Study Title

Determination of Virucidal Activity of Peraspray (H_2O_2/PAA) Ready to Use (RTU) Solution vs. Human Rhinovirus 42

Product Identity

Peraspray (H_2O_2/PAA) Ready to Use (RTU) solutions

Data Requirement

DIS/TSS-7 / Nov. 12, 1981 – EFFICACY DATA REQUIREMENTS: VIRUCIDES

Author

Chuan Wang, Ph.D.
Virology Manager

Study Completion Date

10/20/2010

Testing Facility

Gibraltar Laboratories, Inc.
16 Montesano Road
Fairfield, NJ 07004

Laboratory Project Number (Study File)

GBL Study # GR 2690

GIBRALTAR LABORATORIES, INC.
122 FAIRFIELD RD., FAIRFIELD, NJ 07004-2405 • PHONE: (973) 227-6882 • (973) 227-0812
e-mail: info@gibraltarlabsinc.com • Internet: www.gibraltarlabsinc.com

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B) or (C).

Company Enviro Tech Chemical Services, Inc

Company Agent Michael Harvey Date 10-25-10

President
Title

Michael Harvey
Signature

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122 FAIRFIELD RD., FAIRFIELD, NJ 07004-2405 • PHONE: (973) 227-6882 • (973) 227-0812
e-mail: info@gibraltarlabsinc.com • Internet: www.gibraltarlabsinc.com

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160 with the exception that the test agent stability information, synthesis, and purity analysis, composition and other characteristics of the test product remain with the sponsor.

SUBMITTER: Enviro Tech Chemical Services, Inc.

Ina Rodzus DATE: 10/25/10
Submitter's Name
Lab Manager
Submitter's Title

SPONSOR: Enviro Tech Chemical Services, Inc.

Michael Harvey DATE: 10-25-10
Michael Harvey
Study Sponsor's Name
President
Sponsor's Title

STUDY DIRECTOR:

DATE: 10/20/2010
Chuan Wang, Ph.D.
Acting Manager of Virology & Molecular Biology
Study Director's Title

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QUALITY ASSURANCE STATEMENT

Study Title: Determination of Virucidal Activity of Peraspray (H_2O_2/PAA) Ready to Use (RTU) Solution vs. Human Rhinovirus 42

Study Number: GR 2690

In accordance with the Good Laboratory Practice Standards (EPA 40 CFR Part 160), quality assurance audits of this study were conducted and reported to management and the study director as listed below:

Audit Date	Phase Audited	Date Reported to Study Director	Date Reported to Management
08/23/2010	Procedure	08/23/2010	08/23/2010
08/23/2010	Facilities	08/23/2010	08/23/2010
10/12/2010	Data	10/12/2010	10/12/2010
10/12/2010	Report	10/12/2010	10/12/2010

Chuck Weibel
Quality Assurance Manager

10/20/10
Date

GIBRALTAR LABORATORIES, INC.
122 FAIRFIELD RD., FAIRFIELD, NJ 07004-2405 • PHONE: (973) 227-6882 • (973) 227-0812
e-mail: info@gibraltarlabsinc.com • Internet: www.gibraltarlabsinc.com

STUDY PERSONNEL

Testing Facility Management	Daniel L. Prince, Ph.D. President	10/20/10 Date
Study Director and Supervisory Personnel	Chuan Wang, Ph.D. Virology Manager	10/20/2010 Date
Laboratory Personnel	Agnes T. Berki, Ph.D. Microbiologist/Virologist	10/20/10 Date
Laboratory Personnel	Roselle Ramos, M.S. Microbiologist/Virologist	10/20/10 Date

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e-mail: info@gibraltarlabsinc.com • Internet: www.gibraltarlabsinc.com

STUDY REPORT

STUDY TITLE: Determination of Virucidal Activity of Peraspray (H_2O_2/PAA) Ready to Use (RTU) Solution vs. Human Rhinovirus 42

SPONSOR: Enviro Tech Chemical Services, Inc.
500 Winmoore Way
Modesto, CA 95358
Attn: Michael Harvey
Tel # (209) 581-9576 Ext: 117
Fax # (209) 581-9653
Purchase Order # 740082
Sponsor # (1124)

TEST FACILITY: Gibraltar Laboratories, Inc.
16 Montesano Road
Fairfield, NJ 07004
Tel #: (973) 227-6882
Fax #: (973) 582-1565

TEST SUBSTANCE IDENTIFICATION

TEST SUBSTANCE NAME: Peraspray liquids: Lot TRNB9-1-56 and Lot TRNB11-1-01

LOT/BATCH NUMBER (S):
GBL # 243294/1 = Lot # TRNB9-1-56 Manufacturing Date: 10/28/2009
GBL # 243294/2 = Lot # TRNB11-1-01 Manufacturing Date: 07/01/2010

DESCRIPTION OF TEST SUBSTANCE: Two amber glass bottles, each containing approximately 50 mL of Peraspray (H_2O_2/PAA) Ready to Use (RTU) solution: Lot # TRNB9-1-56 and Lot# TRNB11-1-01. Expiration date is not known. Storage Conditions: The test materials were stored at ambient room temperature at the testing facility. Stability under storage conditions: Stability and purity are the responsibility of the sponsor.

CHEMICAL CHARACTERIZATION: The identity, solubility, stability, strength, purity, and chemical composition were not provided.

STUDY INITIATION DATE: 07/14/2010
EXPERIMENTAL START DATE: 08/23/2010
EXPERIMENTAL END DATE: 08/30/2010
STUDY COMPLETION DATE: 10/20/2010

STUDY OBJECTIVE: To determine the virucidal efficacy of two Peraspray liquid samples against Rhinovirus (common cold): Human Rhinovirus 42, upon ten minutes contact time.

TEST METHOD: Viral Infectivity Assay in Tissue Culture, GLP Protocol No. 3107. Study No.: GR 2690

TEST SYSTEM/STRAINS:
1. African green monkey kidney (VERO) Cells, Passage: 87 on 08/20/10, GBL# 181720/3
2. Rhinovirus (common cold): Human Rhinovirus 42, Pool#: 5, ATCC#: VR-338

STUDY MATERIALS

MEDIA AND REAGENTS

1. Propagation Media
Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 5% of Fetal Bovine Serum (FBS), and 100U/100µg/mL of Penicillin/Streptomycin, GBL Reagent Lot#: 3811

2. Neutralization Media
Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 20% of Fetal Bovine Serum (FBS), and 100U/100µg/mL of Penicillin/Streptomycin, GBL Reagent Lot#: 3811

3. Inoculation Media
3.1. Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 100U/100µg/mL of Penicillin/Streptomycin and 10.0 µg/mL of Trypsin, GBL#: 241769
3.2. Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 5% of Fetal Bovine Serum (FBS), and 100U/100µg/mL of Penicillin/Streptomycin, GBL Reagent Lot#: 3811, and 3814.

EQUIPMENT
Humidified Incubators: 33°C with 5% CO_2, GBL# 121415
BioSafety Cabinet, GBL# 78476
Calibrated Timers, GBL# 136232/1 and GBL# 117125
Multichannel pipettes: 100-1200µL, GBL# 233862, and 20-300µL, GBL# 233859
Single channel pipettes: 1000µL, GBL# 78571, and 200µL, GBL# 78574

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STUDY METHODS

1. PREPARATION OF TEST SUBSTANCE AND TEST SYSTEMS/STRAINS

1.1. Carrier Preparation: 0.2 mL of virus stock of known titer is evenly spread over a marked area of the inner surface of a sterile Petri dish under sterile conditions. The formed fluid-film is allowed to dry at room temperature under a Biosafety cabinet. The subsequent steps of the procedure are carried out immediately after the fluid-film is dried.

1.2. Test Reagent Preparations: The test reagents are prepared according to the sponsor's directions and proposed label claims.

1.3. *In Vitro* anti-viral procedure: 2.0 mL of test article is applied to cover the dried virus film completely in the Petri dish and allowed to be in contact for 10 minutes. The obtained solution is referred to as the 10^0 dilution reaction mixture. The action of the anti-viral test article is stopped by adding it to 10 fold excess of neutralizing medium, and by immediate vortexing. The obtained solution is labeled as the 10^{-1} dilution reaction mixture.

1.4. Dried Virus Recovery Infectivity Control: was prepared as described in Study Methods step 1.3. except by replacing the test article with 2.0 mL of neutralization medium.

1.5. Virus Stock Infectivity Control: 0.2 mL of virus stock is placed into a sterile Petri dish. Without allowing it to dry 1.8 mL of neutralization medium is added making the solution equivalent in virus content with the 10^0-label reaction mixture (Study Methods step 1.3.). The diluted virus stock in the Petri dish is further diluted by adding it to 10 fold excess of neutralizing medium (10^{-1} dilution).

1.6. Cytotoxicity and Neutralization Controls: For the cytotoxicity control the test article is treated the same way as described in Study Methods step 1.3., but with no dried virus present, instead the test sample is placed into a sterile Petri dish. The neutralization control is prepared as the cytotoxicity control and then virus, approximately 100 $TCID_{50}$, is added to all replicates of each dilution, which is obtained in the following step of the procedure (Study Methods step 1.7., 10-fold Serial Dilutions).

1.7. 10-fold Serial Dilutions: The obtained 10^{-1} dilution reaction mixtures are further diluted. The dilutions are carried out using Dilution Medium.

1.8. Infectivity Assay: Confluent sheets of appropriate cells in 96-well or 24-well plates are inoculated with the 10-fold serial dilutions in quadruplicate using 0.2 mL/well or 2.0 mL/well inoculum size, respectively. The cultures are incubated in a humidified incubator with 5% CO_2 at the appropriate temperature.

1.9. Negative Controls: Dilution medium alone is added to culture wells designated to serve as negative controls (Cell Control).

1.10. Microscopy: The morphology of the cell sheets is monitored for cytopathic effect (CPE 0-4) and cytotoxicity (T0-T4). The results of the observations are recorded on several days (0-7).

1.11. Calculations:

$TCID_{50}$ values are calculated according to the Reed-Muench method.

The logarithm of the $TCID_{50}$ of the dried virus control (Log_{10} $TCID_{50}$ of Dried Virus Control) is used in calculations to obtain the log-reduction for each Test Sample for every virus tested.

Log Reduction of the Test Sample = Log_{10} $TCID_{50}$ of Dried Virus – Log_{10} $TCID_{50}$ of Test Sample.

EXPOSURE CONDITIONS

Contact Time: 10 minutes

Test Temperature for 10 minutes contact: room temperature

Temperature for cell culture maintenance: 33°C

PROTOCOL CHANGES

PROTOCOL AMENDMENTS
None

PROTOCOL DEVIATIONS
None

CONTROLS

PREPARATION OF CONTROLS
Quantitative Control
To confirm the titer of the virus pools used a "Virus Stock Infectivity Control" was prepared as detailed under Study Methods step 1.5. To confirm the viable titer of viruses after drying and before in contact with the virucide samples, a "Dried Virus Recovery Infectivity Control" was prepared as described in Study Methods step 1.4.

Negative Control
The "Negative Control" wells (Cell Control) were used and prepared as described above in Study Methods step 1.9.

Neutralization Challenge
The "Neutralization Control" was performed by back inoculation of the virus as described above in Study Methods step 1.6.

STUDY ACCEPTANCE CRITERIA

STUDY REQUIREMENTS
1) Control Requirements: Quantitative Control: Log $TCID_{50}$ of the dried virus control titer is at least 3 logs.
Negative Control: No virus is present in Negative Control wells (Cell Control).
Neutralization Challenge: Neutralization using back inoculation is effective, that is the replication of virus is observable in all dilutions of the disinfectants beyond cytotoxicity.

2) Performance criteria:
1. No survivors at any dilution of the virus-disinfectant mixture upon 10 minutes contact time.
2. The dried control virus infectivity titer is at least 3-logs beyond the highest cytotoxic dilutions of virus disinfectant mixture upon 10 minutes contact time.

DATA ANALYSIS

CALCULATIONS
Basic arithmetic described in Study Methods step 1.11.

STATISTICAL ANALYSIS
None

STUDY RETENTION

Data Retention
The final report of this study as well as all raw data accumulated during the study will be kept in the archives of Gibraltar Laboratories, Inc. for a period of at least 10 years, unless notified by sponsor in writing, after which the documents will be returned to the sponsor.

Specimen Retention
After all studies are complete the remaining test material, if any, will be discarded or destroyed in accordance with GBL policy and State and Federal regulations.

STUDY RESULTS

Quantitative, Negative, and Neutralization Control Results (Tables 2 and 3): The quantitative control requirement is met. The Negative Control requirement is met. The neutralization challenge requirement is met. No survivors were seen at any dilution of the virus-disinfectant mixture. The dried control virus infectivity titer was at least 3-logs beyond the highest cytotoxic dilutions. Negative control wells showed no viral presence.

Performance Criteria (Summary is in Table 1, details in Table 3):
Peraspray liquid lot TRNB9-1-56 and lot TRNB11-1-01 produced virucidal inactivation rates of ≥3.0 and ≥3.0 logs, respectively, after 10 minutes contact time against Rhinovirus type 42 strain in a dried carrier test.

Study Results (Summary in Table 1, details in Table 3):
Peraspray liquid lot TRNB9-1-56 and lot TRNB11-1-01 produced virucidal inactivation rates of ≥3.0 and ≥3.0 logs, respectively, after 10 minutes contact time against Rhinovirus type 42 strain in a dried carrier test.

STUDY CONCLUSION
Under the conditions of this study, Peraspray (H_2O_2/PAA) Ready to Use (RTU) solutions, Lot TRNB9-1-56 and Lot TRNB11-1-01, did pass the DIS/TSS-7 / Nov. 12, 1981 – EFFICACY DATA REQUIREMENTS for VIRUCIDES against strain Rhinovirus type 42.

REPORT SUBMITTED BY:

Chuan Wang, Ph.D. Study Director

10/20/2010
Study Completion Date

Table 1 Collation of Data (Executive Summary)

Virus Strain Log $TCID_{50}$/mL		Human Rhinovirus 42
Dried Virus Control		4.2
Log Reduction	TRNB9-1-56 243294/1	≥ 3.0
	TRNB11-1-01 243294/2	≥ 3.0

Passes EPA Test ☒ Yes ☐ No

Table 2 Virus Controls of **Human Rhinovirus 42**

Viral Recovery Controls								
Dilutions	Virus Stock				Dried Virus			
10^{-1}	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+
10^{-4}	+	+	+	+	+	+	+	+
10^{-5}	+	+	+	+	0	0	0	0
10^{-6}	+	+	+	+	0	0	0	0
Cell Control	0	0	0	0	0	0	0	0
Log_{10} $TCID_{50}$/2mL	≥ 6.5				4.5			
Log_{10} $TCID_{50}$/mL	≥ 6.2				4.2			

Note: + Positive for viral infection, **0** Negative for viral infection or cytotoxicity.

Table 3 Antiviral Efficacy upon 10 minutes contact time

Antiviral Effect	**Human Rhinovirus 42**							
Dilutions	**TRNB9-1-56 243294/1**				**TRNB11-1-01 243294/2**			
10^{-1}	T	T	T	T	T	T	T	T
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
Log_{10} $TCID_{50}$/2mL	≤ 1.5				≤ 1.5			
Log_{10} $TCID_{50}$/mL	≤ 1.2				≤ 1.2			
Log Reduction*	≥ 3.0				≥ 3.0			
Percentage Inactivation	≥ 99.90 %				≥ 99.90 %			
Cytotoxicity	**243294/1**				**243294/2**			
10^{-1}	T	T	T	T	T	T	T	T
10^{-2}	0	0	0	0	0	0	0	0
10^{-3}	0	0	0	0	0	0	0	0
Log_{10} $TCLD_{50}$/2mL	1.5				1.5			
Log_{10} $TCLD_{50}$/mL	1.2				1.2			
Neutralization	**243294/1**				**243294/2**			
10^{-1}	T	T	T	T	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+
Negative Control	**243294/1**				**243294/2**			
Cell Control	0	0	0	0	0	0	0	0

Note: **T** Cytotoxicity, + Positive for viral infection, **0** Negative for viral infection or cytotoxicity.

* Calculated based on the Log_{10} $TCID_{50}$/mL of dried virus recovery control in Table 4.

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REPORT AMENDMENT NUMBER 1

GBL Study #: GR2690
GBL Protocol #: 3107
Report #: R-242368-R0 [Rhinovirus 42]
Study Title: Determination of Virucidal Activity of Peraspray (H_2O_2/PAA) Ready to Use (RTU) Solution vs. Human Rhinovirus 42

Sponsor: Enviro Tech Chemical Services, Inc.
500 Winmoore Way
Modesto, CA 95358

Attn.: Michael Harvey
Phone: (209) 581-9576
Fax: (209) 581-9653

The reason for this amendment is to correct the Submitting laboratory and the sponsor contact. The submitter was changed from Gibraltar Laboratories, Inc. to Enviro Tech Chemical Services, Inc. The sponsor contact was changed from Tina Rodrigues to Michael Harvey.

This report amendment must accompany the final report for this clarification.

Approved by:

Study Director: _____ Date: 10/20/10
Chuan Wang

QA Approved: _____ Date: 10/20/10
Michael Pannullo