ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 2/22)

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:

Resistant	Type of Microbe	Examples
	Prions	Bovine spongiform encephalopathy (Mad Cow) Creutzfeldt-Jakob disease
	Bacterial Spores	*Bacillus subtilis; Clostridium sporogenes, Clostridioides difficile*
	Mycobacteria	*Mycobacterium bovis, M. terrae, and other Nontuberculous mycobacterium*
	Non-enveloped or Small Viruses	Poliovirus; Coxsackievirus; Rhinovirus; Adenovirus
	Fungi	*Trichophyton spp.; Cryptococcus sp.; Candida sp.*
	Vegetative Bacteria	*Pseudomonas aeruginosa; Staphylococcus aureus; Salmonella choleraesuis; Enterococci*
Susceptible	Enveloped or Medium-size Viruses	*Herpes Simplex; CMV; RSV; HIV; HBV; HCV; Hantavirus, Ebola Virus*

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. The Environmental Protection Agency (EPA) maintains a registry (https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants) of disinfectants that have been tested and shown to be effective against certain microorganisms.

Always select an EPA registered disinfectant known to be effective for the microbe(s) in use in your laboratory.

Disinfectant Categories

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. Always review the Safety Data Sheet for chemical disinfectants for hazards, recommended PPE and proper disposal methods. Corrosive, flammable or oxidizing chemicals should never be autoclaved.

1.1 Hypochlorite (Bleach):

Contact time:	10 – 30 minutes
Shelf Life:	5000-6000 ppm NaOCl: 24 hours; 8000 ppm 14 days; >10000 ppm: 30 days

A 10:1 bleach solution/sodium hypochlorite (NaOCl) (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**! Commercially available bleach can contain 5-8.25% (50,000-82,500ppm) sodium hypochlorite. 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.

Two things you must know about the commercial bleach solution you use:

1. **The concentration of sodium hypochlorite.** This must be >5.25%.
2. **The manufacture date of the solution**. Some manufactures print this date on the bottle, others like Clorox print a code on the bottle, which must be deciphered. https://www.clorox.com/how-to/laundry-basics/bleach-101/bleach-expiration-dates/

Sodium hypochlorite solutions are unstable. When open to the air, chlorine evaporates at a high rate from the solution, rapidly reducing the concentration of free chlorine. When stored at room temperature and away from sunlight, bleach loses 20-50% of its sodium

hypochlorite concentration after 6 months; breaking down into salt and water. **Commercial bleach containers must be disposed of within 1 year of the date of manufacture to ensure that the sodium hypochlorite concentration is always at an effective level.**

> *If you are unable to determine the manufacture date, it is acceptable to label the bottle with the date the bottle was received from the supplier. Then use or dispose of the solution within 1 year of that date.*

Hypochlorite solutions are classified as irritant and corrosive. Take appropriate precautions 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.

Bleach Solution Preparation Table

Starting NaOCl %	Ending NaOCl %	Bleach Solution Ratio	Bleach Dilution	Chlorine concentration	Shelf life*
5.25%	0.53%	1:10	1 part bleach, 9 parts water	5,250 ppm	7 days
5.25%	1.05%	1:5	1 part bleach, 4 parts water	10,500 ppm	30 days
6.15%	0.62%	1:10	1 part bleach, 9 parts water	6,150 ppm	7 days
8.25%	0.55%	1:15	1 part bleach, 14 parts water	5,500 ppm	7 days
8.25%	0.825%	1:10	1 part bleach, 9 parts water	8,250 ppm	14 days

**If stored in opaque container away from heat and light. Translucent or clear containers will speed up decomposition of NaOCl into salt and water.*

1.2 Alcohols:

Contact time:	2-5 minutes
Shelf Life:	~3 years; 1 year for lab-made solutions *See mfg. label for purchased solutions*

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 solutions be labeled and dated, with an expiration date of 1 year from the date it is made. If the solution drops below 70%, it will no longer be an effective disinfectant. Discontinue use if the solution changes color from clear.

1.3 Formalin:

Contact time:	10 – 30 minutes
Shelf Life:	10%: 7 days

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.

1.4 Glutaraldehyde: (also called a "cold disinfectant"):

Contact time:	15 – 30 minutes
Shelf Life:	2-3%: 14 days

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.

1.5 Oxidizing Agents

Contact time:	10 – 60 minutes
Shelf Life:	Dilute solutions (<6%): 5-7 days

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, dilute solutions have a short shelf life of just five to seven days. In their diluted form, these agents are relatively safe but may be irritating and damage clothing when concentrated. When stored properly in dark containers, the decomposition rate is less than 2% per year.

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®, Peridox RTU

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. A 1% solution loses half its strength through hydrolysis in 6 days, whereas 40% peracetic acid loses 1%–2% of its active ingredients per month.

WARNING! These compounds have a strong vinegar odor, are acidic, and can cause irritation to skin, eyes, and mucous membranes. Use in well ventilated areas and/or with appropriate respiratory protection.

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.

1.6 Phenolic Compounds:

Contact time:	10 minutes
Shelf Life:	Working solution: 7 days

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 solutions in a chemical fume hood.

1.7 Quaternary Ammonium Compounds (Quats):

Contact time:	10 minutes
Shelf Life:	~1-5 years

Quats are available as pre-made solutions containing one or more quaternary ammonium compounds at concentrations ranging from 0.1 – 2%. This concentration is effective against vegetative bacteria and lipophilic (lipid enveloped) viruses. Quaternary ammonium compounds are not effective against spores and may be neutralized by anionic detergents and organic material (e.g., dirt, blood, etc.). Example: Lysol I.C., Envirocare, Conflikt

1.8 Iodophor Disinfectant:

Contact time:	10 – 30 minutes
Shelf Life:	~3 years

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, 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

Disinfectants and Inactivation Methods for Other Biological Materials

Human Materials (Blood, tissues, body fluids, cell lines, etc.)

OSHA has stated that any EPA-registered disinfectant effective against HIV and HBV or *Mycobacterium tuberculosis* will be effective in disinfecting and inactivating human materials under the Bloodborne Pathogens Standard (29 CFR 1910.1030). Some effective products that meet this requirement include 10% bleach (>5000ppm NaOCl), CaviCide™ solution or wipes, PDI Sani-Cloth® wipes, and Spor-Klenz®. Refer to the product label for dilution instructions and contact times for effective disinfection.

Disinfection of liquids contaminated with human materials such as cell culture media or body fluids can be effectively decontaminated by following these steps:

1. Add concentrated bleach (>5.25% available chlorine) to a final concentration of 10% bleach
2. Let the solution sit for 30-60 minutes. *Larger volumes may require longer contact times.*
3. Dispose of the solution down the sanitary sewer (drain).

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 2N 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.

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

Biological Toxins

Allow at least a 30-minute contact time for complete inactivation of toxin.

Toxin	2.5% NaOCl + 0.25 N NaOH[1]	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl
Abrin	YES	YES	YES	NO
Botulinum neurotoxins A-G [2]	YES	YES	YES	YES
Brevetoxin (PbTx-2)	YES	YES	NO	NO
Conotoxins	YES	YES	YES	NO
Diacetoxyscirpenol (DAS)	YES	NO	NO	NO
Microcystin	YES	YES	YES	NO
Palytoxin	YES	YES	YES	YES
Ricin [2]	YES	YES	YES	NO
Saxitoxin [2]	YES	YES	YES	YES
Shigatoxin	YES	YES	YES	NO
Staphylococcal enterotoxins [2]	YES	YES	YES	NO
Tetrodotoxin [2]	YES	YES	YES	NO
T-2 Toxin [3]	YES	YES	NO	NO

1. *This is a caustic and corrosive material and may damage surfaces and equipment. Wear appropriate PPE. Neutralize after appropriate contact time.*
2. *Inactivation for Saxitoxin, Tetrodotoxin, Ricin, Botulinum toxin, or Staphylococcal Enterotoxins B (SEB), exposure of 60 minutes to 10% sodium hypochlorite is an effective procedure for working solutions, equipment, working area and spills.*
3. *For complete inactivation of T-2 mycotoxins, all liquid samples, accidental spills, and non-burnable waste be soaked in 2.5% sodium hypochlorite with 0.25 N sodium hydroxide for 4 hours. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 2.5% sodium hypochlorite with 0.25 N sodium hydroxide for 4 hours.*

Container Labeling Requirements

Disinfectant containers must be labelled just like any other chemical container, see the **EHS SOP Chemical Container Labelling** for general guidance. There are a few additional labelling requirements for containers of disinfectant working solutions.

1. The concentration of the disinfectant must be included on the label.
2. The date the solution was prepared must be included on the label.
3. The date the solution expires must be included on the label.

10% bleach
Made: 5/1/19, Exp: 5/8/19

Example Label

These additional requirements are important because the concentration of a disinfectant is important to its effectiveness. The concentration tells you how strong the disinfectant is and the date will tell you if the solution will still be an effective disinfectant. While some working solutions of disinfectants are good up to up to a month or longer after preparation (e.g., alcohols, quats), others need to be made daily or weekly (e.g., 10% bleach).

Disinfectant Selection and Preparation

Use the information in this document to assist you in selecting an appropriate disinfectant for the microbes or materials you are working with. Approved disinfectants for the agents used in your lab are listed in the approved IBC protocol for your lab. If you are unsure of the appropriate disinfectant to use or do not have access to the IBC protocol, contact EHS at 402-472-4925 or ehs@unl.edu.

Labs should establish procedures for regularly checking disinfectant solutions and ensuring they are freshly made at the correct interval and concentration.

Disposal of expired disinfectants

Disinfectants that have lost their effectiveness due to use or expiration must be properly disposed. Most disinfectants are hazardous chemicals in their concentrated form and EHS Hazardous Waste management procedures must be followed. Some disinfectants are safe to dispose of down the sanitary sewer at the working solution concentration; others must be collected by EHS for disposal. Please review the EHS SOPs ***Sewer Disposal List*** and ***Hazardous/Radioactive Material Collection Procedures*** to determine the appropriate means of disposal.

Sources:

- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th ed.; Meechan, P., Potts, J., Eds.; U.S. Department of Health and Human Services, Center for Disease Control and Prevention, National Institutes of Health, 2020.
- Rutala, W. A. (1996). APIC guideline for selection and use of disinfectants. *American Journal of Infection Control*, *24*(4), 313-342.
- Rutala W. A., Cole G., Thomann, C. A., Weber, D. J. (1998). Stability and Bactericidal Activity of Chlorine Solutions. *Infection Control and Hospital Epidemiology* 19(5):323-7
- “Disinfection with Bleach”, 3M Tech Talk, June 2011 https://multimedia.3m.com/mws/media/735976O/disinfection-with-bleach-tech-talk.pdf
- Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute Respiratory Infections in Health Care. Geneva: World Health Organization; 2014. Annex G, Use of

disinfectants: alcohol and bleach. Available from: https://www.ncbi.nlm.nih.gov/books/NBK214356/

- Some material is adapted from Fred Hutchinson Hazard Awareness & Management Manual Chapter 5 https://extranet.fredhutch.org/en/u/ehs/hamm/chap5/section4.html
- https://www.cdc.gov/prions/cjd/infection-control.html
- J Hosp Infect. 2013 Dec;85(4):268-73. doi: 10.1016/j.jhin.2013.08.003
- "Disinfection 101," Center for Food Security & Public Health, Iowa State University www.cfsph.iastate.edu
- Guideline for Disinfection and Sterilization in Health Care Facilities (2008) https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html

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
Enveloped viruses	X	X	X	X	X	X	X	X
Tubercle bacilli		X	X	X	X	X	X	X
Non-enveloped viruses		[1]	X	X	[1]	X	X	X
Bacterial spores			X, [6]			X	X	X
Important Characteristics								
Effective Shelf Life [2]	>1 year *See mfg label.*	up to 1 week	24h (5000 ppm); 14d (8000 ppm) 30d (10,000 ppm)	Solution: 7 days	Check with mfg. or 1-3 years	>1 week	14 days	5-7 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.