# FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Porcine Respiratory & Reproductive Syndrome (PRRS) virus**

PRODUCT IDENTITY

West Perox
Lot 070701 and Lot 070704

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

WPC02070723.PRRS

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

September 13, 2023

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PROJECT NUMBER

A38360

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: PIERRE STEWART

MANAGER TECHNICAL SERVICES
Title

Signature

Date: 07-02-2024

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exception(s):

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

Characterization of the compounds was performed by the Sponsor prior to use in the study, however not in accordance with 40 CFR Part 160.

Submitter: _____ Date: 07-02-2024

Sponsor: _____ Date: 07-02-2024

Study Director: _____ Date: 9-13-23
Mary J. Miller, M.T.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

| Phase Inspected | Date of Phase Inspection | Date Reported to Study Director | Date Reported to Management |
|---|---|---|---|
| Critical Phase Audit: Preparation of Test Substance | August 22, 2023 | August 22, 2023 | September 13, 2023 |
| Final Report | September 1, 2023 | September 1, 2023 | |

Quality Assurance Specialist: _____ Date: 09/13/23

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

| | |
|---|---|
| Kelleen Lauer, M.S. | - General Manager |
| Nicole Felicelli, B.A. | - Manager, Study Director Operations |
| Miranda Peskar, B.S. | - Core Services Laboratory Operations Manager |
| Joseph Artus, B.S. | - Virologist |
| Sydney Sawatzke, B.S. | - Associate Virologist |

## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A38360

**Protocol Number:** WPC02070723.PRRS

**Sponsor:** West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**Testing Facility:** Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance Name:** West Perox

**Lot/Batch(s):** Lot 070701 and Lot 070704

**Manufacture Date:** Lot 070701 – July 7, 2023
Lot 070704 – July 7, 2023

**Test Substance Characterization**
Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). Test substance stability testing of the formulation was not performed prior to or concurrent with its use in this study. The Test Substance Certificate of Analysis Report(s) may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

### STUDY DATES

**Date Sample Received:** July 27, 2023
**Study Initiation Date:** August 10, 2023
**Experimental Start Date:** August 22, 2023 (Start time: 12:51 p.m.)
**Experimental End Date:** August 29, 2023 (End time: 9:49 a.m.)
**Study Completion Date:** See Page 1 of Report

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

Test Substance: West Perox, Lot 070701 and Lot 070704

Dilution: 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water

Virus: Porcine Respiratory & Reproductive Syndrome (PRRS) virus, Strain NVSL, Obtained from the University of Kentucky

Exposure Time: 10 minutes

Exposure Temperature: 20±2°C (21.0°C)

Exposure Humidity: 56.87%

Organic Soil Load: 5% fetal bovine serum

Efficacy Result:

| Test Organism | Test Results | | Efficacy Performance |
|---|---|---|---|
| | West Perox | | |
| | Lot 070701 | Lot 070704 | |
| Porcine Respiratory & Reproductive Syndrome (PRRS) virus | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | Requirements met |

## TEST SYSTEM

1. Virus

The NVSL strain of Porcine Respiratory & Reproductive Syndrome (PRRS) virus used for this study was obtained from the University of Kentucky. The 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 ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot PRR-33) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Porcine Respiratory & Reproductive Syndrome virus on MARC-145 cells.

2. Indicator Cell Cultures

Cultures of MARC-145 cells were originally obtained from the National Veterinary Services Laboratory, Ames, IA. The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. 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.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 μg/mL gentamicin, 100 units/mL penicillin, and 2.5 μg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two lots of West Perox (Lot 070701 and Lot 070704) were tested at a 1:64 dilution defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 64.0 mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was at the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 294 ppm) and used on the day of testing.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 21.0°C in a relative humidity of 56.87% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel (16.8%). On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance
For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 56.87% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 56.87% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls
A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into MARC-145 cell cultures. Cytotoxicity of the MARC-145 cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of 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 (approximately 100 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

9. Infectivity Assays
The MARC-145 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Respiratory & Reproductive Syndrome virus, 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. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$) in sterile disposable cell culture labware. The cell cultures were examined approximately 20.25 hours post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PLANNED PROTOCOL CHANGES

**Protocol Amendments:**
No protocol amendments were required for this study.

**Planned Protocol Deviations:**
No planned protocol deviations occurred during this study.

## UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier):

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

### Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

### Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

## RECORD RETENTION

**Study Specific Documents**
All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

## RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

**Key for Control and Results Tables:**

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

## ANALYSIS

Results of tests with two lots of West Perox (Lot 070701 and Lot 070704), diluted 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water, exposed to Porcine Respiratory & Reproductive Syndrome virus in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.0°C) and 56.87% relative humidity for 10 minutes were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.50 $\log_{10}$/100 µL. The titer of the dried virus control was 5.25 $\log_{10}$/100 µL (5.55 $\log_{10}$/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested [≤0.50 $\log_{10}$/100 µL (≤0.80 $\log_{10}$/carrier)]. Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/100 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier, was ≥4.75 $\log_{10}$ for both lots.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, West Perox, diluted 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 $\log_{10}$ reduction in titer of Porcine Respiratory & Reproductive Syndrome virus following a 10 minute exposure time at 20±2°C (21.0°C) and 56.87% relative humidity as required by the U.S. EPA and Health Canada.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

## TABLE 1: Virus Controls and Test Results

### Effects of West Perox (Lot 070701 and Lot 070704) Following a 10 Minute Exposure to Porcine Respiratory & Reproductive Syndrome Virus Dried on an Inanimate Surface

| Dilution | Input Virus Control | Dried Virus Control | Porcine Respiratory & Reproductive Syndrome virus + Lot 070701 | Porcine Respiratory & Reproductive Syndrome virus + Lot 070704 |
|---|---|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | + + | + + + + | 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 0 |
| $10^{-6}$ | + 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | + 0 | 0 0 0 0 | NT | NT |
| $10^{-8}$ | 0 0 | 0 0 0 0 | NT | NT |
| $TCID_{50}$/100 µL | $10^{6.50}$ | $10^{5.25}$ | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |
| $TCID_{50}$/carrier | NA | $10^{5.55}$ | $\leq 10^{0.80}$ | $\leq 10^{0.80}$ |
| Log Reduction[1] | NA | NA | ≥4.75 | ≥4.75 |

[1] This reduction is both per volume inoculated per well and per carrier.

## TABLE 2: Cytotoxicity Control Results

### Cytotoxicity of West Perox on MARC-145 Cell Cultures

| Dilution | Cytotoxicity Control Lot 070701 | Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | 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 |
| $TCD_{50}$/100 µL | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |

## TABLE 3: Neutralization Control Results

### Non-Virucidal Level of the Test Substance (Neutralization Control)

| Dilution | Test Virus + Cytotoxicity Control Lot 070701 | Test Virus + Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + + + | + + + + |
| $10^{-2}$ | + + + + | + + + + |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of $\leq 0.50$ $\log_{10}$ for both lots.

## ATTACHMENT I: Test Substance Certificate(s) of Analysis
## Lot 070701

West Penetone

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070701

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.8 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.5 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.2 |

July 14, 2023
Date

Pierre Stewart
Quality Control

## Test Substance Certificate(s) of Analysis
## Lot 070704

West Penetone®

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070704

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.9 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.4 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.0 |

July 14, 2023
Date

Pierre Stewart
Quality Control

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A38360
Test Substance Tracking # TS072723,WPC02-PRRS 8-10-23

# PROTOCOL

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Porcine Respiratory & Reproductive Syndrome (PRRS) virus

**PROTOCOL NUMBER**

WPC02070723.PRRS

**SPONSOR**

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**PERFORMING LABORATORY**

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**DATE**

July 7, 2023

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

**TEST SUBSTANCE CHARACTERIZATION**

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

**SCHEDULING AND DISCLAIMER OF WARRANTY**

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 23, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of September 20, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

**JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM**

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The MARC-145 cell line, which supports the growth of the Porcine Respiratory & Reproductive Syndrome (PRRS) virus, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.

**TEST PRINCIPLE**
A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

**STUDY DESIGN**
Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

**VIRUS**
The NVSL strain of Porcine Respiratory & Reproductive Syndrome (PRRS) virus to be used for this study was obtained from the University of Kentucky ~~(U of KY (NVSL strain))~~.(1) Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

**INDICATOR CELL CULTURES**
Cultures of MARC-145 cells are received from National Veterinary Services Laboratory, Ames, IA.. Cultures are maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO2. MARC-145 cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

(1) Revised per 8-10-23 email. mm 8-10-23

**TEST MEDIUM**

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

**PREPARATION OF OECD HARD WATER (if applicable)**

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

**PREPARATION OF UN-SOFTENED TAP WATER (if applicable)**

Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

**PREPARATION OF TEST SUBSTANCE**

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

**PREPARATION OF VIRUS FILMS**

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

**TEST METHOD**

**Preparation of Sephadex Gel Filtration Columns**

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

**Input Virus Control**

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

**Treatment of Virus Films with the Test Substance**
For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be 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 will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

**Infectivity Assays**
The MARC-145 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Respiratory & Reproductive Syndrome (PRRS) virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.

## DATA ANALYSIS

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$\text{- Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

**Calculation of Log Reduction**
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**Calculation of Infectious Units**

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

**Statistical Methods**
None used.

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**
The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

**METHOD FOR CONTROL OF BIAS**: N/A

**STUDY ACCEPTANCE CRITERIA**
Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA and Health Canada Submission**
A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

**FINAL REPORT**
The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

**PROTOCOL CHANGES**
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

**TEST SUBSTANCE RETENTION**
Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

**RECORD RETENTION**

**Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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.

**Facility Specific Documents**

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

**PROPOSED STATISTICAL METHODS:** N/A



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • https://www.element.com

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

## STUDY INFORMATION

*(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)*

**Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)**

| Test Substance Name | Lot/Batch Number | Manufacture Date |
|---|---|---|
| West Perox | 070701 | 07/07/2023 |
| West Perox | 070704 | 07/07/2023 |

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**

☑ Quaternary ammonia ☐ Peracetic acid ☐ Sodium hypochlorite
☐ Iodophor ☑ Peroxide ☐ Other _____

**Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):** 5.8 %w/w QUATERNARY AMMONIA + 7.5 %w/w PEROXIDE
*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**Storage Conditions**

☑ Room Temperature
☐ 2-8°C
☐ Other _____

**Hazards**

☐ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
☐ As Follows: _____

**Product Preparation**

☐ No dilution required, Use as received (RTU)
☑ *Dilution(s) to be tested:

1:64 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 64 parts (amount of diluent)

☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
☐ OECD Hard Water: 375 ppm (338-394 ppm)
☑ Other 300 ppm AOAC synthetic hard water

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.***

**Test Virus:** Porcine Respiratory & Reproductive Syndrome (PRRS) virus

**Exposure Time:** 10 minutes

**Exposure Temperature:** ☐ Room temperature
☑ Other: 20±2°C (please specify range)

**Directions for application of aerosol/spray products:**

☑ Spray instructions are not applicable.

Trigger spray application:

☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:

☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**

☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
☑ 5% fetal bovine serum
☐ Other _____

**Number of Carriers to be Tested**

☑ One (typical for U.S. EPA submission)
☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**

*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*

☐ Sprayer(s) and bottle(s) are provided by the Sponsor
☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**

☑ U.S. EPA
☑ Health Canada
☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**COMPLIANCE**

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

**PROTOCOL MODIFICATIONS**

☐ Approved without modification
☑ Approved with modification
Reference 2 is updated to: American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
Reference 12 is updated to: U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

**PROTOCOL ATTACHMENTS**

Supplemental Information Form Attached - ☐ Yes ☑ No

**TEST SUBSTANCE SHIPMENT STATUS**

*(This section is for informational purposes only.)*
☐ Test Substance is already present at Element Materials Technology Eagan.
☑ Test Substance has been or will be shipped to Element Materials Technology Eagan.
Date of expected receipt at Element Materials Technology Eagan: _____

**TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))**

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** (1)

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☐ **Yes** ☑ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

(1) Added per 8-10-23 email. mm 8-10-23

***PROPRIETARY INFORMATION***



## APPROVAL SIGNATURES

***SPONSOR:***

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: [signature] DATE: JUL 20 2023

PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

*For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.*

***Other individuals authorized to receive information regarding this study:*** ☐ See Attached

***Element Materials Technology Eagan:***

NAME: Mary J. Miller
Study Director

SIGNATURE: Mary J. Miller DATE: 8-10-23
Study Director

# FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Porcine Epidemic Diarrhea Virus**

PRODUCT IDENTITY

West Perox
Lot 070701 and Lot 070704

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

WPC02070723.PEDV

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

September 13, 2023

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PROJECT NUMBER

A38361

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: PIERRE STEWART

MANAGER OF TECHNICAL SERVICES
Title

Signature

Date: 07-02-2024

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exception(s):

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

Characterization of the compounds was performed by the Sponsor prior to use in the study, however not in accordance with 40 CFR Part 160.

Submitter: _____ Date: 07-02-2024

Sponsor: _____ Date: 07-02-2024

Study Director: _____ Date: 9-13-23
Mary J. Miller, M.T.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

| Phase Inspected | Date of Phase Inspection | Date Reported to Study Director | Date Reported to Management |
|---|---|---|---|
| Critical Phase Audit: Preparation of Test Substance | August 24, 2023 | August 24, 2023 | September 13, 2023 |
| Final Report | September 5, 2023 | September 6, 2023 | |

Quality Assurance Specialist: _____ Date: 09/13/23

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

| | |
|---|---|
| Kelleen Lauer, M.S. | - General Manager |
| Nicole Felicelli, B.A. | - Manager, Study Director Operations |
| Miranda Peskar, B.S. | - Core Services Laboratory Operations Manager |
| Sydney Sawatzke, B.S. | - Associate Virologist |

# STUDY REPORT

## GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A38361

**Protocol Number:** WPC02070723.PEDV

**Sponsor:** West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**Testing Facility:** Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** West Perox

**Lot/Batch(s):** Lot 070701 and Lot 070704

**Manufacture Date:** Lot 070701 – July 7, 2023
Lot 070704 – July 7, 2023

**Test Substance Characterization**
Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). Test substance stability testing of the formulation was not performed prior to or concurrent with its use in this study. The Test Substance Certificate of Analysis Report(s) may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

## STUDY DATES

**Date Sample Received:** July 27, 2023
**Study Initiation Date:** August 10, 2023
**Experimental Start Date:** August 24, 2023 (Start time: 10:49 a.m.)
**Experimental End Date:** August 31, 2023 (End time: 12:27 p.m.)
**Study Completion Date:** See Page 1 of Report

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

Test Substance: West Perox, Lot 070701 and Lot 070704

Dilution: 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water

Virus: Porcine Epidemic Diarrhea Virus, Strain Colorado 2013 Isolate Obtained from the National Veterinary Services Laboratories, Ames, IA.

Exposure Time: 10 minutes

Exposure Temperature: 20±2°C (21.0°C)

Exposure Humidity: 50.17%

Organic Soil Load: 5% fetal bovine serum

Efficacy Result:

| Test Organism | Test Results | | Efficacy Performance |
|---|---|---|---|
| | West Perox | | |
| | Lot 070701 | Lot 070704 | |
| Porcine Epidemic Diarrhea Virus | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | Requirements met |

## TEST SYSTEM

1. Virus

The Colorado 2013 Isolate strain of Porcine Epidemic Diarrhea Virus used for this study was obtained from the National Veterinary Services Laboratories, Ames, IA. The stock virus was prepared by collecting the supernatant culture fluid from 50-75% 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 ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot PED-66) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Porcine Epidemic Diarrhea Virus on Vero 76 cells.

2. Indicator Cell Cultures

Cultures of Vero 76 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1587). The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. 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.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 10% tryptose phosphate broth, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two lots of West Perox (Lot 070701 and Lot 070704) were tested at a 1:64 dilution defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 64.0 mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was at the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 300 ppm) and used on the day of testing.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 50% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel (16.8%). On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance
For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 50.17% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minute at 20±2°C (21.0°C) and 50.17% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls
A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into Vero 76 cell cultures. Cytotoxicity of the Vero 76 cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of 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 200 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus (approximately 16 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates. Although the calculated value for infectious units used in the neutralization control was lower than the typical target range, the low inoculum increases the stringency of this control. Growth of virus in the neutralization control indicates neutralization of the test substance was demonstrated as required. Therefore, the neutralization control is valid.

9. Infectivity Assays
The Vero 76 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Epidemic Diarrhea Virus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 200 µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$) in sterile disposable cell culture labware. The cell cultures were examined approximately 4 hours post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PLANNED PROTOCOL CHANGES

**Protocol Amendments:**
No protocol amendments were required for this study.

**Planned Protocol Deviations:**
No planned protocol deviations occurred during this study.

## UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier):

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

### Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

### Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus: $10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

## RECORD RETENTION

### Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

## RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

**Key for Control and Results Tables:**

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

## ANALYSIS

Results of tests with two lots of West Perox (Lot 070701 and Lot 070704), diluted 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water, exposed to Porcine Epidemic Diarrhea Virus in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.0°C) and 50.17% relative humidity for 10 minute were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.00 $\log_{10}$/200 µL. The titer of the dried virus control was 5.00 $\log_{10}$/200 µL (5.00 $\log_{10}$/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested [≤0.50 $\log_{10}$/200 µL (≤0.50 $\log_{10}$/carrier)]. Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/200 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/200 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier, was ≥4.50 $\log_{10}$ for both lots.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, West Perox, diluted 1:64 defined as 1 part test substance + 64 parts of 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 $log_{10}$ reduction in titer of Porcine Epidemic Diarrhea Virus following a 10 minute exposure time at 20±2°C (21.0°C) and 50.17% relative humidity as required by the U.S. EPA and Health Canada.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

## TABLE 1: Virus Controls and Test Results

### Effects of West Perox (Lot 070701 and Lot 070704) Following a 10 Minute Exposure to Porcine Epidemic Diarrhea Virus Dried on an Inanimate Surface

| Dilution | Input Virus Control | Dried Virus Control | Porcine Epidemic Diarrhea Virus + Lot 070701 | Porcine Epidemic Diarrhea Virus + Lot 070704 |
|---|---|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | + + | + + + + | 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 0 0 |
| $10^{-6}$ | 0 + | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-8}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-9}$ | 0 0 | NT | NT | NT |
| $TCID_{50}$/200 µL | $10^{6.00}$ | $10^{5.00}$ | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |
| $TCID_{50}$/carrier | NA | $10^{5.00}$ | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |
| Log Reduction[1] | NA | NA | ≥4.50 | ≥4.50 |

[1] This reduction is both per volume inoculated per well and per carrier.

**TABLE 2: Cytotoxicity Control Results**

**Cytotoxicity of West Perox on Vero 76 Cell Cultures**

| Dilution | Cytotoxicity Control Lot 070701 | Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | 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 |
| $TCD_{50}$/200 µL | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |

**TABLE 3: Neutralization Control Results**

**Non-Virucidal Level of the Test Substance (Neutralization Control)**

| Dilution | Test Virus + Cytotoxicity Control Lot 070701 | Test Virus + Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + + + | + + + + |
| $10^{-2}$ | + + + + | + + + + |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/200 µL of $\leq 0.50$ $\log_{10}$ for both lots.

## ATTACHMENT I: Test Substance Certificate(s) of Analysis
## Lot 070701

West Penetone®

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070701

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.8 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.5 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.2 |

July 14 2023
Date

Pierre Stewart
Quality Control

## Test Substance Certificate(s) of Analysis
## Lot 070704

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070704

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.9 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.4 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.0 |

July 14, 2023
Date

Pierre Stewart
Quality Control

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A38361
Test Substance Tracking # TS072723.WPC02 MM 8-10-23

**PROTOCOL**

# Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Porcine Epidemic Diarrhea Virus

**PROTOCOL NUMBER**

WPC02070723.PEDV

**SPONSOR**

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**PERFORMING LABORATORY**

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**DATE**

July 7, 2023

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

**TEST SUBSTANCE CHARACTERIZATION**

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

**SCHEDULING AND DISCLAIMER OF WARRANTY**

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 23, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of September 20, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

**JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM**

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The Vero 76 cell line, which supports the growth of the Porcine Epidemic Diarrhea Virus, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.

**TEST PRINCIPLE**
A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

**STUDY DESIGN**
Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

**VIRUS**
The Colorado 2013 Isolate strain of Porcine Epidemic Diarrhea Virus to be used for this study was obtained from the National Veterinary Services Laboratories (NVSL), Ames, IA. ~~(Colorado 2013 Isolate).~~ (1) Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. Note: If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

**INDICATOR CELL CULTURES**
Cultures of Vero 76 cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CRL-1587). The cells are propagated by Element Materials Technology Eagan personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. The confluency of the cells will be appropriate for the test virus. Vero 76 cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

(1) Revised per 8-10-23 email. mm 8-10-23

**TEST MEDIUM**

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

**PREPARATION OF OECD HARD WATER (if applicable)**

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

**PREPARATION OF UN-SOFTENED TAP WATER (if applicable)**

Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

**PREPARATION OF TEST SUBSTANCE**

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

**PREPARATION OF VIRUS FILMS**

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

**TEST METHOD**

**Preparation of Sephadex Gel Filtration Columns**

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

**Input Virus Control**

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

**Treatment of Virus Films with the Test Substance**
For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be 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 will be inoculated with a 200 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

**Infectivity Assays**
The Vero 76 cell line, which exhibits cytopathic effect (CPE) in the presence of Porcine Epidemic Diarrhea Virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with ~~100 µL~~ 0 of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.
(1) Corrected to 200uL per 8-10-23 email. mm 8-10-23

**DATA ANALYSIS**

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

**Calculation of Log Reduction**
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**Calculation of Infectious Units**

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

**Statistical Methods**
None used.

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**

The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

**METHOD FOR CONTROL OF BIAS**: N/A

**STUDY ACCEPTANCE CRITERIA**

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA and Health Canada Submission**

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

**FINAL REPORT**

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

**PROTOCOL CHANGES**

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

**TEST SUBSTANCE RETENTION**

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.



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**RECORD RETENTION**

**Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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.

**Facility Specific Documents**

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

**PROPOSED STATISTICAL METHODS:** N/A



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## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

## STUDY INFORMATION

*(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)*

**Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)**

| Test Substance Name | Lot/Batch Number | Manufacture Date |
|---|---|---|
| West Perox | 070701 | 07/07/2023 |
| West Perox | 070704 | 07/07/2023 |

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**

☑ Quaternary ammonia ☐ Peracetic acid ☐ Sodium hypochlorite
☐ Iodophor ☑ Peroxide ☐ Other _____

**Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):** 5.8 % QUATERNARY (w/w) + 7.5% PEROXIDE
*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**Storage Conditions**

☑ Room Temperature
☐ 2-8°C
☐ Other _____

**Hazards**

(1)
☐ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
☐ As Follows: _____

**Product Preparation**

☐ No dilution required, Use as received (RTU)
☑ *Dilution(s) to be tested:

1:64 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 64 parts (amount of diluent)

☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
☐ OECD Hard Water: 375 ppm (338-394 ppm)
☑ Other 300 ppm AOAC synthetic hard water

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.**

**Test Virus:** Porcine Epidemic Diarrhea Virus

**Exposure Time:** 10 minutes

**Exposure Temperature:** ☐ Room temperature
☑ Other: 20±2°C (please specify range)

**Directions for application of aerosol/spray products:**

☑ Spray instructions are not applicable.

Trigger spray application:

☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:

☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**

☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
☑ 5% fetal bovine serum
☐ Other _____

**Number of Carriers to be Tested**

☑ One (typical for U.S. EPA submission)
☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

(1) Added per 8-10-23 email. MMS-10-23

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**

*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*

- ☐ Sprayer(s) and bottle(s) are provided by the Sponsor
- ☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
- ☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**

- ☑ U.S. EPA
- ☑ Health Canada
- ☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**COMPLIANCE**

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.

- ☑ Yes
- ☐ No (Non-GLP or Development Study)

**PROTOCOL MODIFICATIONS**

- ☐ Approved without modification
- ☑ Approved with modification

Reference 2 is updated to: American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization. E1482-23.

Reference 12 is updated to: U.S. Environmental Protection Agency. Office of Pesticide Programs SOP Number: MB-30-03. Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products. November 14, 2022.

**PROTOCOL ATTACHMENTS**

Supplemental Information Form Attached - ☐ Yes ☑ No

**TEST SUBSTANCE SHIPMENT STATUS**

*(This section is for informational purposes only.)*

- ☐ Test Substance is already present at Element Materials Technology Eagan.
- ☑ Test Substance has been or will be shipped to Element Materials Technology Eagan.

Date of expected receipt at Element Materials Technology Eagan: _____

**TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))**

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** (1)

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing: ☐ **Yes** ☑ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

(1) Added per 8-10-23 email. mm 8-10-23

***PROPRIETARY INFORMATION***



## APPROVAL SIGNATURES

***SPONSOR:***

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: [signature] DATE: JUL 20 2023

PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

*For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.*

***Other individuals authorized to receive information regarding this study:*** ☐ See Attached

***Element Materials Technology Eagan:***

NAME: Mary J. Miller
Study Director

SIGNATURE: Mary J. Miller DATE: 8-10-23
Study Director



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# FINAL STUDY REPORT

## STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Avian Influenza A (H5N1) virus**

## PRODUCT IDENTITY

West Perox
Lot 070701 and Lot 070704

## TEST GUIDELINE

OCSPP 810.2200

## PROTOCOL NUMBER

WPC02070723.AFLU

## AUTHOR

Kasey Thompson, B.S.
Study Director

## STUDY COMPLETION DATE

August 31, 2023

## PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

## SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

## PROJECT NUMBER

A38370

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: PIERRE STEWART

MANAGER of TECHNICAL SERVICES
Title

Signature Date: 07-02-2024

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exception(s):

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

Characterization of the compounds was performed by the Sponsor prior to use in the study, however not in accordance with 40 CFR Part 160.

Submitter: _____ Date: 07-02-2024

Sponsor: _____ Date: 07-02-2024

Study Director: _____ Date: 8-31-23
Kasey Thompson, B.S.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

| Phase Inspected | Date of Phase Inspection | Date Reported to Study Director | Date Reported to Management |
|---|---|---|---|
| Critical Phase Audit: Preparation of Test Substance | August 14, 2023 | August 14, 2023 | August 31, 2023 |
| Final Report | August 30, 2023 | August 30, 2023 | |

Quality Assurance Specialist: Cody Aay Date: 8/31/23

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Kasey Thompson, B.S.

Professional Personnel Involved:

| | |
|---|---|
| Kelleen Lauer, M.S. | - General Manager |
| Nicole Felicelli, B.A. | - Manager, Study Director Operations |
| Miranda Peskar, B.S. | - Core Services Laboratory Operations Manager |
| Joseph Artus, B.S. | - Virologist |
| Sydney Sawatzke, B.S. | - Associate Virologist |

# STUDY REPORT

## GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A38370

**Protocol Number:** WPC02070723.AFLU

**Sponsor:** West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**Testing Facility:** Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** West Perox

**Lot/Batch(s):** Lot 070701 and Lot 070704

**Manufacture Date:** July 7, 2023 (both lots)

**Test Substance Characterization**
Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). The Test Substance Certificate of Analysis Reports may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

## STUDY DATES

**Date Sample Received:** July 27, 2023
**Study Initiation Date:** August 11, 2023
**Experimental Start Date:** August 14, 2023 (Start time: 2:07 p.m.)
**Experimental End Date:** August 21, 2023 (End time: 2:44 p.m.)
**Study Completion Date:** See Page 1 of Report

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

Test Substance: West Perox, Lot 070701 and Lot 070704

Dilution: 1:64 defined as 1 part test substance + 64 parts 300 ppm AOAC Synthetic Hard Water

Virus: Avian Influenza A (H5N1) virus, Strain VNH5N1-PR8/CDC-RG, (CDC #2006719965)

Exposure Time: 10 minutes

Exposure Temperature: 20±2°C (21.76°C)

Exposure Humidity: 45.62%

Organic Soil Load: 5% fetal bovine serum

Efficacy Result:

| Test Organism | Test Results: West Perox | | Efficacy Performance |
|---|---|---|---|
| | Lot 070701 | Lot 070704 | |
| Avian Influenza A (H5N1) virus | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | Requirements met |

## TEST SYSTEM

1. Virus

The VNH5N1-PR8/CDC-RG strain of Avian Influenza A (H5N1) virus used for this study was obtained from the Centers for Disease control and Prevention (CDC) Atlanta, Georgia (CDC #2006719965). The 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 ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot H5N1-45) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Influenza virus on MDCK cells.

2. Indicator Cell Cultures

Cultures of MDCK (canine kidney) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-34). The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. 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.

3. Test Medium

The test medium used in this study was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 µg/mL TPCK-Trypsin, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two lots of West Perox (Lot 070701 and Lot 070704) were tested at a 1:64 dilution defined as 1 part test substance + 64 parts 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 64.0 mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was at the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 302 ppm) and used on the day of testing.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 21.76°C in a relative humidity of 45.62% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel (16.8%). On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance
For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.76°C) and 45.62% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at 20±2°C (21.76°C) and 45.62% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls
A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into MDCK cell cultures. Cytotoxicity of the MDCK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of 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 (approximately 10 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates. Although the calculated value for infectious units used in the neutralization control was lower than the typical target range, the low inoculum increases the stringency of this control. Growth of virus in the neutralization control indicates neutralization of the test substance was demonstrated as required. Therefore, the neutralization control is valid.

9. Infectivity Assays
The MDCK cell line, which exhibits cytopathic effect (CPE) in the presence of Avian Influenza A (H5N1) virus, 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. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$) in sterile disposable cell culture labware. The cell cultures were examined approximately 23.5 hours post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PLANNED PROTOCOL CHANGES

**Protocol Amendments:**
No protocol amendments were required for this study.

**Planned Protocol Deviations:**
No planned protocol deviations occurred during this study.

## UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

### Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

### Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

## RECORD RETENTION

**Study Specific Documents**
All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

## RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

**Key for Control and Results Tables:**

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

## ANALYSIS

Results of tests with two lots of West Perox (Lot 070701 and Lot 070704), diluted 1:64 defined as 1 part test substance + 64 parts 300 ppm AOAC Synthetic Hard Water, exposed to Avian Influenza A (H5N1) virus in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.76°C) and 45.62% relative humidity for 10 minutes were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 5.50 $\log_{10}$/100 µL. The titer of the dried virus control was 5.00 $\log_{10}$/100 µL (5.30 $\log_{10}$/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested [≤0.50 $\log_{10}$/100 µL (≤0.80 $\log_{10}$/carrier)]. Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/100 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier was ≥4.50 $\log_{10}$ for both lots.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, West Perox, diluted 1:64 defined as 1 part test substance + 64 parts 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 $log_{10}$ reduction in titer of Avian Influenza A (H5N1) virus following a 10 minute exposure time at 20±2°C (21.76°C) and 45.62% relative humidity as required by the U.S. EPA and Health Canada.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

## TABLE 1: Virus Controls and Test Results

### Effects of West Perox (Lot 070701 and Lot 070704) Following a 10 Minute Exposure to Avian Influenza A (H5N1) Virus Dried on an Inanimate Surface

| Dilution | Input Virus Control | Dried Virus Control | Avian Influenza A (H5N1) virus + Lot 070701 | Avian Influenza A (H5N1) virus + Lot 070704 |
|---|---|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | + + | + + + + | 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 0 0 |
| $10^{-6}$ | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-8}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-9}$ | 0 0 | NT | NT | NT |
| $TCID_{50}$/100 µL | $10^{5.50}$ | $10^{5.00}$ | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |
| $TCID_{50}$/carrier | NA | $10^{5.30}$ | $\leq 10^{0.80}$ | $\leq 10^{0.80}$ |
| Log Reduction[1] | NA | NA | ≥4.50 | ≥4.50 |

[1] This reduction is both per volume inoculated per well and per carrier.

**TABLE 2: Cytotoxicity Control Results**

**Cytotoxicity of West Perox on MDCK Cell Cultures**

| Dilution | Cytotoxicity Control Lot 070701 | Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | 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 |
| $TCD_{50}$/100 µL | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |

**TABLE 3: Neutralization Control Results**

**Non-Virucidal Level of the Test Substance (Neutralization Control)**

| Dilution | Test Virus + Cytotoxicity Control Lot 070701 | Test Virus + Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + + + | + + + + |
| $10^{-2}$ | + + + + | + + + + |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of $\leq 0.50$ $\log_{10}$ for both lots.

## ATTACHMENT I: Test Substance Certificate(s) of Analysis
## Lot 070701

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070701

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.8 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.5 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.2 |

July 14 2023
Date

Pierre Stewart
Quality Control

## Test Substance Certificate(s) of Analysis
## Lot 070704

West Penetone®

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070704

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.9 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.4 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.0 |

July 14, 2023
Date

Pierre Stewart
Quality Control

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A38373
Test Substance Tracking # TS072723.WPC02 KIT8-11

element

**PROTOCOL**

# Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Avian Influenza A (H5N1) virus

**PROTOCOL NUMBER**

WPC02070723.AFLU

**SPONSOR**

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**PERFORMING LABORATORY**

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**DATE**

July 7, 2023

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

**TEST SUBSTANCE CHARACTERIZATION**

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

**SCHEDULING AND DISCLAIMER OF WARRANTY**

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 23, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of September 20, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

**JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM**

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The MDCK cell line, which supports the growth of the Avian Influenza A (H5N1) virus, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.

**TEST PRINCIPLE**

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

**STUDY DESIGN**

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

**VIRUS**

The VNH5N1-PR8/CDC-RG strain of Avian Influenza A (H5N1) virus to be used for this study was obtained from the Centers for Disease Control and Prevention (CDC) Atlanta, Georgia (CDC-2006719965). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. Note: If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

**INDICATOR CELL CULTURES**

Cultures of MDCK (canine kidney) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-34). The cells are propagated by Element Materials Technology Eagan personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. The confluency of the cells will be appropriate for the test virus. MDCK cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

**TEST MEDIUM**

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

**PREPARATION OF OECD HARD WATER (if applicable)**

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

**PREPARATION OF UN-SOFTENED TAP WATER (if applicable)**

Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

**PREPARATION OF TEST SUBSTANCE**

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

**PREPARATION OF VIRUS FILMS**

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

**TEST METHOD**

**Preparation of Sephadex Gel Filtration Columns**

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

**Input Virus Control**

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

**Treatment of Virus Films with the Test Substance**
For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be 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 will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

**Infectivity Assays**
The MDCK cell line, which exhibits cytopathic effect (CPE) in the presence of Avian Influenza A (H5N1) virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.

## DATA ANALYSIS

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\log_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\log_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

**Calculation of Log Reduction**
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\log_{10}$ $TCID_{50}$ – Test Substance $\log_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**Calculation of Infectious Units**

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

**Statistical Methods**
None used.

## PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

## METHOD FOR CONTROL OF BIAS: N/A

## STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA and Health Canada Submission**

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

## FINAL REPORT

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

## PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

## TEST SUBSTANCE RETENTION

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

**RECORD RETENTION**

**Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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.

**Facility Specific Documents**

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

**PROPOSED STATISTICAL METHODS:** N/A



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**REFERENCES**

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.



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## STUDY INFORMATION

*(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)*

**Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)**

| Test Substance Name | Lot/Batch Number | Manufacture Date |
|---|---|---|
| West Perox | 070701 | 07/07/2023 |
| West Perox | 070704 | 07/07/2023 |

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**

- ☑ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☑ Peroxide
- ☐ Sodium hypochlorite
- ☐ Other _____

**Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):** 5.8% QUATERNARY AMMONIA + 7.5% w/w PEROXIDE

*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**Storage Conditions**

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other _____

**Hazards**

- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

**Product Preparation**

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:

1:64 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 64 parts (amount of diluent)

- ☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
- ☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
- ☐ OECD Hard Water: 375 ppm (338-394 ppm)
- ☑ Other 300 ppm AOAC synthetic hard water

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.**

**Test Virus:** Avian Influenza A (H5N1) virus

**Exposure Time:** 10 minutes

**Exposure Temperature:**

- ☐ Room temperature
- ☑ Other: 20±2°C (please specify range)

**Directions for application of aerosol/spray products:**

- ☑ Spray instructions are not applicable.

Trigger spray application:

- ☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
- ☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:

- ☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**

- ☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
- ☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
- ☑ 5% fetal bovine serum
- ☐ Other _____

**Number of Carriers to be Tested**

- ☑ One (typical for U.S. EPA submission)
- ☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**

*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*

- ☐ Sprayer(s) and bottle(s) are provided by the Sponsor
- ☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
- ☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**

- ☑ U.S. EPA
- ☑ Health Canada
- ☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**COMPLIANCE**

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.

- ☑ Yes
- ☐ No (Non-GLP or Development Study)

**PROTOCOL MODIFICATIONS**

- ☐ Approved without modification
- ☑ Approved with modification

Reference 2 is updated to: American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
Reference 12 is updated to: U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

**PROTOCOL ATTACHMENTS**

Supplemental Information Form Attached - ☐ Yes ☑ No

**TEST SUBSTANCE SHIPMENT STATUS**

*(This section is for informational purposes only.)*

- ☐ Test Substance is already present at Element Materials Technology Eagan.
- ☑ Test Substance has been or will be shipped to Element Materials Technology Eagan.

Date of expected receipt at Element Materials Technology Eagan: _____

**TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))**

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** (1)

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing: ☐ **Yes** ☑ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

(1) Updated per 8-11-23 email. KLT 8-11-23

***PROPRIETARY INFORMATION***



## APPROVAL SIGNATURES

***SPONSOR:***

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: _____ DATE: JUL 20 2023

PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

*For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.*

***Other individuals authorized to receive information regarding this study:*** ☐ See Attached

***Element Materials Technology Eagan:***

NAME: Kasey Thompson
Study Director

SIGNATURE: _____ DATE: 8-11-23
Study Director



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# FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Poliovirus type 1**

PRODUCT IDENTITY

West Perox
Lot 070701 and Lot 070704

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

WPC02092223.POL

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

November 30, 2023

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PROJECT NUMBER

A38645

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: PIERRE STEWART

MANAGER OF TECHNICAL SERVICES
Title

Signature

Date: 07-02-2024

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exception(s):

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

Characterization of the compounds was performed by the Sponsor prior to use in the study, however not in accordance with 40 CFR Part 160.

Submitter: _____ Date: 07-02-2024

Sponsor: _____ Date: 07-02-2024

Study Director: _____ Date: 11-30-23
Mary J. Miller, M.T.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

| Phase Inspected | Date of Phase Inspection | Date Reported to Study Director | Date Reported to Management |
|---|---|---|---|
| Critical Phase Audit: Preparation of Test Substance | November 20, 2023 | November 20, 2023 | November 30, 2023 |
| Final Report | November 29, 2023 | November 29, 2023 | |

Quality Assurance Specialist: _____ Date: 11/30/23

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

| | |
|---|---|
| Kelleen Lauer, M.S. | - General Manager |
| Nicole Felicelli, B.A. | - Manager, Study Director Operations |
| Miranda Peskar, B.S. | - Core Services Laboratory Operations Manager |
| Joseph Artus, B.S. | - Virologist |
| Sydney Sawatzke, B.S. | - Associate Virologist |

# STUDY REPORT

## GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A38645

**Protocol Number:** WPC02092223.POL

**Sponsor:** West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**Testing Facility:** Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** West Perox

**Lot/Batch(s):** Lot 070701 and Lot 070704

**Manufacture Date:** Lot 070701 – July 7, 2023
Lot 070704 – July 7, 2023

**Test Substance Characterization**
Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). Test substance stability testing of the formulation was not performed prior to or concurrent with its use in this study. The Test Substance Certificate of Analysis Report(s) may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

## STUDY DATES

**Date Sample Received:** July 27, 2023
**Study Initiation Date:** October 25, 2023
**Experimental Start Date:** November 20, 2023 (Start time: 1:55 p.m.)
**Experimental End Date:** November 27, 2023 (End time: 1:25 p.m.)
**Study Completion Date:** See Page 1 of Report

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

| | |
|---|---|
| Test Substance: | West Perox, Lot 070701 and Lot 070704 |
| Dilution: | 1:40 defined as 1 part test substance + 40 parts of 300 ppm AOAC Synthetic Hard Water |
| Virus: | Poliovirus type 1, ATCC VR-1562, Strain Chat |
| Exposure Time: | 10 minutes |
| Exposure Temperature: | 20±2°C (21.0°C) |
| Exposure Humidity: | 27.07% |
| Organic Soil Load: | 5% fetal bovine serum |

Efficacy Result:

| Test Organism | Test Results | | Efficacy Performance |
|---|---|---|---|
| | West Perox | | |
| | Lot 070701 | Lot 070704 | |
| Poliovirus type 1 | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | Requirements met |

## TEST SYSTEM

1. Virus

The Chat strain of Poliovirus type 1 used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-1562). The stock virus was prepared by collecting the supernatant culture fluid from 50-75% 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 ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot PC2-48) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Poliovirus on Vero cells.

2. Indicator Cell Cultures

Cultures of Vero cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-81). The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. 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.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two lots of West Perox (Lot 070701 and Lot 070704) were tested at a 1:40 dilution defined as 1 part test substance + 40 parts of 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 40.0. mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was at the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 305 ppm) and used on the day of testing.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 15.5°C in a relative humidity of 55% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel (16.8%). On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance
For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 27.07% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at 20±2°C (21.0°C) and 27.07% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls
A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into Vero cell cultures. Cytotoxicity of the Vero cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of 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 (approximately 100 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

9. Infectivity Assays
The Vero cell line, which exhibits cytopathic effect (CPE) in the presence of Poliovirus type 1, 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. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$) in sterile disposable cell culture labware. The cell cultures were examined approximately 22.25 hours post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PLANNED PROTOCOL CHANGES

**Protocol Amendments:**
No protocol amendments were required for this study.

**Planned Protocol Deviations:**
No planned protocol deviations occurred during this study.

## UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier):

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

### Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

### Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus: $10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

## RECORD RETENTION

**Study Specific Documents**
All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

## RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

**Key for Control and Results Tables:**

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

## ANALYSIS

Results of tests with two lots of West Perox (Lot 070701 and Lot 070704), diluted 1:40 defined as 1 part test substance + 40 parts of 300 ppm AOAC Synthetic Hard Water, exposed to Poliovirus type 1 in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.0°C) and 27.07% relative humidity for 10 minutes were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 7.50 $\log_{10}$/100 µL. The titer of the dried virus control was 6.00 $\log_{10}$/100 µL (6.30 $\log_{10}$/carrier). Following exposure, test virus infectivity was detected in the virus-test substance mixture for both lots at 2.75 $\log_{10}$/100 µL (3.05 $\log_{10}$/carrier). Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/100 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier, was 3.25 $\log_{10}$ for both lots.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, West Perox, diluted 1:40 defined as 1 part test substance + 40 parts of 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 $log_{10}$ reduction in titer of Poliovirus type 1 following a 10 minute exposure time at 20±2°C (21.0°C) and 27.07% relative humidity as required by the U.S. EPA and Health Canada.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

## TABLE 1: Virus Controls and Test Results

### Effects of West Perox (Lot 070701 and Lot 070704) Following a 10 Minute Exposure to Poliovirus Type 1 Dried on an Inanimate Surface

| Dilution | Input Virus Control | Dried Virus Control | Poliovirus type 1 + Lot 070701 | Poliovirus type 1 + Lot 070704 |
|---|---|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + | + + + + | + + + + |
| $10^{-2}$ | + + | + + + + | + + + + | + + + + |
| $10^{-3}$ | + + | + + + + | 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 0 0 |
| $10^{-7}$ | + + | 0 0 0 0 | NT | NT |
| $10^{-8}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-9}$ | 0 0 | NT | NT | NT |
| $TCID_{50}$/100 µL | $10^{7.50}$ | $10^{6.00}$ | $10^{2.75}$ | $10^{2.75}$ |
| $TCID_{50}$/carrier | NA | $10^{6.30}$ | $10^{3.05}$ | $10^{3.05}$ |
| Log Reduction[1] | NA | NA | 3.25 | 3.25 |

[1] This reduction is both per volume inoculated per well and per carrier.

**TABLE 2: Cytotoxicity Control Results**

**Cytotoxicity of West Perox on Vero Cell Cultures**

| Dilution | Cytotoxicity Control Lot 070701 | Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | 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 |
| $TCD_{50}$/100 µL | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |

**TABLE 3: Neutralization Control Results**

**Non-Virucidal Level of the Test Substance (Neutralization Control)**

| Dilution | Test Virus + Cytotoxicity Control Lot 070701 | Test Virus + Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + + + | + + + + |
| $10^{-2}$ | + + + + | + + + + |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of $\leq 0.50$ $\log_{10}$ for both lots.

**ATTACHMENT I: Test Substance Certificate(s) of Analysis**
**Lot 070701**

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070701

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.8 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.5 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.2 |

July 14, 2023
Date

Pierre Stewart
Quality Control

## Test Substance Certificate(s) of Analysis
## Lot 070704

West Penetone®

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070704

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.9 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.4 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.0 |

July 14, 2023
Date

Pierre Stewart
Quality Control

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A38645
Test Substance Tracking # 1307272 23, WP102 pm 10-25-23

**PROTOCOL**

# Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Poliovirus type 1

**PROTOCOL NUMBER**

WPC02092223.POL

**SPONSOR**

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**PERFORMING LABORATORY**

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**DATE**

September 22, 2023

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

**TEST SUBSTANCE CHARACTERIZATION**

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

**SCHEDULING AND DISCLAIMER OF WARRANTY**

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is October 17, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of November 20, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

**JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM**

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The Vero cell line, which supports the growth of the Poliovirus type 1, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.

**TEST PRINCIPLE**
A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

**STUDY DESIGN**
Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

**VIRUS**
The Chat strain of Poliovirus type 1 to be used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-1562). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

**INDICATOR CELL CULTURES**
Cultures of Vero cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-81). The cells are propagated by Element Materials Technology Eagan personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. The confluency of the cells will be appropriate for the test virus. Vero cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

**TEST MEDIUM**

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

**PREPARATION OF OECD HARD WATER (If applicable)**

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

**PREPARATION OF UN-SOFTENED TAP WATER (If applicable)**

Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

**PREPARATION OF TEST SUBSTANCE**

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

**PREPARATION OF VIRUS FILMS**

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

**TEST METHOD**

**Preparation of Sephadex Gel Filtration Columns**

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

**Input Virus Control**

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

**Treatment of Virus Films with the Test Substance**
For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be 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 will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

**Infectivity Assays**
The Vero cell line, which exhibits cytopathic effect (CPE) in the presence of Poliovirus type 1, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.

## DATA ANALYSIS

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$\text{- Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\log_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\log_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

**Calculation of Log Reduction**
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\log_{10}$ $TCID_{50}$ – Test Substance $\log_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**Calculation of Infectious Units**

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

**Statistical Methods**
None used.

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**
The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

**METHOD FOR CONTROL OF BIAS:** N/A

**STUDY ACCEPTANCE CRITERIA**
Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA and Health Canada Submission**
A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

**FINAL REPORT**
The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

**PROTOCOL CHANGES**
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

**TEST SUBSTANCE RETENTION**
Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

**RECORD RETENTION**

**Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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.

**Facility Specific Documents**

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

**PROPOSED STATISTICAL METHODS:** N/A

**REFERENCES**

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

## STUDY INFORMATION

*(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)*

**Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)**

| Test Substance Name | Lot/Batch Number | Manufacture Date |
|---|---|---|
| West Perox | 070701 | 07/07/2023 |
| West Perox | 070704 | 07/07/2023 |

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**

- ☑ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☑ Peroxide
- ☐ Sodium hypochlorite
- ☐ Other _____

**Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):** 5.8% QUATERNARY Ammonia + 7.5% Peroxide

*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**Storage Conditions**

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other _____

**Hazards**

- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

**Product Preparation**

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:

1:40 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 40 parts (amount of diluent)

- ☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
- ☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
- ☐ OECD Hard Water: 375 ppm (338-394 ppm)
- ☑ Other 300 ppm AOAC synthetic hard water

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.**

**Test Virus:** Poliovirus type 1

**Exposure Time:** 10 minutes

**Exposure Temperature:**

- ☐ Room temperature
- ☑ Other: 20±2°C (please specify range)

**Directions for application of aerosol/spray products:**

- ☑ Spray instructions are not applicable.

Trigger spray application:

- ☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
- ☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:

- ☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**

- ☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
- ☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
- ☑ 5% fetal bovine serum
- ☐ Other _____

**Number of Carriers to be Tested**

- ☑ One (typical for U.S. EPA submission)
- ☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**
*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*
☐ Sprayer(s) and bottle(s) are provided by the Sponsor
☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**
☑ U.S. EPA
☑ Health Canada
☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**COMPLIANCE**
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

**PROTOCOL MODIFICATIONS**
☐ Approved without modification
☑ Approved with modification
Reference 2 is updated to: American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization. E1482-23.
Reference 12 is updated to: U.S. Environmental Protection Agency. Office of Pesticide Programs SOP Number: MB-30-03. Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products. November 14 2022.

**PROTOCOL ATTACHMENTS**
Supplemental Information Form Attached - ☐ Yes ☑ No

**TEST SUBSTANCE SHIPMENT STATUS**
*(This section is for informational purposes only.)*
☑ Test Substance is already present at Element Materials Technology Eagan.
☐ Test Substance has been or will be shipped to Element Materials Technology Eagan.
Date of expected receipt at Element Materials Technology Eagan: _____

**TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))**

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** (1)

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☐ **Yes** ☑ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

(1) Added per 10-24-23 email. mm 10-25-23

***PROPRIETARY INFORMATION***



## APPROVAL SIGNATURES

***SPONSOR:***

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: [signature] DATE: SEP 28 2023

PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

*For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.*

***Other individuals authorized to receive information regarding this study:*** ☐ See Attached

***Element Materials Technology Eagan:***

NAME: Mary J. Miller
Study Director

SIGNATURE: Mary J. Miller DATE: 10-25-23
Study Director

# FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Infectious Laryngotracheitis virus**

PRODUCT IDENTITY

West Perox
Lot 070701 and Lot 070704

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

WPC02092223.ILGT

AUTHOR

Tanner Straus, B.S.
Study Director

STUDY COMPLETION DATE

December 21, 2023

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

PROJECT NUMBER

A38739

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: West Penetone, Inc.

Company Agent: PIERRE STEWART

MANAGER OF TECHNICAL SERVICES
Title

Signature Date: 07-02-2024

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exception(s):

Characterization of the compounds was performed by the Sponsor prior to use in the study, however not in accordance with 40 CFR Part 160.

Stability testing of the compounds was not performed by the Sponsor prior to use in the study or concurrent with the study per 40 CFR Part 160.

Submitter: _____ Date: 07-02-2024

Sponsor: _____ Date: 07-02-2024

Study Director: _____ Date: 12-21-23
Tanner Straus, B.S.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

| Phase Inspected | Date of Phase Inspection | Date Reported to Study Director | Date Reported to Management |
|---|---|---|---|
| Critical Phase Audit: Preparation of Virus Films | December 1, 2023 | December 1, 2023 | December 21, 2023 |
| Final Report | December 20, 2023 | December 20, 2023 | |

Quality Assurance Specialist: _____ Date: 12/21/23

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Tanner Straus, B.S.

Professional Personnel Involved:

| | |
|---|---|
| Kelleen Lauer, M.S. | - General Manager |
| Nicole Felicelli, B.A. | - Manager, Study Director Operations |
| Miranda Peskar, B.S. | - Core Services Laboratory Operations Manager |
| Matt Cantin, B.S. | - Study Director and Client Services Supervisor |
| Miranda Quist, B.S. | - Virology Laboratory Supervisor |
| Kasey Thompson, B.S. | - Virologist |
| Sydney Sawatzke, B.S. | - Associate Virologist |
| Gabriel Blanco-Ruiz, B.S. | - Associate Virologist |

# STUDY REPORT

## GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A38739

**Protocol Number:** WPC02092223.ILGT

**Sponsor:** West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**Testing Facility:** Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** West Perox

**Lot/Batch(s):** Lot 070701 and Lot 070704

**Manufacture Date:** July 7, 2023 (both lots)

**Test Substance Characterization**
Test substance characterization as to identity, strength, purity, and uniformity, as applicable, was performed and documented prior to its use in this study, however not in accordance with 40 CFR Part 160, Subpart F (160.105). Test substance stability testing was not performed prior to or concurrent with its use in this study. The Test Substance Certificate of Analysis Report(s) may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

## STUDY DATES

**Date Sample Received:** July 27, 2023
**Study Initiation Date:** November 30, 2023
**Experimental Start Date:** December 1, 2023 (Start time: 11:20 am)
**Experimental End Date:** December 8, 2023 (End time: 2:10 pm)
**Study Completion Date:** See Page 1 of Report

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

Test Substance: West Perox, Lot 070701 and Lot 070704

Dilution: Dilute to 1:40 defined as 1 part test substance + 40 parts 300 ppm AOAC Synthetic Hard Water

Virus: Infectious Laryngotracheitis virus, Obtained from Poultry Health and Specialties, St. Cloud, MN, Strain LT-IVAX (Modified live vaccine)

Exposure Time: 10 minutes

Exposure Temperature: 20±2°C (21.74°C)

Exposure Humidity: 13.98%

Organic Soil Load: 5% fetal bovine serum

Efficacy Result:

| Test Organism | Test Results | | Efficacy Performance |
|---|---|---|---|
| | West Perox | | |
| | Lot 070701 | Lot 070704 | |
| Infectious Laryngotracheitis virus | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | A ≥3 $\log_{10}$ reduction in titer was demonstrated. **(PASS)** | Requirements met |

## TEST SYSTEM

1. Virus

The LT-IVAX (Modified live vaccine) strain of Infectious Laryngotracheitis virus used for this study was obtained from the Poultry Health and specialties, St.Cloud, MN. The 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 ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Lot LT-36) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The Stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Infectious Laryngotracheitis virus on CEK cells.

2. Indicator Cell Cultures

Cultures of chicken embryo kidney (CEK) cells were originally obtained from the AVS Bio. The cells were propagated by Element Materials Technology Eagan personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. 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.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5 % (v/v) heat-inactivated fetal bovine serum (FBS), 5% (v/v) Tryptose Phosphate Broth, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two lots of West Perox (Lot 070701 and Lot 070704) were tested at a 1:40 dilution defined as 1 part test substance + 40 parts 300 ppm AOAC Synthetic Hard Water (1.00 mL product + 40.0 mL water) as requested by the Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was equilibrated to the exposure temperature prior to use.

The 300 ppm AOAC Synthetic Hard Water was prepared using 3.0 mL of Solution I and 4.0 mL of Solution II. The total volume of hard water was brought to approximately 1 liter using sterile deionized water. The 300 ppm hard water was prepared, titrated (at 311 ppm) and used on the day of testing.

2. Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 40% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel (16.8%). On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance
For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at 20±2°C (21.74°C) and 13.98% relative humidity. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at 20±2°C (21.74°C) and 13.98% relative humidity. Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls
A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into CEK cell cultures. Cytotoxicity of the CEK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
The neutralization control was performed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of 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 (approximately 100 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

9. Infectivity Assays
The CEK cell line, which exhibits cytopathic effect (CPE) in the presence of Infectious Laryngotracheitis virus, 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. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The inoculum was allowed to adsorb for sixty minutes at 36-38°C (36.8-37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$). Following the adsorption period, a 1.0 mL aliquot of test medium was added to each well of the cell cultures, and the cultures were incubated at 36-38°C (37.0°C) in a humidified atmosphere of 5-7% $CO_2$ (6.0% $CO_2$) in sterile disposable cell culture labware. The cell cultures were examined approximately 1 hour post incubation for cytotoxicity, at which time no dilutions were demonstrating cytotoxicity. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PLANNED PROTOCOL CHANGES

**Protocol Amendments:**
Due to inadvertent omission in the Infectivity Assays section on page 6 of the protocol, the following wording is added, prior to the last sentence of the first paragraph:

"The inoculum is allowed to adsorb for ≥60 minutes at the appropriate incubation temperature. Following the adsorption time, a 1.0 mL aliquot of test medium is added to each well of the cell cultures."

**Planned Protocol Deviations:**
No planned protocol deviations occurred during this study.

## UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers will be expressed as -$\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$Log_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $Log_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

### Calculation of Log Reduction

The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $Log_{10}$ $TCID_{50}$ – Test Substance $Log_{10}$ $TCID_{50}$ = Log Reduction

### Calculation of Infectious Units

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

($10^{5.50}$ / $10^{3.00}$) (100 µL / 250 µL) = ~126 infectious units

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4.8 $log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

## RECORD RETENTION

**Study Specific Documents**
All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032.

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-23.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

## RESULTS

For Input Virus Control, Treatment of Virus Films with the Test Substance, and Treatment of Dried Virus Control Film Results, see Table 1.

For Cytotoxicity Control Results, see Table 2.

For Assay of Non-Virucidal Level of Test Substance (Neutralization Control) Results, see Table 3.

**Key for Control and Results Tables:**

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(NT) = Not tested

(NA) = Not applicable

## ANALYSIS

Results of tests with two lots of West Perox (Lot 070701 and Lot 070704), diluted to 1:40 defined as 1 part test substance + 40 parts 300 ppm AOAC Synthetic Hard Water, exposed to Infectious Laryngotracheitis virus in the presence of a 5% fetal bovine serum organic soil load at 20±2°C (21.74°C) and 13.98% relative humidity for 10 minutes were as described below. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 5.50 $\log_{10}$/100 µL. The titer of the dried virus control was 4.75 $\log_{10}$/100 µL (5.05 $\log_{10}$/carrier). Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested [≤0.50 $\log_{10}$/100 µL (≤0.80$\log_{10}$/carrier)]. Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$/100 µL for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier was ≥4.25 $\log_{10}$ for both lots.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, West Perox, diluted to 1:40 defined as 1 part test substance + 40 parts 300 ppm AOAC Synthetic Hard Water, demonstrated a ≥3 $log_{10}$ reduction in titer of Infectious Laryngotracheitis virus following a 10 minute exposure time at 20±2°C (21.74°C) and 13.98% relative humidity as required by the U.S. EPA and Health Canada.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data. Results pertain only to the items tested.

## TABLE 1: Virus Controls and Test Results

### Effects of West Perox Following a 10 Minute Exposure to Infectious Laryngotracheitis Virus Dried on an Inanimate Surface

| Dilution | Input Virus Control | Dried Virus Control | Infectious Laryngotracheitis virus + Lot 070701 | Infectious Laryngotracheitis virus + Lot 070704 |
|---|---|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | + + | + + + + | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | + + | + + + + | 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 0 0 0 |
| $10^{-6}$ | 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | 0 0 | 0 0 0 0 | NT | NT |
| $10^{-8}$ | 0 0 | 0 0 0 0 | NT | NT |
| $TCID_{50}$/100 µL | $10^{5.50}$ | $10^{4.75}$ | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |
| $TCID_{50}$/carrier | NA | $10^{5.05}$ | $\leq 10^{0.80}$ | $\leq 10^{0.80}$ |
| Log Reduction[1] | NA | NA | ≥4.25 | ≥4.25 |

[1] This reduction is both per volume inoculated per well and per carrier.

**TABLE 2: Cytotoxicity Control Results**

**Cytotoxicity of West Perox on CEK Cell Cultures**

| Dilution | Cytotoxicity Control Lot 070701 | Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-3}$ | 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 |
| $TCD_{50}$/100 µL | $\leq 10^{0.50}$ | $\leq 10^{0.50}$ |

**TABLE 3: Neutralization Control Results**

**Non-Virucidal Level of the Test Substance (Neutralization Control)**

| Dilution | Test Virus + Cytotoxicity Control Lot 070701 | Test Virus + Cytotoxicity Control Lot 070704 |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + + + | + + + + |
| $10^{-2}$ | + + + + | + + + + |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of $\leq 0.50$ $\log_{10}$ for both lots.

## ATTACHMENT I: Test Substance Certificate(s) of Analysis
## Lot 070701

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070701

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.8 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.5 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.2 |

July 14 2023
Date

Pierre Stewart
Quality Control

## Test Substance Certificate(s) of Analysis
## Lot 070704

CERTIFICATE OF ANALYSIS

# West Perox

MANUFACTURING DATE: July 7, 2023

LOT NUMBER: 070704

| TEST | METHOD | SPECIFICATION | RESULT |
|---|---|---|---|
| APPEARANCE | Visual | clear, colorless liquid | Conforms |
| pH (as is) | WM 30 | 1.5 – 2.5 | 1.9 |
| Total active quat, % w/w | WM 47-B | 5.2 – 6.5 | 5.4 |
| Hydrogen peroxide, % w/w | WM 193-B | 7.0 – 9.0 | 8.0 |

July 14, 2023
Date

Pierre Stewart
Quality Control

## AMENDMENT TO GLP TEST PROTOCOL

| | |
|---|---|
| **Amendment No.:** | 1 |
| **Effective Date:** | December 1, 2023 |
| **Sponsor:** | West Penetone, Inc.<br>10900 Secant<br>Montreal, QC H1J 1S5<br>Canada |
| **Test Facility:** | Element Materials Technology Eagan<br>1285 Corporate Center Drive, Suite 110<br>Eagan, MN 55121 |
| **Protocol Title:** | Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces |
| **Protocol Number:** | WPC02092223.ILGT |
| **Project Number:** | A38739 |

**Modifications to Protocol:**

Due to inadvertent omission in the Infectivity Assays section on page 6 of the protocol, the following wording is added, prior to the last sentence of the first paragraph:

"The inoculum is allowed to adsorb for ≥60 minutes at the appropriate incubation temperature. Following the adsorption time, a 1.0 mL aliquot of test medium is added to each well of the cell cultures."

Changes to the protocol are acceptable as noted.

Study Director

12-1-23
Date



ELEMENT EAGAN | (877) 287-8378 | contact.us@element.com | Element.com

(For Laboratory Use Only)
Element Materials Technology Eagan Project # A38739
Test Substance Tracking # TS077225, WPC02 11-30-23

element

**PROTOCOL**

# Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Infectious Laryngotracheitis virus

**PROTOCOL NUMBER**

WPC02092223.ILGT

**SPONSOR**

West Penetone, Inc.
10900 Secant
Montreal, QC H1J 1S5
Canada

**PERFORMING LABORATORY**

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**DATE**

September 22, 2023

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

**TEST SUBSTANCE CHARACTERIZATION**

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

**SCHEDULING AND DISCLAIMER OF WARRANTY**

Experimental start dates are generally scheduled on a first-come/first-serve basis once Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is October 23, 2023. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of November 20, 2023. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, because of failure by Element Materials Technology Eagan to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

**JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM**

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The CEK cell line, which supports the growth of the Infectious Laryngotracheitis virus, will be used in this study. The experimental design in this protocol meets these requirements and is guided by ASTM E1053-20.



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**TEST PRINCIPLE**
A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

**STUDY DESIGN**
Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

**VIRUS**
The LT-IVAX (Modified live vaccine) strain of Infectious Laryngotracheitis virus to be used for this study was obtained from the Poultry Health and Specialties, St. Cloud, MN. Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

**INDICATOR CELL CULTURES** AVS B:D. WETAS 11-30-23
Cultures of CEK (chicken embryo kidney) cells are received from ~~Charles River. Cultures~~ are maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. CEK cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

**TEST MEDIUM**

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

**PREPARATION OF OECD HARD WATER (if applicable)**

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to 7.0 ± 0.2. The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.

**PREPARATION OF UN-SOFTENED TAP WATER (if applicable)**

Place the required amount of un-softened tap water in a sterile vessel. Titrate for water hardness per CGT-0001 (Section 6.3.1-6.3.11). The acceptable range is 180-210 ppm. If the un-softened tap water falls above 210 ppm the water will be diluted with deionized water and re-titrated per CGT-0001 (Section 6.3.1-6.3.11).

**PREPARATION OF TEST SUBSTANCE**

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

**PREPARATION OF VIRUS FILMS**

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

**TEST METHOD**

**Preparation of Sephadex Gel Filtration Columns**

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

**Input Virus Control**

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.



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**Treatment of Virus Films with the Test Substance**
For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature and relative humidity will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
The neutralization control will be confirmed concurrently with testing. Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be 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 will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates. The infectious units of the low titer stock virus will be calculated and included in the final report.

**Infectivity Assays**
The CEK cell line, which exhibits cytopathic effect (CPE) in the presence of Infectious Laryngotracheitis virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days.

The cell cultures will be examined approximately 1-36 hours post incubation for cytotoxicity. Test medium will be aspirated from any dilutions demonstrating cytotoxicity and replaced with fresh test medium. Additional dilutions may be treated in the same manner to mitigate risk of additional cytotoxicity. On the final day of incubation, the cell cultures will be microscopically observed for the absence or presence of CPE (virus infectivity), cytotoxicity and for viability. The observations will be recorded on the raw data worksheets. The cell cultures may be observed at other times during incubation; only the results from the final observations will be reported.

## DATA ANALYSIS

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

Per Volume Inoculated ($TCID_{50}$/volume inoculated):

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

Per Carrier ($TCID_{50}$/carrier) :

(Antilog of $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

$\text{Log}_{10}$ of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 $\text{Log}_{10}$)

*$TCID_{50}$ value calculated based on the volume inoculated per well

**Calculation of Log Reduction**
The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier.

Dried Virus Control $\text{Log}_{10}$ $TCID_{50}$ – Test Substance $\text{Log}_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**Calculation of Infectious Units**

$$\left(\frac{\text{input virus titer}}{\text{dilution of test virus used for neutralization control}}\right)\left(\frac{\text{low titer virus inoculation volume}}{\text{input virus inoculation volume}}\right) = \sim \text{infectious units}$$

Example: Titer of the input virus:$10^{5.50}$ ($TCID_{50}$ of $10^{6.00}$), 1:1,000 dilution made from stock virus for use in the neutralization control, 100 µL/well of low titer virus inoculated and 250 µL/well of input virus inoculated)

$(10^{5.50} / 10^{3.00})$ (100 µL / 250 µL) = ~126 infectious units

**Statistical Methods**
None used.

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**
The specialized virucidal testing section of Element Materials Technology Eagan maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

**METHOD FOR CONTROL OF BIAS:** N/A

**STUDY ACCEPTANCE CRITERIA**
Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA and Health Canada Submission**
A valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

**FINAL REPORT**
The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, a conclusion as it relates to the purpose of the test and all other items required by 40 CFR Part 160.185. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

**PROTOCOL CHANGES**
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

**TEST SUBSTANCE RETENTION**
Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.



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**RECORD RETENTION**

**Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. 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.

**Facility Specific Documents**

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

**PROPOSED STATISTICAL METHODS:** N/A

## REFERENCES

1. ASTM E1053-20, Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2020, www.astm.org.
2. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, April 2020. Guidance Document - Disinfectant Drugs.
8. Health Canada, April 2020. Guidance Document - Safety and Efficacy Requirements for Surface Disinfectant Drugs.
9. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
10. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
12. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.

## STUDY INFORMATION

*(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)*

**Test Substance (Name and Lot/Batch Number exactly as it should appear on final report)**

| Test Substance Name | Lot/Batch Number | Manufacture Date |
|---|---|---|
| West Perox | 070701 | 07/07/2023 |
| West Perox | 070704 | 07/07/2023 |

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**

- ☑ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☑ Peroxide
- ☐ Sodium hypochlorite
- ☐ Other _____

**Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):** 5.8% quaternary ammonia + 7.5% peroxide

*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**Storage Conditions**

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other _____

**Hazards**

- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

**Product Preparation**

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:

1:40 (example: 1 oz/gallon) defined as 1 part (amount of test substance) + 40 parts (amount of diluent)

- ☐ AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
- ☐ Un-softened Tap Water: 200 ppm (180-210 ppm)
- ☐ OECD Hard Water: 375 ppm (338-394 ppm)
- ☑ Other 300 ppm AOAC synthetic hard water

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.**

**Test Virus:** Infectious Laryngotracheitis virus

**Exposure Time:** 10 minutes

**Exposure Temperature:**
- ☐ Room temperature
- ☑ Other: 20±2°C (please specify range)

**Directions for application of aerosol/spray products:**

- ☑ Spray instructions are not applicable.

Trigger spray application:
- ☐ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
- ☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:
- ☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**

- ☐ 0% fetal bovine serum *(only for Human Rotavirus, Porcine Epidemic Diarrhea Virus and most Influenza viruses)*
- ☐ 1% fetal bovine serum *(minimum level that can be tested for all other viruses)*
- ☑ 5% fetal bovine serum
- ☐ Other _____

**Number of Carriers to be Tested**

- ☑ One (typical for U.S. EPA submission)
- ☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**
*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*
☐ Sprayer(s) and bottle(s) are provided by the Sponsor
☐ General purpose spray bottle(s) are to be provided by Element Materials Technology Eagan
☐ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Element Materials Technology Eagan

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**
☑ U.S. EPA
☑ Health Canada
☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**COMPLIANCE**
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

**PROTOCOL MODIFICATIONS**
☐ Approved without modification
☑ Approved with modification
Reference 2 is updated to: American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization. E1482-23.
Reference 12 is updated to: U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

**PROTOCOL ATTACHMENTS**
Supplemental Information Form Attached - ☐ Yes ☑ No

**TEST SUBSTANCE SHIPMENT STATUS**
*(This section is for informational purposes only.)*
☑ Test Substance is already present at Element Materials Technology Eagan.
☐ Test Substance has been or will be shipped to Element Materials Technology Eagan.
Date of expected receipt at Element Materials Technology Eagan: _____

**TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))**

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☑ **No*** Per 11-30-23 Email. JLS 11-30-23

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☐ **Yes** ☑ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☐ **Yes** ☐ **No***

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

***PROPRIETARY INFORMATION***



## APPROVAL SIGNATURES

***SPONSOR:***

NAME: Mr. Pierre Stewart TITLE: Manager of Technical Services

SIGNATURE: DATE: SEP 28 2023

PHONE: 1 (514) 355 - 6060 EMAIL: pstewart@westpenetoneinc.com

*For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.*

***Other individuals authorized to receive information regarding this study:*** ☐ See Attached

***Element Materials Technology Eagan:***

NAME: Tamar Strauss
Study Director

SIGNATURE: DATE: 11-30-23
Study Director