Decontamination Methods In Microbiology lab.

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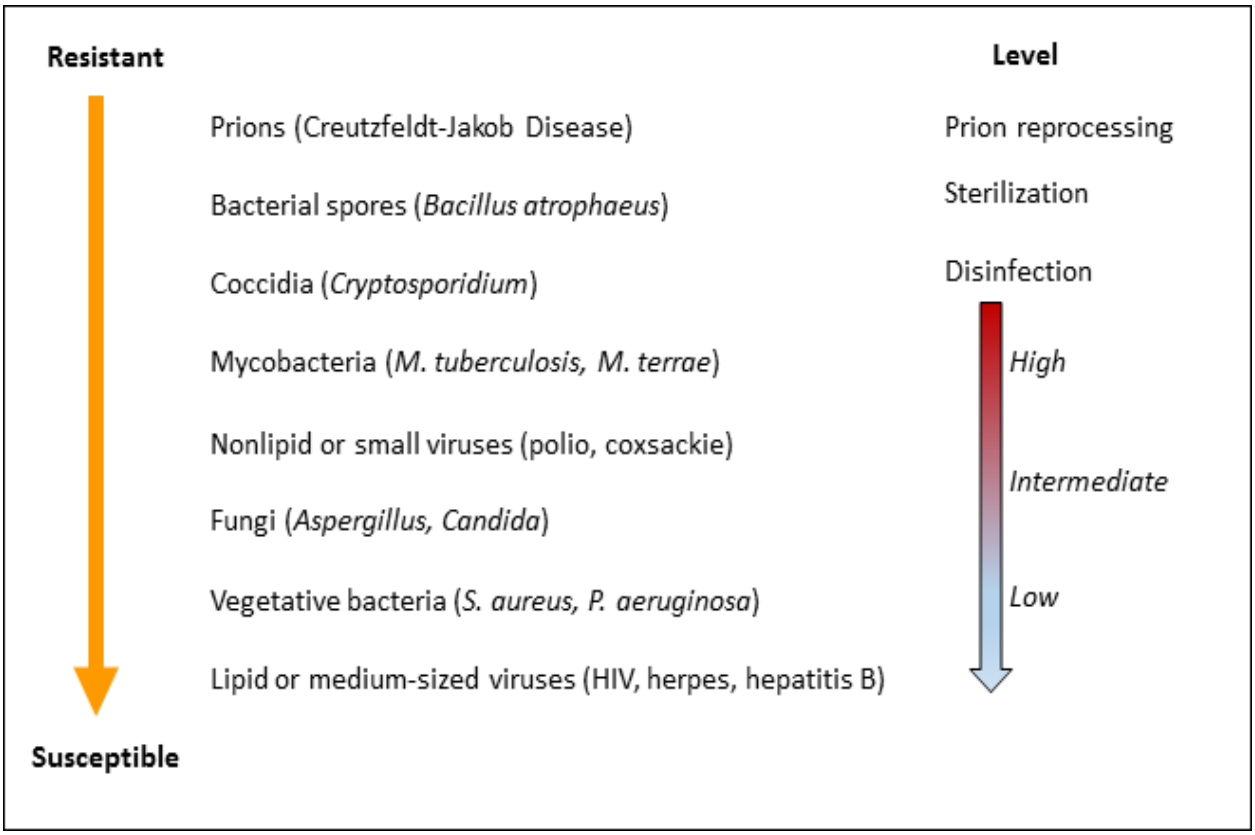
Laboratory personnel is at risk of biological contamination leading to laboratory-acquired infections(LAIs). The use of disinfection products is essential in the prevention of these infections.

What are the common sources of infection?

- Environment : Blood, body fluids, secretions, excretions, placenta, contaminated sharps and other equipments.
- Other clients & Attendants
- People in the community
- Health care delivery personnel

Level of sterilant/ disinfectants according to their microbicidal action



Standard Precautions

1. Hand washing
2. Use of protective attire
3. Processing of used items
4. Proper handling and disposal of sharps
5. Maintaining a clean environment
6. Biomedical waste disposal

2. Standard Precautions: Protective attire

- Gloves
- Masks
- Eye-covers
- Gowns
- Caps
- Footwear

3. Processing of used items

A. Decontamination:

Preparation of Bleaching solution (0.5%)

- Wear utility gloves and plastic apron.
- Take 1 litre of water in plastic bucket.
- Make thick paste with 3 levelled tea spoons of bleaching powder and water in a plastic mug.
- Mix paste in the bucket of water to make 0.5 % chlorine solution.

B. Cleaning:

Involves - scrubbing with a brush, detergent and water. Detergent is important for effective cleaning because water alone does not remove proteins, oils and grease.

C. Sterilization:

Sterilization ensures that items are free of all microorganisms (bacteria, viruses, fungi and parasite) including endospores.

Three methods of sterilisation:

- Steam sterilisation / Autoclaving / Pressure cooker autoclaving
- Dry heat sterilisation
- Chemical / cold sterilization
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1. <u>Physical methods</u>:

- Heat (dry heat & moist heat)
- U.V. Light
- Ionizing Radiation
- Filtration

2. <u>Chemical method</u>: (used for heat sensitive equipments)

- Ethylene Oxide
- Gluteraldehyde .

PHYSICAL METHODS

<u>HEAT</u> : Most important should be used whenever possible , <u>types:</u>

A-Dry heat at temperature of 160˚C for one hour.

B- Moist heat eg. Autoclave at 121 or 134 C for 10 or 15 minute

- Dry Heat- kills microorganisms by destroying their oxidative processes.

– Simplest method is exposing item to be sterilized to the naked flame e.g. Bunsen burner- for sterilizing bacteriological loops, knives, blades.

– **Hot air oven expose items to 160 °C for 1 hour.**

- Has electric element in chamber as source of heat plus a fan to circulate air for even distribution of heat in chamber. Oven without fan is dangerous. Used for items that are lacking water such as:
  -Metals
  -Glassware
  -Ointment / Oils/ Waxes /Powder

Moist Heat

- Uses hot water. Moist heat kills microorganisms by denaturating proteins.
- Autoclaving – standard sterilization method in hospitals.

- The equipment is called Autoclave and it works under the same principle as the pressure cooker where water boils at increased atmosphere pressure i.e. because of increase pressure the boiling point of water is >100 °C.
- The autoclave is a tough double walled chamber in which air is replaced by pure saturated steam under pressure**.**

Advantages of Autoclave

- Temp. > 100 C therefore spores killed.
- Condensation of steam generates extra heat.
- The condensation also allows the steam to penetrate rapidly into porous materials.
- *Note: for all invasive procedures at operating room or clinics, autoclavable equipments should be used.*

Radiation

- **U.V. light**
  - Has limited sterilizing power because of poor penetration into most materials. Generally used in irradiation of air in certain areas such as operating rooms and tuberculosis labs.
- **Ionizing radiation-**
  - e.g. Gamma radiation: has greater energy than U.V. light, therefore more effective. Used mainly in industrial facilities e.g. sterilization of disposable plastic syringes, gloves, specimens containers and Petri dishes.

Chemical Methods

- Some strong chemical substances may be used to achieve sterilization (kill spores) e.g. Gluteraldehyde and Ethylene oxide. Used for heat sensitive equipments.

- Disinfectants /Antiseptics

  eg. phenolics, chlorhexidine, alcohol, etc..

High level disinfectant (HLD)	Intermediate level disinfectant (ILD)	Low-level disinfectants (LLD)
Capable of killing all microorganisms & bacterial spores when used in sufficient concentration under suitable conditions	Destroy all microorganisms, but not bacterial spores	Destroy vegetative bacteria & enveloped viruses; variable action on nonenveloped viruses & fungi, but no action on tubercle bacilli & spores

Chlorine

•Household bleach usually 5-6% Sodium hypochlorite (or ~50,000 ppm)

•In-use dilutions depend on application and amount of organic material present

•Clean surfaces -1,000 ppm Av CL (2% bleach or 0.1% sodium hypochlorite

•General disinfection -5,000 ppm Av Cl (10% bleach or 0.5% sodium hypochlorite)

•Organic material -10,000 ppm Av Cl (20% bleach or 1% sodium hypochlorite).

Alcohols

•Not acceptable for disinfection of Bloodborne Pathogens (per OSHA): i.e., Human tissue culture activities.

•Typically ethyl or isopropyl alcohol: Often used in combination with other disinfectants

•70% in water is most effective concentration: 100% alcohol is not effective!!!

•Should primarily be used in the lab for sanitization:

–Killing environmental organisms

–Removing other disinfectants from surfaces

General Laboratory Cleaning:

•Lab floor ≥weekly with a germicidal detergent

–LPH, 1-Stroke Environ

–Tuberculocidal plus actually clean dirt...

•Lab benches after working

–Disinfectant, such as 2% bleach, Super Sani-Cloth

–Unused areas also need cleaning (dust, mold)

•Sinks ≥weekly

–Disinfectant or normal commercial cleaning products

–Encourage mold growth

•Laboratory equipment (carts, centrifuges, trays) >weekly

Clean all surfaces with a disinfectant before/after working

–Alcohol OK /before work

–Disinfectant after work

•**Human/primate cell culture**

•**Infectious agents**

–2% bleach, Cavicide

-Followed by alcohol

For decontamination of BSCs, occasionally incubators

–Formaldehyde

–Vapor Phase Hydrogen Peroxide

–Chlorine Dioxide

•**Use of these requires training, specialized equipment, special PPE**

•**Algaecides, fungicides:** For water baths and incubators

Factors influencing activity of disinfectants

1. Activity directly proportional to temperature.

2. Directly proportional to concentration up to a point – optimum concentration. After this level no advantage in further increases in concentration.

3. Disinfectants may be inactivated by :

–Dirt
–Organic matter : Proteins, Pus, Blood, Mucus and Feces.
–Non organic: Cork, Hard water and Some plastics.

4. Time : Disinfectants need time to work.

5. Range of Action : Disinfectants not equally effective against the whole spectrum of microbes. e.g. Chlorhexidine less active against Gram negative bacteria than Gram positive cocci.

Hypochlorites and Gluteraldehyde are more active against hepatitis viruses than most other disinfectants**.**

Processing of used items:

D. Storage:

- To prevent contamination after processing
- Do not store instruments or other items such as scalpel blades and suture needles in solution, always store them dry

4. Standard Precautions:
Proper Handling and Disposal of sharps

- Use disposable needle and syringe ONLY ONCE.
- Always wear utility gloves while handling sharps
- Do not disassemble the needle and syringe after use.
- Do not recap, bend or break needles before disposal.
- Make needles unusable after single use by burning them in a needle destroyer
- Never burn syringes
- Dispose off needles and syringes in a puncture-proof container such as metal box, cardboard box or an empty plastic box.
- Finally dispose as follows:

    (i) Dispose the needles and broken vials in pit / tank,

    (ii) Send the syringes and unbroken vials for recycling or landfill

5. Standard Precautions:
Biomedical Waste Disposal

It is the waste that is generated during diagnosis, treatment or immunization of human beings

Purpose of waste disposal:

- Minimize/Prevent the spread of infection to hospital personnel who handle waste
- Prevent the spread of infection to the local community

Steps of waste disposal

A. Segregation

B. Collection and Storage

C. Transportation

D. Treatment and disposal

This workshop recommended:

- Wearing utility gloves
- Transporting and segregate the solid contaminated waste to the disposal site in covered containers
- Disposing of all sharp items in puncture-resistant containers
- Carefully deal with disinfectants according to their types and using the optimum concentration for each type.
- Burning or burying contaminated solid waste
- Washing containers, gloves and hands after disposal of infectious waste