgical glove (latex)	HCoV	Strains 229E and OC43	5×10^3	21°C	≤ 8 h	[24]
Disposable gown	SARS-CoV	Strain GVU6109	10^6	RT	2 d	[26]
			10^5		24 h	
			10^4		1 h	
Ceramic	HCoV	Strain 229E	10^3	21°C	5 d	[23]
Teflon	HCoV	Strain 229E	10^3	21°C	5 d	[23]

MERS = Middle East Respiratory Syndrome; HCoV = human coronavirus; TGEV = transmissible gastroenteritis virus; MHV = mouse hepatitis virus; SARS = Severe Acute Respiratory Syndrome; RT = room temperature.

[21] van Doremalen N, Bushmaker T, Munster VJ. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. Euro Surveill 2013;18.
[22] Casanova LM, Jeon S, Rutala WA, Weber DJ, Sobsey MD. Effects of air temperature and relative humidity on coronavirus survival on surfaces. Appl Environ Microbiol 2010; 76:2712-7.
[23] Warnes SL, Little ZR, Keevil CW. Human Coronavirus 229E Remains Infectious on Common Touch Surface Materials. mBio2015;6:e01697-15.
[24] Sizun J, Yu MW, Talbot PJ. Survival of human coronaviruses 229E and OC43 in suspension and after drying on surfaces: a possible source of hospital-acquired infections. J Hosp Infect 2000; 46:55-60.
[25] Duan SM, Zhao XS, Wen RF, Huang JJ, Pi GH, Zhang SX, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci 2003;16:246-55.
[26] Lai MY, Cheng PK, Lim WW. Survival of severe acute respiratory syndrome coronavirus. Clin Infect Dis 2005; 41:e67-71.
[27] Chan KH, Peiris JS, Lam SY, Poon LL, Yuen KY, Seto WH. The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus. Adv Virol 2011;734690.
[28] Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W,Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol 2005;194:1-6.
[29 van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. Aerosol and Surface Stability of **SARS-CoV-2** as Compared with SARS-CoV-1. NEJM. 2020, Mar 17.

Appendix V

Inactivation of coronaviruses by different types of biocidal agents in carrier tests

Biocidal agent	Concentration	Virus	Strain / isolate	Volume / material	Organic load	Exposure time	Reduction of viral infectivity (log_{10})	Reference
Ethanol	71%	TGEV	Unknown	50 µl / stainless steel	None	1 min	3.5	[39]
	71%	MHV	Unknown	50 µl / stainless steel	None	1 min	2.0	[39]
	70%	TGEV	Unknown	50 µl / stainless steel	None	1 min	3.2	[39]
	70%	MHV	Unknown	50 µl / stainless steel	None	1 min	3.9	[39]
	70%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	62%	TGEV	Unknown	50 µl / stainless steel	None	1 min	4.0	[39]
	62%	MHV	Unknown	50 µl / stainless steel	None	1 min	2.7	[39]
Benzalkoniumchloride	0.04%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	< 3.0	[40]
Sodium hypochlorite	0.5%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	0.1%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
	0.06%	TGEV	Unknown	50 µl / stainless steel	None	1 min	0.4	[39]
	0.06%	MHV	Unknown	50 µl / stainless steel	None	1 min	0.6	[39]
	0.01%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	< 3.0	[40]
Glutardialdehyde	2%	HCoV	Strain 229E	20 µl / stainless steel	5% serum	1 min	> 3.0	[40]
Ortho-phtalaldehyde	0.55%	TGEV	Unknown	50 µl / stainless steel	None	1 min	2.3	[39]
	0.55%	MHV	Unknown	50 µl / stainless steel	None	1 min	1.7	[39]
Hydrogen peroxide	Vapor of unknown concentration	TGEV	Purdue strain type 1	20 µl / stainless steel	None	2–3 h	4.9–5.3*	[41]

TGEV = transmissible gastroenteritis virus; MHV = mouse hepatitis virus; HCoV = human coronavirus; *depending on the volume of injected hydrogen peroxide.

[39] Hulkower RL, Casanova LM, Rutala WA, Weber DJ, Sobsey MD. *Inactivation of surrogate coronaviruses on hard surfaces by healthcare germicides.* Am J Infect Control 2011;39:401-7.

[40] Sattar SA, Springthorpe VS, Karim Y, Loro P. *Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses.* Epidemiol Infect 1989;102:493-505.

[41] Goyal SM, Chander Y, Yezli S, Otter JA. *Evaluating the virucidal efficacy of hydrogen peroxide vapour.* J Hosp Infect 2014;86:255-9.G. Kampf et al. / Journal of Hospital Infection 104 (2020) 246e251251

Appendix VI. SARS-CoV-2 Researcher Exposure and Symptom Reporting Plan:

Title:	*SARS-CoV-2 Researcher Exposure and Symptom Reporting Plan*		
Author: Justin C. Roth, PhD, Assoc. Dir. of Biosafety, RO — Date	Approved: Dr. Timothy Key, Dir EHS Occupational Medicine Program — Date	Distribution: SEBLAB	
Approved: Suzanne Michalek, PhD, UAB Institutional Biosafety Committee (IBC) Chair — Date		Discontinued:	

COVID-19 Reporting and Information for UAB faculty, staff, hospital employees and students:

- UAB Students should call **UAB Student Health Services** at (205) 934-3580
- UAB employees (faculty, staff, paid graduate students, and hospital) should call UAB Employee Health:
  - Monday-Friday (6:30 a.m.-5:00 p.m.): (205) 934-3675
  - After hours and on weekends: call the UAB Call Center at (205) 934-3411 and ask them to page the needlestick/exposure nurse on call.
- UAB Employee Health will offer testing for providers and staff exhibiting possible COVID-19 symptoms. If an employee has been exposed to the virus, or if that employee has fever or symptoms that make them concerned they may have the virus, they can call the UAB Employee Health Triage Line at 205.934.3675 for guidance on whether to seek further evaluation, but anyone who feels like they have been exposed should submit that information on an **exposure reporting form:**

1. **INTRODUCTION**: UAB seeks to promote a safe and healthy workplace. This is accomplished by limiting opportunities for exposure, promptly detecting and addressing symptoms indicative of an exposure, and using information gained from workplace experiences to enhance safety precautions. A SARS-CoV-2 exposure in the laboratory poses significant danger to laboratory workers and represents a potential threat to public health. Therefore, strict medical surveillance requirements are in place for those with any potential contact with research materials containing potentially viable SARS-CoV-2. All personnel working in UAB research laboratories must adhere to enrollment and reporting requirements as set forth in the UAB Occupational Medicine Program. **Due to the ongoing pandemic, individuals who experience symptoms associated with COVID-19 are expected to report and self-isolate, regardless of whether the exposure event is perceived to be laboratory or community acquired.**

2. **PURPOSE**: The purpose of this SOP is to outline the required procedures for any personnel with access to viable SARS-CoV-2 or COVID-19+ patient samples to report ANY respiratory illness, fever, actual or potential exposure, as well as the follow-up medical surveillance and communications necessary to ensure prompt treatment and to prevent transmission.

3. **PROCEDURE**:

A. **For Emergencies call 911, otherwise use the following guide:**

B. **SYMPTOM REPORTING AND CONSULTATION:**
Individuals who work in a laboratory with active SARS-CoV-2 virus or COVID-19+ patient sample research must report **ANY** respiratory infection, shortness of breath, weakness, fever, coughing, or fatigue.

How to report:

a) Has the symptomatic individual worked with SARS-COV-2 or COVID-19+ patient samples within 14 days, or been in contact with personnel who have worked with this material within 28 days and are also experiencing symptoms?

- **No:** Consult primary care physician, if needed, and notify your supervisor.
  Supervisors should notify the UAB Biosafety Officer at 205-996-9469 or 205-996-4402

- **Yes:** Report symptoms to **Employee Health and state you are a COVID-19 Researcher:**
  - If testing is positive, Employee Health will notify the UAB Biosafety Officer, who will conduct a review to determine: 1) potential sources of exposure, 2) whether federal agencies should be notified and 3) whether to initiate workplace notifications and building disinfection protocols

b) **Treatment:** Are you seeking medical treatment (For guidance, see the CDC Coronavirus Self-Checker survey: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/index.html)?

- **No:** isolate at home and avoid close contacts. Report any changes to your supervisor

- **Yes:** Call Employee Health **and state you are a COVID-19 Researcher** to determine appropriate actions, which may depend on the current state of the SARS-CoV-2 pandemic.
  - Prior to any medical intervention On-the-job-injury (OJI) forms must be submitted to UAB HR at http://www.uab.edu/humanresources/home/relations/on-the-job-injury-oji
  - Employee Health will notify the UAB Biosafety Officer, who will conduct a review to determine: 1) potential sources of exposure, 2) whether federal agencies should be notified and 3) whether UAB should initiate workplace notifications and building disinfection protocols

C. **EXPOSURE REPORTING AND CONSULTATION:** Exposures, or events perceived as exposures, should be reported to the following:

a) Laboratory workers who are believed to have had a laboratory exposure to SARS-CoV-2 (eg., overt or suspected spill, aerosolization outside of primary containment, identifiable breaks in laboratory procedures – tear in glove PAPR failure, or tear in coveralls) will be counseled by Employee Health about the risk of SARS-CoV-2 transmission to others, and the requirement for self-monitoring and reporting any symptoms.

b) BSL3 Containment:
The SEBLAB Operations Manager: by phone, email, or verbal communication. All laboratory incidents and "close-calls" shall be reported to the SEBLAB Operations Manager. Emergency response procedures take priority over this procedure. For example, spills of biological material should be properly decontaminated prior to reporting.

c) Employee Health will notify the UAB Biosafety Officer, who will conduct a review to determine potential sources of exposure, whether federal agencies should be notified, and whether isolation protocols should be enacted

d) An on-the-job-injury form must be completed at http://www.uab.edu/humanresources/home/relations/on-the-job-injury-oji

e) In the event symptoms develop within 1-14 days from an exposure or perceived exposure, immediately don a surgical mask, and follow guidance from Step 3, above.

D. **REFERENCES:**

CDC, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th Ed. Washington, DC: US Department of Health and Human Services, Public Health Service, CDC; DHHS publication no. (CDC) 2007.

CDC Guidance for Laboratories: https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html

CDC Coronavirus (COVID-19) main page: https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html

What to do if you are sick: https://www.cdc.gov/coronavirus/2019-ncov/if-you-are-sick/steps-when-sick.html

Symptoms and Testing: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/index.html

UAB Coronavirus Pandemic Updates and Closures: https://www.uab.edu/news/coronavirus

Appendix VII. Door Signage

BIOHAZARD

BSL-2+

RESTRICTED ENTRY-AUTHORIZED PERSONNEL ONLY

AGENTS:
COVID-19^{+} Patient Samples

Appendix VIII. Spill Response Cue Cards

 ***Cut out cue cards and post in a highly visible work area*** 

SPILLS INSIDE THE BIOSAFETY CABINET

1. Make sure the cabinet continues to operate. Wait 5 min. to allow aerosols to be pulled through the HEPA filter.
2. Decontaminate the surfaces within the cabinet wearing protective clothing. Gently cover the spill with absorbent paper towels and apply the appropriate disinfectant starting at the perimeter and working towards the center.
3. Wait for the appropriate contact time _____ (disinfectant:_____)
4. Discard soaked paper towels in a biohazard bag. Wipe up residual fluids. Wipe down surfaces with 70% EtOH, discarding towels in a biohazard bag.

* Note: Examine drain pan for contents of the spill. Disinfect if needed.

SPILLS OUTSIDE THE BIOSAFETY CABINET

Small Spill (<10 mL, localized to small area)

1. Alert personnel in the vicinity.
2. Check for contaminated clothing, including shoes. Decontaminate if necessary.
3. Evacuate the room. Close door. Discard potentially contaminated PPE, remove and decon any contaminated clothing. Wash hands.
4. Notify PI. Wait for 30 minutes to allow for room air exchanges to clear aerosols through room exhaust.
5. Don fresh PPE: lab coat or gown, gloves, N95, eye protection.
6. Cover spill with paper towels.
7. Soak paper towels with the appropriate disinfectant, from perimeter toward the center.
8. Wait for the appropriate contact time _____ (disinfectant:_____)
9. Work can continue during contact time.
10. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
11. Wipe down spill area one final time with appropriate disinfectant.

SPILLS OUTSIDE THE BSC

Major Spill (>10 mL, localized to small area)

1. Alert personnel in the vicinity.
2. Check for contaminated clothing, including shoes. Decontaminate if necessary.
3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands thoroughly.
4. Post warning sign: "DO NOT ENTER: Biological spill!"
5. Wait 30 min. for potential aerosols to clear.
6. Notify PI and a Biosafety Officer/Specialist (934-2487).
7. If assistance is needed, discuss with Biosafety Officer.
8. Don fresh PPE: lab coat or gown, gloves, N95, eye protection.
9. Re-enter the room, cover spill with paper towels.
10. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
11. Wait for the appropriate contact time _____ (disinfectant:_____)
12. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
13. Wipe down spill area one final time with appropriate disinfectant.
14. With PI, write up a report and submit to the Biosafety Officer.

SPILLS INSIDE AN INCUBATOR

Decontaminate water pan via autoclave.

1. Alert personnel in the vicinity.
2. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands thoroughly.
3. Notify PI and wait 30 min for potential aerosols to clear
4. Don fresh PPE: lab coat or gown, gloves, N95, eye protection.
5. Cover spill with paper towels.
6. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
7. Wait for the appropriate contact time _____ (disinfectant:_____)
8. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
9. Wipe down spill area one final time with appropriate disinfectant.

SPILLS INSIDE A CENTRIFUGE

1. Open lid of centrifuge slowly.
2. If there has been no breach of containment, spray rotor with 70% EtOH.
3. If inside of rotor is contaminated, decontaminate in the BSC. As a precautionary measure, decontaminate the centrifuge chamber.
4. If rotor buckets are damaged, close centrifuge lid.
5. Alert personnel in the vicinity, doff and discard PPE, and evacuate room.
7. Wait 30 min. for aerosols to clear.
8. Notify PI and a Biosafety Officer/Specialist (934-2487).
9. If assistance is needed, discuss with Biosafety Officer.
10. Don fresh PPE, including lab coat or gown, gloves, N95, eye protection
11. Open lid slowly and add paper towels.
12. Spray walls of chamber and rotor with 70% EtOH.
13. Close centrifuge lid
14. Wait for the appropriate contact time _____ (disinfectant:_____)
15. Finish centrifuge clean-up as for major spill outside the BSC. Transport rotor to BSC.
16. Open and decontaminate rotor/buckets in the BSC.
17. With PI, write up a report and submit to Biosafety Officer

Appendix IX. Facility-Specific SOPs

The purpose of this section is to develop SOPs that specifically outline protocols where consideration for safety with a BSL2+ agent is paramount. Detailed, step-by-step protocols describing entire experiments with materials and methods are not necessary.

Examples of SOPs where safety is emphasized are bulleted below:

- Experiments that require PPE in addition to minimum BSL2+ PPE
- Experiments that require manipulation of a BSL2+ agent outside a biosafety cabinet (requires a written risk assessment from Biosafety Officer)
- How to properly vortex or sonicate a viable BSL2+ agent
- How to safely centrifuge a sample containing BSL2+ agents
- How to inactivate a sample containing BSL2+ agents for flow cytometry