Hospitals are often required to perform a supplemental disinfection of their water systems to protect individuals from hospital-acquired Legionnaires’ disease. The authors of this article recently studied one hospital where three cases of hospital-acquired Legionnaires’ disease were detected in less than two years. These cases were linked to Legionella colonization of the hospital’s water system. Chlorine dioxide (ClO₂) was considered a cost-effective approach to disinfection given that ClO₂ generators could treat the 23 buildings comprising the hospital complex from one central location. The authors evaluated the efficacy of maintaining a residual of 0.5 to 0.8 mg/L of ClO₂ for Legionella control in the secondary distribution system of this 437-bed hospital over a two-year period. Monthly monitoring showed mean Legionella positivity at hot water outlets and cold building source water areas decreased from 23 to 12% and 9 to 0%, respectively (p < 0.05). ClO₂ residuals decreased with increasing distance from the application point and temperature. Mean ClO₂ concentrations were lowest in hot water outlets (0.08 mg/L) followed by cold water outlets (0.33 mg/L) and reservoirs (0.68 mg/L). Complete eradication (0% positivity) of Legionella was achieved after 1.75 years, and no cases of Legionnaires’ disease were reported during this time.
The hospital focused on in this article, a 437-bed hospital in Pennsylvania, identified 13 cases of Legionnaires’ disease from 1994 to 1999. In an 18-month period from 1998 through 1999, three cases were confirmed as hospital-acquired Legionnaires’ disease. Therefore in 1998, the hospital began to monitor its drinking water supply. Interventions included maintaining a free chlorine residual (approximately 0.40 mg/L) throughout the distribution system and performing thermal eradication (heat and flush) disinfection of identified Legionella-positive areas of the distribution system. Although thermal eradication was temporarily effective, the hospital did not want to use this treatment as a long-term disinfection approach because of its short-term efficacy, logistical difficulties, and high cost. Possible alternative disinfection approaches included chlorine dioxide (ClO₂) or copper/silver ionization.

The hospital monitors water supply after cases of Legionnaires’ disease

COPPER/SILVER IONIZATION PROCESS POSES PROBLEMS FOR 23-BUILDING HOSPITAL COMPLEX

Copper/silver ionization was considered as an option because it has undergone numerous laboratory and field experiments demonstrating effectiveness, both in the United States and abroad (Stout et al, 1997; Lin et al, 1998b). However, because each of the hospital’s 23 buildings would require its own ionization system, this disinfection option was deemed to be cost-prohibitive.

ClO₂ USED IN EUROPEAN HOSPITALS

ClO₂ was presented to the hospital as an alternative disinfection method; the method has been used in European hospitals (Hill et al, 2000; Hood et al, 2000; Harris & Rendell, 1999; Makin, 1998; Hamilton et al, 1996; Walker et al, 1995). The ClO₂ method could be installed at a central location to service the entire secondary distribution system at a lower cost than copper/silver ionization.

The generation system proposed to the hospital was based on an electrochemical oxidation process that safely produced ClO₂ in a convenient unit (Sidari & VanBriesen, 2002). ClO₂ has been used in the United States for many years as a primary water treatment chemical, originally to remove phenol-related compounds. More recently, ClO₂ has been used as an alternative primary disinfectant to reduce the production of trihalomethane and haloacetic acids at primary water treatment works (Gordon, 2001).

The US Environmental Protection Agency (USEPA) regulates the use of ClO₂ as a disinfectant in the treatment of potable water. Specifically, the National Primary Drinking Water Regulations establish the maximum residual disinfectant level of 0.8 mg/L as ClO₂ and the maximum contaminant level (MCL) at 1.0 mg/L for chlorite (USEPA, 1998).

Although ClO₂ has been effectively used to control Legionella in European hospitals, there were no reports in the peer-reviewed literature of its use in US hospitals for this purpose at the outset of this study. Based on the favorable literature review of laboratory experiments (Gao et al, 2000; Pavey & Roper, 1998; Botzenhart et al, 1993) and European field trials, coupled with the cost savings for the Pennsylvania hospital’s particular installation, the decision was made to use ClO₂. Therefore, a ClO₂ system was installed in June 2000.
After its installation, this method was studied to (1) evaluate the efficacy of ClO₂ in controlling *Legionella* in a colonized potable water system, (2) determine whether an effective residual could be maintained throughout the distribution system, (3) provide data that other facilities could use to evaluate disinfection technologies, and (4) assist the hospital with maximizing the disinfection capabilities of its system.

**STUDY SETTING**

A unique attribute of the hospital is its operation of a large secondary distribution system. The hospital receives 80 to 85% of its daily water demand from the local municipality, which provides conventional treatment, including chlorination of a surface water source. The remaining 15 to 20% of daily demand is met by an onsite well, which is also chlorinated. Water is blended together, treated with ClO₂, and stored in a 520,000 gal (1,968 m³) covered reservoir. The treated water is then distributed through approximately 10,000 ft (3,048 m) of 6 and 8 in. (150 and 200 mm) piping to 23 buildings across 60 acres (20 ha). Typical water use is estimated as 400,000 gpd (1,514 m³/d) in the summer and 250,000 gpd (946 m³/d) in the winter.

The installed ClO₂ generation system employed a new technology that generates an almost pure ClO₂ solution (500 mg/L) by the direct oxidation of 25% active sodium chlorite solution across a membrane system. Three ClO₂ generators supplied up to 3.18 lb (1.44 kg) of ClO₂ per day to the reservoir. One generator produced a constant supply of ClO₂ based on the water flow into the reservoir. The second generator produced additional ClO₂ based on feedback from oxidation reduction potential (ORP) probes at the reservoir. A third generator was in standby mode and was only brought into service during maintenance on the first two generators.

**CHARACTERIZING SOURCE WATER IMPORTANT WHEN COMPARING RESULTS**

The water quality of the municipal and onsite well water used by the hospital was monitored in February 2001 and January 2002. The mean concentration of the following parameters was observed as follows: hardness was 110 mg/L as calcium carbonate, alkalinity was 98 mg/L as calcium carbonate, pH was 7.5, total iron was 0.03 mg/L, total manganese was 0.02 mg/L, sulfate was...
52.0 mg/L, turbidity was 0.6 ntu, and total organic carbon was 2.22 mg/L. The mean free chlorine residual entering the reservoir was 0.42 mg/L. Because ClO2 demand, decay, and chlorite formation may depend on water quality, characterizing the source water may be important to compare results between facilities.

Legionella cultures were performed by the hospital before ClO2 disinfection from April 1998 to June 2000 (referred to in this article as pre-ClO2). This data set was used to compare results before and after the ClO2 system was installed. The pre-ClO2 data set sampled only hot water distal sites in patient areas. A distal site is a water tap located in a sink, tub, or shower within a building. The Special Pathogens Laboratory at the Department of Veterans Affairs (VA) Medical Center in Pittsburgh performed all microbiological analysis before and during this study.

**EXPERIMENTAL DESIGN**

Sampling locations were selected throughout the distribution system, with a focus on patient care areas (Figure 1). Samples from the reservoir were collected at the reservoir discharge area where the water enters the secondary distribution system. Where available, incoming cold water to the building (cold building source water areas) was sampled from a valve located at the building pump or on the incoming cold water main. Hot and cold water samples were drawn from a minimum of two distal sites (sinks) at each sampling location. Distal sites in the buildings were selected based on perceived use by the hospital staff. A distal sink in a high-use (regularly used) area and low-use (seldom used) area were selected in each building.

**SAMPLE COLLECTION PROCESS**

The sample collection process started with removing the faucet aerators. Water at each sampling location was then purged for 1 min at a moderate flow rate before collecting samples. At distal locations with hot and cold water taps, the cold water was purged and sampled first. Sampling was performed once a month from February 2001 through April 2002, with the exception of March 2001. The study facility monitored reservoir ClO2 residuals three times a day, every day, during the study.

Temperature and pH measurements were taken directly from the flow stream using a digital pH meter kit. A 10-mL sample was analyzed for ClO2 residual within 10 s of sample collection using the DPD method for ClO2 (0.00 to 5.00 mg/L), as adapted from Standard Methods for the Examination of Water and Wastewater (1998). The sample was processed using a glycine reagent to mask chlorine interference. Testing was performed using the DPD free chlorine reagent, and the colorimetric measurement was made with the spectrophotometer. ClO2 residual measured by the hospital was performed using a pocket colorimeter following the DPD method using the same reagents as the study sampling. The DPD method is a USEPA-approved method for ClO2 analysis.

A 120 mL sample was collected in a sterile wide-mouthed polyethylene container and appropriately labeled for culturing in the laboratory. Environmental samples were refrigerated at 2 to 8°C upon receipt at the VA Medical Center’s Special Pathogens Laboratory and processed within 12 h. Legionella culture was performed according to the laboratory’s standard operating procedures, which include direct (0.1 mL/plate) and filtered (50 mL filtered, resuspended in 5 mL of original sample, 0.1 mL/plate) plating on buffered charcoal yeast extract and differential glycine-vancomycin-polymyxin B media (Ta et al, 1995). Inoculated plates were incubated at 37°C for at least five days before being evaluated for Legionella colony growth. The extent of Legionella colonization was expressed as the number of positive sample locations divided by the total number of samples tested. This is referred to as Legionella percent positivity.

**SAMPLES COLLECTED DAILY FROM RESERVOIR**

On a daily basis (Monday through Friday), 200 mL samples were collected from the reservoir and tested for Complete eradication of Legionella from hot water distal sites was not realized until 1.75 years after implementation of chlorine dioxide disinfection. A trend in declining positivity during phase 2 of the study was observed. Continuing sampling by the hospital following study protocol observed 0% positivity in June 2002.

**FIGURE 2** Declining monthly Legionella positivity in hot water over time

<table>
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<th>Month</th>
<th>Legionella Positivity %</th>
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<tr>
<td>2/01</td>
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<tr>
<td>4/01</td>
<td>35</td>
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</table>

Complete eradication of Legionella from hot water distal sites was not realized until 1.75 years after implementation of chlorine dioxide disinfection. A trend in declining positivity during phase 2 of the study was observed. Continuing sampling by the hospital following study protocol observed 0% positivity in June 2002.
Chlorite. Samples were purged with nitrogen gas in the field to remove residual ClO₂ gas. The samples were immediately tested according to the USEPA-approved method 4500 ClO₂–E (amperometric titration) in Standard Methods (1998). The analysis was performed using an amperometric titrator.

The Pittsburgh Water Authority in Pittsburgh performed the general water quality profile of the raw water. Water samples of the city and well supply as well as the reservoir were collected in 1 L (0.3 gal) opaque polyethylene containers. The samples were stored at 2 to 8°C for no longer than 48 h before transfer to the Pittsburgh Water Authority for analysis under its standard laboratory procedures. The city, well, and reservoir samples were analyzed separately.

**STATISTICAL ANALYSIS**

Statistica® and spreadsheet® software were used for statistical analysis. A correlation coefficient was used to compare ClO₂ residuals between distal sites and the reservoir. A blocked analysis of variance and Tukey multiple contrast (for the pairwise comparisons) were used to evaluate the ClO₂ residuals between the reservoir and cold and hot distal sites. The significant differences were evaluated by t-tests, chi-square, and Fisher’s exact tests.

**RESULTS**

Data were collected during three phases. The hospital collected 208 water samples (186 hot, 0 cold, and 22 source) from April 1998 to June 2000 while using thermal eradication and chlorination before the ClO₂ system was installed (pre-ClO₂). After the ClO₂ system was installed, the hospital collected 146 water samples (124 hot, 8 cold, and 14 source) from June 2000 to January 2001 (phase I). Finally, the research team collected 610 water samples (257 hot, 257 cold, and 96 source) from February 2001 through April 2002 (phase II). Hot water sampling dominated the pre-ClO₂ and phase 1 studies, and these data are used for comparison.

**LEGIONELLA POSITIVITY AND COUNTS**

Hot water distal sites were monitored before and after ClO₂ disinfection was implemented. Cold water distal sites were monitored only after disinfection began. Figure 2 shows the monthly hot water positivity results. The percentage of distal sites positive for Legionella in the hot water and cold building source water areas decreased significantly with use of ClO₂ (p = 0.011 and 0.048, respectively) (Figure 3). Hot water positivity significantly decreased from 23%, observed during use of thermal eradication and chlorination (pre-ClO₂), to 12% following implementation of ClO₂ disinfection (p <0.05). A significant decrease was also observed in the cold building source water areas where Legionella percent positivity significantly decreased from 9% (pre-ClO₂) to 0% during phases 1 and 2 (p <0.05).

CIＯ₂—chlorine dioxide

ClO₂ has provided better control for Legionella compared with thermal eradication and chlorination. After installation of the ClO₂ system, a significant decrease in Legionella percent positivity was observed at hot water distal outlets and in cold building source water (p = 0.011 and 0.048, respectively)

No cases of hospital-acquired Legionnaires’ disease have been detected since initiation of ClO₂ disinfection in June 2000. Although no cases of Legionnaires’ disease were detected after initiation of ClO₂ disinfection, statistical significance has not been attained.
The mean concentration of *Legionella* in positive hot water samples during the pre-ClO₂ phase was 28 cfu/mL (range = 5 to 320 cfu/mL; mode = 5 cfu/mL). There was no significant decrease in the mean concentration of *Legionella* in positive hot water samples during phase 2 (mean = 29 cfu/mL, range = 5 to 500 cfu/mL; mode = 5 cfu/mL). Thus, although overall distal site positivity declined during the study period, the mean concentration of *Legionella* in positive samples was statistically unchanged.

The reservoir was only sampled during phase 2 and revealed 0% positivity (0/14). Cold building source water was sampled before installation: pre-ClO₂ (9% positivity; 2/22), phase 1 (0%; 0/14), and phase 2 (0%; 0/82). Cold distal outlets were only sampled during phase 2 and showed 1% positivity (3/257), a value not significantly different from 0% (p = 0.25).

**NO DETECTIONS OF LEGIONNAIRES’ DISEASE AFTER ClO₂**

Figure 4 shows the cases of Legionnaires’ disease diagnosed at the hospital since 1994. Three cases in 1998 to 1999 were confirmed as hospital-acquired Legionnaires’ disease and occurred before the ClO₂ disinfection system was installed. *Legionella* was isolated from the hospital water system after these cases were diagnosed. Molecular subtyping by pulse-field gel electrophoresis found the patient and environmental *Legionella* strains to be identical. The source for the 10 cases before 1998 was unknown. No cases of hospital-acquired Legionnaires’ disease have been detected at the hospital since the ClO₂ system was installed in June 2000.

**ClO₂ RESIDUALS**

The mean ClO₂ residuals during phase II were 0.68 mg/L, 0.50 mg/L, 0.33 mg/L, and 0.08 mg/L for the reservoir, cold building source, cold distal, and hot distal sample sites, respectively. The difference in mean concentrations from the reservoir to cold distal outlets and to hot distal outlets was significant (p < 0.001). The difference in mean ClO₂ concentration between cold to hot water distal outlets is also significant (p = 0.001). Figure 5 shows mean monthly ClO₂ residuals during phase 2. No significant correlation between cold building source, cold distal, and hot distal mean monthly ClO₂ residual was observed during phase 2. The changes and variability in mean monthly residual are attributed to the imprecise control of the generators by ORP feedback and operational adjustments made based on water demand and incoming water quality changes inherent to any secondary distribution system. The residual at the hot distal sites showed the least variability; this variability was not correlated with changes in positivity (R² = 0.02).

**HOT WATER TEMPERATURES**

The mean distal site hot water temperature during phase 2I was 51°C, range = 26 to 61°C. The phase II mean temperature was significantly lower than the mean temperature observed during the pre-ClO₂ phase of 54°C, range = 37 to 63°C (p = 0.01). The mean hot water temperature during phase 1, 53°C, also was not significantly different from the pre-ClO₂ phase. No correlation between temperature and ClO₂ residual was observed at the hot water distal sites during phase 2 (R² = 0.09). No strong correlation between temperature and ClO₂ residual was observed during phase 1 (R² = 0.57).

Medical center personnel collected samples from the reservoir for chlorite analysis, and data are available from March 2002 through May 2003. A total of 183 samples were tested in 2002. The average chlorite concentration for 2002 was 0.64 mg/L (with a minimum of 0.23 mg/L and a maximum of 1.0 mg/L). At the time this study was conducted, 89 samples had been tested in 2003. The average chlorite concentration for 2003 was 0.63 mg/L (with a minimum of 0.31 mg/L and a maximum of 0.98 mg/L). The measured mean chlorite levels did not exceed USEPA’s MCL for chlorite (1.0 mg/L).

**DISCUSSION**

After the ClO₂ system was installed, a significant decrease in *Legionella* percent positivity was observed in
both cold building source water areas and at hot distal outlets (Figure 3). The percent distal positivity at cold distal outlets was not significantly different from 0% during phase 2. ClO₂ provided better control for *Legionella* at hot and cold distal sites and in cold building source water areas compared with thermal eradication and chlorination during the pre-ClO₂ phase.

A dramatic change in positivity at hot distal sites was observed after August 2001 (Figure 2). The decrease in positivity between the first period (February through August) and the second (September through April), within phase 2, is significant (*p* < 0.001). The positivity from February through August was 27% (28/105), whereas September to April was 3% (4/158). This change cannot be attributed to an increase in hot water ClO₂ residual because the monthly mean residuals were not significantly different: 0.08 versus 0.09 (*p* = 0.57).

Routine engineering interventions to system operation did not appear to have influenced the decrease in positivity between the two periods. The mean monthly hot water temperature increased from 48.7°C during the first period to 52.4°C during the second period of phase II. The difference in these monthly mean temperatures was not significant (*p* = 0.13). Neither the authors nor other researchers in this area have observed that hot water temperatures below 60°C affect *Legionella* colonization (Lin et al, 1998a; Zacheus & Martikainen, 1996; Darelid et al, 1994). Also, the temperature during the low-positivity period 52.4°C was not significantly different (*p* = 0.16) from that during the pre-ClO₂ phase when the mean hot water temperature was 53.7°C and 23% positivity was observed at hot distal sites.

**PLUMBING MODIFICATIONS HAVE LITTLE EFFECT ON LEGIONELLA**

Finally, plumbing modifications including “dead-leg” removal and repairs were performed near seven of the persistently positive sites during the study from June to October of 2001. However only 71% (5/7) of the persistently positive sites near plumbing modifications remained negative afterward, whereas 100% (5/5) of the distal sites where no modifications were performed also remained negative. This result suggests that these plumbing modifications had little immediate effect on *Legionella* colonization in this system.

Complete eradication of *Legionella* from hot water distal sites was not realized until after more than 1.75 years of ClO₂ use; declining positivity was clearly observed during the last eight months of the study (Figure 2). The hospital conducted followup sampling after the study was completed (June 2002 through April 2003), which verified the continuing trend of low *Legionella* positivity in the water system. *Legionella* was isolated from 3.7% (3/81) of hot water samples and 0.8% (1/118) of cold water samples.

**FIELD STUDIES DOCUMENT ClO₂ IN EUROPEAN HOSPITALS**

Published field studies on the efficacy of ClO₂ in European hospitals also document that at least six months of ClO₂ use was required for complete eradication of *Legionella* (Smith et al, 2001; Hill et al, 2000; Hamilton et al, 1996). Three other studies ranging from 39 weeks to 6 years in duration documented suppression of *Legionella* growth in the potable water distribution systems (Hood et al, 2000; Harris & Rendell, 1999; Makin, 1998). It is plausible that sustained application of ClO₂ is necessary for long-term eradication to occur.

No cases of Legionnaires’ disease were detected at the hospital after the ClO₂ system was used (Figure 4), despite continued isolation of *Legionella* at hot water distal sites. As suggested by other authors, complete eradication of *Legionella* may not be necessary to prevent Legionnaires’ disease.

Laboratory studies indicate a ClO₂ residual above 0.1 mg/L is effective in eradicating planktonic *Legionella* (Gao, 2000; Pavey & Roper, 1998; Botzenhart et al, 1993). ClO₂ residuals above 0.5 mg/L provide a 99.99%
Numerous Factors Affect Residual Amounts

Maintaining a sufficient disinfecting residual at distal outlets depends on numerous factors, including residence time and temperature. Retention time is a function of system volume and water use. The hospital’s 10,000-plus ft (3,000-plus m) of piping in its secondary distribution system resulted in a large distance between the ClO2 application point at the reservoir, cold building source water areas, and at cold water distal sites exceeded 0.3 mg/L. The same sites maintaining a residual above 0.3 mg/L also demonstrated eradication of Legionella. The three cold water distal sites in which positivity was observed—all during the same month at the beginning of the study—had a mean ClO2 concentration of 0.26 mg/L (median = 0.07 mg/L) below the reported effective concentration of 0.3 mg/L.

Exceeding ClO2 May Pose Health Risks

Potentially exceeding the regulatory limits for ClO2 is significant because of the possibility of adverse health effects. Historically, disinfectant by-products of chlorine (trihalomethane/haloacetic acids) (Swan et al, 1998; Morris et al, 1993) in potable water have been linked to potentially harmful human health effects. The by-products of ClO2, chlorite and chlorate, have been shown to damage blood by oxidation in animals. In addition, consuming drinking water treated with ClO2 during pregnancy has been implicated in pregnancy complications (Kanitz et al, 1996; Gates, 1998a; Gates, 1998b). Adverse health effects in controlled prospective studies in humans and field situations in community water systems have not produced clear evidence of adverse health effects from ClO2 by-products. Although studies in this area continue, monitoring of these regulated by-products is recommended (Smith & Willhite, 1990). However, the observed chlorite levels did not exceed USEPA’s MCL. Further study is needed to confirm these findings.

ClO2 is similar to copper/silver ionization (the current prominent Legionella control method in hot water systems) when considering regulatory requirements, health impacts on potable water quality, and system operation and maintenance. ClO2 has numerous advantages compared with hyperchlorination and thermal eradication for long-term suppression of Legionella in distribution systems (Sidari & VanBriesen, 2002).

ClO2 appears to be a promising Legionella disinfection technology. This is the first field study in the United States to show that if a ClO2 residual of 0.3 to 0.5 mg/L is maintained at distal outlets, Legionella can be suppressed in potable water.

Need for Further Evaluations

Further evaluations of the hospital studied in this article should be conducted, and updates on the long-term use of ClO2 in European facilities should be reported to provide hospitals and other end users with a better understanding of the benefits and limits of this disinfection method.

Acknowledgment

The authors would like to thank and acknowledge all those individuals who have assisted in collecting samples, processing samples, and gathering information. Included are the engineering and infection control staff at the hospital, the staff at the Special Pathogens Laboratory (Ashley Hangard, Sue Mietzner, Laura Morris, Asia Sagniemi, Pat Sheffer, and Sara Vaccarello), and Ty Drayton, an undergraduate assistant who helped with sample collection. Special thanks are extended to Joseph Hannigan of Klenzoid Inc. for providing technical assistance and support for this study.

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FOOTNOTES

2Digi-Sense, (ATC)/mV/ORP (P/N 5938-52), Cole-Parmer Instrument Co., Vernon Hills, Ill.
3Hach Method 10101, Hach Co., Loveland, Colo.
4Hach 21055-69, Hach Co., Loveland, Colo.
5Hach DR/2010 (P/N 49300-60), Hach Co., Loveland, Colo.
6P/N 46700-51, Hach Co., Loveland, Colo.
10To whom correspondence should be addressed

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