BSL-2 biological decontamination procedure.

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This protocol describes the standard operating procedures (SOPs) required for the decontamination of biological residues and materials potentially contaminated by risk group 2 agents. The procedures relating to risk group 3 agents are described in a separate protocol (see Biological Decontamination for Risk Group 3 Agents.pdf). Biological decontamination is intended to negate the possibility of transmitting infectious or toxic agents to lab workers, general public and surrounding environment. Selection of disinfectant and decontamination procedures depends on the type of agent concerned whereby enveloped viruses are the least resistant and prions and spore forming bacteria are the most resistant. The following procedures are based on those recommended for high-level semi-critical surgical decontamination as well as for critical sterilization of materials and waste products.

High-level semi-critical surgical decontamination relies on the use of liquid or gaseous disinfectants like 2% Glutaraldehyde solutions, 6% stabilized hydrogen peroxide solutions, Peracetic acid solutions, 1000 ppm sodium hypochlorite solutions or on-demand chlorine gas. Critical sterilization, on the other hand, relies on the use of dry heat or high-pressure water vapour sterilization or ethylene oxide gas. Decontamination includes ***cleaning*** to remove organic material, dirt and grease as well as ***disinfecting*** to remove microbial contaminants using a suitable disinfectant. Thorough decontamination of equipment and *personal protective equipment* (PPE) are essential to protect personnel from pathogen exposure and to prevent the spread of pathogens to wildlife, environment and humans.

NOTICE: All work pertaining to the handling of biological samples potentially bearing risk group 2 agents (all human samples and some animal samples) must be carried out in a class II biological safety cabinet following proper BSL2 discipline and standard microbiological practices.

CAUTION: This protocol DOES NOT apply to work with non-lipid enveloped viruses. Follow the appropriate protocol for decontamination of non-enveloped viruses such as: Adenovirus, Human Papillomavirus, JC virus, BK virus, Parvovirus B19, Coxsackievirus, Echovirus, Poliovirus, Rhinovirus, Hepatitis A and E virus, Rotavirus, Norovirus and Rabies virus.

Infectious microorganisms are classified by the WHO, NIH and CDC into four different risk groups which correlate with but do not equate to biosafety levels. **Risk Group 2** agents are defined by the NIH as “Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.” and by the WHO as “A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. These represent a moderate individual risk and a low community risk.”

Disclaimer

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Decontamination of work surfaces

1) Wipe down all work surfaces with 70% ethanol to remove excess NaOCl and residues. Allow to air dry and place paper towels in biological waste bin or bag (red bags)

2) Further decontamination can be accomplished by UV radiation as long as quartz tube is wiped clean before use, is functioning correctly and is of the proper germicidal wavelength and intensity. Do not rely solely on UV lamp decontamination for surfaces or inside of biological safety cabinets.

Decontamination of personal protective equipment (PPE)

1) Use new gloves for all sterile work and dispose of used gloves after manipulating biological samples of any type. Reuse of gloves should be restricted to gloves used for the operation of equipment, washing, opening and servicing refrigerators and freezers and other procedures NOT involving biological material of any type.

2) When working with samples potentially contaminated by risk group 2 agents (all biological samples of human origin and some laboratory animals) gloves should be wiped down with 70% ethanol every 15 minutes. Latex gloves will eventually turn brown and brittle. Change gloves at least hourly.

3) Wipe gloves down with 70% ethanol before removing and changing them. Inspect for ruptures, notify lab manager or supervisor if puncture evident. Document as a lab incident.

4) Wipe goggles or face shield with 70% ethanol before removing and AFTER changing gloves. Allow face shield to dry before removing.

3) Remove N95 or N99 half-face respirators AFTER removing gloves, avoid touching exterior of respirator and dispose of in biological waste bin or bag (red bags).

4) Remove disposable lab gown is used and dispose of in biological waste bin or bag (red bags).

5) Remove lab coat and hang in assigned place or place in red plastic bag if soiled for laundry cleaning.

6) Wash hands thoroughly with soap and water before leaving the lab.

NOTE: On site laundry cleaning of all lab coats is warranted. Do not launder with non-laboratory clothing or linen.

Decontamination of disposable material and waste.

1. Place a suitably sized basin inside the biological safety cabinet and fill with either clorhexidine or 0.1% NaOCl for immersion decontamination of disposable PLASTIC serological pipettes, transfer pipettes and Pasteur pipettes, tubes, vials and flasks. Allow a minimum contact time of 15 minutes before removing basin and contents from biological safety cabinet.

2. Place all plastic micropipette tips and glass transfer or Pasteur pipettes directly into a sharp's container. Do not place in liquid disinfectant; do not add liquid disinfectant to sharps container!

3. After removing liquid disinfectant basin from biological safety cabinet, decontaminate work surface as shown above and place all paper towels in the plastic waste bag located inside the biological safety cabinet.

4. Tie a knot on plastic waste bag, clean exterior with 70% ethanol and remove from cabinet. Place in red biological waste bin.

5. Once sharps container is filled to 75% of its capacity, close, wipe down with 7% ethanol and remove from biological safety cabinet. Store in lab until next scheduled (monthly) biological waste recollection.

6. Pour liquid disinfectant of the basin on which disposable plastic pipettes and material were decontaminated down the drain and rinse material. Place in red biological waste bin bag.

7. Close biological waste bin bag once filled to 75% capacity and store in lab until next scheduled (monthly) biological waste recollection.

8. Once the lab manager indicates that the biological waste bins are scheduled to be recollected, follow the BIOLOGICAL WASTE ROUTE to the biohazardous waste recollection site.

Biohazardous waste route sticker as used at Facultad de Medicina UASLP

Biohazardous waste recollection site used at Facultad de Medicina UASLP

Decontamination of re-usable material.

1. Wipe down exterior of all material, pipettes, tubes and flasks with 70% ethanol BEFORE removing from biological safety cabinet.

2. Re-label tubes accordingly if label has been affected by ethanol.

3. Wipe down all material, pipettes, tubes and flasks with 70% ethanol AFTER removing from

biological safety cabinet.

4. Re-label tubes accordingly if label has been affected by ethanol.

Autoclave decontamination of risk group 2 biohazardous waste.

NOTE: Autoclave sterilization of risk group 2 biohazardous waste is not warranted on site when such waste is processed by a specialized waste management company as employed by Facultad de Medicina UASLP. However, certain special situations MIGHT warrant such decontamination to further reduce the risk of inadvertent exposure to biological hazards. Such is the case of certain bacterial cultures, large volumes of biohazardous waste, long time till recollection and certain human samples known to represent a particularly high risk (HBV, HCV and similar).

VERY IMPORTANT NOTICE: Bleach (NaOCl) or other liquid disinfectants present in materials to be placed in autoclave may either generate toxic gas fumes or cause damage to autoclave itself. Neutralize waste containing bleach with equal amounts of 1% sodium thiosulfate in water prior to autoclaving. Better yet, avoid use of bleach in materials to be autoclaved.

1. Place biohazardous waste bags neatly organized in wire mesh autoclave baskets. Make sure plastic bag is capable of withstanding autoclave program.

Wire mesh autoclave baskets.

2. The 54-litre Biolab BAVT-302-A high-pressure vertical steam sterilizer/autoclave located inside the cell biology laboratory (BSL2) is the only autoclave used to decontaminate risk group 2 contaminated materials. At our facility, these materials are processed within the BSL2 cell biology laboratory following BSL2 discipline and protocols. This autoclave is also used to sterilize equipment and instruments that are normally used within the cell biology lab. Other autoclave/sterilizers located outside of the cell biology laboratory are not to be used for this purpose.

54 Litre Biolab BAVT-302-A autoclave/sterilizer used.

3. Before opening autoclave sterilizer verify chamber pressure is 0 as indicated in the pressure manometer located on the front panel of the autoclave. For extra assurance, purge chamber pressure by pressing on exhaust relief valve located on left-hand side panel.

Pressure manometer.

Exhaust purge valve (below).

4. On the autoclave's right-hand side panel (facing front) find and close the main chamber drainage water valve. When properly closed, the yellow valve should be perpendicular to the nozzle as shown in the picture below. The two hoses do not need to be placed in water basin until after sterilizing has

been accomplished and water has cooled down. When the dry cycle is used, no water should remain in the chamber. As such, these valves are rarely opened after waste decontamination.

Main chamber drainage water valve (in the closed position).

5. Verify that the steam-discharge hose located in rear panel is properly seated in an empty water recollection bottle.

Steam outflow hose properly seated in water recollection bottle.

6. Fill main chamber with distilled water up to the bottom of the removable floor plate.

Main chamber floor-plate (octagonal plate in centre).

7. Load chamber with wire mesh basket and biohazardous waste bags.

8. Turn autoclave/sterilizer on by flipping switch located on right-hand upper side panel.

Thermomagnetic electrical switch.

9. Set autoclaving protocol to **sterilize & dry cycle** for 30 minutes at 121ºC sterilization and a drying step of 100ºC for 10 minutes (drying temperature can not be modified).

Front display panel.

10. After autoclaving, bags are allowed to cool down and either stored locally in the lab or referred to the biohazardous waste recollection site.

11. Each user should annotate the sterilization/decontamination cycle used as well as his name after autoclaving.

Laboratorio de Genómica Viral y Humana
Autoclave log for biohazardous waste decontamination

Date (dd/mmm/yyyy)	RG	Material description	Autoclave program	Operator

User autoclave log as used at Facultad de Medicina UASLP.

12. Excess water in steam-water bottle and main chamber should be purged and the autoclave left open to completely dry out.

13. Red biohazard bags are destined for incineration along as institutional biological waste. The primary responsibility for the safe handling and disposal of infectious waste resides on the laboratory generating the waste. This responsibility extends to the ultimate point of disposal and should consider the possibility that other parties including vulnerable population groups (as happens in developing countries) may be exposed to the waste. Therefore, the waste producing lab should conduct inspections or take measures that ensure that the waste is either being handled and disposed of properly, or is being thoroughly decontaminated before egress. In addition, there may be federal, state, or local regulations controlling medical waste disposal and recordkeeping that must be observed.

Application	Suggested programs			Dry cycle
Surgical instruments	121ºC x 30 min	132ºC x 15 min	135ºC x 10 min	15 - 45 min
Cloth and linen	121ºC x 30 min	132ºC x 25 min	135ºC x 10 min	15 - 30 min
Wrapped material	121ºC x 30 min	132ºC x 15 min	135ºC x 10 min	30 min
Glassware	121ºC x 60 min			30 min
Liquid media	121ºC x 60 min			None
Animal carcasses	121ºC x ≤ 8 hrs	132ºC x ≤ 4 hrs	135ºC x ≤ 2 hrs	15 - 30 min
Biohazardous waste	121ºC x 30 min	132ºC x 30 min	135ºC x 20 min	30 min

Alternate autoclave programs by application.

References

1. James N. Mills *et al*, U.S. Department of Health & Human Services, Centers for Disease Control and Prevention. Methods for Trapping and Sampling Small Mammals for Virologic Testing. 1995.

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3. V. Shelley, S. Kaiser, E. Shelley, T. Williams, M. Kramer, K. Haman, K. Keel, and H.A. Barton - Evaluation of strategies for the decontamination of equipment for Geomyces destructans, the causative agent of White-Nose Syndrome (WNS) Journal of Cave and Karst Studies, v. 75, no. 1, p. 1-10.

Revision history

1.0 Original document.

2.0 Added references and autoclave programs.

3.0 Changes to document format only and protocol title.