TECHNICAL SUMMARY

Aquaox Disinfectant 275 | Aquaox Disinfectant 525

PRODUCT EFFICACY

Aquaox Disinfectant 275 and Aquaox Disinfectant 525 are Hypochlorous Acid solutions generated electrochemically from Sodium Chloride. Both products are EPA registered antimicrobial pesticides bearing a Hospital and a General/Broad Spectrum Disinfectant claims per FIFRA Section 3(c)(5). Using established ASTM standards, AOAC methods and EPA guidelines, a series of studies have been conducted to characterize the solutions' abilities to disinfect and reduce microorganisms through a one-step disinfecting mechanism. These studies are further discussed below.

1. AOAC Use-Dilution Method (AOAC 955.14, 955.15, 964.02)

The AOAC Use-Dilution Test is considered a "high-level" test for disinfectants, i.e., an antimicrobial solution must have appreciable biocidal activity on a relatively short time frame, < 10 minutes, to pass the test.

A culture of the challenge microorganism, listed in Table 1 below, is amended with a 5% organic soil load to mimic a "dirty" surface to challenge test article's one-step cleaning and disinfecting efficacy. The bacteria is then cultured for 48 hours and the 48-hour is dried onto a number of small small, cylindrical, and stainless steel test surfaces test surfaces called penicylinders to create a contaminated surface. At least 10 contaminated surfaces are prepared.

Using a wire hook, each dry, containated test surface is then transferred individually to a test tube filled with the the test article (Aquaox Disinfectant 275 or 525) for the exposure (contact) time of 10 minutes at room temperature (20 – 25°C). After the exposure time has elasped, the treated test surfaces are transferred to test tubes containing a liquid growth medium that will neutralize the action of the disinfectant. The treated test surfaces are then incuated in the neutralizing growh medium for 48 hours to recover the microorganism. After incubation in the neutralization media, the number of test tubes showing recovery of the challenge microorganism is recorded.

TABLE 1. *Aquaox Disinfectant evaluated against Gram+ and Gram- Bacteria in the presence of 5% Organic Soil Load*

Exposure Time: 10 minutes **Sample Dilution:** Ready to Use (RTU)			
Test Organism	**Strain**	**Number of Positive Carriers per Number Tested**	**Test Result**
Pseudomonas aeruginosa	ATCC 15442	0 / 10	Pass
Staphylococcus aureus	ATCC 6538	0 / 10	Pass
Staphylococcus aureus (HA-MRSA)	ATCC 33591	0 / 10	Pass
Salmonella enterica	ATCC 10708	0 / 60	Pass
Escherichia coli (NDM-1)	ATCC BAA-2469	0 / 10	Pass
Vancomydin Resistant Enterococcus feacalis (VRE)	ATCC 700221	0 / 10	Pass

Conclusion: Under the condition of this study, in the presence of 5% organic soil load, Aquaox Disinfectant, ready to use, demonstrated efficacy against the above listed microorganisms following a 10-minute exposure time at room temperature.

2. AOAC Tuberculocidal Activity of Disinfectants Test Method

The AOAC Tuberculocidal Activity of Disinfectants Test is considered a "high-level" test for disinfectants, i.e., an antimicrobial solution must have appreciable biocidal activity on a relatively short (<10 minutes) time frame to pass the test.

A culture of the *Mycobacterium bovis BCG*, an EPA recommended surrogate of Mycobacterium tuberculosis, is amended with a 5% fetal bovine serum to mimic a "dirty" surface to challenge test article's one-step cleaning and disinfecting efficacy. The bacteria is then cultured for 21 days, and dried onto a number of penicylinders to create a test surface. At least 10 contaminated test surfaces are created.

Each dry, containated test surface is then transferred, individually, to a test tube filled with the test article for the exposure (contact) time of 10 minutes near room temperature. After the contact time has elapsed, the treated test surfaces are transferred to test tubes containing a liquid medium that has been amended with chemical agents to immediately neutralize the action of the disinfectant. Immediately after transfer from the disinfectant into the neutralizer, the treated test surfaces are transferred into bacterial growth medium and are incubated for 60 days. After the 60-day incubation, the number of tubes showing growth of *Mycobacterium bovis BCG* is recorded.

TABLE 2. *Aquaox Disinfectant evaluated against Mycobacterium bovis BCG in the presence of 5% Fetal Bovine Serum*

Exposure Time: 10 minutes **Sample Dilution:** Ready to Use (RTU)		
Challenge Suspension Initial Population (CFU/mL)	**Number of Positive Carriers per Number Tested (All Media Types)**	**Test Result**
2.850 x 10^7	0 / 10	Pass
2.850 x 10^7	0 / 10	Pass

Conclusion: Under the condition of this study, in the presence of 5% organic soil load, Aquaox Disinfectant, ready to use, met the required performance criteria versus *Mycobacterium bovis BCG* following a 10-minute exposure time at room temperature.

3. Virucidal Hard Surface Disinfection Evluation using ASTM E1053 Method

This test is performed to verify the performance capability of a test substance as a virucidal agent.

Aquaox Disinfectant has been tested against four different viruses, HIV-1, H1N1, Rhinovirus 16 and Murine Norovirus. The test virus, HIV-1, H1N1 or Rhinovirus 16, is loaded with a 5% organic soil load to mimic a "dirty" surface to challenge test article's one-step cleaning and disinfecting efficacy. An inoculum of the test virus is spread over the carrier surface and allowed to dry. The

test virus is then innoculated onto a hard, nonporous surface (100 x 15 mm glass Petri dish) to create a dried film carrier. Two dried film carriers are prepared per lot of test substance for surrogate viruses and one dried film carrier for non-surrogate viruses.

The dried virus films are treated with the test article for the exposure (contact) time of 10 minutes near room temperature. At the close of the contact time, the test carrier films are neutralized by addition of a neutralizer solution followed by scraping of the carrier surface using a cell scraper. The test suspensions are then plated, cultured, and observed for virus presence or absence.

TABLE 3.1. *Aquaox Disinfectant evaluated against HIV-1 virus in the presence of 5% Fetal Bovine Serum*
Virus / Strain: *HIV-1/Mn (ZeptoMetrix #0810027CF)*
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

	Virus Control		**After Exposure to Test Substance – Lot #1**		**After Exposure to Test Substance – Lot #2**	
Dilution	**Carrier 1**	**Carrier 2**	**Carrier 1**	**Carrier 2**	**Carrier 1**	**Carrier 2**
10^{-2}	Not Tested		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ (log 10)	5.750	5.500	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50
Average $TCID_{50}$ (log 10)	5.625		≤ 1.50		≤ 1.50	
Log 10 Reduction	N/A		≥ 4.125	≥ 4.125	≥ 4.125	≥ 4.125
Average Log 10 Reduction			≥ 4.125		≥ 4.125	
Percent Reduction			> 99.99	> 99.99	> 99.99	> 99.99
Average % Reduction			> 99.99		> 99.99	

Dilution refers to the fold of dilution from virus inoculum
(+) = Positive for the presence of test virus
(0) = No test virus recovered
"≤ " indicates a viral titer at or below the limit of dectection for this assay

TABLE 3.2. *Aquaox Disinfectant evaluated against Swine Influenza A (H1N1) virus in the presence of 5% Fetal Bovine Serum – Virus Controls and Test Results*

Virus / Strain: Swine Influenza A (H1N1) Virus, ATCC VR-333
Strain A / Swine / Iowa / 15 / 30
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Dilution	Input Virus Control	Dried Virus Control	After Exposure to Test Substance
Cell Control	0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	0 0 0 0
10^{-2}	+ +	+ + + +	0 0 0 0
10^{-3}	+ +	+ + + +	0 0 0 0
10^{-4}	+ +	+ + + +	0 0 0 0
10^{-5}	+ +	+ + + +	0 0 0 0
10^{-6}	0 0	+ 0 + 0	0 0 0 0
10^{-7}	0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ (log 10) / 100uL	6.50	6.00	≤ 0.50
Log 10 Reduction	N/A		≥ 5.50

TABLE 3.3. *Aquaox Disinfectant evaluated against Rhinovirus 16 (common cold agent) in the presence of 5% Fetal Bovine Serum – Virus Controls and Test Results*

Virus / Strain: Rhinovirus 16 (Rhino 16), ATCC VR-283
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Test Substance	Log 10 Infectious Units per Carrier	Log 10 Reduction after Exposure	Percent Reduction after Exposure
Control	5.80	N/A	N/A
After Exposure to Test Substance	≤ 1.80	≥ 4.00	≥ 99.99%

Viral stock enumeration demonstrated a titer of 7.00 log 10 per 0.1 mL.

TABLE 3.4. *Aquaox Disinfectant evaluated against Murine Norovirus (without 5% Fetal Bovine Serum) – Virus Controls and Test Results*

Virus / Strain: Murine Norovirus, Strain MNV-G
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Dilution	Virus Stock Titer Control	Plate Recovery Control	After Exposure to Test Substance – Lot #1	After Exposure to Test Substance – Lot #2
10^{-2}	Not Tested	Not Tested	0 0 0 0	0 0 0 0
10^{-3}	Not Tested	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-6}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-7}	0 0 + +	0 0 0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	Not Tested	Not Tested
10^{-9}	0 0 0 0	Not Tested	Not Tested	Not Tested
$TCID_{50}$ (log 10) / mL	7.00	6.50	≤ 1.50	≤ 1.50
$TCID_{50}$ (log 10) per Carrier (0.40mL Challenge)	N/A	6.10	≤ 1.10	≤ 1.10
Log 10 Reduction	N/A		≥ 5.00	≥ 5.00

Conclusion: Under the condition of the above studies, in the presence of 5% organic soil load, Aquaox Disinfectant, ready to use, demonstrated at least a 4-log reduction (> 99.99%) of H1N1 Virus and Rhinovirus 16, and at least a 5-log reduction of the HIV-1 Virus, following a 10-minute exposure time at room temperature. For Murine Norovirus, Aquaox Disinfectant, ready to use, demonstrated at leas a 5-log reduction of the test virus, in the absence of an organic soil load. Aquaox Disinfectant was able to meet the EPA success criteria for virucidal efficacy of a disinfectant, i.e. a minimum of 4-log reduction of the test virus.

TABLE 4 Efficacy Test Summary – Aquaox Disinfectant 275

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
Aquaox Disinfectant 275 (Tested at **10ppm** FAC)	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Staphylococcus aureus,* *Pseudomonas aeruginosa,* *Escherichia coli,* *Serratia marcescens,* *Klebsiella pneumoniae,* *Proteus vulgaris,* *Acinetobacter baumannii*	All **Gram-Negative** Bacteria except for *Staphylococcus aureus* , which is **Gram-Positive**	Log reduction in **15 s**: S. aureus: > 5.25 P. aeruginosa: > 5.00 E. Coli: > 4.85 S. marcescens: > 4.88 K. pneumoniae: > 4.98 P. vulgaris: > 4.98 A. baumannii: > 5.12	NAMSA
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Acinetobacter baumannii* - Multi Drug Resistant, *Enterococcus faecium* - Multi Drug Resistant, Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus faecalis* (VRE)	**Gram-Negative** Bacteria **Gram-Positive** Bacteria **Gram-Positive** Bacteria **Gram-Positive** Bacteria	Log reduction in **15 s**: A. baumannii: > 5.45 E. faecium: > 5.30 MRSA: > 5.36 VRE: > 5.56	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Bacteroides fragilis,* *Haemmophilus influenzae,* *Streptococcus pyogenes*	**Gram-Negative** Bacteria **Gram-Negative** Bacteria **Gram-Positive** Bacteria	Log reduction in **15 s**: B. fragilis: > 5.89 H. influenzae: > 4.44 S. pyogenes: > 5.79	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Staphylococcuss epidermidis,* *Staphylococcus haemolyticus,* *Staphylococcus hominis,* *Staphylococcus saprophyticus*	All **Gram-Positive** Bacteria and of the *Staphylococcus* genus	Log reduction in **15 s**: S. epidermidis: > 5.08 S. haemolyticus: > 5.01 S. hominis: > 5.32 S. saprophyticus: > 5.15	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Enterobacter aerogenes,* *Escherichia coli,* *Klebsiella pneumoniae,* *Micrococcus luteus,* *Proteus mirabilis,* *Serratia marcescens*	All **Gram-Negative** Bacteria except for *Micrococcus luteus* , which is **Gram-Positive to Gram-Variable**	Log reduction in **15 s**: E. aerogenes: > 5.88 E. coli: > 5.61 K. pneumoniae: > 5.42 M. luteus: > 4.46 P. mirabilis: > 5.92 S. marcescens: > 5.43	ATS Lab
Aquaox Disinfectant 275	Testing Disinfectant against *Pseudomonas aeruginos* and *Staphylococcus aureus*	AOAC Official Method, 964.02, 955.15, Use-Dilution Method	*Staphylococcus aureus,* *Pseudomonas aeruginosa*	**Gram-Positive** Bacteria **Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 275	Testing Dieinfectant against *Salmonella enterica*	AOAC Official Method, 955.14, Use-Dilution Method	*Salmonella enterica*	**Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Myobacterium bovis - BCG*	Bacteria that causes **Tuberculosis** in humans	> 5.21 log reduction in **60 s**	ATS Lab

TABLE 4. Efficacy Test Summary – Aquaox Disinfectant 275 (Cont.)

Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	Hepatitis B Virus	**Virus**	> 5.25 log reduction in **30 s**	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	Rhinovirus type 37	**Virus**	> 3.75 log reduction in **60 s**	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	Swine Influenza A (H1N1) Virus	**Virus**	> 5.50 log reduction in 5% organic soil load in 10 minutes	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052	Murine Norovirus	**Virus**	> 5.00 log reduction in 10 minutes	Microbac Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Aspergillus brasiliensis*	**Fungus**	Log reduction in **15 s** A. brasiliensis: = 4.11	NAMSA
Aquaox Disinfectant 275 (Tested at **10ppm** FAC)	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Candida albicans*	**Fungus**	> 4.38 log reduction in **15 s**	NAMSA
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Candida albicans*	**Fungus**	> 5.31 log reduction in **15 s**	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Standard Guideline E2315-03, E2839-11	*Clostridium difficile* - spore form	**Spore**	> 5.35 log reduction in **30 s**	ATS Lab

TABLE 5. Efficacy Test Summary – Aquaox Disinfectant 525

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
Aquaox Disinfectant 525	Testing Disinfectant against *Pseudomonas aeruginos* and *Staphylococcus aureus*	AOAC Official Method, 964.02, 955.15, Use-Dilution Method	*Staphylococcus aureus, Pseudomonas aeruginosa*	**Gram-Positive** Bacteria **Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 525	Testing Disinfectant against Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA)	AOAC Official Method, 964.02, Use-Dilution Method	Hospital Acquired Methicillin Resistant *Staphylococcus aureus (HA-MRSA)*	**Gram-Positive** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Testing Disinfectant against *Salmonella enterica*	AOAC Official Method, 955.14, Use-Dilution Method	*Salmonella enterica*	**Gram-Negative** Bacteria	Killed **60 out of 60** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Testing Disinfectant against NDM-1 *E.Coli and VRE*	AOAC Official Method, 955.15, Use-Dilution Method	*NDM-1 Escherichia coli Vancomycin Resistant Enterococcus faecalis (VRE)*	**Gram-Negative** Bacteria **Gram-Positive** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	
Aquaox Disinfectant 525	AOAC Tuberculocidal Activity of Disinfectants	AOAC Official Method, 965.12, 960.09	*Myobacterium bovis - BCG*	Bacteria that causes **Tuberculosis** in humans	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053, E1482	Swine Influenza A (H1N1) Virus	**Virus**	> 5.50 log reduction in **5% organic soil load** in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053	Human Immunodeficiency Virus Type 1 (HIV-1)	**Virus**	> 4.125 log reduction in **5% organic soil load** in 10 minutes	Bioscience
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053	Rhinovirus 16 (Common Cold Agent)	**Virus**	> 4.000 log reduction in **5% organic soil load** in 10 minutes	ATL Lab
Aquaox Disinfectant 525	Standard Quantitative Disk Carrier Test Method for Determining Sporocidal Activities	ASTM Standard Guideline E2197-11, Standard Quantitative Disk Carrier Test Method	*Clostridium difficile* - spore form	**Spore**	> 5.96 log reduction in 10 minutes in the absernce of organic soil load	ATS Lab

PRODUCT SAFETY

A nonclinical toxicology investigation has been done on the above products as following. The Aquaox Disinfectant products contain Hypochlorous Acid as the active ingredients. The only inactive ingredient in the product solution is residual Sodium Chloride from the electrolysis process. Sodium Chloride (CAS RN 8028-77-1) is listed as an inactive ingredient in FDA CDER database for use in approved drug products. Moreover, the Sodium Chloride used in Aquaox electrolysis process is NSF certified. Therefore, the presence of Sodium Chloride in the Aquaox Disinfectant products does not present a safety concern.

A series of non-clinical toxicology testing has been done on the product solutions to assess their potential local and systemic toxicity. The toxicology studies were conducted at NAMSA and IIT Research Institute (IITRI), both of which being AALAC approved facilities. All toxicology studies conducted were in compliance with Good Laboratory Practice (GLP) regulations.

The GLP toxicology testing program was based on ISO-10993 requirements on biocompatibility testing for a surface device with contact with breached or compromised surface. These studies, together with the study results, are listed in Table 4.

TABLE 6. *Nonclinical Toxicity Testing Summary*

Study Type	Test Species	Route	Result	Testing Facility
In vitro Cytotoxicity	L-929 Mouse Fibroblast Cells	In vitro	Not Cytotoxic / Meet USP Requirement	NAMSA
Repeated-Dose Toxicity	Rats	Dermal	No Local or Systemic Toxicity on Intact or Wounded skin	NAMSA
Maximization Sensitization	Guinea Pigs	Dermal	Not a Sensitizer (Does not induce allergic responses)	NAMSA
Acute Toxicity	Rats	Oral	Non-Toxic	NAMSA
Acute Toxicity	Rats	Inhalation / Nose	Non-Toxic	IIT RI
Skin Irritation	Rabbits	Dermal	Not a Skin Irritant on Intact or Abraded Skin	NAMSA
Eye Irritation	Rabbits	Ocular	Not an Eye Irritant	NAMSA

Conclusion

Exposure to L-929 cells *in vitro* to the product solutions produced a slight cell lysis, which was not considered cytotoxic per USP requirement. Product solutions were also not considered a primary dermal or ocular irritant, and did not show sensitization potential in the dermal and ocular irritation studies. Product was considered non-toxic in both the acute oral toxicity study and the single dose inhalation study when tested at the maximal feasible concentration. In a 28-day repeated dose toxicity study, topical application of the product to intact and wounded skin areas did not result in any treatment-related skin irritation or wound healing issues. Therefore, the results of the toxicology testing program confirmed the biocompatibility and safety profile of the product solutions for its intended use.