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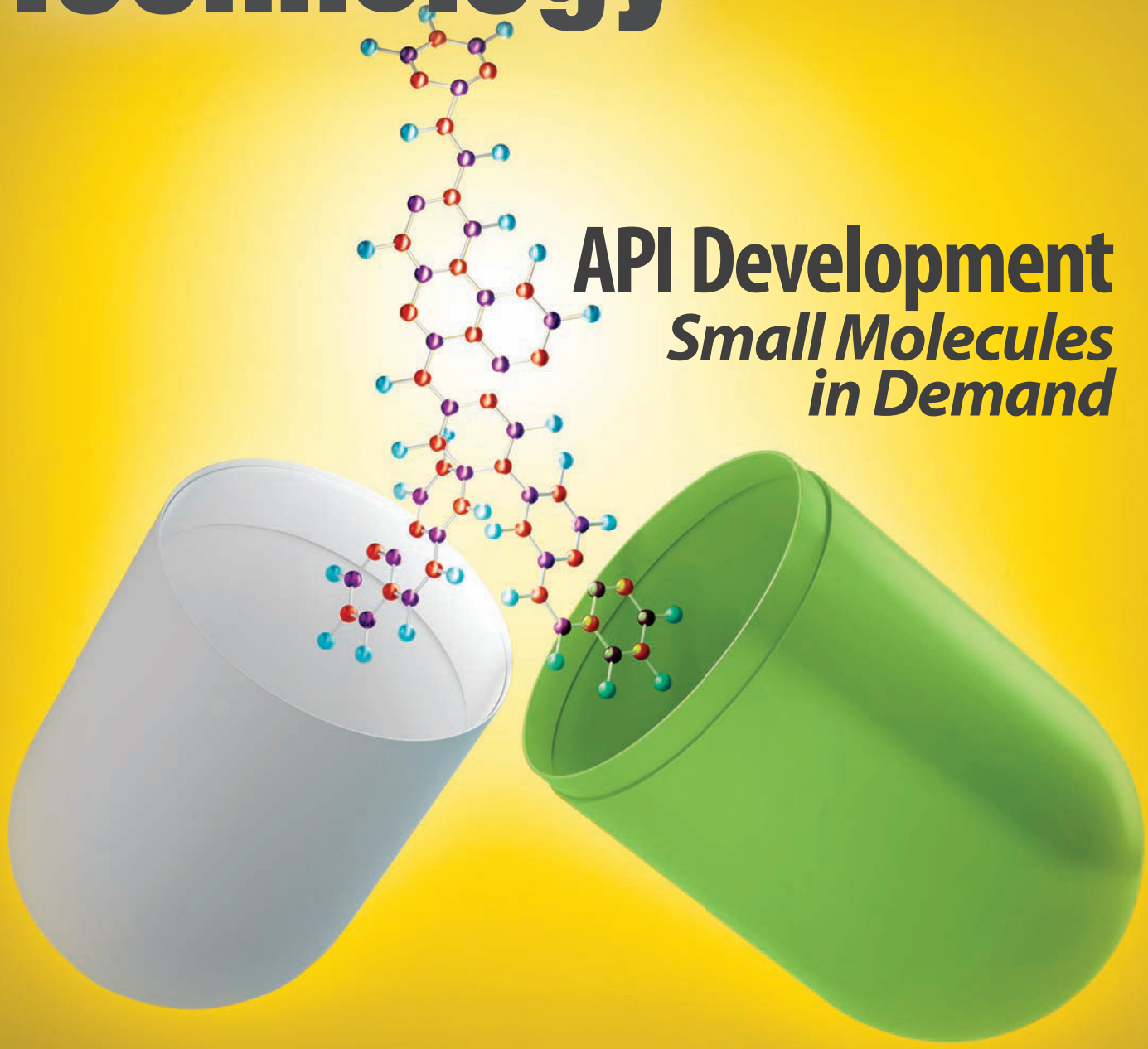

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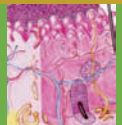

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On the cover

COVER STORY

26 Pharma APIs: It's Still a Small World

Stronger pipelines, the need for complex chemistries, and the rise of small- to mid-size innovators are driving demand for small-molecule APIs.

Art direction by Dan Ward
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FEATURES

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DEPARTMENTS/ PRODUCTS

PEER-REVIEWED RESEARCH

Evaluating Disinfectant Efficacy of a Silver-Based Disinfectant

John M. Lindsay

A disinfectant composed of a low-concentration suspension of silver ions is a quick-acting sporicidal disinfectant that is non-corrosive, is skin-safe (non-toxic), and is not a respiratory irritant. The disinfectant was evaluated at the Aseptic Training Institute (ATI) at the Johnston Community College Work Force Development Center in Clayton, NC. Six microbial preparations were used as challenge organisms for the disinfectant on four different surface materials. In addition, the effectiveness of the disinfectant was evaluated *in situ* by collecting environmental samples after intentionally contaminating and cleaning a facility and equipment. The author reports the results of these evaluations and concludes that the disinfectant is completely sporicidal with only a one-minute contact time.

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Silver's antimicrobial effects have been recognized since ancient times, where it was used in water containers (1). In 1884, the practice of putting silver nitrate drops in newborn's eyes was introduced to prevent infections caused by *Neisseria gonorrhea* transmitted from infected mothers during childbirth (2). In 1928, the "Katadyn Process" for water purification, which is based on low concentrations of silver in water, was introduced (3).

The bactericidal efficacy of silver and its associated ions is through the strong binding with disulfide (S—S) and sulfhydryl (—SH) groups found in the proteins of microbial cell walls. The binding event disrupts normal metabolic processes of the cell, leading to cell death (4). There is also evidence that, after entering a cell, the silver ions displace the hydrogen bonds between adjacent nitrogen molecules of purine and pyrimidine bases, which may stabilize the DNA helix and prevent replication of the DNA and, subsequently, division of the cell (5).

Steriplex, a disinfectant composed of a low concentration (approximately 0.015% by weight) suspension of silver ions was developed after Sept. 11, 2001, in response to the need for a quick-acting sporicidal disinfectant to be used in post offices and other public buildings if potential bioterrorism activity was suspected. It was approved and registered by the US Environmental Protection Agency on April 2, 2010 as an "anthrax disinfectant." It has been used in hospitals for several years and has demonstrated to be effective in reducing the rate of hospital-acquired *Clostridium difficile* infections. Numerous laboratories, including the microbiology department at Brigham Young University, have evaluated the microbial effectiveness of the product (6, 7).

The formulation for Steriplex SD (sBioMed, Utah) has evolved from these earlier formulations into a two-part system. Part A is composed of a suspension of elemental silver (0.015%) in ethanol, inert food-grade ingredients, and water. Part B is the activator and is composed of hydrogen peroxide, peroxyacetic acid, acetic acid, and water. To prepare the disinfectant suspension, Part B is poured into Part A, which activates the suspended silver ions in Part A. **Table I** shows the percentages of the combined ingredients when Part A and Part B are mixed.

The evaluations discussed in this article were performed at the Aseptic Training Institute (ATI) at the Johnston Community College Work Force Development Center in Clayton, NC. Known populations of six different microbial

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suspensions were placed on carriers composed of four different materials commonly found in cleanrooms. The inoculated carriers were then immersed in activated Steriplex SD for one minute. After the one-minute contact time, the carriers were removed and placed in a neutralization broth. The broth and carrier were then rapidly agitated on a vortex mixer to remove any surviving microorganisms. Serial dilutions from the broth were made and different dilutions plated onto growth media to determine the number of survivors and subsequently the log reduction from a positive control. The experimental design was developed from guidance in *United States Pharmacopoeia (USP)* 37 <1072> (8). A 3-log reduction was specified in the protocol for all evaluations, although *USP* 37 <1072> suggests "at least a 2-log" reduction for bacterial spores.

Materials and methods

Microorganisms. Six microbial preparations were used as challenge organisms for the disinfectant on four different surface materials. The following microorganisms were tested: *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Aspergillus niger*, a mixture of *Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)*, and a mixture of *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)*. Suspensions of the organisms were inoculated onto carriers (approximately 1 x 3 cm). This study included the test material combinations described in **Table II**.

Neutralizer broth. After the inoculated carriers were exposed to the disinfectant or saline, they were immediately placed into tubes of Steriplex Neutralization Broth. The following recipe for 100 mL of neutralizer with 1% cysteine was developed by Richard Robison, department chair at the Department of Microbiology, Brigham Young University, Salt Lake City, Utah:

- 12.7 mL Tween 80
- 6.0 g Tamol
- 1.7 g Lecithin
- 1.0 g Peptone
- 1.0 g Cysteine
- 25 mL 2M Tris Buffer, pH 7.0
- 55 mL H_2O.

The neutralizer should be made on the day of use, otherwise some of the ingredients will precipitate out of solution over time, especially if stored at a cool temperature. The recipe was prepared using the following steps:

1. All ingredients, except Tamol, were added to an autoclavable bottle containing a magnetic stir bar, which was placed on a heating stir plate and heated while mixing until ingredients were completely dissolved.
2. The bottle was removed from the hot-plate and, while stirring on another plate, Tamol was added, which causes boiling to occur faster.
3. After boiling temperature was reached, an exterior magnet was used to hold the stir-bar in the bottle and the solution was poured into a graduated cylinder or volumetric flask.
4. Distilled water was added to bring the volume up to 100 mL.
5. Solution was transferred back to the bottle and autoclaved for approximately 30–40 min. (Alternatively, the solution can be filter-sterilized when cooled.)
6. The solution was dispensed into appropriate volumes for testing (usually 9-mL blanks to neutralize 1 mL of disinfectant).

Procedures. Microorganisms were grown on appropriate media and harvested in the log-phase of growth. *Bacillus* species were grown on AK Sporulation Agar and harvested with sterile 70% isopropyl alcohol (IPA). It was confirmed that the suspension was a uniform spore suspension (no

Table I: Percentage of ingredients in activated Steriplex.

Chemical	Weight %
Silver (elemental)	0.015
Ethanol	10.000
Hydrogen peroxide	0.220
Peroxyacetic acid	0.150
Acetic acid	0.150
Inert food-grade ingredients	0.175
Water	89.290

Table II: Disinfectant efficacy test combinations.

Sanitizer
Steriplex SD (four different lots were tested)
Surface types
316L stainless steel
Polycarbonate used for barrier systems (Lexan 9030, Sabic Innovative Plastics)
Vinyl flooring (Mipolam Symbioz, 6043 Wood, Gerflor USA)
Vinyl flexible curtain material
Microorganisms
Bacillus cereus (environmental isolate*)
Bacillus megaterium (environmental isolate*)
Bacillus subtilis (ATCC No. 6633)
Aspergillus niger (ATCC No. 16404)
Gram positive cocktail composed of *Staphylococcus aureus* (ATCC No. 6538) and *Staphylococcus epidermidis* (ATCC No. 12228)
Gram negative cocktail composed of *E. coli* (ATCC No. 25922) and *P. aeruginosa* (ATCC No. 9027)
*Provided by a pharmaceutical company in the Clayton, NC area that supports the Aseptic Training Institute.

Figure 1: Inoculating carriers.

Figure 2: Dried, inoculating carriers in the biosafety cabinet.

vegetative cells) by performing a spore stain on a sample of the suspension. The *Aspergillus* was grown on tryptic soy agar (TSA) and harvested with sterile saline containing 0.1% Tween 80. The surfactant enhances removal of the mold mycelia from the agar surface. A four-day culture of mold was harvested and resulted in substantially dispersed mold filaments and/or *Aspergillus conidia* after vigorous agitation with a vortex mixer. Eighteen-hour cultures of the non-spore-forming bacteria were harvested with sterile saline from TSA plates.

The challenge cultures were enumerated by serial diluting and plate counting using the spread-plate method. Gram negative and gram positive "cocktails" were prepared using mixtures of *E. coli* and *P. aeruginosa* for the former and a mixture of *S. aureus* and *S. epidermidis* for the latter.

The surface carriers were sterilized by autoclaving. The carriers were processed in a Class II biosafety cabinet. With sterilized forceps, 10 carriers were placed into four individual sterile petri dishes and labeled as the following: Steriplex test article—3 carriers; positive control—3 carriers; neutralization control—3 carriers; and negative control—1 carrier. As shown in **Figure 1**, 100 µL of the challenge organism suspension was dispersed onto each of the positive control carriers and each of the test carriers. The suspension was spread evenly on the carriers and air dried in the biosafety cabinet; petri dish covers were left off in the cabinet (see **Figure 2**).

Processing the carriers. Steriplex-test and neutralization-test carriers (three carriers of each for each microorganism and each surface type) were processed using the following procedure:

- Activated Steriplex was poured into the petri dishes covering the surface of each of the three Steriplex-test carriers and each of the three neutralization carriers so that the entire surface of each carrier was covered with the disinfectant (see **Figure 3a**).
- The surface of the carriers remained in contact with the disinfectant for a maximum of one minute, timed with a calibrated stopwatch, which is defined as the contact time.
- Using sterile forceps, each carrier was tapped on its side on the surface of the petri dish to allow excess disinfectant to drain off the carrier (see **Figure 3b**).
- One of the three uninoculated neutralization carriers was placed into a tube containing 4.0 mL sterile saline. The other two carriers were carefully inserted into separate tubes containing 4.0 mL neutralizing broth. Each of the Steriplex-test carriers was placed into a tube of neutralizing broth (see **Figure 3c**). Each carrier was fully immersed in the broth.
- Each tube containing the Steriplex-test carriers and neutralizing broth or saline was then agitated with a vortex mixer vigorously for a minimum of 1 min. at a moderate speed before microbial enumeration was performed.
- Ten-fold dilutions of the vortexed Steriplex-test carrier in neutralizing broth were made (1.0 mL into 9.0 mL sterile saline).

Positive and negative control carriers were processed using the following procedure:

- Sterile saline was poured into the petri dish containing the three inoculated positive control carriers and the petri dish containing the uninoculated single carrier, so that the entire surface of the carriers were immersed in the saline.
- The carriers remained in contact with the saline for a maximum of 1 min.
- Using sterile forceps, each carrier was tapped on its side on the surface of the petri dish to allow excess saline to drain off the carrier.
- Each of the carriers was placed into separate tubes containing 4.0 mL sterile neutralizing broth. Each carrier was fully immersed in the broth.
- Each tube containing the coupon in neutralizing broth was agitated with a vortex mixer vigorously for a maximum of 1 min. at a moderate speed before microbial enumeration was performed.
- Ten-fold dilutions from the positive control neutralization tubes (1.0 mL neutralization broth into 9.0 mL sterile saline) were made until the expected dilution containing 10–100 colony forming units (CFUs) per 0.1 mL inoculum was achieved.

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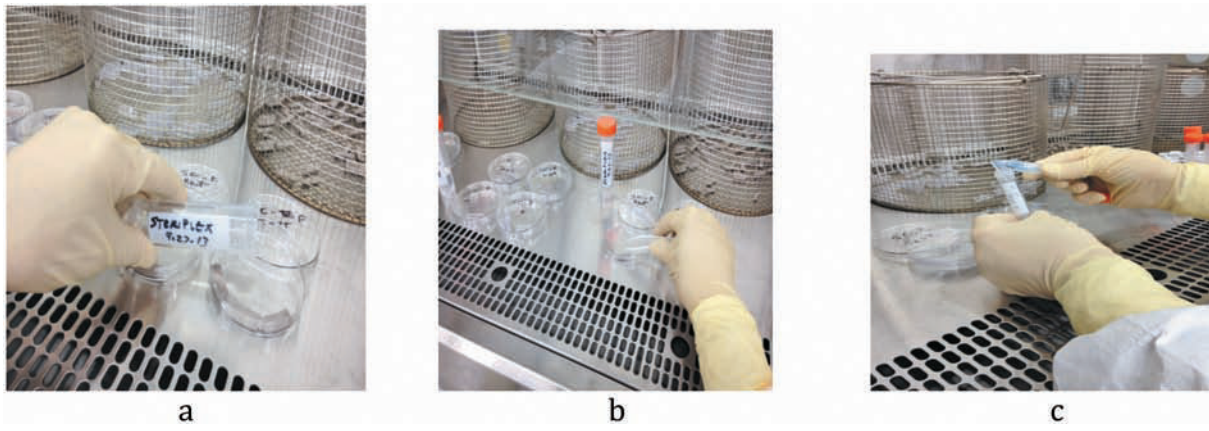
Figure 3: (a) Pouring Steriplex on neutralization test carriers. (b) Retreiving a carrier with forceps after one-minute contact time. (c) Placing carrier in broth.



Microbial enumeration testing. To test the negative control carriers, 0.1 mL of the negative control neutralizing broth was transferred to each of three sterile TSA plates and the inoculum spread over the surface of each plate.

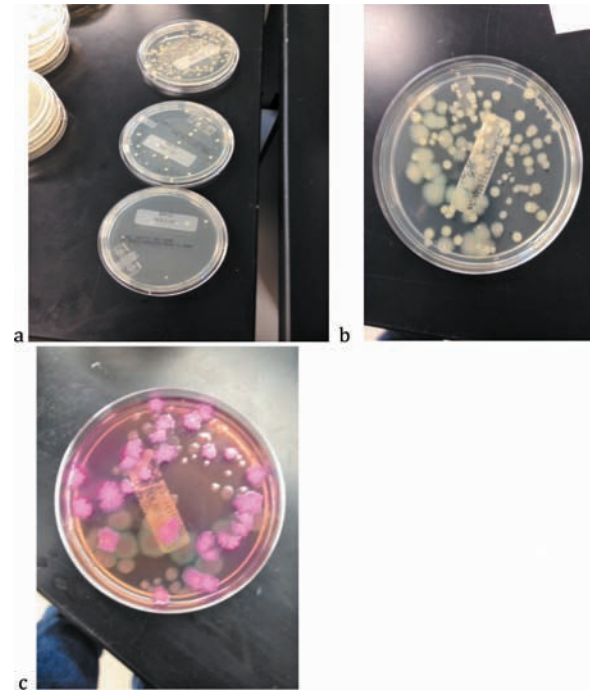
To test the positive control and test carriers, ten-fold dilutions were made from the neutralizing broth for each positive control and Steriplex-test carrier. The 100 and 10^{-1} dilution of the Steriplex-test carriers, and the 10^{-2} through 10^{-4} dilutions of the positive control carriers were plated. A volume of 0.1 mL of each test dilution was transferred onto each of three TSA plates and the inoculum spread over the surface of each plate (see **Figure 4**). The gram-negative cocktail test dilutions were tested on a duplicate set of plates containing MacConkey agar, and the gram-positive cocktail dilutions were tested on a separate set of plates containing 5% sheep blood agar.

Neutralization test carriers were tested using the following method, which is based on *USP* 37 <1227> (9). A volume of 0.1 mL of less than 100 CFU/mL of the test microorganism was transferred into each of the three tubes—2 tubes each containing 4.0 mL of neutralization broth plus carrier and one tube containing 4.0 mL of sterile saline plus carrier. Each tube was then vortexed, and 0.1 mL from each tube was plated onto three TSA plates. The appropriate dilution was calculated from the microbial suspensions prepared as challenge cultures.

As an example, the following procedure would be used for an enumeration culture that contains 10^7 CFU/mL of *B. cereus*:

- Make three ten-fold dilutions to get 10^4 CFU/mL
- Inoculate 0.1 mL of that dilution into each 4.0 mL tube of neutralization broth or saline
- The count in the neutralization broth or saline should be approximately 243 CFU/mL
- Place 0.1 mL onto each of three TSA plates per tube
- The neutralization count should be approximately 24 CFU per plate.

Figure 4: (a) *B. cereus* positive control cocktail (3 dilutions). (b) Gram-negative cocktail, positive control on tryptic soy agar. (c). Gram-negative control on MacConkey agar.



All bacterial plates were incubated at 32 °C + 2 °C for 18–48 h. The *Aspergillus* was incubated at 22 °C + 2 °C for 48–96 h. When colonies were visibly distinct they were countable.

All plates were counted and the minimum population reduction (efficacy) of the test carriers was determined by subtracting the log of the mean surviving population from the Steriplex-test carriers from the log of the mean surviving population of the positive controls.

Table III: Results of one-minute contact time on all tests, NP is not performed, * indicates log difference between positive control and Steriplex test, PC is polycarbonate used for barrier systems. For Lot D, the cocktail was tested and the microorganisms were also tested separately.

	Microorganism challenge					
Surface material	***Bacillus cereus***	***Bacillus megaterium***	***Bacillus subtilis***	***Aspergillus niger***	**Gram (+) cocktail**	**Gram (-) cocktail**
					S. aureus/S. epidermidis	***E. coli/P. aeruginosa***
Lot A (Steriplex Part A Lot 01-132761; Part B Lot 2602-248)						
Stainless steel	*3.14	> 5.40	> 5.34	>4.60	NP	NP
Curtain vinyl	NP	NP	>4.90	NP	NP	NP
Floor vinyl	>5.49	>5.50	>5.15	>5.20	NP	NP
PC	3.39	>5.50	>5.20	>4.52	NP	NP
Lot B (Steriplex Part A Lot 01-132901; Part B Lot 2602-290)						
Stainless steel	*5.50	> 5.54	NP	>4.60	NP	NP
Curtain vinyl	NP	NP	NP	NP	NP	NP
Floor vinyl	>5.50	>5.50	NP	>5.20	NP	NP
PC	4.54	>5.40	>5.50	>4.52	NP	NP
Lot C (Steriplex Part A Lot 01-133121; Part B Lot 2602-290)						
Stainless steel	*>5.50	> 5.40	NP	>4.60	NP	NP
Curtain vinyl	NP	NP	>4.90	NP	NP	NP
Floor vinyl	>4.89	>5.40	NP	>5.20	NP	NP
PC	>5.54	>5.40	>5.20	>4.67	NP	NP
Lot D (Steriplex Part A Lot 01-132761; Part B Lot 2602-276)						
Stainless steel	*>6.88	> 6.56	> 5.34	>4.60	>5.59	>4.83
					5.53/4.00	3.90/4.36
Curtain vinyl	>5.66	>6.56	>4.90	NP	>5.81	>5.38
					5.81/3.95	4.81/4.89
Floor vinyl	>5.90	5.20	>5.15	>5.20	>5.32	>5.40
					5.32/3.04	4.38/4.54
PC	5.95	>6.56	>5.20	>4.52	>5.71	>5.15
					5.71/3.89	4.30/4.69

Acceptance criteria. For a test to be valid, the average recovery from the positive control carrier must be sufficient to demonstrate the required 3-log reduction. The negative control should not contain presence of growth. For a disinfectant to be efficacious against a test organism, the minimum population reduction must be ≥ 3-log reduction for bacteria, molds, and bacterial spores. The *USP* now says a 2-log reduction may be appropriate for bacterial spores and fungi. If the duplicate tests are not within 1 log of each other, the test is considered invalid. The acceptance criteria have been selected based on the sterility assurance level (SAL) of 10^{-3} for a sanitization process. The average count of the neutralization test carriers should be no less than 20% of the count of the saline neutralization control.

Table IV: Results of *in-situ* testing; RODAC is replicate organism detection and counting plates.

Location/sample type	Number sampled	Number positive	% positive	Number mold	% mold
Baseline after detergent cleaning, prior to sanitization					
RODACs	150	106*	71	47	31
Swabs	32	15	47	1	3
After sanitization with Steriplex					
RODACs and swabs	100	0	0	0	0

*mostly spreaders—*Bacillus* species

Results

The results are summarized in **Table III**. There was no growth from negative controls. All neutralization evaluations were as expected. The average count from the neutralization tubes was not less than 20% of the counts from the saline control.

Since Steriplex Part B is composed of hydrogen peroxide and peroxyacetic acid, the active ingredients in some commonly used sporicides, an evaluation was performed to determine if Part B mixed with water would give similar results as with the activated product. That is, the evaluation was intended to demonstrate that the silver component was critical to the efficacy of activated Steriplex. Steriplex Part B, Lot A was combined with four liters of sterile water and tested on stainless-steel coupons. Only a 1.2-log reduction was achieved compared to a 3.8 log-reduction with the same component used in activated Steriplex.

In-situ evaluations

Hands-on aseptic processing training is offered at the Aseptic Training Institute (ATI) in Clayton, NC. During courses in 2012 and 2013, *in-situ* evaluations were performed using Steriplex. The ATI facility is a soft-wall cleanroom, approximately 450 ft^2 in space. Specific *Bacillus* spores were intentionally used to contaminate surfaces, materials, and operator's sterile gloves during media fills performed in the cleanroom at the facility. To evaluate the effectiveness of Steriplex *in situ* after the facility and equipment had been contaminated and subsequently cleaned with a detergent solution, environmental samples from surfaces of equipment and the facility were collected. The facility and equipment were then sanitized once with Steriplex SD and sampled again. The results are shown in **Table IV**. The *in-situ* testing has been performed three times with similar results.

Discussion and conclusion

The data demonstrate anywhere from a 3+ log reduction to over 6+ log reduction of bacterial spores, mold, and both gram-negative and gram-positive bacteria with a one-minute contact time. The *in-situ* data demonstrate that the product was extremely effective and nonoffensive with regard to operator comfort and health in the workplace.

Activated Steriplex has been used directly on hands as a hand sanitizer in the ATI laboratory. During sanitization of the entire cleanroom and filling equipment at the ATI, there is an aroma and slight eye burning during the process (mainly due to the exhausts from the cleanroom being just louvers in the wall where air from inside the cleanroom is pushed out to the adjoining laboratory). That mild irritation vanishes in 15–20 min. However, there are none of the health and safety concerns as there are with the commonly used sporicidal disinfectants containing higher concentrations of chlorine, hydrogen peroxide, and/or peroxyacetic acid.

A one-minute contact time for any chemical that is sporicidal is revolutionary. Many company standard operating procedures specify contact times of 5, 10, 15, or even 30 minutes. To actually maintain a wet surface in a cleanroom for those long periods of time is impossible unless someone is actually applying the disinfectant throughout that timeframe. Verification of the one-minute contact time is therefore extremely important.

One of the most noteworthy features of Steriplex is that it is non-corrosive. That means it could be used daily with no damage to equipment. Ten 316L stainless-steel coupons have been immersed in activated Steriplex at ATI for eight months, and there is no corrosion or rust.

Our data indicate that Steriplex is completely sporicidal with only a one-minute contact time, which should be achievable in any cleanroom. The product can be sprayed, wiped, mopped, or fogged onto surfaces. All data indicates that the product is safe and will not damage equipment or facilities.

References

1. N. Grier, "Silver and its compounds," in *Disinfection, Sterilization, and Preservation*, Block, Ed. (Lea & Febiger, Philadelphia, PA, 3rd Ed., 1983), pp. 375-389.
2. R.M. Slawson et al., *Plasmid* 27 (1) 72-79 (1992).
3. G.A. Krause, *Neue Wege zur Wasserterilisierung* (Bermann, München, 1928).
4. N. Silvestry-Rodriguez et al., *Rev. Environ. Contamination and Toxicology*, 191, 23-45 (2007).
5. U. Klue et al., *J. Biomed. Mat. Res.* 53 (6) 621-631 (2000).
6. M.D. Pratt et al., Abstract A-4 from Intermountain Branch of the American Society for Microbiology Annual Meeting (Logan, Utah, 2008).
7. J. Meyers et al., *J. Antimicrobial Chemotherapy* 69 (6) 1546-1550 (2014).
8. *USP* 37-*NF* 32, Supplement 1 (US Pharmacopeial Convention, Rockville, MD, 2014) pp. 781-785.
9. *USP* General Chapter <1227>, "Validation of Microbial Recovery from Pharmaceutical Articles" (US Pharmacopeial Convention, Rockville, MD, 2014). **PT**