LSUHealthNewOrleans

Standard Operating Procedure (SOP) for Laboratory Disinfection

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Purpose:

This document describes the use of disinfectants for routine laboratory decontamination of surfaces and equipment.

Definitions:

Antiseptic – A substance that inhibits the growth and development of microorganisms without killing them. Usually applied topically to skin.
Decontamination – A process that removes the total burden of all classes of microorganisms, usually using chemicals, heat, and/or pressure.
Disinfectant – A chemical used to reduce the microbial burden on a surface or object. Does not kill spores.
Disinfection – A process that reduces microbial burden on a surface or object.
Inactivation – The process of rendering an organism inert by application of heat or other means.
Microbicide – A chemical that kills all classes of microorganisms. Synonymous with biocide, germicide, and antimicrobial.
Sterilization - A process that removes the total burden of all classes of microorganisms, usually using chemicals, heat, and/or pressure.

Principle:

The effectiveness of disinfectants depends upon the population of organisms present, the concentration of both organism and disinfectant, the duration of contact, and the presence of organic debris.
Common laboratory disinfectants with broad antimicrobial efficacy are working solutions of 70% ethanol and 10% sodium hypochlorite (bleach). It should not be assumed that these or any other common disinfectants are effective against all laboratory pathogens. Prior to selecting a disinfectant for your laboratory, it is important to check the susceptibility and the recommended contact time of the species and strain with which you are working.

Risks:

The potential safety risks for routine laboratory disinfection are:

- Creation of infectious aerosols
- Exposure to respiratory and eye irritants

Proper PPE:

Personal protective clothing and equipment must be worn when using disinfectants:

- The PPE to be worn when working with disinfectants should be commensurate with the highest risk or hazard designation for any single biological agent or material present prior to disinfection.
- Eye and respiratory protection should used whenever the creation of aerosols is possible.

Many disinfectants are respiratory, skin, and/or eye irritants and require the use of appropriate PPE.

Reagents and Solutions:

Alcohols
Ethanol (denatured ethanol, methylated spirits) or isopropanol are used at a dilution of 70%. Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in closed containers to avoid evaporation. Bottles with alcohol-containing solutions must be clearly labeled to avoid autoclaving. Alcohols can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.
To prepare 70% alcohol:
Add 100 ml of 95% ethanol to 39.1 ml of distilled water
Add 100 ml of 90% ethanol to 31.0 ml of distilled water
Add 100 ml of 85% ethanol to 23.1 ml of distilled water
Add 100 ml of 80% ethanol to 15.3 ml of distilled water
Add 100 ml of 75% ethanol to 7.64 ml of distilled water

Glutaraldehyde
Glutaraldehyde does not require dilution, but an activator must be added; the activator is sold separately by the manufacturer). Glutaraldehyde is usually supplied as a 2% solution and the activator is a bicarbonate compound. The activated solution should be used within two weeks and discarded if turbid. It can be reused for 1 – 4 weeks depending on the formulation and type, and the frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions in use. Glutaraldehyde is toxic and a skin and mucous membrane irritant. PPE must be used to prevent contact; it must be used in a fume hood or in well-ventilated areas.

Iodophors
Iodophors are a combination of iodine and inert polymers such as (poly)vinyl-pyrrolidone that reduces surface tension and slowly releases iodine. Iodophors are less irritating than iodine and do not stain. Iodophor preparations should be used at concentrations of 3% to 5% and contact time should be 15 – 30 minutes, depending on the type and volume of material being disinfected. The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter.

Chlorine
Sodium hypochlorite solutions (i.e., bleach) contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively. Bleach, either in stock or in working solutions must be stored in well ventilated dark areas. Under proper storage conditions, stock solution may last as long as 3 months, while diluted solutions should be prepared weekly. Two alternatives should be considered: Granules or tablets of calcium hypochlorite ($Ca(ClO)_2$) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l will contain 1.0 g/l and 5.0 g/l of available chlorine, respectively. Storage of stock or working solutions in open containers releases chlorine gas and weakens their antimicrobial potential. Bleach can be used as a general purpose disinfectant and for soaking contaminated materials that do not contain non-ferrous metals; it is highly alkaline and will corrode non-ferrous metal. It is safe to use on stainless steel.

Phenol
Phenol should be used at a concentration of 2% to 5% in water. Inhalation and dermal exposure to phenol is highly irritating to the skin, eyes, and mucous membranes. Phenol is also designated as highly toxic through oral exposure. Because of its toxicity and odor, phenol derivatives are commonly used in place of phenol. Many phenolic compounds are used for the decontamination of surfaces and some (e.g., triclosan, choroxylenol, orthophenoylphenol) are among the more commonly used antiseptics. Commercially available solutions should be used according to manufacturer's instructions. Phenol solutions can be used for decontaminating equipment and single use items prior to disposal.

Procedure:

Apply disinfectant to contaminated or potentially contaminated area. If visible or gross contamination is present, apply enough disinfectant to saturate the contamination. Let stand for the indicated contact time. Wipe thoroughly and place absorbent material in the biohazardous waste box.
In case of a small (less than 10L) biological spill:

1. **Cover** spill area with paper towels.
2. **Disinfect** by pouring slowly around perimeter and into the center of spill area and let sit for 20 minutes.
3. **Clean** by absorbing with paper towels and place in a biohazard bag.
4. **Disinfect** by re-spraying the spill area with disinfectant; allow to air dry

All work areas and materials that come or may come into contact with biological agents should be disinfected both before and after each use. This provides protection for personnel, the community, the environment, and your experiments.
This procedure should also be followed for spills involving recombinant DNA (rDNA).

Non-routine Disinfection:

For disinfection or decontamination outside of normal operations, such as cleaning a large piece of laboratory equipment prior to disposal, contact the Biological Safety Officer for assistance.
It is necessary to produce an inventory of all biological materials that are or have been stored in the piece of equipment to be decontaminated. Disinfectant(s) will be selected based upon efficacy against the biological agents that are known or suspected to have contaminated the piece of equipment.
The piece of equipment should be saturated with disinfectant to the extent that it's reasonably practicable and allowed to stand the contact time.
If any processing, disassembly, or other modification of the equipment is necessary in order to facilitate decontamination, the Biological Safety Officer will perform a risk assessment and make recommendations on PPE and work practices.

Contact Information
Environmental Health & Safety Office, Biological Safety Officer
(504) 568-6585