

# Disinfection of Water Distribution Systems for *Legionella*

Yu-sen E. Lin, Janet E. Stout, Victor L. Yu, and Radisav D. Vidic

Hospital-acquired legionnaires' disease arises from the presence of *Legionella* in hospital water systems. *Legionella* not only persists in hot water tanks but is also found in the biofilm throughout the entire water distribution system. Conditions within water systems that promote *Legionella* colonization include water temperature, configuration and age of the hot water tank, physicochemical constituents of the water, plumbing materials, and commensal microflora. Hospital-acquired legionnaires' disease has been prevented by instituting control measures directed at the water distribution system. These include superheat-and-flush, copper/silver ionization, ultraviolet light, instantaneous heating systems, and hyperchlorination. Each of the above disinfection methods has been proven to be effective in the short-term, but long-term

**W**ATER DISTRIBUTION systems are the primary reservoirs for hospital-acquired legionnaires' disease.<sup>1,2</sup> Prevention of hospital-acquired legionnaires' disease has been accomplished by disinfecting hospital water distribution systems, especially the hot water recirculating lines.<sup>3-5</sup>

Once cases of legionnaires' disease have been diagnosed, hospitals and institutions are faced with the dilemma of choosing an appropriate control measure. Several disinfection modalities have been used either singly or in combination with variable results. We will review the factors that contribute to *Legionella* colonization of hospital water distribution systems and the disinfection measures used to eradicate *Legionella pneumophila* from hospital water distribution systems. Finally, we review criteria that can be used for selecting optimal eradication measures for individual hospitals.

## LEGIONELLA GROWTH-PROMOTING CONDITIONS IN WATER SYSTEMS

To develop an effective strategy to control *Legionella* in hospital water systems one must first understand the conditions that promote *Legionella* growth in water distribution systems. Our early investigations found higher concentrations of *Legionella* in hot water lines compared to cold water lines. The highest concentrations of *Legionella* were recovered from water collected from the bottom of hot water storage tanks. This led us to the naive belief that the principal reservoir for *Legionella* in the hospital water system was hot water storage tanks. Since then, we and others have

efficacy has been difficult due to limitations associated with each method. The complexities of *Legionella* disinfection, including advantages and disadvantages of each method, are reviewed. A successful *Legionella* prevention program requires cooperation and communication among hospital administrative personnel, engineers, and infection control staff. Routine environmental surveillance cultures for *Legionella* are the critical component for successful long-term disinfection. Culture results document the efficacy of the disinfection method and alert the hospital staff to consider *Legionella* in hospitalized patients with pneumonia.

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defined various factors that promote *Legionella* growth in water distribution systems including hot water temperatures less than 60°C, physicochemical factors, water system materials and design, and commensal microflora. Our current understanding now includes the concept of biofilms. Hot water tanks are only one component of a distribution system that is lined with a nutrient-rich slime layer (biofilm) that is a niche for many microorganisms, including *Legionella*.

Environmental surveys for *Legionella* in the water systems of hospitals have shown that 12% to 70% were colonized by *Legionella* (Table 1).<sup>6-10</sup> The large water distribution systems and high volume hot water storage tanks of hospitals provide *Legionella* with optimal conditions for growth: warm temperatures, nutrients in the form of sediments and biofilms, and commensal microorganisms.

Several investigators have shown an association between *Legionella* positivity and hot water temperature. In in vivo studies using a model plumbing system, *L pneumophila* was able to survive and/or grow at temperatures of 20°, 40°, and 50°C, but *L pneumophila* was not recovered at 60°C.<sup>11</sup> Plouffe

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*From the Departments of Civil & Environmental Engineering and Medicine, University of Pittsburgh, PA; and the VA Medical Center, Pittsburgh, PA.*

*Address reprint requests to Victor L. Yu, MD, Infectious Disease Section, VA Medical Center, University Drive C, Pittsburgh, PA 15240.*

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**Table 1. Results of Environmental Surveys of Hospital Water Distribution Systems for the Presence of *Legionella***

Country/State	Year of Report	No. of Hospitals Surveyed	% Positive for <i>Legionella</i>	Isolate Identification	Reference
England/Wales	1985	40	70% (28/40)	<i>L pneumophila</i> serogroup 1	Second Report of the Committee of Inquiry into the outbreak of legionnaires' disease in Stafford, HMSO 1985 <sup>10</sup>
United States/Pennsylvania	1987	15	60% (9/15)	<i>L pneumophila</i> serogroups 1-6	Vickers et al <sup>6</sup>
Canada/Quebec	1992	84	68% (57/84)	<i>L pneumophila</i> serogroups 1-8, <i>L longbeachae</i> , <i>L micdadei</i>	Alary et al <sup>7</sup>
England/Scotland	1993	17	12% (2/17)	<i>L pneumophila</i> serogroups 1, 4, 6	Liu et al <sup>8</sup>
Nova Scotia	1994	39	23% (9/39)	<i>L pneumophila</i> , <i>L longbeachae</i>	Marrie et al <sup>9</sup>

et al found that hot water tank samples with temperatures of 50°C or less were significantly more likely to be positive for *Legionella*.<sup>12</sup> Similar associations with temperatures less than 60°C were also shown in surveys of hospitals in Quebec<sup>7</sup> and western Pennsylvania.<sup>6</sup> These studies suggested that the average hot water storage temperature (45°C ~ 50°C) in hospitals is ideal for *L pneumophila* colonization.

The configuration of hot water storage tanks has also been predictive of *L pneumophila* contamination. Vertical tanks were significantly more likely to be contaminated than horizontal tanks; 79% of vertical hot water tanks were contaminated with *L pneumophila* whereas only 29% of horizontal tanks were contaminated with *L pneumophila*.<sup>6</sup> Furthermore, the age of the tanks was also significantly associated with the presence of *L pneumophila*.<sup>6,13</sup> Older tanks were found to be contaminated with *L pneumophila* whereas newer tanks (less than 5 years) were generally free of *L pneumophila*. This phenomena may be due to the accumulation of scale and sediment in the older systems after years of service. However, cases of legionnaires' disease have also been diagnosed in newly constructed hospitals.<sup>14,15</sup>

The presence of *L pneumophila* in hot water systems has been associated with physicochemical constituents of the water and certain plumbing materials. Higher concentrations of calcium and magnesium, the principal components of scale and sediment, were found to be associated with presence of *L pneumophila* in hot water tank samples in a prospective study among 15 hospitals.<sup>6</sup>

Various materials found in water systems can support or promote the growth of bacteria, including *Legionella*. Shock absorbers installed within water lines were a reservoir for *Legionella* in one hospital.<sup>16</sup> After removal of these shock absorbers,

the percent of tap water samples positive for *Legionella* decreased from 20% to 5%.

Investigators from London suggested that the presence of *L pneumophila* in the hot water distribution system was due to the design of shower and tap fittings which allowed water to be trapped behind rubber washers and gaskets. These rubber fittings were experimentally shown to support the growth of *Legionella*.<sup>17</sup> Replacement of these rubber materials with "suitable alternatives" was reported to have eradicated *Legionella* from the fittings.<sup>17</sup> However, these results were not reproduced in another hospital that replaced rubber washers with nitrile washers.<sup>18</sup> We are skeptical that simple removal of rubber washers or gaskets can eliminate the growth of *Legionella* because *Legionella* can colonize a variety of plumbing materials, including plastics such as polyvinyl chloride (PVC), stainless steel, wood, and to a lesser degree copper.<sup>11,19,20</sup> Maximum accumulation of *Legionella* occurred on plastics at 40°C.<sup>11</sup>

Other bacteria and protozoa also colonize these surfaces, some of which have been shown to promote *Legionella* replication. Amebae and other ciliated protozoa are natural hosts for *Legionella*. *Legionella* multiply intracellularly within amebae trophozoites.<sup>21</sup> *L pneumophila* is known to infect five different genera of amebae, most notably *Hartmannella vermiformis* and *Acanthamoeba* spp. *Legionella* can also multiply within the ciliated protozoa, *Tetrahymena*. Bacterial species that appear to provide *Legionella* with growth-promoting factors include *Pseudomonas* spp, *Acinetobacter* spp, *Flavobacterium*, and *Alcaligenes* spp.<sup>22,23</sup>

*Legionella* and other microorganisms become attached to surfaces submerged in an aquatic environment forming a biofilm. These biofilms are found on the surface of pipes, distal sites, and stagnant areas of any water distribution system.

*Legionella* has been shown to colonize the surfaces of plumbing materials in concentrations up to  $10^5$  CFU/cm<sup>2</sup>.<sup>11,20</sup> Disturbance of the biofilm after water pressure changes associated with construction may cause water to become brown and the concentration of *Legionella* to dramatically increase.<sup>24</sup> In addition to facilitating growth, the presence of a biofilm can also interfere with disinfection. Bacteria in a biofilm are more resistant to biocides than freely suspended bacteria. For example, water cultures of *Legionella* attached to a stainless-steel surface were 135 times more resistant to iodine than freely suspended *Legionella*, and 210,000 times more resistant than agar-grown strains.<sup>25</sup> Biofilms can also protect microorganisms from disinfectants and harsh environmental conditions such as increased water temperatures. Some chemical disinfectants such as chlorine are even rendered inactive by the organic constituents of biofilms. The presence of *Legionella* in the biofilms throughout hospital hot water systems may explain the rapid recolonization found in some hospitals despite superheat-and-flush procedure or shock hyperchlorination.<sup>26,27</sup>

#### PRINCIPLES OF DISINFECTION

Disinfection modalities can be classified as either focal or systemic in their application.<sup>28</sup> Focal disinfection refers to disinfection directed at a portion of a water distribution system, usually the incoming water and the outlets. Focal disinfection approaches include ultraviolet light and instantaneous heating systems that are modular and easy to install.<sup>3</sup> However, these disinfection modalities are notably less effective if the water distribution system is already heavily colonized with *L pneumophila* that will persist in the biofilm throughout the water distribution system. Therefore, focal disinfection can eliminate *Legionella* only at the point of contact.

Systemic disinfection refers to disinfection directed at the entire water distribution system by providing a disinfectant residual that is bacteriostatic or bactericidal throughout the system, especially the distal sites and stagnant areas.<sup>28</sup> These modalities include continuous hyperchlorination and copper/silver ionization.<sup>29,30</sup> Thermal eradication (superheat and flush) is a systemic disinfection modality, however, the duration of disinfection is only short-term. Each systemic modality is highly dependent on adequate water distribution through-

out the system. Failure of the disinfectant to reach the colonized area will adversely affect the overall success of the method.

#### *LEGIONELLA* DISINFECTION METHODS

##### *Thermal Eradication (Superheat-and-Flush)*

Increasing the temperature of hot water was the first method to be used to control *Legionella* in hospital water distribution systems.<sup>31,32</sup> If *Legionella* must be eradicated from the water distribution system immediately to control an outbreak of legionnaires' disease, the superheat-and-flush method warrants primary consideration. Water temperatures greater than 60°C are inhibitory to *Legionella*. Laboratory studies have shown that the time required to reduce a population of *Legionella* by one log at 45°C, 50°C, 60°C, and 70°C was 2,500, 380, less than 5, and less than 1 minute.<sup>33-35</sup> The inhibitory effects of increased temperatures have been confirmed in hospitals. Hot water systems that are maintained above 50°C are less likely to be colonized by *Legionella*.<sup>6,12,13,36,37</sup>

*Method.* The basic method requires that hot water tank temperatures be increased to 70°C (158°F) followed by flushing of all water outlets, faucets, and showerheads with hot water for a minimum of 30 minutes to kill *Legionella* colonizing these sites.<sup>38</sup> It is critical to document that the water temperature achieved at the distal outlet reaches or exceeds 60°C. If this temperature is not achieved and maintained, the procedure is likely to fail. After the flush procedure, selected sites are recultured; if no *L pneumophila* is recovered, the procedure is considered completed. If *L pneumophila* can be isolated, the entire heat and flush protocol is repeated. Some hospitals have maintained hot water temperature at 60°C after the flushing to delay recolonization of *Legionella*.

*Clinical experience.* Numerous hospitals have used superheat-and-flush as a disinfection measure to control hospital-acquired infections. However, recolonization with *L pneumophila* has been reported to occur after superheat-and-flush procedures, followed by new cases of hospital-acquired legionnaires' disease.<sup>26,39</sup> Maintaining hot water temperature at 60°C after the superheat-and-flush disinfection has been successful in ensuring negative cultures for *Legionella* and disappearance of hospital-acquired legionnaires' disease.<sup>3,24,40</sup> Two hospitals reported that after maintaining the hot water temperature at 60°C, only two cases of

hospital-acquired legionnaires' disease were diagnosed during the subsequent two years.<sup>41,42</sup>

**Advantages.** The superheat-and-flush method requires no special equipment so that it can be initiated expeditiously, a notable advantage in an outbreak situation. The costs are minimal if personnel costs and overtime can be controlled.

**Disadvantages.** The main disadvantage of this method is that it is a time-consuming procedure and numerous personnel are involved to monitor distal sites, water tank temperatures, and flushing times. Scalding can occur, although such incidents have not been reported in hospitals using this method.<sup>26,43</sup> The posting of signs warning employees and patients has been effective in averting scalding incidents.<sup>38</sup> In fact, several hospitals that use superheat-and-flush method do not alert patients and personnel and scalding incidents have not been documented.

Thermal eradication is a temporary systemic disinfection modality. Recolonization of the water system will occur within weeks to months after a superheat-and-flush protocol if circulating temperatures are returned to a baseline of 45°C to 50°C.<sup>3,40,44</sup>

**Cost.** Among the various systemic disinfection methods, the superheat-and-flush is the least expensive. The greatest expense has been personnel costs for overtime. For example, in one 400- to 500-bed hospital, the average cost per superheat-and-flush episode was about \$20,000 (Stout JE, Dec 1994 personal communication). In another hospital with 900 beds, these costs were as high as \$31,000.<sup>26</sup> Surprisingly, fuel and energy costs to maintain higher hot water temperature (60°C) are not increased because at the higher temperature less hot water is used to maintain water at a comfortable temperature for showering, bathing, and washing. In fact, the American Society of Plumbing Engineers noted that maintaining hot water temperature at 60°C (140°F) compared with 43°C (110°F) resulted in less energy use and lower cost.<sup>45</sup>

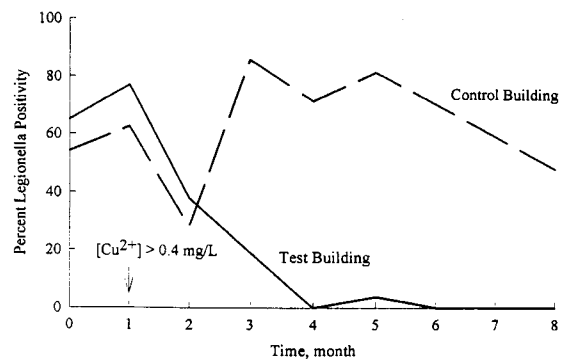
### Copper/Silver Ionization

Heavy metals such as copper and silver ions are known bactericidal agents.<sup>46,47</sup> Positively charged copper ( $\text{Cu}^{2+}$ ) and silver ( $\text{Ag}^+$ ) ions form electrostatic bonds with negatively charged sites on the organism's cell wall. These electrostatic bonds create stresses leading to distorted cell wall permeability. This action, coupled with protein denaturation, leads to cell lysis and death. Copper and

silver ions kill *L pneumophila* in vitro<sup>48,49</sup> and in vivo.<sup>30,50</sup>

**Method.** Electrolytically generated copper and silver ions are introduced into recirculating hot water from a flow cell containing electrodes made of copper/silver metal alloy (LiquiTech, Willowbrook, IL). The dose rate of ions generated is maintained by a microprocessor system. The concentration of copper and silver ions necessary for complete eradication of *L pneumophila* is somewhat dependent on the nature of the system, although the manufacturer recommends copper and silver ion concentrations to be maintained at 0.2 ~ 0.4 and 0.02 ~ 0.04 mg/L, respectively. Such concentrations are well below the maximum contaminant levels for drinking water regulated by the US Environmental Protection Agency. These levels can be maintained by periodic monitoring of copper and silver ions and adjustment of current and voltage. Copper and silver ions can be monitored using atomic absorption spectroscopy while copper ions can also be tested with a sampling kit provided by the manufacturer.

**Clinical experience.** A controlled evaluation of copper-silver ionization proved effective in eradicating *L pneumophila* from a hospital water distribution system.<sup>30</sup> *Legionella* colonization of distal outlets was reduced from 75% to 0% (Fig 1). Although Liu et al observed a rapid decrease in *Legionella* distal site colonization after copper and silver ion concentrations were above 0.4 mg/L and 0.04 mg/L, we point out that lower ion levels do appear to be effective. In fact, we target a range of 0.2 ~ 0.8 mg/L for copper and 0.02 ~ 0.08 mg/L for silver. When the ionization unit was inactivated



**Fig 1. Effect of Copper-Silver Ionization on *Legionella* Positivity.**<sup>30</sup> After copper/silver ion concentrations reached 0.4/0.04 mg/L at the test building, the recovery of *L pneumophila* from distal fixtures decreased while the control building positivity remained around 60%.

in this study, recolonization was delayed and the system was free of *Legionella* for 2 additional months. Colville et al also reported that no *Legionella* were recovered from the hospital water system in 12 months after installing the copper/silver ionization unit, and cases of hospital-acquired legionellosis disappeared.<sup>41</sup> More than 30 hospitals in the United States are now using copper-silver ionization to control *L pneumophila* in hospital water distribution systems.<sup>29,51,52</sup>

**Advantages.** The advantages of copper/silver ionization include relatively low cost and easy installation and maintenance. The efficacy of copper/silver ionization is not affected by higher water temperature, unlike chlorine and ultraviolet light. Copper/silver ions are added only into the hospital hot water recirculating lines such that consumption by human beings is limited (unlike hyperchlorination). In addition, *Legionella* are killed rather than suppressed which can minimize the possibility of recolonization.<sup>49</sup> Liu et al reported that recolonization is delayed by 6 to 12 weeks even after inactivating the ionization system.<sup>30</sup> Thus, an added safety buffer exists for this modality (unlike hyperchlorination in which *Legionella* can rapidly appear in the event of system malfunction).

**Disadvantages.** The electrodes accumulate scale and must be cleaned regularly to ensure maximum performance. In addition, the level of copper and silver in water may fluctuate. Excessive ion levels have led to blackish discoloration of water and lavender discoloration of porcelain sink surfaces. Monitoring of ion levels by atomic absorption must be performed routinely. Long-term treatment with copper and silver ions could theoretically result in the development of resistance to these ions. Although such resistance has not yet been reported.

**Cost.** The costs of installation of the copper/silver ionization units range from \$60,000 to \$100,000 depending on the size of hospital. Annual maintenance cost ranges from \$1,500 to \$4,000 for electrode replacement.

### Ultraviolet Light

Ultraviolet light irradiation is a theoretically attractive alternative for disinfection of potable water. Ultraviolet light (254 nm) kills bacteria by producing thymine dimers in DNA which subsequently hampers DNA replication. The efficacy of ultraviolet light for eradicating *L pneumophila* has

been shown in vitro<sup>53-55</sup> and in vivo.<sup>2,50</sup> The application of ultraviolet light is a focal disinfection modality that is effective if disinfection can be localized.<sup>5,56</sup> It is unsuitable as a sole modality for an entire hospital because *Legionella* persist within biofilms within the deadends and stagnant sections of the system.

**Method.** Ultraviolet light units are installed near the "point-of-use," such as shower heads and faucets. The water flows in one port of the hydraulic chamber and sterilization occurs from exposure to ultraviolet light generated from low-pressure mercury lamps. Heat-and flush or chlorination can be applied before ultraviolet light activation to eradicate existing *L pneumophila* in the system.

**Clinical experience.** In vitro<sup>53-55,57</sup> and in vivo model assessments<sup>50</sup> of ultraviolet light disinfection have established that ultraviolet light is bactericidal for *L pneumophila*. The installation of prefiltration system is necessary to prevent scale accumulation on the ultraviolet light lamps (Fig 2). Continuous ultraviolet light treatment combined with filtration was effective in preventing *Legionella* recolonization in water fixtures of a hospital ward housing renal transplant recipients.<sup>2</sup> Ultraviolet light plus prefiltration can prevent *Legionella* recolonization of a hospital for months after superheat-and-flush if the ultraviolet units are installed near the point-of-use.<sup>56</sup> Ultraviolet light can be used to provide supplemental protection against *Legionella* with chlorination.<sup>5</sup> Installation of ultraviolet units only on the inlets and outlets of hot water tanks failed to prevent colonization of *L pneumophila* because of lack of protection at distal sites.<sup>58</sup>

**Advantages.** The advantages of ultraviolet light include easy installation and no adverse effects on water or plumbing. Unlike other chemical disinfectants, water taste is not affected and there is no chemical by-product. If ultraviolet light is used, it should be combined with another systemic disinfection method. It may be more effective if control of *Legionella* can be localized to a small area, such as transplant or an intensive care units.<sup>28</sup>

**Disadvantages.** The major disadvantage of ultraviolet light is lack of residual protection at distal sites. Frequent systemic disinfection (eg, superheat-and-flush) is required to provide additional protection. The quartz sleeves housing the ultraviolet lamps are susceptible to scale and mineral deposits and must be cleaned regularly. Prefiltration is



Fig 2. Scale accumulation on the quartz sleeves housing the ultraviolet lamps impairs uv transmission (above). Prefiltration prevented scale accumulation on the surface of the quartz sleeve (below).

strongly recommended to prevent accumulation of scale on the quartz sleeves that would compromise the intensity of the ultraviolet irradiation. In one hospital, recolonization of *Legionella* occurred within weeks for ultraviolet light without prefiltration whereas no recolonization was found for 3 months using ultraviolet light with prefiltration.<sup>56</sup>

**Cost.** The cost for ultraviolet light was estimated at \$50,000 for a 500-bed hospital where four large (260 gal/min) and two small (30 gal/min) units were installed (Aquafine, Valencia, CA, Pureflow, Inc, Atlanta, GA). Because prefiltration is required for optimal performance of ultraviolet light system, this would be an additional expense.

#### *Instantaneous Heating System*

Instantaneous heating systems are operated by flash heating water to a temperature greater than 88°C (190°F) to kill *L pneumophila* and then blending the hot water with cold water to achieve the desired temperature. In some hospitals, such systems are more economical than conventional hot water tank systems because no water storage tank is needed and space requirements for installation can be reduced. Instantaneous heating is most effective when installed as the original heating system in a new building. However, it may not be effective in old systems because established biofilms of *L pneumophila* are not affected despite disinfection of the incoming water by the heaters. If an instantaneous heating system is to be installed in

a system which already contain *L pneumophila*, the water system will require decontamination subsequent to installation.

**Clinical experience.** Two hospitals with instantaneous heating systems (Leslie Controls, Tampa, FL) were *Legionella*-free as opposed to 70% (9 of 13) of hospitals with conventional water tank systems.<sup>6</sup> Control of *Legionella* by using instantaneous heaters proved unsuccessful when installed in a 700-bed tertiary-care hospital with pre-existing *Legionella* colonization.<sup>59</sup> In one hospital, replacement of large volume hot water storage tanks with instantaneous heating systems had little effect on the recovery of *Legionella* from the distal fixtures.<sup>52</sup>

**Advantages.** Instantaneous or semi-instantaneous heating systems have advantages in efficiency and space requirements over the conventional hot water tanks. No specialized personnel are needed to operate such system. These heaters also eliminate large volume storage of hot water where temperature stratification and sediment accumulation can support the growth of *Legionella*.

**Disadvantages.** Similar to superheat-and flush and ultraviolet light, no residual protection is provided by an instantaneous heating system because treatment is limited to the incoming hot water. It cannot achieve complete eradication of *L pneumophila* in the system unless the hot water temperature at outlet sites exceeds 60°C. Furthermore, we have found that performing a superheat-

and-flush procedure in buildings with instantaneous or semi-instantaneous heaters is more difficult. These heaters have difficulty in providing the large volume of super-heated water required to flush many outlets for an extended period.

*Cost.* For one 330-bed hospital with three semi-instantaneous heaters, the average cost for each heating unit was \$12,000 to \$15,000 plus installation costs.

### *Hyperchlorination*

Chlorine is an oxidizing agent that has been successfully used as a disinfectant for controlling pathogens in domestic drinking water. Free chlorine concentration of 0.4 mg/L can inactivate *Legionella* in suspension within 15 minutes in vitro.<sup>60</sup> However, *L pneumophila* attached to the surface of pipes are more resistant to chlorine. Inactivation and suppression of *L pneumophila* requires chlorine levels of greater than 3 ppm, while the residual level in domestic water is usually less than 1.0 ppm.<sup>50,61</sup> In addition, chlorine decomposes at increased water temperatures.

*Method.* Hyperchlorination implies that additional chlorine is added to water with an existing chlorine residual. Two approaches have been applied with regard to *Legionella* disinfection, shock hyperchlorination and continuous hyperchlorination. Shock hyperchlorination is used by a pulse injection of chlorine in water to achieve concentration of chlorine at 20 ~ 50 ppm throughout the system.<sup>3,62</sup> After time, the water is drained and the system is mixed with incoming water so that the residual chlorine level returns to normal concentrations (0.5 ~ 1 ppm). Continuous hyperchlorination is accomplished by continuous injection of additional chlorine which may be introduced through calcium hypochlorite, sodium hypochlorite, or gas chlorination.<sup>3</sup> Residual chlorine levels will fluctuate because of changes in incoming water quality, flow rates, and scavenging by system materials or indigenous biofilms. If the system has areas of stagnation or low usage or if there are recirculation problems within the water distribution system, chlorine will not inactivate *Legionella* in these areas. Qualified maintenance personnel are needed to conduct monitoring programs and perform residual disinfectant analysis.

*Clinical experience.* Continuous hyperchlorination has been used with variable success to control the growth of *L pneumophila*.<sup>14,26,52,63-65</sup> Supplemen-

tal chlorination in the range of 2 ~ 6 mg/L has also been combined with the superheat-and-flush method to decontaminate the hospital water systems.<sup>26,39</sup> Snyder et al reported that positive environmental cultures for *L pneumophila* decreased from 43% to 8% in 6 weeks after supplemental chlorination was applied.<sup>26</sup> Recolonization of *L pneumophila* in one hospital using shock hyperchlorination occurred within 2 to 5 months after the chlorine concentrations returned to normal levels.<sup>27</sup>

*Advantages.* Chlorination is a systemic disinfection modality that provides a residual disinfectant concentration throughout the entire water distribution system so that colonization of *L pneumophila* at the distal sites can be minimized.

*Disadvantages.* The unique disadvantage of hyperchlorination is that chlorine is highly corrosive and causes significant pipe damage. The average number of pipes leaks at the University of Iowa Hospital increased 30-fold after 3 years of chlorination.<sup>66</sup> Although this problem can be minimized by chemically coating all hot water pipes with a sodium silicate precipitate, the initial and maintenance costs are high. Furthermore, leaks continued to occur at a rate of one to three leaks per month after the pipes were coated at this hospital.<sup>66</sup> Because of problems associated with corrosion and variable efficacy, at least five major academic centers that initially adopted hyperchlorination as their disinfection modality have now converted to copper-silver ionization systems.

Chlorine may only suppress *Legionella* rather than kill. It has been shown that *Legionella* is relatively chlorine tolerant. Kuchta et al reported that a 99% kill of *L pneumophila* was achieved at 0.1 mg/L of free chlorine residual within 40 minutes, compared with less than 1 minute for *Escherichia coli*.<sup>67</sup> If shock hyperchlorination is used, recolonization of *L pneumophila* will occur after chlorine levels decrease. Because *Legionella* is suppressed but not killed, chlorinator failure can result in rapid emergence of dangerously high levels within days of chlorine withdrawal. Another theoretical reason why *Legionella* may recolonize after chlorine levels decrease is the presence of *Legionella* within the protozoan cysts of *Acanthamoeba*. These cysts survive free chlorine levels of 50 ppm.<sup>68</sup>

Finally, high residual chlorine will react with organic materials and accelerate the production of trihalomethanes, which is a known carcinogen.

Chlorine concentrations used in drinking water purification are usually less than 1 ppm. It is important to emphasize that a positive association has been found for consumption of chlorinated water and cancer in numerous epidemiological studies. For example, 64% of risk estimates derived from 10 epidemiological studies showed a higher risk estimate for cancer with exposure to chlorinated water as compared to controls.<sup>69-78</sup> A meta-analysis of 10 case-control studies<sup>69-78</sup> and two cohort studies<sup>75,79</sup> showed a "clear and significant association between neoplastic disease and consumption of water containing chlorination by-products."<sup>80</sup> The highest risk estimates were for rectal and bladder cancer. Given this strong epidemiological association with a lower chlorine concentration that is used for the municipal water supplies, the risk of acquisition of cancer is probably even higher with hyperchlorination.

**Cost.** Costs will depend on the type of chlorinator, the number and capacity of chlorinators, and any supplemental equipment. Grosserode et al reported the costs of \$75,800 to install chlorinator injectors: \$48,000 for consultant fees, and an annual operating cost of \$7,000. At another hospital with 800 beds, the costs of supplemental chlorination were approximately \$88,000 initially cost plus \$16,000 annually for maintenance costs. One Pittsburgh hospital found the costs of continuous hyperchlorination to be greater than \$100,000 to \$150,000 for the first year.<sup>52</sup> One should expect significant annual maintenance cost for replacement of pipes due to corrosion over time. If the silicate coating is chosen to protect the surfaces of plumbing materials from corrosion, the costs for silicate injection devices were as high as \$54,480 for installation with an annual operating cost of \$10,814.<sup>66</sup>

#### *Redundancy As a Disinfection Approach*

In some hospitals with endemic legionellosis and a high-risk population (especially transplant and intensive care unit patients), multiple disinfection approaches may be desired so that if one fails accidentally, the other one can serve as a backup.<sup>38</sup> For example, ultraviolet light units can be combined with other systemic disinfection modalities at those critical sites to ensure maximum protection. In addition, although hyperchlorination generally has proven to be unsatisfactory, *in vitro* studies have shown synergy between chlorine and ultraviolet light or copper and silver ions such that chlorina-

tion might be combined with other disinfection modalities at a much lower concentration of chlorine.<sup>13,48</sup> Thus, the existing chlorination facilities (eg, the chlorinator) of the hospital should not be dismantled even when it is replaced by other more effective methods. Supplementation with chlorine in the future could be a viable option if there are problems with the new method.

#### UNSUCCESSFUL ERADICATION METHODS

The efficacy of localized disinfection confined to one particular area or group of fixtures is usually temporary. Recolonization often occurs within days to weeks. Immersing contaminated shower heads and faucets in boiling water or chemical disinfectants has proven ineffective; recolonization of *Legionella* occurred when these fixtures were placed back on-line in a contaminated water distribution system. Physical cleaning and chemical sterilization of taps, and replacing rubber washers with "approved" brands were ineffective in one study.<sup>18</sup> Automatic drain valves fitted to showers were also ineffective in maintaining a reduction in the number of *Legionella* in shower water.<sup>81</sup>

There is widespread misconception that good engineering practice and preventive maintenance will prevent *Legionella* colonization.<sup>82</sup> However, hospitals that practiced a preventive maintenance program, including cleaning or flushing the hot water storage tank on a weekly to annual basis, were as likely to be contaminated with *Legionella* as those without such programs.<sup>6</sup> Even after "appropriate" engineering practices for the prevention of legionellosis were instituted in 17 hospitals in England and Wales, 12% (2 of 17) had *Legionella* recovered from their water systems.<sup>8</sup> These investigators concluded that in addition to preventive maintenance programs, regular cultures of water from a limited number of areas in hospital water systems were necessary to evaluate the potential for *Legionella* colonization.

#### POSTDISINFECTION MONITORING

Routine periodic surveillance with environmental cultures is necessary to ensure maximum protection of patients from legionnaires' disease because mechanical failures of disinfection systems and human error are expected with any system. The laboratory techniques for the isolation of *Legionella* are easily performed and inexpensive. Selective dye-containing media should be used.<sup>83</sup>



In a comparison of different selective media formulation, we found the highest concentration of *Legionella* recovered from the selective media containing dyes, glycine, vancomycin, and polymyxin  $\beta$ .<sup>83</sup> We recommend the use of swabs to culture distal fixtures. A swab sample is more likely to recover *Legionella* from a distal fixture than a water sample collected from the same site.<sup>83</sup> Environmental cultures should be performed at 2-month intervals because recolonization is likely to occur within weeks due to malfunction of disinfection. For hospitals using hyperchlorination, cultures at 2-week intervals may be required because *Legionella* are relatively chlorine-resistant and rapid recolonization can occur in the event of system failure. If environmental cultures are positive, a higher index of suspicion by physicians and infection control practitioners should be paid to patients with hospital-acquired pneumonia.

Complete elimination of *Legionella* from a water distribution system is difficult to achieve with any disinfection approach. Although the disinfection modality may inactivate *Legionella* within large portions of the biofilm, small pockets of *Legionella* in protected niches may still be viable but in numbers insufficient to cause infection in most patients. Thus, the end points for environmental surveillance and disinfection should be realistic and clinically relevant.

*Legionella* infections in one Pittsburgh hospital did not occur until the percentage of *Legionella*-positive sites exceeded 30%.<sup>32</sup> Although this percentage will vary among different hospitals, there may be a critical threshold of *Legionella* positivity above which cases will occur. Further studies are necessary to define this "critical" threshold, but it will likely be dependent on inocula of organisms present, access of susceptible patients to water, underlying disease of the patient population, and volume of surgery (especially transplants). If such a critical threshold could be defined empirically, this would provide an objective end point for monitoring.

Outbreaks of legionnaire's disease have been temporally associated with hospital construction.<sup>24</sup> Mermel et al recommended that attention be paid to environmental surveillance and preventive measures during major construction projects that affect water lines, excavation on hospital grounds, or when the water supply is shut down and later repressurized.

Maintenance of the disinfection modality is an important, but underestimated factor for sustaining the efficacy of any disinfection modality. For systemic disinfection modalities, such as hyperchlorination and copper/silver ionization, disinfectant levels must be monitored. The disinfection devices (lamps, electrodes, etc) must be cleaned regularly to ensure maximum efficacy. The efficacy of any disinfection modality is dependent not only on the equipment, but also on the consistency of *Legionella* surveillance, water monitoring, duration of disinfection measure, and cooperation among the engineers and staff.

## RECOMMENDATIONS

Careful planning and analysis based on efficacy, cost, installation, and maintenance is required for choosing a disinfection modality.<sup>84</sup> None of the disinfection techniques can be successful without a conscientious monitoring program and a committed staff.

Environmental surveillance cultures for *L pneumophila* are the cornerstone of prevention of hospital-acquired legionnaires' disease (see Appendix for culture methodology). In a nonoutbreak situation, hospitals should obtain baseline cultures before instituting any disinfection method so that the efficacy of the selected modality can be adequately evaluated. If any cultures are positive, greater attention should be paid to occurrence of hospital-acquired pneumonia. In an outbreak situation, baseline environmental surveillance should be documented before disinfection. The selection of sites for culture of faucets, showerheads, and ice machines should be directed at high-risk patients area including intensive care units, transplantation wards, and at rooms housing infected patients.<sup>85</sup>

In an outbreak situation, remedial action must be undertaken with very short notice and limited resources. The superheat-and-flush procedure can be used for emergency control of *Legionella* because it requires no special equipment and can be initiated expeditiously. Once the crisis is over, long-term solutions can be considered.

For long-term prevention of *Legionella* from water distribution systems, systemic disinfection methods are most reliable. Preliminary assessments suggest that copper-silver ionization is the most efficacious and cost-effective method today. Given the public health implications, the history of experience and service commitment by commercial ven-

dors in *Legionella* disinfection must be reviewed in detail before purchase.<sup>84</sup> It would be prudent to obtain assessments from other hospitals that have used the vendor's product. Communication must involve all hospital personnel who may affect the disinfection procedures, *Legionella* surveillance and disinfection must be coordinated with infection control personnel, engineering staff, and administration.

## APPENDIX

### Culture Methodology for *Legionella* from the Environment

In this appendix, we will review the methods and procedures for the isolation of *Legionella* from environmental samples. We also include information about availability of media and reagents from commercial companies.

The method of sample collection is critical in evaluating *Legionella* colonization of water samples. Because *Legionella* grows within the biofilm, swab samples have a greater likelihood of being positive than water samples collected from the same site.<sup>83</sup> If water samples are collected from distal sites, a 200-mL sample should be collected after the swab sample and filter concentrated. Buffered charcoal yeast extract agar with dyes, glycine, vancomycin, and polymyxin (DGVP) is the primary medium used for isolation of these organisms from the environment. Acid pretreatment of the specimen is used to prevent overgrowth of competing flora.

#### Sample Collection

**Faucets.** Moisten the outlet by allowing hot water to trickle through the opening. A sterile, Dacron swab is inserted and rotated four times around the inner circumference and moving up the faucet as far as the swab will reach. Replace the swab into the container. If the swab system does not contain a transport medium, then allow 0.5 mL of water to flow from the faucet into the container to keep the swab moist. The culturette II system (Becton Dickinson) has a self-contained transport medium, and it is not necessary to add water to the swabs.

**Shower heads.** Moisten the shower head by allowing hot water to trickle through the opening. Rotate the swab over the entire surface of the shower head four times. Replace the swab into the container. If the swab system does not contain a transport medium, then allow 0.5 mL of water to flow from the shower head into the container.

**Hot water tank.** Open the drain valve at the base of the tank. Collect 50 to 100 mL immediately into a sterile specimen container. Let the water drain out of the pipe for 15 to 30 seconds to flush out residual water within the drain pipe. Collect another 50 to 100 mL into a second specimen container. This procedure ensures that both residual water in the drain pipes and water from the tank are sampled. Scale and sediment often harbor *Legionella* bacteria, therefore, it is worthwhile to obtain scale or

sediment from tanks or distal sites. Specimens from the bottom of hot water tanks that contain an excessive amount of sediment may not yield the organism because of the inhibiting effect of a high concentration of minerals.

#### Sample Storage Temperature

Samples should be refrigerated at 2° to 8°C before and after processing.

#### Culture Media

Buffered Charcoal Yeast Extract (BCYE) agar is the primary isolation medium for recovery of *Legionella*. A selective medium, DGVP, is also used. These culture media are commercially available and can be obtained from the following vendors: Remel: BCYE catalog number 07-092; DGVP catalog number 01-338; BBL via Fisher Scientific or Becton Dickinson Microbiology Systems: BCYE catalog number 21808; DGVP catalog number 99645 (orders for DGVP require 1 week advance notice).

#### Sample Processing

Swabs are acid-buffer pretreated and 0.1 mL of the treated sample is inoculated on BCYE and DGVP agar plates (see Acid-buffer Pretreatment for swab sample). Water samples from hot water tanks are inoculated directly (0.1 mL) onto BCYE and DGVP agar plates and spread onto the surface by using a sterile bent glass rod. If water is collected from a distal site, 100 mL of the sample should be concentrated by filtration and inoculated directly and after acid pretreatment onto BCYE and DGVP agar plates (see Acid Pretreatment of water samples).

All plates are incubated in a humidified atmosphere at 37°C and read daily after 3 days for 7 to 10 days.

#### Acid-Buffer Pretreatment

##### Buffer preparation:

1. Prepare 0.2 mol/L potassium chloride (KCl) solution (1.49 g KCl into 100 mL deionized water).
2. Prepare 0.2 mol/L hydrochloric acid (HCl) solution.
3. Mix 3.9 mL HCl with 25 mL KCl and filter sterilize. Check pH: it should be  $2.2 \pm 0.1$ . Adjust pH if necessary.

##### Acid-buffer pretreatment for water samples:

1. Filter 100 mL of specimen using a 100-mL filter funnel assembly with a 0.2- $\mu$ m/47-mm polycarbonate filter (Nucleopore Co, Pleasanton, CA).
2. Aseptically remove the filter pad and resuspend in 10 mL of the original sample by vortexing for 30 seconds.
3. The resuspended sample is treated with equivalent volume of acid buffer for 3 minutes and spread 0.1 mL of the treated specimen onto BCYE and DGVP media.

##### Acid-buffer pretreatment for swab sample:

1. Shake the swab vigorously in 2.0 mL of HCl-KCl acid-buffer, squeeze the swab against the inside of the tube, and mix for 3 minutes.
2. Spread 0.1 mL of treated specimens onto BCYE and DGVP media.

## REFERENCES

1. Johnson JT, Yu VL, Best M, et al: Nosocomial legionellosis uncovered in surgical patients with head and neck cancer: Implications for epidemiologic reservoir and mode of transmission. *Lancet* 2:298-300, 1985
2. Farr BM, Bratz J, Tartaglino J, et al: Evaluation of ultraviolet light for disinfection of hospital works contaminated with *Legionella*. *Lancet* 2:669-672, 1988
3. Muraca P, Yu VL, Goetz A: Disinfection of water distribu-

tion systems for Legionella: A review of application procedures and methodologies. *Infect Control Hosp Epidemiol* 11:79-88, 1990

4. Goetz A, Yu VL: Screening for nosocomial legionellosis by culture of the water supply and targeting of high-risk patients for specialized laboratory testing. *Am J Infect Cont* 19:63-66, 1991

5. Matulonis U, Rosenfeld CS, Shaddock: Prevention of *Legionella* infections in a bone marrow transplant unit: Multifaceted approach to decontamination of a water system. *Infect Control Hosp Epidemiol* 14:571-575, 1993

6. Vickers RM, Yu VL, Hanna SS, et al: Determinants of *L pneumophila* contamination of water distribution systems: 15 hospital prospective study. *Infect Control* 8:357-363, 1987

7. Alary M, Joly JR: Factors contributing to the contamination of hospital water distribution systems. *Appl Environ Microbiol* 165:565-569, 1992

8. Liu WK, Healing DE, Yeomans JT, et al: Monitoring of hospital water supplies for *Legionella*. *J Hosp Infect* 24:1-9, 1993

9. Marrie TJ, Green T, Burbridge S: Legionellaceae in the potable water of Nova Scotia hospital and Halifax residences. *Epidemiol Infect* 112:143-150, 1994

10. Anonymous: Second report of the committee of inquiry into the outbreak of legionnaires' disease in Stafford in April 1985. London, Her Majesty's Stationery Office (HMSO), 1987

11. Rogers J, Doowsett AB, Dennis PJ, et al: Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl Environ Microbiol* 60:1842-1851, 1994

12. Plouffe JF, Webster LR, Hackman B: Relationship between colonization of hospital building with *Legionella pneumophila* and hot water temperature. *Appl Environ Microbiol* 46:769-779, 1983

13. Alary M, Joly JR: Risk factors for contamination of domestic hot water systems by *Legionella*. *Appl Environ Microbiol* 57:2360-2367, 1991

14. Shands K, Ho J, Meyer R, et al: Potable water as a source of legionnaires' disease. *JAMA* 253:1412-1416, 1985

15. Yu VL: personal communication, 1996

16. Memish ZA, Oxley C, Contant J, et al: Plumbing system shock absorbers as a source of *Legionella pneumophila*. *Am J Infect Control* 20:305-309, 1992

17. Colbourne JS, Pratt DJ, Smith MG, et al: Water fittings as sources of *Legionella pneumophila* in a hospital plumbing system. *Lancet* 1:210-213, 1984

18. Robeiro CD, Burge SH, Palmer SR, et al: *Legionella pneumophila* in a hospital water system following a hospital-acquired outbreak: Prevalence, monoclonal antibody subgroup and effect of control measures. *Epidemiol Infect* 98:253-262, 1987

19. Scholfield GM, Locci R: Colonization of components of a model hot water system by *Legionella pneumophila*. *Appl Bacteriol* 58:151-162, 1985

20. Wright JB, Ruseska I, Athar M, et al: *Legionella pneumophila* grows adherent to surfaces in vitro and in situ. *Infect Control Hosp Epidemiol* 10:408-415, 1989

21. Fields BS: *Legionella* and protozoa: Interaction of a pathogen and its natural host. Current status and emerging perspectives of *Legionella*, In: Barbaree JM, Breiman RF, Dufour AP (eds): Washington, DC, American Society for Microbiology, 1993, pp 129-136

22. Wadowsky RM, Yee RB, Mezmar L, et al: Hot water systems as sources of *Legionella pneumophila* in hospital and nonhospital plumbing fixtures. *Appl Environ Microbiol* 43:1104-1110, 1982

23. Stout JE, Yu VL, Vickers RM, et al: Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic legionnaires' disease. *N Engl J Med* 36:466-468, 1982

24. Mermel LA, Josephson SL, Girogio CH, et al: Association of legionnaires' disease with construction: Contamination of potable water. *Infect Cont Hosp Epid* 16:76-81, 1995

25. Cargill KL, Pyle BH, Sauer RL, et al: Effects of culture conditions and biofilm formation on the iodine susceptibility of *Legionella pneumophila*. *Can J Microbiol* 38:423-429, 1991

26. Snyder MB, Siwicki M, Wireman J, et al: Reduction in *Legionella pneumophila* through heat flushing followed by continuous supplemental chlorination of hospital hot water. *J Infect Dis* 162:127-132, 1990

27. Levin AS, Gobara S, Scarpitta CM: Electric showers as a control measure for *Legionella* spp. in a renal transplant unit in San Paulo, Brazil. *J Hosp Infect* 30:133-137, 1995

28. Yu VL, Liu Z, Stout JE, et al: *Legionella* disinfection of water distribution systems: Principles, problems, and practice. *Infect Control Hosp Epidemiol* 14:567-570, 1993

29. Baker RL, Stevens J, Fish L, et al: Nosocomial legionnaires' disease controlled by UV light and low level silver/copper ions. *Third Int Conf Nosoco Infect*, Atlanta, GA, 1990 (No. 72)

30. Liu Z, Stout JE, Tedesco L, et al: Controlled evaluation of copper-silver ionization in eradicating *Legionella pneumophila* from a hospital water distribution system. *J Infect Dis* 169:919-922, 1994

31. Fisher-Hoch SP, Tobin JO, Belson AM: Investigation and control of an outbreak of legionnaires' disease in a district general hospital. *Lancet* 1:932-936, 1981

32. Best M, Yu VL, Stout J, et al: Legionellaceae in the hospital water supply—Epidemiological link with disease and evaluation of a method of control of nosocomial legionnaires' disease and Pittsburgh pneumonia. *Lancet* 2:307-310, 1983

33. Dennis PJ, Green D, Jones BP: A note on the temperature tolerance of *Legionella*. *J Appl Bacteriol* 56:349-350, 1984

34. Stout JE, Best M, Yu VL: Susceptibility of members of the family Legionellaceae to thermal stress: Implications for heat eradication methods in water distribution systems. *Appl Environ Microbiol* 52:396-399, 1986

35. Sanden GN, Fields BS, Barbaree JM: Viability of *Legionella pneumophila* in chlorine-free water at elevated temperature. *Curr Microbiol* 18:61-65, 1989

36. Groothuis DG, Veenendall HR, Dijkstra HL: Influence of temperature on the number of *Legionella pneumophila* in hot water system. *J Appl Bacteriol* 59:529-536, 1985

37. Lee TC, Stout JE, Yu VL: Factors predisposing to *L pneumophila* colonization in residential water systems. *Arch Environ Health* 43:59-62, 1988

38. Best MG, Goetz A, Yu VL: Heat eradication measures for control of hospital-acquired legionnaires' disease: Implementation, education, and cost analysis. *Am J Infect Control* 12:26-30, 1984

39. Heimberger T, Birkhead G, Bornstein D, et al: Control of nosocomial legionnaires' disease through hot water flushing and supplemental chlorination of potable water. *J Infect Dis* 163:413, 1991

40. Furuhashi K, Takayanagi T, Danno N, et al: Contamina-

- tion of hot water supply in office buildings by *Legionella pneumophila* and some countermeasures. *Japan J Public Health* 41:1073-1083, 1994
41. Colville A, Crowley J, Dearden D, et al: Outbreak of legionnaires' disease at a University Hospital, Nottingham. *Epidemiology, microbiology, and control. Epidemiol Infect* 10:105-116, 1993
  42. Darelid J, Bengtsson L, Gastrin B, et al: An outbreak of legionnaires' disease in a Swedish hospital. *Scand J Infect Dis* 26:417-425, 1994
  43. Struelens MJ, Maes N, Rost F, et al: Genotypic and phenotypic methods for the investigation of a hospital-acquired *Legionella pneumophila* outbreak and efficacy of control measures. *J Infect Dis* 166:22-30, 1992
  44. Ezzedine H, VanOssel C, Delmee M, et al: *Legionella* spp. in a hospital hot water system: Effect of control measures. *J Hosp Infect* 13:121-131, 1989
  45. American Society of Plumbing Engineers: Temperature limits in service hot water systems. ASPE Research Foundation, Los Angeles, CA 1989, pp 88-101
  46. Clarke NA: Disinfection of drinking water, swimming pool water, and treated sewage effluents, in Block SS (ed): *Disinfection, Sterilization and Preservation*. Philadelphia, PA, Lea & Febiger, 1983, pp 524-541
  47. Wood JM: Evolutionary aspects of metals ion transport through cell membrane, in Sigel H (ed): *Metal Ions in Biological System*. New York, NY, Marcel Dekker, 1984, pp 223-237
  48. Landeen LK, Yahya MT, Gerba CP: Efficacy of copper and silver ions and reduced levels of free chlorine in inactivation of *Legionella pneumophila*. *Appl Environ Microbiol* 55:3045-3050, 1989
  49. Lin YE, Vidic RD, Stout JE, et al: Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila*. *Wat Res* 30:1905-1913, 1996
  50. Muraca P, Stout JE, Yu VL: Comparative assessment of chlorine, heat, ozone, and UV light for killing *Legionella pneumophila* within a model plumbing systems. *Appl Environ Microbiol* 53:447-453, 1987
  51. Thomson RB, File TM, Plouffe J: Use of Tarn-Pure to eradicate *Legionella pneumophila* from a hospital hot water system. 1990 (No. L18) Proceedings of the Annual Meeting of the Amer Soc Microbiol, Anaheim, CA
  52. Stout JE: personal communication, 1996
  53. Gilpin RW: Laboratory and field application of UV light disinfection on six species of *Legionella* and other bacteria in water, in Thornsberry C (ed): *Legionella—Proceedings of 2nd International Symposium*. Washington, DC, American Society for Microbiology, 1984 pp 337-339, Atlanta, GA
  54. Wadowsky RM, Wolford R, McNamara AM, et al: Effect of temperature, pH, and oxygen level on the multiplication of naturally occurring *Legionella pneumophila* in potable water. *Appl Environ Microbiol* 49:1197-1205, 1985
  55. Martiny H, Seidel K, Ruden H: Use of UV irradiation for disinfection of water—III Communication: UV susceptibility of *Legionella pneumophila* of different age in cold and warm water. *Zbl Hyg* 188:35-46, 1989
  56. Liu Z, Stout JE, Tedesco L, et al: Efficacy of ultraviolet light in preventing *Legionella* colonization of a hospital water distribution system. *Wat Res* 29:2275-2280, 1995
  57. Yamamoto H, Urakami I, Nakano K, et al: Effects of FLONLIZER, ultraviolet sterilizer, on *Legionella* species inhabiting cooling tower water. *Microbiol Immunol* 31:745-752, 1987
  58. Schulze-Robbecke R, Jung KD, Pullmann H, et al: Sanitizing a hospital hot water system contaminated with *Legionella pneumophila*. *Zentralblatt Hyg Umweltmedizin* 190: 84-100, 1990
  59. Sellick JA, Mylotte JM: Nosocomial *Legionella pneumophila* in a hospital with an instantaneous hot water tank, in Barbaree JM, Breiman RF, Dufour AP (eds): *Legionella—Current Status and Emerging Perspectives*. Washington, DC, American Society for Microbiology, 1993, pp 43-45
  60. Yabuuchi E, Wang L, Yamayoshi T, et al: Bactericidal effect of chlorine on strains of *Legionella* species. *J Japan Assoc Infect Dis* 69:151-157, 1995
  61. Skaliy P, Thompson TA, Gorman GW, et al: Laboratory studies of disinfectants against *Legionella pneumophila*. *Appl Environ Microbiol* 40:697-700, 1980
  62. Allegheny County Health Department: Approaches to Prevention and Control of *Legionella* Infection in Allegheny County Health Care Facilities. Allegheny County Health Department, Pittsburgh, PA, 1993, pp 1-13
  63. Baird I, Potts W, Smiley J, et al: Control of endemic nosocomial legionellosis by hyperchlorination of potable water, in Thornsberry C, et al. (eds): *Legionella—Proceedings of the 2nd International Symposium*, Washington, DC, American Society of Microbiology, 1984, pp 333
  64. Witherall LE, Duncan R, Store K, et al: Investigation of *L pneumophila* in drinking water. *J Am Water Works Assoc* 80:87-93, 1988
  65. Helms CM, Massanari M, Wenzel RP, et al: Legionnaires' disease associated with a hospital water system: A five-year progress report on continuous hyperchlorination. *JAMA* 259:2423-2427, 1988
  66. Grosserode M, Helms C, Pfaller M, et al: Continuous hyperchlorination for control of nosocomial legionnaires' disease: A ten-year follow-up of efficacy, environmental effects, and cost, in Barbaree JM, Breiman RF, Dufour AP (eds): *Legionella—Current Status and Emerging Perspectives*. Washington, DC, American Society for Microbiology, 1993, pp 226-229
  67. Kuchta JM, States SJ, McNamara AM: Susceptibility of *Legionella pneumophila* to chlorine in tap water. *Appl Environ Microbiol* 46:1134-1139, 1983
  68. Kilvington S, Price J: Survival of *Legionella pneumophila* within cysts of *Acanthamoeba polyphaga* following chlorine exposure. *J Appl Bacteriol* 68:519-525, 1990
  69. Cantor KP, Hoover R, Hartge P, et al: Bladder cancer, drink water source, and tap water consumption: A case-control study. *J Natl Cancer Inst* 79:1269-1279, 1987
  70. Young TB, Kanarek MS, Tsiatis AA: Epidemiologic study of drinking water chlorination and Wisconsin female cancer mortality. *J Natl Cancer Inst* 67:1191-1198, 1981
  71. Young TB, Wolf DA, Kanarek MS: Case-control study of colon cancer and drinking water trihalomethanes in Wisconsin. *Int J Epidemiol* 16:190-197, 1987
  72. Zierler S, Danley RA, Feingold L: Type of disinfectant in drinking water and patterns of mortality in Massachusetts. *Environ Health Perspect* 69:275-279, 1986
  73. Cragle DL, Shy CM, Struba RJ, et al: A case-control study of colon cancer and water chlorination in North Carolina, in Jolley RL (ed): *Water Chlorination Chemistry*, Environmental

Impact and Health Effects. Chelsea, MI, Lewis Publishers, 1985, pp 153-159

74. Lawrence CE, Taylor PR, Trock BJ, et al: Trihalomethanes in drinking water and human colorectal cancer. *J Natl Cancer Inst* 72:563-568, 1984

75. Wilkins JR, Comstock GW: Source of drinking water at home and site-specific cancer incidence in Washington County, Maryland. *Am J Epidemiol* 114:178-190, 1981

76. Gottlieb MS, Carr JK, Clarkson JR: Drinking water and cancer in Louisiana: A retrospective mortality study. *Am J Epidemiol* 116:281-283, 1982

77. Brenniman GR, Vasilomanolakis-Lagos J, Amsel J, et al: Case-control study of cancer deaths in Illinois communities served by chlorinated or non-chlorinated water, in Jolley RL, Brungs WA, Cumming RB (eds): *Water Chlorination: Environmental Impact and Health Effects*. Ann Arbor, MI, Ann Arbor Scientific Publishers, 1980, pp 1043-1057

78. Alvanja M, Goldstein I, Susser M: A case-control study of gastrointestinal and urinary tract cancer mortality and drinking water chlorination, in Jolley RL, Gorchev H, Hamilton DHJ (eds): *Water Chlorination: Environmental Impact and Health Effects*. Ann Arbor, MI, Ann Arbor Scientific Publishers, 1978, pp 395-409

79. Schulte PA, Ringen K, Hemstreet GP, et al: Risk factors for bladder cancer in cohort exposed to aromatic amines. *Cancer* 58:2156-2162, 1986

80. Morris RD, Audet AM, Angelillo IF, et al: Chlorination, chlorination by-products, and cancer: A meta-analysis. *Am J Pub Health* 82:955-963, 1992

81. Makin T, Hart CA: The efficacy of control measures for eradicating legionellae in showers. *J Hosp Infect* 16:1-7, 1990

82. United Kingdom Department of Health: *The Control of Legionellae in Health Care Premises—A Code of Practice*. London, UK, Her Majesty's Stationary Office, 1991

83. Ta AC, Stout JE, Yu VL, et al: Comparison of culture methods for monitoring *Legionella* species in hospital potable water systems and recommendations for standardization of such methods. *J Clin Microbiol* 33:2118-2123, 1995

84. Freije MR: *Legionellae control in health care facilities, A guide for minimizing risk*. Indianapolis, IN, HC Information Resources, Inc, 1996

85. Marrie TJ, Johnson WM, Tyler SD, et al: Genomic stability of *Legionella pneumophila* isolates recovered from two cardiac transplants with nosocomial legionnaires' disease. *Microbiol* 32:3085-3087, 1994