

**STANDARD OPERATING PROCEDURES**  
**DIVISION OF COMPARATIVE MEDICINE**  
**UNIVERSITY OF SOUTH FLORIDA**

SOP#: 1016.4    Date Issued: 3/16

Date Revised: 2/23

Page 1 of 6

---

<b>TITLE:</b>	<b>Hydrogen Peroxide Vapor Decontamination</b>
<b>SCOPE:</b>	Animal Care Personnel
<b>RESPONSIBILITY:</b>	Facility Manager and Technical Staff
<b>PURPOSE:</b>	To Outline the Proper Procedures for Decontamination of Animal Research Facilities and Equipment

---

**I.     PURPOSE**

1. This procedure outlines the proper procedures to be followed for high-level disinfection of animal facility housing rooms, procedural areas, surgical suites, labs, necropsy areas, and equipment.

**II.    RESPONSIBILITY**

1. It is the responsibility of the Facility Manager or designee to ensure that all technical staff performing decontamination are adequately trained in the following procedures and these procedures are adhered to.
2. It is the responsibility of the technical staff assigned to decontamination procedures to read, understand, and follow the procedures outlined below.
3. It is the responsibility of the technical staff assigned to decontamination procedures to read and understand the information provided in the Bioquell Hydrogen Peroxide Sterilant SDS.

**III.   GENERAL SAFETY PROCEDURES**

1. High-level disinfection of facilities/animal housing is accomplished using a high quality 35% w/w hydrogen peroxide (e.g., medical or food grade) and a Bioquell Z-2 VHP Generator System in accordance with **SOP #1162**.
2. Hydrogen peroxide (HP) is a strong oxidizer and is irritating to the eyes, skin, and mucous membranes. It is imperative that all personnel using HP wear the appropriate personal protection equipment.
  - a. Protective eyewear (e.g., goggles or face shield) must be worn when performing procedures that could result in HP coming in contact with the eyes.
  - b. Protective eyewear, impervious sleeves and gloves (e.g., neoprene or vinyl) are required when handling concentrated HP solutions (i.e., changing or filing bottles).
  - c. Wash hands after handling HP.
  - d. Flush skin/eyes with water if come in contact with HP.
  - e. HP spills should be cleaned-up with water.
  - f. If you must enter an area briefly during or immediately after fogging with HP, Tyvek coverall with hood and boots (or long sleeves, long pants, hair cover and

shoe covers), gloves, snug-fitting goggles, and half-face respirator with organic vapor filters and a particulate filter (e.g., 3M 6000 half face-piece respirator assembly with 3M 6001 Organic Vapor cartridges and a 5n11 or 5P71 particulate filter) are required.

3. HP should be stored in the dark at ambient room temperature.

#### IV. BIO-DECONTAMINATION PROCEDURES

1. Common procedural areas are decontaminated in accordance with **SOP #1015 *Decontamination of Common Procedural Areas*** and as needed using the Bioquell Z-2 Hydrogen Peroxide Vapor Generator System. Animal housing areas are decontaminated in accordance with **SOP #016 *Animal Room Preparation*** as they become empty.
2. In animal housing areas, prior to moving IVC racking systems, evaluate all casters and rack welds to ensure stability of the IVC racking system. If weaknesses or instabilities are detected, remove the rack from rotation and contact your supervisor for directions on how to have rack repairs implemented
3. Thoroughly read and understand all manufacturers' instructions and observe all "Cautions" and "Warnings" prior to using any equipment or solutions. **See SOP #1162 *Bioquell Z-2 Hydrogen Peroxide Vapor Generator System***.
4. HP is a surface decontaminant and may not penetrate dirt and detritus. Heavily soiled surfaces should be cleaned prior to decontamination with VHP. Microorganisms present in gross contamination (e.g., excessive dust, dirt, feces) could possibly survive the bio-decontamination process.
5. Remove all personnel and animals from area to be decontaminated as well as any equipment and materials that are not compatible with VHP and high levels of moisture, including TempTrak wireless monitoring sensors. Trash/waste receptacle contents must be bagged, sealed and left in place for surface decontamination prior to being removed from the room.
6. Determine the cubic volume of space to be decontaminated (length x width x height in feet) using the laser measuring device stored in the lectern. This information will be uploaded and stored, for future use, into the VHP generator.
7. Shut off HVAC to area to be decontaminated when possible. If HVAC cannot be shut off then all HVAC supplies, returns, dampers, and thimbles, where present, must be sealed to ensure no vapor leaves the area.
8. Open all cabinet doors, drawers, and doors to suites and minimize occluded/covered surfaces to facilitate penetration of VHP.
9. While absorbable materials can remain in the room during bio-decontamination (e.g., paper, clothing, fabrics, etc.) the presence of a large quantity of absorbable material will extend gassing time and aeration time to account for peroxide absorbed.
10. Remove any materials that may be incompatible with HP (e.g., open chemicals and medicines).

11. Turn off equipment that may operate above or below ambient room temperature (e.g., autoclaves, incubators, refrigerators, and cold rooms) and allow them to return to ambient room temperature prior to cycle initiation to ensure VHP distribution.
12. **Bioquell VHP-CI (chemical indicators) are used during each room decontamination** process to assess the distribution of VHP within the room. The CI will change color and fades from blue towards white when exposed to the parameters (i.e., time, concentration and micro-condensation) of the VHP surface decontamination process.
  - a. Avoid skin and or liquid contact with the indicator portion of the CI when handling/placing.
  - b. Since VHP is heavy, a CI or CIs (depending on room size and complexity) should be placed in the room, high on the wall in a corner at the wall/ceiling junction, and the farthest distance from the VHP generator. More than one CI may be required at the discretion of the operator depending on room size, configuration, and presence of equipment complexities.
  - c. CIs can also be placed in drawers, biological safety cabinets, and within equipment to evaluate the VHP distribution and surface decontamination. CIs should be placed deep within drawers, and on surfaces not directly exposed to VHP to challenge the efficacy of decontamination.
  - d. CIs are labeled to identify their location in the room with permanent marker on the printed-side of the card, away from the indicator portion. If more than one CI is used for wall/ceiling interface placement, number the CIs beginning with #1 from the corner immediately on your left as you enter the rooming and continuing consecutively around the room to the last corner.
  - e. CI cards should be used within 14 days after opening foil package.
13. **HP biological indicators (BI) are periodically used to validate efficacy of the bio-decontamination cycle, as required by the veterinarian.**
  - a. BI should be stored refrigerated at 2-8°C.
  - b. Since VHP is heavy, BIs should be placed in the room, high on the wall in corners at the wall/ceiling junction, and the farthest distance from the VHP generator.
  - c. BIs can also be placed in drawers, biological safety cabinets, and within equipment to evaluate the VHP distribution and surface decontamination. BIs should be placed deep within drawers, and on surfaces not directly exposed to VHP to challenge the efficacy of decontamination.
  - d. BIs are labeled to identify their location in the room with permanent marker on the upper right-hand corner of printed-side of the Tyvek pouch. For wall/ceiling interface placement, number the BIs beginning with #1 from the corner immediately on your left as you enter the rooming and continuing consecutively around the room to the last corner.
  - e. Tape the BIs on the wall with the writing side of the Tyvek pouch facing up and folded so that the indicator disc is parallel to the floor.
  - f. Masking tape can used to affix BIs to surfaces/equipment.
  - g. One BI will serve as control and should not be exposed to VHP.
14. Place the Z-2 generator in the center of most rooms for even VHP distribution. Other positioning may be required for alcoves or side rooms are connected to the main room.

15. The Bioquell R-30 unit is used to aid in the distribution of VHP and for aeration of the room to remove VHP after decontamination.
  - a. When using the unit for aeration only, place unit so that air can flow, unimpeded into all six filters. Blocking filters will prolong the aeration process.
  - b. When using the unit to aid in VHP distribution, the front of the unit is positioned so that the fans are directed toward side rooms, alcoves or areas not in the line of sight of the VHP generator.
16. Changing stations, Class A2A laminar flow hoods, and **equipment that recirculates air can be left "ON" during the cycle to obtain a more thorough decontamination** of the equipment. However, if equipment contains internal filtration, aeration may need to be extended to counteract absorption into the filters.
17. To decontaminate hard ducted BSCs during room decontamination:
  - a. If sash can be left open without creating excessive draw, leave power "ON".
  - b. Shut "OFF" power at the breaker.
  - c. Turn unit on at breaker when room is fully saturated for a minute or two to draw VHP into the unit.
  - d. Turn unit "OFF" at breaker after a couple minutes.
  - e. At the end of the VHP cycle the BSC can be turned on again at the breaker to aid in aeration of the room.
18. Check the area again for people, ensure all doors are shut and secured, that all possible openings where significant amounts of gas could escape are sealed, and the ventilation is off or the ductwork/thimbles are sealed.
19. Check that the hand-held low level H<sub>2</sub>O<sub>2</sub> sensor (stored in the lectern) is fully charged.
20. Place the flat cable that connects the lectern to the VHP generator under the door carefully, so as not to damage the cable, as you shut the door and leave the room.
21. Seal outer door edges with tape. Bioquell sells tape however most any tape will work (e.g., paper, masking, or duct tape). Use the hand held H<sub>2</sub>O<sub>2</sub> sensor during first 10 minutes of the VHP gassing cycle to test seals at bottom of door to ensure it is properly sealed.
22. Follow the procedures described in **SOP #1162 Bioquell Z-2 Hydrogen Peroxide Vapor Generator System** to start VHP decontamination and aeration.
23. When aeration begins hard ducted biosafety cabinet can be started at the breaker to speed-up the process.
24. After the appropriate aeration time, the screen will display "**Aeration Target Achieved Check Concentration <1ppm Before Ending Cycle.**" Use the hand-held low HP sensor to check the area's actual HP concentration.
25. Place the handheld sensor inside the door for a few minutes, then retrieve the sensor.
26. If the displayed level is <1ppm it is safe to end the cycle and enter the room.

27. If the handheld HP reads >1ppm the sensor will give both audible and visual alarms. Let the unit continue to aerate and repeat testing until <1ppm is achieved.
28. When 1ppm is reached, stop the cycle by pressing “**END CYCLE**” button (password required).
29. Enter room adhering to appropriate practices to prevent re-contamination of the area (e.g., appropriate PPE, essential personnel and work flow).
30. Remove any HVAC plenum and thimble covers.
31. Wearing gloves retrieve all CIs, and BIs when used, from the room.
32. Return the TempTrak wireless monitoring sensor to its appropriate location in the room.

## VI. VALIDATION

1. Inspect CIs to ensure adequate color change. CIs change color in the presence of HP by fading from blue towards white.
  - a. CIs provide a crude assessment of vapor distribution by visually comparing the degree of color change.
  - b. The color of the CI should be compared with each of the 2-log, 4-log, 6-log and >6-log printed reference colors on the product insert sheet.
  - c. The color change gives an indication of the progressive bioburden reduction.
  - d. CIs should be used for indicative purposes only and not as a direct replacement for BIs.
2. BIs are the industry standard method for validation of hydrogen peroxide bio-decontamination. **Preparing BI for incubation:**
  - a. While wearing gloves collect BIs and place in a sealable plastic bag.
  - b. Ideally BI disk transfer to growth media should be conducted in a BSC. Disinfect inside of BSC with a sporicidal disinfectant (e.g. Sporidicin) and allow surface to dry
  - c. **Label trypticase soy broth (TSB) tubes** (Fisher Scientific cat # BB21093, stored between 5-25<sup>o</sup>C) to coincide with BI pouch labeling and date. In addition label one TSB tube “**positive control**” and one tube “**negative control.**”
  - d. Remove BIs from the plastic bag and transfer the BIs to their corresponding TSB tube using the “pinch and peel” method described below:
    1. While wearing gloves, select the corresponding TSB tube.
    2. Unscrew cap until freely spinning, **DO NOT REMOVE CAP** from tube.
    3. With thumb and forefinger, pick up corresponding BI pouch, ensuring the small hole is at the bottom.
    4. Using the other thumb and forefinger, pinch and flex the sides of the indicator pouch so that the metal tab drops to the bottom of the pouch.
    5. Ensuring the BI disc can be felt between both thumbs; hold the flaps of the BI pouch between thumbs and forefingers.
    6. Holding the pouch directly over the top of the tube, use the third and fourth finger of one hand to carefully lift the cap and hold to one side of the tube.
    7. Apply pressure to the wing tabs to open pouch. Use thumbs to control the metal tab and direct it to fall into the tube ensuring that neither the tab nor the pouch touches the tube during the transfer.

8. While still holding the cap between the third and fourth finger, replace cap on tube.
  9. Dispose if tyvek pouch.
  10. Tighten TSB tube cap.
  - e. After all exposed BI have been transferred, **prepare the positive control** by transferring a BI tab from a pouch that has not been exposed to VHP following the “pinch & peel” technique described above.
  - f. The **negative control** is an unopened tube that contains only TSB.
  - g. Incubate all the tubes containing BIs including the negative control at  $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .
  - h. **Record the time and date incubation is initiated directly on the tube.**
3. Contaminated media tubes, caps, or BI tabs (i.e., touched by outside of pouch or dropped during transfer) should be noted in the “Comments” section of the **Bioquell VHP Decontamination Record**. Caps that are dropped can be replaced with a cap from a spare/extra tube.
  4. **Incubation and interpretation:**
    - a. BIs should be examined after overnight (>18 hours) incubation to give an initial indication, and after 7 days to confirm the final results.
    - b. Turbidity indicates spore growth. Turbid media in test vials indicates VHP was not effective in achieving complete bio-decontamination.
    - c. Positive controls should be turbid. Lack of turbidity indicates a problem with the BIs.
    - d. Negative controls should be clear. Turbid liquid indicates a problem with the sterility of the media.
  5. **Results of CIs and BIs are recorded on CMDC # 217 Bioquell VHP Decontamination Record.**
  6. Z-2 printed report tapes and the Bioquell VHP Decontamination Record are maintained with the facility sanitation records for a period of 6 months.
  7. The facility manager or their designee will submit the Bioquell VHP Decontamination Record to the Microbiological Monitoring Program Manager at the end of the month following decontamination procedures.

## VII. REFERENCES

1. *Bioquell Z-2 Hydrogen Peroxide Vapor Generator System User Manual.*
2. *Bioquell R-30 Aeration Unit User Manual.*
3. *Bioquell Hydrogen Peroxide SDS.*
4. Bioquell SOP #7 entitled *Biological Indicator Handling and Positioning.*
5. For additional information contact Bioquell Technical Support at (215) 682-0225

**Approved:**

**Date:**