

This study evaluated the safety and efficacy of chlorine dioxide (ClO₂) fed into the incoming main water line to control *Legionella* bacteria in two hospital water systems. In both hospital A and B, positivity of all distal outlets (sinks and showers) for *Legionella* decreased from 60% to ≤10% after the ClO₂ treatment. In hospital B, the heterotrophic plate count bacteria in hot water were reduced from 15,400 cfu/mL to 2,900 cfu/mL after ClO₂ treatment. Mean concentrations of ClO₂ and chlorite (ClO₂⁻) in cold and hot water did not exceed the maximum residual disinfection level of 0.8 mg/L and the maximum contaminant level of 1.0 mg/L, respectively. No cases of healthcare acquired legionellosis have been identified in the postdisinfection period in these two hospitals. The study indicates that ClO₂ is a promising disinfectant for controlling not only *Legionella*, but also other microorganisms in drinking water.

Legionella control by chlorine dioxide in hospital water systems

Legionnaires' disease is caused by *Legionella* bacteria, which colonize 12–70% of hospital water systems (Lin et al, 1998b). Of the Legionnaires' disease cases reported to the Centers for Disease Control and Prevention (CDC), 25–45% were hospital acquired (Benin et al, 2002). *Legionella* and other opportunistic pathogens colonize water systems; cause pneumonia, wound, and bloodstream infections in hospitalized patients; and cause approximately 25% of all hospital acquired infections (Anaisse et al, 2002). These infections could be prevented through active and supplemental disinfection of hospital water systems.

Disinfection methods that have been used by hospital water systems to control *Legionella* include thermal eradication, hyperchlorination, copper–silver ionization, and point-of-use (POU) filters. Thermal eradication is a short term solution that is time consuming and labor intensive to implement. High levels of free chlorine in distribution systems from hyperchlorination will cause excessive pipe corrosion problems. Although copper–silver ionization has been an effective disinfection method, a recent study showed that *Legionella* could develop resistance to copper and silver ions (Mietzner et al, 2005). POU filters do not provide disinfectant residual protection for the entire water distribution system.

Chlorine dioxide (ClO₂) is a promising alternative disinfectant for hospital water systems to prevent Legionnaires' disease. Chlorine dioxide has been effectively used to control *Legionella* in European hospitals (Hill et al, 2000; Hood et al, 2000; Hamilton, et al, 1996). However, chlorine dioxide has not been extensively evaluated in the US for its disinfection efficacy and safety to control hos-

BY ZHE ZHANG,
CAROLE MCCANN (DECEASED),
JENNIFER HANRAHAN,
ANNETTE JENCSON, DANIEL
JOYCE, STEVEN FYFFE, STEVE
PIESCZYNSKI, ROBERT HAWKS,
JANET E. STOUT, VICTOR L. YU,
AND RADISAV VIDIC

pital acquired Legionnaires' disease (Bova et al, 2004; Sidari et al, 2004; Srinivasan et al, 2003). Chlorine dioxide has long been considered as an alternative drinking water disinfectant in the US (Aieta & Berg, 1986). ClO₂ exists as a stable free radical with an unpaired electron and reacts with organic and inorganic compounds mainly through a one-electron transfer reaction. Unlike chlorine, ClO₂ itself does not react with natural organic matter to form trihalomethanes or haloacetic acids. ClO₂ is a highly selective oxidant with respect to specific functional groups, such as phenolic moieties or tertiary amino groups (Hoigne & Bader, 1994). The biocidal efficiency of ClO₂ equals or is superior to chlorine (Gates, 1998; Korich et al, 1990; Aieta & Berg, 1986). ClO₂ is effective against viruses, bacteria, protozoan cysts, biofilm, and waterborne pathogens in public drinking water systems (Radziminski et al, 2002; Chauret et al, 2001; Walker & Morales, 1997; Walker et al, 1995; Olivieri et al, 1986). Moreover, ClO₂ is an effective biocide over a wide pH range, and is effective for removing iron and manganese and for controlling taste and odor.

Legionella is considered to be a continuing risk and the single most common etiologic agent associated with outbreaks involving drinking water (WSTB, 2006). Healthcare facilities are increasingly faced with the decision of choosing a *Legionella* disinfection method. It is recommended that any such methods undergo a four-step evaluation process to ensure the method's safety and efficacy (Stout & Yu, 2003). These steps are: (1) demonstrate disinfectant efficacy in vitro, (2) document anecdotal experience in preventing disease outbreaks in individual hospitals, (3) implement controlled studies of sufficient duration in single hospitals, and (4) validate confirmatory reports from multiple hospitals over a prolonged period. This study represents step three of the process for chlorine dioxide—a controlled prospective full scale study in two hospitals.

A previous field study showed that an extended time (i.e., 1.75 years) was required for complete *Legionella* eradication from a hospital distribution system, and that the ClO₂ residual in the hot water system was significantly lower than in the cold water system (Sidari et al, 2004). This hospital also had a unique secondary distribution system that included a 520,000-gallon reservoir where ClO₂ was injected and a 10,000-ft pipeline to 23 buildings across 60 acres. Based on this field study, it can be hypothesized that the efficacy of ClO₂ for controlling *Legionella* might be improved for hospitals with smaller secondary distribution systems in which ClO₂ is injected into the incoming water.

Therefore, to check this hypothesis, the objectives of this study were

- to evaluate the efficacy of ClO₂ for controlling *Legionella* and total heterotrophic plate count (HPC) bacteria in two hospitals with smaller secondary water distribution systems and

- to monitor the levels of ClO_2 , chlorite (ClO_2^-) and chlorate (ClO_3^-) to ensure that the maximum residual disinfection level (MRDL) and maximum contaminant level (MCL) were not exceeded.

MATERIALS AND METHODS

Study hospital A. Healthcare acquired legionellosis due to *Legionella pneumophila* was diagnosed in an immunocompromised patient in hospital A. Following the initial case, steps were taken to control *Legionella* in the water distribution system and ClO_2 was chosen to treat the hospital water system. Hospital A has 364 patient beds and 74 skilled nursing beds, and comprises two buildings: building 1 (referred to as B1) and building 2 (referred to as B2). Both buildings have eight floors. Hospital water is supplied by the city water department.

Legionella has been detected in the hot water systems of both buildings of hospital A since October 2002. The extent of *Legionella* colonization is expressed as percent *Legionella* positivity, which is the percentage of all sampling sites that tested positive for *Legionella*. The risk of Legionnaires' disease in hospitalized patients has been shown to be better predicted by the percentage of water system sites testing positive for *Legionella* than by the concentration of *Legionella* bacteria in individual samples (Stout & Yu, 2003; Kool et al, 1999). In January 2003, the hospital began operating a ClO_2 generating system to control *Legionella* in the water system. Before installation of the ClO_2 system, the percentage of *Legionella* positive hot water outlets was 67% (six out of nine samples).

Study hospital B. From March 2001 to January 2002, three cases of hospital acquired *Legionella pneumophila* pneumonia occurred in hospital B. Cultures from the water distribution system reflected the presence of *Legionella pneumophila* serogroup 5 and were identical to *Legionella* cultured from one of the identified patients. Following the initial cases, steps were taken to eliminate *Legionella* in the water distribution system, including superheating and flushing the hot water system and replacing a water tank found to be colonized with *Legionella*. Despite these measures, another case of hospital acquired *Legionella pneumophila* pneumonia was identified in January 2002. The optimal method for long term disinfection has not yet been identified and no recommendations for long term treatment exist at this time (CDC, 2004). To investigate the effectiveness of ClO_2 , this method was chosen to treat the hospital water system and was installed in April 2004. In hospital B, the single 12-floor building that is treated with ClO_2 has 672 operating patient beds. Predisinfection baseline cultures were collected from 2002 to 2004.

ClO_2 generation systems. One ClO_2 generating unit¹ was installed in each of the two buildings of hospital A by a private contractor.² Another ClO_2 generator³ was installed in hospital B by a different private contractor.⁴ Examples of ClO_2 generators are shown on page XXX. The

generators use modular electrochemical cassettes to generate a solution with approximately 500 mg/L of ClO_2 using a 25% sodium chlorite solution. ClO_2 is injected into the incoming cold water main at the target ClO_2 concentration of 0.5–0.7 mg/L based on the flow rate of the incoming cold water.

Sample collection and analyses. Sampling locations for *Legionella* and HPC bacteria were selected throughout the distribution systems in hospitals A and B. In hospital A, 13 sampling locations in B1 and seven sampling locations in B2 were located on the second, fourth, fifth, sixth, and eighth floors of each building. In hospital B, 17 sampling locations were located in the third through the twelfth floors. Hot and cold water samples were collected from distal outlets (sinks and showers) at each sampling location. The hot water storage tank was also sampled at both hospitals.

Sampling in hospital A was performed every two months from June 2004 to August 2005 and then extended to June 2006. Sampling in hospital B was performed from August 2003 to June 2005 and then extended to February 2006. *Legionella* testing was performed in a laboratory,⁵ as described in an earlier study (Sidari et al, 2004).

For *Legionella* cultures, 120-mL water samples were collected immediately after the outlet taps were turned on. Distal outlets were then flushed for 1 min to collect representative water samples for ClO_2 analysis. Temperature measurements were taken directly from the flow stream after the flush. A 10-mL sample was taken for ClO_2 analysis at the time of collection and 100g/L of glycine was added to eliminate free chlorine interference. Levels of ClO_2 were analyzed in both hot and cold water samples using the DPD method for ClO_2 (0.00 to 5.00 mg/L; Hach, 2000) and using a glycine reagent and a DPD free chlorine reagent.⁶ Colorimetric measurements were performed using a spectrophotometer.⁷

Hospital personnel also monitored ClO_2 residual in cold water throughout the distribution system every month in hospital A. Hospital personnel performed ClO_2 residual measurements with a pocket colorimeter⁸ following method 10101 (Hach, 2000) with the same reagents as the study samples.

Samples for ClO_2^- and ClO_3^- analysis were chosen to represent various distances from the ClO_2 injection point (closest, midpoint, and farthest sites). In hospital A, a total of seven hot water samples and five cold water samples were collected for ClO_2^- and ClO_3^- analysis every two months from five locations in B1 and two locations in B2. In hospital B, a total of six hot water samples and four cold water samples were collected for ClO_2^- and ClO_3^- analysis every two months from six locations.

Samples for ClO_2^- and ClO_3^- analysis were sparged with nitrogen gas for 10 min immediately following the collection to remove ClO_2 residual. Next, 30 mL of the sample was filtered through a 0.2- μm filter, followed by

the addition of 50 mg/L of ethylenediamine to each sample. ClO_2^- and ClO_3^- were measured by ion chromatography⁹ with suppressor and conductivity detectors according to method 300.1 (USEPA, 1997). Two samples were also sent to a reference laboratory¹⁰ each time as a quality control measure to ensure the accuracy of ClO_2^- and ClO_3^- analysis.

Water quality of the municipal water supply was evaluated in October 2003 and June 2004. Water samples from the city water supply were collected and stored at 4°C before transfer to the Pittsburgh Water and Sewer Authority for analysis using standard laboratory procedures.

Statistical analysis. Statistical software¹¹ was used for statistical analysis. Significant differences were evaluated by student's *t*-tests and analysis of variance (ANOVA), and a *p* value below 0.05 was considered indicative of a significant difference.

RESULTS AND DISCUSSION

Hospital A. Water quality parameters. Water quality of the municipal water supply was monitored in October 2003 and June 2004. The mean values of water quality parameters were as follows: hardness was 127 mg/L as calcium carbonate, alkalinity was 83 mg/L as calcium carbonate, pH was 7.70, total iron was 0.03 mg/L, total manganese was 0.01 mg/L, total organic carbon (TOC) was 1.96 mg/L, and turbidity was 0.40 ntu. The ClO_2 demand of the drinking water was determined to be 0.20 mg/L after 6 h contact time at 24°C and pH 7.9 using method 2350 C (*Standard Methods*, 1998).

Legionella Positivity. *Legionella* positivity in hot water was reduced from 60% (12 out of 20 samples) in August 2003 to 10% (two out of 20 samples) in February 2006 (Figure 1). Significant reduction in hot water *Legionella* positivity (ANOVA, $p < 0.05$) occurred in 18 months (Figure 1) because of the ClO_2 residual. The authors believe that this decline can be attributed to an increase in ClO_2 residual in the hot water: ClO_2 residual in the hot water increased significantly from 0.04 mg/L in August 2003 to 0.11 mg/L in February 2006 ($p < 0.05$) as shown in Figure 1. The decline in *Legionella* positivity in the hot water can not be attributed to the variation of the hot water temperatures because hot water temperatures below 60°C do not affect *Legionella* colonization (Lin et al, 1998b; Zacheus & Martikainen, 1996; Darelid et al, 1994). The mean distal site hot water temperature was 44°C during the study (range from 27 to 52°C). The mean distal site cold water temperature was 18°C (range from 4 to 31°C). *Legionella* positivity in cold water samples was below 20% with 0.3–0.5 mg/L of ClO_2 residual (Figure 2). The increase in ClO_2 with time in the cold water was not significant ($p > 0.05$).

In February 2005, *Legionella* positivity of hot water samples unexpectedly increased from 10% (in December 2004) to 45%. No malfunction of the ClO_2 genera-

tor was found prior to this increase, and the mean ClO₂ residual remained at 0.36 mg/L in the cold water on the sampling day. The reason for the increase in *Legionella* positivity remained unclear. The feed concentration of ClO₂ was increased to 0.58 mg/L in B1 in April 2005. Samples collected in April and June 2005 showed that *Legionella* positivity returned to 25% (Figure 1).

Although the overall distal site positivity declined during the study, the authors did not observe a significant decrease in the concentration of *Legionella* (mean cfu/mL) in positive samples ($p > 0.05$). No cases of hospital acquired Legionnaires' disease have been detected in this hospital since the ClO₂ system was installed in January 2003.

The feed pump of the ClO₂ generator was changed on June 8, 2004 to allow for additional feed capacity. As shown in Figure 3, the mean monthly ClO₂ residual in the cold water increased significantly ($p < 0.001$) from 0.41 mg/L to 0.54 mg/L after the pump replacement. The changes and variability in mean monthly ClO₂ residual are attributed to operational adjustments and maintenance.

After the ClO₂ system was installed, a significant decrease in *Legionella* percent positivity was observed in the hot water system ($p < 0.05$). However, an extended period (18 months) was needed to achieve this reduction in positivity, which is most likely due to the low concentration of ClO₂ in the hot water. This is significant because *Legionella* species proliferate in hot water (Lin et al, 1998b). It is clear that maintaining sufficient ClO₂ residual in the hot water system is quite challenging. Elevated water temperature hastens the conversion of ClO₂ to ClO₂⁻ through the reactions with organic compounds in the water distribution system (Zhang et al, 2006). This is consistent with the observation that ClO₂ was consumed and converted to ClO₂⁻ to a greater extent in hot water, since the mean ClO₂⁻ concentration in hot water was higher than in cold water (Figure 4, part A). However, this study and other studies have demonstrated that zero positivity is not necessary to prevent hospital acquired Legionnaires' disease (Stout & Yu, 2003).

It may be possible to reduce this lag period by performing shock ClO₂ treatment (Bova et al, 2004). Makin reported (1998) that the successful application of ClO₂ in hot water systems for controlling *Legionella* required increasing the ClO₂ level to 3–5 mg/L in hot water systems. Alternatively, daily flushing of sinks and showers in patient rooms may also be effective (Bova et al, 2004). However, both of these measures need to be evaluated in a controlled study. Another possible approach to achieving a higher ClO₂ residual in hot water includes adding a ClO₂ injection point to the line after the hot water tanks. This may shorten the time needed to achieve a measurable ClO₂ residual at distal outlets. The impact of injecting ClO₂ directly into the hot water for controlling *Legionella* has yet to be evaluated.

ClO₂ and its disinfection by-products. ClO₂, ClO₂⁻,

and ClO_3^- levels were monitored throughout the buildings during the study. The mean ClO_2 residual in the hot water was 0.07 mg/L; the residual rarely exceeded 0.1 mg/L. The mean ClO_2 residual in the cold water was 0.42 mg/L. The difference in the mean ClO_2 concentrations between the cold water distal outlets and hot water distal outlets was significant ($p < 0.05$).

A total of 91 hot water samples and 65 cold water samples were analyzed for ClO_2^- and ClO_3^- . The average ClO_2^- concentrations in the hot and cold water were 0.42 and 0.28 mg/L, respectively. The ClO_2^- concentrations measured in this study were in agreement with those obtained by the reference laboratory¹⁰ (data not shown), as the mean difference was 8% ($p > 0.05$). The mean ClO_2^- concentrations in cold and hot water were below the MCL of 1.0 mg/L (Figure 4). The mean ClO_3^- concentrations in hot and cold water were below the detection limit of 0.10 mg/L.

In Figure 5, parts A and B show the mean ClO_2 and ClO_2^- concentrations between the different sampling locations in hospital A over two years. Chlorine dioxide concentration ranged from 0 to 0.70 mg/L, while chlorite concentration ranged from 0 to 0.82 mg/L. No significant differences ($p > 0.05$) occurred in mean ClO_2 and ClO_2^- concentrations between the sampling locations that represent various distances from the injection point in the hot water (Figure 5, part A) and in the cold water (Figure 5, part B).

Hospital B. Water quality parameters. Water quality of the municipal water supply was monitored in June 2004. The values of water quality parameters were as follows: hardness was 124 mg/L as calcium carbonate, alkalinity was 69 mg/L as calcium carbonate, pH was 8.57, total iron was 0.04 mg/L, total manganese was 0.01 mg/L, TOC was 2.25 mg/L, and turbidity was 0.38 ntu. The ClO_2 demand of the drinking water was determined to be 0.20 mg/L after 6 h contact time at 23°C and pH 7.8 using method 2350 C (*Standard Methods*, 1998).

Legionella positivity. Mean percent positivity of all distal outlets (both hot and cold) for *Legionella* was 60% before the ClO_2 treatment (range from 35 to 88%, $n = 72$). After the ClO_2 treatment, mean percent positivity of all distal outlets for *Legionella* decreased from 60 to 8% (range from 0 to 24%, $n = 165$, $p < 0.05$) as shown in Figure 6. *Legionella* positivity in the hot and cold water was reduced to 0% after 6 months of ClO_2 treatment and remained at 0% for three consecutive sampling events after August 2005 (Figure 6). *Legionella* positivity unexpectedly increased to 19% in June 2006. One possible explanation for the observed increase is that the ClO_2 generator malfunctioned between the two sampling events. The ClO_2 residual in hot and cold water was 0.09 and 0.29 mg/L, respectively, which was lower than the average values. No cases of healthcare acquired legionellosis have been identified in the postdisinfection period.

The mean concentration of *Legionella* in positive hot

water samples decreased from 166 cfu/mL (range from 10 to 520 cfu/mL) to 43 cfu/mL (range from 10 to 100 cfu/mL). The mean concentration of *Legionella* in positive cold water samples decreased from 20 cfu/mL (range from 10 to 20 cfu/mL) to 0 cfu/mL. The decrease in mean concentration of *Legionella* in positive samples was not significant, but the overall distal site positivity decreased significantly. The mean distal site water temperatures for the hot and cold water during the study were 43°C (range from 34 to 52°C) and 17°C (range from 4 to 25°C), respectively.

Legionella positivity in the hospital B water system was reduced to 0% in a much shorter period of time (six to 10 months) as compared to hospital A. The mean ClO₂ residual in the hot water system of hospital B reached above 0.10 mg/L in a much shorter time, and the mean ClO₂ residual in hot water of hospital B was significantly higher than in hospital A (Table 1). The percentage of samples positive for *Legionella* in hot water has been shown to decrease as ClO₂ residual increased to above 0.1 mg/L (Zhang et al, 2007). It is hypothesized that reducing the time it takes to achieve ClO₂ residual in hot water above 0.10 mg/L leads to faster reduction of *Legionella* positivity.

It was also found that the incoming drinking water in hospital B contained high levels of free chlorine (range from 0.75 to 1.02 mg/L) because the hospital was very close to the water treatment plant. The mean chlorine in the incoming water of hospital B was higher than in hospital A (Table 1). It can be hypothesized that the coincident reaction of two disinfectants (chlorine and chlorine dioxide) provided synergistic effects in controlling *Legionella* in the water distribution system. It is also possible that the high levels of free chlorine in the drinking water could meet some oxidant demand so that ClO₂ injected in this healthcare facility can be maintained at a stable residual concentration in both hot and cold water systems. Katz showed (Katz et al, 1994) that the combination of two disinfectants (chlorine dioxide and chlorine) applied to the effluent from a municipal sewage treatment plant produced a relatively stable high residual of both disinfectants and reduced the concentration of the undesirable disinfection by-product (i.e., chlorite ion) despite increasing chlorine dioxide concentration. Several studies showed that mechanically mixed oxidants achieved considerable disinfection efficiency for selected microorganisms (Son et al, 2005). However, the level of the enhanced disinfection efficiency remains unclear and the synergistic effect of the mixed oxidants also needs to be confirmed. One study (Cho et al, 2006) showed that sequential disinfection with chlorine dioxide followed by free chlorine is an effective approach to treating *Bacillus subtilis* spores while another (Corona-Vasquez et al, 2002) found no such synergy for treating *Cryptosporidium parvum* oocysts. The synergistic effect of sequential treatment may be caused by the unique activity of each dis-

infection agent reacting with specific chemical groups of the cell walls and needs to be investigated further in the case of chlorine dioxide, free chlorine, and *Legionella*.

HPC bacteria. The efficacy of ClO_2 for controlling HPC bacteria in the hot water system was also evaluated in hospital B. Although most of the HPC bacteria in drinking water are not human pathogens, HPC bacteria in drinking water may include bacterial species that are pathogenic for immunocompromised patients in hospitals, such as *Pseudomonas* species and different fungi (Glasmacher et al, 2003). The efficacy of ClO_2 for controlling HPC bacteria in hospital B was also evaluated to assess the potential for reducing the risk of infection from waterborne opportunistic pathogens. The average concentration of HPC bacteria was significantly reduced ($p < 0.05$) from 15,400 cfu/mL before chlorine dioxide treatment to 2,900 cfu/mL after treatment (Figure 7). This suggests that ClO_2 may be an effective disinfectant not only for controlling *Legionella* but also against opportunistic bacteria in hospital drinking water systems. Further study would be needed to evaluate the effects of chlorine dioxide on specific opportunistic bacteria in hospital water systems.

ClO_2 and its disinfection by-products. The mean ClO_2 residuals in the hot and cold water samples from hospital B were 0.11 and 0.36 mg/L, respectively (Table 1). The difference in mean ClO_2 concentrations between the cold water distal outlets and hot water distal outlets was significant ($p < 0.05$). A total of 54 hot water samples and 36 cold water samples were analyzed for ClO_2^- and ClO_3^- . The mean ClO_2^- concentrations in cold and hot water were 0.42 and 0.38 mg/L, respectively (Figure 4, part B). The ClO_2^- concentrations measured in this study were in agreement with those obtained by the reference laboratory¹⁰ (data not shown) as the mean difference was only 8% ($p > 0.05$). The mean ClO_3^- concentrations in hot and cold water were below 0.10 mg/L.

In Figure 5, parts C and D show mean ClO_2 and ClO_2^- concentrations on different floors in the hot and cold water system of hospital B during the two-year study. Chlorine dioxide concentration ranged from 0.04 to 0.74 mg/L, while chlorite concentration ranged from 0.19 to 0.66 mg/L. ANOVA analysis showed that there was no significant difference in ClO_2 and ClO_2^- levels between different sampling locations representing various distances from the injection point in the hot water and cold water ($p > 0.05$).

Srinivasan compared (Srinivasan et al, 2003) the ClO_2 and ClO_2^- levels at different sampling locations between two time points in a hospital water system. The ClO_2 and ClO_2^- levels at the lower floor were higher than the ClO_2 and ClO_2^- levels at the higher floor one month after treatment started. The differences disappeared after 17 months. One explanation for this phenomenon was that the background ClO_2 demand in the system had been met after 17 months. In the author's study, no significant difference was found for the mean ClO_2 and

ClO_2^- concentrations in the hot water between sampling locations that represented various distances from the ClO_2 injection point in hospital A and B (Figure 5, parts A and C). Also, no significant difference was found in ClO_2 and ClO_2^- levels with increasing distance from the point of injection in the cold water in hospital A and B (Figure 5, parts B and D). The change in ClO_2 and ClO_2^- levels with time could be attributed to mechanical modifications to the ClO_2 feed system and operational adjustments. For hospital A, the study began six months after the installation and operation of the ClO_2 unit. The initial demand may have been met and the system may have reached equilibrium within the first six months of continuous treatment with ClO_2 . For hospital B, the high level of free chlorine in the incoming cold water may have met some of the oxidant demand and helped to maintain the ClO_2 residual at relative stable levels.

The operation of the ClO_2 system for controlling *Legionella* in two hospital water systems was found to be safe based on the MRDL for ClO_2 and MCL for ClO_2^- . ClO_3^- is currently not regulated but its levels in hot and cold water never exceeded 0.30 mg/L. Users of chlorine dioxide systems must comply with current regulations for municipal water systems. These monitoring requirements address concerns that chlorine dioxide and its disinfection by-products (chlorite and chlorate ions) may pose health risks to consumers. Chlorite may cause congenital cardiac defects and hemolytic anemia through oxidative damage to the red blood cell membrane (Condie, 1986). US Environmental Protection Agency (USEPA) has set the MRDL for ClO_2 of 0.8 mg/L and the MCL for ClO_2^- of 1.0 mg/L (USEPA, 1998). Chlorate is currently not regulated because of the lack of health data. ClO_2 and the persistence of its disinfection by-products in water treatment plants and large distribution systems has been studied (Hoehn et al, 2003; Baribeau et al, 2002), however, few data exist for small secondary water systems.

Monitoring of a hospital water system involves daily monitoring of ClO_2 at no less than three different sites, and monthly monitoring of ClO_2^- at no less than three different sites. ClO_2^- monitoring can be reduced to quarterly monitoring after monthly monitoring results show that the ClO_2^- level in the distribution system has not exceeded the MCL of 1.0 mg/L for one year (USEPA, 1998). The author's data suggest that the chlorite level in hot and cold water of an open water distribution system is unlikely to exceed the MCL when 0.5–0.7 mg/L of ClO_2 is injected in the incoming cold water. Less frequent monitoring of the disinfection by-products would satisfy the safety concerns in hospital water systems.

Cost of *Legionella* control. *Legionella* remediation efforts are not inexpensive. Cost estimates in the range of \$70,000–\$80,000 for continuous hyperchlorination, and \$60,000–\$100,000 for copper–silver ionization systems have been reported (Lin et al, 1998a). One hospital estimated the cost for engineering measures with chlorine

dioxide at approximately \$50,000 per annum (Hosein et al, 2005). For 438-bed hospital A, the annual cost for operation and maintenance of two chlorine dioxide units was approximately \$34,000 per year; installation costs are not included because the hospital leased the units. Hospital personnel installed the required flowmeters, filters, injection points, and piping. The annual cost for monitoring the chlorine dioxide residual and chlorite level in the hospital water system ranged from \$3,000 to \$5,000, which brings the total annual cost for the chlorine dioxide system in hospital A close to \$40,000. For hospital B, the capital cost for the chlorine dioxide unit was close to \$50,000.

CONCLUSIONS

This study conducted in two hospitals showed that *Legionella* can be successfully controlled by chlorine dioxide. However, the exact amount of time needed to achieve requisite reduction in percent positivity is site specific. Chlorine dioxide and its disinfection by-products were successfully maintained below the regulatory limits. Specifically, when ClO_2 was injected into the cold water main at 0.5–0.7 mg/L, mean concentrations of ClO_2 and ClO_2^- in cold and hot water samples did not exceed the MRDL of 0.8 mg/L for ClO_2 and MCL of 1.0 mg/L for ClO_2^- , respectively. Distance from the ClO_2 injection point did not significantly affect mean concentrations of ClO_2 and ClO_2^- in both hot and cold water systems. In addition to *Legionella*, ClO_2 is also a promising disinfectant for controlling other opportunistic waterborne pathogens in hospital drinking water systems.

ACKNOWLEDGMENT

The authors thank Carole McCann (posthumously), Jim McElroy, Ray Bisson, and Marian Przybysz of Mercy Hospital; Sue Mietzner, Laura Morris, Asia Obman, Pat Sheffer, and Sara Vaccarello of the Special Pathogens Laboratory; Stanley States of the Pittsburgh Water and Sewer Authority; and Joseph Hannigan of Klenzoid Inc. for their assistance on the project. This study was supported in part by Halox Inc. and Klenzoid Inc., and was also part of PhD research conducted by Zhe Zhang at the University of Pittsburgh.

ABOUT THE AUTHORS



Zhe Zhang is special pathogens director at SanAir Technologies Laboratory, Powhatan, Va. He has a BS degree from Wuhan University of Technology in Wuhan, China; an MS degree from Wuhan University in Wuhan, China; and a PhD degree from the University of Pittsburgh, Pa.

He has five years of experience evaluating chlorine dioxide for Legionella control in hospital water systems. At the time this article was written, Carole

McCann was an infection control professional at Mercy Hospital, Buffalo, N.Y. Jennifer Hanrahan is a medical doctor, Annette Jencson is a staff member, Daniel Joyce is a staff member, and Steven Fyffe is a staff member at MetroHealth System, Cleveland, Ohio. Steve Piesczynski is the maintenance department supervisor and Robert Hawks is the director of facility services at Mercy Hospital, Buffalo, N.Y. Janet E. Stout is a research associate professor and Victor L. Yu is a professor of medicine at the University of Pittsburgh, Pa. Radisav Vidic (to whom correspondence should be addressed) is a professor at the University of Pittsburgh, 949 Benedum Hall, Pittsburgh, PA 15261; vidic@pitt.edu.

Date of submission: 02/25/08

Date of acceptance: 07/16/08

FOOTNOTES

- ¹Chlorine dioxide generator, Halox Inc., Bridgeport, Conn.
- ²Environmental Hygiene Services, Nalco Co., Naperville, Ill.
- ³Chlorine dioxide generator, Diox, Klenzoid Inc., Conshohocken, Pa.
- ⁴Klenzoid Inc., Conshohocken, Pa.
- ⁵Special Pathogens Laboratory, Pittsburgh, Pa.
- ⁶Hach DPD Free Chlorine Reagent, Hach Co., Loveland, Colo.
- ⁷Hach DR/2010 Spectrophotometer, Hach Co., Loveland, Colo.
- ⁸Hach Pocket Colorimeter, Hach Co., Loveland, Colo.
- ⁹DX-500, Dionex Inc., Sunnyvale, Calif.
- ¹⁰Novachem Laboratories Inc., Oxford, Ohio
- ¹¹Stata 9.0, StataCorp, College Station, Texas

If you have a comment about this article,
please contact us at journal@awwa.org.

REFERENCES

- Aieta, M.F. & Berg, D.J., 1986. A Review of Chlorine Dioxide in Drinking Water Treatment. *Jour. AWWA*,78:6:62.
- Anaisse, E.J.; Penzak, S.R.; & Dignani, C., 2002. The Hospital Water Supply as a Source of Nosocomial Infections: A Plea for Action. *Archives of Internal Medicine*, 166:1483.
- Baribeau, H.; Prevost, M.; Desjardins, R.; LaFrance, P.; & Gates, D. J., 2002. Chlorite and Chlorate Ion Variability in Distribution Systems. *Jour. AWWA*, 94:7:96.
- Benin, A.L.; Benson, R.F.; & Besser, R.E., 2002. Trends in Legionnaires' Disease, 1980-1998: Declining Mortality and New Patterns of Diagnosis. *Clinical Infectious Diseases*, 35:10:1039.
- Bova, G.; Sharpe, P.; & Keane, T., 2004. Evaluation of Chlorine Dioxide in Potable Water Systems for *Legionella* Control in an Acute Care Hospital Environment. Proc. 65th International Water Conference, Pittsburgh, Pa.
- CDC (Centers for Disease Control and Prevention), 2004. Guidelines for Preventing Health-Care-Associated Pneumonia, 2003. *Morbid. & Mortality Weekly Rept.*, 53:RR-3:1.
- Chauret, C.P.; Radziminski, C.Z.; Lepuil, M.; Creason, R.; & Andrews, R.C., 2001. Chlorine Dioxide Inactivation of *Cryptosporidium parvum* Oocysts and Bacterial Spore Indicators. *Appl. Envir. Microbiol.*, 67:7:2993.
- Cho, M.; Kim, J.H.; & Yoon, J., 2006. Investigating Synergism During

- Sequential Inactivation of *Bacillus subtilis* Spores With Several Disinfectants. *Water Res.*, 40:15:2911.
- Condie, L.W., 1986. Toxicological Problems Associated With Chlorine Dioxide. *Jour. AWWA*, 78:6:73.
- Corona-Vasquez, B.; Rennecker, J.L.; Driedger, A.M.; & Marinas, B.J., 2002. Sequential Inactivation of *Cryptosporidium parvum* Oocysts With Chlorine Dioxide Followed by Free Chlorine or Monochloramine. *Water Res.*, 36:1:178.
- Darelid, J.; Bengtsson, L.; Gästrin, B.; Hallander, H.; Löfgren, S.; Malmvall, B.E.; Olinder-Nielsen, A.M.; & Thelin, A.C., 1994. An Outbreak of Legionnaires' Disease in a Swedish Hospital. *Scand. Jour. Infectious Diseases*, 26:417.
- Gates, D., 1998. *The Chlorine Dioxide Handbook*. AWWA, Denver.
- Glasmacher, A.; Engelhart, S.; & Exner, M., 2003. *Heterotrophic Plate Counts and Drinking Water Safety: The Significance of HPCs for Water Quality and the Human Health*. (J. Bartram, J. Cotruvo, M. Exner, C. Fricker, and A. Glasmacher, editors). IWA Publishing, London, http://www.who.int/water_sanitation_health/dwq/hpc/en/index.html (accessed Mar. 5, 2009).
- Hach Co., 2000. DR/2010 Spectrophotometer Procedures Manual. Hach Co., Loveland, Colo.
- Hamilton, E.; Seal, D.V.; & Hay, J., 1996. Comparison of Chlorine and Chlorine Dioxide Disinfection for Control of *Legionella* in a Hospital Potable Water Supply. *Jour. of Hospital Infection*, 32:2:156.
- Hill, W.; Boyes, C.E.; & Hosein, I.K., 2000. Continuous Chlorine Dioxide Dosing of Hospital Hot Water System Following a Case of Nosocomial Aspiration *Legionella* Pneumonia. Proc. 5th International Conference on *Legionella*, Ulm, Germany.
- Hoehn, R.C.; Ellenberger, C.S.; Gallagher, D.L.; Wiseman, E. Jr.; Benninger, R.W.; & Rosenblatt, A., 2003. Chlorine Dioxide and By-product Persistence in a Drinking Water System. *Jour. AWWA*, 95:4:141.
- Hoigne, J. & Bader, H., 1994. Kinetics of Reactions of Chlorine Dioxide (OCIO) in Water. Part 1: Rate Constants for Inorganic and Organic Compounds. *Water Res.*, 28:1:45.
- Hood, J.; Cheape, G.; Mead, A.; & Curran, E., 2000. Six Years' Experience With Chlorine Dioxide in Control of *Legionella pneumophila* in Potable Water Supply of Glasgow Royal Infirmary. *Amer. Jour. of Infection Control*, 28:1:86.
- Hosein, I.K.; Hill, D.; Tan, T.; Butchart, E.; Wilson, K.; Finlay, G.; Burge, S.; & Ribeiro, C., 2005. Point-of-care Controls for Nosocomial Legionellosis Combined With Chlorine Dioxide Potable Water Decontamination: A Two-year Survey at a Welsh Teaching Hospital. *Jour. of Hospital Infection*, 61:2:100.
- Katz, A.; Narkis, N.; Orshansky, F.; Friedland, E.; & Kott, Y., 1994. Disinfection of Effluent by Combinations of Equal Doses of Chlorine Dioxide and Chlorine Added Simultaneously Over Varying Contact Times. *Water Res.*, 28:10:2133.
- Kool, J.L.; Bergmire-Sweat, D.; Butler, J.C.; Brown, E.W.; Peabody, D.J.; Massi, D.S.; Carpenter, J.C.; Pruckler, J.M.; Benson, R.F.; & Fields, B.S., 1999. Hospital Characteristics Associated With Colonization of Water Systems by *Legionella* and Risk of Nosocomial Legionnaires' Disease: A Cohort Study of 15 Hospitals. *Infection Control and Hospital Epidemiol.*, 20:12:798.
- Korich, D.G.; Mead, J.R.; Madore, M.S.; Sinclair, N.A.; & Sterling, C.R., 1990. Effects of Ozone, Chlorine Dioxide, Chlorine, and Monochloramine on *Cryptosporidium parvum* Oocyst Viability. *Appl. & Envir. Microbiol.*, 56:5:1423.
- Lin, Y.E.; Stout, J.E.; Yu, V.L.; & Vidic, R.D., 1998a. Disinfection of Water Distribution Systems for *Legionella*. *Seminars in Respiratory Infections*, 13:2:147.

- Lin, Y.E.; Vidic, R.D.; Stout, J.E.; & Yu, V.L., 1998b. *Legionella* in Water Distribution Systems. *Jour. AWWA*, 90:1:112.
- Makin, T., 1998. Control of *Legionella* in Domestic Water Systems and Potential Energy Savings Resulting From the Control of *Legionella* With Chlorine Dioxide. 15th International Federation of Hospital Engineering Congress, Edinburgh, Scotland.
- Mietzner, S.M.; Hangard, A.; Stout, J.E.; Rohr, U.; Pedro-Botet, M.L.; Samore, M.H.; & Yu, V.L., 2005. Reduced Susceptibility of *Legionella pneumophila* to the Antimicrobial Effects of Copper and Silver Ions. Proc. 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington.
- Olivieri, V.P.; Snead M.C.; Krusé, C.W.; & Kawata, K., 1986. Stability and Effectiveness of Chlorine Disinfectants in Water Distribution Systems. *Envir. Health Perspect.*, 69:11:15.
- Radziminski, C.; Ballantyne, L.; Hodson, J.; Creason, R.; Andrews, R.C.; & Chauret, C., 2002. Disinfection of *Bacillus subtilis* Spores With Chlorine Dioxide: A Bench-scale and Pilot-scale Study. *Water Res.*, 36:6:1629.
- Sidari, F.P.; Stout, J.E.; VanBriesen, J.M.; Bowman, A.M.; Grubb, D.; Neuner, A.; Wagener, M.M.; & Yu, V.L., 2004. Keeping *Legionella* Out of Water Systems. *Jour. AWWA*, 96:1:111.
- Son, H.; Cho, M.; Kim, J.; Oh, B.; Chung, H.M.; & Yoon, J., 2005. Enhanced Disinfection Efficiency of Mechanically Mixed Oxidants With Free Chlorine. *Water Res.*, 39:4:721.
- Srinivasan, A.; Bova, G.; Ross, T.; Mackie, K.; Paquette, N.; Merz, W.; & Perl T.M., 2003. A 17-Month Evaluation of a Chlorine Dioxide Water Treatment System to Control *Legionella* Species in a Hospital Water Supply. *Infection Control and Hospital Epidemiol.*, 24:8:575.
- Standard Methods for the Examination of Water and Wastewater*, 1998 (20th ed.). APHA, AWWA, and WEF, Washington.
- Stout, J.E. & Yu, V.L., 2003. Experiences of the First 16 Hospitals Using Copper-silver Ionization for *Legionella* Control: Implications for the Evaluation of Other Disinfection Modalities. *Infection Control and Hospital Epidemiol.*, 24:80:563.
- USEPA (US Environmental Protection Agency), 1997. Method 300.1. Determination of Inorganic Anions in Drinking Water by Ion Chromatography. Office of Water, Washington.
- USEPA, 1998. National Primary Drinking Water Regulations: Disinfectant Disinfection Byproducts. Final Rule. *Fed. Reg.* 63:241:69390.
- Walker, J.T.; Mackerness, C.W.; Mallon, D.; Makin, T.; Williets, T.; & Keevil, C.W., 1995. Control of *Legionella pneumophila* in a Hospital Water System by Chlorine Dioxide. *Jour. Indust. Microbiol.*, 15:4:384.
- Walker, J.T. & Morales, M., 1997. Evaluation of Chlorine Dioxide for the Control of Biofilms. *Water Sci. Technol.*, 35:11-12:319.
- WSTB (Water Science and Technology Board), 2006. *Drinking Water Distribution Systems: Assessing and Reducing Risks*, The National Academies Press, Washington.
- Zacheus, O.M. & Martikainen, P.J., 1996. Effect of Heat Flushing on the Concentrations of *Legionella pneumophila* and Other Heterotrophic Microbes in Hot Water Systems of Apartment Buildings. *Canadian Jour. Microbiol.*, 42:8:811.
- Zhang, Z.; McCann, C.; Hawks, R.; McElroy, J.; Piescznski, S.; Bisson, R.; Vidic, R.; & Stout, J. E., 2007. Prospective Study of the Safety and Efficacy of Chlorine Dioxide for *Legionella* Control in a Hospital Water System. *Infection Control and Hospital Epidemiol.*, 28:8:1012.
- Zhang, Z.; Stout, J.E.; & Vidic, R.D., 2006. The Impact of Temperature and TOC on Chlorine Dioxide Decay in Drinking Water. Proc. 2006 AWWA Ann. Conf., San Antonio, Texas.

A flow meter monitored the incoming cold water main in one of the hospital water systems. ClO₂ is injected into the incoming cold water main at the target ClO₂ concentration of 0.5–0.7 mg/L based on the flow rate of the incoming cold water.

Monitoring for chlorine dioxide was conducted using method 10101 (Hach, 2000).

The chlorine dioxide generators used in this study employ an electrical source and membrane technology to produce chlorine dioxide from a sodium chlorite precursor. The membranes are housed in removable cassettes shown here in the upper portion of the unit. The generators eliminate the need for handling hazardous chlorine gas or strong acids.

The chlorine dioxide generator used in hospital B was capable of generating chlorine dioxide at a rate of 5.0 lb/d and a concentration of 500–600 mg/L. System software allowed for remote monitoring via an Internet connection.

Depending on the size of the facility, multiple chlorine dioxide generators can be installed to accommodate larger demand or to provide backup units.

With this chlorine dioxide generator installed in each building of hospital A, chlorine dioxide was injected into the incoming cold water main at a target concentration of 0.5–0.7 mg/L, depending on the flow rate of the incoming cold water. Probes for oxidation–reduction potential were installed to provide an indirect monitor for chlorine dioxide levels.

L*egionella* is considered to be a continuing risk and the single most common etiologic agent associated with outbreaks involving drinking water.

The study indicates that chlorine dioxide is a promising disinfectant for controlling not only *Legionella*, but also other microorganisms in drinking water.

Chlorine dioxide is a promising alternative disinfectant for hospital water systems to prevent Legionnaires' disease.

No cases of healthcare acquired legionellosis have been identified in the postdisinfection period in these two hospitals.

It is clear that maintaining sufficient ClO_2 residual in the hot water system is quite challenging.

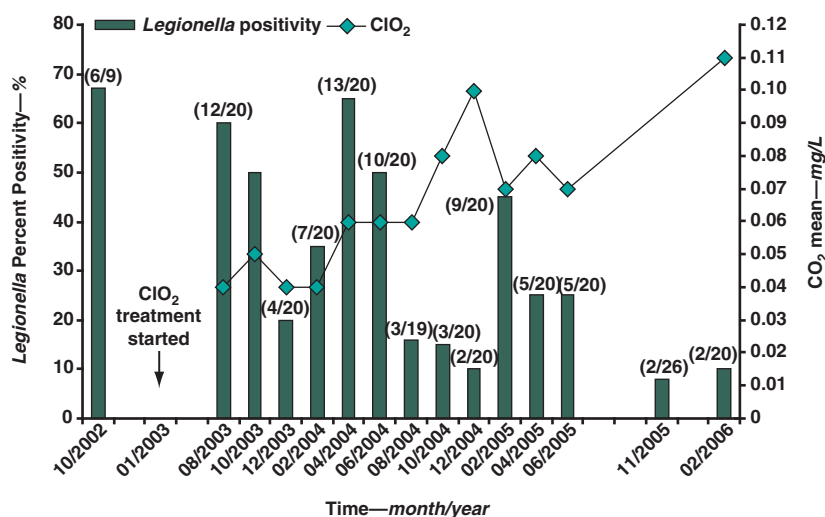
TABLE 1 Comparison of study parameters of two hospitals using ClO₂ for *Legionella* control

Parameter	Hospital A	Hospital B
Size	438 beds	672 beds
	Two buildings	One building
ClO ₂ injection point	Cold water main	Cold water main
Mean ClO ₂ in hot water—mg/L	0.07	0.12 (<i>p</i> < 0.05)
Mean ClO ₂ in cold water—mg/L	0.42	0.36 (<i>p</i> > 0.05)
Mean Cl ₂ in cold water—mg/L	0.55	0.91 <i>p</i> < 0.05)
Months to achieve 0% <i>Legionella</i> positivity	> 24 months	6–10 months

Cl₂—chlorine, ClO₂—chlorine dioxide, *p*—probability, 0–1, unitless

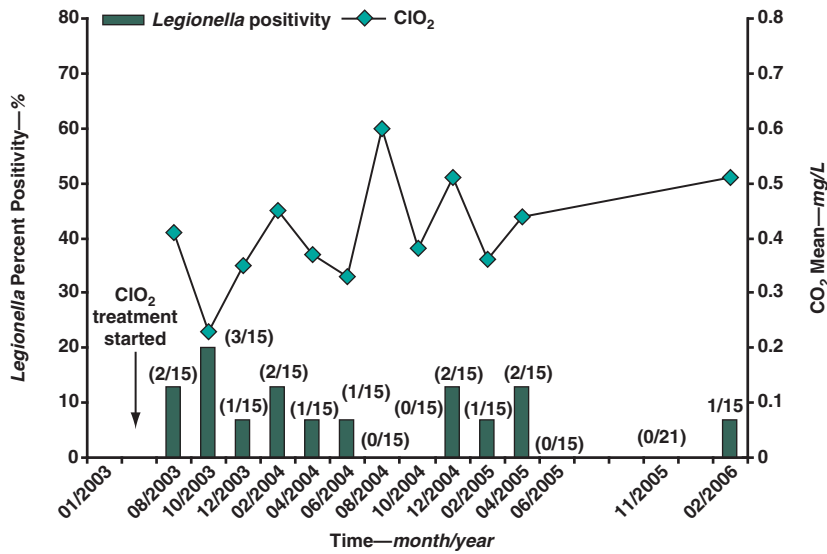
Mean ClO₂ and Cl₂ in hospitals A and B were compared by *t*-test

FIGURE 1 Percent distal site *Legionella* positivity and mean ClO₂ concentrations over 40 months in hot water of hospital A



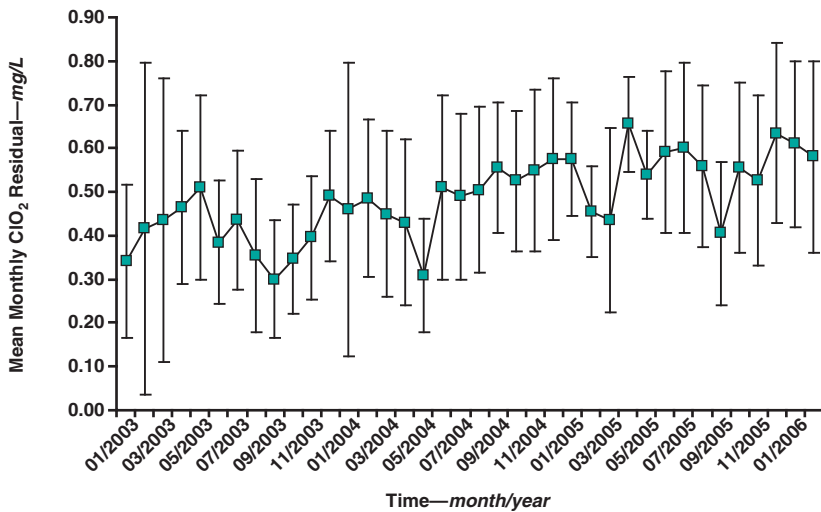
ANOVA—analysis of variance, ClO₂—chlorine dioxide
 Within the figure, numbers in parentheses represent positive *Legionella* samples/total samples for month indicated.
 A significant reduction in percent *Legionella* positivity (ANOVA, *p* < 0.05) was observed after the first 18 months of ClO₂ treatment (Zhang et al, 2007).

FIGURE 2 Percent distal site *Legionella* positivity and mean ClO₂ concentrations over 40 months in cold water of hospital A



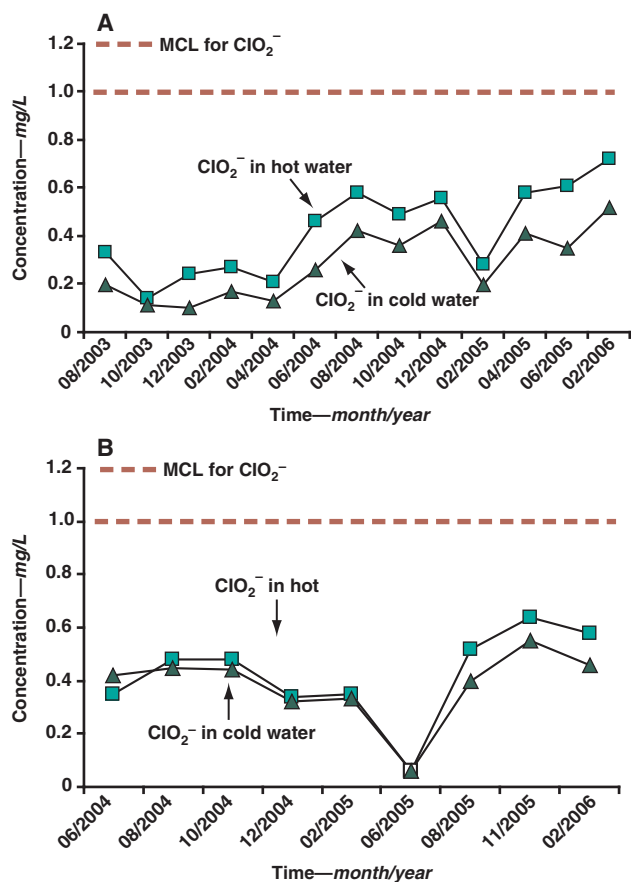
ClO₂—chlorine dioxide
 Within the figure, numbers in parentheses represent positive *Legionella* samples/total samples for month indicated.
Legionella positivity was maintained below 20% with ClO₂ residual of 0.3–0.5 mg/L.

FIGURE 3 Mean monthly ClO₂ residual in cold water samples of hospital A
 ClO₂—chlorine dioxide



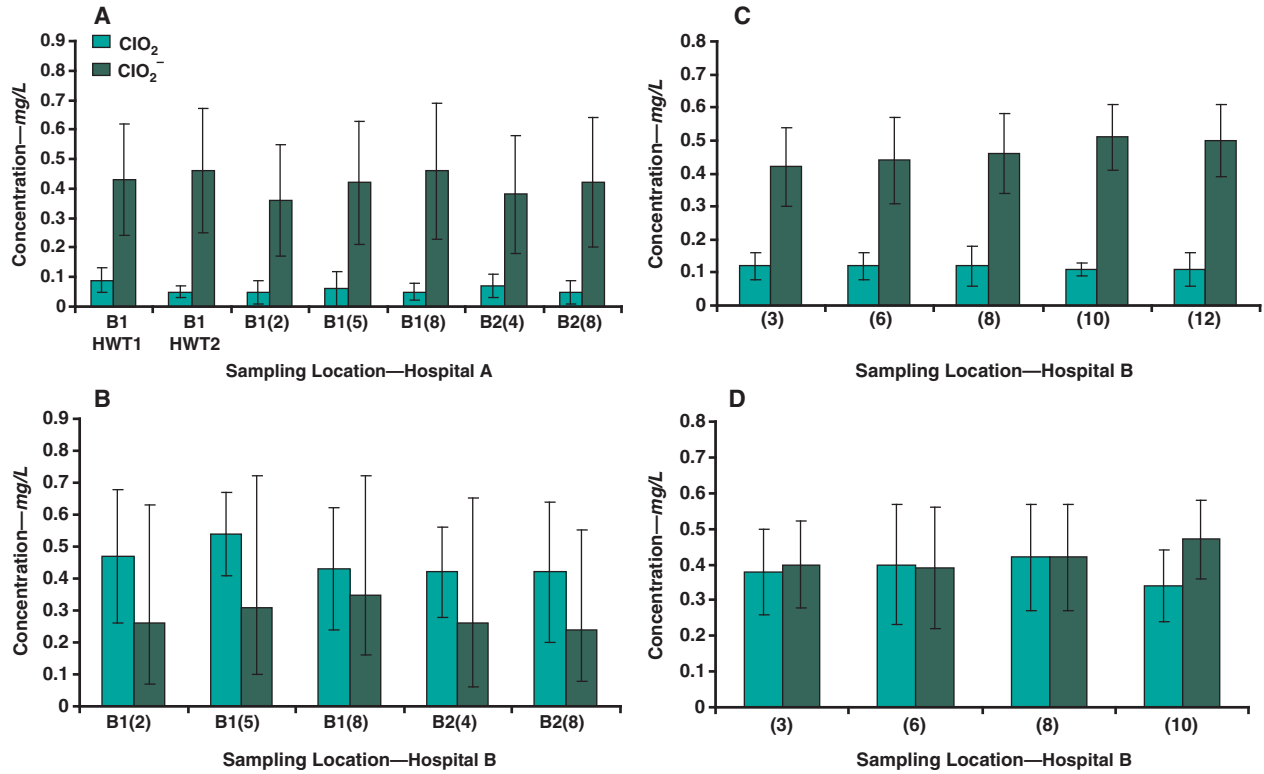
Error bars represent standard deviation.
 The changes and variability in mean monthly residual are attributed to operational adjustments and maintenance.

FIGURE 4 Mean ClO_2^- level in hot and cold water of (A) hospital A and (B) hospital B



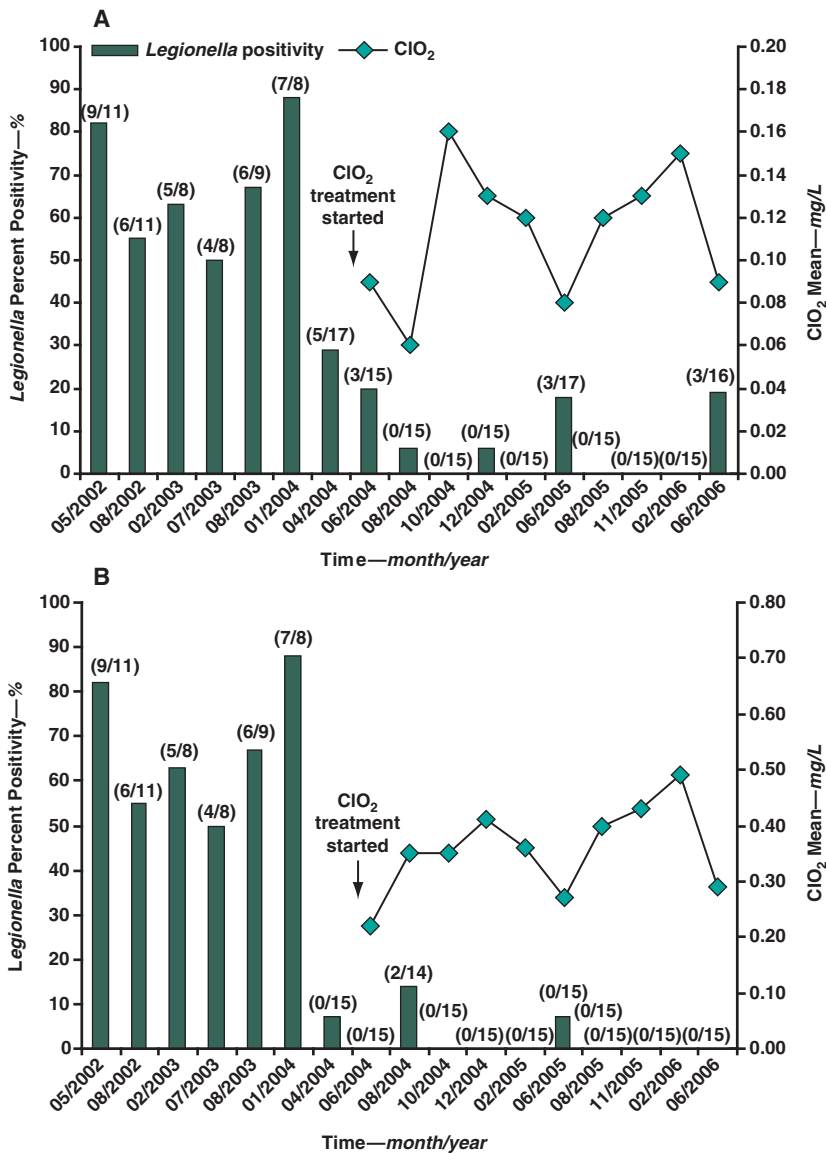
ClO_2^- —chlorite, MCL—maximum contaminant level
 ClO_2^- level in hot and cold water systems of hospitals A and B did not exceed the MCL.

FIGURE 5 ClO_2 residual and ClO_2^- level in hot water (A and C) and cold water (B and D) at different locations in hospitals A and B



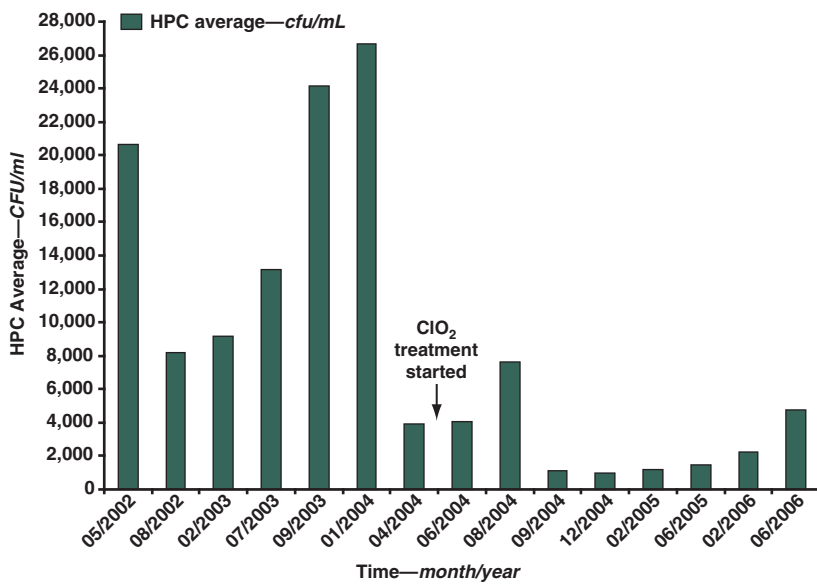
ANOVA—analysis of variance, B—building, ClO_2 —chlorine dioxide, ClO_2^- —chlorite, HWT—hot water tank
 Error bars represent standard deviation. Numbers in parentheses indicate floor.
 Distance from the ClO_2 injection point did not significantly affect mean concentrations of ClO_2 and ClO_2^- in hot and cold water of hospitals A and B (ANOVA, $p > 0.05$).

FIGURE 6 Percent distal site *Legionella* positivity and mean ClO₂ concentrations in (A) hot water and (B) cold water of hospital B



Within the figure, numbers in parentheses represent positive *Legionella* samples/total samples for month indicated. *Legionella* positivity from May 2002 to January 2004 represents average positivity for both hot and cold water sites. Significant reduction in *Legionella* positivity (ANOVA, $p < 0.05$) was accomplished after six months of ClO₂ treatment.

FIGURE 7 HPC bacteria concentration in hot water of hospital B before and after ClO₂ treatment



ClO₂—chlorine dioxide, HPC—heterotrophic plate count
HPC bacteria concentration in hot water samples decreased significantly after ClO₂ treatment (*t*-test, *p* < 0.05).