

Video Transcript – Animal BSL3 (<https://vimeo.com/9476256>)

Time: 22m 33s

Work at Animal Biosafety level three is suitable for work with animals infected with indigenous or exotic biohazardous agents capable of causing serious or potentially lethal disease whose route of transmission includes the inhalation of aerosols, ingestion, cuts in the skin, or braided skin, and splashes to the eyes, nose, or mouth.

Agents that fall into this category are often referred to as risk group three pathogens. The standard practices, procedures, containment equipment and facility requirements utilized at animal biosafety level three. Build on those required at animal biosafety level two.

The unique features of a facility housing animal biosafety level three research physically isolate or seal the animal room from adjacent spaces. These features, which include a double door entry system and a special ventilation system, provide a secondary barrier to the escape of biohazardous agents should the agents escape primary containment.

The secondary containment features protect individuals working adjacent to these rooms. The room's primary containment devices such as these microisolator cages, and biological safety cabinet serve as the primary barrier and protect individuals working within the room. The double door entry system consists of an anteroom or a clean corridor system that serves multiple animal rooms.

The ventilation system creates an airflow pattern that draws air from clean areas, such as the anteroom or adjacent spaces, and into the animal room. An instrument monitors this directional flow and alarms if there is a failure in the system. The air exhausted from the animal room is typically filtered or otherwise treated before being exhausted safely outside. None of this air is recycled back into the facility. Other potential routes for pathogens to escape the animal room are also addressed. Any penetrations in floors, walls, or ceilings are sealed. All interior surfaces are water resistant to facilitate cleaning. A hands-free sink is available for washing. Sink and floor drains are filled with disinfectant.

Vacuum services are fitted with disinfectant traps and inline HEPA filters. An autoclave is available in the animal room or exit corridor system so waste can be decontaminated before exiting the facility.

The door leading into an animal facility is secured and only authorized personnel may enter. Entry into the animal room is through the anteroom. The door leading into the anteroom and the door leading into the animal room should never be open at the same time. This ensures that air cannot be drawn out of the animal room and into adjacent spaces.

These doors are typically interlocked. Personal protective equipment and clothing is donned in the entry anteroom and what is worn is dependent on the nature of the work conducted and its associated risks. A disposable back fastened gown is worn over your scrubs, a uniform, or your personal clothes for frontal splash protection. Booties are worn to prevent the contamination of the shoes. A disposable N95 or N100 respirator is typically worn to prevent the inhalation of aerosols when conducting low risk procedures such as routine checks of animals or when work will be conducted in a biological safety cabinet. Double gloving is strongly recommended and is often required. Double gloving allows one to strip off the outer glove and replace it should it become contaminated. A second glove also adds another layer of protection. The gloves should be long enough so that they extend over the cuff of your gown, protecting your wrist. Check for any holes or breaks in the gloves as you don them.

Safety glasses or a face shield are required to protect the eyes from splashes. This is the standard attire typically worn when conducting work at Animal Biosafety level three. Additional or alternative protective clothing and equipment may be specified in some instances. This will be determined during the protocol's risk assessment. For example, a disposable coded Tyvek coverall may be worn to provide additional splash protection for the entire body. A second pair of booties may be donned along with disposable sleeves, if there is a high likelihood of contamination to the feet or forearms.

And a powered air purifying respirator or P A P R should be worn if a higher level of respiratory protection is warranted, such as when procedures that may generate infectious aerosols must be conducted outside of a biological safety cabinet.

or other physical containment device. When wearing a P A P R check to see that the airflow is sufficient before donning the respirator.

Please note that the wearing of disposable protective clothing is preferred over reusable clothing due to the need to decontaminate and launder reusable clothing. If the risk assessment has determined that a shower out is required when exiting the facility then personal clothes must be removed and scrubs donned. In these instances, disposable protective clothing must be worn.

Because of the high risk associated with working in an animal biosafety level three animal room access to animal rooms is limited to the fewest number of individuals possible. These individuals must typically be approved for entry by the laboratory or animal facility director, and must receive additional training on the potential hazards associated with the work the appropriate precautions to follow to prevent exposures, emergency and post-exposure response procedures, and the signs and symptoms of infection. The training is repeated regularly, typically, annually, or when necessary to communicate changes in the procedures or policies. Investigators working at this biosafety level must also demonstrate that they are proficient in the techniques to be used prior to beginning their work.

Medical clearance is also required for entry. Those at increased risk of acquiring infection or for whom infection might be unusually hazardous, are not allowed to enter unless the risk to them can be adequately controlled. This is determined during a consult with the occupational health physician. Depending on the biohazardous agents handled personnel entering the facility may also receive immunizations and participate in a serum storage program. The biohazard sign posted on the entrance of the room will list the room entrance requirements, the agent in use and contact information for the investigator responsible for the work.

When entering the animal room, you should not bring anything in with you that you do not need for the activities you are about to conduct. At this containment level, animals are housed in a primary containment device such as this negative pressure ventilated caging system or this static microisolator cage.

These caging systems prevent biohazardous aerosols from infected animals or their bedding from being dispersed into the animal room and exposing personnel. Prior to beginning your work, interior surfaces of the biological safety cabinet or other work surfaces should be wiped down with a disinfectant specific to the biohazardous agent under study. At this bio-safety level, animal handling and procedures with animals are conducted in a biological safety cabinet or other physical containment device. When using a biological safety cabinet, it is essential that you carefully plan out your activities, placing all of the materials you will need to conduct your work in the cabinet before introducing a cage or animal. Lay out your materials and supplies so you will have easy access to them.

Cages are opened in the cabinet and once opened everything within the cabinet, including your gloves are considered contaminated and are not removed without being decontaminated first. It is especially important that all aerosol generating procedures such as an necropsy, the harvesting of tissues or fluids or intranasal inoculation of animals be conducted in the cabinet. Special containment devices such as a class three biosafety cabinet or a HEPA filtered glove box can be used to completely isolate procedures that involve a very high risk of aerosol generation of a risk group three agent. The aerosolized delivery of multi-drug resistant TB is an example of such a procedure.

Cage changing also takes place in the cabinet. After the animals have been transferred to the new cage, the cage is reassembled, wiped down, and returned to the rack after allowing sufficient disinfectant contact time. The cage containing the spent bedding is sprayed down with disinfectant and sealed in a biohazard bag. This outer bag is also sprayed with disinfectant before being removed from the cabinet. This bagged cage is autoclaved with other waste.

One of the most important work practices to remember when conducting work at this biosafety level is to avoid the use of sharps whenever possible. If a sharp must be used, select the safest, sharp possible and follow safe, sharp practices. Safe sharps are designed to immediately cover the needle or blade after use, making it less likely for a puncture or cut to occur between use and disposal.

Blunt tipped instruments are also examples of safer sharps. When working at Animal Biosafety level three, try to limit the use of sharps to the inoculation of animals or the drawing of samples. And if possible, replace glass containers or tubes with plastic substitutes or Mylar wrap tubes. If you must inoculate an animal, load the syringe in the cabinet, wipe the injection area with disinfectant, inoculate the animal, and immediately discard the needle and syringe in a sharps disposal container. Wipe the injection area again with disinfectant to absorb any inoculum. Safe, sharp techniques are illustrated during this animal necropsy.

The animal is humanely euthanized. The euthanized animal is secured to the disposable cutting board with tape rather than pins. Pins are considered sharps and can cause punctures. Blunt tipped instruments are used. Forceps keep the fingers away from the cutting area and from touching the animal. The necropsy is being conducted in a biological safety cabinet to control aerosols that are generated as tissues are manipulated or sliced.

A scalpel with a sliding shield is used and the shield is activated between uses. Disposable sharps are discarded in the sharp's disposal container located in the cabinet, and reusable sharps are placed in a container of disinfectant. Reusable sharps, such as scissors and forceps, must be disinfected and cleaned after use.

This can be done by placing them in an unbreakable container with an appropriate disinfectant. Arrange the sharps so the sharp ends are separated. If disinfecting a large number of reusable sharps, place the sharp ends on one end of the container. Scissors should be opened so the disinfectant reaches all surfaces.

After soaking for the appropriate contact time, any residual debris may be removed with a brush. After removing the debris, return the sharps to the disinfectant and allow them to soak again.

Whenever possible animals should be anesthetized when performing hazardous procedures such as inoculations with biohazardous materials or blood draw on an animal infected with a biohazardous agent. If the animal cannot be anesthetized due to the nature of the procedure, sedate the animal and use a mechanical restraint. Such as this plastic tube. At this biosafety level, it is recommended that investigators work with an assistant. This allows the investigator to focus on the anesthetized or restrained animal and their interactions with the animal.

Once you have completed your procedure and you are ready to return the cage to the rack, reassemble the cage and decontaminate its outer surfaces by spraying or wiping down the cage with a disinfectant specific to the organisms present. All sides of the cage, including behind the card holder, along with its top and bottom, must be disinfected. When finished set the cage aside on disinfectant soaked matting. This facilitates the disinfecting of the cage bottom. After allowing the appropriate amount of contact time, the cage may be removed by an assistant. You must also remove or disinfect your outer gloves before withdrawing your hands from the cabinet or if they should become contaminated with infectious material while working in the cabinet.

Before exiting the cabinet, this researcher is removing his outer gloves and placing them correctly in the biomedical waste. An alternative technique is to decontaminate the outer gloves by spraying them with disinfectant and rubbing the gloves together as if washing your hands. When using this technique, it is essential that you have selected an outer glove that is resistant to permeation by the disinfectant. Always allow the appropriate amount of disinfectant contact time before removing your gloves, cage, or any other equipment from the cabinet.

All items in the biological safety cabinet, as well as its interior surfaces should be completely decontaminated with an effective disinfectant before you exit the animal room. If you are using some other containment device or work surface, it should be completely decontaminated. The first step is decontaminating any samples and waste that will be transported out of the animal room. If biohazardous samples will be removed, the primary containers should have a screw top. All sides, top and bottom of the sample containers should be decontaminated and then placed into a labeled secondary container with screw top. All outer surfaces of the secondary container should also be decontaminated before it is removed from the cabinet. All wastes are placed in biohazard bags with sufficient water to generate steam when autoclaved, sprayed with disinfectant, double bagged, taped, and decontaminated again. Animal tissues and carcasses are typically separated from other waste but bagged in a similar way.

The second step is decontaminating the cabinet and its contents. To do this place all items within the cabinet on one side, and then disinfect the opposite side. Surfaces that must be disinfected include the back, the side, the work surface, and the view screen. Once half the cabinet is disinfected items within the cabinet are disinfected one at a time and placed on the clean side.

All surfaces, including the top and bottom of each item should be disinfected. Finally, the second side of the cabinet is disinfected. The cabinet's grate is disinfected last. Once the interior surfaces of the cabinet, the items within it and your gloves have been decontaminated and the disinfectant has been allotted sufficient contact time, the cabinet is unloaded. Please note that the transport of infected animals outside the animal biosafety level three animal room is not recommended. But if necessary, special arrangements must be made. Special HEPA filtered transport containers or carts are required to transport these animals.

The exact exit procedure for exiting the animal room will depend on the layout of your facility. The exit may be back through the entry anteroom, or in some instances through an exit corridor system or exit anteroom. Upon exiting, animal carcasses are typically placed in cold storage and later incinerated. Other waste are sterilized using pass through autoclaves. Equipment that cannot be autoclaved, such as this lamp must be wiped down with a disinfectant specific to the organisms present before it leaves the facility. Proper removal of protective clothing and equipment when exiting the animal room prevents contamination from spreading to other areas. The procedure followed will vary from facility to facility, but to minimize the potential for contamination. The most contaminated items are always removed first and protective clothing is always removed before exiting the anteroom or exit corridor system. In this facility, the outer booties, outer gloves and sleeve covers are removed as one exits the animal room and placed in the biomedical waste. In the anteroom. The reusable respirator is removed, decontaminated and stored for future use.

Next, the inner booties and disposable coveralls are removed and disposed of as biomedical waste. When removing clothing, try to avoid contaminating your inner gloves or skin by touching uncontaminated areas of the clothing and turning it inside out. If reusable protective clothing were worn, it would be placed in a contaminated clothing bin for decontamination and laundering by a commercial laundry that accepts garments potentially contaminated with biohazardous materials. Finally, remove your inner gloves and your hair protection, which in this case was protected by the respirator. Remove your scrubs or uniform and shower out if required by the protocol or wash our hands thoroughly, if not.