SteraMist™ BIT™ Good Laboratory Practice (GLP)

Disinfectant Study Summaries

Written by: Dr. Helene Paxton, MS, MT(ASCP), PhD, CIC, Infection Preventionist, Bio Guidance, LLC.

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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)*

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. The EPA rules apply to liquids and mist/fogs and wipes. The EPA has not had (or has had few) regulatory oversights to date with Ultraviolet systems (UVC) on the market creating an uneven regulatory field. The claims for UVC are generally disseminated by the owner of the technology or through written manuscripts and reports. The EPA program is described as follows: (1)

Pesticide Registration

Program Overview

The Antimicrobial Testing Program ensures (ATP) that EPA-approved hospital disinfectants and tuberculocides in the marketplace continue to meet stringent efficacy standards.

Under the ATP, we have tested samples of EPA-registered products from manufacturers, distributors and sellers of hospital sterilants, disinfectants and tuberculocides. The ATP's efficacy test methods provide a rigorous challenge to the product. We adopted high standards to ensure that products will be effective even when extremely high pathogen levels are present.

Hospital sterilants, disinfectants and tuberculocides that do not meet the ATP standards are brought into compliance through regulatory or enforcement measures. (2)

What Products Are Tested?

The ATP tests:

- Registered hospital disinfectants.
- Products for use in dialysis facilities.
- Tuberculocides.

At the time of registration, the registrant of each primary product must provide efficacy data. A primary product may support tens or hundreds of "supplemental distributor" products. You can identify a product by the EPA registration number listed on the product label:

- The primary product carries a two-part EPA registration number – the first number is assigned to the company; the second number is unique to the product.

- A distributor product has a third number following the primary EPA registration number.
- For example, EPA registration Number 12345-12 is the primary registration number; EPA registration number 12345-12-2567 refers to a distributor product.

Under the ATP, primary or supplemental distributor products may be tested. The testing results are reflected under the primary registration number. (2)

A company must fulfill the testing under Good Laboratory Practices (GLP) and must follow the appropriate study standard, OCSPP 810.2200 Disinfectant for Use on Hard Surfaces-Efficacy Data recommendations, (Office of Chemical Safety and Pollution Prevention). The nature of the testing varies as to the whether the product is a liquid, wipe, spray, or fog/mist. The GLP studies and protocols are designed to meet the above standards.

The goal of these studies is to demonstrate the efficacy of the required log kill or other stated efficacy EPA criteria by the TOMI Environmental Solutions, Inc.'s SteraMist™ BIT™ systems for *Staphylococcus aureus, Methicillin Resistant Staph aureus (MRSA), Pseudomonas aeruginosa, Influenza A (H_1N_1), and Clostridium difficile* spores

Materials and Methods

All studies were performed by contracted laboratories meeting the GLP requirements as defined in 40 CFR Part 160 and substance characterization as defined in Subpart F (160.105) (3) apply to studies to support disinfection on hard, non-porous surfaces. Note: 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). Any modifications must be approved by the EPA prior to start of 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 Product test modified for viruses or ASTM E1053 for virus claimed on label. In this study *H_1N_1 Influenza A* (ATCC VR-1469). Additionally, *S. aureus* (MRSA, ATCC 6538) and *Clostridium difficile* (*C. diff*) *spores* (ATCC 43598) were tested.

Methods

The GLP laboratory followed established protocol as defined on page 3-4 of protocol TEST 01122314 GS. (4) for SA and PA; Protocol TES01062615.RDT (5) pages 8-16 for *C. diff*; and Protocol TES01120614 GS pages 8-10 for MRSA (6); TES01120614.FLU A for H_1N_1 pages 3-12 modifications included (7).

All lots of BIT™ 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.

a. For each 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 BIT Solution were tested for efficacy for each organism. For SA, MRSA, PA and H_1N_1.

BIT Solution was applied to the carriers using the SteraMist™ Surface Unit for 5 seconds/ ft^2 at a distance of 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.

b. In the case of the *C. diff* spores these were tested using the SteraMist™ Environment System and carriers were systematically placed in a location according to room dimension as described on page 26 of protocol TES01062615.RDT.(5)

BIT Solution for C. diff spores was tested in a 3663.7 ft^3 room at a dosage of 0.5 ml/cu ft. with a 15 minute contact time and a 26-29-minute aeration time.

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

Results

Criteria of acceptability as Hospital Disinfectant:

1. ***Pseudomonas aeruginosa* and *Staphylococcus aureus*:**

The efficacy performance requirements for label claim states that the test substance must kill the microorganism in 59/60 carriers. All 60 carriers had a complete kill.

Table 1: Test Results: PS *Table 4* Page 17 project A17822 2/05/15 (4)

Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed as Test Organism
Binary Ionization Technology® (BIT™) Solution Batch# OJ30A1	*Pseudomonas aeruginosa (ATCC 15442)*	Ready to Use	60	0	0
	Staphylococcus aureus (ATCC 6538)		60	0	0
Binary Ionization Technology® (BIT™) Solution Batch# OJ02A1	*Pseudomonas aeruginosa (ATCC 15442)*	Ready to Use	60	0	0
	Staphylococcus aureus (ATCC 6538)		60	0	0

Binary Ionization Technology® (BIT™) Solution Batch# 20131231	*Pseudomonas aeruginosa (ATCC 15442)*	Ready to Use	60	0	0
	Staphylococcus aureus (ATCC 6538)		60	0	0

2. MRSA:

The efficacy performance requirements 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 CLSI.

Table 2: Test Results: MRSA *Table 4* Page 16 Project A17825 3/04/2015 (6)

Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed as Test Organism
Binary Ionization Technology® (BIT™) Solution Batch# OJ02A1	Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592)	Ready to Use	10	0	0
Binary Ionization Technology® (BIT™) Solution Batch# OJ30A1			10	0	0

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Table 3: Test Results: *Table 5* MRSA Project A17825 3/04/2015
Verification of Antibiotic Resistance:

Quality Control Organism	**Zone of Inhibition (mm)**	**CLSI* Acceptable Range (mm)**
Staphylococcus aureus (ATCC 25923)	19	18-24
Test Organism	**Zone of Inhibition (mm)**	**CLSI* Resistant Range (mm)**
Methicillin Resistant *Staphylococcus aureus*-MRSA (ATCC 33592)	6	≤10

***CLSI = Clinical and Laboratory Standards Institute**
Interpretation of result and acceptable range are from the Clinical and Laboratory Standards Institute, Performance Standards and Antimicrobial Susceptibility Testing; Twenty-Second Information Supplement January 2012, Volume 21 Niumber1, Approved Standard M02-A11 and M07-A9, Wayne Pennsylvania.

3. *Clostridium difficile* spores:

In order to be considered effective for disinfection of *C. difficile* spores, the test substance must demonstrate a minimum of 6 Log 10 reduction in numbers of test organism as compared to the carrier population control (post testing). Controls must perform according to protocol criteria.

Table 4: Test Results: CD *Table 7* page 23 Project A18948 9/16/2015 (5)

CFU on Filter: CFU/Carrier (Log $_{10}$)		
Binary Ionization Technology® (BIT™) Solution Lot: OJ30A1	**Binary Ionization Technology® (BIT™) Solution Lot: OJ02A1**	**Binary Ionization Technology® (BIT™) Solution Lot: OL11A1**
#1: 0: <1 (<0.00)	#1: 1: 1 (0.00)	#1: 1: 1 (0.00)
#2: 0: <1 (<0.00)	#2: 20: $2x10^1$ (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 Log_{10}: <0.00	**Average Log_{10}: <0.08**	**Average Log_{10}: <0.03**
Log_{10} Reduction: >6.33	**Log_{10} Reduction: >6.42**	**Log_{10} Reduction: >6.47**

CFU = Colony Forming Unit

Table 5: Test results: *Table* 8: CD Chemical Indicator Results: page 23 Project A18948 9/16/2015

Test Substance or Cycle Identifier	Identity of Carrier(s) the Indicator was Paired with	Result
Binary Ionization Technology® (BIT™) Solution Lot: OJ30A1	4	Color Change Present
	5	Color Change Present
	7	Color Change Present
Binary Ionization Technology® (BIT™) Solution Lot: OJ02A1	4	Color Change Present
	5	Color Change Present
	7	Color Change Present
Binary Ionization Technology® (BIT™) Solution Lot: OL11A1	4	Color Change Present
	5	Color Change Present
	7	Color Change Present

4. ***Influenza A - H_1N_1*:**

Results

i. A valid test requires that at least a 4 Log 10 of infectivity be recovered from the dried virus control film;
ii. That when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level;
iii. That the cell control be negative for infectivity. Note: an efficacious product must demonstrate complete inactivation of the virus at all dilutions.

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Table 6: Input Virus Control Results: *Table 1*: H_1N_1, Page 15 Project TES01120614.FLUA 3/02/15 (7)

Dilution	Input Virus Control
Cell Control	00
10^{-1}	++
10^{-2}	++
10^{-3}	++
10^{-4}	++
10^{-5}	++
10^{-6}	++
10^{-7}	+0
10^{-8}	00
10^{-9}	00
$TCID_{50}$/100uL	$10^{7.00}$

(+) = Positive for the Presence of Test Virus
(0) = No test Virus Recovered and/or No Cytoxcisity Present

Table 7: Test results: Effects of Binary Ionization Technology® (BIT™) Solution (Batch# OJ30A1 & Batch# OJ02A1) Following a 5 second application per ft^2 time and 7 minute contact time to Influenza A (H_1N_1) Virus Dried on an Inanimate Surface.

Dilution	Dried Virus Control	Influenza A (H_1N_1) Virus + Batch# OJ30A1	Influenza A (H_1N_1) 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
$TCID_{50}$/100uL	$10^{6.30}$	$\leq 10^{0.80}$	$\leq 10^{0.80}$

(+) = Positive for the Presence of Test Virus
(0) = No test Virus Recovered and/or No Cytoxcisity Present

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Discussion

GLP studies are highly structured and must meet the standards as defined and listed in this paper. There is no room for modification and the substance being tested must meet the criteria as stated and these must be reflected in the product labeling. The GLP studies do not reflect the actual use of the product in a hospital or healthcare setting. The studies do in fact give the user confidence that the claims made by the manufacturer are in fact supported by independent laboratories.

The above studies demonstrate the efficacy of both the hand held SteraMist™ 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 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 disinfection solution and the effect of the dispersal method and the resulting effect on the killing efficacy for multiple organisms.

The above studies also demonstrate the difficulty in creating an even playing field in the evaluation of products by the end user due to the different modes of application of a product whether wipe, spray, mist or fog. This is even more complicated by the recent addition of Ultraviolet disinfection systems which are not evaluated by the EPA at this time. Claims for performance thus are hard to compare between disinfectant technologies.

The GLP and other studies have demonstrated the effectiveness of kill for Gram positives, Gram negatives, spore formers, *C. diff*, and Influenza A, H_1N_1, virus with the combination of BIT Solution and dispersal system whether hand-held or as an environmental robot controlled system.

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References

1. EPA 712-C-07-074 OCSPP 810.2200, EPA, Office of Chemical and Safety and Pollution Prevention (7510P), 2012.
2. *(https://www.epa.gov/pesticide-registration/antimicrobial-testing-program)*
3. 40 CFR Part 160.
4. Accuratus Lab Services. Project number A17822, protocol number TES01122314.GS, 2/2015, Pseudo/SA; (Accuratus Lab Services, Egan MN 55121)
5. Accuratus Lab Services. Project number A18948, protocol number TES01062615.RDT, 9/2015/C.diff
6. Accuratus Lab Services. Project number A17825, protocol number TES01120614.GS, 3/2015/MRSA
7. Accuratus Lab Services. Project number A17824, protocol number TEST01120614.FLUA, 3/2015,H_1N_1

Note: Table numbers in italics refers to original study result in protocol findings.

Binary Ionization Technology® (BIT™) is EPA registered for use as a Hospital-Healthcare Disinfectant and Effective Broad-Spectrum Surface Disinfectant (EPA Reg. No. 90150-2).

SteraMist™ Surface Unit:
Bactericide*
*Staphylococcus aureus (Staphylococcus) (Staph) (ATCC #6538), Pseudomonas aeruginosa (Pseudomonas) (ATCC #15442), Methicillin Resistant Staphylococcus aureus (MRSA) (ATCC #33592)
Virucide**
**Influenza A (H1N1) virus (ATCC VR-1469)
For use in mold and mildew control and remediation

SteraMist™ Environment System:
Bactericide+
+Staphylococcus aureus (Staphylococcus) (Staph) (ATCC #6538), Pseudomonas aeruginosa (Pseudomonas) (ATCC #15442), and Clostridium difficile spores (C. diff) (ATCC #43598)