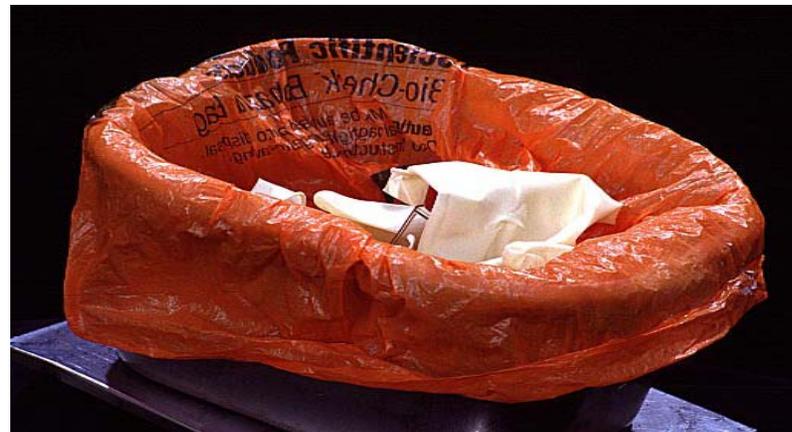


Decontamination, Waste Handling



Definitions

Sterilization

The use of a physical or chemical procedure to destroy all microbial life*, including large numbers of highly resistant bacterial spores.

***Typically measured as a kill of at least 10^6 .**

Decontamination

Reduction of microbial burden so that material is safe to handle.

Definitions

Disinfection

The use of a physical or chemical procedure to virtually eliminate all recognized pathogenic microorganisms but not all microbial forms (bacterial endospores) on inanimate objects.

Antisepsis

A germicide that is used on skin or living tissue for the purpose of inhibiting or destroying microorganisms.

Biosafety Practices

- ⚠ Decontaminate equipment and work surfaces routinely
 - ⚠ **With appropriate disinfectant for agent**
 - ⚠ **After spills or contamination**
 - ⚠ **When work is finished**
 - ⚠ **Decontaminate equipment before removal from lab**
- ⚠ Decontaminate potentially infectious materials before disposal or reuse

Factors to Consider

Agent Selection

- ⚠ Degree of microbial killing required
- ⚠ Nature of item/surface to be treated
- ⚠ Is it clean?
- ⚠ Ease of use
- ⚠ Safety
- ⚠ Cost

Factors to Consider

Agent Efficacy

- ⚠ Type of organism
- ⚠ Number of organisms
- ⚠ Amount of organic material present
- ⚠ Type & configuration of material to be treated
- ⚠ Type & concentration of germicide
- ⚠ Time and temperature or exposure
- ⚠ pH
- ⚠ Humidity

Decontamination

Methods

 **Heat (dry, wet, incineration *)**

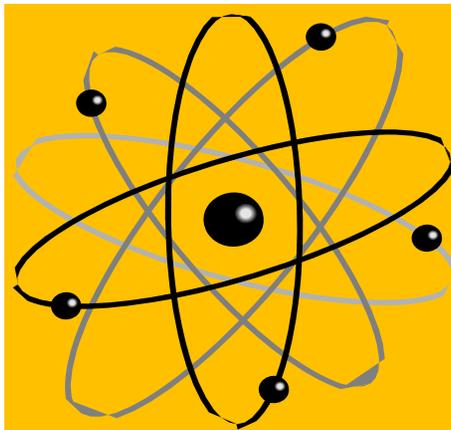
 **Chemical (liquid, gas, vapor)**

 **Radiation (limited use)**

*The most effective method of sterilization

Radiation

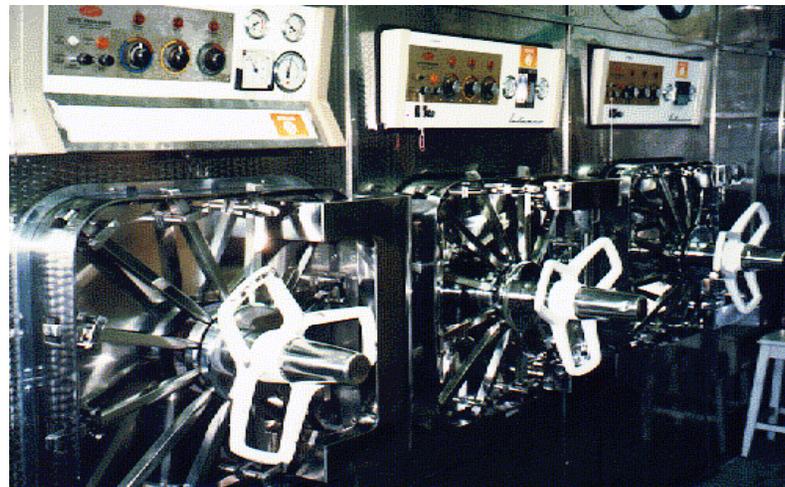
- ☣ For laboratory use, radiation is generally only used to inactivate infectious materials being removed from maximum containment labs for further manipulation at a lower BSL – e.g., pathological studies, electron microscopy, Polymerase Chain Reaction, etc.



Decontamination

Heat

- ❖ Steam sterilization practices
 - ❖ **Ensure proper functioning of autoclave**
 - ❖ **Determine appropriate loading and conditions to ensure microbial kill**



Decontamination

Heat

- ❖ Steam sterilization verification
 - ❖ Direct assay
 - ❖ Thermocouples
 - ❖ Chemical indicators
 - ❖ Biological indicators
 - ❖ (*Bacillus stearothermophilis*)



Decontamination

Heat

☣ Dry heat sterilization

☣ Denaturation of proteins: 160⁰ – 170⁰ C/2-4 hours

☣ Effective on impervious non-organic materials like glass

Decontamination

Heat

- ⚠ Incineration
 - ⚠ Method of choice for animal carcasses
 - ⚠ Requires certified incinerator



Chemical Decontamination



Decontamination

Chemical

☠ Types

- ☠ Liquids, i.e., bleach, alcohols, etc
- ☠ Gases, i.e., ethylene oxide, formaldehyde, etc
- Vapors, i.e., vapor phase hydrogen peroxide, etc



Decontamination

Chemical

- ⚠ Agent selection – complexity of chemicals
 - ⚠ Over 14,000 registered products
 - ⚠ Over 300 active ingredients
 - ⚠ 14 ingredients present in 92% of products
- ⚠ Agent selection - activity
 - ⚠ HLD – high level disinfection
 - ⚠ ILD – intermediate level disinfection
 - ⚠ LLD – low level disinfection

Decontamination

Chemical

- ⚠ High level disinfection - sporocides
 - ⚠ **Kills all microorganisms except high numbers of bacterial spores**
 - ⚠ **Require 5-10 min. exposure**
 - ⚠ **Examples: aldehydes, hydrogen peroxide, peracetic acid**

Decontamination

Chemical

- ⚠ Intermediate level disinfection - tuberculocides
 - ⚠ **Kills *M. tuberculosis var. bovis* and all vegetative bacteria, fungi, and most viruses**
 - ⚠ **Require minimum 20 min. exposure**
 - ⚠ **Examples: phenolics, iodophores, chlorine compounds, alcohols**

Decontamination

Chemical

- ⚠ Low level disinfection – hospital germicides used for housekeeping
 - ⚠ **Kills most vegetative bacteria and some fungi, but not *M. tuberculosis var. bovis***
 - ⚠ **Require minimum 20 min. exposure**
 - ⚠ **Examples: quaternary ammonium compounds**



For Consideration

- ⚠ Decontamination is not the same as cleaning.
- ⚠ Effective decontamination requires removal of organic materials *before* applying the specific decon procedure.

Decontamination

Bacterial Spores

B. subtilis

Mycobacterium

MTB var. bovis

Non-lipid Viruses

Polio-

Rhino-

Fungi

Cryptococcus sp.

Candida sp.

Vegatative Bacteria

Pseudomonas sp.

Staphylococcus sp.

Salmonella sp.

Lipid Viruses

Herpes

CMV

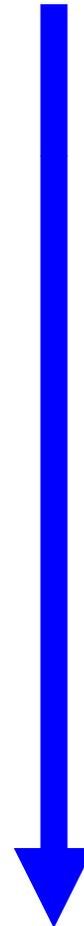
HBV

HIV

Sterilization



HLD



ILD



LLD



Space Decontamination

(Paraformaldehyde)

Properties

 **PEL = 9.75 ppm; TLV/STEL = 0.3 ppm; flashpoint = 70 C**

Advantages

 **Powerful decontaminant**

 **Non-corrosive to metals**

 **Relatively easy to generate**

Disadvantages

 **Probable human carcinogen**

 **Acute respiratory irritant**

 **Must neutralize**

 **Requires high relative humidity**

Space Decontamination

Vapor Hydrogen Peroxide

Properties

 **PEL = 1.0 ppm; STEL = 75 ppm**

Advantages

 **Effective against viruses, bacteria, spores**

 **End products are water and oxygen**

Disadvantages

 **Acute toxicity at high concentrations**

 **Vapor reactive, may break down with certain surfaces, such as galvanized steel or porous surfaces like paper**

Space Decontamination

Chlorine dioxide (ClO₂)

Properties

 PEL and TLV = 0.1 ppm; TLV/STEL = 0.3 ppm

Advantages

 Effective against viruses, bacteria, spores

 Rapid natural breakdown; no residue

 Effective on porous & non-porous surfaces

Disadvantages

 Unstable, requires continuous generation

 Maintenance of humidity critical

 May affect certain metals



Testing for Effectiveness

- ⚠ Whatever the space decontamination method used, testing with spore strips is necessary to monitor effectiveness.

Chemical Decontamination

 **General Lab Use - *Hypochlorite Solutions***

 **Large Spills/Large Organic Load**

 undiluted from bottle

 **Small Spills/Virus Inactivation**

 10% - 1:9

 **General Surface Disinfection**

 1% - 1:99

 **Clean before decontamination**



Decontamination

Disinfectants do not replace
standard microbiological
practices or good hygiene!

Biological Waste

Decontamination

☠ To render the object/material safe by reducing or removing the bioburden

☠ Methods

☠ **chemical ... match, contact time**

☠ **physical ... Heat, steam and pressure**

☠ **incineration**

☠ **other choices, i.e. shredding + chemical**

Biological Waste

Types

 **cultures, stocks, isolates**

 **materials containing or contaminated with blood**

 **sharps**

 **pipettes, wrappers, tips**

 **All materials used in the lab that are not re-usable and are to be discarded.**

Microbiological Laboratories

All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.



Biological Waste

■ Disposal

- *Never place lab waste into office waste containers*
- *Line discard containers with autoclave bag*
- *Decontaminate discard p... from the lab:*

1. Disinfect outside
2. Label
3. Tape ends with autoclave tape
4. Secure for transport



Sharps

- ⚠ Segregate wastes (chem, bio, rad, sharps)
- ⚠ *Place sharps into “sharps” container*
- ⚠ Effectively decontaminate
 - ⚠ **Autoclaving, chemical disinfection**



Sharps

- ⚠ Packaged to remove for incineration
- ⚠ Protect personnel handling waste



Personal Decontamination

Wash hands for 20-30 seconds after:

- ☠ Handling infectious materials or animals
- ☠ Removing gloves
- ☠ Before leaving lab

