Successful Sterilization Using Chlorine Dioxide Gas

Part One: Sanitizing an Aseptic Fill Isolator

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Chlorine dioxide (CD) is an oxidizing agent used extensively in solution for bleaching pulp, for treating water systems, and for disinfecting food processing equipment and food including fresh fruits and vegetables. It has also been used in the formulations of disinfectant mouthwashes and toothpaste (1, 2). Most visibly in recent years, chlorine dioxide in both its gaseous and aqueous forms was used to successfully decontaminate the Hart Office Building and Brentwood postal sorting facility in Washington, DC, in response to their contamination with Anthrax spores (3–5). Chlorine dioxide gas is approved for use as a sterilant by the United States Environmental Protection Agency (US EPA). Its sporicidal effects are well documented and can be compared with those of vaporized hydrogen peroxide and formaldehyde, agents commonly used for surface disinfection in the pharmaceutical industry.

Here we describe the development of a process for disinfecting the surfaces of an isolator using CD. The approach to developing sanitization cycles was modeled after cycle development in more typical sterilization and disinfection procedures (such as steam sterilizers) for pharmaceutical equipment.

In the past study, gaseous CD was typically prepared by vaporization of a solution (6). For our study, CD was generated at the point of use by a method in which small plastic cartridges containing solid sodium chloride were perfused by a 2%

Photo 1: Aseptic fill isolator undergoing chlorine dioxide disinfection

Photo 2: Prototype chlorine dioxide gas generator showing CD-generating cartridges

PRODUCT FOCUS: ASEPTIC PARENTERAL PRODUCTS

PROCESS FOCUS: FILL/FINISH

WHO SHOULD READ: MANUFACTURING, QUALITY, VALIDATION, ENVIRONMENTAL MONITORING

KEYWORDS: STERILIZATION, CLEANING METHODS, ASEPTIC PROCESSING, ISOLATORS

LEVEL: INTERMEDIATE

Table 1: Location of biological indicators

No.	Location	No.	Location
1	Front of half-suit	11	Stopper bowl location
2	Armpit of half-suit	12	Fill station
3	Exhaust Vent at CV001	13	Exhaust vent at CV013 (deep vent)
4	Vial Tray	14	Vial location
5	Isolator bench top	15	HEPA fan grill
6	Accumulator	16	Window
7	Under disk of accumulator	17	Window
8	Front of second half-suit	18	Window
9	Armpit of second half-suit	19	Window
10	Exhaust vent at CV009	20	Positive Control (not exposed to chlorine dioxide)

Table 2: Disinfection cycle parameter

Run Code	Humidification Time (minutes)	CD Concentration (mg/L)	CD Exposure Time (minutes)
1a	15	5.0	60
1b	15	5.0	60
2a	30	5.0	30
2b	30	5.0	30
3a	15	5.0	30
3b	15	5.0	30
4a	15	7.5	30
4b	15	7.5	15
5a	15	5.0	45
5b	15	5.0	45

MATERIALS USED

Aseptic fill isolator, prototype, 240-ft^3 volume; Specialty Metal Works, Oxnard, CA (www.specialtymetalworks.com)

Chlorine dioxide gas generator, prototype; Advanced Sterilization Products, Irvine, CA (www.sterrad.com), now fabricated by ClorDiSys Solutions, Inc., Lebanon, NJ (www.clordisys.com)

CD-Cartridge set, Advanced Sterilization Products, Irvine, CA (www.sterrad.com), now supplied by ClorDiSys Solutions, Inc., Lebanon, NJ (www.clordisys.com)

2% chlorine–98% nitrogen compressed gas, 200 SCF/cylinder; Praxair, Inc., Oxnard, CA (www.praxair.com)

Amsco biological indicators (*Bacillus subtilis*), paper, 10^6 spores/carrier; Steris Corporation, Mentor, OH (www.steris.com)

Tyvek/film pouches for repackaging biological indicators, Steris Corporation, Mentor, OH (www.steris.com)

Heat impulse sealer HS-8; Fetpak Inc., Commack, NY (www.fetpack.com)

chlorine–98% nitrogen gas mixture. The chemical reaction products are CD and sodium chloride. The target CD concentration in this application was 5 mg/L; with a 20-minute exposure once that concentration was reached. The prototype generates up to 8,000 L of chlorine dioxide at 100 mg/L, under a pressure of typically 50 Pascals above atmospheric. The isolator chamber (with spore strips) was preconditioned for 30 minutes at 70% relative humidity based on prior work (1). The gas was later disposed of by aeration under positive pressure assist using 0.22-µm filtered pharmaceutical grade air through an exhaust fan into the atmosphere at limits approved by the EPA for exhaust of CD.

No corrosion is observed when using pharmaceutical-type materials such as high-grade 316SS (stainless steel) and 304SS, Lexan, and various other plastics such as Delrin, Teflon, and UHMWPE (7). No impact was observed on the exhaust system in this study. In a separate experiment, postexposure rinses of 304SS coupons in water for injection (WFI) showed no residual CD as measured using an HPLC method for detection of chlorides (8).

MATERIALS AND METHODS

Materials used are listed in the sidebar on this page. Photo 1 shows testing of CD for its sporicidal activity on a prototype aseptic fill isolator equipped with two half-suits (La Calhene); Photo 2 shows the chlorine dioxide gas generator prototype. Twenty biological indicators were used as the microbial challenge for each test. They were placed throughout the isolator on representative surfaces, described in Table 1 and marked in Figure 1. The indicators were removed from their original glassine envelopes, transferred into Tyvek/film pouches, and sealed using a heat impulse sealer. Gas impedance of the Tyvek was found to be negligible for the diffusion of chlorine dioxide, unlike for glassine, which exhibited slower diffusion of gas into the envelope. Data

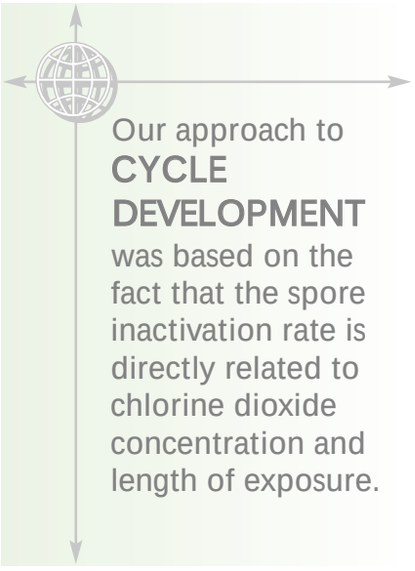


Table 3: Results of chlorine dioxide disinfection using *B. subtilis* spore strips (population 1×10^6)

Run Code	Half-suit Arm Placement	Humidification Time (minutes)	CD (mg/L)	CD Exposure Time (minutes)	Biological Indicators in Tyvek Pouch (Location for Positives)
1a	Down	15	5	60	All Negative
1b	Down	15	5	60	All Negative
2a	Up	30	5	30	All Negative
2b	Down	30	5	30	(Positive 2: Half-Suit Armpit) (Positive 9: Half-Suit Armpit)
3a	Down	15	5	30	(Positive 2: Half-Suit Armpit)
3b	Up	15	5	30	All Negative
4a	Down	15	7.5	30	All Negative
4b	Up	15	7.5	15	All Negative
5a	Down	15	5	45	All Negative
5b	Up	15	5	45	All Negative

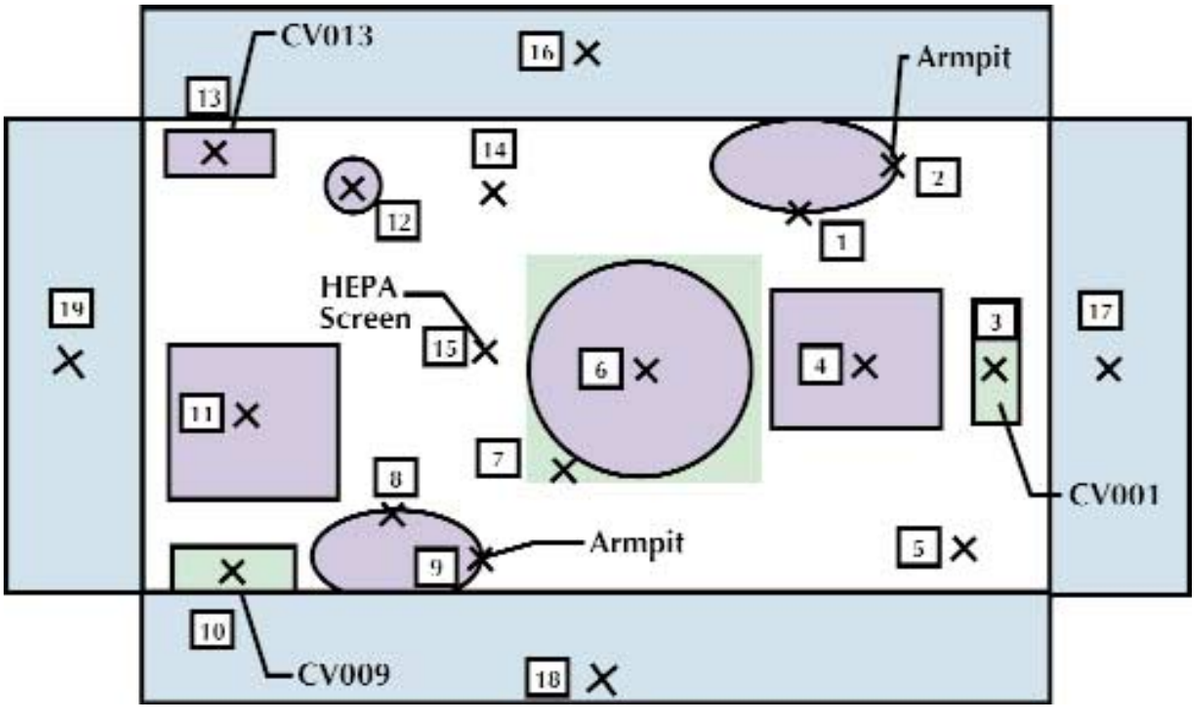
presented here came from the Tyvek-enclosed biological indicators, which were considered to represent a realistic yet rigorous challenge.

We manipulated the variables of humidification time, CD concentration, and CD exposure time (Table 2). Our approach to cycle development was based on the fact that the spore inactivation rate is directly related to CD concentration and length of exposure. Preexposure humidification also affects inactivation kinetics, so the preexposure relative humidity was fixed at 70% for 30 minutes based on previously published work (9).

Test Results and Discussion

CD distribution was excellent in this study, with variability throughout the isolator and its associated ducts measured at less than 0.2 mg/L at an exposure concentration of 5 mg/L. CD distribution was measured using a 385-nm

Figure 1: Location of biological indicators in the isolator, as detailed in Table 1



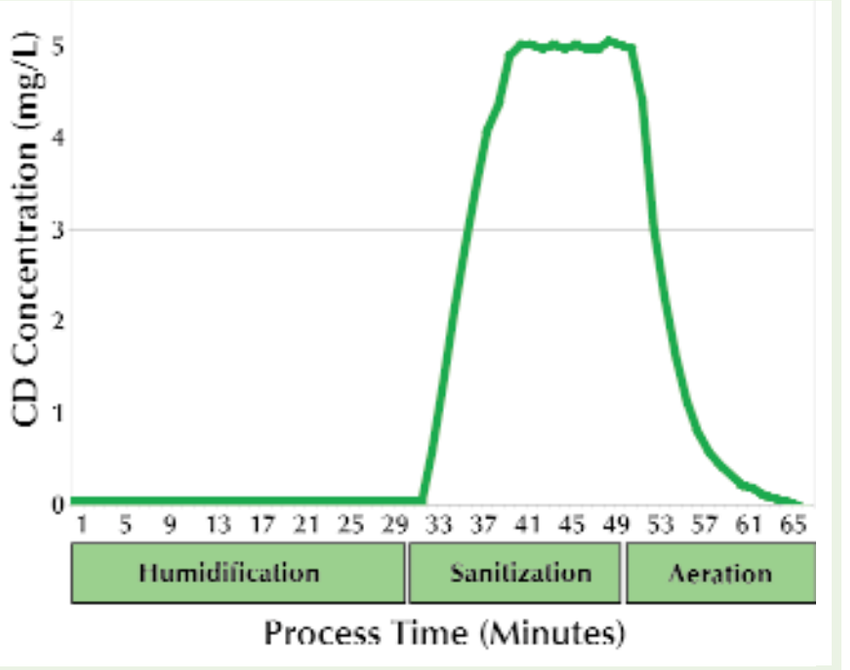
wavelength spectrophotometer connected to a manifold of plastic PVC tubing placed at high and low locations throughout the isolator. The charging time up to an exposure concentration of 5 mg/L took about 9 minutes. Aeration time to safe human exposure levels of 0.1 ppm took less than an hour. Several exposure cycles were shown to be successful using biological indicator spore strips with a population of 10^6 spores each. Disinfection of the half-suit armpit areas was improved when the arms were raised during the disinfection cycle, although inactivation of biological indicators in the armpits was also achieved with lowered arms over an extended cycle time or with increased CD concentration. Biological test results are reported in Table 3.

CD is easy to use. It has a distinct odor, making even minor leaks self-alerting, which is a significant safety feature. However, because it is a green gas, direct measurement of the CD concentration was readily performed using a spectrophotometer. The concentration profile for the decontamination process is illustrated in Figure 2.

Conclusions

CD generated with a prototype chlorine dioxide gas generator was used to successfully disinfect the interior surfaces of an isolator. Several exposure cycles were shown to be successful using biological indicator spore strips. Additionally, CD penetrated into dead-leg and hard-to-reach areas of the isolator such as deep vents, half-suit armpits, and beneath other structures. In the conclusion of this two-part article, we will describe a similar study involving process vessels. We believe that CD has proven itself to be a practical and effective method for disinfecting isolators, as demonstrated by the high-level spore reduction we achieved in both studies.

Figure 2: Example of chlorine dioxide process data (concentration profile)



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