University of Nebraska Lincoln

Safe Operating Procedure

(Revised 9/17)

CHEMICAL DISINFECTANTS FOR BIOHAZARDOUS MATERIALS

Chemicals used for biohazardous decontamination are called sterilizers, disinfectants, sanitizers, antiseptics and germicides. These terms are sometimes equivalent, but not always, but for the purposes of this document all the chemicals described herein are disinfectants.

The efficacy of every disinfectant is based on several factors: 1) organic load (the amount of dirt and other contaminants on the surface), 2) microbial load, 3) type of organism, 4) condition of surfaces to be disinfected (i.e., porous or nonporous), and 5) disinfectant concentration, pH, temperature, contact time and environmental humidity. These factors determine if the disinfectant is considered a high, intermediate or low level disinfectant, in that order.

Prior to selecting a specific disinfectant, consider the relative resistance of microorganisms. The following table provides information regarding chemical disinfectant resistance of various biological agents.

Microbial Resistance to Chemical Disinfectants:

More Resistant

Less Resistant

Type of Microbe	Examples
Prions	Bovine spongiform encephalopathy (Mad Cow) Creutzfeldt-Jakob disease
Bacterial Spores	*Bacillus subtilis; Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis; Mycobacterium bovis*
Hydrophillic Viruses (non-lipid, non-enveloped)	*Rhinovirus; Adenovirus*
Fungi	*Cryptococcus sp.; Candida sp.*
Vegetative Bacteria	*Streptococcus pneumonia; Staphylococcus aureus*
Lipophillic Viruses (lipid containing, enveloped)	*Herpes Simplex; Cytomegalovirus; HIV*

The microorganisms listed in the above table are examples, it is always best to perform kill determinations in your own laboratory with your own strains of microorganisms to ensure effective disinfection/sterilization.

Below some common disinfectants are described and they are compared in ***Table 1, Liquid Disinfectant Comparison***.

IMPORTANT: Always refer to the product label for use directions and for the list of agents against which the chemical is effective. Corrosive, flammable or oxidizing chemicals should never be autoclaved.

1. Hypochlorite (Bleach):

Contact time: 10 – 30 minutes.
A 10:1 bleach solution/sodium hypochlorite (also called 10% bleach solution) is made by adding nine parts water to one part laboratory bleach (sodium hypochlorite). Bleach solution is corrosive to stainless steel; therefore, thorough rinsing must follow its use in the biosafety cabinet. Do not autoclave bleach solutions. The present stock bleach solution is 8.25% sodium hypochlorite, so a 10:1 solution will result in a final concentration of 0.825%. The diluted solution should be labeled and dated, with an expiration date of 14 days. Note that household bleach is 5.25% sodium hypochlorite and can be used in a 10:1 solution, but has an expiration date of 24 hours. When used as a broad-spectrum disinfectant it is recommended to use a solution that contains at least 5000 ppm, but not more than 10,000 ppm available chlorine. See the preparation instructions below for how to obtain these solution concentrations.

Hypochlorite solutions are classified as irritant and corrosive. Appropriate precautions should be taken when using hypochlorite products: read labels carefully, adhering to cautionary warnings and following usage directions. Chlorine solutions should never be mixed or stored with cleaning products containing ammonia, ammonium chloride, or phosphoric acid. Combining these chemicals will result in the release of a chlorine gas, which can cause nausea, eye irritation, tearing, headache, and shortness of breath. These symptoms may last for several hours. If you are exposed to an unpleasantly strong odor following the mixing of a chlorine solution with a cleaning product, leave the room or area immediately until the fumes have cleared completely.

To prepare disinfectant bleach solutions:

- Using **8.25% hypochlorite** (Clorox Concentrated) **in a 1:10 dilution** (one part household bleach and nine parts water) yields 8,250 ppm or a 0.825% hypochlorite solution. **Use within 14 days.**
- Using **8.25% hypochlorite** (Clorox Concentrated) in a **1:15 dilution** (one part household bleach and fourteen parts water) yields 5,500 ppm or a 0.55% hypochlorite solution. **Use within 24 hours.**

- Using **5.25% hypochlorite** (Clorox) in a **1:5** dilution (one part Clorox and four parts water) yields 10,500 ppm or a 1.05% hypochlorite solution. **Use within 30 days.**
- Using **5.25% hypochlorite** (Clorox) in a **1:10** dilution (one part Clorox and nine parts water) yields 5,250 ppm or a 0.53% hypochlorite solution. **Use within 24 hours.**

2. Alcohols:

Contact time: 2 - 5 minutes.
70% ethanol or isopropyl alcohol is better than 95% ethanol as a disinfectant. These chemicals can be purchased already diluted or you can prepare your own solutions. A 70% ethanol or isopropyl solution is made by adding 2.5 parts water to 7.5 parts 95% ethanol. Methanol should not be substituted for ethanol or isopropyl, because it is not as effective and is a health hazard. Alcohols evaporate rapidly, so extended contact times are difficult to achieve without immersion. Ensure surfaces stay wet for the minimum contact time. When a longer contact time is required, select a different disinfectant.

These solutions are flammable. Always keep ethanol and isopropyl solutions away from potential sources of ignition. Prolonged and repeated use of alcohol as a disinfectant can also cause discoloration, swelling, hardening and cracking of rubber and certain plastics. Check with the manufacturer for the effective shelf life if buying undiluted solutions. It is recommended that lab-made be labeled and dated, with an expiration date of 6 months from the date it is made.

3. Formalin:

Contact time: 10 – 30 minutes.
Formalin is a 37% solution of formaldehyde gas in water. A 10% formalin solution is roughly equivalent to 4% formaldehyde; at this concentration it is an effective disinfectant. Formaldehyde (formalin) has good disinfectant properties against vegetative bacteria, spores and viruses. It has an irritating odor and is a human carcinogen. Formaldehyde is not recommended for daily disinfection. Use only with proper ventilation control (e.g., chemical fume hood). The shelf life for 10% formalin solution is about 1 week; shelf life is moderately extended in brands that use methanol to prevent polymerization. Breakdown of the solution can be determined by the appearance of precipitate forming. Make a fresh solution if precipitate is visible.

4. Glutaraldehyde: (also called a "cold disinfectant"):

Contact time: 15 – 30 minutes.
Two-percent (2%) solutions exhibit good activity against vegetative bacteria, spores and viruses. Glutaraldehyde is toxic, a sensitizer and is generally not used for laboratory surface disinfectant, and is capable of eye damage. Concentrated glutaraldehyde maintains its concentration for up to one (1) year. Temperature, pH and contamination can adversely affect shelf life. Working solutions of 2-3%

glutaraldehyde can be used for up to 14 days. Store solutions at or below room temperature. Use only with proper ventilation control, such as a chemical fume hood or specially designed slot hood. Example: Cidex.

5. Oxidizing Agents

Contact time: 10 - 60 minutes.
Like chlorine, hydrogen peroxide (H2O2) and peracetic acid are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment. However, they have a short shelf life of just five (5) days for dilute solutions. In their diluted form, these agents are relatively safe but may be irritating and damage clothing when concentrated

Hydrogen peroxide can be purchased as a 30% aqueous solution to be diluted to a working solution. However, it should be noted that dilute solutions of < 6% hydrogen peroxide alone are relatively slow and limited as germicides. Hydrogen peroxide is effective against bacteria, viruses, and fungus and at higher concentrations (>15%) is sporicidal. It has limited activity against mycobacteria. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets.

Peracetic acid is a strong oxidizing agent and is a formulation of hydrogen peroxide and acetic acid. It is effective against bacteria, fungi, spores and viruses. It is also effective against mycobacteria and algae and has some activity in the presence of organic material. Example: Spor-Klenz, OxySept 333®

Hydrogen peroxide and peracetic acid can be corrosive to metals such as aluminum, copper, brass, and zinc, and can also decolorize fabrics, hair, skin, and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

Virkon® S (potassium peroxymonosulfate and sodium chloride) is a peroxygen molecule, organic acid and surfactant combination, with a wide microbial spectrum of activity and some efficacy in the presence of organic material. This comes in powder or tablet form and mixed solutions are good for up to 7 days. The powder is corrosive; use appropriate PPE when preparing solutions. Maximum contact time required is 10 minutes.

6. Phenolic Compounds:

Contact time: 10 minutes.
At a concentration of 0.2-5%, phenolic compounds are effective against vegetative bacteria, fungi and lipid-containing viruses. Phenolic compounds are not suitable for bacterial spores and some hydrophilic viruses. They have an unpleasant odor, can easily be absorbed through the skin and are irritants to the mucous membrane and respiratory tract. The shelf life of working solutions is up to 1 week. Examples: Amphyl, Vesphene IIse, Tek-Trol

WARNING! Phenolic compounds can cause rapid and permanent eye damage--always wear safety glasses, nitrile gloves and lab coat when using or preparing phenolic solutions. Prepare in chemical fume hood.

7. Quaternary Ammonium Compounds (Quats):

Contact time: 10 minutes.
Quats are available as solutions containing one or more quaternary ammonium compounds at concentrations ranging from 0.1 - 2%. This concentration is effective against vegetative bacteria and lipophillic (lipid enveloped) viruses. Quaternary ammonium compounds are not effective against spores and may be neutralized by anionic detergents and organic material. Example: Lysol I.C., Envirocare, Conflikt

8. Iodophor Disinfectant:

Contact time: 10 - 30 minutes.
Iodine compounds are broad spectrum and considered effective for a variety of bacteria, mycobacteria, fungi and viruses. Iodines function by denaturing proteins to interfere with the enzymatic systems of microorganisms. Iodine compounds are often formulated with soaps and considered relatively safe. Concentrated iodine compounds can be irritating to the skin, can stain clothes and damage rubber and some metals. Iodine agents are inactivated by organic material. Prepare iodine solutions according to the instructions on the label. Shelf life is approximately 3 years, but should be marked on the bottle by the manufacturer.

Iodophors are iodine complexes that have increased solubility and sustained release of iodine. One of the more commonly used iodophors is povidone-iodine. They are good for general use and are less readily inactivated by organic matter than elemental iodine compounds. The dilution of iodophors actually increases the free iodine concentration and antimicrobial activity. The final concentration listed in the table reflects the active iodine concentration, which is commonly 1% titratable iodine. Example: Wescodyne, Betadine, Povidone-iodine

Liquid Disinfectant Comparison Table 1

Disinfectant	Quaternary ammonium compounds	Phenolic compounds	Chlorine compounds [5]	Peroxygen Compounds (Virkon® S)	Alcohol (ethyl or isopropyl)	Formaldehyde (liquid) (*Formalin*)	Glutaraldehyde	Hydrogen peroxide (liquid)
Use Requirements								
Final Concentration for use	0.1-2%	0.2-5%	500-10000 ppm available chlorine	1-2%	70-85%	10% formalin solution	2%	6-30%
Contact Times								
Lipo viruses Only	10 min	10 min	10 min	10 min	2-10 min	10 min	15 min	10 min
Broad spectrum	**N/E**	**N/E**	30 min	10 min	**N/E**	30 min	30 min	60 min
Inactivates								
Vegetative bacteria	X	X	X	X	X	X	X	X
Lipo viruses	X	X	X	X	X	X	X	X
Tubercle bacilli		X	X	X	X	X	X	X
Hydrophillic viruses		[1]	X	X	[1]	X	X	X
Bacterial spores			X, [6]			Y	X	X
Important Characteristics								
Effective Shelf Life [2]	>1 week	up to 1 week	24h (5000 ppm); 14d (8000 ppm) 30d (10,000 ppm)	Solution: 7 days	Check with mfg. or 180 days	>1 week	14 days	5 days
Inactivated by organic matter	X		X		X			
Residual		X	X			X	X	
Corrosive		X	X	X				X
Flammable					X			
Irritant: Skin/eye/respiratory	X / X / X	X / X / X	X / X / X	X / X / X	* / X / *	X / X / X	X / X / X	X / X / X
Toxic	X	X	X		X	X	X	
Applications								
Work Surfaces	X	X	X	X	X	X	X	X
Equipment Surfaces	X	X	X & [4]	X	X	X	X	X
Lens Compatible [3]	X						X	
Other considerations	**[8]**	**[7], [8] Unpleasant odor**	**[7]**	**Can be used in foot baths**		**Carcinogen**	**[9]**	**[4] Oxidizer**

KEY:

N/A = Not applicable
N/E = Not effective
X = Effective disinfectant/characteristic
[1] = Variable results dependent on virus
[2] = When protected from light and air
[3] = Refers to microscope and camera lenses
[4] = Will corrode stainless steel and other metals
[5] = 10:1 dilution of 5.25% bleach = 5000 ppm
[6] = >2500 ppm
[7] = Effectiveness reduced by alkaline Ph
[8] = Effectiveness influenced by hard water and detergents
[9] = Usable on plastics, rubber, lenses, and other items that cannot be autoclaved.

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Biological Toxins

- 2N sodium hydroxide (NaOH) for 1 hour (This is a caustic and corrosive material and may damage surfaces and equipment. Wear appropriate PPE.). Neutralize after appropriate contact time.

Prions:

Current recommendations from both the CDC and World Health Organization (WHO) for inactivation of prions include:

Instruments

- Immerse in 1N NaOH or 2.5% NaOCl (sodium hypochlorite; 20,000 ppm available chlorine) for 1 hour; remove and rinse in water, and then transfer to open pan and treat in a gravity displacement (121°C) or porous load (134°C) autoclave for 1 hour; clean; and subject to routine sterilization.

Surfaces

- Spray or pour 1N NaOH or 2.5% NaOCl (sodium hypochlorite; 20,000 ppm available chlorine) on surface and let sit for 1 hour. Ensure surfaces stay wet for entire period, and then rinse twice with water. Surfaces should be clean of any gross contamination as organic material can reduce the effectiveness of the solutions.

Caution: These solutions are corrosive, wear appropriate PPE when using and be aware that they may damage stainless steel surfaces if not rinsed properly.

Solutions of NaOH and NaOCl should be made fresh daily to ensure maximum effectiveness.

Sources:

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