Protocol for Disinfection of Cell Culture and Tissue Culture in Media:

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DECONTAMINATION OF CELL CULTURE WASTE

Cell culture has become a common laboratory technique indispensible to current research. The liquid aspirated off during media changes can contain cells that must be inactivated prior to disposal.

1. Commercial bleach is the leading choice for the decontamination of cell culture waste because its mechanism of action has been proven effective against containment level 1 and 2 animal cell lines. The hypochlorite ions are strong oxidants that irreversibly react with thiol groups of essential enzymes rendering them ineffective.

2. Once you have collected a volume of liquid that fills ¾ of the collector flask (Flask A), add enough commercial bleach to create a 10% solution (1:10 v/v)). It is imperative that you add the disinfectant once the flask is filled with waste water due to the shelf life/stability of the hydroxide ion. The concentration of available chlorine diminishes by approximately 50% after 30 days of storage. Undiluted commercial bleach can be stored for 6 months at room temperature. After 6 months, bleach will degrade at a rate of 20% each year.

3. Wear the appropriate gloves, lab coat and eye protection whenever using chemical disinfectants.

4. In order to avoid harm caused by the inhalation of fumes, the best practice is to place the waste container or flask, in the fume hood while being filled and throughout the contact period.

5. A minimum contact time of 30 minutes is necessary to ensure its efficacy.

6. After 30 minutes, the solution can be poured into the 4L Aqueous Waste Container.

7. Once the 4L waste is full, it is taken to the Chemistry Stockroom water waste container for proper disposal.

SURFACE DISINFECTION

1. Initially, and wearing the proper gloves, goggles, and lab coat, remove any soiled material from the workspace. This should be performed using absorbent towels, and disposed in the biohazard waste container.

2. A 70% ethanol solution is used best for surface cleaning. !0% bleach is best not used on metal surfaces, due to the ability to oxidize and corrode the surfaces. Stick with the 70%ethanol solution. It is broad-spectrum and is compatible with most materials.

3. Cover the surface with the disinfectant.

4. Ensure the surface remains wet for the specified contact time.

5. Wipe the surface with absorbent material until dry. If the chemical disinfectant is autoclave safe, the absorbent material must be disposed of in an autoclave bag. If the chemical disinfectant can not be autoclaved, imbibed absorbent material can be properly disposed of in regular waste upon a sufficient contact time.

DISINFECTION OF CONTAMINATED PIPETTE AND NANOSPRAY TIPS

1. Place tips in a properly labeled beaker in the fume hood. Add a 10% solution of bleach, and let soak for 30 minutes.

2. Be sure the solution covers the tips completely.

3. 100% microbial kill requires a contact time of 30 minutes.

4. Empty solution into 4L Aqueous Waste Container. Place disinfected tips in Biological Waste Sharps Container.

DISINFECTION OF CONTAMINATED GLASSWARE

1. Wear the appropriate gloves, lab coat and eye protection whenever using chemical disinfectants.

2. When needed for glassware decontamination, prepare the appropriate microbicidal solution as in bleach, prepare a 10% v/v solution. After 30 days of storage, the available chlorine in a bleach solution diminishes by approximately 50%. With low levels of organic material, a 5% bleach solution

is sufficient for disinfection of glassware. Therefore, 10% bleach baths must be changed on a monthly basis and more frequently if the organic load is high.

3. Completely submerge the glassware and leave soaking for an appropriate contact time.

4. Do a final wash with Alconox or Versa-Clean solution and rinse thoroughly.

Safety Aspects of Cell Culture

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Fundamental Techniques in Cell Culture Laboratory Handbook-2nd Edition

3.2 Biohazards

Viruses pathogenic for humans are one of the most likely biohazards presented by cell cultures. Where infection with an agent pathogenic for humans is known or suspected, the cell culture should be handled at a containment level appropriate for the agent concerned. Other potential biohazards should also be considered. These relate to components of the cell culture medium, other adventitious agents (e.g. contaminating mycoplasmas), and cell products, some of which may be biologically active molecules with pharmacological, immunomodulating or sensitising properties. In addition, the generation and use of modified cells, for example, hybrids, transformed cells and cells containing recombinant DNA can be hazardous. These procedures could potentially result in the appearance of modified or reactivated viruses, novel fusion/hybrid proteins (especially in cross-species hybrids) and the expression of viral or cellular oncogenes.

Laboratory workers should never culture their own cells. *In vitro* transformation or genetic modification could result in malignant disease or expression of an unusual pharmacologically active protein if they were to be accidentally inoculated into the donor. Therefore, human cells should be obtained from individuals having no association with the experimental work.

Biohazardous waste should be disposed of according to the methods described under ‘3.5 Waste Disposal’.

3.4 Disinfection

Methods designed for the disinfection/decontamination of culture waste, work surfaces and equipment represent important means for minimising the risk of harm. Always wear appropriate personal protective equipment (PPE) such as gloves and eye protection when using concentrated forms of disinfectants. The selected gloves should protect against the substance being handled and meet the European standard EN374-3. Manufacturers’’ charts will help to identify the best gloves for the work.

The major disinfectants fall into four groups and their relative merits can be summarised as follows:

Hypochlorites (e.g., Sodium Hypochlorite)

- Good general purpose disinfectant
- Active against viruses
- Corrosive against metals and therefore should not be used on metal surfaces e.g. centrifuges
- Readily inactivated by organic matter and therefore should be made fresh daily
- Should be used at 1000ppm for general use surface disinfection, 2500ppm in discard waste pots for disinfecting pipettes, and 10,000ppm for tissue culture waste and spillages

Note: When fumigating a cabinet or room using formaldehyde all the hypochlorites must first be removed as the two chemicals react together to produce carcinogenic products.

Phenolics
Phenolic based disinfectants should never be used as they are not supported as part of the EU Biocidal Products Directive review programme.

Alcohol (e.g. Ethanol, Isopropanol)

- Effective concentrations: 70% for ethanol, 60-70% for isopropanol
- Their mode of activity is by dehydration and fixation
- Effective against bacteria. Ethanol is effective against most viruses but not non-enveloped viruses
- Isopropanol is not effective against viruses

Aldehydes (e.g. Formaldehyde)

- Aldehydes are irritants and their use should be limited due to problems of sensitisation
- Should only be used in well ventilated areas.

Formaldehyde is used to fumigate laboratories. The formaldehye is heated in a device so it will vaporise and all exposed surfaces are coated with the disinfectant.

Generally the use of aldehydes for disinfection and fumigation purposes can be hazardous. Check local regulations and with your safety advisor.

3.5 Waste Disposal

Any employer has a ‘duty of care’ to dispose of all biological waste safely in accordance with national legislative requirements. Given below is a list of ways in which tissue culture waste can be decontaminated and disposed of safely. One of the most important aspects of the management of all laboratory-generated waste is to dispose of waste regularly and not to allow the amounts to build up. The best approach is ‘little and often’. Different forms of waste require different treatment.

- Tissue culture waste (culture medium) – inactivate for at least 2 hours in a solution of hypochlorite (10,000ppm) prior to disposal to drain with an excess of water.
- Contaminated pipettes should be placed in hypochlorite solution (2500ppm) overnight before disposal by autoclaving and incineration.
- Solid waste such as flasks, centrifuge tubes, contaminated gloves, tissues, etc., should be placed inside heavy-duty sacks for contaminated waste and incinerated.
- If at all possible waste should be incinerated rather than autoclaved.
- Waste from specially licensed laboratories e.g. those handling genetically modified level 3 (GM3) organisms requires specific treatment and tracking.