BSL-3 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 3 agents. The procedures relating to risk group 2 agents are described in a separate protocol (see Biological Decontamination for Risk Group 2 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 3** agents are defined by the NIH as “Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.” and by the WHO as “A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.” These represent “a high individual risk but low community risk” in both classifications.

Disclaimer

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Decontamination of work surfaces

1) Wipe down all work surfaces with soap and water and sponge. Dry with paper towels and place towels in biological waste bin or bag (red bags).

2) Wipe down all work surfaces with 0.1% sodium hypochlorite (NaOCl) and allow 5 minutes contact time for proper decontamination. Dry with paper towels and place towels in biological waste bin or bag (red bags).

3) 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)

4) 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 work and dispose of used gloves after all and every procedure within a Biological Safetly Level 2 Plus (BSL2+) environment.

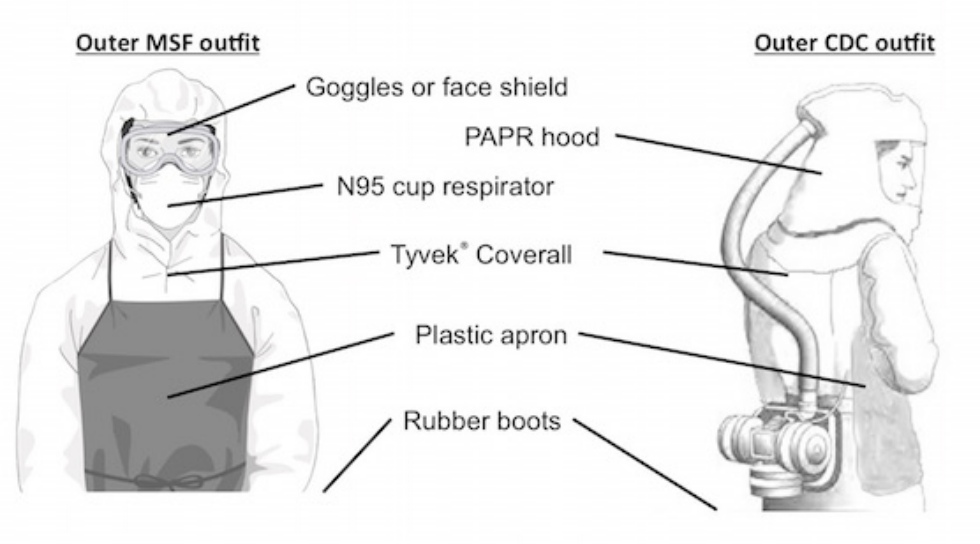
2) Gloves should never be reused.

3) When working with samples potentially contaminated by risk group 3 agents (some human biological samples and most wild animal samples) gloves should be wiped down with 70% ethanol every 15 minutes. Latex gloves will eventually turn brown and brittle. Change outer gloves at least hourly. Do not remove inner gloves within the BSL2+ lab.

4) 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 and considere post-exposure prophylaxis.

5) When using either the Médecins Sans Frontières (MSF) or Centers for Disease Control and prevention

(CDC) biological safety attire for work with BSL3 discipline, spray down entire outer garment with freshly prepared 0.1% NaOCl solution. Allow a minimum of 15 minutes of contact time for proper decontamination.



Two different BSL3 protective garments used at Facultad de Medicina UASLP

6) After 15 minutes of contact time, wipe dry with paper towels and place towels in red biohazardous waste bag.

7) Wash outer gloves with soap and dry with paper towels. Place towels along with outer gloves in red biohazardous waste bag.

8) Wash inner gloves with soap and dry with paper towels. Place towels in red biohazardous waste bag.

9) Exit BSL2+ laboratory and wash inner gloves again in the third (Personal Protective Equipment, PPE antechamber).

10) Once in the third (PPE antechamber), remove the plastic apron while leaning forward, avoid contaminating hands. Hang apron up in corresponding hook or dispose of in red biohazardous waste bag.

11) Wash gloved hands (inner gloves) with soap and water or with alcohol rub.

12) Remove PAPR hood without turning motor off, avoid touching exposed facial skin or mucous membranes. Disconnect air supply hose, turn motor off and hang PAPR hood and tube in the corresponding hook.

13) Wash gloved hands (inner gloves) with soap and water or with alcohol rub.

14) Remove PAPR motor belt and wipe clean with 70% ethanol. Remove battery and place them in

corresponding place.

15) Wash gloved hands (inner gloves) with soap and water or with alcohol rub.

16) Remove coverall by unzipping front without touching inner clothing or exposed skin, “crawl-off” the suit from top to bottom and place in red biohazardous waste bag.

17) Wash gloved hands (inner gloves) with soap and water or with alcohol rub.

18) Remove rubber boots carefully without touching inner clothing or exposed skin. Place them in their allocated place.

19) Wash gloved hands (inner gloves) with soap and water or with alcohol rub.

20) Remove inner gloves carefully with WHO discipline without touching exposed skin with outer part o gloves.

World Health Organisation (WHO) inner glove doffing procedure

21) Wash hands thoroughly with soap followed by alcohol rub.

22) Dry hands with paper towel and use a different paper towel to open door separating third antechamber from second (shower) antechamber.

23) Shower thoroughly with soap and proceed to exit through to first (clean) antechamber.

Decontamination of disposable material and waste.

1. Place a suitably sized basin inside the biological safety cabinet and fill with either clorhexidine or

0.5% 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 sharps 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. Biological safety cabinet waste bag will later be steam sterilized before removing from BSL2+ laboratory.

5. Once sharps container is filled to 75% of its capacity, close, wipe down with 7% ethanol and remove from biological safety cabinet. Sharps container will later be steam sterilized before removing from BSL2+ laboratory.

6. Pour liquid disinfectant of the basin on which disposable plastic pipettes and material were decontaminated down the drain and rinse material off clear of NaOCl residues. All material will later be steam sterilized before removing from BSL2+ laboratory.

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.

Decontamination of non-disposable and re-usable material.

1. Wipe down exterior of all material, pipettes, tubes and flasks with freshly prepared 0.1% NaOCl solution, allow contact time of 15 minutes for proper decontamination.

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

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

4. Wipe down all material, pipettes, tubes and flasks with 70% ethanol AFTER removing from biological safety cabinet.

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

Autoclave decontamination of risk group 3 biohazardous waste.

NOTE: Autoclave sterilization of risk group 3 biohazardous waste is WARRANTED in spite of the fact that biohazardous waste might be ultimately processed by a specialized waste management company as employed by Facultad de Medicina UASLP. However, certain special situations MIGHT not require such decontamination. Such is the case of certain NaOCl containing material (criovials, flasks or NaOCl-decontaminated liquid media or absorbent material. In this case, the items must be decontaminated by submersion in 0.5% NaOCl for 30 minutes and/or placed in red biohazardous waste bags which should be tied and thoroughly sprayed with 0.5% NaOCl solution and, after a minimum contact time of 15 minutes, be wiped dry with paper towels and then placed into yet another red biohazardous bag for removal from BSL2+ lab.

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, sharps containers and animal carcasses to be sterilized in with either wire mesh autoclave basket or autoclave metal container.

Wire mesh autoclave basket and autoclave metal container.

2. The 36 litre Yamato SM300 high-pressure steam sterilizer/autoclave located inside the enhanced biocontainment laboratory (BSL2+) is the only autoclave used to decontaminate BSL3 risk group materials. At our facility, these materials are normally processed and produced within the BSL2+ laboratory itself. This autoclave is also used to sterilize equipment and instruments that are normally used within the BSL2+ lab as well. Other autoclave/sterilizers located outside of the BSL2+ laboratory are not to be used for this purpose. In general, no material used, processed or produced inside the BSL2+ laboratory should ever leave such installation without prior decontamination.

36 Litre Yamato SM300 autoclave/sterilizer used.

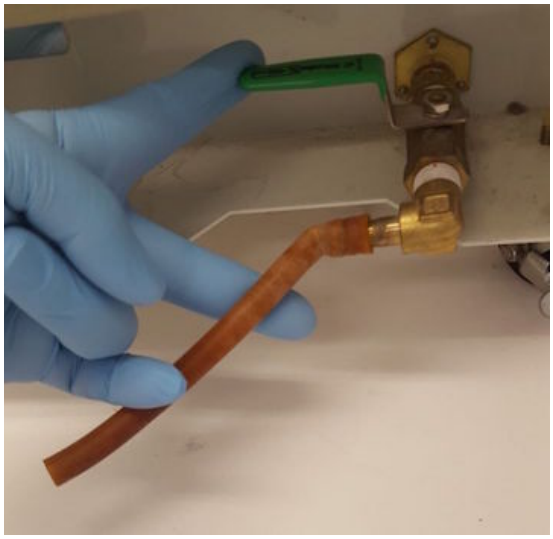
3. Before opening autoclave sterilizer verify chamber pressure is 0 as indicated in the pressure manometer located on the front panel of the autoclave. For extra assurance, purge chamber pressure by pressing on exhaust relief valve located on left-hand side panel.

Pressure manometer.

Exhaust purge valve.

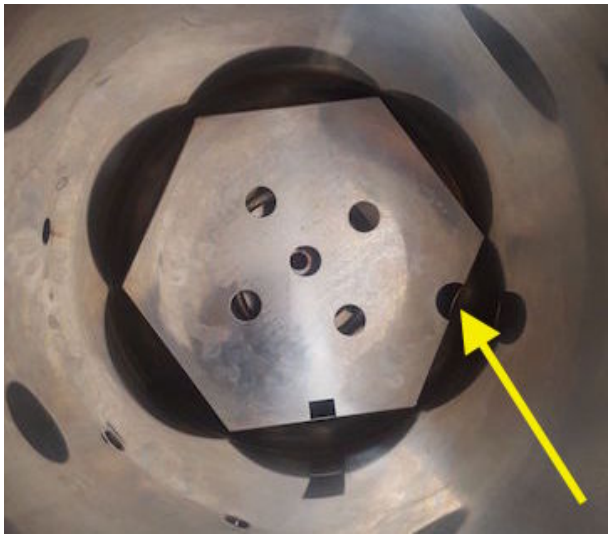
4. Open the autoclave's front panel door and close main chamber water valve. Verify that the steam-water bottle is filled with distilled water to the indicated mark. If above this level, retrieve bottle from panel and discard water directly to the drain. Replace bottle and make sure the bottles rubber stopper is tightly fitted.

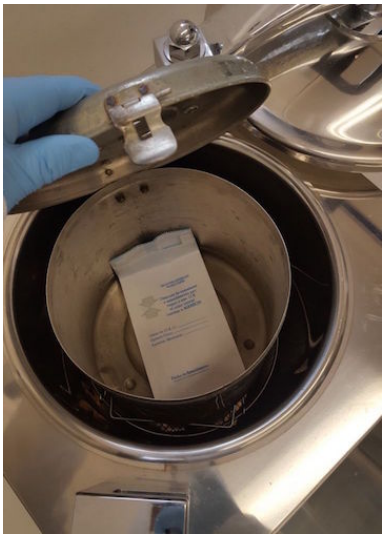

Front panel door.


Main chamber water valve (closed position).


Steam-water bottle

5. Fill main chamber with distilled water up to the corresponding level mark (1 cm below the main chamber floor plate and indicated by yellow arrow in picture below).


Main chamber water level indicator notch.


Autoclave container with carcass bag.

6. Load chamber with the wire mesh basket and/or metal container. As depicted in the picture above right.

7. Turn autoclave/sterilizer on by flipping switch located on left hand side panel. Make sure steam-catch reservoir located on right-hand side panel is empty and place beneath excess steam nozzle (this fitting is magnetically attached to side of autoclave).

Thermomagnetic electrical switch.


Steam-catch reservoir.

8. Set autoclaving protocol to **sterilize & dry cycle** for 90 minutes at 124ºC sterilization and a drying step of 150ºC for 32 minutes as shown in the picture below (although the picture below indicates 40 minutes only). Other programs may apply based on type of material to be sterilized, see detailed and most current bat or rodent carcass decontamination.

9. After autoclaving bags are allowed to cool down and placed into red biohazard bags. These bags are sealed tight and spray exterior of bag with 0.5% NaOCl, allow a minimum decontamination contact time of 15 minutes before removing from BSL2+ laboratory.

10. Each user should annotate the sterilization/decontamination cycle used as well as his name after autoclaving. Excess water in steam-water bottle and main chamber should be purged and the autoclave left open to dry out until future use.

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.

11. Red biohazard bags are destined for incineration along with institutional biological waste and are now considered decontaminated. The primary responsibility for the safe handling and disposal of infectious waste resides with 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 generator 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.

2. Blehert, David S. White-Node Syndrome Diagnostic Laboratory Network. Ver. 1.3 (1 Mar 2015) www.whitenosesyndrome.org/.

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.