

## Discussion and Notes

*Keep a copy of these safety training notes and a signed attendance sheet to verify regular safety training. Regulatory inspectors will usually request proof of safety training.*

*Check with your state and local agencies for specific regulations regarding biohazardous waste disposal.*

*For increased safety using bleach disinfection, open plates while they are under solution, not in air.*

*Check with local authorities to determine if autoclaved bags may be disposed of as general waste.*

## Autoclave, Bleach or Biohazard Bag?

Biology, microbiology and biochemistry labs may generate wastes that must be managed as potentially infectious, biohazardous or regulated medical waste. Disposal of biohazardous wastes is subject to regulation by numerous authorities, including federal or state OSHA (for bloodborne pathogens), state environmental protection agencies, and local wastewater treatment plants. Review the following general guidelines and essential equipment needed for the sterilization and ultimate disposal of biological wastes *before* planning laboratory activities involving the use of microorganisms, body fluids or recombinant DNA.

### Types of Biological Waste

- Microbiological cultures and stocks, including all bacterial cultures and culture tubes
- Contaminated media and culture vessels (Petri dishes, inoculating loops) and personal protective equipment (PPE) such as disposable gloves
- Blood and other bodily or biological fluids
- Sharps and broken glass, including needles, razor or dissecting blades, glass pipets and glass tubing

### Universal Precautions

Use only non-pathogenic microorganisms, that is, those that are known NOT to cause disease in humans. It is prudent practice, however, to treat all microbial cultures and contaminated materials as if they may cause infection or pose an environmental risk if released. Microorganisms cultured directly from the environment should NOT be incubated at temperatures higher than 25°C and cultures should not be opened after they have been plated on agar. After incubation, a single microbial cell may multiply to more than one million, and at that level may present a risk if a culture is broken or carelessly handled. Universal precautions for microbiological wastes, biological fluids, and contaminated labware include autoclaving, dry-heat sterilization or chemical disinfection.

### Sterilization

Sterilization is defined as the death of all living organisms, including spores, in or on an object. Autoclaving with steam and dry heat sterilization are the preferred methods for achieving this objective. Chemical disinfection with diluted household bleach is also effective at killing bacteria, fungi and algae, including bacterial spores and viruses. The required concentrations and time will vary for different organisms and spores. Use only fresh household bleach, dilute 1:10 with water immediately prior to disinfection, and immerse materials for at least 6 hours (overnight is best).

### Autoclave Use, Safety and Verification

Objects to be autoclaved should be placed into autoclave or biohazard bags without opening the containers (Petri dishes, culture tubes, etc.). *The following materials should NEVER be placed in an autoclave: flammable, combustible or volatile liquids, and any liquid in a sealed container.* Do not place sharp objects into an autoclave bag. Loosely close but do not seal the bags—steam must penetrate the materials for effective sterilization—and place the bags on trays inside the autoclave to capture potential spills. Depending on the load density, typical sterilization conditions are 30 minutes at 121°C and 15 psi pressure. The requirements for length of autoclaving and temperature increase at higher altitudes. Carefully follow the manufacturer's instructions and all safety precautions, including the use of PPE. The use of a biological or chemical indicator, such as autoclave indicator tape, is highly recommended. Some state authorities may require periodic validation of autoclave operation using bacterial strips or cultures.

## Disposal of Sharps

Sharp objects such as needles, razor blades and glass pipets must be collected in a labeled, puncture-proof container. Sharps that are contaminated with potentially hazardous biological materials or fluids should be sterilized prior to collection and/or disposal.

## References

1. *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition; Centers for Disease Control and Prevention (2009). The full edition is available online at <http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf>
2. OSHA Standard for Occupational Exposure to Bloodborne Pathogens, 29 CFR §1910.1030. May be accessed online at [https://www.osha.gov/pls/oshaweb/owadisp.show\\_document?p\\_id=10051&p\\_table=STANDARDS](https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS)
3. National Research Council; Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards; The National Academies Press: Washington, D.C. (2011)
4. The use of bleach for chemical disinfection is described in the CDC publication “Guideline for Disinfection and Sterilization in Healthcare Facilities,” which is available online at <https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf>

## Biology Lab Safety—Free Online Video

Please visit the Flinn Scientific website at the following link to view a free online video on proper procedures, instruments and safe methods for dissection and microbiology lab activities. <http://labsafety.flinnsci.com/Chapter.aspx?ChapterId=118&UnitId=8>

This video is part of the comprehensive Flinn Scientific Laboratory Safety Course. The table of contents for this free certification course is available at <http://labsafety.flinnsci.com/Assets/Documents/High%20School%20Laboratory%20Safety%20Course.pdf>

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