Biosafety Guidelines for Saliva and Oral Swab Collection and Handling

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The following guidance is intended for research programs where saliva or oral swabs are collected and handled by OSU personnel for research purposes. Saliva is ordinarily considered a non-hazardous body fluid unless visibly contaminated with blood. However, the Covid-19 pandemic has greatly changed the risk profile for collection and handling of saliva. Recent studies have found that the saliva of infected persons had high levels of virus and were comparable to standard nasal swabs in sensitivity and specificity, as determined by RT-PCR.[1] Therefore, saliva must be considered a high risk source of viral exposures, and the collection and handling of saliva must implement biosafety procedures to mitigate the risk of exposure.

For research studies involving the collection of saliva for purposes other than measuring SARS-CoV-2 viral burden, study participants should wear masks except when samples are actually being collected. Participants should first be screened for fever and other signs / symptoms of Covid-19. Fever screening can easily be accomplished using a no-touch device, and screening for other signs / symptoms by use of an oral or written questionnaire. Those individuals testing positive should not participate in saliva collection, and should be instructed to contact their physician.

Sample Collection

Self-Collection (preferred method): Sample collection of saliva is easily accomplished by study participants themselves. Study participants should be instructed in proper use of the self-collection tube or kit supplied by the research team. Participants should also be supplied with disinfecting wipes and instructed to wipe the outside surfaces of the tube or collection device after sample collection and sealing. The sample tube or device should be bagged for transport to the lab.

During self-collection, the participant should be alone in a room or other secluded area to avoid aerosol exposures of other persons nearby.

Researcher-Collected Saliva: While self-collection is preferred, if research staff must collect saliva, this should be done following Standard Precautions (see attachment).

- Personal protective equipment (PPE) shall be worn similar to what would be expected for swab collection by medical professionals: face mask, eye protection, disposable gown and gloves.
- Gloves should be changed and hand hygiene performed between contacts with study participants.
- Gloves must be removed inside out without touching the outer surface of the glove with bare hands. The Beaking Method is recommended.
- All used PPE must be discarded after use as medical waste for treatment.

[1] Zhu J, Guo J, Xu Y, Chen X. Viral dynamics of SARS-CoV-2 in saliva from infected patients. *J Infect*. 2020;81(3):e48-e50. doi:10.1016/j.jinf.2020.06.059

Specimen Handling: BSL-2 Facilities and Practices

Handling of saliva specimens should follow CDC Interim Laboratory Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019.

- Personnel conducting manipulations must be familiar with BSL-2 work practices and competent in aseptic technique.
- Open specimen tubes being manipulated should be handled in a BSL-2 laboratory, in a certified class II biological safety cabinet (BSC). Personnel working in a BSC should complete EH&S online training on the proper use of a BSC here; alternatively, EH&S can provide on-site evaluation and training for lab personnel.
- Standard precautions, including PPE must be worn at all times, and hand hygiene strictly adhered to. PPE must be removed before leaving the laboratory.
- Centrifugation procedures should utilize aerosol containment technology such as safety cups, sealed rotors, or HEPA filter aerosol capture.
- Follow all standard BSL-2 laboratory safety practices (attached).

Standard or Universal Precautions

Key elements:

1. Hand hygiene
    a. Hand washing method (20-30 seconds): wet hands and apply soap; rub all surfaces; rinse hands and dry thoroughly with a paper towel; use towel to turn off faucet.
    b. When to wash hands:
        i. before and after any direct hand contact with cultures, untreated wastes, or potentially contaminated surfaces or equipment
        ii. immediately after removal of gloves
        iii. before leaving the laboratory
2. Gloves
    a. Wear gloves when touching blood, body fluids, secretions, animals, cultures or untreated wastes. Wear gloves when cleaning spills.
    b. Change gloves whenever they become soiled, if small holes or tears develop, or if you think the glove has become compromised.
    c. Remove gloves inside out without touching the outer surface with bare hands.
    d. Remove gloves before touching non-contaminated items and surfaces or any common-use touch points such as doorknobs or drinking fountains.
    e. Perform hand hygiene after glove removal.
3. Facial protection (eyes, nose, mouth)
    a. Wear a surgical or procedure mask and eye protection or a face shield to protect mucous membranes of the eyes, nose and mouth during activities that have the potential to generate splashes or sprays of potentially infectious liquids.
4. Protective Clothing: Gown or Laboratory Coat or similar
    a. Wear to protect skin and prevent soiling of clothes during lab activities. Many activities have the potential to create small – droplet aerosols that can contaminate clothing.
    b. Remove protective clothing as soon as possible and perform hand hygiene.
5. Prevention of needle stick and other sharps accidents[1]
    a. Use care when handling needles, scalpels, and other sharp instruments or devices.
    b. Avoid use of re-usable sharps to the extent possible. If re-usable sharps are needed, they should be handled carefully during cleaning and transported in closed containers.
    c. Discard used disposable sharps into commercially available hard-sided, leak-proof sharps containers.

[1] For more extensive information, please see the OSU Sharps Safety Plan, at https://ehs.oregonstate.edu/bio/resources

6. Environmental cleaning
    a. Clean and disinfect work surfaces, equipment and other solid materials after use with an effective disinfectant.
        i. The EPA has a website for registered disinfectants.[2]
    b. Clean and disinfect work surfaces at the end of each workday.
7. Waste disposal
    a. Potentially infectious wastes must be segregated at the point of generation and treated with an effective method prior to release into the normal waste stream.
        i. Potentially infectious wastes must be collected into autoclavable bags inside hard-sided, leak-proof secondary containers with fitted lids and a biohazard symbol clearly visible on the exterior of the container.
        ii. In Oregon, waste treatment is limited to incineration or steam sterilization by autoclaving, and a few other methods.
        iii. Liquid wastes should be autoclaved prior to discard. Treatment with bleach or other disinfectants followed by drain disposal is not allowed.
        iv. For more information, please see the OSU Biological Waste Management web page.[3]

[2] https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants
[3] https://ehs.oregonstate.edu/biological-waste-management

Control of Biohazards in the BSL-2 Laboratory

General Information

The most important element of containment of infectious materials is consistent adherence to standard microbiological practices and techniques, and frequent handwashing. Persons working with infectious agents or infected materials must be aware of potential hazards and be trained and proficient in the practices and techniques for safely handling such material. Inexperienced lab workers must be supervised by more experienced persons until they have achieved competency in microbiological aseptic technique and demonstrated to the satisfaction of the supervising scientist the ability to safely handle potential biohazards.

Universal or standard precautions should be used for handling of all samples, even established cell lines, since cell lines and other routine lab cultures can become contaminated if not properly handled using aseptic technique.[1]

Each laboratory supervisor must develop or adopt safety and operational procedures to identify the hazards that will, or may likely, be encountered. They must also specify practices and procedures designed to minimize or eliminate identified risks, as well as the procedures to be used in the event of an accidental exposure. Personnel and students must be required to read and follow the established practices and procedures and must be advised of any special hazards present in the laboratory.

Routes of Transmission and Reduction of Risks

Respiratory / Aerosol Routes of Infection

A variety of agents infect by the respiratory or aerosol route. Airborne transmission differs from aerosol transmission in that the organisms are inhaled and initiate infection in the lower respiratory tract and can travel long distances, carried by air currents. Aerosols are larger micro-droplets that settle out quickly, landing on surfaces, clothing and vulnerable mucous membranes – eyes, nose and mouth. Aerosol droplets generally travel only short distances, usually about 3 feet or less from the source. Aerosol transmission generally initiates infections in the upper respiratory tract, conjunctival membranes, or gastrointestinal tract. Aerosol, but not airborne transmission, is a hazard for the agents usually handled at BSL-2. Airborne transmission is associated with agents handled at BSL-3.

Aerosols are produced by most common laboratory manipulations: transferring liquids, flaming inoculation loops, streaking plates for isolation, harvesting liquids from cell culture or embryonated eggs; large amounts of aerosols are produced by chopping, grinding and sonication of infectious materials. Aerosols are thought to be a major source of laboratory associated infections, and there are a number of well documented cases where aerosols have been identified as the source of laboratory

[1] For example, see Shioda et. al., Royal Society Open Science 5: 172472 (2018).

infections; for many other cases, aerosols are suspected. Aerosol generation and dissemination can be reduced following some relatively simple guidelines consistently:

1. Use of a properly operating laminar-flow biological safety cabinets (BSC) for protection against aerosols generated during manipulation of cultures or other potentially infectious materials.
2. Thorough decontamination of work surfaces before and after work and following spills of biohazardous material. This is particularly effective in preventing secondary aerosols generated by agents resistant to drying.
3. Use of absorbent lab matting on immediate work surfaces to contain splashes, micro-droplets, and drips. Discard after each use.
4. If manipulations must be done outside the BSC, then increased personal protective equipment to protect eyes, nose and mouth from aerosols.
5. Always disinfect the work area and equipment immediately after use and at the end of the day to remove infectious materials deposited by aerosols created during work activities.
6. If vacuum lines are used to suction potentially infectious materials, there must be one or two traps for collection of liquids, preferably with disinfectant in them. An inline HEPA filter must be present between the terminal liquid trap and the source of the vacuum (house or vacuum pump) to prevent aerosol contamination and dispersal.
7. Always perform vortexing, sonication, and homogenization steps involving biohazards inside the BSC. If using a blender, it is a good idea to place a cloth impregnated with disinfectant over the blender chamber during use.
8. Employ good aseptic technique when streaking plates, inoculating tubes or flasks, and opening culture tubes or vessels.

Ingestion Route of Infection

A variety of organisms used in the laboratory are enteric pathogens which use ingestion as the primary route of infection. Infection by these organisms generally occurs in the following ways:

1. Direct ingestion of culture or other infectious materials by mouth pipetting. Mouth pipetting was at one time a standard practice in microbiological laboratories but is now prohibited because of the risk of ingestion. Pipetting should always be done with a pipetting device slowly and, whenever possible, leaving the last small amount in the pipet tip rather than discharging it to avoid creating aerosols.
2. “Hand-to-mouth” contact where infectious materials are transmitted indirectly by the hand to the oral cavity. Activities such as smoking, eating and drinking are prohibited in laboratories for this reason. Frequent handwashing between activities is required to minimize risk.

Needlesticks and Other Sharps Accidents / Contact with Non-intact Skin

Transmission of any pathogen capable of initiating an infection in blood or soft tissues can easily occur as a result of sharps accidents or contact between non-intact skin and infectious materials. Some important ways to prevent these types of exposures are listed below:

1. Only use sharps when no suitable alternative exists. Sharps being used to suspend microbial pellets or other materials can usually be replaced with thin-bore plastic pipet tips that fit onto syringes. Breakable glass containers should be replaced with plastic where possible.
2. Syringes must be disposed of in sharps containers in Oregon, even if no needle is attached. Syringes should be LEUR-LOK type if used for attachment of needles or filter devices.
3. Use disposable sharps wherever possible. If non-disposable sharps are used, place into hard-sided containers after use for transport for decontamination before re-use, preferably by autoclaving.
4. Always keep a sharps disposal container as close to the work area where sharps are used as possible. Containers must be hard-sided, leak-proof commercially available red sharps containers with a biohazard symbol, and should not be over-filled.
5. Never carry a used sharp from one location to another, or hand used sharps to another person.
6. Do not recap used needles or sharps, just discard into a sharps container. OSHA will issue citations if the inspectors find recapped needles in a sharps container. If sharps must be recapped, use a one-handed method or replace caps with hemostats or forceps.
7. Substitute safety-engineered sharps wherever possible.
8. Report all sharps injuries to EH&S using the Sharps Injury Log, available on the EH&S website.

Other Considerations

Potentially Infectious Wastes: Segregation Requirements

1. Wastes must be segregated in the laboratory (at the point of generation) into potentially hazardous and the normal waste stream. In a BSL-2 laboratory, all cultures and associated materials (used gloves, tubes, pipet tips, pipettes, etc.) must be collected for disposal into hard-sided, leak-proof containers with fitted lids and biohazard symbols, lined with autoclavable bags. Basically, anything medical in appearance should go into these bags with the exceptions of used sharps and contaminated broken glass.
2. Contaminated broken glass, used microscope slides, and contaminated glass tubes must be discarded into sharps containers.
3. Non-hazardous wastes such as paper or plastic wrappers and used paper towels can be discarded into the regular trash waste streams.
4. Uncontaminated broken glass should be picked up using mechanical methods such as broom and dustpan or tongs, and disposed of in lined cardboard boxes. When the boxes are full, they should be taped closed and deposited in the dumpster.

Potentially Infectious Wastes: Treatment Requirements

In Oregon, there are only two viable treatment methods readily available to OSU facilities allowed: autoclaving and incineration. Incineration is mainly used for human body parts and animal carcasses where the animals have been infected with some type of infectious agent for research purposes. Most laboratory wastes are decontaminated by autoclaving. The following are requirements for autoclave treatment of wastes:

1. Waste bags should not be over-full and should be secured in the lab using string, autoclave tape or other heat resistant methods. Leave the bags closed during transport and autoclaving steps.

2. Transport secured bags inside tubs or other suitable secondary containment to the autoclave for decontamination. Leave bags in secondary containment at all times; do not place bags onto floors or other surfaces without secondary containment.
3. Place bags into the autoclave (in autoclavable tubs or trays). Do not overfill the autoclave, as steam needs to have access to all surfaces of the bags ideally. A metal rack in the bottom of a tub or tray works well, but is not necessary.
4. Autoclave on dry cycle for the appropriate length of time (determined by challenge testing), which is typically about 1 hour for a medium to large size bag of waste.
5. Autoclaves must have SOPs posted and be regularly challenge tested using *Geobacillus stearothermophilus* endospore vials. EH&S can provide the kits for this testing.
6. After the bags are cool, they should be placed inside black plastic bags and deposited into the dumpster. The custodial contractors will not generally dispose of these.
7. Liquid culture wastes should be autoclaved before disposal to sanitary sewer drains. Never pour melted medium containing agar into drains. Keep in mind that large volumes of liquid wastes will require longer times in the autoclave.

Centrifuge Guidelines

Centrifuges have great potential for the creation of aerosols if there is any leakage or breakage during centrifugation. The following guidelines will minimize risk during centrifugation:

1. Use tubes that are capable of withstanding the forces generated during the run, seal well, and are compatible with the dimensions of the rotor. Tubes with O-ring seals are superior in preventing leaks during centrifugation. Conical tubes should be used with rubber adaptors that provide cushioning of the conical tip of the tube.
2. Avoid filling tubes completely unless using an ultracentrifuge.
3. Make sure all tubes are well sealed and appropriately balanced in mass.
4. If using a hanging-bucket type of rotor, make sure the buckets are secured with well sealing safety cups after loading and before starting the centrifuge. If using a fixed-angle rotor, make sure the O-ring in the lid of the rotor is in good condition and lubricated to achieve a good seal.
5. After centrifugation, remove tubes and decant supernatants inside the BSC. If using safety cups in a hanging bucket rotor, take the whole bucket into the BSC for opening.

Storage / Labeling Guidelines

Laboratories should always know what is in storage (keep an inventory) and periodically verify the accuracy of the inventory, and discard unwanted or unnecessary materials from storage. The following guidelines should be followed:

1. Refrigerators, freezers, N_2 storage devices and other storage areas of labs should periodically be defrosted, disinfected and organized in such a way that materials are easily retrieved when needed. Samples that have broken during storage should be removed and discarded. Tubes removed from liquid nitrogen can sometimes explode as they warm up, so these should be placed inside secondary containment during thawing.
2. Equipment containing potentially biohazardous materials should be secured against unauthorized access. If storage is in common-use areas, these devices should have locks and be

labeled with the name and telephone number of a laboratory contact person. A universal biohazard symbol as well as a warning indicating “no food or drink” should be affixed to the door.

3. Flammable solvents in refrigeration should only be stored in refrigerators approved for these materials.
4. All materials in storage, chemical and biological, should be labeled in plain English, with dates. Labile materials should also be labeled with an expiration date.
5. Materials that are being transported should be appropriately identified and in secondary containment during transport. If potentially infectious, a biohazard symbol is required.

Personal Protective Equipment Guidelines

Personal protective equipment is required in laboratories. Personal protective equipment should not be worn outside the lab because it may be contaminated. For a BSL-2 lab, the following guidance should be consistently followed:

1. Use of the university – wide lab coat service is recommended. Lab coats must be worn while working in the laboratory. Cloth lab coats need to be regularly laundered. If the lab coats are to be laundered outside the lab, they must be bagged prior to removal and only opened when being deposited into a washing machine. Wash with warm or hot water and bleach. Disposable lab coats are preferable in many ways, cooler, and should be discarded after a few days of wearing or whenever visibly soiled.
2. Eye protection must be worn whenever handling liquids or conducting procedures where there is a potential for splashes or sprays, or projectiles. A good habit to develop is to put on eye protection when entering the lab and leave it on while working at all times. Eye protection should be regularly disinfected and left in the lab when not in use.
3. Gloves must be worn at all times when handling potentially infectious materials, cultures, wastes, or equipment. Gloves should be regularly inspected during use for tears or holes and discarded when compromised or contaminated. Gloves should be changed regularly and never re-used. Gloves should be removed inside-out without touching the outer surfaces. Gloves must be removed before touching common – use touch points such as doorknobs, drinking fountains, elevator buttons and freezer latches or doors. If you must carry potentially infectious materials through a lab door, place the materials inside a clean transport container, remove gloves and carry the container to the final destination. Wash hands after removing and discarding gloves.

Effective Use of the Biological Safety Cabinet (BSC)

The class II BSC is the most effective primary containment device for handling infectious materials and cultures in a BSL-2 laboratory. Cabinets must be used properly and certified annually. The following steps should be taken when working in the BSC:

1. Turn on the BSC and run it for at least 4-5 minutes prior to beginning work. Wipe down all work surfaces inside the cabinet with disinfectant[2] or alcohol. Plastic backed absorbent toweling on the work surface facilitates routine cleanup and reduces splatter and aerosol generation.
2. Prepare a written checklist of material for a particular activity and place all materials inside the BSC before beginning to work. Include discard containers for tips, loops, tubes and pipettes on one side of the BSC. If the user is right handed, the right side works well for discard containers. Left handed users will probably want the discards on the left side of the cabinet. Extra supplies should be stored outside the cabinet; only those materials needed for the tasks to be performed should be inside the cabinet.
3. Adjust the height of the chair so that arms at a 90^{o} angle to the body are inserted into the sash opening of the BSC about midway between the front grill and the bottom of the sash.
4. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce the risk of compromising the containment barrier provided by the air currents.
5. When working, do not occlude the front grill with supplies or your arms.
6. All operations should be performed on the work surface at least 4 inches from the front grill towards the center of the work surface.
7. The surfaces of all materials and containers placed into the cabinet should be wiped with 70% alcohol or alcohol/QAC wipes to reduce introduction of contaminants to the cabinet environment.
8. Upright pipette collection containers should not be used in the BSC nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place used pipettes in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Use a horizontal pipet discard inside the BSC.
9. When working with open containers or tubes, these should not be held in a vertical position. The lid should only be opened for brief periods of time on tubes or Petri dishes.
10. Open flames should not be used, and are not required, in the BSC.
11. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.
12. Spills should be cleaned up as described in the OSU *Response Guide for BSL-1 and BSL-2 Laboratories and Recombinant DNA Laboratories*, available on the EH&S / biosafety website.
13. Disinfect all work surfaces after completion of work and before turning off the blower.
14. If your BSC is equipped with a UV light and users want to make use of this feature, only use the UV light if the sash is completely closed (no opening beneath the sash) and only for 10-12 minutes after each use and after chemical decontamination has been completed. UV lights should never be used in cabinets that cannot be completely closed.

This information is based on current federal, state and international standards and regulations.
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[2] Do not use bleach or other strong oxidizers in the BSC unless immediately followed by deionized or distilled water because bleach and some other strong oxidizing agents will damage the stainless steel.