*This document is under review, if you have any input, please email safety@unsw.edu.au.

HS324 Disinfection of Tissue-Culture Waste Guideline

Introduction

The purpose of this document is to provide a practical approach to the chemical disinfection of tissue-culture waste. The Australian / New Zealand Standard for Safety in Laboratories Part 3: Microbiological safety and containment (AS/NZS 2243.3:2010) states that all waste that is contaminated or potentially contaminated with microorganisms 'shall be decontaminated' before disposal. This includes wastes containing genetically modified organisms.

Scope

At UNSW, tissue-culture waste from all facilities, irrespective of the physical containment (PC) level shall be considered potentially contaminated and therefore must be appropriately decontaminated before disposal. If the culture has been chemically decontaminated, it must be disposed of as chemical waste.

This Guideline provides information on the two most common disinfectants, **chlorine, and iodine**, used to chemically decontaminate tissue-culture waste, and provides information on the appropriate dilutions required for each method. These two disinfectants have a broad spectrum of activity against microorganisms and may be suitable for decontamination of liquid tissue-culture waste, depending upon the type of work being undertaken. The effectiveness of most disinfectants is reduced in the presence of organic matter so the suitability of any disinfectant should first be determined by a risk assessment.

The properties of these two disinfectants are more fully described in AS/NZS 2243.3:2010 appendix F.

The use of disinfectants such as Virkon, Viraclean, F10 and such, is increasing around the University but these products are intended to be used as a surface disinfectant only. There is no supporting documentation from the manufacturers to support their use for disinfecting tissue-culture waste. Unless you can provide

the evidence of validation of their effectiveness, including concentration, conditions and duration, the use of these as tissue-culture decontaminants is not supported.

Disinfectants

2.1. Chlorine

Effectiveness

The most effective concentration of available chlorine is in the range 0.5 - 5%. A higher concentration of chlorine may be required if there is a large amount of organic material in the waste.

The most effective pH is in the range of 6-8. If the pH is too acidic (<pH 6), the solution will fail to oxidize the cell lipids and proteins, and therefore you will not have achieved effective decontamination.

Depending on where it is purchased, the % available chlorine can vary significantly. For example, it may be 4%, 10% or 12.5% available chlorine.

You must check the concentration of the stock solution each time new stock is received into the laboratory. If the stock is greater than 4%, dilute the stock down to 4 - 5% available chlorine in order to begin at the correct dilution and ph. A higher concentration of chlorine is *not* necessarily better as this affects the pH.

See APPENDIX A Table 1, Chlorine dilution, for the volume of **4%** sodium hypochlorite (4% starting concentration) required to achieve a final concentration of 1% available chlorine.

Classed as a high-level disinfectant, Chlorine is suitable for the decontamination of waste containing gram positive and negative bacteria, mycobacteria (a possible infective agent in all cell lines), fungi, enveloped and non-enveloped viruses, and prions.

For blood / serum or viral cultures, a concentration of 0.5 - 1% and a minimum contact time of 10 minutes is recommended.

It is ***NOT effective*** for the decontamination of waste containing bacterial endospores.

If used as a surface disinfectant, requires the addition of a surfactant.

Chlorine combines rapidly with proteins and other organic material and therefore the amount of organic material in the tissue-culture waste needs to be considered. The concentration of chlorine needs to be increased accordingly to ensure there is sufficient available to decontaminate the waste.

For mycobacteria or prions

2% chlorine and a minimum contact time of 60 minutes is required.

Stability

Chlorine is unstable and therefore it needs to be prepared fresh each time it is used for decontamination. The effective strength of the diluted chlorine decreases over time and degrades on exposure to sunlight.

To purchase

Sodium hypochlorite stock solutions can be purchased from any supermarket and hardware store, and from the UNSW web store.

2.2. Iodine

Effectiveness

The effective concentration of iodine is in the range 0.5 – 2.5% with a minimum contact time of 10 minutes.

It is most effective at a neutral to acidic pH.

Classed as an intermediate level disinfectant, it is suitable for the decontamination of waste containing gram positive and negative bacteria, fungi, and enveloped viruses.

Iodine is ***NOT effective*** for the decontamination of waste containing bacterial endospores, mycobacteria (a possible infective agent in all cell lines), non-enveloped viruses or prions.

See APPENDIX A Table 2, Povidone-Iodine dilution, to achieve a final concentration of 1% iodine from a 10% stock solution.

Stability

Iodine will decompose above 40C, releasing iodine vapour which is toxic; otherwise, it is considered an irritant.

Iodine reacts more slowly with organic material and so the level of inactivation is likely to be less significant (compared to chlorine) unless the iodine is in a dilute solution.

To purchase

Iodine solutions are more conveniently handled as a povidone-iodine solution (polyvinylpyrrolidone iodine), which is usually a 10% solution.

Povidone-iodine solution (10%) can be purchased from the UNSW web store or other suppliers. Check the starting concentration of the solution by reading the label on the container.

A 10% povidone-iodine solution is not a hazardous chemical or substance.

Procedure for Chlorine and Iodine

3.1. Validation

Any process used to decontaminate waste should be validated periodically (as determined by a risk assessment) to ensure that the process continues to work effectively and as expected.

3.2. Labelling

All containers used for the collection of waste must be labelled in accordance with the Laboratory Hazardous Waste Disposal Guideline (HS321). For example, a label for tissue-culture waste to be treated with bleach would be as follows (example):

Waste Category:	Chemical waste
Specific hazard information: (including dangerous good class, if relevant)	1% Chlorine Bleach-treated tissue-culture waste Non-hazardous
Waste Generator:	*Person responsible for the waste*
Date:	*Date or period over which the waste was generated*
Room:	*Where the waste was generated (laboratory/facility room number)*

3.3. Chlorine/tissue-culture waste label

Labels must include:

- "Non-hazardous, chlorine-treated tissue-culture waste"; or
- "Non-hazardous, chlorine-treated GMO tissue-culture waste"

3.4. Iodine/tissue-culture waste label

Labels must include:

- "Non-hazardous, iodine-treated tissue-culture waste"; or
- "Non-hazardous, iodine-treated GMO tissue-culture waste"

3.5. Method

Any containers used for collection should be emptied and decontaminated between collections of waste. The contents should not merely be 'topped up' with disinfectant.

Collect the tissue-culture media waste in an empty, labelled, graduated bottle/container of the appropriate capacity. For example, for volumes less than 350 ml waste, use a bottle with capacity for at least 500 ml, and for 350 – 500 ml of waste, use a bottle with capacity 1L.

To adequately disinfect the waste, the final volume (waste plus disinfectant) must have the appropriate *final* concentration. Refer to the relevant table in Appendix A for the volume of disinfectant to be added to a given volume of waste in order to arrive at the effective % concentration of disinfectant.

Mix well and leave for at least 10 minutes.

For bleach disinfection, if the tissue-culture waste was initially a pink colour (due to the presence of phenol red in the media) it should change to a yellow/clear colour when chlorine has effectively decontaminated the waste.

Transfer the treated waste to a labelled plastic waste container that will be collected by the chemical waste contractor. Plastic waste cubes are available from the Faculty of Science web store in a variety of volumes. When selecting a suitable volume container, consider the volume of waste generated and the weight of the full container, which will impact upon safe manual handling of the waste container.

3.6. Disposal

When the plastic waste container is full, it is disposed of as chemical waste through UNSW Chemical Waste System.

For waste collection enquiries, contact emgeneralservices@unsw.edu.au.

3.7. Waste labelling

Label waste containers in accordance with the Laboratory Hazardous Waste Disposal Guideline (HS321). For example, a label for bleach treated non-GMO tissue-culture waste would be as follows:

Waste Category:	Chemical waste
Specific hazard information: (including dangerous good class, if relevant)	1% Chlorine Bleach-treated tissue-culture waste Non-hazardous
Waste Generator:	*Person responsible for the waste*
Date:	*Date or period over which the waste was generated*
Building:	*Name your building*
Room:	*Where the waste was generated (laboratory/facility room number)*

References

4.1. References

Australian Standards:

- AS/NZS2243. Safety in laboratories:
- Part 2: chemical aspects; and
- Part 3: microbiological safety and containment.

4.2. Contacts:

- UNSW Stores
- Waste collection enquiries
- emgeneralservices@unsw.edu.au
- EM General Services Ph 9385 5111

Appendix A: dilution tables

Table1 Chlorine dilutions: the volume of 4% sodium hypochlorite to be added to waste to achieve a final concentration of 1% sodium hypochlorite**.**

Volume of Tissue-Culture Waste (ml)	Volume of 4% hypochlorite required (ml) (final conc 1% hypochlorite)
10	3.5
20	7
30	10
40	14
50	17
100	34
150	50
200	67
250	84
300	100
350	117
400	134
450	150
500	167

Note: if the volume of waste generated is not on this table, use the volume of 4% hypochlorite for the next highest volume of tissue-culture waste.

Bleach dilution table courtesy St George Clinical School, Faculty of Medicine & Health, UNSW

Table 2 Iodine dilutions: the volume of 10% povidone-iodine to be added to waste to achieve a final concentration of 1% iodine.

Volume of Tissue-Culture Waste (ml)	Volume of 10% povidone-iodine required (ml) (final conc 1% iodine)
10	1.5
20	2.5
30	3.5
40	4.5

50	6
100	11.5
150	17
200	22.5
250	28
300	33.5
350	39
400	45
450	50
500	56

Note: if the volume of waste generated is not on this table, use the volume of 10% povidone-iodine for the next highest volume of tissue-culture waste.

Iodine information courtesy of ACP, Faculty of Medicine & Health, UNSW

Appendix B: References

Australian Standards:

- AS/NZS2243. Safety in laboratories:
- Part 2: chemical aspects; and
- Part 3: microbiological safety and containment.

Contacts:

- UNSW Stores
- Waste collection enquiries
- emgeneralservices@unsw.edu.au
- EM General Services Ph 9385 5111

Appendix C: Version control

Version	Authorised by	Approval Date	Effective Date	Sections modified
1.0	Director, Human Resources	10 October 2012	10 October 2012	New document
1.1	Director, Human Resources	14 April 2013	14 April 2013	Updated Branding Logo in accordance with UNSW Branding Guidelines. Modified the document identifier from OHS to HS in accordance with WHS legislation review
1.2	Director, UNSW Safety and Sustainability	30 April 2014	30 April 2014	Reviewed for administrative updates
1.3	Director, UNSW Safety and Sustainability	5 August 2015	5 August 2015	Revise re: National Audit Tool and update links to new HS website
2.0	Director, Risk & Safety Management	8 August 2022	8 August 2022	Reviewed for administrative updates, template update and removed from Governance website.

Updates to this document

Any suggestions, recommendations or updates to this document should be emailed to safety@unsw.edu.au with the email header stating *GUIDELINES UPDATE HS324*.