



Policy Hierarchy link	Work Health and Safety Policy HS323 Biosafety Procedure		
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Superseded Documents	HS324 Disinfection of Tissue-Culture Waste Guideline Version 1.2, approved 30 April 2014		
File Number	2015/19789		
Associated Documents	HS321 Laboratory Hazardous Waste Disposal Guideline		
Version	Authorised by	Approval Date	Effective Date
1.3	Director, UNSW Safety and Sustainability	5 August 2015	5 August 2015

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1. Introduction and Scope

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.

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 use of chlorine and iodine as chemical disinfectants to decontaminate tissue-culture waste by providing 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 Virkon and F10 disinfectants is increasing in use around the University. These are intended to be used as a surface disinfectant and 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 Virkon and/or F10 as tissue-culture decontaminants is not supported.

2. Definitions/Synonyms

- **Chlorine** – sodium hypochlorite or bleach.
- **Iodine** - Iodine/Iodophor, such as povidone-iodine.

3. Disinfectants

3.1 Chlorine

3.1.1 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. Therefore the amount of organic material in the tissue-culture waste needs to be considered and the concentration of chlorine 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.

3.1.2 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.

3.1.3 To purchase

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

3.2 Iodine

3.2.1 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.

3.2.2 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.

3.2.3 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.

3.3 Virkon

3.3.1 Effectiveness

Virkon is a peroxygen compound disinfectant with surfactants. It is primarily used as a surface treatment for non-metallic surfaces, and is suitable for the disinfection of medical devices, textiles, laboratory benches and equipment. As a surface disinfectant, the Virkon solution needs a pH of approximately 2 - 3, and contact time of at least 10 minutes.

It can also be used in powder form directly onto spills of liquid biological hazards, then scooped up for disposal as chemical waste.

Virkon is effective against a wide variety of viruses, bacteria and fungi, but it is **NOT effective** for the decontamination of prions.

For surface decontamination, make up a 1% solution of Virkon as described by the manufacturer using tepid water: Note that a concentration higher than 4% Virkon physically cannot be achieved due to solubility.

There is no supporting data as yet for the effectiveness of Virkon to decontaminate liquid tissue-culture waste. For example, it is not known whether diluting Virkon, such as a 2% Virkon solution 1:1 with tissue culture waste will allow the required pH and chemical pathway that leads to successful decontamination.

Unless you can provide the evidence of validation of effectiveness use, including concentration, conditions and duration, the use of Virkon as tissue-culture decontamination is not supported.

3.3.2 Stability

1% solutions of Virkon should be made up weekly however, to ensure good infection control, it is recommended that solutions should be discarded daily, especially when the colour fades from pink towards clear.

It is not known whether the stability is affected by the concentration.

4. Procedure for chlorine and iodine

4.1 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.

4.2 Labelling

All containers used for the collection of waste must be labeled 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:	<i>Person responsible for the waste</i>
Date:	<i>Date or period over which the waste was generated</i>
Room:	<i>Where the waste was generated (laboratory/facility room number)</i>

4.2.1 Chlorine/tissue-culture waste label

Labels must include:

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

4.2.2 Iodine/tissue-culture waste label

Labels must include:

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

4.3 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, labeled, 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 labeled 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.

4.4 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 fmgeneralservices@unsw.edu.au.

4.4.1 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:	<i>Person responsible for the waste</i>
Date:	<i>Date or period over which the waste was generated</i>
Building:	<i>Name your building</i>
Room:	<i>Where the waste was generated (laboratory/facility room number)</i>

5. References and Appendices

5.1 References

[Virkon S– broad spectrum disinfectant information sheet](#)
[Australian Standards:](#)

- AS/NZS2243. Safety in laboratories:
 - Part2: chemical aspects; and
 - Part 3: microbiological safety and containment.

5.2 Acknowledgement

- Bleach dilution table courtesy St George Clinical School, Faculty of Medicine, UNSW; and
- Iodine information courtesy of ACP, Faculty of Medicine, UNSW.

5.3 Contacts for UNSW Stores:

- Upper campus Ph: 9385 2007, Fax: 9385 1568
Located in the Lowy Cancer Research Centre (Building C25) on the Lower Ground Floor room LG08. Items can be ordered by logging in to the [online store](#).

Middle/lower campus 9385 4695
Located in the Heffron Building (G5), Ground Floor

5.4 Appendix A: dilution tables

5.4.1 Table1 Chlorine dilutions

Table 1 shows the correct volume of 4% sodium hypochlorite to be added to tissue-culture 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.

5.4.2 Table 2 Iodine dilutions

Table 2. Correct volume of 10% povidone-iodine to be added to tissue-culture 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.

5.5 Appendix B: Document History

The authorisation and amendment history for this document must be listed in the following table. Refer to information about [Version Control](#) on the Governance Support website.

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