
Decontamination and Waste Management

Laboratory Biosecurity and Biosafety
for BSL3 Laboratories
Bogor, Indonesia
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Surface Decontamination



Definitions

- **Germicide:** Any antimicrobial chemical agent used for disinfection, antisepsis 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
- **Antisepsis:** Destruction of vegetative forms of microorganisms but not their permanent forms
- **Sterilization:** Complete killing of all microorganisms

Effectiveness of Germicides

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

Surface Disinfectants

- Alcohols (Ethyl alcohol)
- Halogens (Sodium and Calcium hypochlorite)
- Quaternary Ammonium Compounds
- Phenolics (Lysol)
- Aldehydes (Formalin)
- Hydrogen peroxide



Autoclaves

Autoclaves



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

Why Autoclave

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



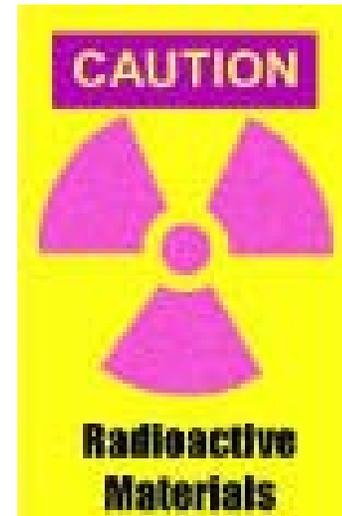
What can be Autoclaved?

- 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



What should NOT be Autoclaved?

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



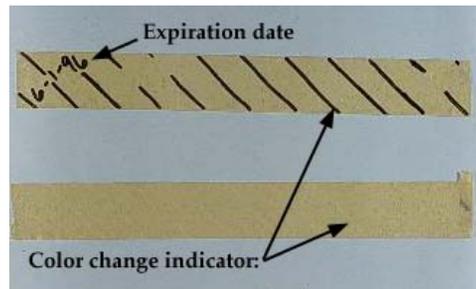
Autoclave Safety Procedures

- **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



Chemical Indicators for Autoclaves

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



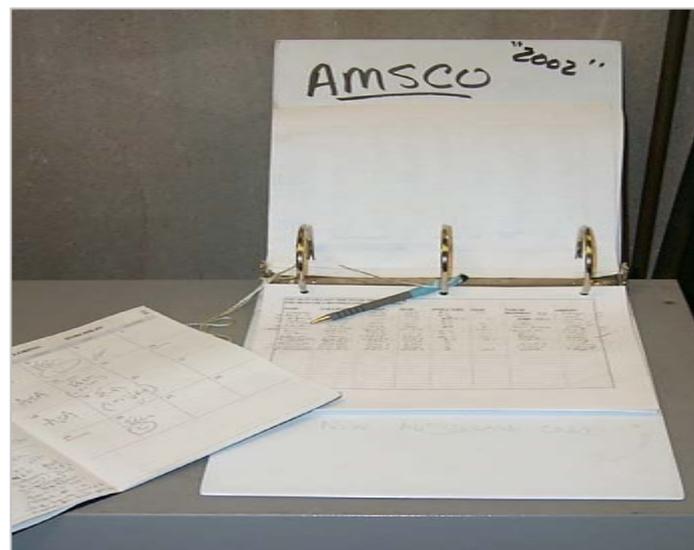
Biological Indicators for Autoclaves

- 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



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



Cleaning Autoclaves

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



Area Decontamination

AMSCO VHP 1000

Vaporized Hydrogen Peroxide Generator

Front View

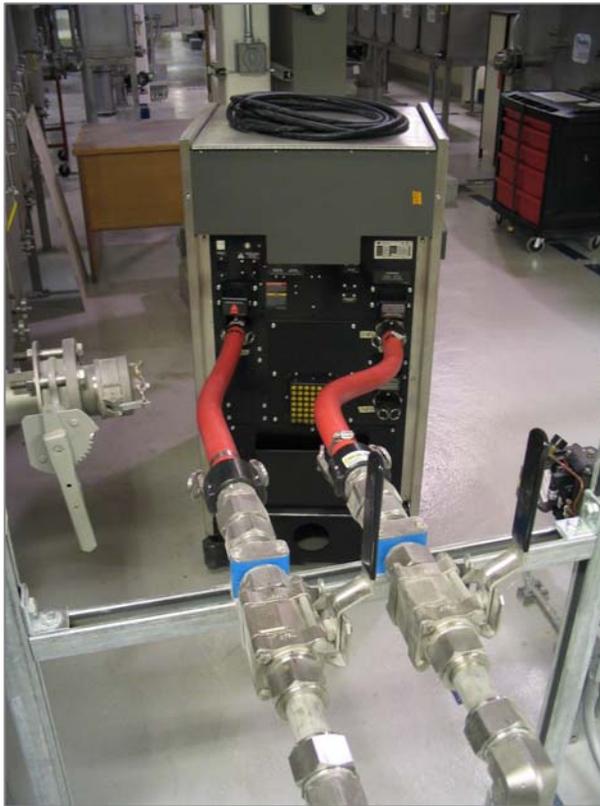
- Control Panel
- Printer
- Sterilant Cartridge
- Break release
- Warning Lights



CSCHAH, Winnipeg, Canada

Connections outside Containment

Connect to Room via external piping



CSCHAH, Winnipeg, Canada

Connections inside Containment



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

CSCHAH, Winnipeg, Canada

Chlorine Dioxide - Alternative Sterilant



Cloridox-B or GMP Sterilization System

Alfa Medical, Hempstead, NY



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

Sabre Technical Services, Reston, VA

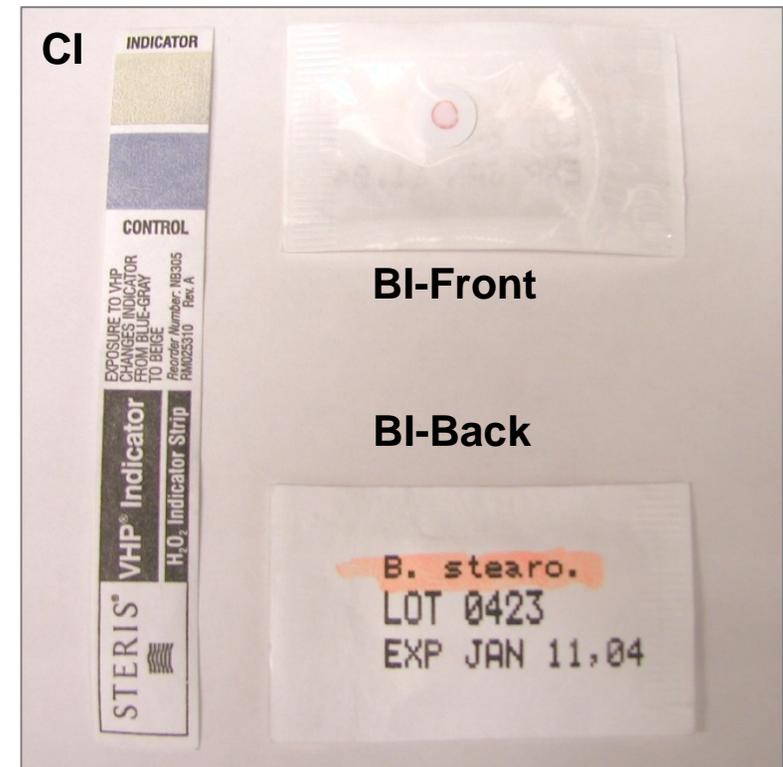
Biological and Chemical Indicators (VHP)

- **Chemical Indicators (CI)**

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

- **Biological Indicator (BI)**

- Spordex®-VHP™ from STERIS
- *Geobacillus stearothermophilus*
- Population of 10^5



CSCHAH, Winnipeg, Canada

Formaldehyde Vapor Decontamination Procedure

- **Biological Indicator (spore strips) containing at least 106 CFU (colony forming units) of *Bacillus atrophaeus* (formerly *B. subtilis* var *niger*) and at least 105 CFU of *Geobacillus stearothermophilus* (formerly *B. stearothermophilus*).**
- **pHCHO: Volume of the area to be decontaminated in ft³ X 0.3 g/ft³ (m³ x 10.6 g/m³) and ÷ by the % of HCHO in the pHCHO to be used.**

$$0.3 \text{ g/ ft}^3; \text{ therefore } 1 \text{ ft}^3/0.0283 \text{ m}^3 = 0.3 \text{ g} \div 0.0283 \text{ m}^3 = 10.6 \text{ g/m}^3$$

Formaldehyde Vapor Decontamination Procedure

E.g., An area of 10 ft (length) (3.048 m) by 15 ft (width) (4.572 m) by 12 ft (height) (3.658 m) = 1800 ft³ (50.97 m³). The pHCHO contains 91% HCHO. The amount of pHCHO needed is (1800 ft³ X 0.3 g/ft³) ÷ 0.91 = 593.4 g; or

$$50.97 \text{ m}^3 \times 10.6 \text{ g/ m}^3 \div 0.91 = 593.7 \text{ g}$$

- ▶ In areas where *Coxiella burnetii* was used,
↑ pHCHO to 0.6 g/ft³ (21.2 g/m³).
- ▶ If Relative Humidity (RH) = < 60%, add H₂O
to fry pans designated for water.

Formaldehyde Vapor Decontamination Procedure

- **NH₄HCO₃**: Calculate the amount of NH₄HCO₃:

Multiply g of pHCHO determined by the formula
[area x 0.3 g/ft³ or 10.6 g/m³] by 1.1.

E.g., the amount of NH₄HCO₃ is:

$$593.4 \text{ g} \times 1.1 = 652.7 \text{ g; or}$$

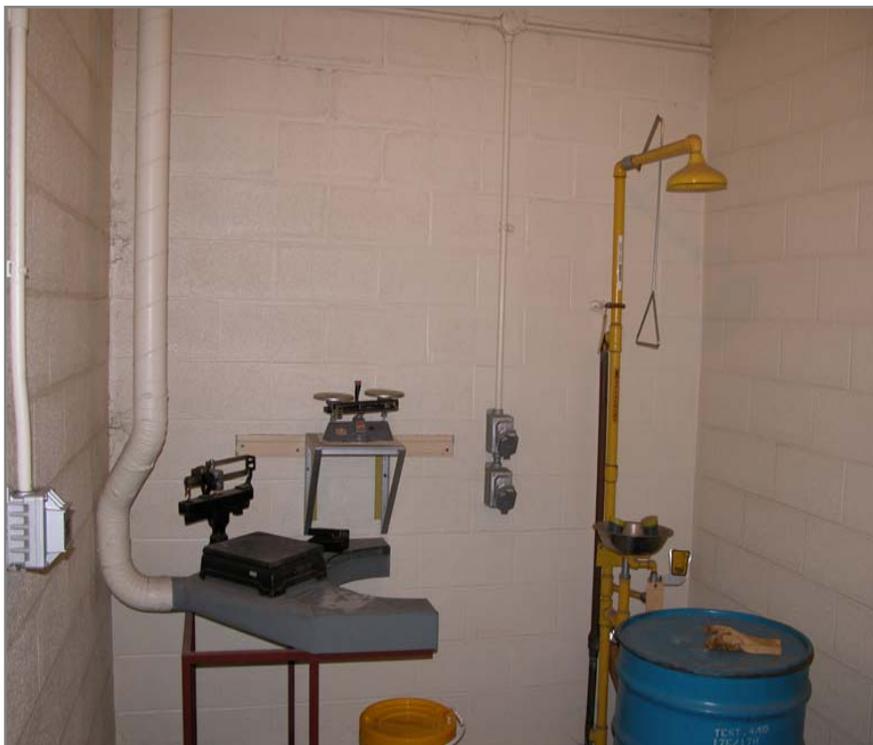
$$\mathbf{593.7 \text{ g} \times 1.1 = 653.1 \text{ g}}$$

In lieu of pHCHO prills or flakes, use 37%
formalin at a rate of 1 ml/ft³ or 35 ml/m³

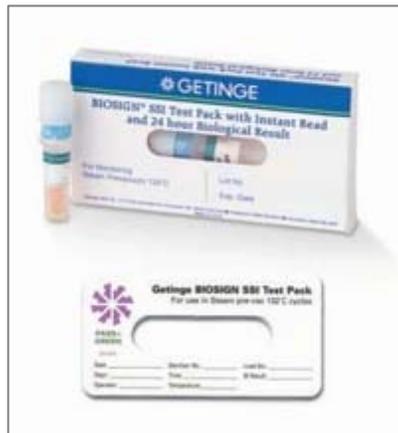
Decontamination Equipment (HCHO)



Formaldehyde Decontamination Chemicals



Facility Decontamination Validation (HCHO)



Containment Suite Exhaust Plenum



Biological Waste

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)

Solid Biowaste Collection



Waste Storage and Transport



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



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
 - 121° C - 123° C (250° F - 254° F) 15 pounds per square inch (psi); 1.05 kg/cm²
 - 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