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Working in the eBSL-3

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We use this protocol and it's working

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Disclaimer

It is not an SOP but rather a guide/peak/reference into what goes into working in an enhanced facility.

Some specific details may be omitted for privacy reasons. There are far far more details involved that can only be explained/experienced through a physical walkthrough/shadowing experience.

Exact details may vary between institutions depending on size of the facility, number of rooms, autoclave set up, biosafety level of agents in use, etc.



Abstract

This information has been recorded based on my experience with an eBSL-3. It is not an SOP but rather a guide/peak/reference into what goes into working in an enhanced facility. I did not have this sort of guide and want to provide it to others that may need it. I also recorded this guide for our lab/myself to reference later on.



Regulatory Paperwork

- 1 The enhanced BSL-3 facility requires extensive documentation. This documentation can and will be inspected at random by the CDC, so it must be filled out properly every single time you enter the facility.

You will fill out the following information in blue or black ink:

Date

Pressure reading

Temperature reading

Legal names of those entering

Room(s) you will enter in the facility

Reason(s) you will enter the room(s) in the facility

Project ID

Time of entry

Water collection tank status

Time of exit

You will also fill out a separate print out sheet detailing the status of the facility (water tanks, alarms, etc).

Mistakes on the paperwork must be crossed out, initialed, and dated.

Autoclave

- 2 Before entering the facility you will need to check the clean side of the autoclave.
Unseal the autoclave and remove any bags/cages/coffins.
Put bioindicators from the autoclave cycle on the heatblock, once these indicators are confirmed to be negative for bacteria growth (incubate them for __ hours), you may unbag the coffins and discard the liquid and solid wastes.
Put the empty coffins back into the autoclave, seal it, and turn it on to retrieve them on the inside of the facility.

PPE

- 3 eBSL-3 work requires extensive PPE, especially for select agents. You will need the following:
scrubs
Tyvek suit
N95
2 sets of different colored gloves
PAPR set up

additional PPE is required for in vivo work:



shoe covers
solid front gown
third pair of gloves

3.1 PAPR set up:

After donning your PPE you will enter the facility and immediately enter the PAPR donning room/area. This area is the 'cleanest' part of the facility since this is where PAPRs are stored. The hose of the PAPR will have a bag clamped onto it to keep it isolated from the facility since this is where clean air will be supplied to your Tyvek suit.

You will get your PAPR out from its box, insert a fully charged battery pack, and turn on the PAPR.

Once air is running out of the hose you will unclamp the bag and insert the hose into the opening sleeve on the back of your hood. You will clamp the hose down here.

Take your hood at an angle, hold it open, and put your head inside. There is a helmet piece inside that will sit on your head like a crown. You may adjust the tightness.

Buckle your PAPR around your waist.

Tuck the excess material from the hood (there will be a lot of excess material in the form of a front and back bib to work on creating a sealed environment) into your tyvek suit.

Zip up your sui.

You and your partner(s) should then inspect your suits to see if it is properly inflated or if there are any holes before you exit the room.

Entering the facility

- 4 Once you've donned the PAPR and entered the facility you should immediately open up the autoclave and retrieve any clean coffins from earlier. It's so important to get the autoclave ready before you enter since you will generate waste that will need to be autoclaved ASAP.

You should then prepare a fresh coffin with PV solution for your work and proceed to your designated room. You will need to scan into your room to enter, your movements are recorded in the facility. Each room has a set of double doors.

Prepping for Work

- 5 Set up the coffin in the BSC. This is where you will discard most waste, even for in vivo studies. Do not block any vents inside the BSC.
Ensure that you have both ethanol and PV spray bottles since you will constantly be disinfecting your gloves, sleeves, and surfaces.
Ensure that you have small biohazard bags on each side of the BSC since you will be collecting used gloves, paper towels, etc.
Keep items in the BSC limited. Having excess items in the hood increases the chances for spills.
Organize your items in a way that makes work flow easy.
Always slide items to your partner, never lift and pass them.



- 5.1 The ideal scenario for work in the BSC is to have one 'clean' person and one 'dirty' person. The dirty person will be handling the virus/infected samples and will remain in the hood, while the clean person is free to grab materials, start timers, bring plates to the incubator, etc.

However, this is not always possible.

Regardless, you should constantly be disinfecting your gloves and/or the BSC surface-
after handling virus aliquots or infected samples
before removing hands from the hood
whenever you find you have a bit of 'free time'

You may also change to fresh outer gloves at any time you'd like. This is recommended during in vivo work as urine and feces are considered infectious.

5.2 Small Spills

Spills happen. They can be as small as a single droplet. You must always assume that this is infectious. When you notice this you should immediately place a paper towel on top of the droplet(s) and add PV solution on top. Let this sit for a few minutes before wiping up. Then go ahead and disinfect the surface with a fresh paper towel.

Large Spills

Large spills must be reported. There is a spill button in each room.

- 6 Once you've finished your work you will double bag the coffin and tape it closed. Spray the outside of the bags and remove from the BSC.
Repeat for the small biohazard bags containing used gloves and paper towels. Replace these bags for the next user
Disinfect the BSC surface well.
Disinfect and remove your first layer of gloves, put them in the fresh biohazard bags in the BSC.
Immediately put on a new pair of gloves!
You may now exit the room.

Exiting

- 7 Bring your waste to the autoclave and load it.
Always include 2 biological indicators and a chemical indicator. Seal the autoclave and start the cycle.
Always add small amount of liquid (could be water) to dry waste (PPE waste).
- 7.1 Disinfect your outer gloves again. Remove them and discard in the used PPE bin. You and your partner will take turns using ethanol to disinfect the following (while still in the PAPR suit):
Inner gloves
arms
PAPR hood, including under the front flap
torso
legs



bottom of the feet
directly on the vent of the PAPR

Once you have disinfected each other you will enter the PAPR room to remove it.
unbuckle the PAPR and angle your head to the side. Carefully slide your hood off.
You will now only be wearing the tyvek, N95, and inner gloves.
Undo the PAPR set up and store them away.

- 7.2 Exit the PAPR room and stand with your back to the exit door.
disinfect your gloves
Unzip the tyvek suit
shimmy out of the tyvek without touching the 'clean' inside of the suit
disinfect your ID
disinfect the bottom of your shoes
take a small step back into door (it will open). do not drag the tyvek suit with you.
fold up the suit and place into the PPE trash
carefully remove your inner gloves and place in the PPE trash
Go fully into the next room

- 7.3 In this mini room there is a doffing area, a shower, and a locker area.

Remove your N95 and place in the N95 trash
Remove your scrubs and place in the scrub bin
Shower
Exit and change into a new pair of scrubs

You are then ready to exit the facility and wait for your partner in the entryway.

- 7.4 Once you are both done you will scan out of the facility and finish the paperwork detailing the status of the facility.
- 7.5 You are responsible for unloading your own waste from the autoclave once the cycle is done.