Chlorine Dioxide Gas Decontamination of Large Animal Hospital Intensive and Neonatal Care Units

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Abstract

In May of 2004, the large animal intensive care and neonatal intensive care units (ICU/NICU) of the University of Pennsylvania's New Bolton Center were temporarily closed to all admissions following an outbreak of salmonellosis that had affected its patients. Environmental testing continued to recover a multi-drug resistant organism identified as Salmonella enterica serovar Newport following repeated liquid disinfection treatments of the facility. Based upon various environmental considerations, it was decided that the most feasible and effective process for disinfecting the facility would be achieved using chlorine dioxide (ClO$_2$) gas. The facility was appropriately sealed, supplied with gas distribution mechanisms and demonstrated to have met the specification for humidity before introduction of ClO$_2$. The total exposure for this 4800 cubic meters structure was approximately 400 ppm-hr. Efficacy of the decontamination was monitored by the placement of 20 Bacillus atrophaeus spore, 40 Geobacillus stearothermophilus spore, and 40 Salmonella Newport vegetative cell strips. Microbiological testing demonstrated greater than 5.5 and 6.1 log reduction for the G. stearothermophilus and B. atrophaeus spore strips, respectively. Log reductions of the S. Newport bacterial strips were also within acceptable levels. The success of this project demonstrates the utility of ClO$_2$ gas as a biological decontaminant approach for mid-sized commercial and public facilities.

Introduction

The George D. Widener Large Animal Hospital, located within the New Bolton Center in Kennett Square, Pennsylvania, is one of the busiest, large animal hospitals in the United States, having approximately 6000 patient visits annually with a primarily (82%) equine caseload. As part of the University of Pennsylvania, this hospital also serves as an integral part within the University's School of Veterinary Medicine and is important to the teaching, research, and service missions of the school. Subclinical and clinical infections with *Salmonella* are reasonably common in large animal patients (Dunowska et al., 2004; Morley et al., 2004; & Smith et al., 2004) and major outbreaks of nosocomial *Salmonella* infections have been documented at large animal veterinary teaching hospitals and other veterinary facilities over the past several decades (Anon., 2001; Castor et al., 1989; Dargatz & Traub-Dargatz, 2004; Hartmann et al., 1996; Parè et al., 1996; Schott et al., 2001; & Tillotson et al., 1997). Among equine patients, horses presenting with gastrointestinal disturbances, such as diarrhea and colic, may be at highest risk of shedding *Salmonella* (Ernst et al., 2004; House et al., 1999; Kim et al., 2001; Morley et al., 2004; & Palmer et al., 1985). As a tertiary care referral center, the Widener hospital admits a large number of critically ill and emergency cases. The largest proportion of emergency admissions, about half of approximately 1,200 emergency admissions per year from 1998-2003, have colic or diarrhea as their primary problem. As is the case with other large animal veterinary teaching hospitals, Widener Hospital is at greater risk of having animals that are actively shedding *Salmonella* within its environs than many other animal housing facilities.

A possible *Salmonella* outbreak was detected at the Widener Hospital in mid-March 2004. A weekly environmental surveillance program of various sites around the Widener Hospital had been employed for several years and would occasionally have a sample test positive for *Salmonella*. In response to the perception of increased cases, environmental and patient surveillance was increased in March, but gross contamination of the environment was still not indicated. A number of major steps were taken during the ensuing 58 days to determine the precise nature of the contamination and bring it under control. Surveillance of patients was increased to include submission of fecal samples from all animals (including

those with no clinical signs of infection) housed in high-risk areas. Efforts were made to clean and decontaminate high-risk areas while maintaining essential hospital services. Specific parts of the hospital were temporarily closed to patients. After all disposables were discarded, these areas were cleaned, disinfected and restocked before reopening. During these 58 days, the intensive care unit and neonatal intensive care unit (ICU/NICU) itself was subject to two such rounds of cleaning and disinfection.

In mid-April, the environmental monitoring and culture techniques employed were changed in two ways. First, electrostatic dust collection wipes (Swiffer®; Procter and Gamble, Cincinnati, OH) were used for sample collection (Burgess et al., 2004). The charge on the wipes attracts debris and bacteria and thus the wipes can be used to sample large surface areas. Secondly, a more sensitive technique for the isolation of *Salmonella*, utilized by the PADLS (Pennsylvania Animal Diagnostic Laboratory System) microbiology laboratories for isolating low numbers of stressed Salmonellae from environmental samples and foodstuffs, was adopted. This method was an adaptation of the International Standards Organization method for the detection of *Salmonella* spp. (ISO, 2002).

Toward the end of the 58-day period, the hospital was closed to elective in-patients; however, out-patients were seen, but only emergency cases were admitted to the hospital. Despite these extensive control efforts, culture-positive animals continued to be identified. Retrospective analysis of medical records revealed that between January 1, 2004, and closure of all parts of the hospital on May 10, 2004, 37 animals were positive for a group C_2 *Salmonella* identified as *Salmonella enterica* serovar Newport. There was significant morbidity and mortality among the animals that were shedding *Salmonella* in their feces, although some of the culture-positive animals never showed clinical signs of salmonellosis.

During the last two weeks of April, 37 of 140 cultures taken throughout the hospital complex were found to be positive for *S. Newport*. The causative agent was identified as a multi-drug resistant form of *S. Newport*, a gram-negative, non-spore forming bacterium. In light of the positive fecal cultures obtained from patients and the environmental findings, it was deemed necessary in early May 2004 to discharge all remaining patients and close the entire Widener hospital until the adverse bacterial population could be brought under control.

After closing, decontamination efforts were made throughout the Widener Hospital. Rigorous, multistage cleaning and disinfection was performed which included four hospital wards, particularly for horses; a ward for large ruminants; an isolation facility; a sports medicine building; a treadmill facility; and all operating rooms and diagnostic areas including paths connecting such buildings. In many animal-housing areas, grounds and/or floors were sandblasted and/or resurfaced. Some flooring surfaces were completely removed and replaced with concrete plus a polyurethane-based monolithic flooring system, where appropriate. Flooring surfaces in stalls and other areas of the ICU/NICU facility were removed and replaced and not subject to sandblasting. Equipment and supplies were cleaned or discarded. Liquid disinfectants were applied on most surfaces throughout the hospital. Particular use was made of an aqueous solution containing potassium peroxomonosulfate, sodium dodecylbenzenesufonate and sulfamic acid (Virkon-S®; Antec International, Sudbury, UK), an AOAC-approved oxidizing detergent sanitizer. While the bacterial population was successfully reduced at many locations, the population persisted within the ICU/NICU. After several failed attempts at controlling the *Salmonella* population within this facility, it was decided that a gas-phase space decontamination would be the most effective route to a successful decontamination.

Physical Site

The ICU/NICU comprises a nearly isolated building, adjoined through one hallway to an equine orthopedic ward (Figure 1). The total volume of space considered for decontamination is approximately 4800 m³ (170,000 ft³). Its main floor is comprised of fifteen patient units with wooden sliding doors and appropriate facilities for providing food and waste disposal. This floor also has two nursing stations, a laboratory, a pharmacy, three restrooms, several offices and other smaller rooms. Two weighing stations were built into the floor and an overhead hoist system is present to facilitate the movement of patients and equipment. A second floor contains additional offices, a restroom, kitchenette, and a mechanical room housing the exhaust and supply blowers. Two basement rooms house additional mechanical and storage space, and two elevators and an internal stairway adjoin the floors. Although much of the space had been disinfected, office rooms remained furnished and contained files, as well as other personal effects. Interstitial spaces were visibly soiled. The interstitial space above the basement is open to the earth foundation under part of the building. The HVAC (heating, ventilation and air conditioning) system is 100% in/out. Most of the offices have window air conditioners.

Decontaminant Choice

Three fumigants were considered for the space decontamination of the ICU/NICU—formaldehyde gas, hydrogen peroxide vapor, and chlorine dioxide gas. All three were known to be effective decontaminants for spore and non-spore forming bacteria under standard laboratory conditions, i.e., clean flat surfaces lacking porous materials or potential dead-legs with which fumigant

Figure 1
George D. Widener Large Animal Hospital

penetration might be retarded.

Standard application of formaldehyde (Taylor et al., 1969) typically involves the use of ammonia gas following the decontamination cycle to neutralize the remaining formaldehyde (Luftman, 2005). A residue consisting of polymerized formaldehyde (paraformaldehyde) and the neutralization product (methenamine) is commonly left after such treatment. The removal of such a residue was considered problematic for this facility. Residual formaldehyde from off-gassing was also of concern, due to its odor and its toxicity. Formaldehyde is considered a potential carcinogen by the EPA and an actual carcinogen by the International Agency for Research on Cancer (IARC, 2004).

Hydrogen peroxide vapor, when used as a space fumigant, is typically generated at a concentration that is close to or above its condensation point under typical temperature and humidity conditions (Watling et al., 2002). Different systems attempt to either prevent or promote this condensation. Nevertheless, as a result of this physical instability, there is a need to have rapid and very efficient recirculation of the hydrogen peroxide if one is trying to prevent condensation, or to inject the vapor at nearly line-of-sight to all surfaces if condensation is desired. These conditions were believed to be too restrictive for the current application, particularly when all interstitial space and ductwork were to be included within the decontamination. Furthermore, hydrogen peroxide is known to be chemically unstable in the presence of cellu-

lose-based material, and the ICU/NICU space contained many wooden surfaces within the clinical areas and paper within the offices. It was believed that it would have been difficult to maintain an appropriate concentration of active hydrogen peroxide within the space.

The performance of disinfection by chlorine dioxide (Knapp & Battisti, 2001; & Knapp et al., 1986) had recently gained attention by virtue of its application against anthrax spore contaminations at the Hart Senate Building and the Brentwood Mail Facility in Washington, DC and of the U.S. Postal Service Trenton Mail Processing and Distribution Center in Hamilton, New Jersey (Haas, 2001; & U.S. EPA, 2005). It has also been employed with sterilizers, isolators, and small rooms (Leo et al., 2005). Chlorine dioxide (ClO_2) is a water soluble, yellow-green gas with a boiling point of 10°C. Dissolved in water, it has been used as a germicide in water and food treatment and as a bleaching agent by the paper industry. ClO_2 is a selective oxidant reacting primarily with organics that are highly reduced (e.g., alcohols, aldehydes, ketones, tertiary amines and sulfur-containing amino acids) and thus is generally not as adversely affected by typical organic loads, as are other oxidants, such as hydrogen peroxide (Knapp & Battisti, 2001). Its deleterious effect on bacterial endospores is believed to be directed primarily toward the cell membrane rather than DNA (Young & Setlow, 2003). Unlike bleach or chlorine gas, ClO_2 is known not to form chlorinated by-products (Knapp & Battisti, 2001). As a selective oxidant, it has also been shown to be

compatible with most standard materials including stainless steel, anodized aluminum, Teflon, Viton, polyethylene, polypropylene, and nylon (Eylath et al., 2003; Kowalski, 1998; & Leo et al., 2005). Some discoloration of uncoated copper and cold roll steel had been observed, comparable to what is seen when those materials are exposed to high humidity environments.

Several issues needed be addressed in planning for this method of decontamination. As with typical sporicidal fumigation protocol, the space would require sealing in order to maintain a sufficiently high concentration of the fumigant for efficacy, as well as to maintain a safe perimeter outside the building. A relative humidity greater than 60% would need to be maintained within the space for optimal ClO_2 potency. ClO_2 would need to be generated on site due to its short-term chemical instability. Because chlorine gas (Cl_2), is highly corrosive to many materials, the method of preparation would need to ensure its absence from being present within the ClO_2 flow. Furthermore, because ClO_2 can dissociate releasing Cl_2 in the presence of ultraviolet light, ultraviolet sources such as sunlight need to be avoided.

Method and Materials

Site Preparation

Sealing the facility proved to be the most time-consuming, labor-intensive part of the project. In part, this was because the ICU/NICU building had not been designed with the intent of ever requiring fumigation. Most of the sealing was performed on exterior surfaces. All vents or potentially leaky seams along the roof, which included vents adjacent to roof gutters and ridge vents, were sealed using combinations of caulk, expanding foam, duct tape and adhesive films. The exhaust and air supply units for the building were deactivated and their associated vents on the exterior walls and roof were sealed. External switches for these units were supplied to enable reenergizing them following the decontamination without having to enter the gas-filled space. Office windows that were capable of being opened were sealed, as were office air conditioning units. Obvious external wall damage and cracks were sealed. Vents for the two basement rooms were sealed. All external doors were ultimately sealed, as was an internal door leading from the ICU/NICU building to the adjacent 17-stall equine orthopedic ward. Several cover plates and plumbing fixtures on the outer walls and floors were sealed. The two dump holes on the underside of the building were sealed.

Aside from the door leading to the adjacent barn, all internal doors were propped open. All drawers and cabinet doors were opened and light diffusers were removed. All access panels to interstitial spaces were opened within the main and second floors. Access panels within the basement rooms were sealed, because the adjacent inter-

stitial space opened to an earth foundation and was considered not capable of being disinfected and a potential source of loss of the fumigant by absorption or reaction. Internal supply and exhaust vents were left open. Water was poured into all floor and sink drains that had traps, while other drains were capped.

Over 40 fans and blowers were distributed throughout the facility to ensure ClO_2 circulation. Several of these were placed at open access panels, within ceiling interstitial regions. A blower, connected by flexible duct, was inserted between openings within the main building exhaust and return units to force circulation throughout the HVAC system. The blower within the one biological safety cabinet in the building was turned on during the decontamination.

The efficacy of ClO_2 as a decontaminant is greatly enhanced at a relative humidity greater than 60% (Jeng & Woodworth, 1990). Thirty-five pans on hot plates were placed throughout the facility, with the intention of boiling water just before decontamination. Gauges to measure relative humidity and temperature were placed at several windows, allowing the internal conditions to be monitored during the decontamination.

A test of the containment of the ICU/NICU was performed. All circulating fans were energized. All except one of the entrances to the building were sealed. Three pans with 200 g isoamyl acetate and approximately 100 ml water were place upon hotplates within the ICU/NICU and the hot plates were energized. Leaking sites were then sought along all external surfaces, including the roof and basement areas of the ICU/NICU, by scenting for the isoamyl acetate. One major leak at the wall adjoining the ICU/NICU to the adjacent 17-stall equine ward and several minor leaks along the roof were detected. Specific leak sites were visualized using smoke generated by a glycol fogger, and subsequently repaired.

Biological indicators were aseptically placed a few hours before the decontamination throughout the building, including within ceiling interstitial spaces and the HVAC exhaust, to corroborate the efficacy of the decontamination (Figure 2). The indicators included (a) 40 unwrapped strips each with approximately 2 x 10^6 spores of *G. stearothermophilus* (Spordex), which are considered to be the most difficult challenge for ClO_2 decontamination (Leighton et al., 2004); (b) 20 unwrapped spore strips of *B. atrophaeus* (Spordex), previously known as *Bacillus subtilis var. niger*; and (c) 40 strips inoculated with approximately 10^9 colony forming units (CFU) of the bacterium *Salmonella* Newport. [The *S.* Newport test strips were prepared by the PADLS *Salmonella* Reference Center (SRC). They consisted of 1 x 1.5 inch absorbent paper with an impermeable plastic backing (Benchkote™), inoculated with 1 ml of a 10^9 ml^{-1} suspension of the bacteria. The strain of *Salmonella* had previously been isolated from an environmental sample submitted to the PADLS laborato-

Figure 2

Biological indicator placements. One *Geobacillus stearothermophilus* endospore and one *Salmonella* Newport test strip were placed at each location (·). One *Bacillus atrophaeus* endospore strip was placed at half of these locations (not indicated).
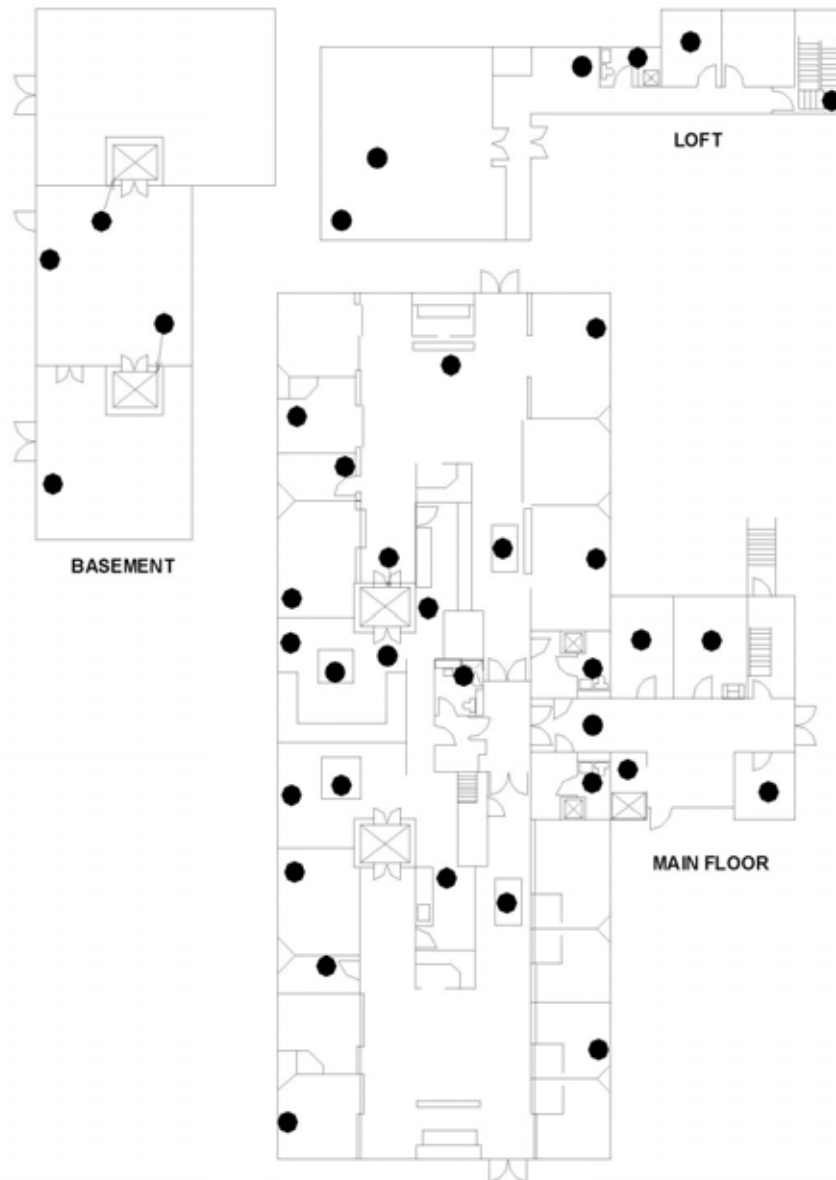

ries for culture. A representative batch of the test strips was tested for long-term viability prior to use. The culture work was performed in the PADLS/UP Clinical Microbiology laboratories and the serogrouping and serotyping was undertaken by the SRC.]

It was intended that the actual decontamination take place during nighttime hours, primarily to minimize the amount of photo-dissociation that is known to occur with ClO_2. Circuit breakers for all emergency lighting within the ICU/NICU were identified so that all internal lighting would be off during the decontamination. Pressure manometers with tubes extending under external doors into the building were placed at four locations about the ICU/NICU to ensure that the differential pressure from outside to inside the building remained as close to neutral as possible throughout the decontamination.

Chlorine Dioxide Preparation

Chlorine dioxide was to be generated from chlorine gas and sodium chlorite by the reaction:

$$Cl_2 \text{ (g)} + 2NaClO_2 \text{ (s)} \rightarrow 2ClO_2 \text{ (g)} + 2NaCl \text{ (s)}$$
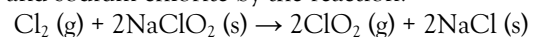
2% Cl_2 gas in nitrogen was passed through columns packed with solid sodium chlorite and other stabilizing material. Columns were appropriately sized such that 100

g Cl_2 was fully consumed by the above reaction with no measurable (<1 ppm) Cl_2 exiting from the column. Ten such generative systems were placed outside the ICU/NICU, with delivery tubes leading to various locations within the site. Several delivery tube lines were branched within the facility to further enhance gas distribution. Real-time concentration analysis of the ClO_2 was performed by ultraviolet/visible absorption measurements near the peak absorbance for ClO_2. A spectrophotometer (ClorDiSys Solutions, Inc.) was situated outside the ICU/NICU with 10 tubes for gas sampling running from the spectrophotometer to various locations within the ICU/NICU. A sampling manifold was utilized to switch detection from one area to another.

The intent was to attain a concentration of 1 mg ClO_2 per liter of air within the ICU/NICU after 4 hours of delivery, followed by an additional 1 hour of contact time at the targeted level, and then aeration. Prior experience by ClorDiSys Systems, Inc. indicated that the overall dose would be capable of delivering an 8-log kill to spores of *B. atrophaeus*, which is substantially more resistant to decontamination than gram-negative vegetative bacteria, such as *S. Newport*.

The Decontamination Event

The generation and introduction of the ClO_2 was initiated at 9:00 p.m. Exterior temperature at the time was approximately 75°F with a relative humidity of 70%. Due to the high humidity, the use of the hot plates for further humidification became unnecessary. The intended schedule consisted of building up gas concentration to a level of 1 mg ClO_2 per liter of air (~350 ppm) until 1:00 a.m., maintaining same concentration until 3:00 a.m., and then aerating until 9:00 a.m. The intent was to have a total exposure (the product of concentration and time) of 3.0 mg (ClO_2)–hour/liter (air) or 1050

ppm-hours. It was estimated that the exhaust capability of the ICU/NICU was on the order of 170 m^3/min (6000 ft^3/min) and the building volume was approximately 4800 m^3 (170,000 ft^3). A 6-hour aeration was estimated to bring the concentration of ClO_2 from its target level of 350 ppm to safely below its PEL limit of 0.1 ppm.

Internal temperature, relative humidity and pressure differential to the outside were monitored at the beginning and during the decontamination. Values were acceptable throughout, with the exception of one low relative humidity reading, later attributed to the meter being incorrectly set. There was no measurable amount of ClO_2 detected outside the decontamination area, although the gas could be smelled within the barn in the area adjacent to the sealed ICU/NICU. A fan was placed within this area to prevent gas accumulation. The concentration of ClO_2 was monitored serially from 10 locations regularly during the treatment. The spectrophotometer was sensitive to approximately 0.1 mg ClO_2/liter air. The raw measurements are shown in Table 1.

After 2 hours, it was ascertained that the concentration of ClO_2 was not increasing at the anticipated rate. Previously sealed points about the ICU/NICU were again investigated. A minor amount of ClO_2 leakage was detected and immediately sealed at points along the roof and at plumbing fixtures on the eastern wall. Nevertheless, the level of ClO_2 still did not increase at the desired rate and it was evident that the target concentration level would not be attained. It was decided to modify the schedule by continuing the introduction of ClO_2 until 8:00 a.m. (660 min), allowing for an overall increase of ClO_2 exposure. The end time was set, in part, to minimize the effect of photo-disassociation of the ClO_2 from sunlight. The total integrated doses of ClO_2, calculated from the data in Table 1, are shown in Table 2.

The average ClO_2 dose of 1.13 mg-hour/liter air (or

Table 1
Concentration Measurements of ClO_2 (mg/liter air).

Sample Site #	Time after Initiation (min)												
	40	75	95	150	215	270	330	390	450	510	555	600	640
1	0	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.05
2	0	0.1	0.1	0.1	0.2	0.2	0	0	0	0.2	0.2	0.2	0.2
3	0	0	0	0.1	0.1	0.1	0	0	0.1	0.1	0.1	0.1	0
4	0	0	0	0.1	0.1	0.1	0	0	0	0.1	0.15	0.1	0.1
5	0.1	0.2	0.2	0.2	0.3	0.2	0.1	0.1	0.1	0.3	0.3	0.2	0.2
6	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0	0.1	0.3	0.3	0.2	0.2
7	0	0.1	0.1	0.1	0.2	0.2	0.1	0	0	0.2	0.2	0.1	0.1
8	0	0	0	0	0.1	0.1	0	0	0	0.1	0.1	0.1	0.1
9	0	0	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1
10	0	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1

~400 ppm-hr) was 38% that of the target level of 3.0 mg-hour/liter air. It was estimated at the time that this exposure would be sufficient for at least a 5-log population reduction for the spore strips and a greater reduction for the S. Newport strips.

Chlorine Dioxide Aeration

Personnel wearing self-contained breathing apparatuses commenced aeration that morning and the sealing materials from exhaust and intake fans on the roof were removed and the fans were energized. A whitish emission was seen rising from the vent points and a chlorine odor was detected at ground level north of the ICU/NICU, ceasing within 50 minutes. Dräger measurements in these vicinities indicated that the level of ClO_2 and/or Cl_2 remained below the detection limit of 0.1 ppm. An external door was opened after an hour to aid with ventilation. The concentration of ClO_2 within the ICU/NICU was monitored periodically during the following hour. At the onset of aeration, the interior space was foggy and ClO_2 levels up to 10 ppm were measured. After 2.5 hours, the level of ClO_2 was no longer measurable and the space could be entered without personnel protective equipment. The reduction of required aeration time from the anticipated 6 hours was consistent with the lower than planned concentration of ClO_2 at the onset of the aeration.

Biological Indicator Analysis

Biological indicators were then aseptically collected throughout the ICU/NICU. All of the exposed spore strips impregnated with *Geobacillus stearothermophilus* and *Bacillus atrophaeus* and two unexposed strips were delivered to Microbiological Environments for determination of remaining viability. The two unexposed indicators served as controls to verify recovery efficacy and to calculate the logarithmic population reduction. The strips of S. Newport were delivered to the PADLS (University of Pennsylvania Clinical Microbiology Laboratories) for culture.

The *B. subtilis* and *G. stearothermophilus* biological indicators were processed the same day they had been received. Determination of the viable spore population was accomplished by aseptically transferring each spore strip to 30 ml of a sterile Butterfield's buffer solution. A cell disruptor was used to completely macerate the paper carriers and 10 ml aliquots were plated. The control samples were diluted 10-fold to the predicted population and 1 ml aliquots were plated. The plates were incubated under growth-permissive conditions.

Forty-two S. Newport test strips (including one unexposed control and one uninoculated strip) were each placed in 100 ml of Buffered Peptone Water (BPW) Oxoid CM0509 (Oxoid; Ogdensburg, NY). The BPW cultures were incubated at 37°C for 18 hours. Following incubation, 0.1 ml aliquots of BPW were transferred to 10 ml of Rappaport Vassiliadis (RV) Oxoid CM0669 enrichment medium. The RV broths were incubated at 42°C for 18 hours. After incubation, the RV cultures were each sub-cultured on McConkey's Agar (MC) Oxoid CM507, Difco XLD medium (XLD) and Desoxycholate-Citrate Agar (DCA) Oxoid CM0227.

Results

The plate counts for the two *Geobacillus stearothermophilus* control strips, each diluted by a factor of 3.0×10^4, were 16 and 27 colony forming units (CFU). These correspond to viable spore populations of 4.8×10^5 and 8.1×10^5. The mean value of 6.5×10^5 spores was used as the

Table 2
Calculated ClO_2 Dose

Site #	Location	Dose (mg ClO_2—hour/liter air)
1	Main Floor, east end	1.44
2	Main Floor, west end	1.13
3	Room 154	0.63
4	Room 157	0.60
5	Loft stairwell	1.93
6	Office 201	1.73
7	Loft mechanical room	1.13
8	HVAC return	0.46
9	Office 144	1.28
10	Eastern elevator, basement	1.01
Mean		1.13

reference for reduction calculations. Of the 40 plates produced from the *G. stearothermophilus* biological indicators placed within the ICU/NICU before the ClO₂ decontamination, 32 yielded no growth, seven yielded one CFU, and one yielded two CFU. There was no discernable pattern as to the location of the biological indicators that corresponded to non-zero CFU plates. Attributing no growth to an upper limit of 0.9 CFU and given a dilution factor of 3 for the test plate studies, no growth, 1 and 2 CFU correspond to log reduction values of >5.5, 5.4, and 5.1, respectively.

The plate counts for the two *Bacillus atrophaeus* control measurements, each diluted by a factor of 3.0 x 10⁴, were 109 and 112 CFU. These correspond to viable spore populations of 3.27 x 10⁶ and 3.36 x 10⁶, respectively. The mean value of 3.32 x 10⁶ spores was used as the reference for reduction calculations. Of the 20 plates produced from the *B. atrophaeus* biological indicators placed within the ICU/NICU before the ClO₂ decontamination, all 20 yielded no growth. Attributing no growth to an upper limit of 0.9 CFU and given a dilution factor of 3 for the test plate studies, no growth corresponds to log reduction values of >6.14.

Of the 40 *Salmonella* test strips processed, S. Newport was recovered from 2, with the remaining 38 testing negative for the presence of *Salmonella*. The inoculated but unexposed strip was positive for S. Newport and the uninoculated control strip was negative. No attempt was made to perform viable counts in order to calculate log reductions.

Within 3 hours following the onset of the aeration of the ICU/NICU from ClO₂, no residual odors within the ICU/NICU were detected. Furthermore, the pre-existing "animal" odors were also absent. No physical residue corresponding to the ClO₂ was observed and there was also no visible indication of material degradation—specifically, no obvious indication of corrosion of any of the metal-containing equipment that had been left within the ICU/NICU. Mechanical and electrical items that had been present within the ICU/NICU during the decontamination all continue to be functional at this time.

After receipt of the analytical results from the biological indicators, sealing material was removed from the ICU/NICU one week following the decontamination event. Six sets of hand and foot area Swiffer® tests for *Salmonella* (noted in the introduction) were taken throughout the ICU/NICU during the four months following the decontamination. A typical sample set included hand and foot samples for the nursing station (encompassing the two nursing stations, laboratory and pharmacy); main floor of the ICU; stall composite for the ICU; main floor of the NICU; stall composite for the NICU; and the link corridor. Hand and floor samples composited from areas like bathrooms, offices, the upper floor and the basement were included on a rotating basis.

All results were negative. Following decontamination, the flooring of the ICU/NICU nursing stations, laboratory, pharmacy and those in the muck rooms and feed storage areas were replaced with a poured epoxy surface that was coved up the walls. A monolithic polyurethane-based equine flooring system was installed in each of the stalls. Flooring that had been present in these areas (tile and rubber mats) had all been removed before the decontamination event. In addition, casework and plumbing fixtures, most of which had been removed before decontamination, were reinstalled. Automatic waterers formerly present in each stall were not replaced, but repairs to the block walls and repainting were undertaken before reopening. Ceiling tiles which had been removed in preparation for the ClO₂ decontamination were replaced, and damaged dry-wall ceiling or wall areas due to the liquid disinfection phases were repaired and repainted. New laundry facilities were also put in place; modifications and repairs to electrical and telephone systems were made and the entire area was restocked. Immediately prior to reopening, the air handling system of the building was rebalanced and culture sets taken both before and after air balancing were all found to be negative for *Salmonella*. Although the main part of the hospital initially reopened for cases on August 2, 2004, and the office spaces within the ICU/NICU were back in use shortly after decontamination, the extensive remediation and remodeling efforts undertaken in the clinical and animal housing areas of the building delayed facility reopening to patients. The building was, however, declared fully operational as of January 18, 2005. Since reopening and starting to accept patients, weekly environmental sample sets have been collected. At the time of writing, all samples have been negative for *Salmonella*.

Discussion

The targeted concentration and dosage of ClO₂ used for the ICU/NICU decontamination were based upon prior experience of two of the authors (Lorcheim and Czarneski) and upon studies within the literature. Substantially greater concentration and dosages have been used in other reported work. Han et al., (2003) experimented with initial concentrations of 1500 to 10,000 ppm for up to 12 hours exposure, concluding that if the ClO₂ were not replenished, the higher limit of concentration might be required. The Environmental Technology Verification study of ClO₂ (Rogers et al., 2004) employed a relatively constant concentration of 2000 ppm with a 6-hour exposure. Based upon studies of the anthrax spore remediation in Washington, DC, the U.S. EPA issued a crisis exemption for the use of ClO₂ at a total dosage of 9000 ppm-hr. (U.S. EPA, 2005). Leighton et al. (2004) estimated spore survival probabilities of well below one out of a million from laboratory studies using strips with

10^6 spores of *B. atrophaeus* and *G. stearothermophilus* with four-hour exposures of ClO_2 at 500 ppm. Their statistical modeling indicated that the survival fraction for *G. stearothermophilus* spores would be below 5×10^{-6} for biological indicators with 10^6 spores with a dose of 500 ppm-hr at 80% relative humidity. Jeng and Woodworth (1990) demonstrated a D value (the amount of time required for a 90% or 1-log reduction in viability) for *B. atrophaeus* of 1.6 min using 2500 ppm ClO_2, with 10^6 spores deactivated within 15 min exposure. Leo et al. (2005) demonstrated 4-log reduction of *B. atrophaeus* after a 30 min exposure to 1.0 mg/l and a similar reduction in 10 min with 3.0 mg/l ClO_2 (1000 ppm). Lorcheim (Kowalski, 1998) used unwrapped paper carriers impregnated with *B. atrophaeus* spores to obtain D values of 4.9 and 3.6 min. with ClO_2 concentrations of 3 and 5 mg/l (1000 and 1600 ppm), respectively. The targeted level of ClO_2 exposure for this project, approximately 1000 ppm-hr. for an 8-log kill, had been based largely upon these latter data.

With the actual exposure of ClO_2 in this work at approximately 400 ppm-hr., we anticipated a log reduction of *B. atrophaeus* spores to be on the order of 5 to 6, a level borne out by the results. A significantly greater population reduction was anticipated for the species of concern, S. Newport, as bacterial spores are known to be substantially more resistant to the gas than are vegetative cells (Knapp & Battisti, 2001).

As previously noted, the concentration of ClO_2 within the ICU/NICU did not increase at the anticipated rate given the rate that Cl_2 was introduced into the ClO_2 reactors. Furthermore, as may be inferred from Table 1, the concentration of ClO_2 within the ICU/NICU decreased at a significant rate when the Cl_2 flow was interrupted. Three potential causes for this loss have been considered: 1) leakage of ClO_2 to the ambient; 2) absorption of ClO_2 by various materials within the ICU/NICU; and/or 3) decomposition of ClO_2 within the ICU/NICU by interaction with materials or other environmental factors, such as ultraviolet light. Major leakage sites were found and remediated before the decontamination. Minor leakage sites detected during the initial hours of the decontamination were remediated as discovered. While some ClO_2 continued to be sensed outside of the space near the junction between the ICU/NICU and its adjoined building during the decontamination, the local level did not exceed the Dräger tube sensitivity of 0.1 ppm. The mean concentration within the ICU/NICU during the decontamination was approximately 40 ppm. Thus, leakage does not appear to have been significant enough to account for much of the loss.

Accelerated decomposition of ClO_2 would be anticipated to occur either through reductive reactions or photolysis. Light-induced decomposition can be ruled out as a significant source of gas loss as the process was per-

formed during the night and there were no lights energized within the space. As noted previously, ClO_2 is a fairly selective oxidant, which is not expected to react with typical building materials (including wood) or soil. Buttner et al. (2004) noted that a soiling load had no notable effect on the efficacy of ClO_2 on *B. atrophaeus* endospores, although they did not report whether more gas generation was required to maintain their target concentration. Han et al. (2003) noted significant loss of ClO_2 during experiments with various materials within a sealed 10-liter vessel, which the authors attributed to decomposition or absorption. As noted previously, there was a substantial soil load within the ICU. The decontamination space encompassed two elevator shafts, ductwork, mechanical spaces and interstitial spaces that had not been previously cleaned. Interstitial space associated with the basement was contiguous to a soil foundation. While an attempt was made to isolate this particular space from the decontamination, it was imperfect. The space encompassed multiple offices containing file cabinets and desks containing paper, cardboard, and personal items. ClO_2 would also preferentially dissolve into open water sources associated with restrooms within the space. Future experiments within controlled spaces are in order to isolate the most significant absorption and/or reaction sites to aid in the practical design of decontaminations in the field.

Independent of the cause for the loss of ClO_2, the importance of being able to monitor the concentration of the gas in real time was accentuated, particularly for spaces having such a variety of potential issues. Flexibility in the decontamination, such that modification of the amount of introduced decontaminant managed, would also be useful.

Conclusion

Large animal intensive care and neonatal intensive care units were contaminated with *Salmonella* Newport, thereby resulting in the infection of multiple patients and the ultimate quarantining of the facility. Chlorine dioxide gas was used to decontaminate the 4800 m^3 facility. After the facility had been appropriately sealed, a total ClO_2 dosage of ~400 ppm-hr was applied over a single evening. The process was monitored with 100 biological indicator strips inoculated with *Geobacillus stearothermophilus* spores, *Bacillus atrophaeus* spores, or *Salmonella* Newport vegetative cells. Subsequent analysis indicated better than 6-log viable reductions for the *B. atrophaeus* and *S.* Newport and better than 5-log reductions for the *G. stearothermophilus*. No deleterious material effects were noted. The facility has been reopened and no similar infections have been reported to date. ClO_2 has been demonstrated to be an effective decontaminant under non-laboratory conditions.

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