APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1987, p. 447–453
0099-2240/87/020447-07$02.00/0

Vol. 53, No. 2

Comparative Assessment of Chlorine, Heat, Ozone, and UV Light for Killing *Legionella pneumophila* within a Model Plumbing System

PAUL MURACA, JANET E. STOUT, AND VICTOR L. YU*

Special Pathogens Section, Veterans Administration Medical Center and the University of Pittsburgh, Pittsburgh, Pennsylvania 15240

Received 23 September 1986/Accepted 21 November 1986

Nosocomial Legionnaires disease can be acquired by exposure to the organism from the hospital water distribution system. As a result, many hospitals have instituted eradication procedures, including hypercholorination and thermal eradication. We compared the efficacy of ozonation, UV light, hyperchlorination, and heat eradication using a model plumbing system constructed of copper piping, brass spigots, Plexiglas reservoir, electric hot water tank, and a pump. *Legionella pneumophila* was added to the system at 10^7 CFU/ml. Each method was tested under three conditions; (i) nonturbid water at 25°C, (ii) turbid water at 25°C, and (iii) nonturbid water at 43°C. UV light and heat killed *L. pneumophila* most rapidly and required minimal maintenance. Both UV light and heat (60°C) produced a 5 log kill in less than 1 h. In contrast, both chlorine and ozone required 5 h of exposure to produce a 5 log decrease. Neither turbidity nor the higher temperature of 43°C impaired the efficacy of any of the disinfectant methods. Surprisingly, higher temperature enhanced the disinfecting efficacy of chlorine. However, higher temperature accelerated the decomposition of the chlorine residual such that an additional 120% volume of chlorine was required. All four methods proved efficacious in eradicating *L. pneumophila* from a model plumbing system.

Legionella pneumophila in the water distribution system has been epidemiologically linked to nosocomial Legionnaires disease (6, 12, 18). As a result, many hospitals have been compelled to implement disinfection procedures for their water distribution systems. The two most widely used methods of *L. pneumophila* disinfection are hyperchlorination and heat eradication. Both methods have proven effective in eradicating *L. pneumophila*; however, each has its unique disadvantages.

Hyperchlorination involves the installation of a chlorinator and raising of the chlorine concentration to 2 to 6 mg/liter (12, 21). The notable drawback of hyperchlorination is its inability to completely eradicate the organism from the water distribution system of the building. Recontamination easily occurs when the chlorine residual drops below the recommended levels. Thus, for this technique to be used successfully, stringent monitoring of chlorine levels, as well as full-time personnel for monitoring and maintaining equipment, is a necessity. Chlorine also has a notable corrosive impact on distribution pipes over time. In addition, chlorine is a precursor to halogenated organic compounds known to be carcinogenic (13).

Another proven and more widely used method is heat eradication (5, 6). The temperature of the hot water tanks is raised to 60 to 80°C for several days. Since colonization of *L. pneumophila* occurs throughout the water distribution system, distal sites (faucets, shower heads) may also harbor *L. pneumophila*. Effective disinfection, thus, requires flushing of the distal sites with hot water.

Although heating and flushing are simple to implement and inexpensive compared with hyperchlorination, a major disadvantage is the need for periodic disinfection since the system may be recolonized over time. The potential for scalding incidents exists, although none has yet been reported.

Given the drawbacks associated with each technique, an investigation of new modalities is warranted. We evaluated the efficacy of four disinfection modalities (chlorine, heat, ozone, and UV light), using a model plumbing system constructed of copper piping, brass fixtures, a centrifugal pump, a Plexiglas reservoir, and an electric hot-water tank (Fig. 1).

MATERIALS AND METHODS

Inoculum and specimen processing. An environmental isolate of *L. pneumophila*, serogroup 1, was frozen at −20°C in 50% (vol/vol) Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and glycerol. A subculture of this isolate was prepared on buffered charcoal-yeast extract agar and incubated for 72 h at 37°C. Growth was transferred to two tubes which contained 10 ml of sterile water to match a 1.0 McFarland standard. Each tube was added to 500 ml of buffered yeast extract broth. After incubation for 60 to 72 h on a shaking incubator, the cells from each broth culture were concentrated by centrifugation. The packed cells were resuspended in 300 ml of sterile water. This suspension was introduced to the model system via the Plexiglas reservoir (Fig. 1) and allowed to circulate for 45 to 60 min. The final concentration of *L. pneumophila* in the model system was approximately 1.0×10^7 CFU/ml.

Bacterial analysis for each disinfection trial was performed as follows. All water samples were collected from the sample port preceding the UV light unit (Fig. 1), serially diluted, and plated in duplicate onto buffered charcoal-yeast extract agar and dye-containing selective media (9, 17, 23). The culture plates were incubated at 37°C, and subsequent *L. pneumophila* growth was recorded 5 days later.

Model design and construction. The components used in construction of the model were characteristic of those found

* Corresponding author.

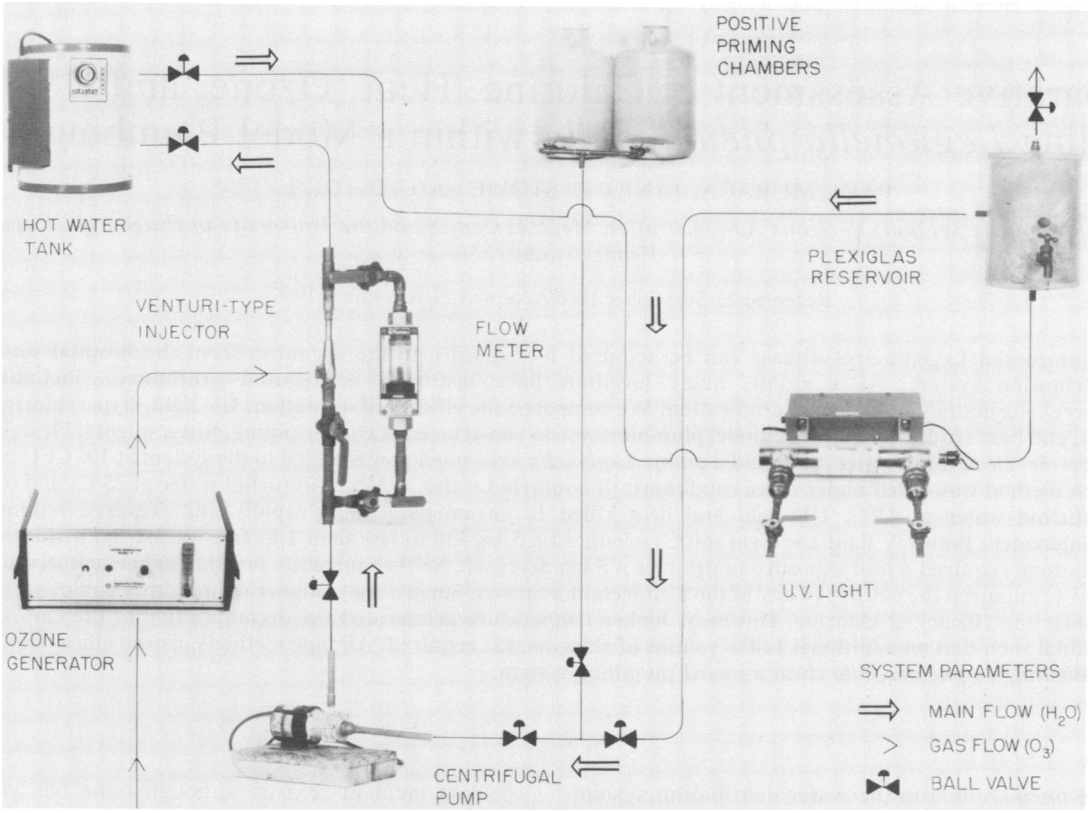


FIG. 1. A schematic representation of the in vitro model plumbing system. The system recirculates approximately 38 liters of water, which is introduced via two positve priming chambers (19 liters each). The priming chambers are elevated to maintain a positive pressure on the centrifugal pump which fills the system. Water discharged from the pump is monitored via the flow meter and regulated by the ball valve preceding the flow meter. Flow rate is adjusted to 3.0 liters/min. Water flows into and out of the hot-water tank (23 liters) and to the Plexiglas reservoir (11 liters). Once the system is filled, the valves from the priming chambers are closed and the water circulates throughout the remainder of the system. Samples were taken from the sampling port on the influent side of the UV light unit and monitored for *L. pneumophila* and disinfectant residuals. Disinfectants were tested individually and applied to the system as follows: chlorine was added via the Plexiglas reservoir, heat was regulated via the hot-water tank thermostat, ozone was injected via a venturi-type injector, and UV energy was generated within the self-contained, flow-through UV chamber. Figure components are not presented in scale.

in an actual building system. The model system parts consisted of 7.5 m of 12.5-mm-diameter copper piping and fittings, brass spigots (globe type) and valves (ball type) containing rubber seats; an 11-liter cylindrical, Plexiglas reservoir (Rohm and Haas Co., Philadelphia, Pa.); a 23-liter, variable-temperature, electric hot-water tank (115 to 120 V); and an electric, magnetically coupled, centrifugal recirculation pump (115 to 120 V) rated at 9.5 liters/min at 4.6 m of head.

Model preparation. Before each experimental run, the system was filled with hot, sterile tap water (80°C), each sample port was purged, and the water was recirculated for no longer than 24 h. This served to flush the system of all bacterial contaminants and provide a "sterile" baseline environment. At the beginning of each disinfection trial, the flush water was removed and fresh, nonsterile tap water was introduced to the system. The flow rate of the water was adjusted to 3.0 liters/min via a ball valve on the discharge port of the pump and monitored with a rotometer-type, in-line flow meter. A suspension of *L. pneumophila* was added to the system and allowed to circulate for approximately 45 to 60 min. At this flow rate, *L. pneumophila* was not adversely affected by system hydraulics (as demonstrated by the experimental control; Fig. 2). A recycle rate of approximately 12 min provided steady, laminar flow through each vessel as well as sufficient mix to achieve an average *L. pneumophila* concentration of 10^7 CFU/ml at time zero (t = 0) from each sample port.

Experimental water parameters. Turbid water was prepared by making a 1:10 dilution from concentrated hot-water tank effluent samples. The circulating turbid water was determined to have a suspended solids concentration of 4 to 5 mg/liter (1). Each disinfection experiment was conducted in duplicate for 6 h under conditions of nonturbid tap water at 25°C, turbid tap water at 25°C, and nonturbid tap water at 43°C. Smooth curves were drawn through each data point.

Control experiment. Nonturbid tap water at 25°C was introduced to the system and contained approximately 1.0×10^7 CFU of *L. pneumophila* per ml. This was allowed to circulate for 6 h in the absence of any disinfectant techniques. Samples were collected at predetermined intervals over the 6 h.

Chlorine disinfection experiments. The system was inoculated with *L. pneumophila* as previously described. Predetermined volumes of chlorine (Clorox bleach, 5.25% chlorine by weight) were added to the system via the Plexiglas reservoir as needed over the 6 h. The residual concentration was maintained between 4 and 6 mg/liter by multiple addi-

tions of chlorine and was monitored via a chlorine residual electrode (Orion Research Inc., Cambridge, Mass.). Chlorine (8 ml) was initially added to the system and when the residual concentration fell below 4 mg/liter, additional 2-ml volumes of chlorine were added to the system as needed (the total amount of chlorine added was 18 ml at 25°C and 40 ml at 43°C). Samples for chlorine determination and bacterial analysis were taken simultaneously at predetermined intervals over the 6 h.

Additional experiments were conducted in nonturbid water at 25 and 43°C to investigate the effect of diminishing chlorine residual on the concentration of *L. pneumophila* during disinfection. The system was inoculated with *L. pneumophila* as previously described, and after a time zero (t = 0) sampling, a single addition of chlorine (8 ml) was injected into the system. No additional chlorine was added, and samples for chlorine determination and bacterial analysis were taken simultaneously at predetermined intervals over the 6 h. All sample tubes contained 1 ml of a 10% sodium thiosulfate solution to neutralize the chlorine.

Heat inactivation experiments. The system was inoculated with *L. pneumophila* as previously described. The hot water tank temperature was set at 77°C; system temperature was monitored at the Plexiglas reservoir (Fig. 1). Water samples were taken every 2 min after the water temperature reached 45 to 60°C and then periodically after the water temperature reached 60°C (Fig. 2).

Ozone inactivation experiments. The system was inoculated with *L. pneumophila* as previously described. Ozone was generated from 95% O_2 + 5% CO_2 by a portable laboratory unit (model 1-T; ARCO Environmental, Inc., Vandergrift, Pa.) (Fig. 1). Ozone was injected continuously via a venturi-type injector at a rate of 0.5 liters/min to maintain an ozone residual of 1 to 2 mg/liter during the 6 h. The ozonator was switched on after a time zero sampling, and successive samples for both residual ozone and bacterial analysis were taken at predetermined times. Each sample tube contained 1 ml of a 10% sodium thiosulfate solution to neutralize the residual ozone. All ozone analyses were performed via the iodometric technique as described previously (1).

UV light inactivation experiments. The system was inoculated with *L. pneumophila* as previously described. *L. pneumophila* was irradiated with a flowthrough, stainless steel-enclosed, UV light treatment apparatus rated at 30,000 μW-s/cm^2 at 254 nm (Fig. 1). After a time zero (t = 0) sample was obtained, the water was irradiated for the entire 6 h.

RESULTS

The efficacies of all four disinfectant techniques under conditions of nonturbid water at 25°C are compared in Fig. 2. The efficacies of all four disinfectant techniques under conditions of turbid water are compared in Fig. 3. The efficacies of the disinfectant techniques under conditions of increased temperature are compared in Fig. 4. Each experiment was performed in duplicate, and the data points depicted represent the mean of two experiments. If the surviving fraction of *L. pneumophila* for each set of experiments varied by more than 1 log, the experiments were repeated.

Chlorine disinfection. Continuous chlorination at a concentration of 4 to 6 mg/liter produced a 5 to 6 log decrease of *L. pneumophila* over 6 h (Fig. 2). The effect of nonturbid and turbid water on chlorine disinfection is compared in Fig. 3A; turbidity did not impair the efficacy of chlorine disinfection.

The effect of temperature on chlorine disinfection is presented in Fig. 4A; water temperature of 43°C resulted in both more rapid and complete *L. pneumophila* killing as compared with 25°C. On the other hand, the addition of approximately 120% more chlorine was required at 43°C than at 25°C. This additional chlorine was necessary to overcome thermal decomposition and maintain a chlorine residual of 4 to 6 mg/liter (Fig. 5).

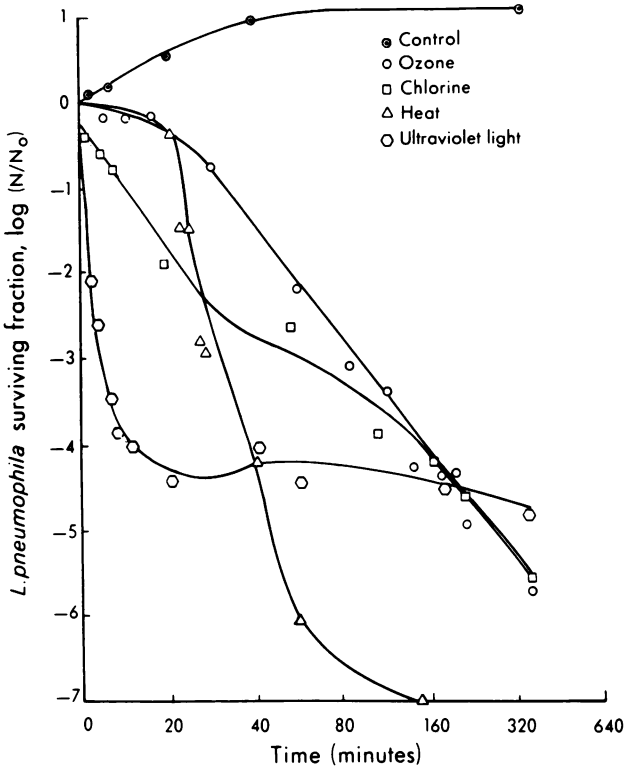


FIG. 2. The comparative efficacy of chlorine, ozone, heat, and UV light in eradicating *L. pneumophila* from a model plumbing system. Each disinfectant technique was evaluated individually in nonturbid water at 25°C. Mean disinfectant levels were as follows: chlorine (4 to 6 mg/liter), heat (50 to 60°C), ozone (1 to 2 mg/liter), and UV light (30,000 μW-s/cm^2). The control plot represents a suspension of *L. pneumophila* circulating in the absence of disinfectant methods. The plots are presented in the form log (N/N_0) vs. time (t), where N = *L. pneumophila* in CFU per milliliter at any time (t) and N_0 = *L. pneumophila* in CFU per milliliter at t = 0.

The adverse effect of a decreasing chlorine residual is shown in Fig. 5. The plots designated (m) depict *L. pneumophila* death by multiple additions of chlorine. A greater decrease in the concentration of *L. pneumophila* was achieved by maintaining a stable residual concentration of 4 to 6 mg/liter. The total amount of chlorine added to the system was 18 ml at 25°C and 40 ml at 43°C.

The plots designated (s) in Fig. 5 depict *L. pneumophila* survival when chlorine was added as a single addition (8 ml). The *L. pneumophila* concentration decreased by 2 logs within 20 min; however, no further decrease in the concentration of *L. pneumophila* was observed after 20 to 40 min when the chlorine residual fell below 4 mg/liter.

Heat disinfection. Temperatures in the range of 50 to 60°C completely eradicated *L. pneumophila* from the model system in less than 3 h (Fig. 2). The effects of nonturbid and turbid water on heat eradication are compared in Fig. 3B. Turbidity did not impair the efficacy of heat eradication.

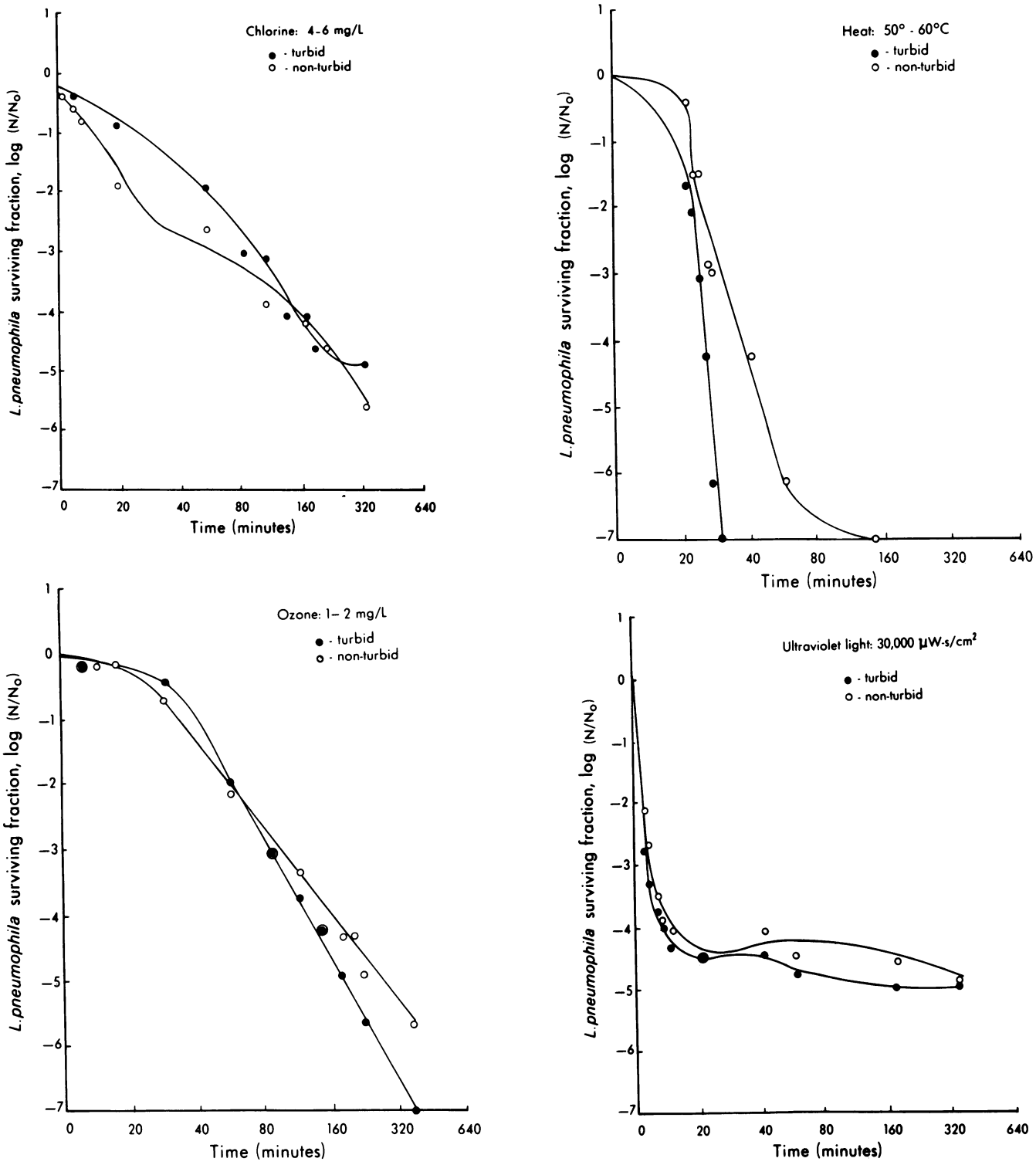
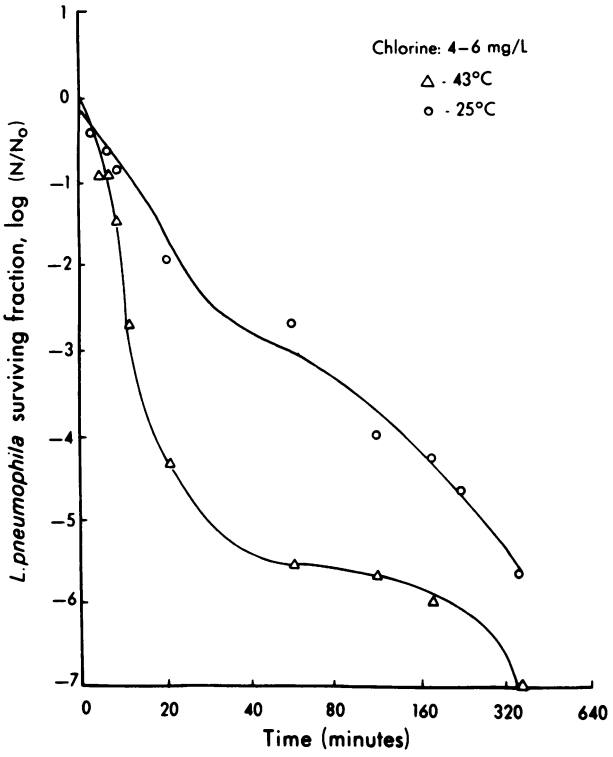
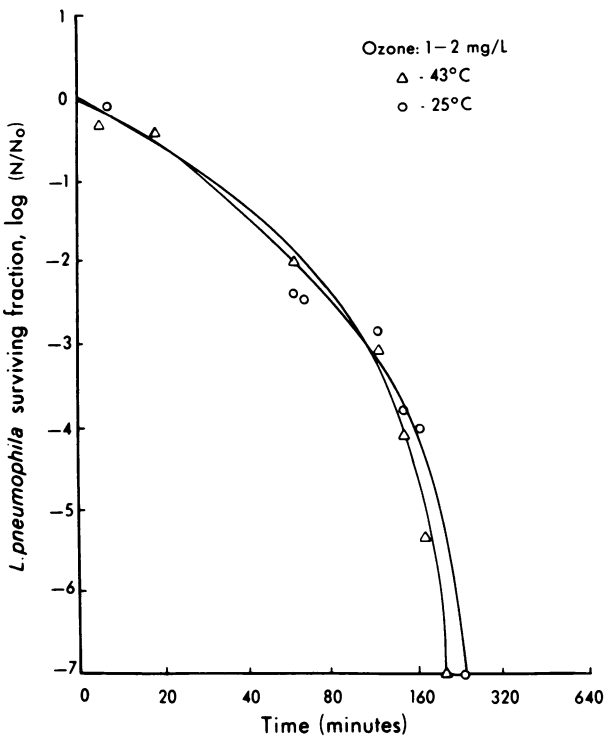


FIG. 3. Turbidity had no effect on the efficacy of chlorine (A), heat (B), ozone (C), or UV light (D). Turbid water was prepared by making a 1:10 dilution from concentrated hot-water tank effluent samples. This water was determined to have a suspended solids concentration of 4 to 5 mg/liter. Tap water was used as the nonturbid medium. The plots are presented in the form log (N/N_0) vs. time (t), where N = *L. pneumophila* in CFU per milliliter at any time (t) and N_0 = *L. pneumophila* in CFU per milliliter at $t = 0$.





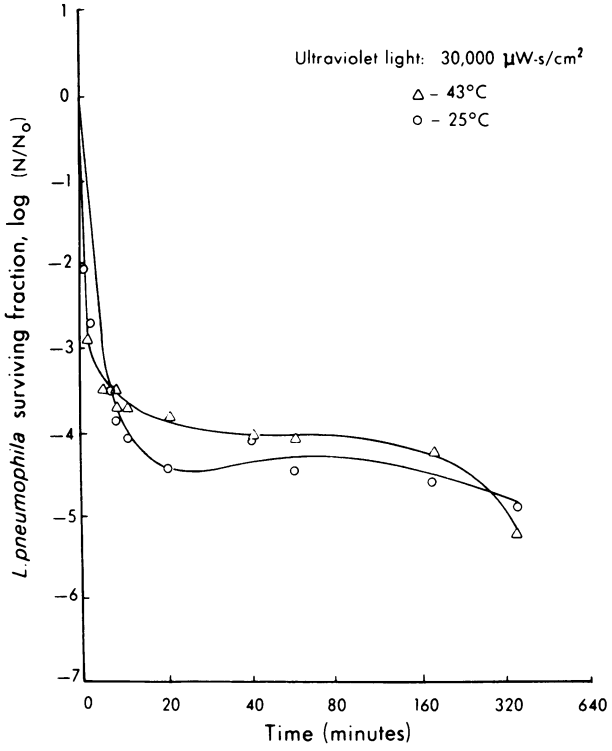


FIG. 4. The effect of temperature on the efficacy of chlorine (A), ozone (B), and UV light (C). Increasing the water temperature (25 vs. 43°C) enhanced the efficacy of chlorine, whereas ozone and UV light were unaffected. The plots are presented in the form log (N/N_0) vs. time (t), where N = *L. pneumophila* in CFU per milliliter at any time (t) and N_0 = *L. pneumophila* in CFU per milliliter at $t = 0$.

Ozone disinfection. Continuous ozonation at a concentration of 1 to 2 mg/liter produced a 5 log decrease from the initial *L. pneumophila* concentration (Fig. 2). Turbidity did not impair the efficacy of ozone disinfection (Fig. 3C). Increasing the water temperature to 43°C also did not impair the efficacy of ozone (Fig. 4B).

UV light disinfection. Continuous UV irradiation, at 30,000 μW-s/cm^2, produced a 5 log decrease in the concentration of *L. pneumophila* within 20 min. No further *L. pneumophila* inactivation was observed after 20 min. The concentration of *L. pneumophila* remained stable at approximately 1×10^2 to 2×10^2 CFU/ml despite 6 h of continuous UV light exposure

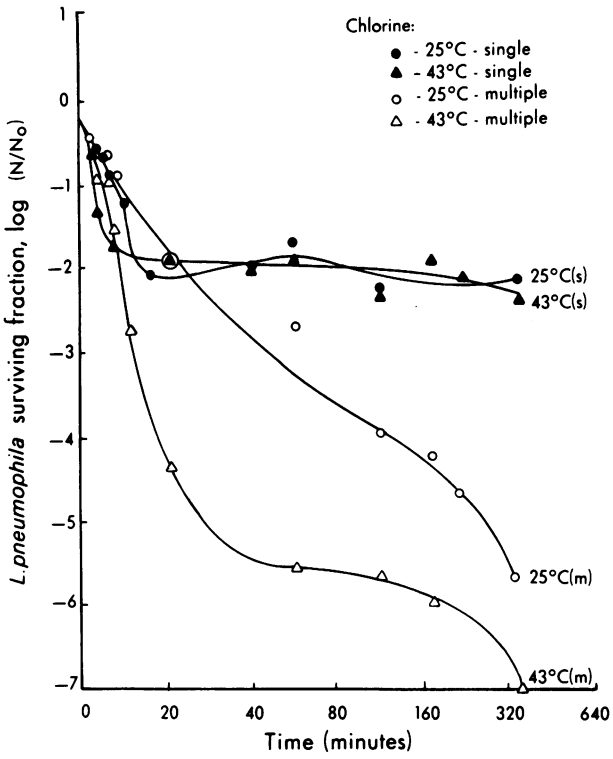


FIG. 5. The efficacy of chlorine disinfection is dependent upon maintaining a residual chlorine concentration. The plots designated (s) depict legionella survival when chlorine was administered as a single injection of 8 ml. After 20 to 40 min, *L. pneumophila* numbers remained stationary because of diminishing chlorine residual concentration. The plots designated (m) depict a 5 to 6 log decrease of *L. pneumophila* when the chlorine residual concentration was maintained at 4 to 6 mg/liter by multiple addition. To maintain a chlorine residual of 4 to 6 mg/liter for 6 h, 18 ml of chlorine was necessary at 25°C, whereas 40 ml of chlorine was necessary at 43°C. The plots are presented in the form log (N/N_0) vs. time (t), where N = *L. pneumophila* in CFU per milliliter at any time (t) and N_0 = *L. pneumophila* in CFU per milliliter at $t = 0$.

(Fig. 2). UV irradiation was not affected by conditions of turbidity or increased temperature (Fig. 3D and 4C).

DISCUSSION

The focal point of *L. pneumophila* colonization within hospitals is the hot-water distribution systems (7, 10, 24). These systems provide environments favorable for *L. pneumophila* growth, which include dead-end sections of pipe risers, scale and sediment aggregates, corrosion sites, cross-connections, and commensal microflora (22).

Currently, disinfection procedures are being evaluated by trial and error within a given hospital, which is time consuming and costly. Most in situ disinfection methods require the employment of specialized personnel to maintain the necessary equipment. Years of experimentation and observation are needed to assess the efficacy of the disinfectant technique (16). Furthermore, parameters such as quantity of legionellae in incoming water, amount of water usage, and ambient temperature would be impossible to adequately control. To overcome these obstacles, we constructed a model water distribution system in which disinfection methods could be evaluated in vitro. Schofield and Wright (20) also constructed a model water system mainly from glass and rubber tubing; this model was designed to evaluate materials which predisposed to *L. pneumophila* colonization rather than methods of disinfection. Our model was constructed of materials commonly found throughout the water system of a building and consisted of a network of copper pipes, brass fixtures, a hot-water tank, and a cylindrical, Plexiglas reservoir (Fig. 1). The design allowed control of operational (flow rates, pressures, recycle rates, etc.) as well as physicochemical (turbidity, temperature, etc.) water parameters.

We evaluated the efficacy of four disinfection modalities in a controlled and comparative fashion: chlorine (4 to 6 mg/liter), heat (50 to 60°C), ozone (1 to 2 mg/liter), and UV light (30,000 μW-s/cm^2). Each disinfectant was tested under three conditions: (i) nonturbid water at 25°C, (ii) turbid water at 25°C, and (iii) nonturbid water at 43°C.

Our results show that all four methods tested were efficacious in eradicating *L. pneumophila* from the model plumbing system (Fig. 2). The application of chlorine, ozone, and UV light showed a 5 to 6 log decrease of *L. pneumophila* within 6 h of continuous disinfection. Heat disinfection eliminated all legionellae within 3 h; in contrast, viable numbers of *L. pneumophila* were present after 3 h of disinfection with chlorine, ozone, and UV light (Fig. 2). UV light produced a 5 log kill within 20 min. Chlorine, ozone, and heat required considerably more time to achieve the same degree of killing (Fig. 2).

In addition to comparing these methods in nonturbid tap water at 25°C, we also sought to determine their efficacy under conditions associated with hot-water distribution systems, i.e., turbidity and higher water temperatures. For example, it was postulated that increasing the turbidity of the water would impair the efficacy of ozonation, chlorination, and UV irradiation by increasing the presence of oxidizable organic material and reducing the transmissibility of irradiant light. In this model, however, turbidity was not shown to impair the efficacy of any of the four disinfection methods (Fig. 3).

Higher water temperature (43°C) was expected to impair the disinfecting efficacy of chlorine, ozone, and UV light by affecting the stability of the chemical disinfectant or by attenuating irradiant light energy. Somewhat surprisingly, the higher temperature enhanced the disinfecting efficacy of chlorine, whereas ozone and UV light were unaffected (Fig. 4). Enhanced efficacy of chlorine in killing *L. pneumophila* at higher temperatures was also noted by Kuchta et al. (15). This may be a result of accelerated binding of chlorine to the cell surface (4, 11). However, it should also be noted that the addition of approximately 120% more chlorine was necessary at the higher temperature of 43°C to overcome the thermal decomposition of the chlorine residual (Fig. 5). If chlorine residual levels were to drop or if the chlorination equipment were to fail, legionellae would survive within the system. Thus, our data demonstrate that hyperchlorination of hot-water systems, as compared with cold-water systems, is more difficult to regulate.

The weakness of any laboratory model is the inherent difficulty in extrapolating laboratory data to actual field conditions. However, some parallels from our experimental results to the actual hospital situation can be drawn. As shown above, hyperchlorination was efficacious in the model system, provided that adequate chlorine concentrations could be maintained. These results have been duplicated in hospital water systems in which hyperchlorination at 4 to 6 mg/liter proved efficacious in suppressing *L. pneumophila*

contamination (3, 12, 21), but when the chlorine residual dropped below 4 mg/liter, cases of nosocomial legionellosis reappeared.

The application of heat (50 to 60°C) eradicated *L. pneumophila* from the model system within 3 h. These results have been duplicated in hospital water systems where heat has been used as a primary disinfection modality (6, 19).

An ozone residual of 1 to 2 mg/liter was shown to effectively control *L. pneumophila* within this model system. Although one study of ozonation in a hospital was inconclusive, the data suggested that ozone could suppress *L. pneumophila* in a large water distribution system (8). Because of the rapid decomposition of the ozone residual in water, its main utility may be limited as a supplemental disinfectant to other agents such as chlorine or heat.

The efficacy of UV light for eradication of *L. pneumophila* has been demonstrated in vitro (2, 14). In our model system, *L. pneumophila* concentrations decreased by 4 to 5 logs with UV irradiation within 20 min, whereas chlorine and ozone required at least 3 h to achieve the same degree of killing (Fig. 2). UV light disinfection was not impaired by conditions of turbidity or increased temperature. Compared with chlorine, heat, or ozone, UV light methodology in this model system required the least maintenance or monitoring. From the results of this study, UV irradiation appears to have potential as a primary or supplemental in situ disinfectant method.

In conclusion, all four disinfectant methods were effective in eradicating *L. pneumophila* from the model plumbing system. However, the application of any eradication method to an actual building water system is dependent upon many parameters besides the efficacy of the disinfectant, including the initial capital expense, the operating and maintenance costs, and the ease of installing a specific disinfection unit in the water distribution system; these related issues are addressed in detail elsewhere (25).

ACKNOWLEDGMENTS

This research was supported in part by the Veterans Administration Medical Research Service.

We thank Richard M. Vickers for microbiologic consultation, Ronald D. Neufeld for engineering consultation, Saul Weiss and Aphia Abdou (Medical Media) for assistance with figures, and Shirley Brinker for manuscript preparation.

LITERATURE CITED

1. **American Public Health Association.** 1976. Standard methods for the examination of water and waste-water, 14th ed. American Public Health Association, Washington, D.C.
2. **Antopol, S. C., and P. D. Ellner.** 1979. Susceptibility of *Legionella pneumophila* to ultraviolet radiation. Appl. Environ. Microbiol. **38**:347–348.
3. **Baird, I. M., W. Potts, J. Smiley, N. Click, S. Schleich, C. Connole, and K. Davison.** 1984. Control of endemic nosocomial legionellosis by hyperchlorination of potable water, p. 333. *In* C. Thornsberry, A. Balows, T. C. Feeley, and W. Jakubowski (ed.), Legionella: proceedings of the 2nd International Symposium. American Society for Microbiology, Washington, D.C.
4. **Banerjee, K., and P. N. Cheremisinoff.** 1985. Sterilization systems, p. 11. Technomic Publishing Co., Lancaster, Pa.
5. **Best, M. G., A. Goetz, and V. L. Yu.** 1984. Heat eradication measures for control of nosocomial Legionnaires' disease: implementation, education, and cost analysis. Am. J. Infect. Control. **12**:26–30.
6. **Best, M., V. L. Yu, J. Stout, A. Goetz, R. R. Muder, and F. Taylor.** 1983. 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 **ii**:307–310.
7. **Dennis, P., J. Taylor, R. Fitzgerald, C. L. R. Bartlett, and G. Barrow.** 1982. *Legionella pneumophila* in water plumbing systems. Lancet **ii**:949–951.
8. **Edelstein, P. H., R. E. Whittaker, R. L. Kreiling, and C. L. Howell.** 1982. Efficacy of ozone in eradication of *Legionella pneumophila* from hospital plumbing fixtures. Appl. Environ. Microbiol. **44**:1330–1334.
9. **Feeley, J. C., R. J. Gibson, G. W. Gorman, N. C. Langford, J. K. Rasheed, D. C. Mackel, and W. B. Baine.** 1979. Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol **10**:437–441.
10. **Fisher-Hoch, S., P. M. G. Smith, D. Harper, and I. Colbourne.** 1984. Source of *Legionella pneumophila* in a hospital hot water system, p. 302–304. *In* C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowski (ed.), Legionella: proceedings of the 2nd International Symposium. American Society for Microbiology, Washington, D.C.
11. **Gaudy, A. F., and E. T. Gaudy.** 1980. Microbiology for environmental scientists and engineers, p. 690. McGraw-Hill Book Co., New York.
12. **Johnson, J. T., V. L. Yu, M. G. Best, R. M. Vickers, A. Goetz, R. Wagner, H. Wicker, and A. Woo.** 1985. Nosocomial legionellosis uncovered in surgical patients with head and neck cancer: implications for epidemiologic reservoir and mode of transmission. Lancet **ii**:298–300.
13. **Jolley, R. L., R. J. Bull, W. P. Davis, S. Katz, M. H. Roberts, and V. A. Jacobs.** 1985. Water chlorination chemistry, environmental impact and health effects, vol. 5, p. 187–277. Lewis Publications, Inc., Chelsea, Mich.
14. **Knudson, G. B.** 1985. Photoreactivation of UV-irradiated *Legionella pneumophila* and other *Legionella* species. Appl. Environ. Microbiol. **49**:975–980.
15. **Kuchta, J. M., S. J. States, A. M. McNamara, R. B. Wadowsky, and R. B. Yee.** 1983. Susceptibility of *Legionella pneumophila* to chlorine in tap water. Appl. Environ. Microbiol. **46**:1134–1139.
16. **Muder, R., V. L. Yu, and A. Woo.** 1986. Mode of transmission of *L. pneumophila*: a critical review. Arch. Intern. Med. **146**: 1607–1612.
17. **Pasculle, A. W., J. C. Feeley, R. J. Gibson, L. G. Cordes, R. L. Myerowitz, C. M. Patton, G. W. Gorman, C. L. Carmack, J. W. Ezzell, and J. N. Dowling.** 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. J. Infect. Dis. **241**: 727–732.
18. **Plouffe, J. F., M. F. Para, W. E. Maher, B. Hackman, and L. Webster.** 1983. Subtypes of *Legionella pneumophila* serogroup 1 associated with different attack rates. Lancet **ii**:649–650.
19. **Plouffe, J. F., L. R. Webster, and B. Hackman.** 1983. Relationship between colonization of hospital buildings with *Legionella pneumophila* and hot water temperatures. Appl. Environ. Microbiol. **46**:769–770.
20. **Schofield, G. M., and H. E. Wright.** 1984. Survival of *Legionella pneumophila* in a model hot water distribution system. J. Gen. Microbiol. **130**:1751–1756.
21. **Shands, K., J. Ho, R. Meyer, G. Gorman, G. Mallison, S. Finegold, and D. Fraser.** 1985. Potable water as a source of Legionnaires' disease. J. Am. Med. Assoc. **253**:1412–1416.
22. **Stout, J. E., V. L. Yu, and M. G. Best.** 1985. Ecology of *Legionnella pneumophila* within water distribution systems. Appl. Environ. Microbiol. **49**:221–228.
23. **Stout, J. E., V. L. Yu, R. M. Vickers, and J. Shonnard.** 1982. Potable water supply as the hospital reservoir for Pittsburgh pneumonia agent. Lancet **i**:471–472.
24. **Stout, J. E., V. L. Yu, R. M. Vickers, J. Zuravleff, and M. Best.** 1982. Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. N. Engl. J. Med. **306**:466–468.
25. **Yu, V. L.** 1986. Nosocomial legionellosis: current epidemiologic issues, p. 239–253. *In* M. Swartz and J. Remington (ed.), Current clinical topics in infectious diseases, 7th ed. McGraw-Hill Book Co., New York.