EQUIPMENT AND EQUIPMENT STERILIZATION PROCEDURES 7.1.1

Specific equipment and supplies are needed for collection and analysis of indicator bacteria by membrane filtration procedures. The equipment listed in table 7.1–2 should be sufficient to begin analysis of fecal indicator bacteria using these procedures.

Table 7.1–2. Equipment and supplies used for membrane filtration analysis

[mL, milliliters; °C, degrees Celsius; µm, micrometers; TTC, triphenyltetrazolium chloride; mm, millimeters; TD, to deliver; NIST, National Institute of Standards and Technology; NA-MUG, nutrient agar-4-methylumbelliferyl-β-D-glucuronide; nm, nanometers; lb/in^2, pounds per square inch; cm, centimeters]

Item	Description
✓Absorbent pads	For use with total coliform and *Escherichia coli* methods
✓ Alcohol burner	Glass or metal, containing ethanol for flame sterilizing forceps
✓Alcohol bottle	Wide mouth, 100 mL, containing ethanol for forceps
✓Autoclave	For sterilization, capable of maintaining 121°C
✓Bottles	Milk dilution, 99 mL with autoclavable screwcaps
✓Counter	Handheld for counting bacterial colonies
✓Filter disk	Sterile, disposable, 0.2 µm, for sterilizing TTC, to fit on 5-mL barrel syringe
✓Filtration assembly	Filter funnel, filter base, and stainless steel, glass, or plastic filter holder; wrapped in aluminum foil, autoclavable bag, or kraft paper, sterile; autoclavable
✓Flasks	Narrow mouth, Erlenmeyer type with stir bars for media preparation, 250 or 500 mL
✓Forceps	Stainless steel, smooth tips
✓Hot plate	With magnetic stirrer or boiling water bath for media preparation
✓Graduated cylinders	Borosilicate glass or plastic, 100 and 25 mL, wrapped in **sterile** aluminum foil, autoclavable bag, or kraft paper
✓Incubator	Aluminum heat sink (heater block) or water bath, capable of maintaining specified temperature ranges during incubation
✓Membrane filters	Sterile, white, gridded, 47-mm diameter, either 0.45- or 0.7-mm pore size, depending on test method
✓Microscope	Wide field type with 10–20 x magnifications, dissecting type with fluorescent lamp
✓Pipets	Sterile, TD, bacteriological or Mohr, glass or plastic, 1 and 10 mL
✓Pipettor or pipet bulb	For use with pipets (**no pipetting by mouth**)
✓Petri dishes	Sterile, plastic, disposable, 50 x 12 mm
✓Syringe	5 mL, disposable for delivering TTC
✓Thermometer	Range of 40–110°C, glass mercury or dial, calibrated in 0.2°C increments checked against a NIST-certified thermometer
✓Ultraviolet lamp, long wave	For use with NA-MUG test, 366 nm, 6-watt bulb
✓Vacuum source	Either a hand pump with gage or electric vacuum or peristaltic pump, vacuum pressure not to exceed 5 lb/in^2 or 25 cm of mercury

Equipment for collection and analysis of bacterial samples must be clean and sterile (table 7.1–3). Wrap equipment in kraft paper, autoclavable bags, or aluminum foil. Sterilize and store the equipment in a clean area. Resterilize equipment if foil, bag, or kraft paper is torn.

Add sodium thiosulfate ($Na_2S_2O_3$) to sample bottles before sterilization if the water to be collected contains residual chlorine or other halogens added for disinfection. Residual chlorine can be found in samples collected from sources such as treated potable-water taps, in effluents, and surface-water samples collected from the mixing zones of wastewater-treatment plants. A 10-percent solution of $Na_2S_2O_3$ is prepared in the following manner. In a volumetric flask, dissolve 100 g $Na_2S_2O_3$ into 500 mL of deionized or distilled water; stir until dissolved, and fill flask to 1,000 mL (Bordner and Winter, 1978, p. 6; American Public Health Association and others, 1992, p. 9–18). Add 0.1 mL of 10-percent $Na_2S_2O_3$ solution for every 100 mL of sample. Keep $Na_2S_2O_3$ refrigerated and in a dark bottle; after 6 months prepare a fresh solution.

Add ethylenediaminetetraacetic acid (EDTA) to sample bottles when water to be collected contains trace elements such as copper, nickel, and zinc at concentrations greater than 10 µg/L (Britton and Greeson, 1989, p. 5–6; Bordner and Winter, 1978, p. 6; American Public Health Association and others, 1992, p. 9–18). A 15-percent solution of EDTA is prepared by dissolving 372 mg in 1,000 mL of distilled or deionized water. Before sterilization, add 0.3 mL of the EDTA solution per 100 mL of sample to sample bottles. EDTA can be combined with the $Na_2S_2O_3$ solution in the sample bottle before sterilization.

Autoclaving is the preferred method for sterilizing equipment.

Sterilize the filtration apparatus between sites or for each sample collected at the same site at different times. Autoclaving is the preferred method of sterilization. Use only autoclaves that have temperature, pressure, and liquid- and dry-utensil-cycle controls. Steam sterilizers and vertical autoclaves are not recommended because the temperature cannot be held constant.

Table 7.1–3. Equipment cleaning and sterilizing procedures

[$Na_2S_2O_3$, sodium thiosulfate; >, greater than value shown; µg/L, micrograms per liter; EDTA, ethylenediaminetetraacetic acid; °C, degrees Celsius; mg/L, milligrams per liter]

Equipment	Cleaning and sterilizing procedures
All equipment	Wash equipment thoroughly with a dilute nonphosphate, laboratory-grade detergent. Rinse three times with hot tap water. Rinse again three to five times with deionized or glass-distilled water.
Glass, polypropylene, or Teflon™ bottles	If sample will contain residual chlorine or other halogens, add $Na_2S_2O_3$. If sample will contain >10 µg/L trace elements, add EDTA. Autoclave at 121°C for 15 minutes, or bake glass jars at 170°C for 2 hours.
Stainless-steel field units	Flame sterilize with methanol (Millipore Hydrosol™ units only), or autoclave, or bake at 170°C for 2 hours.
Portable submersible pumps and pump tubing	Autoclavable equipment (preferred): Autoclave at 121°C for 15 minutes. Non-autoclavable equipment: Submerge sampling system in a 200-mg/L laundry bleach solution and circulate solution through pump and tubing for 30 minutes; follow with thorough rinsing, inside and out, with sample water pumped from the well. DO NOT USE THIS METHOD TO DISINFECT EQUIPMENT USED TO COLLECT SAMPLES FOR SUBSEQUENT DETERMINATIONS OF TRACE ELEMENTS AND ORGANIC SUBSTANCES.

Take care to ensure that materials to be autoclaved, such as tubing and containers, are thermally stable. Polymers (such as polycarbonate, polypropylene, polyallomer, and polymethylpentene) and Teflons™ and Tefzel™ (such as perfluoroalkyoxy-polymers or PFA™, ethylenetetrafluoro-ethylene or ETFE™, fluorinated ethylene propylene or FEP™, and polytetrafluoroethylene polymers or PTFE™) can be autoclaved. Each has different thermal characteristics and tolerances to repeated autoclaving.

CAUTION: when flame sterilizing, have proper safety equipment such as a fire extinguisher on hand, and implement procedures carefully.

Only Millipore Hydrosol™ field filtration units are designed to be flame sterilized with methanol. Formaldehyde gas, a by-product of methanol combustion, kills all microorganisms in the unit. The following sterilization procedure is acceptable for the Hydrosol unit in field situations where other sterilization techniques are not practicable (Millipore, 1973, p. 48–49).

Carefully:

1. Remove the stainless steel flask from the base of the filter-holder assembly.
2. Saturate the asbestos ring (wick) around the base assembly with methanol.
3. Ignite the methanol on the asbestos wick and allow to burn for 30 seconds.
4. Invert the stainless steel flask over the funnel and the burning asbestos ring, and seat the flask on the base of the filter-holder assembly. Leave the flask in place for 15 minutes. Before filtering the next sample, rinse flask and funnel thoroughly with sterile buffered water to remove all residues of formaldehyde.
5. Repeat sterilization procedure before processing the next sample.

Quality control. Use a sterilization indicator, such as autoclave tape or StreamClox™, to help determine whether adequate temperature and pressure have been attained during autoclaving. Keep a log book of equipment and include quality-control procedures with the date, the results, and the name of the analyst. The 18th edition of *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association and others, 1992, p. 9–9, Table 9020:III) contains specifications for the length of time and temperature for autoclave sterilization of various media, apparatus, and cultures to be discarded. Overloading the autoclave with equipment or materials will result in incomplete sterilization. Make sure that steam can circulate around all equipment, utensils, and bottles. Cultures of bacteria to be discarded must be autoclaved for at least one-half hour.