Biological Risk Management and Containment

Biological Risk Management and Containment - Chemical Decontamination of Liquid Biohazardous Waste

Cleaning and Decontamination

Containment Laboratory Guidelines

Version 5.1 - July 2023

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Record of Amendments to Version 5

Date	Page number	Nature of amendment
May 2023	8	Correction of disinfectant names
May 2023	9	Changes to stability of dilutions
May 2023	10	Corrections to definitions
May 2023	All	University Logo updated

Contents

1. Who are these guidelines for?

These guidelines are intended for **principal investigators (PIs), designated persons in charge, designated laboratory persons (DLPs)**, technical staff and students trained in the safe use of **risk biologicals** in appropriate containment facilities.

2. When should chemicals be used to decontaminate liquid biohazardous waste?

In general, liquid waste from PC1 and PC2 laboratories containing GMOs **must** be autoclaved before disposal (see exceptions below). Autoclaves **must** be used for the decontamination of high organic load liquid wastes or large volumes of liquid waste.

Small volumes of PC1 liquid waste (<10 mls) containing GMOs may be sent out in medical waste provided the solutions have been chemically decontaminated and the container is tightly capped to prevent leakage.

It is acceptable to use approved chemical disinfectants instead of an autoclave:

- To decontaminate solutions containing uncleared goods from PC1 laboratories.
- To decontaminate liquid waste from vacuum traps.
- To decontaminate virus cultures or media involved in packaging or viral transduction before removal from Class 2 Biosafety cabinet.
- To decontaminate small volumes (<10ml) of PC1 waste containing GMOs which are to be sent for further treatment as medical waste
- To decontaminate solutions containing non-GM, non-sporulating fungi (use sodium hypochlorite at a final concentration of 0.5%)

Note that this process is specific to the chemical treatment of liquid biohazardous waste, or where instruments/pipette tips are soaked in decontaminating agent. For information on decontaminating surfaces such as benchtops, please refer to:

Benchtop/Surface Decontamination. Do not chemically disinfect materials that will be autoclaved, as this may damage sterilising equipment and expose users to hazardous fumes (especially if sodium hypochlorite is used).

- Solutions containing sporulating and filamentous fungi should be autoclaved. Some approved decontamination agents have limited efficacy against sporulating fungi.
- Take care with solutions with organic load. Chlorine-based decontaminating agents in particular become markedly weaker in the presence of organic material.
- Use approved decontamination agents strictly according to these guidelines, with correct observance of final concentrations and contact times.
- All containers containing diluted solutions are to be labelled with the identity of the decontamination agent, its concentration and the expiry date.

3. How to use approved chemical decontamination agents

Approved decontamination agents for the treatment of liquid biohazardous waste are chlorine bleach (sodium hypochlorite), Virkon, Trigene Advance, Chemgene HLD_4L and Accel Prevail.

3.1. Chlorine bleach

Chlorine bleach is a readily obtainable source of sodium hypochlorite (see Appendix 1 for efficacy references). Chlorine bleach is typically supplied as 4% Sodium hypochlorite (4000ppm chlorine).

- To disinfect low organic load wastes, add chlorine bleach to give a final concentration of 0.25% (i.e. add 65 mL of 4% sodium hypochlorite per 1L of waste).
- Chlorine bleach is effective against enveloped and non-enveloped viruses and may be used to treat solutions contaminated with viruses.

- To disinfect organic load wastes (i.e. those containing protein and/or media), add chlorine bleach to give a final concentration of 0.5% (i.e. add 125 mL of 4% sodium hypochlorite per 1 L of waste). Mix and leave to stand for a minimum of 1 hour.
- Treated waste may be disposed of down a laboratory sink *if there are no other hazardous materials in the solution.*

Precautions

- Sodium hypochlorite has a relatively short half-life (approximately nine months for a 5% solution and 3 months for 12% solution) as the hypochlorite degrades over time. Use stock bleach solutions within 12 months of purchase. Refer to Figure 1.
- The efficacy of sodium hypochlorite is severely limited by organic matter in media. For high organic load wastes, the final concentrations of hypochlorite can be increased as high as 1% (although a final concentration of 0.5% is normally used in such cases).
- Handle sodium hypochlorite with care as it is corrosive and will damage stainless steel surfaces and clothing. When you discharge solutions containing hypochlorite into the sewer, rinse stainless steel sinks with copious amounts of water afterwards.
- Make up fresh solutions of hypochlorite each week. Hypochlorite dilutions are stable for **only one week**.
- Store dilutions of hypochlorite (especially diluted solutions) in containers that exclude contact with sunlight. Ultraviolet light significantly hastens the breakdown of hypochlorite.

3.2. Virkon

- Virkon has an active ingredient of Potassium peroxymonosulfate (21.45%) and is supplied as powder or tablets.
- Virkon solutions are to be prepared fresh.
- Virkon is suitable for use with gram negative and positive bacteria and enveloped and non-enveloped viruses, when used according to manufacturer's instructions. See Appendix 1 for efficacy references.

- To disinfect low organic load wastes, dissolve Virkon in waste to a concentration of 1% (e.g. by adding 10 g/L waste), then leave to stand for a minimum of one hour.
- Treated waste may be disposed of down a laboratory sink *if there are no other hazardous materials in the solution.*

3.3. Trigene Advance

- Trigene Advance is a halogenated tertiary amine composed of chlorides, amines and sulfamic acids. It is safe for use on metals.
- Proven effective against a wide range of bacteria and viruses and has a more limited effectiveness against fungi. See Appendix 1 for efficacy references.
- To disinfect low organic load wastes, mix Trigene in liquid waste to a concentration of 1:100 dilution then leave to stand for a minimum of 60 minutes.
- Treated waste may be disposed of down a laboratory sink *if there are no other hazardous materials in the solution*.

3.4. Chemgene HLD_4

- HLD_4 is effective against a wide range of bacteria and viruses and has a more limited effectiveness against fungi. (See Appendix 1 for efficacy references).
- To disinfect low organic load wastes, mix Chemgene HLD_4L in liquid waste at 1:100 dilution, then leave to stand for a minimum of 60 minutes.
- Treated waste may be disposed of down a laboratory sink *if there are no other hazardous materials in the solution*.
- HLD_4L is safe for use on metals.

3.5. Accel

- Accel TB, Accel Prevent and Accel Prevail are activated hydrogen peroxide solutions. All are United States Environmental Protection Authority (EPA)-approved high level disinfectants (see Appendix 1 for efficacy references).

- Accel TB, Accel Prevent and Accel Prevail are effective against a wide range of bacteria, mycobacteria and viruses, when used according to manufacturer's instructions.
- To disinfect liquid biohazardous waste, use Accel Prevail Concentrate in a ratio of 1:40, then leave to stand for a minimum of 20 minutes.
- Treated waste may be disposed of down a laboratory sink *if there are no other hazardous materials in the solution*

4. Limitations

- Use all stocks of liquid decontaminating agents before the manufacturer's expiry dates.
- Hypochlorite dilutions are stable for only one week.
- Virkon is to be used directly in its powdered form.
- Check manufacturer guidelines for stability of diluted solutions of Trigene and Chemgene

5. Verifiable outcomes

1) Use only approved decontamination agents.
2) Ensure the directions for use are posted in the laboratory.
3) Label all containers with the identity of the decontamination agent, its concentration and the expiry date.
4) Ensure all laboratory users understand how to use these agents.

6. Definitions

Approved chemical disinfectants are those disinfectants (listed in Section 4) with proven efficacy as ratified by the United States Environmental Protection Authority (EPA), peer reviewed journals or by European testing regimes. Refer to Appendices 1 and 2 for more information about US and European standard tests.

High organic load liquid waste means waste such as bacterial and eukaryotic cell cultures (where there are $>10^5$ eukaryotic cells per ml), and blood/body fluids that have a high organic load, making chemical sterilisation difficult.

Low organic load liquid waste means waste such as low-density cell cultures (as a guideline $<10^5$ cells per ml), trap waste, used media and culture supernatants amenable to chemical treatment.

Risk biologicals are New Organisms, Unwanted Organisms, genetically modified organisms (GMOs), biological materials that present a potential biosecurity risk and micro-organisms with a risk classification of Risk Group 2 or higher, as defined by the United States National Institute of Health "Guidelines for Research Involving Recombinant DNA Molecules".

Designated laboratory person (DLP) means the trained person in each research group who has been given the authority to receive purchase requests made in SciTrack and to make a formal request for a purchase order via the Shared Transaction Centre. In containment and transitional facilities DLPs will have additional training to enable them to scrutinise documentation for restricted items and provide support to researchers.

Designated person in charge means a staff member in any of the following roles: sector manager, facility manager, floor manager, technical manager or an appointed delegate.

Principal Investigator (PI): In the context of hazard containment and transitional facilities, a principal investigator is the holder of an independent grant administered by the University and the lead researcher for the grant project, usually in the sciences, such as a laboratory study or a clinical trial. The phrase is also often used as a synonym for "head of the laboratory" or "research group leader." The PI is responsible for assuring compliance with applicable University standards and procedures, and for the oversight of the research study and the informed consent process. Although the PI may delegate tasks, they retain responsibility for the conduct of the study.

7. References

Block SS, ed. (2001). Disinfection, Sterilization and Preservation, 5th ed. Lippincott, Williams & Wilkins, Philadelphia. ISBN: 0683307401.
Perez, Justo *et al*. Activity of Selected Oxidizing Microbicides Against the Spores of Clostridium difficile: Relevance to Environmental Control. American Journal of Infection Control. 2005.

8. Appendix 1: Efficacy of approved decontamination agents

Sodium hypochlorite

Sodium hypochlorite is a broad-spectrum disinfectant that is active against gram positive and gram- n e g a t i v e vegetative bacteria, enveloped and non-enveloped viruses, fungi, mycobacteria, and bacterial endospores. (Disinfection, Sterilization, and Preservation. Block, S. S., 2001). A six log10 reduction of *Bacillus subtilis* endospores by a 5000ppm solution of hypochlorite was accomplished after a contact time of under ten minutes.

A hypochlorite solution of 1000ppm was still active against *Bacillus subtilis* spores at a reduction rate of at least six log10 reduction (Perez, Justo *et al*. Activity of Selected Oxidizing Microbicides Against the Spores of Clostridium difficile: Relevance to Environmental Control. 2005).

Virkon

Virkon is a United States Environmental Protection Authority (US EPA)-approved disinfectant passing the AOAC Use Dilution Test Method (refer to Appendix 3) for norovirus, MRSA and VRE, and HCV when used according to manufacturer's instructions.

Virkon is thus suitable for use with gram negative and positive bacteria and enveloped and non-enveloped viruses, when used according to manufacturer's instructions. Virkon has been shown to be an effective fungicide passing the AOAC Use Dilution Test Method.

Trigene Advance/ Sterigene

Using tests detailed in Appendix 2, Trigene has been shown to achieve:

- >Log 5 bactericidal activity against a range of gram negative and positive bacteria under European test methodologies including EN 13727 and EN 1276 when used at 1:200 dilution
- >Log 4 fungicidal activity against a range of fungi using European test methodologies including EN 13624 and EN 1667 when used at 1:200 dilution

- >Log 6 sporicidal activity against a range of spore forming bacteria such as Bacillus subtilis and Clostridium spp using European test methodologies including EN 13704 when used at 1:100 dilution
- Complete inactivation of a range of enveloped and non-enveloped viruses at 1:100 dilution, using EPA approved AOAC Use Dilution Test Method (refer to Appendix 2)

Chemgene HLD_4

Using tests detailed in Appendix 2, HLD_4 has been shown to achieve:

- >Log 5 bactericidal activity against a range of gram negative and positive bacteria under European test methodologies including EN 13727 and EN 1276 when used at 1:100 dilution
- >Log 4 fungicidal activity against a range of fungi using European test methodologies including EN 1650 and EN 1275 when used at 1:100 dilution
- >Log 6 sporicidal activity against a range of spore forming bacteria such as Bacillus subtilis and Clostridium spp, using European test methodologies including EN 13704 when used at 1:50 dilution
- Complete inactivation of a range of enveloped and non-enveloped viruses at 1:50 dilution using EN 14476:2005

Accel Prevail

Accel Prevail is an activated hydrogen peroxide solution which is US EPA-approved high-level, passing the AOAC Use Dilution Test Method (refer to Appendix 3) for a wide range of resistant gram positive and gram negative bacteria.

9. Appendix 2: Test methods for efficacy of disinfectants

CEN TC 216 European Union Tests for Efficacy of Disinfectants

1) EN 1276 Quantitative Suspension Test of Bactericidal Activity of Chemical Disinfectants

Test organisms	Purpose of test	Typical performance criteria (requirements may vary by claim)
Staphylococcus aureus Pseudomonas aeruginosa Enterococcus hirae Escherichia coli	Suspension-based study formally used to evaluate bactericidal activity.	5 log reduction in ≤5 minutes

1) EN 13727 Chemical Disinfectants and Antiseptics: Quantitative Suspension Test for Bactericidal Activity for Instruments Used in the Medical Arena

Test organisms	Purpose of test	Typical performance criteria (Requirements may vary by claim)
Staphylococcus aureus Pseudomonas aeruginosa Enterococcus hirae	Suspension-based study formally used to evaluate bactericidal activity of products that are used in the medical area (e.g. hygienic hand rub, hygienic hand wash, surgical hand rub, surgical hand wash, instrument disinfection etc.)	3-5 log reduction in 1-5 minutes depending on claim

2) **EN 13624 Chemical Disinfectants and Antiseptics: Quantitative Suspension Test for Fungicidal Activity for Instruments Used in the Medical Area**

Test organisms	**Purpose of test**	**Typical performance criteria (requirements may vary by claim)**
Candida albicans *Aspergillus brasiliensis*	Suspension-based study formally used to evaluate fungicidal activity of products that are used in the medical area for disinfecting instruments by immersion.	4 log reduction in ≤60 minutes

10. Appendix 3: United States Tests for Efficacy of Disinfectants: The AOAC Use Dilution Test Method

Summary of test

The AOAC Use Dilution Test Method is a carrier-based method used to evaluate disinfection efficacy of water- soluble powders and non-volatile liquid products. In this method, a series of stainless steel cylinders ("carriers") are inoculated with a representative test organism and the carriers are dried. The carriers containing the dried organism film are then sequentially immersed into 10 mL of disinfectant and are exposed to the disinfectant for a pre-determined exposure time. After exposure, the carriers are sequentially transferred to a liquid subculture medium specifically selected to neutralize the test substance active and to recover any surviving test organism.

The carriers are incubated and visually examined for the presence or absence of growth.

Method name	Method number	Test organisms	Test parameters	Carrier count acceptance criteria	Performance criteria for efficacy
AOAC Official use dilution method	955.15	*Staphylococcus aureus* (ATCC 6538) Salmonella choleraesuis (ATCC 10708)	Three separate batches tested on three independent test days 60 replicate carriers for each test ≤ 10 minute exposure time	Mean Log10 Density 6.0-7.0	≤3 positive (growth) carriers out of 60 tested
	946.02	*Pseudomonas aeruginosa* (ATCC 15442)			≤6 positive (growth) carriers out of 60 tested

Figure 1 Decay of hypochlorite