EOI
eoi-oxygen.com

TECHNOLOGY NOTES
SB100-Industrial Bacterial Efficacy Testing

Ozone Bacterial Efficacy and SB100 Testing

EOI Sanitizing Spray Bottle SB100-Industrial

The broad-spectrum antimicrobial properties of ozone are well documented. Ozone has been used to treat municipal drinking water for more than 100 years. Ozone as a surface sanitizer has been validated in food processing plants, including meat and fish processing facilities. It is used to keep fruit and vegetables fresher, and is used in wine-making and the majority of water and prepared-drink bottling. Many industries sanitize surfaces with ozonated water. It can be sprayed directly on floors, drains, walls, tanks (inside and outside), fruit bins, and any other wet-able, non-rubber, non-fiberglass surfaces.

EOI has harnessed the power of ozone and made ultra-compact ozone applications possible. The EOI SB100-Industrial Sanitizing Spray Bottle employs a proprietary electrolytic ozone cell made with boron-doped synthetic-diamond technology. The handheld battery-powered spray unit produces low-concentration dissolved ozone (O_3) from water on demand. This powerful yet 'green' sanitizer is ideal for a wide variety of surface sanitization applications.

This document summarizes some of the results gathered during testing of the SB100 and seeks to answer commonly asked questions about testing and efficacy. Other results may be available. Contact the manufacturer for more information.

Efficacy Testing - ASTM E1153-03

The microbial efficacy of the SB100 has been confirmed by an accredited lab [i] using the EPA OCSPP [ii] 810.2300 "*Product Performance Test Guidelines*". In this guideline EPA recommends the ASTM[iii] E1153-03 "*Sanitizers for Use on Hard Surfaces, Efficacy Data Recommendations*". This guideline describes test methods that the EPA believes will generally satisfy testing requirements of the FIFRA [iv] and the FFDCA [v]. The guideline addresses effectiveness testing of antimicrobial pesticides bearing claims for use as sanitizers.

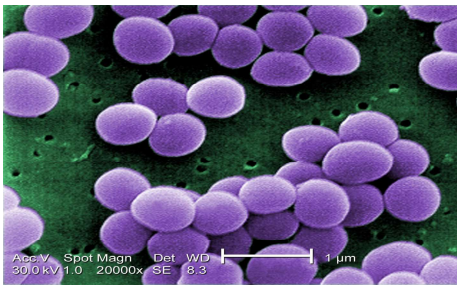

Figure 1. Staphylococcus aureus

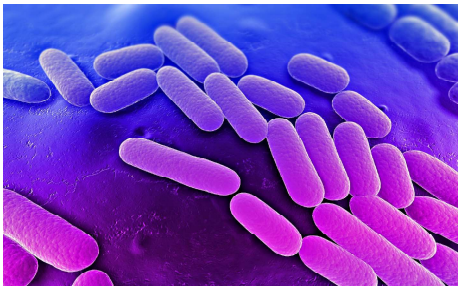
Figure 2. Klebsiela pneumonias

The EOI product sample was tested per ASTM E1153-03 against each test bacterium on stainless steel carrier or glass slide, using 5 test carriers and 3 control carriers. The test microorganisms are: (*S. aureus*) (ATCC 6538) and (*K. pneumoniae*) (ATCC [vi] 4352). The sample should demonstrate a reduction of 99.9% (a 3-$\log_{10}$ reduction) in the number of each test microorganism over the parallel control count within a time period of 5

minutes. The test results indicate that the ozone-enriched water generated by EOI ozone spray bottle meets the requirements of the ASTM test method and can be used to sanitize hard surfaces.

EOI Sanitizing Spray (ASTM E1152-03)

Pathogen	Starting Count	Count After 5 Min	Reduction (min. 99.9%)
Staphylococcus aureus	1,000,000	10	99.999%
Klebsiela pneumonias	1,000,000	25	99.997%

Efficacy Testing on Various Surfaces – Modified AOAC 961.02

EOI and Franke have conducted several tests with a third party accredited lab[i] that have demonstrated the efficacy of the spray bottle on a range of surfaces. The tests followed a modified AOAC[vii] 961.02 "Germicidal Spray Products as Disinfectants". In the AOAC test, the bacteria are dried onto a surface and exposed to the sanitizer for the time period prescribed by the manufacturer (30 sec, 2 min, and 5 min). The remaining live bacteria are counted as colony forming units (CFU). The percentage 'kill' or 'log reduction' is calculated by comparing the results of the same test using tap water without ozone as the control.

The table below lists the efficacy of the SB100 spray bottle on two common surfaces, against a representative sample of pathogens. Similar results were achieved with other surfaces such as, PVC vinyl, Formica®, Corian®, granite, silicone, chrome, and porcelain.

EOI Sanitizing Spray (Modified AOAC 961.02)

Bacteria	Time	% Reduction Glass	% Reduction Stainless Steel
E. coli	0.5 min	99.9	99.9
	2 min	99.99	99.99
	5 min	99.999	99.996
Salmonella	0.5 min	99.9	99.9
	2 min	99.99	99.99
	5 min	99.996	99.999
Staph. aureus	0.5 min	99.95	99.9
	2 min	99.99	99.98
	5 min	99.999	99.996
Listeria	0.5 min	99.9	99.9
	2 min	99.99	99.99
	5 min	99.993	99.993
E. faecium	0.5 min	99.9	99.96
	2 min	99.99	99.99
	5 min	99.996	99.999
Pseudomonas	0.5 min	99.9	99.9
	2 min	99.99	99.99
	5 min	99.990	99.993
Clostridium difficile	0.5 min	99.7	99.8
	2 min	99.93	99.92
	5 min	99.979	99.972

Commonly Asked Questions About Efficacy

Q: Does the quality of the water make a difference to the ozone efficacy?

A: Yes. Typically 'better water better ozone.' The microbial load, water hardness, temperature, and pH all can be factors. Higher kill rates may be achieved when filtered drinking water is used. Cooler temperatures and a neutral pH also contribute to higher kill rates. Distilled water should not be used.

Q: How do I know that the unit is spraying ozone?

A: The lighted indicators tell the user when the unit is making ozone. There is a slight delay in the blue light since the water must have a small amount of time to reach the ozone cell in the head of the sprayer. See the users guide for specifics on indicator lights.

Q: Is there an easy test to prove the concentration of the ozone is enough to give the kill rates in the tables?

A: We are working on a kit similar to those used for swimming pool chemical loading. Look for that solution in 2Q13.

Q: Will an ATP[viii] test kit work for testing the bacteria kill efficacy of the spray bottle?

A: Some industries use ATP testers as indicators of how 'clean' or free of organic material a surface is. This test is effective to show that bacteria have been wiped from a surface. However, many ATP testers show both living and dead bacteria or organics, so even the bacteria killed by ozone may show in this test.

Q: Why are there so many different time periods shown in the efficacy results?

A: The ASTM E1153-03 standard calls for 5 min. Exposure times may also be recommended by the manufacturer. AOAC 961.02 was modified for the recommended contact times of 30 seconds, 2 minutes, and 5 minutes.

---

[i] *Lapuck Laboratories, www.lapucklabs.com*
[ii] *OCSPP, Office of Chemical Safety and Pollution Prevention, www.epa.gov/ocspp*
[iii] *ASTM, American Society for Testing and Materials, offers test methodologies, some of which are recommended by the EPA.*
[iv] *FIFRA, the Federal Insecticide, Fungicide and Rodenticide Act, categorizes SB100 and its on-demand production of a pesticide as a pesticide device.*
[v] *Federal Food, Drug, and Cosmetic Act (FFDCA) authorizes EPA to set tolerances, or maximum residue limits, for pesticide residues on foods.*
[vi] *ATCC, American Type Culture Collection, identifies a strain of microorganism.*
[vii] *AOAC, Association of Official Analytical Chemists, offers test methodologies, some of which are recommended by the EPA.*
[viii] *ATP (Adenosines Tri-Phosphate) test, measures the concentration of microorganisms.*