STUDY TITLE

Virucidal Efficacy of The Sani-TEST Disinfectant Formulation for Use on Inanimate Non-Porous Surfaces Using Human Coronavirus 229E

Product Identity

Hypochlorous Acid 200
Lot <180-1; Lot <180-2; Lot <180-3

Test Guidelines

EPA 810.2000 and 810.2200

Protocol Identification Number

BD-2100229-1

Author

Absar Alum

Experimental Work Completion Date

May 22, 2021

Performing Laboratory

BioDetek Laboratory
1815 W 1st Avenue, Suite 125, Mesa, AZ 85202

Sponsor

Sani-TEST LLC
7911 7th Street, Slatington, PA 18080

Project Identification Number

SanT-1

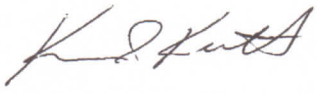
STATEMENTS OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Sani-TEST LLC

Company Agent/ Submitter: Kevin Kutcel

Title:

Signature  Date: 6/11/21

GOOD LABORATORY PRACTICE STATEMENT

This study meets the U.S. Environmental Protection Agency's Good Laboratory Practices Standards and requirements for the 40 CFR Part 160 with the following exceptions.

1. Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) are maintained by the Study Sponsor. The study Sponsor conducted test substance characterization as to identity, strength, purity, solubility and composition, as applicable according to 40 CFR Part 160 subpart F [160, 105] prior to its use in the study. The test substance certificate of analysis may be found attached to this report for reference

Study Director: Absar Alum
Company: Biodetek
Title: Managing Director
Signature: _____ Date 6/3/2021

Study Sponsor: Scott Anderson
Company: Sani-TEST LLC, 7911 7th Street, Slatington, PA 18080
Title: President
Signature: _____ Date 6/10/21

Study Submitter: Kevin Kutcel
Company: KRK Consulting
Title: President
Signature: _____ Date 6/11/21

QUALITY ASSURANCE STATEMENT

Study Title: Virucidal Efficacy of The Sani-TEST Disinfectant Formulation for Use on Inanimate Non-Porous Surfaces Using Human Coronavirus 229E

Study ID#: SanT-1

The following quality assurance audits were conducted with Good Laboratory Practices Standards outlined in 40CFR Part 160 and reported to management and the Study Director

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
In Progress	May 5, 2021	May 5, 2017 (Tuesday)	May 5, 2017 (Tuesday)
In Progress	May 21, 2021	May 21, 2021	May 21, 2021
Draft Report	May 28, 2021	May 28, 2021	May 28, 2021
Final Report	June 3, 2021	June 3, 2021	June 3, 2021

PERSONNEL INVOLVED IN THE STUDY

STUDY DIRECTOR
Name: Absar Alum, Ph.D.
Company: BioDetek
Title: Managing Director

ASSISTING PERSONNEL
Name: Hina Absar, B.S.
Company: BioDetek
Title: Microbiologist

ASSISTING PERSONNEL
Name: Ahmad Abdul Qadar, B.S.
Company: BioDetek
Title: Microbiologist

ASSISTING PERSONNEL
Name: Omer Alsabbry, B.S.
Company: BioDetek
Title: Microbiologist

ASSISTING PERSONNEL
Name: Galahad Z Isaacs, B.S.
Company: BioDetek
Title: Microbiology Technician

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FINAL STUDY SUMMARY

Study Title

Virucidal Efficacy of The Sani-TEST Disinfectant Formulation for Use on Inanimate Non-Porous Surfaces Using Human Coronavirus 229E

Study Identification Number

SanT-1

Protocol Number

BD-2100229-1

Test Microorganism

Human Coronavirus Strain 229E

Study Sponsor

Sani-TEST LLC, 7911 7th Street, Slatington, PA 18080

Testing Facility

Biodetek, 1815 W 1st Avenue, #125, Mesa AZ 85202

Study Director

Absar Alum, Ph.D.

Experiment Completion Date

May 22, 2021

Study Objective

The purpose of this study was to evaluate the virucidal efficacy of a test substance against Human coronavirus strain 229E 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 complies with the requirement of and may be submitted to the U.S. Environmental Protection Agency (EPA).

Study Conclusion in Brief

Three lots of Sani-TEST Disinfectant hypochlorous acid 200 (Lot # <180.1, <180.2 <180.3 met the test criteria specified in the study protocol. Under these test conditions the results indicate a ≥ 3 $\log_{10}$ reduction in the titer of human coronavirus strain 229E as required by the U.S. EPA

FINAL STUDY REPORT

Important Dates

Study Contract Date:	February 14, 2021
Protocol Approval:	February 14, 2021
Study Initiation Date:	March 24, 2021
Experimental Start Date:	April 23, 2021
Experimental End Date:	May 22, 2021

Test Substance Information

Name:	Hypochlorous Acid 200
Lots:	Lot <180-1, Lot <180-2 and Lot <180-3
Date Received:	3/24/2021
Form:	Liquid – Ready to use
Storage Conditions:	Refrigerator

Test Parameters

Microorganism:	Human Corona Virus 229E, ATCC VR-740 (Original Lot 70037543)
Lab Virus Stock Lot:	HuCV–12MAR2021
Host Cell Line:	MRC-5, ATCC CCL-171 (Original Lot 70031696)
Subculture Passage Number:	10
Cell Culture Medium:	EMEM ATCC @ 30-2003(Lot 81023200 Exp 28/FEB/2022)
Carrier Type:	Glass Petri dishes (60 x 15 mm)
Carrier Dry Time:	30 minutes
Carrier Dry Temperature:	23.0°C
Carrier Dry Humidity:	45 – 46% relative humidity
Test Substance Application:	Pipette delivery of test substance
Primary Neutralizer:	0.1% lecithin and 0.5% sodium thiosulfate in EMEM
Test Contact Time:	10 minutes ± 5 seconds
Test Temperature:	23.0 – 24.0°C
Test Humidity:	45 – 46% relative humidity
Organic Soil Load:	5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS)
Neut. Control Hold Time:	15 minutes
Hold Time Temperature:	24.0°C
Hold Time Humidity:	45 – 46% relative humidity
Assay Conditions:	37 ± 2°C; 5 ± 1% CO2
Assay Period:	10 days

TEST PROCEDURETEST PROCEDURES

ASTM International E1053-11 "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces"

Indicator Cells: MRC-5 cells were obtained from ATCC and maintained in cell culture at 36 ± 2°C with 5 ± 3% CO2 prior to seeding. The indicator cell plates were prepared 12 – 30 hours prior to inoculation with test sample. The cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1 mL per well.

Virus Inoculum: Frozen viral stock was thawed on the day of the test. Stock virus contained an organic load of 5.0% FBS.

Test Substance: The test substance was received ready to use.

Test Carriers: 0.2 mL of virus inoculum was spread uniformly over the bottom of a glass petri dish (carrier). The carriers were dried for 30 minutes at 23°C with 45-46% Relative Humidity (RH).

Test Substance Application and Exposure Conditions: 2.0 mL of test substance was pipette delivered making sure to thoroughly wet to the dried virus inoculum and held for the contact time of 10 minutes at 23°C with 45% RH.

Recovery of Samples: After the contact time, the test substance was neutralized with 2.0 mL of neutralizer. The mixture was scraped from the surface of the carrier with a cell scraper. This post-neutralized sample (PNS) was considered the 10-1 dilution. An aliquot of the PNS was ten-fold serially diluted in DM

Infectivity Assay:
Selected dilutions of the sample were inoculated onto the plates at 100 µL per well, 4 wells per dilution, and incubated at 36 ± 2°C with 5 ± 3% CO2 for 1 hour with occasional shaking (every 15 minute). After 1 hour, plates were refed using 100 µL per well of fresh EMEM and then returned to the incubator for the remainder of the incubation period. After 10 days, the plates were removed from incubation, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

Neutralizer Effectiveness and Viral Interference Control (NE/VI):
The control was performed to assess whether residual active ingredient was present after neutralization (Neutralizer Effectiveness) or if the neutralized test substance interferes with virus infectivity (Viral Interference). The NE/VI was prepared identically to the test sample except DM was used in lieu of virus inoculum to inoculate the carrier. After test substance application and neutralization, the PNS was divided into two portions, one for NE/VI and one for Cytotoxicity (see below). For the NE/VI, a 0.5 mL aliquot of the PNS was ten-fold serially diluted and 100 µL of virus stock was added individually to selected dilutions and held for at least the contact time. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

Cytotoxicity Control (CT):
This control was performed to assess the cytotoxic effects of the test substance on indicator cells. The CT (obtained from the NE/VI) was prepared identically to the NE/VI except no virus was added to the selected dilutions inoculated onto indicator cells plates and incubated in an identical manner as the test samples.

Plate Recovery Control (PRC):
This control was performed to establish the input viral load to compare with the test substance results to evaluate the viral reduction by the test substance. The PRC was prepared identically to the test sample except DM was used in lieu of test substance to treat the dried virus inoculum during test substance application. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

Cell Viability Control (CVC):
This control was performed to demonstrate that the indicator host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. Indicator cell plates were aspirated, and 1.0 mL of DM was added to 4 wells of indicator cells and incubated in an identical manner as the test samples.

Virus Stock Titer Control (VST):
This control was performed to demonstrate that the titer of the stock virus was appropriate for use and that the viral infectivity assay was performed appropriately. An aliquot of the virus inoculum used in the study was ten-fold serially diluted in DM. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

CALCULATIONS
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint infectivity (TCID50), respectively, as calculated by the method of Spearman Karber.

– Log of 1st dilution inoculated – [(Sum of % mortality at each dilution/100) – 0.5) x (logarithm of dilution)]

TEST ACCEPTANCE CRITERIA
The test was considered acceptable for test substance evaluation due to the criteria below being satisfied:

- The infectious virus recovered from the PRC was ≥ 4.8 Log10 TCID50 units.
- Viral-induced CPE was distinguishable from test substance induced cytotoxicity (if any).
- Virus was recovered from dilutions of the NE/VI control not exhibiting cytotoxicity.
- The CVC did not exhibit CPE.

PROTOCOL CHANGES

Protocol Amendments

1. Protocol "Miscellaneous Information", Section A: The test substance storage conditions are listed as "Ambient". Per the Sponsor, it should be "Refrigerated". This amendment serves to correct the Protocol.

Protocol Deviations

- No protocol deviations were present in this study

RESULTS

Results are presented in Tables 1 – 7.

Table 1 Virus Titer

Dilution	**Cell showing CPE**
10^{-3}	+ + + +
10^{-4}	+ + + +
10^{-5}	+ + + +
10^{-6}	+ + + +
10^{-7}	+ + + 0
10^{-8}	0 0 0 0
$TCID_{50}$/0.1mL (Log_{10})	**7.25**

Table 2 Plate Recovery Control (PRC)

Dilution	**Cell showing CPE**
10^{-3}	+ + + +
10^{-4}	+ + + +
10^{-5}	+ + + +
10^{-6}	+ + + 0
10^{-7}	+ 0 0 0
10^{-8}	0 0 0 0
$TCID_{50}$/0.1mL (Log_{10})	**6.50**

Table 3 Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution	**Sani-TEST HOCl200**	
	Lot# <180-1	
	NE/VI	**CT**
10^{-2}	+ + + +	0 0 0 0
10^{-3}	+ + + +	0 0 0 0
10^{-4}	+ + + +	0 0 0 0

Table 4 Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution	**Sani-TEST HOCl200**	
	Lot# <180-2	
	NE/VI	**CT**
10^{-2}	+ + + +	0 0 0 0
10^{-3}	+ + + +	0 0 0 0
10^{-4}	+ + + +	0 0 0 0

Table 5 Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution	**Sani-TEST HOCl200**	
	Lot# <180-3	
	NE/VI	**CT**
10^{-2}	+ + + +	0 0 0 0
10^{-3}	+ + + +	0 0 0 0
10^{-4}	+ + + +	0 0 0 0

Table 6 Test Substance

Dilution	**Sani-TEST HOCl200**					
	Lot# <180-1		**Lot# <180-2**		**Lot# <180-3**	
	Replicate 1	**Replicate 2**	**Replicate 1**	**Replicate 2**	**Replicate 1**	**Replicate 2**
10^{-2}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Log_{10} $TCID_{50}$ per 0.1 mL	**≤1.50**	**≤1.50**	**≤1.50**	**≤1.50**	**≤1.50**	**≤1.50**
Log_{10} Reduction	**5**					

CONCLUSIONS

The Sani-TEST HOCl 200, Lot Nos <180-1; <180-2; and <180-3 were tested as described. All three lots passed the Virucidal Hard-Surface Efficacy Test when Human Coronavirus 229E, containing 5.0% Fetal Bovine Serum, was exposed to the test substance for 10 minutes at 23°C and 45% RH

All controls met the criteria for a valid test. These conclusions are based on observed data.

STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention
The study report and corresponding data will be held in the archives of the Biodetek Laboratory for at least 2 years after the date of the final report submission and then may be destroyed. If the study is used by the sponsor, Sani-TEST, in support of label claims, documentation may be returned to the Study Sponsor for archiving. The original data include, but not limited to the following:

- Raw data
- Documentation
- Records
- Protocol and protocol amendments
- Final Report and Final Report Amendments
- Correspondence and other documents relating to the interpretation and evaluation of data

Substance Retention
The test substances will be retained for 30 days after study completion and may be returned to the study Sponsor at their request and expenses. If study sponsor does not request return of the sample, it may be destroyed >30 days after study completion.

REFERNCES

- Annual Book of ASTM Standards, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428. 2

- U.S. EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations.

CERTIFICATE OF ANALYSIS

Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: Hypochlorous Acid, 200 ppm

Batch #: PSL-1, lot <180-1

PSL Reference No.: 210318-10R

Date of Analysis: March 22-23, 2021

Result:

Free Available Chlorine (FAC): 152.8 ppm

HOCl: 113.1 ppm

Approval: _____ March 29, 2021

Lyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs

Date

QA Release: _____ March 29, 2021

Quality Assurance
Product Safety Labs

Date

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 55541

PRODUCT SAFETY LABS
2394 US Highway 130
Dayton, NJ 08810
USA

732-438-5100
psl@productsafetylabs.com
www.productsafetylabs.com

Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: Hypochlorous Acid, 200 ppm

Batch #: PSL-1, lot <180-2

PSL Reference No.: 210318-11R

Date of Analysis: March 22-23, 2021

Result:

Free Available Chlorine (FAC): 159.7 ppm

HOCl: 118.2 ppm

Approval: _____ March 29, 2021

Lyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs

Date

QA Release: _____ March 29, 2021

Quality Assurance
Product Safety Labs

Date

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 55541

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Product Safety Labs

CERTIFICATE OF ANALYSIS

Product: Hypochlorous Acid, 200 ppm

Batch #: PSL-1, lot <180-3

PSL Reference No.: 210318-12R

Date of Analysis: March 22-23, 2021

Result:

Free Available Chlorine (FAC): 158.2 ppm

HOCl: 117.1 ppm

Approval:

Lyla Ansah-Johnson, BS, MBA
Analytical Services
Product Safety Labs

March 29, 2021
Date

QA Release:

Quality Assurance
Product Safety Labs

March 29, 2021
Date

This material was analyzed in compliance with Good Laboratory Practice (40 CFR 160) standards. Data are reported in PSL GLP Study No. 55541

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