

## 1.0 Purpose

To provide information on the selection and use of disinfectants in Biosafety Level 2 (BSL-2) laboratories handling human-sourced, infectious and/or recombinant materials.

## 2.0 Scope

This procedure applies to all individuals, including principal investigators (PI), researchers, instructors, laboratory/clinical managers, students or other personnel working with human-sourced, potentially infectious, or recombinant materials in a BSL-2 laboratory.

## 3.0 Definitions

- Antiseptic - Chemical germicide formulated to be used on skin or tissue.
- Decontamination - Process that renders a surface or object safe to use or handle. Sterilization and disinfection procedures are often used for decontamination.
- Detergent - a synthetic, organic, liquid or water-soluble cleaning agent that has wetting-agent and emulsifying-agent properties and can be used to remove organic material prior to disinfection.
- Disinfection - Process that eliminates nearly all forms of disease-causing microorganisms (viruses, bacteria, or pathogenic fungi, but not necessarily spores) on inanimate surfaces.
- Germicidal Detergent - A detergent that is also EPA-registered as a disinfectant.
- Spore - Relatively water-poor round or elliptical resting cell consisting of condensed cytoplasm and nucleus surrounded by an impervious cell wall or coat. Spores are relatively resistant to disinfectant and sterilant activity and drying conditions (specifically in the genera *Bacillus* and *Clostridium*).
- Sterilization - Use of chemical or physical procedures to destroy all microbial life, including bacterial spores.
- Tuberculocide - Compound that can kill *M. tuberculosis* under stated usage conditions.

## 4.0 Responsibilities

- It is the responsibility of anyone working in the BSL-2 laboratory to clean and disinfect work surfaces and equipment as required by the BSL-2 working procedures.
- It is the responsibility of anyone working in the BSL-2 laboratory to know how to treat/respond to a spill of potentially infectious material, and to know the notification procedure.
- It is the responsibility of the laboratory users to provide/prepare disinfectants for use in the BSL-2 laboratory as directed by manufacturer's instructions and as provided in this document.
- It is the responsibility of the Biosafety Officer (BSO) to review and approve new disinfectants and to remove disinfectants that are no longer approved for a particular use by the EPA/FDA, etc.

## 5.0 Procedure

### 5.1 Selection criteria

- Per OSHA (and Cal/OSHA) interpretation in the United States (U. S.), in the case of human blood/serum, disinfectants must be either:
  - Registered as a tuberculocide with the EPA (EPA list B);
  - Registered as a sterilant with the EPA (EPA List A); or,
  - Registered with the EPA as effective against Hepatitis B virus and HIV-1 (EPA Lists D&E).
- In general, disinfectants suitable for work with human-sourced materials will be appropriate for working with most risk group (RG) 2 agents, with the exception of spore-forming organisms. Work with prions requires unique treatment with disinfecting agents to denature the prion proteins.
- The product must be approved for the specific use and must be used according to the manufacturer's directions. In the U.S., it is illegal to use disinfectants except as specified by the manufacturer.
- All disinfectants used for decontamination purposes must be approved by the BSO if not included in this SOP.

### 5.2 Cautions

- Many disinfectants contain harsh chemicals. Suitable personal protective equipment (PPE) must be worn. Refer to SDS sheets (hard copies or online access required).
- Preparations must be discarded after the expiration date established by manufacturer.  
**NOTE:** Chlorine bleach solutions must be prepared on the day they are used unless:

- Documentation of available free chlorine levels (with < 5% drop in levels) over time is generated by the laboratory, and,
- The testing is specific for the brand of bleach, water (distilled, deionized, tap) and containers that will be used.

### 5.3 Labeling

- Disinfectants placed in a secondary container must be labeled with the following:
  - Product name;
  - Concentration;
  - Expiration date; and,
  - Relevant hazard data.

5.4 The following are approved disinfectants for use where blood, serum, tissues and / or infectious viral, parasitic and / or bacterial agents may be present:

- Surface disinfectants:
  - **0.1% Sodium Hypochlorite**
    - Chlorine Bleach  
**NOTE:** OSHA has approved a 1:100 or stronger dilution of household chlorine bleach containing 5.25% sodium hypochlorite as a tuberculocidal disinfectant. This is equivalent to 500 ppm of available chlorine. It is recommended to use a 2% bleach (1:50, 1000 ppm chlorine) solution, prepared daily, as follows:
      - i. Check the bleach label for the concentration of sodium hypochlorite. Concentrations of household bleach may range from 5.25–8.25%
      - ii. Use the following formula to calculate how much bleach to add to a given volume of water:

$$\frac{\text{Final concentration}}{\text{Concentration of bleach}} \times \text{Total Volume} = \text{Volume of bleach to add}$$

- iii. To prepare a 1 liter or 1000 ml of a 0.1% Sodium Hypochlorite solution from common household bleach containing 5.25% sodium hypochlorite, the proportions are: 19 ml of full strength bleach out of the bottle, add water to 1 liter.

$$[0.1\% / 5.25\% \times 1000 \text{ ml} = 19 \text{ ml}]$$

- **Cavicide or Envirocide**  
EPA Reg.: 46781-6; Metrex Research Corp., Romulus, MI
- **Caviwipes**  
EPA Reg.: 46781-8; Metrex Research Corp., Romulus, MI

- **Clorox Healthcare Bleach Germicidal Cleaner (formerly “Dsipatch”)**  
EPA Reg.: 56392-7; Clorox Professional Products Co., Oakland, CA
  - **Clorox Healthcare Bleach Germicidal Wipes**  
EPA Reg.: 67619-12; Clorox Professional Products Co., Oakland, CA
  - **LYSOL Brand I.C. Quaternary Disinfectant Cleaner (formerly “FORMULATION HWS-256”)**  
EPA Reg.: 47371-129-675; Reckitt Benckiser LLC, Parsippany, NJ  
**NOTE:** Use at a 1/256 dilution as directed by manufacturer.
  - **Super Sani-Cloth Germicidal Disposable Wipe**  
EPA Reg.: 9480-4; PDI, Nice-Pak Products, Inc., Orangeburg, NY
  - **Sani-Cloth Plus Germicidal Disposable Cloth**  
EPA Reg.: 9480-6; PDI, Nice-Pak Products, Inc., Orangeburg, NY
  - **Sporicidin Brand Disinfectant Solution (pump spray and refill)**  
EPA Reg.: 8383-3; Contec, Spartanburg, SC
  - **Sporicidin Brand Disinfectant Towelette**  
EPA Reg.: 8383-7; Contec, Spartanburg, SC
- Liquid Disinfection
    - For bulk liquids (non-radioactive):
      - Dilute waste with bleach or sodium hypochlorite to achieve an approximate final 0.5% sodium hypochlorite solution (5000 ppm, ~ 10% bleach in waste). Treat for a minimum of 30 minutes prior to disposal down the sanitary sewer drain with the water running. These quantities can be approximated (measuring via a pipet is not recommended).
        - **NOTE:** Check the label of the bleach you are using for the concentration of sodium hypochlorite. Concentrations of household bleach may range from 5.25–8.25%
        - **NOTE:** Commercial bleach has a shelf-life of about 1 year from the date of manufacture, NOT the date of purchase. Date of manufacture can be determined from a code on the bottle that shows the manufacture site, year, and day of year (see references below for detail). Use the following formula to calculate how much bleach to add to a given volume of water:  
$$\frac{\text{Final concentration}}{\text{Concentration of bleach}} \times \text{Total Volume} = \text{Volume of bleach to add}$$
      - If the bleach contains ~6% sodium hypochlorite, one liter of waste liquid would require ~83 ml of bleach to achieve a 0.5% hypochlorite solution. After appropriate treatment, the material can be disposed of down the sanitary sewer drain, with the water running.  
[0.5% / 6% X 1000 ml = 83 ml]

- **Wescodyne:** EPA Reg: 4959-16, Steris Corp., St. Louis, MO.
- Add to waste in the ratio of ~10 ml of Wescodyne Plus into 1-liter liquid waste. Mix gently and treat for a minimum of 2 hours prior to disposal down the drain with the water running.
  - **NOTE:** Do not use a metal container to collect bulk liquid for treatment with Wescodyne, as it will corrode metal.
  - **NOTE:** Wescodyne's active ingredient is iodine – **Do not use if allergic to iodine.**

### 5.5 Cleaning solutions

- These solutions are appropriate for reducing environmental contamination in the laboratory and for removing disinfectant residual from equipment (e.g., after using bleach on stainless steel or centrifuge rotors). They are not appropriate for disinfection of work surfaces (when used alone) after handling potentially infectious materials.
  - 70% alcohol solutions, either prepared in the laboratory or purchased (e.g., Septihol Ready To Use, 70% USP grade, non-sterile isopropyl alcohol solution filtered to 0.22 micron (Steris Corporation)).
  - Laboratory cleaning solutions such as Alconox, Luminox, Liqui-Nox, SoluJet, 7-X and any brand of dish detergent are all acceptable for cleaning laboratory equipment and surfaces and as the aqueous detergent used in the first step of decontaminating a contaminated instrument (see Appendix A: Equipment Decontamination Form) or cleaning up a biological spill (for removal of proteins before application of the disinfectant).

### 5.6 Recommended disinfectants for use when working with bacterial spores

- **Spor-Klenz Ready to Use**  
EPA Reg.: 52252-7-1043  
Steris Corporation, St. Louis, MO
- **Spor-Klenz Concentrate**  
EPA Reg.: 52252-4-1043  
Steris Corporation, St. Louis, MO
- **0.5% Sodium Hypochlorite solution** (~10% bleach or 5,000 ppm chlorine)
- **Clorox Healthcare Bleach Germicidal Cleaner and Wipes** (see above)

## 6.0 References

- *Selected EPA-registered disinfectants*  
(<http://www.epa.gov/oppad001/chemregindex.htm>).  
Many of the disinfectants listed have different or a variety of commercial names today. To check a disinfectant against the list, use the EPA Registration number and search the lists. The EPA Registration number can be found on the product of interest website under “technical information”.
- *29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens:*  
[http://www.osha.gov/pls/oshaweb/owadisp.show\\_document?p\\_table=standards&p\\_id=10051](http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10051)
- *Cal OSHA Bloodborne Pathogens, California Code of Regulations, Title 8, Section 5193:*  
<https://www.dir.ca.gov/title8/5193.html>
- *Bleach and Expiration Dates*  
(<https://www.clorox.com/dr-laundry/bleach-expiration-dates/>) On the Clorox® Regular-Bleach bottle shoulder there is a 2 line ink-jet code. The topline has a letter followed by a 7 digit code. The letter and first number are producing plant identification; the next 4 digits are a Julian production code and the final 2 digits are a shift identification. The second line is the EPA registration number (5813) followed by a state identification code. Thus, a code A8809507 would be Clorox® Regular-Bleach made in plant A8 on 8095 (8 for 2008 and 095 for the 95th day or April 4th).
- *Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, Fifth edition:*  
<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>
- *FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices:*  
<https://www.fda.gov/medical-devices/reprocessing-reusable-medical-devices-information-manufacturers/fda-cleared-sterilants-and-high-level-disinfectants-general-claims-processing-reusable-medical-and>

APPENDIX A  
Equipment Decontamination Form



**Equipment Decontamination Form**

Building: \_\_\_\_\_ Room Number(s): \_\_\_\_\_ Property Tag #: \_\_\_\_\_  
Dept: \_\_\_\_\_  
Principal Investigator: \_\_\_\_\_ Office/Lab Ext: \_\_\_\_\_  
Equipment Name: \_\_\_\_\_  
Model: \_\_\_\_\_ Serial #: \_\_\_\_\_

**I certify that the lab equipment mentioned above has been cleaned and decontaminated of all asbestos / chemical / biological / radioactive contaminants.**  
(Circle all that apply)

Principal Investigator's Signature (if available) \_\_\_\_\_ Date \_\_\_\_\_

EHS Staff (Signature) \_\_\_\_\_ EHS Staff (Printed Name) \_\_\_\_\_ Date \_\_\_\_\_

**Additional Notes:**