Appendix C. Water

Guidelines for Environmental Infection Control in Health-Care Facilities (2003)

1. Biofilms

On this Page

Microorganisms have a tendency to associate with and stick to surfaces. These adherent organisms can initiate and develop biofilms, which are comprised of cells embedded in a matrix of extracellularly produced polymers and associated abiotic particles.[1438] It is inevitable that biofilms will form in most water systems. In the health-care facility environment, biofilms may be found in the potable water supply piping, hot water tanks, air conditioning cooling towers, or in sinks, sink traps, aerators, or shower heads. Biofilms, especially in water systems, are not present as a continuous slime or film, but are more often scanty and heterogeneous in nature.[1439] Biofilms may form under stagnant as well as flowing conditions, so storage tanks, in addition to water system piping, may be vulnerable to the development of biofilm, especially if water temperatures are low enough to allow the growth of thermophilic bacteria (e.g., *Legionella* spp.). Favorable conditions for biofilm formation are present if these structures and equipment are not cleaned for extended periods of time.[1440]

Algae, protozoa, and fungi may be present in biofilms, but the predominant microorganisms of water system biofilms are gram-negative bacteria. Although most of these organisms will not normally pose a problem for healthy individuals, certain biofilm bacteria (e.g., *Pseudomonas aeruginosa, Klebsiella* spp., *Pantoea agglomerans*, and *Enterobacter cloacae*) all may be agents for opportunistic infections for immunocompromised individuals.[1441, 1442] These biofilm organisms may easily contaminate indwelling medical devices or intravenous (IV) fluids, and they could be transferred on the hands of health-care workers.[1441–1444]

Biofilms may potentially provide an environment for the survival of pathogenic organisms, such as *Legionella pneumophila* and *E. coli* O157:H7. Although the association of biofilms and medical devices provides a plausible explanation for a variety of health-care associated infections, it is not clear how the presence of biofilms in the water system may influence the rates of health-care– associated waterborne infection.

Organisms within biofilms behave quite differently than their planktonic (i.e., free floating) counterparts. Research has shown that biofilm-associated organisms are more resistant to antibiotics and disinfectants than are planktonic organisms, either because the cells are protected by the polymer matrix, or because they are physiologically different.[1445–1450] Nevertheless, municipal water utilities attempt to maintain a chlorine residual in the distribution system to discourage microbiological growth. Though chlorine in its various forms is a proven disinfectant, it has been shown to be less effective against biofilm bacteria.[1448] Higher levels of chlorine for longer contact times are necessary to eliminate biofilms.

Routine sampling of health-care facility water systems for biofilms is not warranted. If an epidemiologic investigation points to the water supply system as a possible source of infection, then water sampling for biofilm organisms should be considered so that prevention and control strategies can be developed. An established biofilm is is difficult to remove totally in existing piping. Strategies to remediate biofilms in a water system would include flushing the system piping, hot water tank, dead legs, and those areas of the facility's water system subject to low or intermittent flow. The benefits of this treatment would include

a. elimination of corrosion deposits and sludge from the bottom of hot water tanks,
b. removal of biofilms from shower heads and sink aerators, and
c. circulation of fresh water containing elevated chlorine residuals into the health-care facility water system.

The general strategy for evaluating water system biofilm depends on a comparision of the bacteriological quality of the incoming municipal water and that of water sampled from within facility's distribution system. Heterotrophic plate counts and coliform counts, both of which are routinely run by the municipal water utility, will at least provide in indication of the potential for biofilm formation. Heterotrophic plate count levels in potable water should be <500 CFU/mL. These levels may increase on occasion, but counts consistently >500 CFU/mL would indicate a general decrease in water quality. A direct correlation between heterotrophic plate count and biofilm levels has been demonstrated.[1450] Therefore, an increase in heterotrophic plate count would suggest a greater rate and extent of biofilm formation in a health-care facility water system. The water supplied to the facility should also contain <1 coliform bacteria/100 mL. Coliform bacteria are organisms whose presence in the distribution system could indicate fecal contamination. It has been shown that coliform bacteria can colonize biofilms within drinking water systems. Intermittant contamination of a water system with these organisms could lead to colonization of the system.

Water samples can be collected from throughout the health-care facility system, including both hot and cold water sources; samples should be cultured by standard methods.[945] If heterotrophic plate counts in samples from the facility water system are higher than those from samples collected at the point of water entry to the building, it can be concluded that the facility water quality has diminished. If biofilms are detected in the facility water system and determined by an epidemiologic and environmental investigation to be a reservoir for health-care associated pathogens, the municipal water supplier could be contacted with a request to provide higher chlorine residuals in the distribution system, or the health-care facility could consider installing a supplemental chlorination system.

Sample collection sites for biofilm in health-care facilities include

a. hot water tanks
b. shower heads; and
c. faucet aerators, especially in immunocompromised patient-care areas.

Swabs should be placed into tubes containing phosphate buffered water, pH 7.2 or phosphate buffered saline, shipped to the laboratory under refrigeration and processed within 24 hrs. of collection. Samples are suspended by vortexing with sterile glass beads and plated onto a nonselective medium (e.g., Plate Count Agar or R2A medium) and selective media (e.g., media for *Legionella* spp. isolation) after serial dilution. If the plate counts are elevated above levels in the water (i.e. comparing the plate count per square centimeter of swabbed surface to the plate count per milliliter of water), then biofilm formation can be suspected. In the case of an outbreak, it would be advisable to isolate organisms from these plates to determine whether the suspect organisms are present in the biofilm or water samples and compare them to the organisms isolated from patient specimens.

2. Water and Dialysate Sampling Strategies in Dialysis

In order to detect the low, total viable heterotrophic plate counts outlined by the current AAMI standards for water and dialysate in dialysis settings, it is necessary to use standard quantitative culture techniques with appropriate sensitivity levels.[792, 832, 833] The membrane filter technique is particularly suited for this application because it permits large volumes of water to be assayed.[792, 834] Since the membrane filter technique may not be readily available in clinical laboratories, the spread plate assay can be used as an alternative.[834] If the spread plate assay

is used, however, the standard prohibits the use of a calibrated loop when applying sample to the plate.[792] The prohibition is based on the low sensitivity of the calibrated loop. A standard calibrated loop transfers 0.001 mL of sample to the culture medium, so that the minimum sensitivity of the assay is 1,000 CFU/mL. This level of sensitivity is unacceptable when the maximum allowable limit for microorganisms is 200 CFU/mL. Therefore, when the spread plate method is used, a pipette must be used to place 0.1–0.5 mL of water on the culture medium.

The current AAMI standard specifically prohibits the use of nutrient-rich media (e.g., blood agar, and chocolate agar) in dialysis water and dialysate assays because these culture media are too rich for growth of the naturally occurring organisms found in water.[792] Debate continues within AAMI, however, as to the most appropriate culture medium and incubation conditions to be used. The original clinical observations on which the microbiological requirements of this standard were based used Standard Methods Agar (SMA), a medium containing relatively few nutrients.[666] The use of tryptic soy agar (TSA), a general purpose medium for isolating and cultivating microorganisms was recommended in later versions of the standard because it was thought to be more appropriate for culturing bicarbonate-containing dialysate.[788, 789, 835] Moreover, culturing systems based on TSA are readily available from commercial sources. Several studies, however, have shown that the use of nutrient-poor media, such as R2A, results in an increased recovery of bacteria from water.[1451, 1452] The original standard also specified incubation for 48 hours at 95°F–98.6°F (35°C–37°C) before enumeration of bacterial colonies. Extending the culturing time up to 168 hours, or 7 days and using incubation temperatures of 73.4°F– 82.4°F (23°C–28°C) have also been shown to increase the recovery of bacteria.[1451, 1452] Other investigators, however, have not found such clear cut differences between culturing techniques.[835, 1453] After considerable discussion, the AAMI Committee has not reached a consensus regarding changes in the assay technique, and the use of TSA or its equivalent for 48 hours at 95°F–98.6°F (35°C–37°C) remains the recommended method. It should be recognized, however, that these culturing conditions may underestimate the bacterial burden in the water and fail to identify the presence of some organisms. Specifically, the recommended method may not detect the presence of various NTM that have been associated with several outbreaks of infection in dialysis units.[31, 32] In these instances, however, the high numbers of mycobacteria in the water were related to the total heterotrophic plate counts, each of which was significantly greater than that allowable by the AAMI standard. Additionally, the recommended method will not detect fungi and yeast, which have been shown to contaminate water used for hemodialysis applications.[1454] Biofilm on the surface of the pipes may hide viable bacterial colonies, even though no viable colonies are detected in the water using sensitive culturing techniques.[1455] Many disinfection processes remove biofilm poorly, and a rapid increase in the level of bacteria in the water following disinfection may indicate significant biofilm formation. Therefore, although the results of microbiological surveillance obtained using the test methods outlined above may be useful in guiding disinfection schedules and in demonstrating compliance with AAMI standards, they should not be taken as an indication of the absolute microbiological purity of the water.[792]

Endotoxin can be tested by one of two types of assays

a. a kinetic test method [e.g., colorimetric or turbidimetric] or
b. a gel-clot assay.

Endotoxin units are assayed by the *Limulus* Amebocyte Lysate (LAL) method. Because endotoxins differ in their activity on a mass basis, their activity is referred to a standard *Escherichia coli* endotoxin. The current standard (EC-6) is prepared from *E. coli* O113:H10. The relationship between mass of endotoxin and its activity varies with both the lot of LAL and the lot of control standard endotoxin used. Since standards for endotoxin were harmonized in 1983 with the introduction of EC-5, the relationship between mass and activity of endotoxin has been approximately 5–10 EU/ng. Studies to harmonize standards have led to the measurement of endotoxin units (EU) where 5 EU is equivalent to 1 ng *E. coli* O55:B5 endotoxin.[1456]

In summary, water used to prepare dialysate and to reprocess hemodialyzers should not contain a total microbial count >200 CFU/mL as determined by assay on TSA agar for 48 hrs. at 96.8°F (36°C), and ≤2 endotoxin units (EU) per mL. The dialysate at the end of a dialysis treatment should not contain >2,000 CFU/mL.[31, 32, 668, 789, 792]

3. Water Sampling Strategies and Culture Techniques for Detecting Legionellae

Legionella spp. are ubiquitous and can be isolated from 20%–40% of freshwater environments, including man-made water systems.[1457, 1458] In health-care facilities, where legionellae in potable water rarely result in disease among immunocompromised patients, courses of remedial action are unclear.

Scheduled microbiologic monitoring for legionellae remains controversial because the presence of legionellae is not necessarily evidence of a potential for causing disease.[1459] CDC recommends aggressive disinfection measures for cleaning and maintaining devices known to transmit legionellae, but does not recommend regularly scheduled microbiologic assays for the bacteria.[396] However, scheduled monitoring of potable water within a hospital might be considered in certain settings where persons are highly susceptible to illness and mortality from *Legionella* infection (e.g., hematopoietic stem cell transplantation units and solid organ transplant units).[9] Also, after an outbreak of legionellosis, health officials agree monitoring is necessary to identify the source and to evaluate the efficacy of biocides or other prevention measures.

Examination of water samples is the most efficient microbiologic method for identifying sources of legionellae and is an integral part of an epidemiologic investigation into health-care associated Legionnaires disease. Because of the diversity of plumbing and HVAC systems in health-care facilities, the number and types of sites to be tested must be determined before collection of water samples. One environmental sampling protocol that addresses sampling site selection in hospitals might serve as a prototype for sampling in other institutions.[1209] Any water source that might be aerosolized should be considered a potential source for transmission of legionellae. The bacteria are rarely found in municipal water supplies and tend to colonize plumbing systems and point-of-use devices. To colonize, legionellae usually require a temperature range of 77°F–108°F (25°C–42.2°C) and are most commonly located in hot water systems.[1460] Legionellae do not survive drying. Therefore, air-conditioning equipment condensate, which frequently evaporates, is not a likely source.[1461]

Water samples and swabs from point-of-use devices or system surfaces should be collected when sampling for legionellae (Box C.1).[1437] Swabs of system surfaces allow sampling of biofilms, which frequently contain legionellae. When culturing faucet aerators and shower heads, swabs of surface areas should be collected first; water samples are collected after aerators or shower heads are removed from their pipes. Collection and culture techniques are outlined (Box C.2). Swabs can be streaked directly onto buffered charcoal yeast extract agar (BCYE) plates if the pates are available at the collection site. If the swabs and water samples must be transported back to a laboratory for processing, immersing individual swabs in sample water minimizes drying during transit. Place swabs and water samples in insulated coolers to protect specimens from temperature extremes.

Box C.1. Potential sampling sites for *Legionella* spp. in health-care facilities*

- Potable water systems
  incoming water main, water softener unit, holding tanks, cisterns, water heater tanks (at the inflows and outflows)
- Potable water outlets, especially those in or near patient rooms
  faucets or taps, showers
- Cooling towers and evaporative condensers
  makeup water (e.g., added to replace water lost because of evaporation, drift, or leakage), basin (i.e., area under the tower for collection of cooled water), sump (i.e., section of basin from which cooled water returns to heat source), heat sources (e.g., chillers)
- Humidfiers (e.g., nebullizers)
  bubblers for oxygen, water used for respiratory therapy equipment
- Other sources
  decorative fountains, irrigation equipment, fire sprinkler system (if recently used), whirlpools, spas

* Material in this box is adapted from reference 1209.

Box C.2. Procedures for collecting and processing environmental specimens for *Legionella* spp.*

1. Collect water (1-liter samples, if possible) in sterile, screw-top bottles.
2. Collect culture swabs of internal surfaces of faucets, aerators, and shower heads in a sterile, screw-top container (e.g., 50 mL plastic centrifuge tube). Submerge each swab in 5–10 mL of sample water taken from the same device from which the sample was obtained.

3. Transport samples and process in a laboratory proficient at culturing water specimens for *Legionella* spp. as soon as possible after collection. (Samples may be transported at room temperature but must be protected from temperature extremes. Samples not processed within 24 hours of collection should be refrigerated.)
4. Test samples for the presence of *Legionella* spp. by using semiselective culture media using procedures specific to the cultivation and detection of *Legionella* spp.
   - Detection of *Legionella* spp. antigen by the direct fluorescent antibody technique is not suitable for environmental samples.
   - Use of polymerase chain reaction for identification of *Legionella* spp. is not recommended until more data regading the sensitivity and specificity of this procedure are available.

Material in this table is compiled from references 1209, 1437, 1462–1465.

4. Procedure for Cleaning Cooling Towers and Related Equipment

I. Perform these steps prior to chemical disinfection and mechanical cleaning.
   A. Provide protective equipment to workers who perform the disinfection, to prevent their exposure to chemicals used for disinfection and aerosolized water containing *Legionella* spp. Protective equipment may include full-length protective clothing, boots, gloves, goggles, and a full- or half-face mask that combines a HEPA filter and chemical cartridges to protect against airborne chlorine levels of up to 10 mg/L.
   B. Shut off cooling tower.
      1. Shut off the heat source, if possible.
      2. Shut off fans, if present, on the cooling tower/evaporative condenser (CT/EC).
      3. Shut off the system blowdown (i.e., purge) valve.
      4. Shut off the automated blowdown controller, if present, and set the system controller to manual.
      5. Keep make-up water valves open.
      6. Close building air-intake vents within at least 30 meters of the CT/EC until after the cleaning procedure is complete.
      7. Continue operating pumps for water circulation through the CT/EC.

II. Perform these chemical disinfection procedures.
   A. Add fast-release, chlorine-containing disinfectant in pellet, granular, or liquid form, and follow safety instructions on the product label. Use EPA-registered products, if available. Examples of disinfectants include sodium hypochlorite (NaOCl) or calcium hypochlorite (Ca[OCl]2), calculated to achieve initial free residual chlorine (FRC) of 50 mg/L: either
      a. 3.0 lbs [1.4 kg] industrial grade NaOCl [12%–15% available Cl] per 1,000 gallons of CT/EC water
      b. 10.5 lbs
      c. [4.8 kg] domestic grade NaOCl [3%–5% available Cl] per 1,000 gallons of CT/EC water; or
      d. 0.6 lb [0.3 kg] Ca[OCl]2 per 1,000 gallons of CT/EC water. If significant biodeposits are present, additional chlorine may be required. If the volume of water in the CT/EC is unknown, it can be estimated (in gallons) by multiplying either the recirculation rate in gallons per minute by 10 or the refrigeration capacity in tons by 30. Other appropriate compounds may be suggested by a water-treatment specialist.
   B. Record the type and quality of all chemicals used for disinfection, the exact time the chemicals were added to the system, and the time and results of FRC and pH measurements.
   C. Add dispersant simultaneously with or within 15 minutes of adding disinfectant. The dispersant is best added by first dissolving it in water and adding the solution to a turbulent zone in the water system. Automatic-dishwasher compounds are examples of low- or nonfoaming, silicate-based dispersants. Dispersants are added at 10–25 lbs (4.5–11.25 kg) per 1,000 gallons of CT/EC water.
   D. After adding disinfectant and dispersant, continue circulating the water through the system. Monitor the FRC by using an FRC-measuring device with the DPD method (e.g., a swimming-pool test kit), and measure the pH with a pH meter every 15 minutes for 2 hours. Add chlorine as needed to maintain the FRC at ≥10 mg/L. Because the biocidal effect of chlorine is reduced at a higher pH, adjust the pH to 7.5–8.0. The pH may be lowered by using any acid (e.g., nuriatic acid or sulfuric acid used for maintenance of swimming pools) that is compatible with the treatment chemicals.
   E. Two hours after adding disinfectant and dispersant or after the FRC level is stable at ≥10 mg/L, monitor at 2-hour intervals and maintain the FRC at ≥10 mg/L for 24 hours.
   F. After the FRC level has been maintained at ≥10 mg/L for 24 hours, drain the system. CT/EC water may be drained safely into the sanitary sewer. Municipal water and sewerage authorities should be contacted regarding local regulations. If a sanitary sewer is not available, consult local or state authorities (e.g., a department of natural resources or environmental protection) regarding disposal of water. If necessary, the drain-off may be dechlorinated by dissipation or chemical neutralization with sodium bisulfite.
   G. Refill the system with water and repeat the procedure outline in steps 2–7 in I-B above.

III. Perform mechanical cleaning.
   A. After water from the second chemical disinfection has been drained, shut down the CT/EC.
   B. Inspect all water-contact areas for sediment, sludge, and scale. Using brushes and/or a low-pressure water hose, thoroughly clean all CT/EC water-contact areas, including the basin, sump, fill, spray nozzles, and fittings. Replace components as needed.
   C. If possible, clean CT/EC water-contact areas within the chillers.

IV. Perform these procedures after mechanical cleaning.
   A. Fill the system with water and add chlorine to achieve an FRC level of 10 mg/L.
   B. Circulate the water for 1 hour, then open the blowdown valve and flush the entire system until the water is free of turbidity.
   C. Drain the system.
   D. Open any air-intake vents that were closed before cleaning.
   E. Fill the system with water. The CT/EC may be put back into service using an effective water-treatment program.

5. Maintenance Procedures Used to Decrease Survival and Multiplications of *Legionella* spp. in Potable-Water Distribution Systems

Wherever allowable by state code, provide water at ≥124°F (≥51°C) at all points in the heated water system, including the taps. This requires that water in calorifiers (e.g., water heaters) be maintained at ≥140°F (≥60°C). In the United Kingdom, where maintenance of water temperatures at ≥122°F (≥50°C) in hospitals has been mandated, installation of blending or mixing valves at or near taps to reduce the water temperature to ≤109.4°F (≤63°C) has been recommended in certain settings to reduce the risk for scald injury to patients, visitors, and health care workers.[726] However, *Legionella* spp. can multiply even in short segments of pipe containing water at this temperature. Increasing the flow rate from the hot-water-circulation system may help lessen the likelihood of water stagnation and cooling.[711, 1465] Insulation of plumbing to ensure delivery of cold (<68°F [<20°C]) water to water heaters (and to cold-water outlets) may diminish the opportunity for bacterial multiplication.[456] Both dead legs and capped spurs within the plumbing system provide areas of stagnation and cooling to <122°F (<50°C) regardless of the circulating water temperature; these segments may need to be removed to prevent colonization.[704] Rubber fittings within plumbing systems have been associated with persistent colonization, and replacement of these fittings may be required for *Legionella* spp. eradication.[1467]

Continuous chlorination to maintain concentrations of free residual chlorine at 1–2 mg/L (1–2 ppm) at the tap is an alternative option for treatment. This requires the placement of flow-adjusted, continuous injectors of chlorine throughout the water distribution system. Adverse effects of continuous chlorination can include accelerated corrosion of plumbing (resulting in system leaks) and production of potentially carcinogenic trihalomethanes. However, when levels of free residual chlorine are below 3 mg/L (3 ppm), trihalomethane levels are kept below the maximum safety level recommended by the EPA.[727, 1468, 1469 228]

Page last reviewed: November 5, 2015
Content source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)