

**Aquaox Supplemental Data**  
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# Human Coronavirus, strain 229E, ATCC VR-740

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid  
Batch: AX-13196-0210

## SUMMARY OF RESULTS

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Human Coronavirus, strain 229E, ATCC VR-740  
Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Human Coronavirus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>.**

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**FINAL STUDY REPORT**

**STUDY TITLE**

Evaluation of Antiviral Properties of a Product  
Using a Virucidal Suspension Assay

**Virus:** Human Coronavirus

**PRODUCT IDENTITY**

AQUAOX  
Batch #AX-13196-0210

**AUTHOR**

Shanen Conway, B.S.  
Study Director

**STUDY COMPLETION DATE**

December 11, 2013

**PERFORMING LABORATORY**

**ATS Labs**  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

**PROJECT NUMBER**

A15626

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### **GOOD LABORATORY PRACTICE STATEMENT**

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

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## QUALITY ASSURANCE UNIT SUMMARY

Study: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice Regulations (21 CFR Part 58) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

<b>Phase Inspected</b>	<b>Date of Phase Inspection</b>	<b>Date Reported to Study Director</b>	<b>Date Reported to Management</b>
Critical Phase Audit	October 1, 2013	October 1, 2013	October 1, 2013
Draft Report	October 28, 2013	October 28, 2013	October 28, 2013
Final Report	December 11, 2013	December 11, 2013	December 11, 2013

The findings of these inspections have been reported to Management and the Study Director.

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## STUDY PERSONNEL

STUDY DIRECTOR: Shanen Conway, B.S.

Personnel Involved:

Kelleen Gutzmann, M.S.

Katherine A. Paulson, M.L.T.

Matthew Cantin, B.S.

Erica Flinn, B.A.

- Director, Virology & Microbial ID Operations

- Senior Virologist

- Lead Virologist

- Associate Virologist

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

**Project Number:** A15626

**Protocol Number:** INI01091313.COR

**Testing Facility:** ATS Labs  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance:** Aquaox Hypochlorous Acid Batch # AX-13196-0210

**Batch:** AX-13196-0210

### **Test Substance Characterization**

Test substance characterization as to content, stability, solubility, storage, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor.

### **STUDY DATES**

**Date Sample Received:** September 11, 2013  
**Study Initiation Date:** September 23, 2013  
**Experimental Start Date:** October 1, 2013  
**Experimental End Date:** October 11, 2013  
**Study Completion Date:** December 11, 2013

### OBJECTIVE

The objective of this study was to evaluate the antiviral properties of a product against Human Coronavirus when exposed (in suspension) for the specified exposure period. This protocol is a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E 1052).

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## SUMMARY OF RESULTS

Test Substance:	Aquaox Hypochlorous Acid Batch # AX-13196-0210
Dilution Tested:	Ready to use
Virus:	Human Coronavirus, strain 229E, ATCC VR-740
Exposure Time:	30 seconds
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	1% fetal bovine serum
Efficacy Result:	Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log <sub>10</sub> -

## TEST SYSTEM

1. Virus  
The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-740). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at 50-70°C until the day of use. On the day of use an aliquot of stock virus (ATS Labs Lot HCV-69) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Human Coronavirus on WI-38 cells.
2. Indicator Cell Cultures  
Cultures of WI-38 (human lung) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-75). The cells were propagated by ATS Labs personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

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3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures per dilution. See the report text for a more detailed explanation.

<b>PARAMETERS TESTED FOR VIRUCIDAL EFFICACY ASSAY</b>		
Test or Control Group	Dilutions Assayed (10010)	Cultures per Dilution
Cell Control	N/A	4
Virus Control	-2,-3,-4,-5,-6,-7,-8	4
Test Batch+ virus	-2,-3,-4,-5,-6,-7,-8	4
Cytotoxicity Control	-2,-3,-4	4
Neutralization Control	-2,-3,-4	4

**TEST METHOD**

1. Preparation of Jest Substance

Aquaox (Batch# AX-13196-0210) was used as it was received from the Sponsor. The test substance removed from the original container was in solution as determined by visual observation. The test substance was at the exposure temperature prior to use in testing.

2. Treatment of Virus Suspension

For the exposure time assayed, a 1.80 ml aliquot of the test substance was dispensed into a sterile tube and mixed with a 200 µl aliquot of the stock virus suspension. The mixture was vortex mixed for 10 seconds and held for the remainder of the specified exposure time at room temperature (20.0°C). The exposure time assayed was 30 seconds. Following the exposure time, a 100 µl aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilutions (100 µl + 0.9 ml test medium) and assayed for the presence of virus. Note: To decrease the test substance cytotoxicity, the first dilution was made in FBS with the remaining dilutions in test medium.

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3. Treatment of Virus Control

For the exposure time assayed, a 200 µl aliquot of stock virus suspension was exposed to a 1.80 ml aliquot of test medium in lieu of test substance and treated as previously described. Following the exposure time, a 100 µl aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilutions (100 µl + 0.9 ml test medium) and assayed for the presence of virus. All controls employed the FBS neutralizer as described in the Treatment of Virus Suspension section. A virus control was performed for the exposure time tested. The virus control titer was used as a baseline to compare the percent and log reductions of the test parameter following exposure to the test substance.

4. Cytotoxicity Control

A 1.80 ml aliquot of the test substance was mixed with a 200 µl aliquot of test medium containing the Sponsor requested organic soil load in lieu of virus and treated as previously described. The cytotoxicity control was held for the longest exposure time. The cytotoxicity of the cell cultures was scored at the same time as virus-test substance and virus control cultures. Cytotoxicity was graded on the basis of cell viability as determined microscopically. Cellular alterations due to toxicity were graded and reported as toxic (T) if greater than or equal to 50% of the monolayer was affected.

5. Neutralization Control

Each cytotoxicity control mixture (above) was challenged with low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100 µl aliquot of each dilution in quadruplicate. A 100 µl aliquot of low titer stock virus was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

6. Infectivity Assay

The WI-38 cell line, which exhibits cytopathic effect (CPE) in the presence of Human Coronavirus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µl of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell culture labware. The cultures were microscopically scored periodically for ten days for the absence or presence of CPE, cytotoxicity, and for viability.

7. Statistical Methods: Not applicable

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## **PROTOCOL CHANGES**

### **Protocol Amendments:**

No protocol amendments were required for this study.

### **Protocol Deviations:**

No protocol deviations occurred during this study.

## **TEST CRITERIA**

A valid test requires 1) that stock virus be recovered from the virus control and 2) that the cell controls be negative for virus.

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## **RECORD RETENTION**

### **Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

### **Test Substance Retention**

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test substance.

## **REFERENCES**

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1052 (current version).
  2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482 (current version).
  3. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A., and Lennette, E.T. editors. Seventh edition, 1995.
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## STUDY RESULTS

### Cytotoxicity and Neutralization Controls

Test substance cytotoxicity was not observed in any dilution assayed (S1.50 10910). The neutralization control demonstrated that the test substance was neutralized at s:1.50 IOQ10-

### 30 Second Exposure Time

The titer of the virus control was 5.75 log<sub>10</sub>. Following exposure, test virus infectivity was not detected in the virus-test substance mixture at any dilution tested (s;1.50 10910).

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Human Coronavirus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 LOG<sub>10</sub>.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.



## **SANITIZING/DISINFECTING PRODUCTS WITH HOCL (pH-neutral Hypochlorous Acid) AS ACTIVE INGREDIENT.**

**LABEL CLAIMS CERTIFIED BY EPA.**

### **SANITIZER FOR FOOD-CONTACT SURFACES**

**Efficacy >100PPM HOCL - 1 Minute**

*Escherichia coli* - ATCC 11229

*Klebsiella pneumoniae* – ATCC 10031

*Proteus mirabilis* – ATCC 7002

*Salmonella Enterica* –(Subspecies *Enterica* Serovar *Typhi*) - ATCC 6539

*Salmonella typhi* – ATCC 19430

*Salmonella enterica* (tested as *Salmonella choleraesuis*) – ATCC 14028

*Staphylococcus Aureus* - ATCC 6538

*Streptococcus pneumoniae* – ATCC 6305

### **SANITIZER FOR FOOD-CONTACT SURFACES**

**Efficacy <200PPM HOCL - 2 Minutes**

*Escherichia coli* - ATCC 11229

*Salmonella typhi* – ATCC 6539

### **SANITIZER FOR FOOD-CONTACT SURFACES**

**Efficacy <200PPM HOCL - 5 Minutes**

*Enterobacter aerogenes* – ATCC13048

*Escherichia coli* - ATCC 11229

*Salmonella entérica* – ATCC 10708

*Staphylococcus aureus* – ATCC 6538

*Staphylococcus aureus* (HA-MRSA) ATCC 33591

*Streptococcus pneumoniae* – ATCC 19615

*Vancomycin Resistant Enterococcus Faecalis* (VRE) – ATCC 51575



## **DISINFECTANT FOR HARD NON-POROUS SURFACES**

**Efficacy >250PPM HOCL - 10 Minutes**

*Acinobacter baumannii* – ATCC 19606

*Escherichia coli* O157: H7 - ATCC 35150

*Escheria Coli* – ATCC 11229

*Escherichia coli* (NDM) - ATCC BAA-2469

*Clostridium difficile* spore (C. Diff) - ATCC 43598

*Herpes Simplex virus Type 1* – ATCC VR-733

*Herpes Simplex virus Type 2* – ATCC VR-734

*HIV Type 1* (HIV) – Strain HTLV-IIIb

*Human Immunodeficiency Virus Type 1* (HIV-1), strain IIIB (clade B); ZeptoMetrix

*Human corona virus* – ATCC VR-740, stain 229E

*Influenza A* (H1N1) – ATCC VR-1469

*Klebsiella pneumonia New Delhi MetalloBeta Lactamase* (NDM-1) Carbapenem Resistant, CDC 10002

*Listeria monocytogenes* - ATCC 19111

*Listeria monocytogenes* - ATCC 7644

*Mycobacterium bovis*, BCG (Tuberculosis or TB) – ATCC 35734

*Propionibacterium acnes* – ATCC 6919

*Pseudomonas aeruginosa* - ATCC 15442

*Rhinovirus Type 37* - ATCC VR1147, Strain 151-1

*Respiratory Syncytial Virus* (RSV) -ATCC VR-26

*Salmonella entérica* – ATCC 10708

*Staphylococcus aureus* – ATCC 6538

*Staphylococcus aureus* (HA-MRSA) - ATCC 33591

*Staphylococcus aureus* (HA-MRSA) - ATCC 33592

*Serratia marcesens* – ATC 14756

*Streptococcus pyogenes* – ATCC 19615

*Trichophyton mentogrophytes* – ATCC 9533

*Swine influenza Virus* (H1N1) - ATCC VR 99

*Vancomycin Resistant Enterococcus faecalis* (VRE) - ATCC 700221

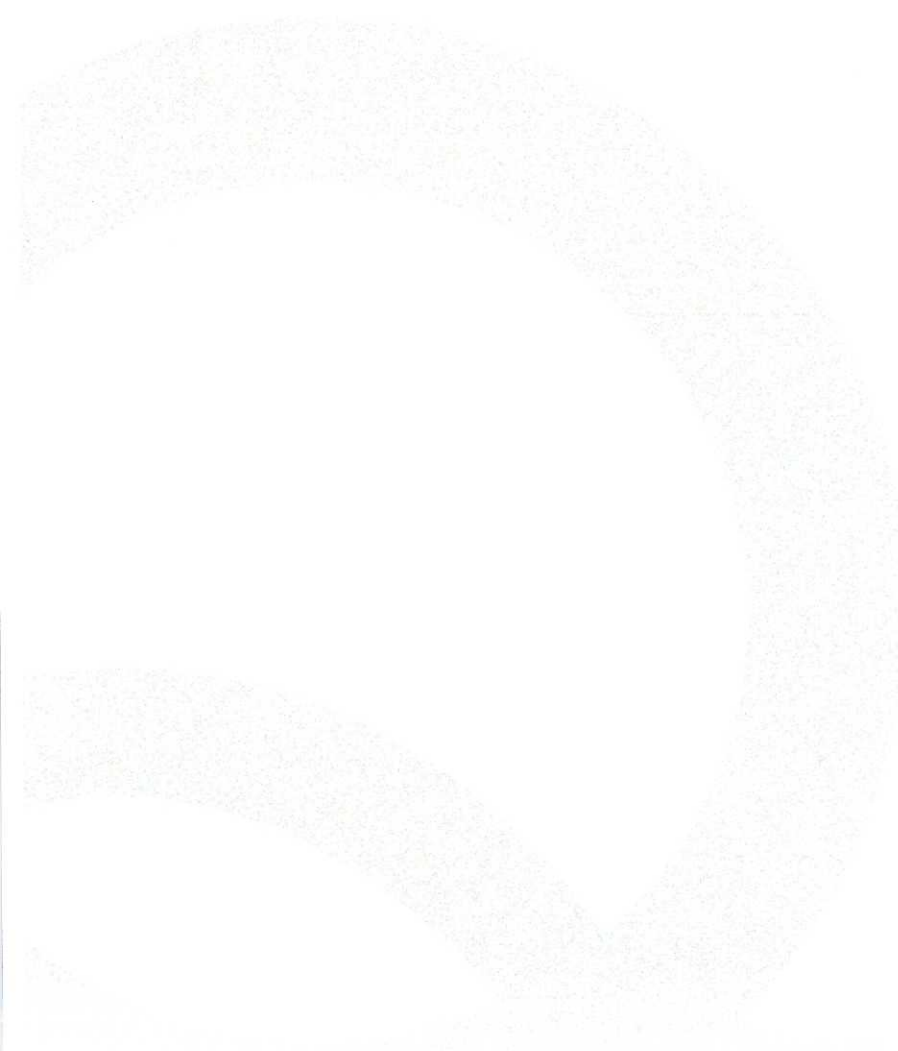
*Vancomycin Resistant Enterococcus faecalis* (VRE)- ATCC 51229

### **Disclaimer:**

Although every reasonable effort has been made to insure the accuracy of the information contained in this document, absolute accuracy cannot be guaranteed. All information are presented to the user "as is" without warranty of any kind, either express or implied. Not responsible for typographical or data errors. See EPA (<https://www.epa.gov/pesticides>) for accurate and updated information



AQUAOX Disinfectant  
Virucidal Efficacy - Test Summary





# Human Coronavirus, strain 229E, ATCC VR-740

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid  
Batch: AX-13196-0210

## SUMMARY OF RESULTS

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Human Coronavirus, strain 229E, ATCC VR-740  
Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Human Coronavirus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>.**

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# Respiratory syncytial virus, Strain Long, ATCC VR-26

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Respiratory syncytial virus, Strain Long, ATCC VR-26

## SUMMERY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Respiratory syncytial virus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>.**

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# Adenovirus type 2, Strain Adenoid 6, ATCC VR-846

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Adenovirus type 2, Strain Adenoid 6, ATCC VR-846

## SUMMERY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.9997% reduction in the stock virus titer as compared to the titer of the virus control. The log reduction in viral titer was 6.50 log<sub>10</sub>

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.9997% reduction in viral titer following a 30 second exposure time to Adenovirus type 2 at room temperature (20.0°C), as compared to the titer of the virus control. The log reduction in viral titer was 6.50LOG<sub>10</sub>**

# Human Immunodeficiency Virus type 1, Strain HTLV-IIIe

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: A Human Immunodeficiency Virus type 1, Strain HTLV-IIIe

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.999% reduction in the stock virus titer as compared to the titer of the virus control. The log reduction in viral titer was  $\geq 5 \log_{10}$

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.999% reduction in viral titer following a 30 second exposure time to Human Immunodeficiency Virus type 1, at room temperature (20.0°C), as compared to the titer of the virus control. The log reduction in viral titer was  $\geq 5 \log_{10}$**



# Duck Hepatitis B virus as a surrogate virus for human Hepatitis B virus

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Duck Hepatitis B virus as a surrogate virus for human Hepatitis B virus

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.9994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 5.25 log<sub>10</sub>.

## STUDY CONCLUSION

**Under the conditions of this investigation, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.9994% reduction in viral titer following a 30 second exposure time to duck Hepatitis B virus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 5.25 log<sub>10</sub>.**

# Poliovirus type 1, strain Chat, ATCC VR-1562

## GENERAL STUDY INFORMATION

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Project Number: A15626  
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Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Poliovirus type 1, strain Chat, ATCC VR-1562

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.999% reduction in the stock virus titer as compared to the titer of the virus control. The log reduction in viral titer was  $\geq 5 \log_{10}$

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.9998% reduction in viral titer following a 30 second exposure time to Poliovirus type 1, at room temperature (20.0°C), as compared to the titer of the virus control and a 99.9994% . reduction in viral titer following a 60 second exposure time to Poliovirus type 1, at room temperature (20.0°C), as compared to the titer of the virus control. The log reduction in viral titer was  $\geq 5.75 \log_{10}$  and  $5.25 \log_{10}$  respectively.**

# Herpes simplex virus type 2, strain G, ATCC VR-734

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Herpes simplex virus type 2, strain G, ATCC VR-734

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>.

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Herpes simplex virus type 2 as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>**

# Herpes simplex virus type 2, strain G, ATCC VR-734

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
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## TEST SUBSTANCE IDENTITY

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Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Herpes simplex virus type 2, strain G, ATCC VR-734

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>.

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Herpes simplex virus type 2 as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25LOG<sub>10</sub>**



# **Bovine viral diarrhea virus as a surrogate virus for Hepatitis C virus, strain Oregon C24v, genotype 1, cytopathic**

## GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay  
Project Number: A15626  
Protocol Number: INI01091313.COR  
Testing Facility: ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210  
Dilution Tested: Ready to use (RTU)  
A near neutral Hypochlorous Acid solution with 225ppm Free Available Chlorine produced by Aquaox.  
Virus: Bovine viral diarrhea virus as a surrogate virus for Hepatitis C virus, strain Oregon C24v, genotype 1, cytopathic

## SUMMARY OF RESULTS

Exposure Time: 30 seconds  
Exposure Temperature: Room temperature (20.0°C)  
Organic Soil Load: 1% fetal bovine serum  
Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.97% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 3.50log<sub>10</sub>.

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.97% reduction in viral titer following a 30 second exposure time to Bovine viral diarrhea virus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 3.50LOG<sub>10</sub>.**

**FINAL STUDY REPORT**

**STUDY TITLE**

Evaluation of Antiviral Properties of a Product  
Using a Virucidal Suspension Assay

**Virus:** Human Coronavirus

**PRODUCT IDENTITY**

AQUAOX  
Batch #AX-13196-0210

**AUTHOR**

Shanen Conway, B.S.  
Study Director

**STUDY COMPLETION DATE**

December 11, 2013

**PERFORMING LABORATORY**

**ATS Labs**  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

**PROJECT NUMBER**

A15626

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### **GOOD LABORATORY PRACTICE STATEMENT**

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

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## QUALITY ASSURANCE UNIT SUMMARY

Study: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice Regulations (21 CFR Part 58) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

<b>Phase Inspected</b>	<b>Date of Phase Inspection</b>	<b>Date Reported to Study Director</b>	<b>Date Reported to Management</b>
Critical Phase Audit	October 1, 2013	October 1, 2013	October 1, 2013
Draft Report	October 28, 2013	October 28, 2013	October 28, 2013
Final Report	December 11, 2013	December 11, 2013	December 11, 2013

The findings of these inspections have been reported to Management and the Study Director.

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**STUDY PERSONNEL**

STUDY DIRECTOR:

Shanen Conway, B.S.

Personnel Involved:

Kelleen Gutzmann, M.S.

Katherine A. Paulson, M.L.T.

Matthew Cantin, B.S.

Erica Flinn, B.A.

- Director, Virology & Microbial ID Operations

- Senior Virologist

- Lead Virologist

- Associate Virologist

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

**Project Number:** A15626

**Protocol Number:** INI01091313.COR

**Testing Facility:** ATS Labs  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance:** Aquaox Hypochlorous Acid Batch # AX-13196-0210

**Batch:** AX-13196-0210

### **Test Substance Characterization**

Test substance characterization as to content, stability, solubility, storage, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor.

### **STUDY DATES**

**Date Sample Received:** September 11, 2013  
**Study Initiation Date:** September 23, 2013  
**Experimental Start Date:** October 1, 2013  
**Experimental End Date:** October 11, 2013  
**Study Completion Date:** December 11, 2013

### OBJECTIVE

The objective of this study was to evaluate the antiviral properties of a product against Human Coronavirus when exposed (in suspension) for the specified exposure period. This protocol is a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E 1052).

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## SUMMARY OF RESULTS

Test Substance: Aquaox Hypochlorous Acid Batch # AX-13196-0210

Dilution Tested: Ready to use

Virus: Human Coronavirus, strain 229E, ATCC VR-740

Exposure Time: 30 seconds

Exposure Temperature: Room temperature (20.0°C)

Organic Soil Load: 1% fetal bovine serum

Efficacy Result: Under these test conditions, Aquaox (Batch # AX-13196-0210) demonstrated a 99.994% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 log<sub>10</sub>-

## TEST SYSTEM

1. Virus  
The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-740). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at 50-70°C until the day of use. On the day of use an aliquot of stock virus (ATS Labs Lot HCV-69) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Human Coronavirus on WI-38 cells.
2. Indicator Cell Cultures  
Cultures of WI-38 (human lung) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-75). The cells were propagated by ATS Labs personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

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3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures per dilution. See the report text for a more detailed explanation.

PARAMETERS TESTED FOR VIRUCIDAL EFFICACY ASSAY		
Test or Control Group	Dilutions Assayed (10010)	Cultures per Dilution
Cell Control	N/A	4
Virus Control	-2,-3,-4,-5,-6,-7,-8	4
Test Batch+ virus	-2,-3,-4,-5,-6,-7,-8	4
Cytotoxicity Control	-2,-3,-4	4
Neutralization Control	-2,-3,-4	4

**TEST METHOD**

1. Preparation of Test Substance

Aquaox (Batch# AX-13196-0210) was used as it was received from the Sponsor. The test substance removed from the original container was in solution as determined by visual observation. The test substance was at the exposure temperature prior to use in testing.

2. Treatment of Virus Suspension

For the exposure time assayed, a 1.80 ml aliquot of the test substance was dispensed into a sterile tube and mixed with a 200 µl aliquot of the stock virus suspension. The mixture was vortex mixed for 10 seconds and held for the remainder of the specified exposure time at room temperature (20.0°C). The exposure time assayed was 30 seconds. Following the exposure time, a 100 µl aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilutions (100 µl + 0.9 ml test medium) and assayed for the presence of virus. Note: To decrease the test substance cytotoxicity, the first dilution was made in FBS with the remaining dilutions in test medium.

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3. Treatment of Virus Control

For the exposure time assayed, a 200 µl aliquot of stock virus suspension was exposed to a 1.80 ml aliquot of test medium in lieu of test substance and treated as previously described. Following the exposure time, a 100 µl aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilutions (100 µl + 0.9 ml test medium) and assayed for the presence of virus. All controls employed the FBS neutralizer as described in the Treatment of Virus Suspension section. A virus control was performed for the exposure time tested. The virus control titer was used as a baseline to compare the percent and log reductions of the test parameter following exposure to the test substance.
  4. Cytotoxicity Control

A 1.80 ml aliquot of the test substance was mixed with a 200 µl aliquot of test medium containing the Sponsor requested organic soil load in lieu of virus and treated as previously described. The cytotoxicity control was held for the longest exposure time. The cytotoxicity of the cell cultures was scored at the same time as virus-test substance and virus control cultures. Cytotoxicity was graded on the basis of cell viability as determined microscopically. Cellular alterations due to toxicity were graded and reported as toxic (T) if greater than or equal to 50% of the monolayer was affected.
  5. Neutralization Control

Each cytotoxicity control mixture (above) was challenged with low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100 µl aliquot of each dilution in quadruplicate. A 100 µl aliquot of low titer stock virus was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.
  6. Infectivity Assay

The WI-38 cell line, which exhibits cytopathic effect (CPE) in the presence of Human Coronavirus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µl of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell culture labware. The cultures were microscopically scored periodically for ten days for the absence or presence of CPE, cytotoxicity, and for viability.
  7. Statistical Methods: Not applicable
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## **PROTOCOL CHANGES**

### **Protocol Amendments:**

No protocol amendments were required for this study.

### **Protocol Deviations:**

No protocol deviations occurred during this study.

## **TEST CRITERIA**

A valid test requires 1) that stock virus be recovered from the virus control and 2) that the cell controls be negative for virus.

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## **RECORD RETENTION**

### **Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

### **Test Substance Retention**

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test substance.

## **REFERENCES**

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1052 (current version).
  2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482 (current version).
  3. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A., and Lennette, E.T. editors. Seventh edition, 1995.
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## STUDY RESULTS

### Cytotoxicity and Neutralization Controls

Test substance cytotoxicity was not observed in any dilution assayed (S1.50 10910). The neutralization control demonstrated that the test substance was neutralized at s:1.50 IOQ10-

### 30 Second Exposure Time

The titer of the virus control was 5.75 log<sub>10</sub>. Following exposure, test virus infectivity was not detected in the virus-test substance mixture at any dilution tested (s;1.50 10910).

## STUDY CONCLUSION

**Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, Aquaox (Batch # AX-13196-0210), ready to use, demonstrated a 99.994% reduction in viral titer following a 30 second exposure time to Human Coronavirus as compared to the titer of the corresponding virus control. The log reduction in viral titer was 4.25 LOG<sub>10</sub>.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.



**ATTACHMENT I – Certificate of Analysis**

Issued: July 16, 2013  
Last Revised: July 29, 2013

FORM CQA-02

AQUAOX INDUSTRIES INC  
18165, Sierra Lakes Parkway,  
Suite 160-714,  
Fontana, CA 92336. USA.



**Certificate of Analysis**


Date of Manufacture: 07 / 15 / 2013  
Product Name: AX250  
Batch / Lot #: AX-13196-0210  
Production Facility: Innovacyn, Inc.  
3546 N. Riverside Ave. Rialto, CA 92377  
Testing Facility: Innovacyn, Inc.  
3546 N. Riverside Ave. Rialto, CA 92377

TEST	ANALYSIS	UNITS
FAC	226	ppm
pH	6.03	n/a
Conductivity	1225	µS/cm
ORP	943	mV
Osmolality	22	mOsm/kg

This certification states that the Intermediate product AX250, bearing the above description and lot number, has been found to conform to the internal specifications established for this product. The above lot was made in accordance with our internal specifications and current good manufacturing practices under controlled procedures.

This lot has been appropriately inspected and tested, and, to the best of our knowledge, conforms to all applicable test methods, standards and internal specifications.

This certification does not constitute any written or expressed warranty or guarantee of any kind.

Rebecca Lei   
QA Regulatory Specialist

Date: 7/29/13

EXACT COPY  
INITIALS/mm DATE 12-10-13

# GLP REPORT

**TEST FACILITY**

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NAMSA  
6750 Wales Road  
Northwood, OH 43619  
419.666.9455

**SPONSOR**

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Michel van Schaik  
Aquafox Industries, Inc.  
16155 Sierra Lakes Pkwy  
Suite 160-714  
Fontana, CA 92336

CONFIDENTIAL

**STUDY TITLE**

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ISO Ocular Irritation Study in Rabbits

**TEST ARTICLE NAME**

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AX250

**TEST ARTICLE IDENTIFICATION**

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Lot AX-13065-0210



## Summary

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The test article, AX250, was evaluated for the potential to produce ocular irritation in rabbits based on the requirements of ISO 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization.

A single 0.1 mL dose of the test article was placed in the right eyes of three male New Zealand White rabbits. The left eyes remained untreated and served as the control condition. Ocular reactions based on a modified Draize classification were evaluated immediately postdose and at 1, 24, 48, and 72 hours after dosing.

There was no irritation or other ocular effects on the cornea, iris, or conjunctiva observed in the treated eyes as compared to the untreated control eyes. The test article was not considered an irritant to the ocular tissue of the rabbit.

Supervisory Personnel:            Lisa A. Severhof, BA  
   Manager, Toxicology

   Colleen M. Stevenson, AA  
   Supervisor, Toxicology

Study Director Approval:

*Laura R. Ott*  
\_\_\_\_\_  
Laura R. Ott, BS, LAT  
Medical Research Manager

*13 May 2013*  
\_\_\_\_\_  
Date Completed

Authorization for duplication of this report, except in whole, is reserved pending NAMSA's written approval.

# TECHNICAL SUMMARY – BOEING D6-7127 PROTOCOL

## Aquaox Disinfectant 275 | Aquaox Disinfectant 525

- I. Protocol: Boeing D6-7127 Rev P incorporating PDD 6-8 –  
Cleaning Interiors of Commercial Transport Aircraft  
Category: Disinfectants

- II. Test Liquids / Properties (as shown on Certificate of Analysis):

### Aquaox Disinfectant 275

TEST	ANALYSIS	UNITS
Free Available Chlorine	302	ppm
pH	6.72	n/a
Conductivity	2544	µS/cm
ORP	878	mV

### Aquaox Disinfectant 525

TEST	ANALYSIS	UNITS
Free Available Chlorine	546	ppm
pH	6.86	n/a
Conductivity	2099	µS/cm
ORP	913	mV

- III. Summary of Test Protocol

The above mentioned liquids have been evaluated according to the Boeing D6-7127 Test Protocol. The test protocol includes 11 different tests as mentioned in a) – k), and each test will be summarized in the subsequent paragraphs. The chemicals, Aquaox Disinfectants 275 and 525, tested for each test are stated under the result table and conclusion of each section.

- a) Sandwich Corrosion Test
- b) Immersion Corrosion Test
- c) Rubber Test
- d) Sealant Test
- e) Painted Surface Test
- f) Tedlar Surface Test
- g) Viyle Surface Test
- h) Fabric and Carpet Test
- i) Leather and Naugahude Test
- j) Polycarbonate Crazing Test
- k) Flash Point Test

a) **SANDWICH CORROSION TEST (Reference: ASTM F1110)**

This test method is intended to be used to qualify and approve chemicals employed in aircraft maintenance operations. The method determines whether aircraft structural aluminum alloys are liable to be corroded or damaged by application of the test chemicals during routine maintenance operations. It evaluates the corrosiveness of test chemicals when present between faying surfaces of aluminum alloys commonly used for aircraft structures. Clad 7075-T6 Aluminum Alloy (AMS 4049) and



Bare 7075-T6 Aluminum Alloy (AMS 4045) anodized per MILA-8625 Type I are used as the test surfaces for this test.

Interpretation of the test results is based on a comparison of the appearance of faying surfaces of three sets of coupons. One set of test coupons is exposed with reagent water only in the faying surfaces to establish the baseline controls. The surfaces exposed to the test chemicals are compared with those exposed to reagent water only. Any corrosion in excess of that shown by the control group is considered as non-conformed.

The relative corrosion severity rating system below is used to allow for a numerical classification of the test results.

Relative corrosion severity rating system:

0—No visible corrosion and no discoloration present

1—Very slight corrosion or very slight discoloration, and/or up to 5 % of area corroded

2—Discoloration and/or up to 10 % of area corroded

3—Discoloration and/or up to 25 % of area corroded

4—Discoloration and/or more than 25 % of area corroded, and/or pitting present

(A) “Area” refers to area where the test material was applied.

**Aquaox Test Results:**

Test Chemical	Clad 7075-T6 Aluminum Alloy	Bare 7075-T6 Aluminum Alloy	Test Result
Aquaox Disinfectant 275	1	1	Conforms
Test Control	1	1	

**Conclusion:**

Test result of Aquaox Disinfectant 525 does not conform on the Clad 7075 T6 Aluminum Alloy surface because corrosion caused by the test chemical is in excess of that caused by the test control.

Test results of Aquaox Disinfectant 275 conform for all test surfaces on all test criteria.

**b) IMMERSION CORROSION TEST (Reference: ASTM F483)**

This method determines the corrosiveness of chemicals on aircraft metals with time under conditions of total immersion through determining the weight change of the test metals after they are immersed with the test chemicals. This method screens test chemicals to ensure compliance with specified weight change criteria. Test chemicals are evaluated on the following panels, 1) Clad 2024-T3 Aluminum (QQ-A-250/5), 2) Bare 2024-T3 Aluminum (QQ-A-250/4) alodined per MIL-C-5541, 3) Bare 2024-T3 Aluminum (QQ-A-250/4) anodized per MIL-A-8625 Type I, and 4) Bare 7178-T6 Aluminum (QQ-A-250/14) anodized per MIL-A-8625 Type I.

Small sections of the above materials are exposed to the test chemical and dried. The weight of the test panel is measured before and after the exposure and drying times. The test chemical shall neither show evidence of corrosion of the test panels nor cause a weight change of the test panels greater than ± 10mg in a 24-hour immersion period per each 1” x 2” test panel.

**Aquaox Test Results:**

Test Chemical	Test Panel	Weight Loss in mg (per 1" x 2" panel)	Test Result
<b>Aquaox Disinfectant 525</b>	Clad 2024-T3 Aluminum (QQ-A-250/5)	0.1	Conforms
	Bare 2024-T3 Aluminum (QQ-A-250/4) alodined per MIL-C-5541	2.3	Conforms
	Bare 2024-T3 Aluminum (QQ-A-250/4) anodized per MIL-A-8625 Type I	0.3	Conforms
	Bare 7178-T6 Aluminum (QQ-A-250/14) anodized per MIL-A-8625 Type I	2.9	Conforms

**Conclusion:** Test results of the Aquaox Disinfectant 525 conform on all test panels for all test criteria.

**c) RUBBER TEST (Reference: ASTM D471)**

This test method evaluates the comparative ability of rubber and rubber-like compositions to withstand the effect of test liquids. It is designed for testing: (1) specimens of vulcanized rubber cut from standard sheets, (2) specimens cut from fabric coated with vulcanized rubber, or (3) finished articles of commerce. Rubber specimens are immersed in the test chemical for 24 hours and are evaluated on the following property changes. Changes in properties shall not exceed the following criteria.

**Aquaox Test Results:**

Test Chemical	Property	Maximum Change Allowed	Test Result
<b>Aquaox Disinfectant 525</b>	Tensile Strength	25 % Loss	< 5 %
	Elongation	25 % Loss	< 5 %
	Volume	± 15 % Loss	< 5 %

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**d) SEALANT TEST**

This test method evaluates a sealed surface to withstand the effect of the test liquids. An Aluminum surface primed with paint (that is normally used in Boeing aircrafts) is smeared with the BMS 5-95 Sealant, a sealant commonly used in aircraft materials. The aircraft surface is sealed with 4" x 1" x 0.25" (length x width x thickness) sealant strips, and is immersed in the test liquid for 70 ± 2 hours for 120 ± 5 °F. No lifting or loss of adhesion shall be observed on the test surface after immersion.

**Aquaox Test Results:**

Test Chemical	Test Result
<b>Aquaox Disinfectant 525</b>	Sealant did not lift at edges or lose adhesion.
<b>Test Control</b>	No lifting or loss of adhesion when pried away from edge.

**Conclusion:** Test result of Aquaox Disinfectant 525 conforms on all test surfaces for all test criteria.

**e) PAINTED SURFACE TEST (Reference: ASTM F502)**

This test method covers the determination of the effects of cleaning solutions and chemical maintenance materials on painted aircraft surfaces. Plate and sheet specimens of aluminum alloy are examined under the test liquids. This test method is applicable to any painted film that is exposed to cleaning materials. Test liquid is heated to  $149 \pm 4$  °F and applied to a painted surface having an initial surface temperature of  $72 \pm 2$  °F. Following exposure, streaking, discoloration, and blistering will be determined visually on the test surface. Softening will also be determined with a series of specially prepared pencils wherein determination of the softest pencil to rupture the paint film on the test surface is made. Test liquid shall not produce any color change and shall not decrease the paint film hardness for more than 2 pencil hardnesses.

**Aquaox Test Results:**

Test Chemical	Property	Test Result
Aquaox Disinfectant 525	Pencil Hardness Change	0
	Color Change	None

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**f) TEDLAR SURFACE TEST**

This method is used to ensure that test liquids do not leave any scratching, color change or staining on the test tedlar surfaces after exposure to the test liquids. Visual observation is used to determine any scratching or permanent stains which require polishing to remove. Test surfaces are exposed to the test liquid for a specific amount of time in room temperature and then rinsed. Exposed surfaces shall not show any scratching, any greater-than-minimal color change or any staining.

**Aquaox Test Results:**

Test Chemical	Test Result
Aquaox Disinfectant 525	No Scratching, Color Change or Staining of specimens is observed.

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**g) VINYL SURFACE TEST**

This method is used to ensure that test liquids do not leave any cracking, brittleness, color change or staining on the test vinyl surfaces after exposure to the test liquids. Test surfaces are exposed to the test liquid for a specific amount of time in room temperature and then rinsed. Exposed surfaces then are visually examined and shall not show any of this above mentioned signs.

**Aquaox Test Results:**

Test Chemical	Test Result
Aquaox Disinfectant 525	No Scratching, Color Change or Staining of specimens is observed.

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**h) FABRIC AND CARPET TEST**

This method is used to ensure that test liquids do not cause any color change or staining on the test fabric and carpet surfaces after exposure to the test liquids. Test surfaces are exposed to the test liquid for a specific amount of time in room temperature and then rinsed. Exposed surfaces are then visually evaluated to check for any color change or staining after exposure to the test liquid.

The test fabric and carpet surfaces are also evaluated on its flammability after being immersed into the test liquid and dried. Test surfaces are completely coated with the test liquid, let soaked for a specific amount of time and then allowed to dry. The dried surfaces are then hung, applied with a flame and allowed for a vertical burn for 12 seconds. Self-Extinguishing time, Burn Length and Drip Extinguish Time will then be determined on the test surfaces. Each of these parameters shall not exceed the maximum value as stated in the table below.

**Aquaox Test Results:**

Test Chemical	Test Surface	Property	Maximum Value	Test Result	
Aquaox Disinfectant 525	Upholstery	Color Change	N/A	None	
		Staining	N/A	None	
		Flammability	Extinguishing Time	15 seconds	< 3 seconds
			Burn Length	8 inches	7 inches
			Drip Extinguish Time	5 seconds	< 3 seconds
	Carpet	Color Change	N/A	None	
		Staining	N/A	None	
		Flammability	Extinguishing Time	15 seconds	< 3 seconds
			Burn Length	8 inches	4 inches
			Drip Extinguish Time	5 seconds	< 3 seconds

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**i) LEATHER AND NAUGAHYDE TEST**

This practice is used to evaluate the compatibility of the test liquids with the test surfaces, i.e. lather and naugahyde surfaces. Test surfaces are exposed to the test liquid for a specific amount of time in room temperature and then rinsed. Visual observation is used for determining any signs of crackling or brittleness, as well as any color change or staining of exposed surfaces. Exposed surfaces shall not show any of the above mentioned signs after exposure to the test liquid.

**Aquaox Test Results:**

Test Chemical	Property	Test Result
Aquaox Disinfectant 525	Cracking or Brittleness	None
	Color Change or Staining	None

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**j) POLYCARBONATE CRAZING TEST (Reference: ASTM F484)**

This test method covers the procedure for determining the crazing effect caused by test liquids on the test materials under bending stress. The materials to be tested include Lexan 9600 and BMS8-400 BAC 70913 plastics, which are commonly used in aircraft structures. Each test surface is bent under a strain of 0.008 and the stressed materials are then exposed to the test liquid for 10 minutes. Exposed surfaces are then visually examined on any signs of cracking or crazing after exposure to test liquids.

**Aquaox Test Result / Conclusion:**

Test Chemical	Test Surface	Test Result
Aquaox Disinfectant 525	Lexan 9600	No cracking or crazing
	BMS8-400 BAC 70913	No cracking or crazing

**Conclusion:** Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

**k) FLASH POINT TEST (Reference: ASTM D93)**

This test is done for information only. The flash point of the test liquid is determined following the ASTM D93 method, all cleaning candidates having a flash point not lower than 212°F shall be approved by the Fire Protection Engineering before they can be evaluated to be used.

**Aquaox Test Result / Conclusion:** No flash point is observed to 212°F for the test liquid.

**IV. Summary of all Test Results**

Test results of Aquaox Disinfectant 525 conform for all test criteria on all the tests included in the Boeing D6-7127 Protocol except for the Clad 7075 T6 Aluminum Alloy surface of the Sandwich Corrosion Test. This test was later repeated with the Aquaox Disinfectant 275, with a passing test result.

**V. References**

- SMI Test Report, Boeing D6-7127 Protocol, Aquaox Disinfectant 525, SMI/REF # 1412-370
- SMI Test Report, Boeing D6-7127 Protocol, Aquaox Disinfectant 275, SMI/REF # 1503-629
- Aquaox Certificate of Analysis, Aquaox Disinfectant 525, dated 011415
- Aquaox Certificate of Analysis, Aquaox Disinfectant 275, dated 032715