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CHAPTER 2

CLEANSING LABORATORY WARE AND EQUIPMENT

1. INTRODUCTION

Laboratory ware (laboratory glassware and plasticware) and equipment must be carefully cleaned to avoid trace chemical contaminants that may introduce toxic substances. When laboratory ware is not chemically clean, laboratories often experience considerable losses in personnel time and supplies. These losses may temporarily suspend research activities with down time occurring when experiments clearly have been adversely affected. The failure to ensure the absence of residues on laboratory glassware, plasticware or equipment also may result in fluctuations in data that are often attributed to experimental error.

Deionized distilled water should be used as the final rinse in the cleansing process. However, distilled water may be used in place of deionized distilled water for rinsing whenever quality control tests show that distilled water is adequate.

Chemical contaminants that adversely affect experimental results can be difficult to detect. The problem of improper washing is usually worse in large laboratories with central preparation facilities that are staffed with personnel ineffectively trained in processing virological laboratory ware and equipment. The key to an effective preparation facility lies in the careful training of hands-on personnel who must understand that a preparation facility is not a kitchen. It

is, of course, imperative that the supervisor of the preparation facility understands and appreciates the need to meet established specifications for chemically clean laboratory ware. Competent supervisors who understand the need can achieve the necessary quality of cleanliness, even with personnel who do not grasp the concepts involved. High performance standards in the cleansing of laboratory ware and equipment are essential to assure the validity of the product in all subsequent laboratory operations.

All personnel within their particular laboratories must exercise independent judgment to determine the adequacy of the safety activities currently in practice. Consequently, they must be made aware of the hazards associated with the monitoring for waterborne viruses and of each worker's responsibility for his or her own safety.

1.1 Precautions.

1.1.1 Sterilize contaminated laboratory ware and equipment before cleansing them (see Chapter 3).

1.1.2 Soiled laboratory ware and equipment are difficult to clean when allowed to dry.

1.1.3 During the washing process, do not allow laboratory ware or equipment to dry until after the final rinse in deionized distilled water. Detergent that has dried on laboratory ware or equipment is difficult to remove.

1.1.4 Monitor routinely for proper operation of the distilled and deionized water sources. See Chapter 4 for servicing and monitoring of the distilled and deionized water sources.

1.1.5 Select detergent which is compatible with area water and leaves behind no undesirable residues on the cleansed laboratory ware and equipment. See Section 1.1.8 for testing for undesirable residues.

1.1.6 Transport strong acids only in appropriate safety carriers.

1.1.7 Once detergent solution or acid used to clean a vessel has been rinsed away, do not touch lip or inside of vessel with hands. Detergent or acid on hands or gloves and oil even from clean skin are sources of contamination.

1.1.8 Check cleansed laboratory ware and equipment for acid residues in accordance with procedures given in Section 2.5. Detergents used in washing may contain inhibitory substances.

As necessary, test for the presence of inhibitory residues (e.g. a new supply of detergent). For appropriate procedure, see Standard Methods for the Examination of Water and Wastewater, 17th Edition, American Public Health Association, Washington, D.C., 1989, p. 9-8.

1.1.9 Use nontoxic stainless steel, nontoxic glass, nontoxic nonbreakable plastic, or other nontoxic materials for plumbing that carries deionized distilled water. Do not use copper plumbing. Do not use plumbing that contains any ions that may be toxic.

1.1.10 If a washing machine is used, ensure that jets are strong enough to reach all parts of deep vessels. Ensure also that jets are not so powerful they fill narrow-necked vessels and prevent draining during the time that water is being delivered. Check daily that jets and drains are not clogged. Ensure that washing machine operates properly. Check weekly timing of wash and rinse cycles. Descale lime deposits with descaler when necessary.

1.1.11 Use only cold water for tap water rinsing. Hot water may contain grease or oil removed from plumbing.

1.1.12 Use only cold water to wash laboratory ware heavily contaminated with proteinaceous material. Hot water may coagulate such material.

1.1.13 Inspect washed laboratory ware and equipment for cleanliness. Recleanse unclean laboratory ware. Overnight soaking or brush-washing with hot detergent solution may be required prior to machine washing.

1.1.14 Check laboratory ware and equipment for damage. Laboratory ware and equipment that is chipped, cracked, excessively scratched or otherwise damaged beyond repair should be replaced.

1.1.15 In a multipurpose laboratory in which different levels of cleanliness are required, user must code all laboratory ware and equipment to cleansing specifications required for laboratory studies. Cleansing problems, breakage, and damage must be reported to user.

1.2 Alternate Procedures.

1.2.1 Disposable laboratory ware may be used when available.

1.2.2 Cleansing procedures described herein are adequate for most laboratory situations. These procedures focus

specifically at rendering laboratory ware and equipment suitable for cell culture preparation.

1.2.3 Less rigorous procedures may be used when quality control tests show they are adequate for laboratory's needs. When cleansing with detergents (Section 3.1), all vessels may not require overnight soaking or brush-washing with hot detergent solution prior to machine washing.

1.2.4 Some critical laboratory ware are washed without detergent or acid to avoid the possibility of retaining undesirable residues. Cleaning reagents may render the surface of laboratory ware unsatisfactory for propagating cell monolayer or render them unsuitable due to difficulties removing residues that are toxic to cells.

1.2.5 When contaminants refractory to detergent procedures are encountered on acid-resistant laboratory ware or equipment, nitric acid or, if necessary, chromic acid may be used to cleanse them. Spent chromic acid, being a hazardous waste, is not disposed of by conventional means. Contact safety or waste control officer for waste management guidelines for its environmentally safe disposal. Nitric acid must be neutralized before disposal into the sewage system. Neutralize by adding acid to a large volume of an ice water solution of sodium hydroxide. For concentrated acid use 6 M sodium hydroxide.

2. PREPARATION OF CLEANSING COMPOUNDS AND REAGENTS

2.1 Liquid Detergent Compound for Machine-Washing Laboratory Ware and Equipment (MIR-A-KOL, product no. 03030, Du Bois Chemical Co., or equivalent). Use according to manufacturer's instructions.

2.2 Detergent Powder for Hand-Washing Laboratory Ware and Equipment (Buell Cleaner, product no. 222, Polychem Corp., or equivalent). Use according to manufacturer's instructions.

2.3 Nitric Acid, 10 percent. To prepare 10 percent nitric acid, pour 100 mL of concentrated nitric acid slowly into 900 mL of cold deionized distilled water. To avoid dangerous splatters, never pour water into concentrated acid (see also precautions noted under Section 3.2).

2.4 Chromic Acid (dichromate solution), 1 percent. Chromic acid should be used only when stubborn contaminates are not effectively removed by other cleaning reagents. Replacement products for chromic acid have been reported by several manufacturers (examples of such products are: Nochromix, Godax Laboratories; PCC-54 concentrate or RBS-35 concentrate, Pierce

Chemical Company; Isoclean concentrate, Isolab Incorporated). To prepare 1 percent chromic acid, dissolve 25 g of sodium dichromate ($Na_2Cr_2O_7$) or potassium dichromate ($K_2Cr_2O_7$) in 2.5 liters of concentrated sulfuric acid. Potassium and sodium dichromate are strong oxidizing agents and must be handled cautiously. Take care to avoid exposure to acid (see precautions noted under Section 3.2).

2.5 Test for Acid Residues on Laboratory Ware.

2.5.1 Select several pieces of clean glassware or plasticware for testing.

2.5.2 Add a few drops of 0.04 percent bromthymol blue into each of the selected pieces. Prepare 0.04 percent bromthymol blue indicator by adding 16 mL of a 0.01 M solution of NaOH to 0.1 g of bromthymol blue and dilute to 250 mL with distilled deionized water.

2.5.3 Observe color reaction. Color reaction should be blue-green in the neutral pH range. A yellow color reaction identifies the presence of an acid residue. The presence of an alkaline residue is distinguished by a blue color reaction.

3. PROCEDURES FOR CLEANSING LABORATORY WARE AND EQUIPMENT

All laboratory ware and equipment must be decontaminated before cleansing. A summary of decontamination procedures is given in Section 5. See Chapter 3 for the detailed steps to decontaminate laboratory ware and equipment. Laboratory ware and equipment may be cleansed in several ways. Those used for cell cultures may require special care (see Section 1.2.4).

3.1 Cleansing with Detergent.

3.1.1 General Laboratory Ware and Washable Equipment.

(a) Mechanical washing procedure. Lightly soiled laboratory ware and washable equipment may not require Steps (a.1) and (a.2). Equip washing machine so as to deliver four deionized distilled water rinses. Additional deionized distilled water rinses may require machine operation using manual cycle. The water jets in some washing machines are not strong enough to reach all walls inside tall vessels. This results in poor washing and rinsing. The water jets in other washing machines are too strong for test tubes and similar vessels and for many other narrow-necked vessels. Jets that are too powerful hold detergent and rinse water in place and do not allow such vessels to drain properly. If washing machine is unable to wash or rinse adequately, use manual washing procedure

described in Section 3.1.1, Step (b).

(a.1) Immerse decontaminated laboratory ware and washable equipment in detergent solution and soak them overnight. If any laboratory item is too large to immerse, fill it to brim with detergent solution and soak it overnight.

(a.2) Brush-wash soaked items with hot detergent solution. Hot tap water that exceeds 50 degrees C is adequate for preparing detergent solution.

(a.3) Machine-wash vessels. Follow manufacturer's instructions carefully. Add four deionized distilled water rinses if not included in manufacturer's instructions. The added four deionized distilled water rinses may require machine operation using manual cycle.

(a.4) Drain and dry washed items at ambient temperature or dry in hot-air chamber.

(a.5) Sterilize the cleansed item by appropriate method. See Chapter 3 for sterilization procedure.

(b) Manual washing procedure. Use fresh detergent solution daily. Solutions that are saved may become heavily contaminated with bacteria.

(b.1) Immerse decontaminated laboratory ware and washable equipment in detergent solution. Soak lightly soiled laboratory ware and washable equipment for 1 hour and heavily soiled items overnight.

(b.2) Brush-wash soaked items with hot detergent solution. Hot tap water that exceeds 50 degrees C is adequate for preparing detergent solution.

(b.3) Swish-rinse washed laboratory ware and washable equipment ten times with cold tap water. To swish-rinse, pour into the vessel or apparatus a volume of tap water equal to about 10 percent of its volume and swish water around entire surface with each rinse.

(b.4) Swish-rinse vessel or apparatus five times with deionized distilled water.

(b.5) Drain and dry rinsed items at ambient temperature or dry in hot-air chamber.

(b.6) Sterilize the cleansed item by appropriate method. See Chapter 3 for sterilization procedure.

3.1.2 Test Tubes.

Test tubes may be washed by procedure described in Section 3.1.1, Step (a) unless a washing machine is unavailable or washing machine jets are so powerful they do not allow adequate evacuation of tubes and thus interfere with washing and rinsing. In either event, the procedure that follows may be used instead of the washing machine procedure.

(a) Place decontaminated test tubes open end up into covered wire basket, place basket into stainless steel or plastic vessel sufficient in size to allow complete immersion of tubes, and fill vessel with hot detergent solution (50-60 degrees C).

(b) Brush-wash test tubes by hand or machine. Heavily soiled test tubes should be soaked overnight before brush washing.

(c) Empty tubes and flush with cold tap water. To achieve thorough rinsing, introduce tap water into bottom of vessel with a hose connected to tap.

(d) Fill and empty tubes ten times with cold tap water.

(e) Fill and empty tubes five times with deionized distilled water. Run hose from deionized distilled water line to bottom of vessel to achieve thorough rinsing.

(f) Drain and dry tubes at ambient temperature or dry in hot-air chamber.

(g) Sterilize screw-cap or cotton-plugged test tubes.

(g.1) Place screw-cap or cotton-plugged tubes in test tube racks.

(g.2) Sterilize test tubes in dry heat oven for one hour. Oven temperature is maintained at 170 degrees C.

(h) Sterilize test tubes with caps or semipermeable plastic inserts.

(h.1) Protect mouths of tubes with caps or with semipermeable plastic inserts.

(h.2) Place tubes with caps or plastic inserts in test tube racks.

(h.3) Sterilize tubes by autoclaving at 121 degrees C for 30 minutes.

3.1.3 Pipettes.

(a) Remove cotton plugs from decontaminated pipettes. If necessary, remove cotton plugs by forcing a jet of air or water through delivery tips of pipettes.

(b) Place pipettes, with tips up, into pipette holder.

(c) Place pipette holder into a pipette jar and fill jar with hot detergent solution. Hot tap water that exceeds 50 degrees C is adequate for preparing detergent solution. Pipettes must be completely immersed. If air bubbles are present in pipettes, raise and lower pipette holder several times to remove bubbles.

(d) Soak pipettes in detergent solution overnight. Raise and lower pipette holder five or six times during this time period to agitate detergent solution and thus help remove soil and debris from pipettes. Pipettes that are lightly soiled may not require soaking overnight in detergent solution.

(e) Place pipette holder into automatic pipette washer and rinse pipettes through ten cycles of cold tap water.

(f) Rinse pipettes through five cycles of deionized distilled water.

(g) Remove pipettes from automatic pipette washer and allow pipettes to drain and dry at ambient temperature.

(h) Plug pipettes with cotton.

(i) Place pipettes in pipette canisters and sterilize in dry heat oven for one hour. Oven temperature is maintained at 170 degrees C.

3.1.4 Automatic Pipettor (Brewer-type).

To ensure continual proper operation of the automatic pipettor, fill reservoir with tap water immediately after pipettor has been used and carefully pump sufficient water through the system to remove cellular debris and other materials that might adhere to apparatus. Water pumped through automatic pipettor is returned to reservoir. Decontaminate automatic pipettor and reservoir as a unit. Cleanse apparatus according to instructions given in Steps (a) through (d). To prevent contamination of cell cultures, do not use detergent or acid to clean pipettor.

(a) Disassemble decontaminated automatic pipettor from

reservoir. Cleanse automatic pipettor as a unit -- syringe, tubing, valve, and cannula (see Step (d) for cleansing reservoir).

(b) Force 250 to 500 mL of deionized distilled water through automatic pipettor.

(c) Allow pipettor to drain and dry at ambient temperature.

(d) Reservoir.

(d.1) Fill reservoir with hot detergent solution. Hot tap water that exceeds 50 degrees C is adequate for preparing detergent solution.

(d.2) Brush-wash reservoir with hot detergent solution. If reservoir remains soiled, rinse with cold tap water and treat with 10 percent nitric acid or if necessary with 1 percent chromic acid (see Section 3.2.1). Take care to avoid exposure to acid (see precautions noted under Section 3.2).

(d.3) Swish-rinse reservoir ten times with cold tap water. To swish-rinse, pour into the vessel a volume of tap water equal to about 10 percent of the volume of the vessel, and swish water around entire surface with each rinse.

(d.4) Machine-wash reservoir. Follow manufacturer's instructions carefully. Add four deionized distilled water rinses if not included in manufacturer's instructions. The added four deionized distilled water rinses may require machine operation using manual cycle. If reservoir cannot be mechanically washed, proceed to swish-rinse vessel an additional five times with cold tap water and five times with deionized distilled water. Drain and dry reservoir at ambient temperature or dry in hot air chamber.

(e) Pour 10 to 20 mL of deionized distilled water into reservoir.

(f) Cover opening of reservoir with aluminum foil or Kraft paper.

(g) Protect cannula with glass tube cover, and wrap syringe, interconnecting tubing and protected cannula in cloth or Kraft paper.

(h) Reconnect tubing to reservoir.

(i) Autoclave assembled apparatus at 121 degrees C for 60 minutes. Use slow exhaust.

(j) Allow apparatus to cool and store in closed cabinet until needed.

3.1.5 Automatic Syringe (Cornwall-type).

To ensure continual proper operation of the automatic syringe, fill reservoir with tap water immediately after pipettor has been used and carefully pump sufficient water through the system to remove cellular debris and other materials that might adhere to apparatus. Water pumped through automatic syringe is returned to reservoir. To prevent chemical contamination of cell culture preparations, do not use detergent or acid to clean pipettor.

(a) Disassemble decontaminated automatic syringe from reservoir. Reservoir is generally a flask or bottle which is cleaned as regular glassware by the method described in Section 3.1.1. Cleanse decontaminated automatic syringe as a unit, syringe, tubing, valve, and cannula. If syringe has not been delivering properly, check inlet and outlet valves. Replace either valve, or both valves, if damaged or hardened.

(b) Force 250 to 500 mL of deionized distilled water through automatic syringe.

(c) Allow syringe to drain and dry at ambient temperature.

(d) Protect cannula with glass tube cover, and wrap syringe, interconnecting tubing and protected cannula in cloth or Kraft paper.

(e) Autoclave apparatus at 121 degrees C for 30 minutes. Use slow exhaust.

(f) Allow apparatus to cool and store in closed cabinet until needed.

3.1.6 Disc Filter Holder.

(a) Disassemble decontaminated filter holder and discard membrane.

(b) Rinse filter holder components with 2 to 5 liters of cold tap water. If debris remains after tap water rinse, brush-wash filter holder with hot (50-60 degrees C) detergent solution. Remove refractory debris with nonabrasive scrubber. Use fine grade steel wool only if absolutely necessary. Rinse again with 2 to 5 liters of cold tap water.

(c) Rinse filter holder components with 1 to 2 liters of

deionized distilled water.

(d) Allow filter holder components to drain and dry at ambient temperature. Check gaskets for distortion (flattened areas), and replace gaskets if necessary. If filter holder is to be used for the VIRADEL disc filter procedure in Chapter 5, proceed to Step (e). If filter holder is to be used in the preparation of media for cell culture, proceed to Step (i).

(e) Reassemble filter holder.

(f) Cover the ports of filter holder with aluminum foil or Kraft paper.

(g) Autoclave filter holder at 121 degrees C for 30 minutes.

(h) Allow filter holder to cool and store in closed cabinet until needed.

(i) Attach tube to outlet port of filter holder.

(j) Attach glass filling bell to outlet port tubing.

(k) Place filter support on base of filter holder.

(l) Add 10 to 20 mL of deionized distilled water to base of filter holder.

(m) Place membrane filter or filters on filter support.

(n) Reassemble filter holder. Loosely tighten down top of filter holder.

(o) Add 10 to 20 mL of deionized distilled water through inlet port of filter holder.

(p) Cover filling bell and inlet port with aluminum foil or Kraft paper.

(q) Autoclave filter and filter holder at 121 degrees C for 30 minutes. Use slow exhaust.

(r) Allow filter holder to cool.

(s) Loosely secure top of holder and store in closed cabinet until needed. If bolts are kept tightly fastened during storage, filter may subsequently crack.

3.1.7 Dispensing Pressure Vessel.

(a) Remove lid from decontaminated dispensing pressure vessel.

(b) Rinse pressure vessel and lid with 4 to 5 liters of cold tap water. If debris remains after tap water rinse, brush-wash vessel and lid with hot (50-60 degrees C) detergent solution. Remove refractory debris with nonabrasive scrubber. Use fine grade steel wool only if absolutely necessary. Rinse again with 4 to 5 liters of cold tap water.

(c) Swish-rinse pressure vessel and lid five times with deionized distilled water. To swish-rinse, pour into the vessel a volume of water equal to about 10 percent of the volume of the vessel, and swish water around entire surface with each rinse.

(d) Allow vessel and lid to drain and dry at ambient temperature.

(e) Wrap lid and cover vessel opening and inlet and outlet ports with aluminum foil or Kraft paper. Be certain vent/relief valve on vessel is open.

(f) Autoclave vessel and lid at 121 degrees C for 30 minutes.

(g) Close vent/relief valve on vessel.

(h) Store in closed cabinet until needed.

3.1.8 Plastic Screw-Caps.

(a) Pour water from vessel containing decontaminated caps and rinse caps with 5 to 10 liters of cold tap water. Run hose from tap to bottom of vessel to achieve thorough rinsing.

(b) Add deionized distilled water to vessel containing rinsed caps and autoclave at 121 degrees C for 15 minutes.

(c) Rinse caps with 4 to 5 liters of deionized distilled water. Run hose from deionized distilled water line to bottom of vessel to achieve thorough rinsing.

(d) Place caps with opening positioned downward on towel and allow caps to drain and dry at ambient temperature.

(e) Place caps with opening positioned downward in glass petri plates.

(f) Place petri plates in petri plate canister.

(g) Autoclave caps at 121 degrees C for 45 minutes. Use fast

exhaust and a 15 minute dry cycle. Leave top off canister during autoclaving to allow penetration of steam.

(h) Secure cover on canister, allow canister and contents to cool, and store in closed cabinet until needed.

3.1.9 Cartridge Filters and Filter Holders.

(a) Filter and filter holder from VIRADEL cartridge filter procedures. The VIRADEL cartridge filter procedures are described in Chapter 6.

(a.1) Remove filter from cartridge filter holder and decontaminate filter and filter holder. Cartridge filters are decontaminated by steam sterilization. Filter holders are decontaminated chemically with either ethylene oxide or chlorine.

(a.2) Discard filter.

(a.3) Rinse filter holder components with 10 to 15 liters of cold tap water. If debris remains after tap water rinse, brush-wash filter holder with hot (50-60 degrees C) detergent solution. Remove refractory debris with nonabrasive scrubber. Rinse again with 10 to 15 liters of cold tap water.

(a.4) Rinse filter holder components with 5 to 10 liters of deionized distilled water.

(a.5) Allow filter holder components to drain and dry at ambient temperature.

(a.6) Reassemble filter holder.

(a.7) Cover the ports of filter holder with Kraft paper if holder is to be chemically sterilized with ethylene oxide. Proper aeration after gas sterilization is essential. Do not cover ports if holder is to be treated with chlorine. After chlorine treatment cover ports with sterile aluminum foil or sterile Kraft paper.

(a.8) Chemically sterilize filter holder according to procedure given in Chapter 3.

(b) Prefilter and filter holder from media preparation. Media preparation is described in Chapter 9. Until clogged, the prefilter may be reused for media preparation. Dispose of clogged filters in plastic-lined trash can.

(b.1) Backwash cartridge prefilter with 100 liters of deionized

distilled water.

(b.2) Disassemble filter holder.

(b.3) Remove prefilter from cartridge holder and drain and dry prefilter at ambient temperature.

(b.4) Resterilize prefilter according to manufacturer's instructions.

(b.5) Swish-rinse filter holder ten times with cold tap water. To swish-rinse, pour into apparatus a volume of tap water equal to about 10 percent of its volume and swish water around entire surface with each rinse.

(b.6) Swish-rinse filter holder five times with deionized distilled water.

(b.7) Allow filter holder components to drain and dry at ambient temperature.

(b.8) Reassemble filter holder.

(b.9) Cover the ports of filter holder with Kraft paper if holder is to be chemically sterilized with ethylene oxide. Sterilization of filter holder with ethylene oxide is an optional step. If gas sterilizer is not available, distilled water rinse and air drying is sufficient treatment for filter holder used for housing of prefilter. Proper aeration after gas sterilization is essential.

3.2 Cleansing with Acid.

Either 10 percent nitric acid or, if necessary, 1 percent chromic acid may be used to cleanse glassware. Cleansing with nitric acid necessitates longer contact time than is required with chromic acid, but residual nitric acid is not as likely to be toxic to cell cultures as residual chromic acid. Also spent chromic acid is not disposed of by conventional means. Replacement products for chromic acid have been reported. Examples of such products are given in Section 2.4. Do not expose metals or other materials to acids unless certain that those substances are acid-resistant. Acids may react violently with organics or other oxidizable substances. Take care to avoid such reactions. Cleanse laboratory ware with detergent solutions before cleansing them with acids. Nitric and chromic acids are strong acids capable of producing severe burns even when used in relatively dilute solutions. Safety procedures are described in part 1090 of the 17th edition of Standard Methods for the Examination of Water and Wastewater and in Material

Safety Data Sheets provided by chemical manufacturers in compliance with OSHA's Hazard Communication Standard. When working with these or with other strong acids, avoid inhalation of fumes. Protect eyes with safety goggles or with full face mask. Protect clothing with acid-resistant laboratory coat or apron. If eyes are accidentally exposed to acid, immediately flush them with copious quantities of water for at least 15 minutes. Consult a physician immediately thereafter. If other parts of the body are exposed to acid, immediately remove clothing over exposed areas and wash exposed areas with copious quantities of water. Consult a physician if affected area is large or if exposure has been lengthy.

3.2.1 General Acid-resistant Laboratory Ware and Equipment.

(a) Nitric acid procedure.

(a.1) Fill vessel to brim with 10 percent nitric acid. Small laboratory ware and equipment components may be immersed directly in a vat of acid. Take care to avoid splatter and do not allow acid to contact skin. When necessary, wear acid-resistant gloves. Gloves must possess good gripping qualities, because acid makes vessels slippery.

(a.2) Allow 10 percent nitric acid to remain overnight in contact with vessel surface.

(a.3) Pour neutralized nitric acid down sewer drain and flush with tap water. Neutralize by adding acid to a large volume of an ice water solution of sodium hydroxide. For concentrated acid use 6 M sodium hydroxide.

(a.4) Fill and empty acid-washed vessel ten times with cold tap water. Be certain that all acid is removed from outside of vessel and that the initial rinse is neutralized before disposal down sewer drain.

(a.5) Swish-rinse vessel five times with deionized distilled water. To swish-rinse, pour into the vessel a volume of water equal to about 10 percent of the volume of the vessel, and swish water around entire surface with each rinse.

(a.6) Drain and dry vessel at ambient temperature or dry in hot-air chamber.

(a.7) Sterilize vessel by appropriate method (see Chapter 3).

(b) Chromic acid procedure. Chromic acid should be used only when other cleansing procedures fail. Glassware and other acid-resistant laboratory ware cleansed with chromic acid may

retain some chromium ions even after extensive rinsing. For some work, these ions may be undesirable. Chromic acid may be toxic to cells. Glassware and other laboratory ware used for cell culture work, if washed with chromic acid, may subsequently need to be washed with detergent solution to remove chromium ions (see Section 3.1).

(b.1) From an acid reservoir pour into vessel a volume of 1 percent chromic acid equal to about 10 percent of the capacity of the vessel. Small laboratory ware and equipment components may be immersed in a vat of acid. Take care to avoid splatter. Do not allow acid to contact eyes or skin. Wear eye protection and an acid-resistant laboratory coat or an acid-resistant apron. Wear acid-resistant gloves. Gloves must possess good gripping qualities, because acid makes vessels slippery.

(b.2) Rotate vessel so that acid covers entire inside area of vessel.

(b.3) Allow chromic acid to remain in contact with vessel for about five minutes.

(b.4) Pour acid from vessel into acid reservoir. Chromic acid is reusable until oxidized (green). If chromic acid is oxidized, dispose of it safely as with other hazardous wastes. Contact safety or waste control officer for waste management guidelines for its environmentally safe disposal.

(b.5) Fill and empty acid-washed vessel ten times with cold tap water. Be certain that all acid is removed from outside of vessel and that the initial rinse water is disposed of safely.

(b.6) Swish-rinse vessel five times with deionized distilled water. To swish-rinse, pour into the vessel a volume of water equal to about 10 percent of the volume of the vessel, and swish water around entire surface with each rinse.

(b.7) Drain and dry vessel at ambient temperature or dry in hot-air chamber.

(b.8) Sterilize vessel by appropriate method (see Chapter 3).

3.2.2 Test Tubes.

(a) Place tubes open end up into covered acid-resistant basket and place basket into acid-resistant vessel.

(b) Fill vessel with 10 percent nitric acid or, if necessary, with 1 percent chromic acid. Take care to avoid splatter. Do not allow acid to contact eyes or skin. Wear eye protection and

an acid-resistant laboratory coat or an acid-resistant apron. Wear acid-resistant gloves. Gloves must possess good gripping qualities, because acid makes tubes slippery. If 10 percent nitric acid is used, allow acid to remain overnight in contact with tubes. If 1 percent chromic acid is used, allow acid to remain in contact with tubes for about five minutes.

(c) Pour acid from tubes. Chromic acid is reusable until oxidized (green) and may be poured back into reservoir. If chromic acid is oxidized, dispose of it safely as with other hazardous wastes. Contact safety or waste control officer for waste management guidelines for its environmentally safe disposal. Pour neutralized nitric acid down sewer drain and flush with tap water. Neutralize by adding acid to a large volume of an ice water solution of sodium hydroxide. For concentrated acid use 6 M sodium hydroxide.

(d) Run cold tap water into vessel to flush acid from tubes.

(e) Fill and empty tubes in vessel ten times with cold tap water. Be certain that the initial rinse water is disposed of safely.

(f) Fill and empty tubes in vessel five times with deionized distilled water. Tubes for cell culture work that have been cleansed with chromic acid must be cleansed with detergent solution. For such tubes, proceed to Section 3.1.2.

(g) Drain and dry tubes at ambient temperature or dry in hot-air chamber.

(h) Sterilize screw-cap or cotton-plugged tubes.

(h.1) Place screw-cap and cotton-plugged tubes in test tube racks.

(h.2) Sterilize tubes in dry heat oven for one hour. Oven temperature is maintained at 170 degrees C. See Section 3.1.8 for processing plastic screw caps.

(i) Sterilize tubes with caps or semipermeable plastic inserts.

(i.1) Protect mouths of tubes with caps or with semipermeable plastic inserts.

(i.2) Place tubes with caps or plastic inserts in test tube racks.

(i.3) Sterilize tubes by autoclaving at 121 degrees C for 30 minutes.

3.2.3 Pipettes.

(a) Remove cotton plugs from decontaminated pipettes. If necessary, remove cotton plugs by forcing a jet of air or water through delivery tips of the pipettes.

(b) Place pipettes, with tips up, into an acid-resistant plastic pipette holder.

(c) Carefully place pipette holder into pipette jar filled with 10 percent nitric acid. Take care to avoid acid splatter. Do not allow acid to contact eyes or skin. Wear eye protection and an acid-resistant laboratory coat or an acid-resistant apron. Wear acid-resistant gloves. Gloves must possess good gripping qualities, because acid makes pipettes and pipette holders slippery.

(d) Carefully raise and lower pipette holder several times to force air bubbles from pipettes.

(e) Soak pipettes overnight in acid. Carefully raise and lower pipette holder five or six times during this time period to agitate acid and thus help remove contaminants and debris from pipettes.

(f) Carefully remove pipette holder from pipette jar and place holder and pipettes in automatic pipette washer. Take care to avoid acid splatter.

(g) Immediately rinse pipettes through fifteen cycles of cold tap water. Do not allow acid dripping from pipettes to remain in contact with metal parts of automatic pipette washer. Acid may damage metal.

(h) Rinse pipettes through five cycles of deionized distilled water.

(i) Remove pipettes from automatic washer and allow pipettes to drain and dry at ambient temperature.

(j) Plug pipettes with cotton.

(k) Place pipettes in pipette canisters and sterilize in dry heat oven for one hour. Oven temperature is maintained at 170 degrees C.

4. WASTE DISPOSAL GUIDELINES

Reasonable waste disposal measures must be taken to protect workers, the public and the environment. Emphasis should be

placed on recycling both nonhazardous and decontaminated hazardous wastes. Where effective, reduce waste at the generating source by reuse of materials.

Some localities have standards for generators of infectious wastes. Contact the local authority for hazardous waste management to determine whether infectious waste regulations have been enacted.

4.1 Disposal of Infectious Waste.

4.1.1 Supplies.

(a) Place disposable solid contaminated waste materials in plastic bag certified for infectious waste use by manufacturer. Waste materials include clothing, towels, and plastic and nonbroken glass laboratory ware. Plastic bags must be autoclavable, leakage- and tear-resistant and imprinted with biohazard symbol.

(b) Place disposable contaminated sharp waste materials in rigid container certified for sharp material use by manufacturer. Sharp waste material include hypodermic needles, broken glass and other laboratory ware with cutting or piercing edges. Sharp waste containers must be autoclavable, rigid, and puncture and leakage resistant and must be marked with a biohazard symbol.

(c) Decontaminate filled plastic bags or sharp waste containers. Decontamination procedures are summarized in Section 5. See Chapter 3 for the detailed steps to decontaminate waste materials. Use care while handling bags so as not to puncture them. Containers should be closed tightly to prevent loss of contents while handling them. If bags or containers must be moved from laboratory area for decontamination, they must be securely sealed to prevent leakage and transported in rigid, sturdy receptacles that are taped closed. The receptacles must be properly labelled with the biohazard symbol and the location and name of the person disposing of the infectious waste.

(d) After decontamination, label plastic bags or containers NONHAZARDOUS.

(e) Minimize waste production by recycling waste materials whenever applicable; otherwise dispose of waste in appropriate trash receptacle.

4.1.2 Disposal of Agar Media.

(a) Use autoclavable, leakage- and tear-resistant plastic bags imprinted with biohazard symbol.

(b) Place plastic bags in a durable leakproof receptacle. Containers to be recycled may be placed in a metal pan. After rendering the waste noninfectious, spent agar media from containers may be flushed with tap water into sewer system.

(c) Decontaminate filled plastic bags Decontamination procedures are summarized in Section 5. See Chapter 3 for the detailed steps to carry out decontamination of the agar media. Use care while handling the bag so as not to puncture the bag. If bags must be moved from laboratory area for decontamination, they must be securely sealed to prevent leakage and transported in rigid, sturdy receptacles that are taped closed. The receptacles must be properly labelled with the biohazard symbol and the location and name of the person disposing of the infectious waste.

(d) After decontamination, label plastic bags NONHAZARDOUS.

(e) Dispose of waste in appropriate trash receptacle.

4.1.3 Disposal of Liquid Media.

(a) Decontaminate liquid media. Decontamination procedures are summarized in Section 5. See Chapter 3 for the detailed steps to decontaminate liquid media.

(b) Flush with tap water decontaminated spent liquid media into sewer system.

4.2 Disposal of Nonhazardous Waste.

4.2.1 Glassware.

(a) Place broken or disposable glassware in plastic-lined puncture-resistant container.

(b) Minimize waste production by recycling waste glassware whenever possible; otherwise dispose of waste in appropriate trash receptacle.

4.2.2 Plasticware.

(a) Place disposable plastic laboratory ware in plastic-lined trash can.

(b) Minimize waste production by recycling disposable plastic laboratory ware whenever possible; otherwise dispose of waste

in appropriate trash receptacle.

4.3 Disposal of Chemical Wastes.

4.3.1 Hazardous Chemicals.

(a) Collect chemical in a suitable container. Container must be sturdy, nonleaking and sealable.

(b) Label container with the name of the chemical waste, its hazardous properties (e.g. corrosive, caustic, toxic, flammable, irritant), quantity, date of disposal, and location and name of person disposing of the waste.

(c) Transfer chemical to hazardous waste storage facility for ultimate disposition by safety or waste control officer.

4.3.2 Acids.

(a) Add ice water solution of 6 M sodium hydroxide until pH is above 6.

(b) Flush with tap water neutralized solution into sewer system.

5. SUMMARY OF DECONTAMINATION PROCEDURES

See Figures 1 and 2 for an overview of procedures that are used for packaging, decontaminating and disposing of infectious agents.

5.1 Decontamination Procedures.

5.1.1 Decontamination by autoclaving at 121 degrees C for 30 minutes.

(a) glass and plastic laboratory ware.

(b) towels, lab coats, and gloves.

(c) spent media, sewage, sludge, wastewaters, effluents, and samples.

(d) filters, and autoclavable equipment.

5.1.2 Chemical Decontamination Procedures.

(a) Ethylene oxide. Decontaminate by gas sterilizing with ethylene oxide for 4 hours and then aerating for 4 hours.

(a.1) Reusable filters.

(a.2) Filter housings.

(a.3) Hoses.

(a.4) Nonautoclavable equipment.

(b) Chlorine. Decontaminate with 10 to 15 mg of chlorine per liter by holding in chlorine solution for 30 minutes or by recirculating solution through equipment for 30 minutes.

(b.1) Hoses.

(b.2) Filter housings.

(b.3) Heat-sensitive equipment (e.g. pumps).

(b.4) Large vessels (e.g. drums).

(b.5) pH electrodes.

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FIGURES

Figure 1. Overview of temperature decontamination and disposal procedures for infectious microbiological wastes.

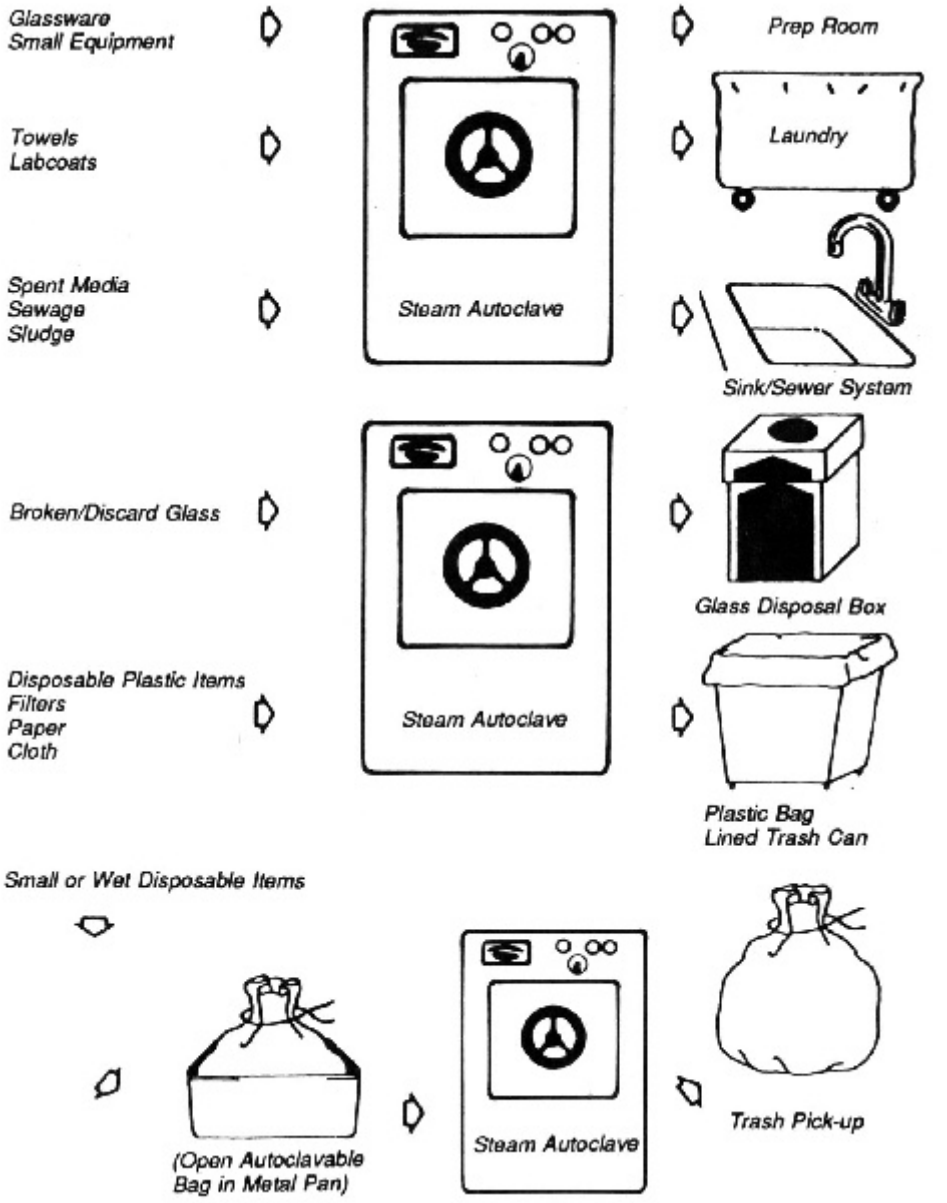
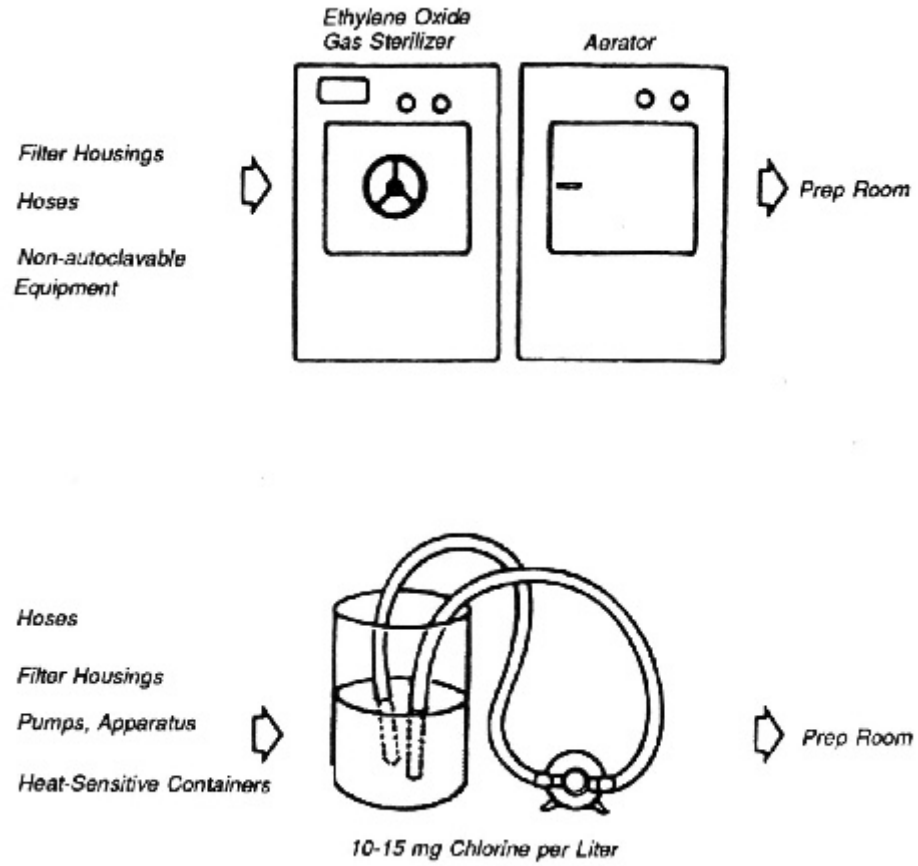


Figure 2. Overview of chemical decontamination and disposal procedures for infectious microbiological wastes.



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