



Decontamination of Facilities that Have Held Rinderpest Virus-Containing Materials

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Contacts: **FAO Secretariat**

Email: Rinderpest-Secretariat@fao.org

OIE Secretariat

Email: Rinderpest@oie.int

Changes to previous version

Section

Annex B	Changed square and cubic feet to square and cubic meters.
All	Formatting document



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1. Purpose

The purpose of this document is to define how facilities must be decontaminated after the destruction or removal of all rinderpest virus (RPV)-containing material (RPV or material likely or suspected to contain RPV), as defined in Chapter 8.13 of the OIE Terrestrial Animal Health Code¹, in the facility. The SOP does not cover decontamination of water used for handwashing, clothes-washing or showering after handling RPV-containing material.

2. Background

RPV is a negative-sense RNA genome virus of the morbillivirus genus. It is the causative agent of rinderpest, a fatal disease of cattle capable of devastating epidemic spread. The incubation period ranges from 8 – 11 days and the disease is characterized by pyrexia, nasal and ocular discharges and necrosis and erosion of the nasal and oral mucosae. Animals develop diarrhoea, and death generally occurs between 7 and 12 days after onset of signs. RPV has poor environmental stability and is sensitive to inactivation by heat, desiccation and exposure to sunlight. The last known case of rinderpest was diagnosed in Kenya in 2001, since which time the world has been free of the disease. Interruption of the chain of transmission and spread of infection was achieved by a global eradication campaign organized by the Food and Agriculture Organization of the United Nations (FAO). FAO and the World Organization for Animal Health (OIE) have formally accredited global freedom from this disease. The vaccine used against rinderpest is an attenuated strain of RPV, and the possibility of reversion to virulence means that despite its widespread use, the vaccine strain must be handled under the same constraints as virulent strains in the post-eradication era.

The cost and effort of eradication, and the global emergency and severe consequences that are likely to accompany a re-introduction or release dictate that the containment procedures for handling RPV-containing material must be enhanced in the post-eradication era, and that the number of facilities holding RPV should be reduced. RPV must now be handled at Biosafety Level 3 (BSL 3) Laboratory

Vaccine stocks must be maintained until all RPV material has been destroyed or gathered into internationally regulated repositories. However, the possibility of cross-contamination of vaccine stocks or seed-stocks with virulent virus dictate that vaccine and non-vaccine strains should be stored and handled separately.

¹ “RPV-containing material” means field and laboratory strains of RPV; vaccine strains of RPV including valid and expired vaccine stocks; tissues, sera and other clinical material from infected or suspect animals; and diagnostic material containing or encoding live virus. Recombinant morbilliviruses (segmented or non-segmented) containing unique rinderpest virus nucleic acid or amino acid sequences are considered to be rinderpest virus. Full length genomic material including virus RNA and cDNA copies of virus RNA is considered to be RPV-containing material. Sub-genomic fragments of morbillivirus nucleic acid that are not capable of being incorporated in a replicating morbillivirus or morbillivirus-like virus are not considered as RPV-containing material



RPV is non-infectious for humans and poses no direct hazard to human health. Containment and handling regulations are to prevent the accidental transport and introduction of the virus to susceptible animals.

3. Training

Training is the responsibility of the Director of the institute/organization where the RPV-containing materials are held and in accordance with the type of holding facility (i.e. training of vaccine production staff will be different from those doing research on the virus). The training proficiency should be in accordance with FAO/OIE approved guidelines. Persons directly involved in the destruction of RPV must be appropriately trained in the handling of dangerous infectious agents and according to the FAO/OIE approved guidelines.

4. Preparation of the Material

RPV-containing material should be destroyed or shipped in accordance with the SOPs for destruction and shipment. After destruction and/or shipment, when there is no more RPV-containing material stored or retained in the facility, the facility should be decontaminated for RPV. Refrigerators and freezers that have been used solely for storing RPV-containing material will be empty after removal of all RPV-containing material, and should remain empty until decontamination is complete.

Wherever possible refrigerators and freezers used for RPV-containing material should not be simultaneously used to store non-RPV samples. Where refrigerators and freezers used to store RPV-containing material are simultaneously used to store other, non-RPV material an inventory of the co-located non-RPV material should be made. Non-RPV material that is not unique and/or is replaceable or of low value should be destroyed by autoclaving. Examples of such material are commercial reagents or diagnostic kits, archived experimental samples, cell lines or virus isolates that can be replaced from other sources. Examples of unique or high value material are non-RPV virus (e.g. foot-and-mouth disease, peste-des-petit ruminants) or bacterial isolates that are not held in other locations in the same or another institution. If the laboratory head or other institutional authority deems that such materials should not be destroyed, the vessels (e.g. vial, ampoule or tube) in which they are held must be surface decontaminated as described below, and then removed to storage that has not been used for RPV or which has been decontaminated since last being used to store RPV.

The interior of refrigerators and freezers must be surface decontaminated with a disinfectant. The outer surfaces of vessels holding non-RPV material, but which have been co-located with RPV-

containing material must also be surface decontaminated with a disinfectant. Suitable disinfectants for surface decontamination are²:

- 5% Virkon™ solution. Virkon™ solution must be made fresh on the day of use.
- 10% Chlorox solution. Chlorox has a manufacturer's shelf life and should only be used if it is "within date" as specified on the container. 10% Chlorox must be made fresh on the day of use. Note: Chlorox can corrode some types of metal surface.
- 2% Sodium Hydroxide.
- Some disinfectants may be chemically incompatible with each other. Wherever possible, one disinfectant should be chosen and used for all the purposes described.

5. Witnessing and Confirmation of Decontamination

Decontamination is important in ensuring that a former RPV holding facility is clear of the virus, and the laboratory head and the Chief Executive/overall head of the site or organization must be satisfied that decontamination has been satisfactorily carried out. To this end, the laboratory head, biosafety officer and the Chief Executive/overall head of the site or organization (or his/her nominated representative) must directly supervise the decontamination process.

The laboratory head, and the Chief Executive/overall head of the site or organization (or his/her nominated representative) must confirm to the veterinary authorities and the FAO³ and OIE⁴ that decontamination has been completed. This confirmation should include:

- The address of the facility
- The building(s) and room(s) where RPV-containing material was stored/used and how they have been decontaminated
- The number of refrigerators and freezers used solely to store RPV-containing material and how they have been decontaminated
- The number of refrigerators and freezers where non-RPV material was co-located with RPV-containing material and how they have been decontaminated

² Manual on procedures for disease eradication by stamping out (FAO).
(<http://www.fao.org/docrep/004/y0660e/y0660e03.htm>)

³ The Chief Veterinary Officer,
FAO HEADQUARTERS
Viale delle Terme di Caracalla
00153 Rome, Italy
Telephone: (+39) 06 57051
Fax: (+39) 06 570 53152
Email: Rinderpest-Secretariat @fao.org

⁴ The Director General
OIE World Organisation for Animal Health 12, rue de Prony 75017 Paris, France
Email: oie@oie.int

The fate of non-RPV materials that have previously been co-located with RPV-containing material

- The number, identity and a general description of retained non-RPV materials that have been co-located with RPV-containing material and how they have been decontaminated
- Completion date of decontamination
- Signatures of the laboratory head, the biosafety officer, and the Chief Executive/overall head of the site or organization

6. Decontamination

6.1 non-RPV materials that have been co-located with RPV-containing material in a fridge

Where a racking or storage system has been used in which non-RPV materials have been stored in closed or lidded boxes and RPV-containing material has never been co-located in the same box, the box must be surface decontaminated by wiping with a cloth impregnated with disinfectant. The box may then be transferred to a fridge that has never held RPV-containing material or which has been decontaminated since removal of RPV-containing material.

Where non-RPV materials have not been stored in a closed or lidded box which has not held RPV material; or where RPV has been stored in the same box, the individual non-RPV vessels must be surface decontaminated by wiping with a cloth impregnated with disinfectant. The vessels may then be transferred to a fridge that has never held RPV-containing material or which has been decontaminated since removal of RPV-containing material.

6.2 Non-RPV materials that have been co-located with RPV-containing material in an electric freezer

Where a racking or storage system has been used in which non-RPV materials have been stored in closed or lidded boxes and RPV-containing material has never been co-located in the same box, the box should be removed from the freezer and placed on a cloth impregnated with disinfectant. If there is no accumulation of ice on the box it must be surface decontaminated by wiping with a cloth impregnated with disinfectant. If there is an accumulation of ice on the box, it should be opened and the contents transferred to a replacement clean box, which may then be transferred to a freezer that has never held RPV-containing material or which has been decontaminated since removal of RPV containing material

Where non-RPV materials have not been stored in a closed or lidded box which has not held RPV-containing material, or where RPV-containing material has been stored in the same box; the box or individual vessels should be removed from the freezer and placed on a cloth impregnated with disinfectant. The individual non-RPV vessels, whether stored in a box or not, must be surface decontaminated by wiping with a cloth impregnated with disinfectant. The

vessels may then be transferred to a freezer that has never held RPV-containing material or which has been decontaminated since removal of RPV-containing material.

6.3 Non-RPV materials that have been co-located with RPV-containing material in a liquid nitrogen freezer or dewar/tank

Viruses have previously been shown to transfer between vessels co-located in the liquid phase of a liquid nitrogen freezer. Consequently, non-RPV materials co-located with RPV materials in the liquid phase of a liquid nitrogen freezer or dewar should be destroyed by autoclaving.

6.4 Refrigerators that have been used to store RPV-containing material

Refrigerators that have been used to store RPV-containing material should be emptied of all non-RPV material as described above. When empty, the refrigerator should be turned off and allowed to equilibrate to room temperature. The shelves should be removed and must be surface decontaminated by wiping with a cloth impregnated with disinfectant. The inside surfaces of the refrigerator must be decontaminated by wiping with a cloth impregnated with disinfectant. The door handles and the outside surfaces of the refrigerator, excluding the rear face where the compressor and radiator are found, should be wiped with a damp cloth impregnated with a commercial soap or cleaning fluid.

6.5 Electric Freezers that have been used to store RPV-containing material

Electric freezers that have been used to store RPV-containing material should be emptied of all non-RPV material as described above. When empty, the freezer should be turned off and allowed to equilibrate to room temperature. For upright freezers care should be taken to prevent possible contamination of the room as the freezer defrosts. This may be achieved by applying liberal quantities of a powder-based disinfectant such as Virkon to the inside of the floor of the freezer, and to the floor of the room around the door area of the freezer. If Virkon is used, it should in this instance be applied as a powder. Ice that is removed from the freezer should be placed in a bucket containing enough Virkon powder to make a 5% solution when the bucket is filled. Any melt water should be transferred to the bucket either from a collecting tray, a cloth or a mop as appropriate. Although the bucket will have sufficient Virkon powder for the volume it will eventually hold, there must still be Virkon powder in the collecting tray and anywhere melt water is likely to flow to prevent any possible contamination beyond the freezer. Shelves must be removed from the freezer after equilibrating to room temperature and decontaminated by wiping with a cloth impregnated with disinfectant. When the freezer has equilibrated to room temperature the inside surfaces must be decontaminated by wiping with a cloth impregnated with disinfectant. The door handles and the outside surfaces of the freezer, excluding the rear face where the compressor and radiator are found, should be wiped with a damp cloth impregnated with a commercial soap or cleaning fluid.

For chest freezers, the empty freezer should be turned off and allowed to equilibrate to room temperature. The volume of any melt water should be estimated, and an appropriate amount of concentrated disinfectant or disinfectant powder added to give a concentration as described in section 4. After 30 minutes the melt water may then be removed and disposed of.

Baskets/shelves must be removed and decontaminated by wiping with a cloth impregnated with disinfectant. The inside surfaces must be decontaminated by wiping with a cloth impregnated with disinfectant. The door handles and the outside surfaces of the freezer should be wiped with a damp cloth impregnated with a commercial soap or cleaning fluid.

6.6 Liquid nitrogen freezers/dewars that have been used to store RPV-containing material

The empty freezer or dewar should be disconnected from the automatic fill system if there is one. If there is no automatic fill system, the person responsible for the freezer or dewar should ensure that it is not refilled. The freezer/dewar should be left until all the liquid nitrogen has evaporated and the freezer has equilibrated to room temperature. If a racking system is employed the empty racks must be removed and decontaminated by wiping with a cloth impregnated with disinfectant. The inside surfaces must be decontaminated by wiping with a cloth impregnated with disinfectant if that is possible; for very large dewars this might be done with a mop. For smaller, narrow-necked dewars where it is not possible to wipe inside with a cloth, liquid disinfectant should be poured into the dewar and the dewar then physically agitated and swirled before upending the dewar to decant the disinfectant. Excess disinfectant may subsequently be removed by rinsing with water. The outside surfaces of the freezer or dewar should be wiped with a damp cloth impregnated with a commercial soap or cleaning fluid. The freezer/dewar should be completely dry inside before refilling with liquid nitrogen.

6.7 Microbiological safety cabinets (MSCs) that have been used to handle RPV-containing material

Where RPV-containing material has previously been handled in an MSC the MSC should be decontaminated by Formaldehyde fumigation or Hydrogen Peroxide fumigation. If the user is familiar with the chosen fumigation procedure then the appropriate procedure should be followed, and validated by inactivation of spore strips. If the user is not familiar with the chosen fumigation procedure, they should seek advice from the FAO and/or OIE before proceeding. Fumigation is a hazardous procedure, which requires appropriate safety protocols to prevent exposure of staff to the dangerous fumes. Effectiveness of fumigation is affected by the design of the microbiological safety cabinet, the venting system, and the procedure must be appropriate for the particular microbiological safety cabinet in use. An example of fumigation procedures is given in annex A.

Fumigation procedures involve hazardous chemicals that can be easily inhaled. Application and removal of the fumigation kit should be performed by persons qualified in the safe handling of such laboratory chemicals and equipment, and equipped with appropriate protective devices and



with appropriate measures to prevent accidental exposure to fumes of themselves and other personnel working in the vicinity.

6.8 Rooms and laboratories where RPV-containing material has been stored or used

The most effective way to decontaminate a room or laboratory is to fumigate the room with Formaldehyde or Hydrogen Peroxide. If the user is familiar with the chosen fumigation procedure then the appropriate procedure should be followed, and validated by inactivation of spore strips. If the user is not familiar with the chosen fumigation procedure, they should seek advice from the FAO and/or OIE before proceeding. It should be noted that the quantities of Formaldehyde or Hydrogen Peroxide vapour produced during room fumigation are extremely hazardous, and unless the room was designed to be sealable for fumigation, then sealing it appropriately will be difficult or impossible. It is not recommended to fumigate rooms unless they are already routinely fumigated and the facility manager is well versed in the process. Examples of fumigation with Formaldehyde and Hydrogen Peroxide procedures are given in annexes B and C, respectively.

To decontaminate a room where RPV-containing material has historically been used or stored but where RPV-containing material has not been used since the accreditation of global freedom, it is sufficient that the room should be thoroughly cleaned. Floors, work-surfaces, chairs etc. should be cleaned with soap and water or a suitable commercial cleaning fluid. The outer surfaces of equipment should be cleaned with a damp cloth impregnated with soap or a commercial cleaning fluid if this is compatible with the equipment. Equipment may alternatively be wiped down with a disinfectant or 70% ethanol; or where laboratories possess decontamination chambers, equipment may be placed in such a chamber and decontaminated with Formaldehyde gas or Hydrogen Peroxide vapour as appropriate.

7. Annexes for Fumigation Procedures

Annex A

Decontamination of Class II Biosafety Cabinets⁵

1. Purpose

- 1.1. This procedure is to provide uniform guidance for implementing the decontamination of Class II Biological Safety Cabinets and their HEPA filtration systems. The procedure contains specific information, requirements and procedures for Safety Technicians to use to accomplish decontamination.

2. Prerequisites

- 2.1. In conducting decontamination procedures the goal is to provide the safest working conditions possible and wear appropriate personnel protection. Only properly trained personnel should perform the procedures.
- 2.2. Materials needed for decontamination:
 - 2.2.1. Two (2) frying pans
 - 2.2.2. Two (2) extension cords
 - 2.2.3. Paraformaldehyde – 20 grams per cubic meter x total cubic meter of the unit. (The cubic meter is determined by length x width x height in meter)
 - 2.2.4. Ammonium Carbonate – 1.2 grams x total amount of Paraformaldehyde used
 - 2.2.5. Biological indicator spore strips – two (2) test + one (1) control for each two biological indicator strips used
 - 2.2.6. Petri dishes – one needed for each biological indicator strip gassed
 - 2.2.7. Hygrometer/Thermometer
 - 2.2.8. Two (2) “Danger Formaldehyde in the Area” warning signs
 - 2.2.9. Personal Protective Equipment – Full Face Negative Pressure Respirator with new Formaldehyde cartridges and Neoprene gloves
 - 2.2.10. Plastic film and duct tape
 - 2.2.11. Plastic bag or containers
 - 2.2.12. Appropriate Formaldehyde dosimetry
 - 2.2.13. Appropriate air monitoring equipment
 - 2.2.14. Formaldehyde and Ammonia detector tubes
- 2.3. Personal Protective Equipment (PPE)
 - 2.3.1. Safety Shoes
 - 2.3.2. Chemical resistant (neoprene) gloves
 - 2.3.3. Full face Negative Pressure Respirator with new Formaldehyde cartridges

3. Definitions

- 3.1. BSSMs – Bio-Systems Safety Mechanics
- 3.2. Class II Biological Safety Cabinet – enclosed, ventilated workspace for safely working with materials contaminated (or potentially contaminated) with pathogens

⁵ Source, Plum Island Animal Disease Center, NY, USA

- 3.3. HEPA Filter – High efficiency particulate air filter, having a nominal efficiency of 99.97% for removal of 0.3µm (microns) sized particles from air
- 3.4. Paraformaldehyde – the chemical used in the fumigation process, when heated produces Formaldehyde gas
- 3.5. Ammonium Carbonate – the chemical used to neutralize Formaldehyde gas, when heated produces ammonia gas
- 3.6. Biological Indicator – spore strips used to determine a successful decontamination. (i.e. Bacillus subtilis spores, AMSCO – 764271-00)
4. **Responsibilities**
 - 4.1. Only properly trained Bio-Safety Systems Mechanics should conduct these procedures. These procedures are to be followed by all Safety Technicians. Proposed changes in these procedures need approval by a qualified safety officer or manager.
5. **Process**
 - 5.1. Shutdown Procedures
 - 5.1.1. Notify appropriate personnel, person in charge of or responsible for affected area of the need to shutdown, decontaminate, and replace filters of the Class II Biological Safety Cabinet.
 - 5.1.2. Prepare a work request or procedure to schedule the procedure.
 - 5.1.3. Coordinate an appropriate time for the procedure with appropriate parties scientists, security, and operation and maintenance personnel.
 - 5.2. Decontamination Procedure
 - 5.2.1. Place warning sign at all entrances to the area (secure additional signs if needed).

FORMALDEHYDE HAS BEEN DETERMINED TO BE A SUSPECT CARCINOGEN. THEREFORE, NONESSENTIAL PERSONNEL MUST VACATE NEARBY AREAS AND ALL BSSM's PERFORMING THE GASSING ARE REQUIRED TO WEAR A FULL FACE RESPIRATOR WITH THE APPROPRIATE CARTRIDGES!

- 5.2.2. Complete the Paraformaldehyde Gas Set-up Checklist.
- 5.2.3. Log Formaldehyde amount into Paraformaldehyde Usage Report.
- 5.2.4. Ensure that the Bio-Safety cabinet is isolated using dampers and/or 6 mil plastic film secured with duct tape.
- 5.2.5. Install the frying pans in the biosafety cabinet.
- 5.2.6. Determine the temperature and relative humidity of the cabinet. A temperature of between 70 and 80 degrees Fahrenheit and a relative humidity of between 70% and 80% is desired. If the relative humidity must be raised to 70%, this can be accomplished by boiling water in the frying pan.
- 5.2.7. Establish monitoring devices (biological indicators and Formaldehyde indicators). Leave biological indicator(s) in its envelope(s) and place in separate sterile petri dish(es). The envelope allows the gas to penetrate the spore strip and keeps out other contaminants.
- 5.2.8. Place the petri dish inside the cabinet. The control strip is maintained outside of the cabinet. Place the Formaldehyde indicator outside the cabinet.

- 5.2.9. Don respiratory protection.
- 5.2.10. When temperature and humidity are within acceptable limits, initiate gassing by setting the temperature on the Paraformaldehyde frying pan to 450 degrees Fahrenheit. Ensure that the frying pan can be energized. While off, add the measured amount of Paraformaldehyde to the pan. Place the measured Ammonium Carbonate in the other frying pan. Seal the entrance to the cabinet and post signage 5.2.11. Plug in the extension cord to heat the pan containing the Paraformaldehyde. Periodically check the pan and when 25% has depolymerized to Formaldehyde gas, turn on the blower for 15 seconds. Repeat this again at 50%, 75%, and 100%.
- 5.2.12. Unplug the frying pan after Paraformaldehyde has converted to Formaldehyde gas.
- 5.2.13. Allow a minimum of 16 hours Formaldehyde contact time. Typically BSSMs allow for a 48 contact time. Periodically turn on and off the blower.
- 5.2.14. The biosafety specialist initiates neutralization by setting the temperature on the Ammonium Carbonate frying pan to 450 degrees Fahrenheit and ensuring that the frying pan can be energized.
- 5.2.15. Plug in the cord to heat the pan. Check the pan and when 25% has converted to gas, turn the blower on for 15 seconds. Repeat this again at 50%, 75%, and 100%.
- 5.2.16. Continually check room area for extraneous Formaldehyde gas by using the Formaldehyde detector tubes.
- 5.2.17. The trained personnel conducting this procedure, if exposed to Formaldehyde gas, will shower and change work clothing.
- 5.2.18. Turn off the frying pan after Ammonium Carbonate has converted to gas.
- 5.2.19. Allow a minimum of 100 minutes contact time for neutralization.
- 5.2.20. Inspect the Formaldehyde indicator (Q.C.) for evidence of exposure.
- 5.2.21. After completion of contact, don PPE and open the plastic enough in order to cover and retrieve the Petri dishes. The dishes are then secured in a plastic bag.
- 5.2.22. If biological indicators indicate that decontamination was not complete, have reviewed by a biosafety specialist to determine if procedure problems. Repeat if required.
- 5.2.23. If biological indicators indicate successful decontamination, remove signage, collect frying pans, tools, equipment, and materials. Return tools and equipment to their proper location and dispose of all consumables.
- 5.2.24. Release the cabinet from control after Formaldehyde detector tube testing does not indicate any Formaldehyde residues.



PARAFORMALDEHYDE GAS SETUP CHECK LIST

Area to be Decontaminated: _____

Size of Area

Length _____ x Width _____ x Height _____ = _____ m³.

Amount of Chemicals

Content (m³) _____ x 20 = _____ Grams of Paraformaldehyde →

Grams of Paraformaldehyde _____ x 1.2 = _____ Grams of Ammonium
Carbonate

Items Required

Warning Signs	
Frying Pans	
Extension Cords	
Paraformaldehyde Amount	
Ammonium Carbonate Amount	
Thermometer	
Biological Indicators	
Media Broth	
Respirators	
Personnel Protection Apparatus	
Plastic Bags	
Notifications	
6 Mill Plastic Sheeting	
5 – 10 cm Duct Tape	
Timer	



Paraformaldehyde Usage Report

Building: _____ **Room/Filter Unit #:** _____ **Date:** _____

Equipment: _____ **ID#** _____

Para formaldehyde Amount _____ **Ammonium Carbonate Amount** _____

Items being Treated: _____

Drager Tube Type _____ **Batch/Lot #** _____ **Exp. Date** _____

Activation Tube _____ **Batch/Lot #** _____ **Exp. Date** _____

Number of Strokes _____ **Leak Test Pre** _____ **Leak Test Post** _____

Remarks



AIR MONITORING RESULTS

Date: _____ **Time:** _____ **PPM:** _____ **Action Taken** _____ **By:** _____

Date: _____ **Time:** _____ **PPM:** _____ **Action Taken** _____ **By:** _____

Date: _____ **Time:** _____ **PPM:** _____ **Action Taken** _____ **By:** _____

Date: _____ **Time:** _____ **PPM:** _____ **Action Taken** _____ **By:** _____

Unexpected Adverse Effects: _____

BIT # _____ **Biological Indicator Results:** _____ **Approved By:** _____

Comments: _____

Decontamination Performed By: _____

Person Conducting Air Sampling _____

Area Released For Use Yes / No _____ **Released By:** _____

Annex B

Biological Decontamination of Rooms by Paraformaldehyde Fumigation⁶

Introduction

A contaminated laboratory room that has been identified for space decontamination is reviewed and whenever possible made compatible for the particular process selected. For example, for a Formaldehyde gas application, porous materials such as paper and other materials would be containerized and removed from the space and decontaminated by an alternative method. Once the room is adequately sealed (supply/exhaust ducting, known penetrations etc) such that the space can a) maintain an effective concentration of decontaminating agent and b) not allow leakage of the agent beyond specified safety limits. Of course decontaminating agents should always be used in accordance with appropriate local, state and federal laws.

1. Purpose

- 1.1. This procedure is to be utilized for decontamination of spaces such as rooms by Formaldehyde fumigation. The Safety Staff conducting this procedure will provide the safest working conditions possible for all employees. All regulatory requirements will be followed in performance of these procedures. The Safety Staff will comply with the Paraformaldehyde exemption, currently held by the facility. This procedure is to provide uniform guidance for implementing the decontamination of airlocks* and other spaces such as rooms by Paraformaldehyde fumigation. It contains specific information, procedures and requirements for the properly trained person(s) to use to accomplish decontamination and describes the procedures to be observed in decontamination.

2. Prerequisites

- 2.1. For any non-conformances, develop a non-conformance report in compliance with the procedures used in the laboratory to manage non-conformance reporting and follow-up.
- 2.2. Personal Protective Equipment (PPE)
 - 2.2.1. Protective eyewear
- 2.3. Printed Forms and Checklists.
- 2.4. Review the material safety data sheets (MSDS) for Paraformaldehyde and Ammonium Carbonate.
- 2.5. Take the necessary measurements in preparation for organizing the necessary materials.
 - 2.5.1. Measure and record the cube meter of the unit on the "Work to be Conducted" on the Paraformaldehyde Decontamination Checklist (PDC) attached below.

⁶ Source is Plum Island Disease Center,
NY USA

* Airlocks are traditionally used in BSL3 & BSL4 laboratories. If your laboratory does not have airlocks, please refer to this for the remainder of the document: All entrances and exits into the room, including doors and windows, must be sealed appropriately with duct tape and temporary sealant, which needs to be airtight, preventing any air escape.

2.5.2. Measure and record the square meters of the floor on the Work to be Conducted
PDC Checklist below.

2.6. Materials required

- 2.6.1. Danger Formaldehyde warning signs.
- 2.6.2. Frying pans (4).
- 2.6.3. Extension cords (at least ½ of the number of frying pans to be used).
- 2.6.4. Measuring device to measure Paraformaldehyde and Ammonium Carbonate in grams.
- 2.6.5. Paraformaldehyde.
- 2.6.6. Ammonium Carbonate.
- 2.6.7. Plastic or glass containers (for Paraformaldehyde).
- 2.6.8. Duct tape (to secure plastic bags).
- 2.6.9. Plastic bags (for Ammonium Carbonate).
- 2.6.10. Tin foil.
- 2.6.11. Biological indicator spore strips.
- 2.6.12. Petri dishes or vials.
- 2.6.13. Hydrometer/Thermometer-to test temperature and R/H.
- 2.6.14. Full Face Negative Pressure Respirator with a Formaldehyde cartridge for setup only.
- 2.6.15. SCBA for spore strip retrieval.
- 2.6.16. Neoprene rubber gloves.
- 2.6.17. Tyvek type clothes
- 2.6.18. Appropriate air monitoring equipment, Drager Pump with Formaldehyde and Ammonia detector tubes.
- 2.6.19. Water (copious amounts to fill drains and traps).
- 2.6.20. Trypticase Soy Broth/Media Broth.
- 2.6.21. Obtain the People to Notify list for Airlock Shutdown from the front desk.
- 2.6.22. Airlock Decontamination form (if applicable).
- 2.6.23. The use of an incubator in the BSL-2.

2.7. Time Allotted

Number of Hours	For
72	Contact Time (After all product is burned off)
168	Incubation
16	Fumigation
232	TOTAL

2.8. Training

- 2.8.1. How to read Biological Indicator Strips
- 2.8.2. How to read a Hydrometer/Thermometer
- 2.8.3. How to properly utilize a SCBA
- 2.8.4. How to properly utilize a Respirator
- 2.8.5. How to properly utilize a Drager Pump

3. Definitions

- 3.1. Cubic meter is a unit is determined by the length x width x height in meter.
- 3.2. Hydrometer / thermometer - to measure humidity and temperature.

- 3.3. Trypticase Soy Broth / media broth - noted in Materials Required checklist for Biological Indicator Strips (at least 5; 3 for gassing, 1 for positive control, and 1 for negative control).

4. Responsibilities

- 4.1. Safety Manager-to be notified if decontamination is not completed.
- 4.2. Biological Safety Officer-to be notified if decontamination is not completed.
- 4.3. Safety Manager-to determine the amount of contact time

5. Process

5.1. Shutdown Procedures

- 5.1.1. Notify appropriate personnel of the need to shut down and decontaminate the airlock.
Provide the intended date and time that the process will begin.
- 5.1.2. Prepare and submit work request.
- 5.1.3. Coordinate an appropriate time for the procedure with appropriate parties.
- 5.1.4. Ensure that the Air Handling Systems are operational.

5.2. Prepare materials needed for decontamination

- 5.2.1. Calculate and record the amount of Paraformaldehyde needed. Will need 20 grams per cubic meter of area.
- 5.2.2. Place the Paraformaldehyde in plastic or glass container and cover with tin foil.
- 5.2.3. Calculate the amount of Ammonium Carbonate needed. Will need 1.2 grams per (x) amount of Paraformaldehyde used.
- 5.2.4. Place the Ammonium Carbonate into two plastic bags with an equal amount in each, securing the bags with duct tape.
- 5.2.5. Calculate and record the number of biological indicator spore strips needed. Will need a minimum of one per 9 square meters of floor space, plus one control in the same lot number. Additional biological indicator strips are required in accessible areas.
- 5.2.6. Calculate the number of Petri dishes or vials needed. One needed for each biological indicator strip being gassed.
- 5.2.7. Place frying pans on secure footing in the room, utilize extension cords when necessary.
- 5.2.8. Test the frying pans.

5.3. Decontamination Procedure

- 5.3.1. Display “Danger Formaldehyde” warning signs on the inside airlock door facing the corridor and on the outside of the door, on the hanger that swings over the corridor, and coming down the stairway from the second floor.
- 5.3.2. Notify Security to release the security system on the airlock door, informing them how long the door will remain open.
- 5.3.3. Notify all personnel on the list of time the fumigation will commence.
- 5.3.4. Fill all floor drains and traps with copious amounts of water.
- 5.3.5. Determine the temperature and relative humidity of the airlock. A temperature of between 21 and 27 degrees Celsius and a relative humidity of 70% is desired. If the relative humidity must be raised to 70%, this is accomplished by boiling water in the frying pan first. Continue to recheck the humidity level until it reaches the optimum level.
- 5.3.6. Ensure that all lids, compartments, and doors are open. Lids on chemical containers should be tightly closed.
- 5.3.7. Vessels should be in the horizontal position.

- 5.3.8. Remove any items which should not be gassed, i.e., wood, paper, or cloth. Remember:
No porous materials are to be treated.
- 5.3.9. Check the contents of the airlock for tags or labels.
- 5.3.10. Ensure that all oils and fluids have been drained.
- 5.3.11. Record all information required by the Airlock Decontamination Form (if applicable).
- 5.3.12. Place biological indicators in separate Petri dish(s) or vial(s). Place the Petri dish or vial at varying heights.
- 5.3.13. Don a full face respirator and neoprene rubber gloves.
- 5.3.14. Trypticase Soy Broth / media broth stays in BSL-2 for inoculating spore strips.
- 5.3.15. Ensure that the control switches are in the off position.
- 5.3.16. When temperature and humidity are within acceptable limits, initiate the steps for gassing by setting the temperature on each frying pan at 232 degrees Celsius. Do NOT turn on the control switch to the frying pans at this time.
- 5.3.17. Place tin foil in each pan that will hold the Ammonium Carbonate. Place the plastic bags containing the Ammonium Carbonate on the tin foil in each frying pan.
- 5.3.18. Place the pre-measured amount of Paraformaldehyde into the frying pans.
- 5.3.19. Close the Airlock door and inflate the air gasket.
- 5.3.20. Again ensure that all warning signs have been posted.
- 5.3.21. Turn on the switch that controls the frying pans that contain the Paraformaldehyde.
- 5.3.22. Turn off the frying pans when all the Paraformaldehyde has converted to Formaldehyde gas.
- 5.3.23. Allow a minimum of 72 hours Formaldehyde contact time, some application may take longer, each case will be determined by Safety Office.
- 5.3.24. Upon completion of the allowable contact time, Ammonium Carbonate frying pans should be turned on for 24 hours after complete burn of product.
- 5.3.25. Properly trained personnel will don PPE including Self Contained Breathing Apparatus and stand by the outside airlock door.
- 5.3.26. When the persons are ready to enter the airlock they will notify the person inside to deflate the outside air gasket and notify security to release the security and restriction to the door, informing them how long the door will be open.
- 5.3.27. The person entering the airlock or room wearing an SCBA will locate and remove the biological indicator. After removing the biological indicator from the airlock, the person will close the outside door and notify the inside person to inflate the air gasket.
- 5.3.28. The person that entered the airlock or room and his backup will remove their PPE and SCBA and properly store them.
- 5.3.29. One trained person from will go to the BSL-2 lab location and start processing the biological indicators.
- 5.3.30. The biological indicators will be incubated for 7 days at 51 degrees Celsius.
- 5.3.31. If the biological indicator indicates that decontamination was not complete, notify the Safety Manager and the Biological Safety Officer immediately. Stop the test.
- 5.3.32. If the biological indicators indicate successful decontamination, aerate the airlock.
- 5.3.33. Test the air in and around the airlock to ensure that the entire product has been aerated using the Drager pump with Formaldehyde and Ammonia tubes to ensure the area is safe for employees to enter. Area should be in compliance with OSHA limit- consult website for current limits (www.osha.gov).
- 5.3.34. If area is clear, remove signage, collect frying pans, and remove items that were decontaminated.

Paraformaldehyde Decontamination Checklist (PDC)

MATERIALS REQUIRED	DONE
Warning Signs- Place appropriately in all clearly visible areas	
Frying Pans- (4) 2 for the Paraformaldehyde and 2 for the Ammonium Carbonate. Test the frying pans before using.	
Extension Cords- 2 each for two frying pan	
Paraformaldehyde- $[\text{Treated Volume (m}^3) \times \text{Application Rate (20 g/m}^3)] / [\% \text{ Product Purity} \times 100] = \text{Paraformaldehyde (g)}$	
Ammonium Carbonate- $1.2 \times \text{Paraformaldehyde (g)} = \text{Ammonium Carbonate (g)}$	
Thermometer- To measure temperature and relative humidity	
Biological Indicators- 1- each per 9 square meter (4) 3 for the gassing and 1- for the positive control	
Media Broth- 5- each, 3-for the gassing strip, 1-for the positive control strip and 1 for negative control	
Respirators- with Formaldehyde filters	
Self-Contained Breathing Apparatus- 2 outside	
Personal Protection Equipment- Gloves, Tyvek type clothes	
Tin Foil- The tin foil is to be placed in the frying pans that will hold the bags of Ammonium Carbonate	
Plastic Bags- Two plastic bags are needed to hold equal amounts of Ammonium Carbonate	
Plastic or Glass Containers with tin foil cover- The containers will hold equal amounts of Paraformaldehyde	

Procedure
PDC (cont)

WORK TO BE CONDUCTED	DONE
<p>Notification-: 1- Obtain appropriate identification and contact information from operations manager number or other responsible officials</p>	
<p>Ensure that Air Handling Systems are operational</p> <p>Post Warning Signs at all entrances and exits or other applicable openings including: Outside Airlock or room door outside the building Airlock or room Door, inside the building Doorway from any other floor to the area -Ensure all warning signs are clearly visible.</p>	-
<p>Notify Security that you need the door security system released. Inform them of how long the door will be open.</p>	
<p>Fill floor drains and traps with copious amount of water</p>	
<p>Measure proper amount of Paraformaldehyde and Ammonium Carbonate</p>	
<p>Place frying pans in the airlock. 2-near the outlets and 2-away from the outlets using extension cords.</p>	
<p>Check temperature and relative humidity. Temperature should be 21 to 27 degrees Celsius and the relative humidity of about 70% is desired. May need to raise temperature and R/H by placing water in the frying pans and turning them on.</p>	
<p>Place biological indicators in Petri dishes (3). More may be needed if there are areas that are in-accessible. Placed in hard-to-reach areas and no higher than a foot off the ground.</p>	
<p>Ensure all items that are being gassed have all their lids, compartments, and doors left open. Note: Lids on chemical containers to remain closed.</p>	
<p>Ensure that all oils and fluids have been drained.</p>	
<p>All vessels should be horizontal, if possible.</p>	
<p>Ensure that there are no paper products, wood, or cloth to be gassed No porous materials are to be treated.</p>	
<p>Inventory all items being decontaminated.</p>	
<p>Ensure all power to the frying pans are off prior to placing the chemicals into them.</p>	



Place tin foil in the two frying pans that will contain the bags of Ammonium	
Carbonate. Place the two bags of Ammonium Carbonate in the frying pans as indicated.	
When temperature and R/H are at acceptable levels, place equal amounts of Paraformaldehyde in the frying pans.	
Secure the airlock doors, turn on the Paraformaldehyde switch. And leave on until all the product has turned to a gas.	
Again ensure all warning sign are posted.	



After the Decontamination Cycle is Completed

WORK TO BE CONDUCTED	DONE
Allow about three (3) days contact time before entering	
Ensure that the frying pans are off	
Prior to opening the outside door, notify Security that you need the security system released to the door. Inform them how long the door will be open.	
Don proper PPE and an Self-Contained Breathing Apparatus	
Have the inside person deflate the outside door gasket (or break the seal into the room). Open the outside door wide enough to allow you to enter safely. Ensure that you have a backup person with you prior to entry.	
Remove the petri dishes that contain the biological indicators	
Exit the airlock, closing the airlock door and have the inside person inflate the gasket.	
Remove the SCBA and the PPE and properly store them.	
Deliver the biological indicator to the BSL-2 lab for processing.	
Incubate the biological indicator for seven (7) days @ 51 degrees Celsius.	
If the biological Indicator indicates successful decontamination, aerate the room or airlock.	
After a few hours of aeration, test the airlock using the Drager indicator tubes for Formaldehyde to determine if the airlock is safe for personnel to enter	
If the biological indicator indicates that the decontamination was not complete, notify the Safety Officer immediately. Stop the test.	

Checklist Conducted By:____Date:

Decontamination of BSL3 Rooms by Hydrogen Peroxide (H₂O₂)⁷

This standard operation procedure (SOP) is for decontaminating high security rooms which are closed to the public and with dedicated air circulation!

- Make sure all air vents are closed (including fire flaps)
- Bring the distribution ventilator (200 m³/h) to the room(s) that are to be sterilized
- Have Seal tape available for doors and other outlets
- Have 3 spore tests readily available

(1) Preparation of H₂O₂ disinfection:

- Calculate the amount of H₂O₂ (35%) that has to be injected for disinfection (400 ml/50 m³)⁸ⁱ
- Start all equipment which should be disinfected (Ventilation, Cages, etc.)
- Detach the screw caps from the inner disinfection vent lines
- Connect the Steris 1000 to the outside connectors of the room to be sterilized
- Set the distribution ventilator on medium speed in the center of the room
- Fix spore tests to 3 separate points (i.e. Wall, Cage, etc.)
- Close door and seal with the tape; Attach the Disinfection Warning Sign
- Switch on STERIS 1000; enter volume of H₂O₂ and the program (i.e. Disinfection VUW-L3, etc.)
- Press START button

(2) Monitoring the disinfection:

- Check the print out and status of the program hourly
 - Drying cycle (to reduce humidity in the room)
 - Disinfection cycle (~ 3 hours/15m² room)
 - H₂O₂ inactivation cycle (~ 2 hours)

(3) Post disinfection

- Open HEPA ventilation in the room and wait for 2 hours for 20 times air change
- Open the door to the room and remove the spore tests for analysis and the ventilator
- Re-cap the air ducts and close the disinfection valves.
- Run the ventilation for 1 day on standard rate and commence work.

⁷ Courtesy of Veterinary University of Vienna

⁸ Concentration for 35% H₂O₂ should be minimum 1g/ m³ i.e. 3 ml/ m³; Max 3g/ m³