

Review of Necessary Practices for EPA Submission of a Hospital Disinfectant Using Good Laboratory Practice (GLP) Disinfectant Study Summaries of the SteraMist™ Bit™ Disinfection System

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Abstract

All disinfectants and pesticides marketed for use in United States must meet safety requirements as described in OCSPP 810.2200. The Antimicrobial Testing Program (ATP) ensures that the Environmental Protection Agency (EPA) approved hospital disinfectants and tuberculocides in the marketplace continue to meet stringent efficacy standards. This paper addresses the issues of getting a hospital disinfectant approved by the EPA, some of the challenges encountered and results achieved for a number of hospital related pathogens including MRSA, *Pseudomonas* sp., H1N1, etc. using a novel approach. The paper also addresses the differences between hands-free and wipes technologies, sprays, and their approval pathways. The paper presents the results from Good Laboratory Practice Studies (GLP) submitted to EPA for a novel technology using The SteraMist™ Surface and Environmental systems. SteraMist™ Binary Ionization Technology® (BIT™), (TOMI, Beverly Hills, CA), converts a 7.8% hydrogen peroxide solution into a Hydroxyl Radical mist. This EPA registered solution is passed through an atmospheric cold plasma arc where activation occurs. Activation creates a mist/fog containing a high concentration of Reactive Oxygen Species (ROS), mainly the Hydroxyl Radical. Nine human pathogens are discussed including Gram positive and negative bacteria, virus species and mycobacterium. The mist/fog referred to as Activated Ionized Hydrogen Peroxide (AIHP) is delivered via a handheld application system or a standalone computerized environmental system.

Keywords

vaporous hydrogen peroxide, infectious agent, decontamination, disinfection, environmental protection agency (EPA), good laboratory practices (GLP)

Introduction

All disinfectants and pesticides marketed for use in United States must meet safety requirements as described in OCSPP 810.2200,¹ *Applicability. This guideline describes test methods that EPA believes will generally satisfy testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)* (7 U.S.C. 136, *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a). It addresses testing to demonstrate the effectiveness of antimicrobial pesticides bearing claims as disinfectants, fungicides, virucides, and tuberculocides. (EPA 712-C-07-074)

This paper will demonstrate the process a company must follow to register a product and will demonstrate the stringency of performance encountered. required. The paper will review studies performed to clear a manufacturer's products through EPA using the current statutes. The author hopes to make the reader and user of hospital grade disinfectant products more

aware as to the differences between claims made by manufacturers and distributors. This knowledge will potentially impact on their use of these products in a hospital or healthcare setting.

The Environmental Protection Agency (EPA) is charged with monitoring an antimicrobial's efficacy and every company must submit their product for approval before marketing the product or making claims about the product's efficacy as a hospital grade disinfectant. The EPA rules apply to liquids, mist/fogs and wipes.

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Table 1. Methodology used for testing organisms as defined. TOMI® EPA registered products only.^{1,2}

Organism	Study Title	Study Completion Date	EPA Product Performance Guidelines	method
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	AOAC Germicidal Spray Method	05 FEB 2015	OCSPP 810.2200	AOAC 961.02
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Disinfection	05 FEB 2015	OCSPP 810.2200	Modified Method AOAC 961.02
<i>Staphylococcus aureus</i> (ATCC 6538)	AOAC Germicidal Spray Method	05 FEB 2015	OCSPP 810.2200	AOAC 961.02
<i>Staphylococcus aureus</i> (ATCC 6538)	Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Disinfection	06 FEB 2015	OCSPP 810.2200	Modified Method AOAC 961.02
Methicillin Resistant <i>Staphylococcus aureus</i> -MRSA (ATCC 33592)	AOAC Germicidal Spray Method	04 MAR 2015	OCSPP 810.2200	Modified method AOAC 961.02
<i>Clostridium difficile</i> (C. diff) spores (ATCC 43598)	Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting, or Vaporizing Device for Disinfection of <i>C. difficile</i> spores	16 SEP 2015	OCSPP 810.2100	QCT2 Method: ASTM E2197, ASTM E2839 ²
H1N1 Influenza A (ATCC VR-1469)	Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces	02 MAR 2015	OCSPP 810.2200	ASTM E1053
<i>Salmonella enterica</i> (ATCC 10708)	Hard Surface Room Disinfection via a Fogging Device against Salmonella	13 OCT 2016	OCSPP 810.2200	Modified Method AOAC 961.02
<i>Salmonella enterica</i> (ATCC 10708)	AOAC Germicidal Spray Products Test Method Modified for Handheld Spraying Device	12 SEP 2016	OCSPP 810.2200	Modified Method AOAC 961.02
Feline calicivirus, Strain F-9, (ATCC VR-782)	AOAC Germicidal Spray Products Test Method Modified for Use with a Fogging Device and Viruses	13 OCT 2016	OCSPP 810.2200	Modified Method AOAC 961.02

Note. MB methods from EPA are specifically referenced in OCSPP citations and are not separately listed.

The EPA program is described as follows (section 40 CFR, 160)⁴.

Pesticide Registration

An overview of the registration program is available at <https://www.epa.gov/pesticide-registration/antimicrobial-testing-program#overview>³. In summary, the EPA Antimicrobial Testing Program evaluates the product claims for disinfectant products. Only products that meet stringent testing requirements can be registered with the EPA and marketed using terms such as “hospital disinfectant” or “tuberculocidal”. Registration of a disinfectant for a specific use requires submission of data that demonstrates efficacy using standardized procedures. The testing must be conducted using Good Laboratory Practices (GLP) (https://ntp.niehs.nih.gov/icc/vam/suppdocs/feddocks/epa/epa_glp40_160.pdf) and (<https://www.fda.gov/ohrms/dockets/98fr/980335s1.pdf> 21 CFR Part 58)^{4,5} and must follow the appropriate study standard, OCSPP 810.2200 (Office of Chemical Safety and Pollution Prevention), etc.¹ See Table 1, Disinfectant for Use on Hard Surfaces-Efficacy Data recommendations. The nature of the testing varies as to the whether the product is a liquid, wipe, spray, or a fog/mist. The GLP studies and protocols are

designed to meet the above standards and must be approved by the EPA before starting.

If approved, the primary product will be issued an EPA registration number that must appear on any label listing disinfectant claims. A second number may appear on the label—this indicates a registered “supplemental distributor product” based on the primary product. For example, EPA registration Number 12345-12 is the primary registration number; EPA registration number 12345-12-2567 refers to a distributor product.

Updates to Registration Procedures

In April, 2013, the EPA issued a certified letter to all companies with registered antimicrobial products. In 2013, the Sterimist product only had fungal EPA claims Reg. No. 90150.1 at that time. All other studies were performed at a later date as shown in Table 1.

Fogger and Mister Final EPA Signed Letter

“As explained in the letter, we are concerned that fogging/misting products may not be as effective as claimed, and we want to ensure that these fogging/misting products are accurately labeled. By this letter, we are asking companies either to provide existing efficacy data, or to commit to provide new data, that address the public health claims for the fogger/mister products. Alternatively, the

registrant may elect to revise the labeling of the affected products and registrations to delete the public health claims for the fogger/mister products.”

“The Agency now considers the claims made for disinfection and sanitization for products applied by fogging/misting to be public health-related. This would include fogging/misting products that bear label claims that the product can be used as “an adjunct” to sanitization or disinfection.”

“The fear is that the differences in particle size may affect the amount of active ingredient on the surface. “A surface treated by fogging/misting does not receive the same amount of active ingredient per unit area as the standard methods of application and, as a result, the level of efficacy actually achieved may not be the same level claimed on the label.”⁶

In 2016, the EPA OCSPP issued a memorandum indicating that all products claiming to be hospital disinfectants be re-evaluated by the EPA’s ATP.⁷

EPA Sept 19, 2016 report 16-p 0316, states that the memorandum is in response to the Office of Inspector General’s (OIG) Draft Report Entitled “EPA Needs a Risk-Based Strategy to Assure Continued Effectiveness of Hospital-Level Disinfectants.”

The 2016 notification adds a significant regulatory hurdle for sponsors as it will require retesting of every hospital disinfectant by the companies. The time-line for this re-evaluation of products is to be determined by the agency but it is anticipated that re-testing will be completed by 2021.⁷ The EPA originally intended that, once the companies submitted the product registration data, the ATP would do testing on product samples voluntarily submitted by the sponsoring companies. However, this re-testing has been very limited, thus potentially causing adverse risks to end-users. There were over 300 EPA-registered products at the time of the September 16, 2016 report; however, manufacturers had only submitted 12 samples in the last three years. Additionally, some of the organisms of concern, such as *C. difficile*, are not within the criteria of the ATP testing program for listing a product as a hospital disinfectant.⁷

The product data presented demonstrates the efficacy of the required log kill or other stated EPA criteria for the TOMI Environmental Solutions’, Inc. SteraMist™ BIT™ systems for *Staphylococcus aureus*, *Methicillin Resistant Staph aureus* (MRSA), *Pseudomonas aeruginosa*, *Influenza A (H1N1)*, *Clostridium difficile* spores, *Salmonella enterica*, *Geobacillus stearothermophilus*, and *Mycobacterium bovis*. Additionally, data for Norovirus is presented which has recently cleared EPA (July 2017).

The studies presented here are only for classification of the SteraMist™ BIT™ system as a hospital disinfectant. They do not represent the Food and Drug Agency (FDA) requirements for high level disinfection and those studies are not considered in this work. The disinfectant system as used in these studies is not classified as a medical device and is not regulated as such.

Materials and Methods

Companies using the dip test for spray and wipe technologies must follow the guidelines found in the *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Use-dilution methods (955.14, 955.15, & 964.02).⁸ In these studies carriers are inoculated with specific organisms with three different lots of disinfectant, one lot being older than 60 days old; 60 carriers per organism used, with 59/60 carriers being negative for the organism after disinfectant treatment. In summary, carriers are inoculated with challenge organism and fully submersed in the disinfectant for the time listed on the label, at which time carriers are removed, neutralized, incubated, and reviewed for growth. The test is also run where the challenge organism is put on the carrier, and then the disinfectant solution is placed on the carrier via pipette, immersing the carrier/inoculation site. Details are found in cited guideline and were not used by the GLP laboratories in testing the SteraMist™ Bit Solution system(s) as the studies done for the spray/fog systems use different methodologies compared to wipe technologies as detailed in Table 1 methods.

A novel technology using The SteraMist™ Surface and Environmental systems, SteraMist™ Binary Ionization Technology® (BIT™), (TOMI®, Beverly Hills, CA), converts a 7.8% hydrogen peroxide solution into a hydroxyl radical mist. This EPA registered solution is passed through an atmospheric cold plasma arc where activation occurs. Activation creates a mist/fog containing a high concentration of reactive oxygen species (ROS), mainly the hydroxyl radical (OH·). The mist/fog referred to as Activated Ionized Hydrogen Peroxide (AIHP) is delivered via a handheld application system or a standalone environmental system with the same properties monitored by robotic software using three units identical to the handheld units but placed on tripods.

All studies presented in the paper were performed for TOMI by contracted laboratories meeting the GLP requirements as defined in 40 CFR Part 160 and substance characterization as defined in Subpart F (160.105)⁴ that apply to studies for determination of disinfection on hard, non-porous surfaces. A sponsor may not conduct the study. The methods used to do the studies are defined by The Association of Official Analytical Chemicals (AOAC) recommended tests and must be performed as written (OCSPP Test guideline 810.2000 and 810.2100 for general testing considerations must be met prior to initiating test). Table 1^{1,8} and any modifications must be approved by the EPA prior to start of the study. Hospital or healthcare disinfectant/hard non-porous surfaces spray products must use *Staphylococcus aureus* (SA) (ATCC 6538) and *Pseudomonas aeruginosa* (PA) (ATCC 15442) as part of their testing for acceptance by EPA. For viruses, the protocol was modified for viruses to include a chemical neutralizer per ASTM E1053. This standard was used for viruses as claimed on the label. In this study *H1N1 Influenza A* (ATCC VR-1469) and Norovirus surrogate *feline calicivirus* was tested. Additionally, *S. aureus* (MRSA, ATCC 6538), *Clostridium difficile* (*C. diff*) spores (ATCC 43598), *Salmonella enterica* (ATCC 10708),

Geobacillus stearothermophilus (ATCC 12980), and *Mycobacterium bovis* (BCG) were tested.

Methods (see Table 1 for specific protocols as defined by EPA requirements)

Handheld Unit

- The GLP laboratory followed established protocol as defined on pages 3-4 of protocol TEST01122314 GS for SA and PA⁹;
 - Protocol TES01120614 GS for MRSA pages 8-10¹⁰;
 - Protocol TES01120614.FLU A for *H1N1* pages 3-12 modifications included¹¹;
 - Protocol Number P1619 pages 25-38 for *Salmonella enterica* and protocol modifications page¹²; and
1. All lots of BITTM Solution being used had to meet acceptance criteria as defined by the manufacturer and accompanied with a certificate of analysis. The Solution is “ready to use” and does not require dilution.
 2. For the handheld unit, for each bacterial and viral organism, the cultures were grown per protocol and coated onto carriers, usually glass microscope slides. Carriers were inoculated, and dried per protocols. (ref above) Three lots of BITTM Solution were tested for efficacy for each organism as noted above. In testing *Norovirus*, protocols required maintenance of 4-log virus on control carriers during trial and a minimum of $\geq 3 \log_{10}$ reduction in viral titer.¹³
 3. BITTM Solution was applied to the carriers using the SteraMistTM Surface Unit for 5 seconds/ 0.0929 m² (1 ft²) at 24 inches and held at 16.7-27% relative humidity for 7 minutes at room temperature 20.9 - 21.8 degrees C depending on organism being tested by GLP laboratory after a trigger release of two seconds. The carriers were transferred using sterile technique to neutralizing solution. All controls were tested according to protocols^{9,10,13-15} as detailed below and the success of the testing was evaluated by the following parameters:
 - a. The initial carrier numbers control enumerations must demonstrate a geometric mean density $\geq 1 \times 10^5$ CFU/carrier unless the product fails, in which case the geometric mean may be lower.
 - b. The final carrier numbers control enumerations must demonstrate a geometric mean density $\geq 1 \times 10^4$ CFU/carrier unless the product fails, in which case the geometric mean may be lower.
 - c. In the event that the final numbers control is lower than that specified above, the mean density of control substance treated carriers (10 carriers treated with control substance) may be used in place of the final carrier numbers control. The control substance treated carrier enumerations

must demonstrate a geometric mean density $\geq 1 \times 10^4$ CFU/carrier unless the product fails, in which case the geometric mean may be lower.

- d. The subculture/neutralization sterility control test tube is negative for growth.
- e. Both of the viability growth control test tubes are positive for growth.
- f. * The neutralization control subculture/neutralization test tube is positive for growth.
- g. *The neutralization control inoculum demonstrates between 10 and 100 CFU.
- h. Carrier sterility control tube is negative for growth.
- i. Test microorganism purity control demonstrates growth and lack of contaminant species.

*The neutralization control data is not shown as the criteria were met as specified in parts e and f. If the criteria had not been met, the study would have failed.

Environmental Unit

- Protocol TES01062615.RDT pages 8-16 for *C. difficile*¹⁴;
 - Protocol TES01030716.RDT page 3 for *Mycobacterium bovis*¹⁵;
 - Protocol MRID 488313-03 (field study) pages 2-4 for *Geobacillus stearothermophilus*¹⁶;
 - Protocols NG7535 and GLP 1507 for *Norovirus*.¹³
1. In the case of the *C. diff* spores these were tested using the SteraMistTM Environment System and carriers were systematically placed in a location according to room dimension as described on page 26 of protocol.-TES01062615.RDT.¹⁴ and Table 1. The BITTM Solution for *C. diff* spores was tested in a 103.74 m³ (3663.7 Ft³) room at a dosage of 0.5 ml / .0283 m³ (0.5 ml/ Ft³.) with a 15 minute contact time and a 26-29 minute aeration time.
 2. *Mycobacterium bovis* was tested with the SteraMistTM Environment System, but did not follow GLP protocols per sponsor.¹⁵ The data represented here were feasibility studies and GLP studies are now being undertaken and are in progress.
 - a. Briefly, the 20 inoculated glass carriers were placed at diverse locations in a sealed testing room. They were fogged for 23 minutes, with a 17 minute dwell time, followed by 50 minute aeration time. All appropriate sterility, purity, and recovery controls were included.
 3. For the Field Study for *Geobacillus stearothermophilus* (ATCC 12980)¹⁶
 - a. 62 inoculated stainless steel carriers were placed in a sealed, 103.74 m³ (3663.7 ft³) room and fogged for 24 minutes, with a 15 minute dwell

Table 2. Test Results: PS/SA (^aTable 4, page 17 project A17822 2/05/15)⁹.

Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed as Test Organism
Binary Ionization Technology [®] (BIT [™]) SolutionBatch# OJ30A1	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Ready to Use	60	0	0
	<i>Staphylococcus aureus</i> (ATCC 6538)		60	0	0
Binary Ionization Technology [®] (BIT [™]) SolutionBatch# OJ02A1	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Ready to Use	60	0	0
	<i>Staphylococcus aureus</i> (ATCC 6538)		60	0	0
Binary Ionization Technology [®] (BIT [™]) SolutionBatch# 20131231	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Ready to Use	60	0	0
	<i>Staphylococcus aureus</i> (ATCC 6538)		60	0	0

Note. ^aTable numbers in italics refers to original study result in protocol findings.

and 90 minute aeration time. Three lots of BIT[™] solution > 60 days old were used.

- b. Carriers were transferred in a sterile manner and tested for growth per protocol. The SteraMist[™] Environment System was positioned within the enclosure.

Any deviations from protocols were recorded for all the above studies. All controls were tested according to protocols as with handheld unit above.

Results

Criteria of acceptability as a “hospital disinfectant” are defined for each organism as it varies as to requirements per “accepted” EPA protocol used by the GLP laboratory.

1. *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Efficacy Performance: For label claim states that the test substance must kill the microorganism in 59/60 carriers.

Results: All 60 carriers had a complete kill. (Table 2)

2. *MRSA*

Efficacy of Performance: For label claim states that the test substance must kill the microorganism on 10 out of the 10 inoculated carriers. Additionally, organism must meet verification of antibiotic resistance as defined by Clinical Laboratory Standard Institute (CLSI).¹⁷ For *Staphylococcus aureus* the zone must be between 18-24 mm and the range for MRSA must be <10 mm.

Results: The achieved zones were 19 and 6 mm respectively for 10 out of 10 carriers.¹⁰

3. *Clostridium difficile* spores

Efficacy of Performance: In order to be considered effective for disinfection of *C. difficile* spores:

- a. The test substance must demonstrate a minimum of 6 Log₁₀ reduction in numbers of test organism as compared to the carrier population control (post testing).

- b. Controls must perform according to protocol criteria. (Table 3).
- c. All lots of solution must have color change indicator present.

Results: All criteria were met (Table 3).

4. *Influenza A -H1N1*

Efficacy of Performance: To pass the test:

- a. A valid test requires that at least a 4 Log₁₀ of infectivity be recovered from the dried virus control film;
- b. That when cytotoxicity is evident, at least a 3 Log₁₀ reduction in titer is demonstrated beyond the cytotoxic level;
- c. That the cell control be negative for infectivity. Note: an efficacious product must demonstrate complete inactivation of the virus at all dilutions.

Results: All virus controls were met with TCID₅₀ results starting 10^{6.30} and reduced to ≤ 10^{0.80} for 2 lots of BIT[™] solution (Table 4).

5. *Salmonella enterica*

Efficacy of Performance: 59 out of 60 carriers tested must be negative for growth per lot of the proposed contact time.

Results: Study Conclusion in Brief using SteraMist[™] Surface Unit. Binary Ionization Technology[®] (BIT[™]) Solution “met U.S. EPA OCSPP 810.2200 for disinfection success criteria when tested against *Salmonella enterica* (ATCC 10708) at a 5 second application per 0.0929 m² (1 Ft.2) ± 7 minutes contact time.”¹³ The three lots of BIT[™] solutions showed carrier results of 1QE09A1, 1/60; PL04A1, *2/60; PL15A2, 0/60. * Lot PL04A1 carrier showed a contaminant but not the organism being tested due to placement of carriers near floor. All subculture/neutralizer test tubes showed zero growth respectively. The average inoculum for each lot was 138.5, 113.5, and 144.

All controls had appropriate results. Conclusion: 60/60 carriers were disinfected within 7 minutes +/- 5 seconds using three lots of BIT[™] Solution (Table 5a, b, c).

Table 3. Test Results: *C. diff* Spores (^aTable 7, page 23 Project A18948 9/16/2015).¹¹

Colony Forming Unit (CFU) on Filter: CFU/Carrier (Log10)		
Binary Ionization Technology® (BIT™) Solution Lot: OJ30A1	Binary Ionization Technology® (BIT™) Solution Lot: OJ02A1	Binary Ionization Technology® (BIT™) Solution Lot: OLI1A1
#1: 0: <1 (<0.00)	#1: 1: 1 (0.00)	#1: 1: 1 (0.00)
#2: 0: <1 (<0.00)	#2: 20: 2x101 (1.30)	#2: 0: <1 (<0.00)
#3: 0: <1 (<0.00)	#3: 0: <1 (<0.00)	#3: 0: <1 (<0.00)
*#4: 0: <1 (<0.00)	*#4: 0: <1 (<0.00)	*#4: 0: <1 (<0.00)
*#5: 0: <1 (<0.00)	*#5: 0: <1 (<0.00)	*#5: 1: 1 (0.00)
#6: 0: <1 (<0.00)	#6: 0: <1 (<0.00)	#6: 0: <1 (<0.00)
*#7: 0: <1 (<0.00)	*#7: 0: <1 (<0.00)	*#7: 0: <1 (<0.00)
#8: 0: <1 (<0.00)	#8: 0: <1 (<0.00)	#8: 0: <1 (<0.00)
#9: 1: 1 (0.00)	#9: 0: <1 (<0.00)	#9: 0: <1 (<0.00)
#10: 0: <1 (<0.00)	#10: 0: <1 (<0.00)	#10: 1: 1 (0.00)
#11: 0: <1 (<0.00)	#11: 0: <1 (<0.00)	#11: 0: <1 (<0.00)
#12: 0: <1 (<0.00)	#12: 0: <1 (<0.00)	#12: 0: <1 (<0.00)
#13: 0: <1 (<0.00)	#13: 0: <1 (<0.00)	#13: 2: 2 (0.30)
#14: 0: <1 (<0.00)	#14: 0: <1 (<0.00)	#14: 0: <1 (<0.00)
#15: 0: <1 (<0.00)	#15: 2: 2 (0.30)	#15: 0: <1 (<0.00)
#16: 0: <1 (<0.00)	#16: 0: <1 (<0.00)	#16: 2: 2 (0.30)
#17: 0: <1 (<0.00)	#17: 0: <1 (<0.00)	#17: 0: <1 (<0.00)
#18: 0: <1 (<0.00)	#18: 0: <1 (<0.00)	#18: 0: <1 (<0.00)
#19: 0: <1 (<0.00)	#19: 0: <1 (<0.00)	#19: 0: <1 (<0.00)
#20: 0: <1 (<0.00)	#20: 1: 1 (0.00)	#20: 0: <1 (<0.00)
Average Log10: <0.00	Average Log10: <0.08	Average Log10: <0.03
Log10 Reduction: >6.33	Log10 Reduction: >6.42	Log10 Reduction: >6.47

*Chemical indicator results were matched with 3 carriers for each lot of BIT™ solution: Carriers 4, 5, and 7 each had color change present validating the results. Note. ^aTable numbers in italics refers to original study result in protocol findings.

Table 4. H1N1 Test results: Effects of Binary Ionization Technology® (BIT™) Solution (Batch# OJ30A1 & Batch# OJ02A1) Following a 5 second application per 0.0929 m² (1 Ft²) time and 7 minute contact time to Influenza A (H1N1) Virus dried on an Inanimate Surface.¹²

Dilution	Dried Virus Control	Influenza A (H1N1) Virus + Batch# OJ30A1	Influenza A (H1N1) Virus + Batch# OJ02A1
Cell Control	0000	0000	0000
10--1.3	++++	0000	0000
10--2.3	++++	0000	0000
10--3.3	++++	0000	0000
10--4.3	++++	0000	0000
10--5.3	++++	0000	0000
10--6.3	0++0	0000	0000
10--7.3	0000	0000	0000
TCID50/100uL	106.30	≤100.80	≤100.80

(+) = Positive for the Presence of Test Virus

(0) = No test Virus Recovered and/or No Cytoxicity Present

6. *Geobacillus stearothermophilus*

Efficacy of Performance: No growth 0/62 carriers, after 7 days of incubation.

Results: 3 lots of BIT™ solution 110911, 010412, 022912 exhibited no growth 0/62 for each starting with 2.4 and 3.1 x 10⁶ CFU/carrier.¹⁶ Study Conclusion: The submitted field efficacy data (MRID 488313-03) supports the use of the product, SteraMist™, as a sporicidal against *Geobacillus stearothermophilus* spores (ATCC 12980) on hard non-porous surfaces, when applied by fogging using BIT™ application in a room not larger than 103.7 m³ (3663.7 Ft³) and not higher than 3.35 meters (10.99 Ft.). This study is non-GLP and was a test application for feasibility using the environmental unit (Table 6).

7. *Mycobacterium bovis*

Efficacy of Performance: 20/20 carriers must be negative for growth at the end of 90 days incubation in three types of mycobacterium growth media.

Results: BIT™ solution Lot QA25A1 used with BIT fogger in a room 103.74 m³ (3663.7 Ft³) at an expected concentration of 150 ppm demonstrated no growth of *M. bovis* – BCG in any of 20 subcultures following a 23 minute fog time, 17 minute dwell time and 50 minute aeration time when tested at room temperature (22.5-22.7 °C). Zero growth occurred in each of three growth mediums at day 30,60 and 90. All color indicators changed color during testing. All controls were appropriate. (Table 7)

Table 5. Test Results: a,b,c Carrier Enumeration for *Salmonella enterica* tested against 3-lots of BIT™ Solution.¹⁵

Test Microorganism	Test Substance	Carriers	CFU/Carrier	Log10 Density	Mean Log10 Density
<i>S. enterica</i> ATCC 10708	Binary Ionization Technology (BIT™) Solution (Lot: QE09A1)	Pre Treatment	2.41E+05	5.38	5.11
		Post Treatment	6.76E+04	4.83	
a.					
<i>S. enterica</i> ATCC 10708	Binary Ionization Technology (BIT™) Solution (Lot: PL04A1)	Pre Treatment	3.93E+05	5.59	5.57
		Post Treatment	3.48E+05	5.54	
b.					
<i>S. enterica</i> ATCC 10708	Binary Ionization Technology (BIT™) Solution (Lot: PI15A2)	Pre Treatment	2.89E+05	5.46	5.42
		Post Treatment	2.41E+05	5.38	
c.					

a. The following were the carrier enumeration results for Binary Ionization Technology (BIT™) Solution (Lot: QE09A1). Testing was conducted on 22 August 2016.

b. The following were the carrier enumeration results for Binary Ionization Technology (BIT™) Solution (Lot: PL04A1). Testing was conducted on 23 August 2016.

c. The following were the carrier enumeration results for Binary Ionization Technology (BIT™) Solution (Lot: PI15A2). Testing was conducted on 23 August 2016

Table 6. *Mycobacterium bovis* Carrier population control results.¹³

Test Microorganism	Carrier Set	CFU/Carrier	Log10
<i>Mycobacterium bovis</i> (MCB)	Pre Treatment	8.3 × 10 ⁴	4.92
	Post Treatment	3.22 × 10 ⁴	4.51

CFU = Colony Forming Unit.

8. Norovirus

Efficacy of Performance: To be scientifically defensible these criteria have to be met:

- A minimum of 4 log₁₀ reduction infectious viruses are recovered from the virus control carrier.
- Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
- Neutralization effectiveness is demonstrated by recovery of comparable levels of infectious viruses from control.
- Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing criteria as noted before includes complete inactivation of test virus at all dilutions with a minimum of ≥ 3 Log₁₀ reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Results: Percent reduction was achieved, see Table 8.

The following is a summary of the results gathered from the test substance lots evaluated, as well as a statement of their ability to meet the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200 and the success criteria detailed in the approved Protocol GLP 1507, MicroChem.

- Test substance Binary Ionization Technology (BIT) Solution (Lot: QE09A1) met the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200 and the success criteria detailed in the approved protocol against Feline calicivirus, Strain F-9, ATCC VR-782 at the reported exposure times. No infectious viral particles were recovered from any of the locations tested with this lot of test substance.
- Test substance Binary Ionization Technology (BIT) Solution (Lot: PL04A1) met the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200 and the success criteria detailed in the approved protocol against *Feline calicivirus*, Strain F-9, ATCC VR-782 at the reported exposure times. No infectious viral particles were recovered from any of the locations tested with this lot of test substance.

Discussion

GLP studies are highly structured and must meet the standards as defined and listed in this paper. There is little to no room for modification and the substance being tested must meet the criteria as stated for efficacy and approved by the EPA. These claims must be reflected in the product labeling. The stringent requirements of the GLP studies may not reflect the “actual use” of the product in a hospital or a healthcare setting as healthcare settings are not GLP controlled environments. The end-users of the technology do not generally have the expertise or the resources to do the necessary testing to support the claims of the manufacturers as demonstrated by the GLP studies. But, the studies do give the user confidence that the claims made by the manufacturer are in fact supported by independent GLP laboratories and reviewed for efficacy of log kill by the

Table 7. *G. stearothermophilus* Carrier Results.¹⁴

MRID#	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population Count (CFU/carrier)
		Lot No. 110911	Lot No. 010412	Lot No. 022912	
488313-03	<i>G. stearothermophilus</i>	0/62	0/62	0/62	2.4 & 3.1 x10 ⁶

Table 8. Test Results *Norovirus* Surrogate, NG7535.¹⁶

Microorganism	Test Device	Treatment	Log10/carrier	Mean Log10	Percent Reduction vs Mean Control	Log10 Reduction vs Mean control
Feline Calicivirus F--9 ATCC VR-782. <i>Norovirus</i> Surrogate ^a	SteraMist™ Surface Unit & BIT™ Solution	Initial # controls	6.55	6.43	n/a	n/a
		Final # control	6.30			
		3 passes, 7 minutes	5.05		95.78%	1.38
		3 passes, 7 minutes	4.80		97.63%	1.63
		5 passes, 7 minutes	4.80		97.63%	1.63

Since human *Norovirus* has no defined cell culture system, a surrogate was used. CDC/US EPA approved surrogate is *feline calicivirus*, and is in the same viral group as human *Norovirus*.¹⁶

EPA. Additionally, the previously registered disinfectants may now be subject to ongoing re-testing as required by recent EPA actions⁸ to further assure product continued efficacy.

The above studies demonstrate the efficacy of both the SteraMist™ handheld Surface Unit and the SteraMist™ Environment System with BIT™ Solution for a large number of clinically significant organisms. These studies are very difficult to perform as compared to the dip test technology where carriers are simply immersed in the disinfectant solution for a period of time. These studies are the first reported studies to the authors knowledge that demonstrate the combination of a disinfection solution and the effect of the dispersal method with the resulting effect on the killing efficacy for multiple organisms as now required by EPA.⁶

The GLP and other feasibility studies presented in this report have demonstrated the effectiveness of kill for Gram positive and Gram negative bacteria; spore forming organisms such as, *C. difficile* and *Geobacillus stearothermophilus*; Acid Fast Bacilli, *Mycobacterium sp.*; and viruses *Influenza A-H1N1*, and *Norovirus* with the combination of TOMI™ BIT™ Solution and cold plasma arc dispersal system whether handheld or as an environmental robot controlled system. The studies presented have also demonstrated the rigorous process of EPA registration for a product to be classified as a hospital disinfectant.

Declaration of Conflicting Interests

The author reports she is on the scientific advisory board of TOMI, Beverly Hills, CA.

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*All referenced protocol numbers (9-16) are the actual files of the laboratory methods and results submitted to the EPA and data is extracted from these protocols with their permission as obtained by TOMI by Elizabeth Battaglia, regulatory. These are proprietary to the GLP laboratories represented in the documents and are not published.