



Decontamination and Waste Management



Laboratory Biosecurity for BSL3 Laboratories
Nashville, TN
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www.biosecurity.sandia.gov



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Surface Decontamination



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Definitions

- **Germicide:** Any antimicrobial chemical agent used for disinfection, antiseptis or sterilization – regardless of whether its action is microbicidal or microbistatic; any microbicidal disinfectant, antiseptic or sterilant
- **Contamination:** Introduction of microorganisms into tissues or sterile materials
- **Decontamination:** Disinfection or sterilization of infected articles to make them suitable for use
- **Disinfection:** Selective elimination of certain undesirable microorganisms in order to prevent their transmission
- **Antiseptis:** Destruction of vegetative forms of microorganisms but not their permanent forms

Sterilization: Complete killing of all microorganisms




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Effectiveness of Germicides

- Concentration of the Germicide
- Concentration of the Agent
- Type of Agent
- Time of Contact
- Environmental Conditions Present

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Surface Disinfectants

- Alcohols (Ethyl alcohol)
- Halogens (Sodium and Calcium hypochlorite)
- Quaternary Ammonium Compounds
- Phenolics (Lysol)
- Aldehydes (Formalin)
- Hydrogen peroxide
- Not all Lysol products are created equal





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Autoclaves

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Autoclaves

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Principles of Autoclave Sterilization

- Direct exposure of each item to steam at the required temperature and pressure for a specific time
- 121° C - 123° C (250° F - 254° F)
- 15 pounds per square inch (psi); 1.05 kg/cm²
- Time
 - Minimal time required depends on the weight and specific nature of the objects to be sterilized
- Steam must contact all areas of a load
- Loosely gathered biohazard bags
- Add 500 ml of water to bags prior to packaging and transport to allow for steam saturation

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Why Autoclave

- Properly used autoclaves may be used to sterilize instruments, other media, and biohazard waste

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What can be Autoclaved?

- Pathogenic plant matter
- Culture and stocks of infectious agents (bacteria, mold, viruses)
- Contaminated solids (paper towel, cloth, plastic pipette tips, glassware)
- Discarded live and attenuated vaccines
- Recombinant DNA, plant and animal specimens
- Animal tissues
- Animal cage waste





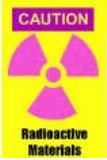

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What should NOT be Autoclaved?

- Items containing solvents, volatiles or corrosive chemicals
- Radioactive material (s)



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Autoclave Safety Procedures

- Follow manufacturers' guidelines
- Do not open when chamber is pressurized
- Avoid standing directly in front of autoclave door when opening
- Divide and Conquer
 - Divide small volumes into small
 - Autoclaving dense materials is not recommended
- Do not place sealed containers into autoclave
- Careful - liquids are hot
- Use shallow metal pans for best results and heat transfer
- Place autoclave on preventive maintenance schedule to ensure it is working according to specifications of the manufacturer
 - Annual inspection by manufacturer-certified technician





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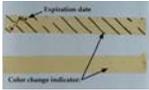


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Chemical Indicators for Autoclaves

- Show proper parameters of:
 - Time
 - Temperature
 - Pressure
- Each load of biohazard waste should be monitored using chemical indicators





Expiration date

Color change indicator



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Biological Indicators for Autoclaves

- When the bioburden is unknown, the most appropriate method to validate sterilization is the overkill method.
- This method involves demonstrating that 10^6 spores (*Geobacillus stearothermophilus*) will be killed in a half cycle.
- Thus a full cycle would result in a 12-log reduction of spores and produce a sterility assurance level (SAL) of 10^{-6} or a one-in-a-million chance of a non-sterile sample.
- Monitoring of biohazard waste using biological indicators should be performed weekly



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Record Keeping for Autoclaves

- Record all information in a Log book
 - Name
 - Laboratory Location
 - Phone Number
 - Date
 - Dwell Time
 - Temperature
 - Type and amount of material



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Cleaning Autoclaves

- Clean trap at least once a week
- Clean surrounding area after every use
- Remove broken glass
- Clean up overflowing liquids immediately



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Area Decontamination



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Area Decontamination

- Vapor Phase Hydrogen Peroxide (VHP)
- Formaldehyde Vapor
- Chlorine Dioxide



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AMSCO VHP 1000 Vaporized Hydrogen Peroxide Generator

Front View

- Control Panel
- Printer
- Sterilant Cartridge
- Break release
- Warning Lights



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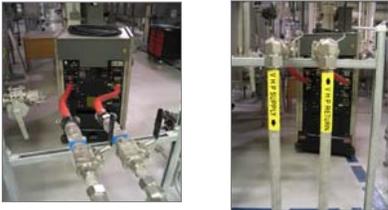
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Connections outside Containment

Connect to Room via external piping



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Connections inside Containment



- Piping in room
- Ability to Connect:
 - Pass through
 - Class III BSC
 - Extension hose for room decontamination

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Chlorine Dioxide - Alternative Sterilant MRI



Cloridox-B or GMP Sterilization System

Alfa Medical, Hempstead, NY



ClO₂ Generator System
(up to 100,000 pounds of ClO₂/ day)

Sabro Technical Services, Reston, VA

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Biological and Chemical Indicators (VHP) MRI

• Chemical Indicators (CI)

- VHP® Indicator from STERIS
- Change color from blue/grey to beige when exposed to Hydrogen Peroxide Vapors

• Biological Indicator (BI)

- Sporidex® VHP™ from STERIS
- Geobacillus stearothermophilus*
- Population of 10⁸



CI

BI-Front

BI-Back

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Decontamination Equipment (HCHO) MRI









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Formaldehyde Decontamination Chemicals








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Facility Decontamination Validation (HCHO)






Canadian Science
Centre for Human
and Animal Health

Centre scientifique
canadien de santé
humaine et animale



Canada

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Containment Suite Exhaust Plenum








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Types of Biological Waste

- Solid, non-sharp waste**
 - Plastic labware (flasks, tubes, plates, bottles, vials)
 - Lab waste (stocks, specimens, cultures, swabs)
 - Tissue or carcass waste (pathological)
 - Gloves, apparel, wipes
 - Pipettes (could also be sharps!)
- Liquids**
 - Aspirates, culture fluids, rinses, washes, etc.
 - Sera, body fluids
 - Spill clean-up waste
- Sharps**
 - Anything with a point or edge capable of piercing or cutting human skin
 - Glass labware (sometimes also broken plastic)
 - Needles, scalpels, blood tubes, Pasteur Pipettes
 - Syringes (*with* and sometimes *without* needles)

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Solid Biowaste Collection

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Waste Storage and Transport

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Intermediate vs. Final Treatment

- **Intermediate Treatment**
 - Usually performed for worker protection
 - Autoclaving most common method
 - Standard microbiology lab practice
 - Performed before transport to final treatment
- **Final Treatment**
 - On-site treatment by facility staff
 - Off-site treatment by disposal contractor

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Summary

- Decontamination is disinfection or sterilization of infected articles to make them suitable for use or disposal.
- Disinfection is the selective elimination of certain undesirable microorganisms in order to prevent their transmission.
- The effectiveness of a germicide (disinfectant) is dependent upon the concentration of the germicide, concentration of the agent, the type of agent, the time of contact, and the environmental conditions present.
- The effectiveness of any decontamination process should be validated.
- Autoclave = Pressure vessel
 - Heat hazard; distribution of load
 - Preventive maintenance schedule
 - Use chemical indicators and biological indicators
- There are different procedures for surface and area decontamination
 - Choice of disinfectants for surfaces
 - Formaldehyde vapor or Vaporized Hydrogen Peroxide for areas
 - ALL decontamination procedures must be validated
- Types of biological waste, disposal

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