Wild bat & rodent carcass decontamination.

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Several emerging infectious diseases are currently recognized as threats to human health. A substantial number of these are zoonoses because animals are their natural reservoirs. One of the most crucial points of identifying and minimizing the impact of emerging infectious disease outbreaks is intensive and continuous surveillance. In the case of zoonoses, surveillance can be carried out in reservoir host populations where infection prevalence and population characteristics of reservoir species can be used to assess risk to humans and ameliorate or prevent outbreaks of EIDs. This protocol describes our local guidelines for the proper biological decontamination of wild bat and rodent carcasses processed as part of exotic viral pathogen surveillance measures.

Disclaimer

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General considerations

1) All personnel involved in capturing, handling, or sampling of bat and rodent samples are responsible for knowing and adhering to the institutional biosafety guidelines and procedures relevant to their tasks.

2) The principal investigator is responsible for ensuring that all personnel involved in the handling of bats or rodents and their organs have had the appropriate biosafety training.

3) All personnel handling bats or rodents or their biological specimens must be vaccinated against rabies virus before being involved in such activities. This should be documented in the workers medical follow-up history.

4) All personnel must agree to follow post-exposure guidelines for any injury obtained from handling bats that may represent a risk of exposure to rabies.

5) Current biosafety recommendations should be revised before the sample collection or organ harvest event to decide the level of personal protection required to prevent contamination of personnel. This risk assessment should be adapted for different biomes, geographic regions, biological specimen types, weather and season of the year.

6) 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 other wildlife **or human populations**.

7) First aid protocols for a bite, scratch or needle-stick injuries should include:

   a) Immediately cease work and notify principal investigator of accident.

   b) Wash lesion with household soap and water for full 5 minutes and then apply betadine or benzalkonium chloride to lesion. Benzalkonium chloride is known for its potency against rabies viruses. It is recommended that benzalkonium be kept readily available for such purposes.

   c) Post-exposure rabies vaccination should be applied immediately if bats, rodents, skunks, foxes, raccoons, coyotes or other feral canines caused lesion. The field team should carry refrigerated doses of rabies vaccine if working in a remote location so as to administer a booster dose immediately after exposure. Otherwise, exposed personnel should report to a medical clinic for administration of the booster doses according to published WHO recommendations. http://www.who.int/rabies/human/postexp/en/

Wild bat or rodent carcass decontamination in a field-station setting.

1. After due samples and organ harvest has taken place, place a dry paper towel inside abdominal cavity of animal and wrap in another paper towel. Spray the entire carcass wrap with 0.5% NaOCl and place along with other materials, plastics and disposable instruments (if any) in an autoclave resistant paper bag.

Autoclave resistant bag used for carcass decontamination

2. Spray entire paper bag with 0.5% NaOCl and set aside until all other bats/rodents have been processed. Allow a minimum NaOCl contact time of 15 minutes for the last animal that is processed.

3. Build a fire large enough for the incineration of all animals and disposable materials used during wild bat or rodent processing using collected wood.

4. After fire is well established, place individual animal bags in centre of pyre until all animal bags and materials have been thoroughly incinerated. Do not leave fire unattended!

5. After incineration is completed, inspect pyre to ensure all materials and animals have been incinerated.

6. Use of additional combustible materials or even liquid fuel should be considered if evidence of uneven incineration of materials or animals is found.

7. General fire precautions and safety should be followed; experience with fire craft IS expected from field-team members.

8. A dry chemical powder fire extinguisher should be available on site as a precaution.

Wild bat or rodent carcass decontamination in laboratory setting.

1. After due samples and organ harvest has taken place, place a dry paper towel inside abdominal cavity of animal and wrap in another paper towel. DO NOT SPRAY CARCASSES WITH 0.5% NaOCl !

   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.

2. Place individual animal bags neatly organized in either wire mesh autoclave basket or autoclave metal container.

Wire mesh autoclave basket and autoclave metal container.

3. 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.

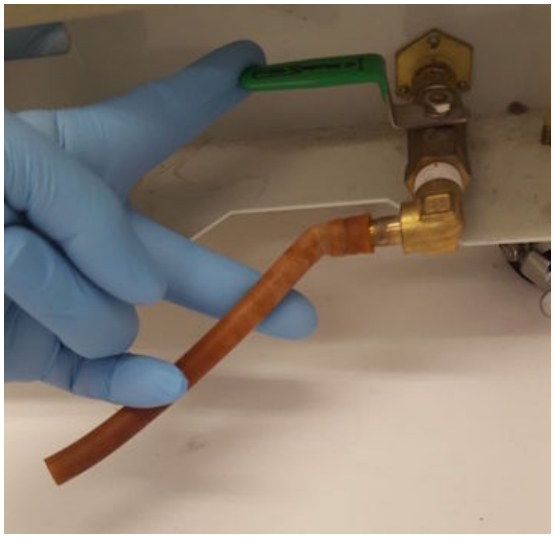
4. Before opening autoclave sterilizer verify chamber pressure is 0 as indicated in the pressure manometer located on the front panel of the autoclave. For extra assurance, purge chamber pressure by pressing on exhaust relief valve located on left-hand side panel.

Pressure manometer.

Exhaust purge valve.

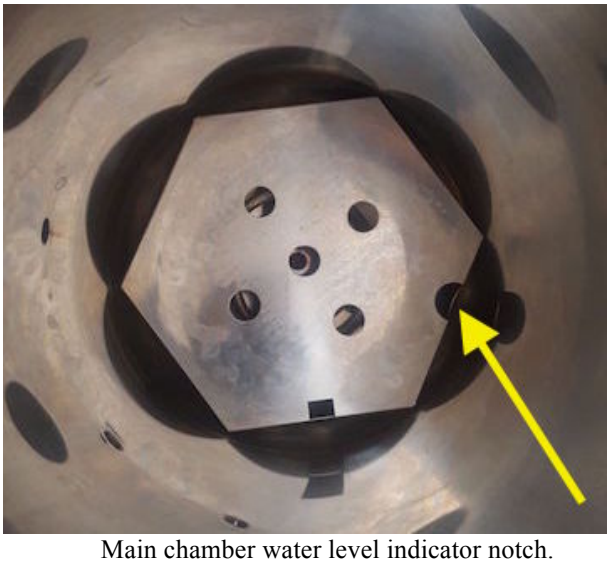
5. Open the autoclave's front panel door and close main chamber water valve. Verify that the steam-water bottle is filled with distilled water to the indicated mark. If above this level, retrieve bottle from panel and discard water directly to the drain. Replace bottle and make sure the bottles rubber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


Front panel door.


Main chamber water valve (closed position).


Steam-water bottle

6. 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.

7. Load chamber with either wire mesh basket and carcass bags or metal container. As depicted in the picture above right.

8. 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.

9. 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). See detailed and most current bat or rodent carcass decontamination.

10. After autoclaving carcass 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 and allow a minimum decontamination contact time of 15 minutes before retrieving from BSL2+ laboratory.

11. 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.

12. 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.

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 Changes to document format only.