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CHAPTER 3

STERILIZATION AND DISINFECTION

1. GENERAL PROCEDURES

1.1 Use aseptic techniques for handling test waters, sewages, sludges, and cell cultures.

1.2 Sterilize apparatus and containers that will come into contact with test waters, sludges, or elutants, all solutions added to test waters unless otherwise indicated, and all elutants.

1.3 Sterilize all contaminated materials (including all blood and blood products) before discarding.

1.4 Disinfect all spills and splatters.

2. STERILIZATION TECHNIQUES

2.1 Solutions. Unless otherwise indicated, sterilize all solutions except those used for cleansing, standard buffers, hydrochloric acid (HCl), sodium hydroxide (NaOH), Freon and other organic materials, and disinfectants by autoclaving them at 121 degrees C for 15 minutes. HCl, NaOH, Freon, and disinfectants as used herein are self-sterilizing (bactericidal and fungicidal). When autoclaving buffered beef extract, use a vessel large enough to accommodate foaming.

2.2 Autoclavable Glassware, Autoclavable Plasticware, Dialysis

Tubing, and Equipment. Water may speed heat transfer in larger vessels during autoclaving and thereby speed the sterilization process. Add deionized distilled water to vessels in quantities indicated in Table 1. Lay large vessels on sides in autoclave, if possible, to facilitate displacement of air in vessels by flowing steam.

2.2.1 Cover openings into glassware, autoclavable plasticware, and equipment with aluminum foil before autoclaving.

2.2.2 Sterilize glassware, unless otherwise noted, in a dry heat oven at a temperature of 170 degrees C for one hour (see Table 2 for acceptable alternative time-temperature couplings).

2.2.3 Autoclave at 121 degrees C for one hour plasticware that can withstand autoclaving. Plasticware requires more time to sterilize than glass-ware because plastic transfers heat more slowly than glass.

2.2.4 Sterilize stainless steel vessels in an autoclave at 121 degrees C for 30 minutes. Vent-relief valves on vessels so equipped must be open during autoclaving and closed immediately when vessels are removed from autoclave.

2.2.5 Sterilize with ethylene oxide tubing and plasticware that cannot withstand autoclaving. CAUTION: Avoid exposure to ethylene oxide fumes. Ethylene oxide is toxic. Sterilize materials in 12 percent ethylene oxide (20-50 percent relative humidity). Expose dry materials to ethylene oxide for two hours. Expose materials that are not dry for four hours.

2.2.6 Autoclave dialysis tubing at 121 degrees C for 15 minutes. Fill dialysis bag two-thirds full with deionized distilled water before autoclaving.

2.2.7 Autoclave membrane filters in situ in filter holders at 121 degrees C for 20 minutes. To speed sterilization and to prevent filters from cracking, seat (and cover) filters in a small volume of deionized distilled water. Open vent/relief valves on filter holders before autoclaving, and close vent/relief valves immediately after autoclaving.

2.2.8 Sterilize cartridge filters according to manufacturer's instructions.

2.2.9 Autoclave apparatus except pumps, cartridge filter holders, and combination-type pH electrodes at 121 degrees C for 30 minutes.

2.2.10 Sterilize drums, other vessels, and other apparatus too

large for autoclaves by chlorination. Fill vessels or apparatus with deionized distilled water containing 10-15 mg of chlorine (NaOCl) per liter, adjusted to pH 6-7 with HCl. Dechlorinate chlorinated water in vessels or apparatus after 30 minutes by adding sufficient sodium thiosulfate ($Na_2S_2O_3$) solution to yield 50 mg per liter. Allow 15 minutes for dechlorination, and drain water from vessels or apparatus.

2.2.11 Sterilize pumps and cartridge filter holders with chlorine or with ethylene oxide.

(a) Chlorine procedure.

(a.1) For 30 minutes, recirculate through pumps and cartridge filter holders 4 liters of deionized distilled water containing 10-15 mg of chlorine (NaOCl) per liter, adjusted to pH 6-7 with HCl.

(a.2) Dechlorinate pumps and cartridge filter holders by passing through them 1 liter of a solution containing 50 mg of sodium thiosulfate ($Na_2S_2O_3$) per liter of deionized distilled water.

(b) Ethylene oxide procedure. CAUTION: Avoid exposure to ethylene oxide fumes. Ethylene oxide is toxic.

(b.1) Sterilize pumps and cartridge filter holders in a gas sterilizer by exposing them to 12 percent ethylene oxide (30-50 percent relative humidity) for four hours at 55-60 degrees C.

(b.2) Aerate pumps and cartridge filter holders in a gas aerator to remove residual ethylene oxide (as recommended by the sterilizer manufacturer), or maintain pumps and cartridge filter holders at 37 degrees C for a minimum of three days before using them.

2.2.12 Sterilize pH electrodes with chlorine or with HCl. Sterilize electrodes before and after each use.

(a) Chlorine procedure.

(a.1) Immerse tip of electrode for one minute into deionized distilled water that contains 10-15 mg of chlorine (NaOCl) per liter, adjusted to pH 6-7 with HCl.

(a.2) Dechlorinate electrode by immersing tip into sterile deionized distilled water that contains 50 mg of $Na_2S_2O_3$ per liter and then rinsing tip with sterile deionized distilled water.

(b) HCl procedure.

(b.1) Immerse tip of pH electrode into 1 M HCl for one minute. Use only fresh 1 M HCl prepared daily from concentrated HCl.

(b.2) Rinse electrode tip with sterile deionized distilled water.

2.2.12 Sterilize working instruments such as scissors and forceps by autoclaving them at 121 degrees C for 30 minutes. Working instruments such as scissors and forceps may be sterilized between uses by immersing them in 70 percent ethanol and flaming them.

2.3 Contaminated materials.

Autoclave contaminated materials for one hour at 121 degrees C. Be sure that steam can enter contaminated materials freely. If volume of contaminated materials is unusually large, exposure time at 121 degrees C must be increased appropriately.

3. DISINFECTION TECHNIQUES

3.1 Disinfect spills and other contamination on surfaces that do not stain with a solution of 0.5 percent I2 in 70 percent ethanol.

3.2 Disinfect spills and other contamination on surfaces that stain with a solution of 0.1 percent HOCl. 0.1 percent HOCl may be prepared by appropriately diluting an NaOCl solution (Clorox, The Clorox Co., or equivalent) and adjusting its pH to 6-7 with dilute HCl.

4. BIBLIOGRAPHY

Block, S. S. ed., Disinfection, Sterilization, and Preservation. Philadelphia, 2nd edition, Lee and Febiger, 1977.

Davis, B. D., Dulbecco, R., Eisen, H.N., and Ginsberg, H.S., Microbiology, 3rd edition, Harper and Row, Publishers, New York, 1980.

TABLES

Table 1. Quantities of deionized distilled water to be added to vessels to facilitate sterilization during autoclaving. (note: Add to vessel the volume of deionized distilled water indicated, cover mouth of vessel with aluminum foil, lay vessel on its side if possible, and autoclave.)

Vessel Size (Liter)	Quantity (mL) of Deionized Distilled Water
2 and 3	25
4	50
8	100
24	500
54	1000

Table 2. Time-temperature couplings for dry sterilization.

Temperature (degrees)		Hours
C	F	
140	285	3
150	300	2.5
160	320	2
170	340	1

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