BSL-2 ENHANCED WORK PRACTICES FOR SPECIMEN PROCESSING AND CELL CULTURE

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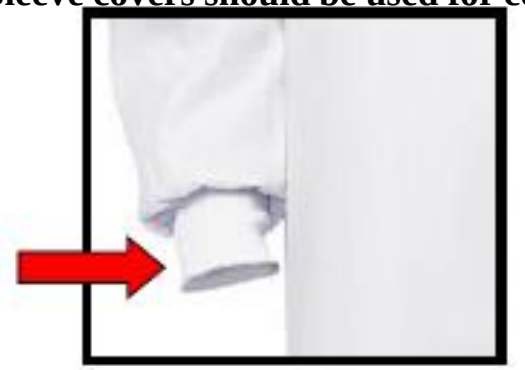
Personal Protective Equipment

Wear a buttoned-up lab coat with cuffed sleeves.

Sleeve covers should be used for coats with uncuffed sleeves



Have dedicated lab coats for cell culture work.
Place hooks in the tissue culture room.

Use disposable sleeve covers to minimize contamination.

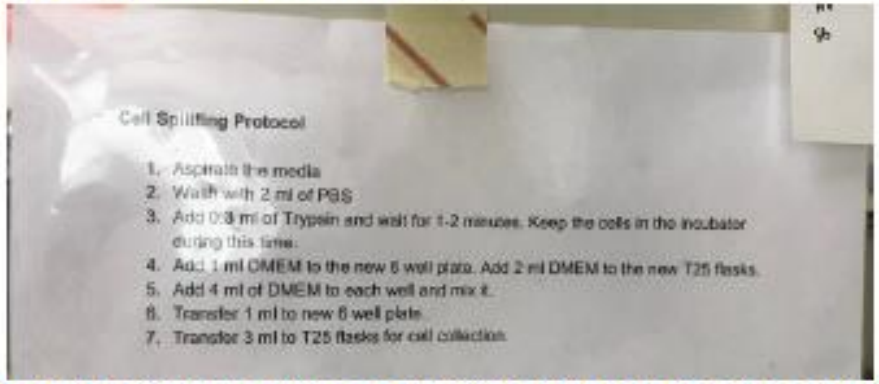
Double gloves are recommended for BSL-2 experiments.

Disinfect or remove outer pair of gloves prior to exiting the biosafety cabinet.

Consider the use of gloves with extended cuffs to ensure your gloves extend over lab coat sleeves.

Setup of Biosafety Cabinet (BSC)

Place the protocol with step-by-step instructions on the outside of the BSC. (Never place it inside the BSC)



Note: Avoid having unnecessary items that you do not need for your experiment inside the BSC; this helps you with the proper decontamination process of the BSC.

This includes a list of things you need to bring inside the BSC.

Cell splitting

____ 5ml pipet

____ Pipetman

____ 1000ul Pipet

____ 1000ul pipet tips

____ T25 Filter flasks

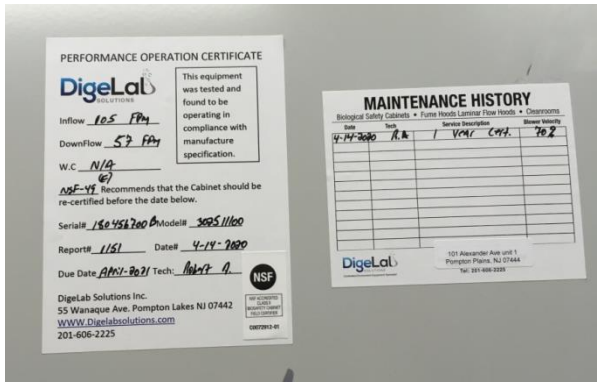
____ 6well plate

____ PBS

____ Trypsin

____ DMEM

Place disinfectant spray bottles inside the BSC.



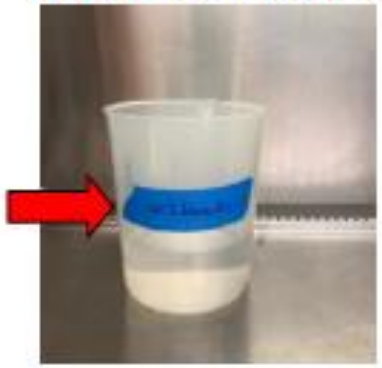
Notes:

- *Leave the bottles inside the BSC for the remaining time until your experiment is done.*
- *This ensures that you can disinfect your items prior to removal without removing hands out of the BSC.*
- *It also prevents disruption of the protective front air barrier and spread of contamination by gloves outside the BSC.*
- ***BSC should be certified annually and have a current certification sticker attached.***

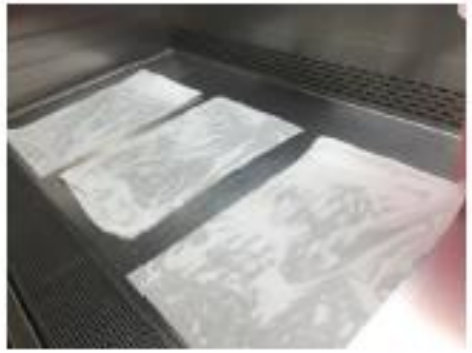
Setup of Biosafety Cabinet (BSC)-Continued

Set up containment to collect waste inside the BSC.

Set up a beaker or horizontal tray containing disinfectant or serological pipets.
Label the container with the name of disinfectant.



If you have large items, consider placing disinfectant-soaked paper towels down in work area which helps to disinfect the bottom of these items.



Set up a biohazard bag for solid waste.

Follow the instructions on the BSC checklist before starting your work.

Setup of Biosafety Cabinet (BSC)-Continued

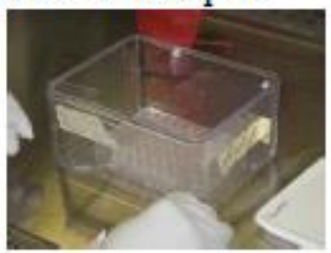
- Here is an example of an appropriate set up of the BSC with a "clean" vs "dirty" area.
- Take time to organize the BSC prior to starting work.
- Notice the location of the waste collection containers, cleaning supplies and disinfectant bottles in the picture.

Engineering Controls (Safety Equipment and Supplies)

Sealable filtered flasks

Sealable secondary containers for transport

e.g., for transport out of lab



e.g., for transport of tissue culture plates from BSC to incubator

Sealable aerosol-tight secondary containers for centrifuge

Note: All major centrifuge manufacturers have designed secondary containers for biohazardous materials available for purchase

Engineering Controls (Safety Equipment and Supplies)-Continued

Make sure to use tubes as primary containers that are also sealable (e.g., screw cap tubes with O-ring).

Ensure that the samples are coded and labeled in a way that identifies their contents to the laboratory. The lab must implement an easy to understand tracking system to discriminate live from inactive material. This could be different colored tubes, colored labels ("cryodots"), colored sharpies etc. Maintain your inventory in a secure manner.

Use filtered serological pipets and filtered pipette tips.

Avoid the use of sharp items (e.g., glass Pasteur pipettes, needles and syringes) for cell culture experiments. *If possible, use the plastic alternatives and/or safe sharp devices.*

e.g., plastic aspirating pipets

Note: If you must use a sharp such as a needle and/or syringe, use a blunt end needle for aspiration of liquid. Please notify EHS for an evaluation of sharps use in cell culture experiments before initiation.

Follow Safe Work Practices

- Work slowly to minimize the amount of aerosols generated.
- Minimize airflow disruption by moving hands in and out of the BSC less often.
- Never block the front or rear grills of the BSC.

Rear airflow grill

Front airflow grill

Follow Safe Work Practices-Continued

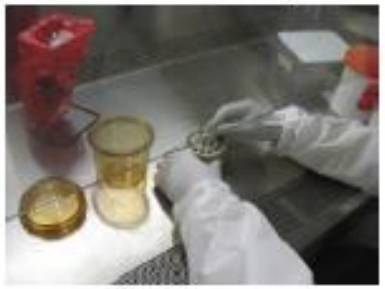
Pipetting can generate aerosols. To minimize aerosols:

- Release liquids against the side wall of the flask/tube.
- Re-suspend cells by carefully pipetting up and down.
    - Do not release all liquid from pipet. Never forcibly expel liquids from pipettes.
- Never dispense liquid from a height as this creates more aerosols.

To reduce potential spread of contamination outside the BSC:

- Disinfect exterior of tubes and tissues culture plates and then load into secondary container inside the BSC.
- Disinfect exterior of secondary container prior to removal from BSC.

- Load and unload centrifuge rotors and/or safety buckets within the BSC.
- Never load secondary container outside of the BSC.



Follow Safe Work Practices-Continued

- Disinfect safety buckets prior to removal from BSC.

Transport to and from the BSC

- Use secondary containers for safe transport of tissue culture plates to the incubator.

- Use safety buckets for safe transport of centrifugation tubes to the centrifuge.

- Open transport container inside incubator.

Collecting Waste Inside the BSC

Use a beaker or tray containing fresh* disinfectant for collection and disinfection of pipets

- Draw disinfectant up inside serological pipet and allow to run down into beaker
- Spray the top of the pipet in the beaker before removal from BSC

- Allow at least a 10-minute contact time for full decontamination.

- Prepare fresh 10% bleach in water each day. Label with date
- Add concentrated bleach to aspiration flasks before use (add 10% of final volume).
- Media should turn pink to clear on contact

- Use small biohazard bags for dry waste (pipet wrappers, used gloves, used paper towels) for items that cannot puncture a bag.
- Do not use this waste bag for items that can puncture a red bag (e.g. pipette tips, serological pipettes)
- Seal biohazard bag with dry waste prior to decontamination of the BSC.

- Spray or wipe down the outside of the biohazard bag wth disinfectant.

Collecting Waste Inside the BSC-Continued

- For items that <u>can</u> puncture a biohazard dry waste bag, use an empty 500ml media bottle to collect pipette tips in 10% household bleach.

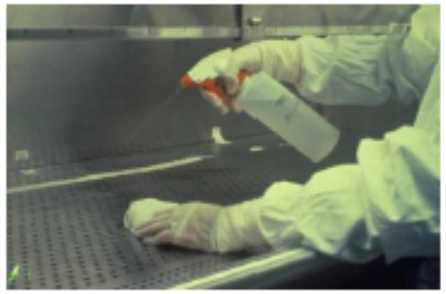
•A plastic beaker can also be used instead and can also be used to collect pipette tips and serological pipettes. Rinse these once with bleach in beaker
•Dispose by decanting 10% bleach down drain
•Pipette tips and serological pipettes are transferred to a sharps container

- Allow sufficient contact time (based on your biohazards) prior to removal of items from BSC.

Disinfection of BSC

- Disinfect the BSC with 1-10% household bleach in water, followed with 70% Ethanol to remove any bleach residues, which corrode the BSC.
- Disinfect all surfaces (back, sides, inside front view screen, grilles and work surface) by spraying disinfectant.

Note: Alternative disinfectants for decontamination of the biosafety cabinet may be used. Consult EHS to verify the effectiveness of new disinfectants.



Disinfection of BSC-Continued

- Use disinfectant-soaked paper towels to spread the disinfectant in order to get the best surface coverage.


- Consider using a metal swiffer or extension cleaning tool for difficult to reach spots.

After Your Experiment

- Remove your PPE before leaving the tissue culture room.
  - First, remove your outer gloves, then your lab coat, followed by your inner gloves.


- Wash your hands with soap and water after removing your PPE.

Content generously shared by Yale University EH&S

ABSA INTERNATIONAL
The Association for Biosafety and Biosecurity

Considerations for Handling Potential SARS-CoV-2 Samples

BSL2

- Whole blood, serum and urine
- Synthetic messenger RNA-based or recombinant protein-based products
- Rapid respiratory testing performed at the point of care (no nucleic acid isolation)
- Viral vector-based products
- Using automated instruments and analyzers (if aerosol containment is a feature) some devices might be older and not contained
- Staining and microscopic analysis of fixed smears
- Examination of bacterial cultures
- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
  - Inactivation methods should be validated
- Molecular analysis of extracted nucleic acid preparations
- Final packaging of specimens for transport to diagnostic laboratories for additional testing
  - Specimens should already be in a sealed, decontaminated primary container
- Using inactivated specimens, such as specimens in nucleic acid extraction buffer
- Performing electron microscopic studies with glutaraldehyde-fixed grids

*CDC Source

Additional procedures

- Cytometry – fixed samples

Laboratory Practices and Technique

- Good (Standard) Microbiological Practices
- Access to the laboratory is restricted when work is being conducted
- All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment
- Use safety cups whenever possible to avoid exposure to aerosols.

Primary & Secondary Barriers and PPE

- PPE: gown/lab coat, single gloves, surgical mask, eye protection, face shield
- Work behind plexiglass screen in an isolated designated area (minimum)
- Notify others in the lab
- Work with samples done over a plastic-backed benchtop pad
- Surface decontamination at every step using EPA List N disinfectants and contact times.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility
- BSCs, if available, are properly maintained and certified
- Mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory (recommended)
- A method for decontaminating all laboratory wastes should be available in the facility

Administrative Controls:

- Training and competency verification on donning and doffing required PPE
- Training and competency verification for each procedure performed
- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
- Occupational health
  - mandatory reporting of any symptoms, any laboratory exposure
  - consider baseline blood, baseline questionnaire, emergency wallet card
- Demonstrated competency on working in a BSC (if available)

BSL2 with BSL3 practices

- Aliquoting and/or diluting specimens
- Inoculating bacterial or mycological culture media
- Performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
- Nucleic acid extraction procedures involving potentially infected specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis

*CDC Source

Additional procedures

- Respiratory samples and secretions
- Stool***
- Cytometry – non-fixed samples
- Inactivated virus lysate
- Work with ANY sample that may produce an aerosol

Laboratory Practices and Technique

Good (Standard) Microbiological Practices as indicated for BSL2

Primary & Secondary Barriers and PPE

- PPE: surgical mask (blood)/N-95 (respiratory secretions), double gloves, impervious gown, eye protection with side shields
- All samples opened inside the BSC in case of spills/leakage. If BSC is not available, don N95 and face shield and work behind plexiglass screen in an isolated designated area, notify other laboratorians, work with samples done over a plastic-backed benchtop pad
- Surface decontamination at every step using EPA List N disinfectants and contact times.

Administrative Controls:

- Scheduled time for handling SARS-CoV-2 samples (best practice)
- Two-person rule for minimizing withdrawing hands from BSC
- Centrifuging of blood specimens is in safety cups or sealed rotor, loaded and unloaded in a BSC
- Training and competency verification on donning and doffing required PPE
- Specific training on use of N95 respirators, if applicable (includes pulmonary function, medical clearance, and fit testing)

BSL3

- Virus isolation/propagation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens
- Infectious clone-derived SARS-CoV-2 virus, infectious cDNA SARS-CoV-2 clones and recombinant derivatives.
- Infection of experimental animals with any of the above

Additional procedures

- FACS/High Speed Cell Sorting
- Transfer of inactivated samples outside BSL3
- Inactivation by validated methods with any of the above

Laboratory Practices and Technique

- Good (Standard) Microbiological Practices
  - Follow BSL3 practices and procedures according to CDC BMBL 6th ed.
- Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures.

Primary & Secondary Barriers and PPE

- All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices.
- Respiratory protection is required (N95 or PAPR/CAPR)
- Autoclave waste before disposal
- HEPA filtration of exhaust air is recommended for certain situations **

Transfer of samples outside BSL3- based on risk assessment by biosafety officer

- Ensure lids are tight
- Decontaminate outside of tubes
- Wrap sample with absorbent material
- Individually place into zip-lock bag
- Seal zip-lock bag and change gloves
- Add all wrapped samples into second bag
- Wrap bagged samples in ample packaging and place into designated carrier, firmly attach lid

*CDC Guidance for Laboratory https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html
**WHO Laboratory Guidance https://apps.who.int/iris/handle/10665/339056?search-result=true&query=covid+biosafety&scope=&rpp=10&sort_by=score&order=desrder=desc
***The Lancet https://www.thelancet.com/journals/langas/article/PIIS2468-1253(20)30124-2/fulltext

These considerations do not supersede any regulatory or country-specific in your locale.
Laboratory practices, techniques, and administrative controls build upon the previous level. Additional controls are indicated at each level.