December 12, 2016

FINAL REPORT #1608407-402

EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIVIRAL PROPERTIES AT THREE EXPOSURE TIMES USING AN IN-VITRO TIME-KILL METHOD

Prepared for:

OCULUS INNOVATIVE SCIENCES (SPONSOR)
1129 North McDowell Boulevard
Petaluma, California 94954

Prepared by:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718
(406) 587-5735

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EXECUTIVE SUMMARY

STUDY NUMBER:	**1608407-402**
TITLE:	**EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIVIRAL PROPERTIES AT THREE EXPOSURE TIMES USING AN IN-VITRO TIME-KILL METHOD**
SPONSOR:	**OCULUS INNOVATIVE SCIENCES** 1129 North McDowell Boulevard Petaluma, California 94954
TESTING FACILITY:	**BIOSCIENCE LABORATORIES, INC.** 1755 South 19th Avenue Bozeman, Montana 59718
STUDY INITIATION DATE:	09/12/2016
STUDY COMPLETION DATE:	12/12/2016

This study evaluated the virucidal properties of one test product when challenged with Feline Infectious Peritonitis virus strain WSU79-1683(3) (ATCC # VR-989), Feline Immunodeficiency virus strain NCSU1 (ATCC #VR-2333), and Canine Distemper virus strain Snyder Hill (ATCC #VR-1587) based on the ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension.* The test product was evaluated at a final concentration of 90% (v/v). The percent and $\log_{10}$ reductions from the initial population of each viral strain were determined following 30-second, 1-minute, and 2-minute exposure times to the product. All testing was performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58, with the exception that the characterization of the identity, strength, purity, composition, stability, and solubility of the test product remained the responsibility of the Study Sponsor and was not be performed by the Testing Facility (GLP 58.105).

STUDY CONCLUSIONS

The Virucidal Suspension Test, based on the ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension*, performed for Feline Infectious Peritonitis virus strain WSU79-1683(3) (ATCC #VR-989), showed that the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179), reduced infectivity of the Feline Infectious Peritonitis virus strain WSU79-1683(3) (ATCC #VR-548) by $\geq$5.00 $\log_{10}$ (>99.99% reduction) following 30-second, 1-minute, and 2-minute exposure times.

The Virucidal Suspension Test, based on the ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension*, performed for Canine Distemper virus strain Snyder Hill (ATCC #VR-1587), showed that the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179), reduced infectivity of the Canine Distemper virus strain Snyder Hill (ATCC #VR-1587) by $\geq$4.25 $\log_{10}$ (>99.99% reduction) following 30-second, 1-minute, and 2-minute exposure times.

The Virucidal Suspension Test, based on the ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension*, performed for Feline Immunodeficiency virus strain NCSU1 (ATCC #VR-2333), showed that the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179), reduced infectivity of the Feline Immunodeficiency virus strain NCSU1 (ATCC #VR-2333) by $\geq$4.25 $\log_{10}$ (>99.99% reduction) following 30-second, 1-minute, and 2-minute exposure times.

December 12, 2016

FINAL REPORT #1608407-402

1.0 TITLE: **EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIVIRAL PROPERTIES AT THREE EXPOSURE TIMES USING AN IN-VITRO TIME-KILL METHOD**

2.0 SPONSOR: **OCULUS INNOVATIVE SCIENCES**
1129 North McDowell Boulevard
Petaluma, California 94954

3.0 TESTING FACILITY: **BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

4.0 STUDY DIRECTOR: Volha Teagle [Dzyakanava], Ph.D.

5.0 PURPOSE:

The purpose of this study was to evaluate the virucidal properties of one test product when challenged with Feline Infectious Peritonitis virus strain WSU79-1683(3), Feline Immunodeficiency virus strain NCSU1, and Canine Distemper virus strain Snyder Hill, using a Virucidal Suspension Test (In-Vitro Time-Kill method). This evaluation was based on ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension.* All testing was performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58, with the exception that the characterization of the identity, strength, purity, composition, stability, and solubility of the test product remained the responsibility of the Study Sponsor and was not performed by the Testing Facility (GLP 58.105).

6.0 SCOPE:

This study evaluated the virucidal properties of one test product when challenged with Feline Infectious Peritonitis virus strain SWU79-1683(3) (ATCC # VR-989), Feline Immunodeficiency virus strain NCSU1 (ATCC #VR-2333), and Canine Distemper virus strain Snyder Hill (ATCC #VR-1587) based on the ASTM E1052-11, *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension.* The test product was evaluated at a final concentration of 90% (v/v). The percent and $\log_{10}$ reductions from the initial population of each viral strain were determined following 30-second, 1-minute, and 2-minute exposure times to the product. Testing of the product was conducted at ambient temperature (nominally, 20°C to 25°C). Plating was performed in four replicates.

The Study Protocol, included in Addendum 1 of this Final Report, presents the study methodology, in detail, as do the General Data Gathering Forms in Addendum 3 of this Final Report. No deviations from the methodology described in the Study Protocol and one deviation from an applicable BioScience Laboratories Standard Operating Procedure occurred during the course of this evaluation as described in Section 16.0 of this Final Report.

7.0 STUDY DATES:

STUDY INITIATION DATE:	09/12/2016
EXPERIMENTAL START DATE:	10/28/2016
EXPERIMENTAL END DATE:	12/07/2016
STUDY COMPLETION DATE:	12/12/2016

8.0 TEST MATERIAL:

The test product evaluated was provided to the Testing Facility by the Study Sponsor. Responsibility for determination of the identity, strength, purity, composition, solubility, and stability of the test product, as well as responsibility for retention of the test product, remained with the Study Sponsor. All documentation provided with the test product is included in Addendum 2 of this Final Report.

Test Product:	Microcyn AH® Wound and Skin Care (RD68-129)
Active Ingredients:	Hypochlorous Acid
Lot Number:	16F179
Manufacture Date:	06/2016
Expiration Date:	06/2018

9.0 CHALLENGE VIRAL STRAINS:

9.1 Feline Infectious Peritonitis virus strain WSU79-1683(3) (ATCC #VR-989)
9.2 Feline Immunodeficiency virus strain NCSU1 (ATCC #VR-2333)
9.3 Canine Distemper virus strain Snyder Hill (ATCC #VR-1587)

10.0 HOST CELLS:

10.1 CRFK (ATCC #CCL-94; *Felis catus* kidney cortex epithelial cells)
10.2 MYA-1 (ATCC #CRL-2417; *Felis catus* peripheral blood T lymphoblast)
10.3 Vero (ATCC #CCL-81; green monkey epithelial kidney cells)

11.0 SUPPLIES AND EQUIPMENT:

The equipment and supplies used in this study are as described in the Study Protocol in Addendum 1 of this Final Report. Additional details are recorded on Virology Equipment and Supplies Tracking Forms (Form No. 07-L-011) in Addendum 3 of this Final Report. All applicable equipment and instrumentation were calibrated in accordance with BioScience Laboratories, Inc., Standard Operating Procedures.

12.0 MEDIA:

The growth media and diluting fluids used in this study are as described in the Study Protocol in Addendum 1 of this Final Report. Additional details are recorded on Virology Equipment and Supplies Tracking Forms (Form No. 07-L-011) and the Virology Media Production Data Sheet (Form No. 03-L-013) in Addendum 3 of this Final Report.

13.0 HOST CELL PREPARATION:

CRFK and Vero cells were maintained as monolayers in disposable cell culture labware and were used for the Virucidal Suspension Test of Feline Infectious Peritonitis virus and Canine Distemper virus, respectively. Prior to testing, host cell cultures will be seeded onto the appropriate cell culture plates. Cell monolayers were 90% confluent and 24-48 hours old before inoculation with the virus. The growth medium (GM) and maintenance medium (MM) was 1X Minimum Essential Medium (MEM) with appropriate for each virus supplements. MYA-1 cells were maintained as a suspension lymphoblast cell culture and were seeded onto multi-well plates on the day of testing. The growth medium (GM) and maintenance medium (MM) was 1X RPMI 1640 with supplements.

14.0 TEST VIRUS PREPARATION:

The test viruses used for this study was from BSLI high titer virus stock. On the day of use, aliquots of the stock virus were removed from a -70°C freezer and thawed prior to use in testing.

15.0 TEST PRODUCT PREPARATION:

The test product was evaluated as provided by the Sponsor. The final test concentration was 90% (v/v).

16.0 DEVIATION FROM BIOSCIENCE LABORATORIES, INC., STANDARD OPERATING PROCEDURE:

Section 3.6 of SOP G-0001, *DOCUMENTATION PROGRAM-GENERAL,* states: "All data will be recorded by the individual conducting the test at the time of testing." The time out from a CO2 incubator was not recorded on 10/28/16 for CRFK and Vero cells. The deviation was due to technician error and did not affect the study outcome. The cells were logged out on test day, 10/28/16, prior to 11:00 am as indicated by the log in record.

17.0 RESULTS – TABLES 1 THROUGH 3:

17.1 Table 1 presents the data from the Virus Control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the log_{10} and percent reductions observed following exposure of Feline Infectious Peritonitis virus strain WSU79-1683(3) to the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179).

TABLE 1

Test Product: Microcyn AH® Wound and Skin Care (RD68-129) Lot # 16F179
Virus / Strain: Feline Infectious Peritonitis virus ATCC # VR-989
Host Cell Line: CRFK Host Cell Line ATCC # CCL-94
Volume Plated per Well: 1.0 mL

Dilution (- Log_{10})	Virus Control	Test exposure time			Cytotoxicity Control	Neutralization Control	Cell Control
		30 seconds	1 minute	2 minutes			
-2	NT	0000	0000	0000	0000	NT	0000
-3	++++	0000	0000	0000	0000	++++	
-4	++++	0000	0000	0000	0000	++++	
-5	++++	0000	0000	0000	NT	++++	
-6	+0++	0000	0000	0000	NT	++0+	
-7	000+	0000	0000	0000	NT	0000	
$TCID_{50}$ (log_{10})	6.50	≤1.50	≤1.50	≤1.50	≤1.50	6.25	
Log_{10} Reduction	N/A	**≥5.00**	**≥5.00**	**≥5.00**	N/A		
Percent Reduction	N/A	**>99.99**	**>99.99**	**>99.99**			

+ CPE (cytopathic/cytotoxic effect) present
0 CPE (cytopathic/cytotoxic effect) not detected
NT Not tested
N/A Not applicable
CTC Cytotoxicity

17.2 Table 2 presents the data from the Virus Control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the log_{10} and percent reductions observed following exposure of Canine Distemper virus strain Snyder Hill to the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179).

TABLE 2

Test Product: Microcyn AH® Wound and Skin Care (RD68-129) Lot # 16F179
Virus / Strain: Canine Distemper virus ATCC # VR-1587
Host Cell Line: Vero Host Cell Line ATCC # CCL-81
Volume Plated per Well: 1.0 mL

Dilution (- Log_{10})	Virus Control	Test exposure time			Cytotoxicity Control	Neutralization Control	Cell Control
		30 seconds	1 minute	2 minutes			
-2	NT	0000	0000	0000	0000	NT	0000
-3	++++	0000	0000	0000	0000	++++	
-4	++++	0000	0000	0000	0000	++++	
-5	++++	0000	0000	0000	NT	++++	
-6	000+	0000	0000	0000	NT	0+00	
-7	0000	0000	0000	0000	NT	0000	
$TCID_{50}$ (log_{10})	5.75	≤1.50	≤1.50	≤1.50	≤1.50	5.75	
Log_{10} Reduction	N/A	**≥4.25**	**≥4.25**	**≥4.25**	N/A		
Percent Reduction	N/A	**>99.99**	**>99.99**	**>99.99**			

\+ CPE (cytopathic/cytotoxic effect) present
0 CPE (cytopathic/cytotoxic effect) not detected
NT Not tested
N/A Not applicable
CTC Cytotoxicity

17.3 Table 3 presents the data from the Virus Control infectivity ($TCID_{50}$) and the post-exposure infectivity ($TCID_{50}$); the log_{10} and percent reductions observed following exposure of Feline Immunodeficiency virus strain NCSU1 to the Test Product, Microcyn AH® Wound and Skin Care (RD68-129) (Lot Number 16F179).

TABLE 3

Test Product: Microcyn AH® Wound and Skin Care (RD68-129) Lot # 16F179
Virus / Strain: Feline Immunodeficiency Virus ATCC # VR-2333
Host Cell Line: MYA-1 Host Cell Line ATCC # CRL-2417
Volume Plated per Well: 1.0 mL

Dilution (- Log_{10})	Virus Control	Test exposure time			Cytotoxicity Control	Neutralization Control	Cell Control
		30 seconds	1 minute	2 minutes			
-2	NT	0000	0000	0000	0000	NT	0000
-3	++++	0000	0000	0000	0000	++++	
-4	++++	0000	0000	0000	0000	++++	
-5	++++	0000	0000	0000	NT	++0+	
-6	+000	0000	0000	0000	NT	0000	
-7	0000	0000	0000	0000	NT	0000	
$TCID_{50}$ (log_{10})	5.75	≤1.50	≤1.50	≤1.50	≤1.50	5.25	
Log_{10} Reduction	N/A	**≥4.25**	**≥4.25**	**≥4.25**	N/A		
Percent Reduction	N/A	**>99.99**	**>99.99**	**>99.99**			

\+ CPE (cytopathic/cytotoxic effect) present
0 CPE (cytopathic/cytotoxic effect) not detected
NT Not tested
N/A Not applicable
CTC Cytotoxicity

18.0 STATISTICAL ANALYSIS:

A statistical analysis was not performed on the data derived from this study.

19.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) conducted an in-phase audit of the critical test procedures over the course of testing, and advised the Study Director and Management of the outcomes of these. On completion of testing, the QAU performed an audit of the raw data and of the Final Report, in its entirety. No deviations from the Study Protocol occurred during the course of this evaluation. One deviation from an applicable BioScience Laboratories, Inc., Standard Operating Procedure occurred and was documented appropriately.

20.0 LABORATORY PERSONNEL:

The following employees of BioScience Laboratories, Inc., were involved in the testing or ancillary support of this Study. The laboratory personnel have been appropriately trained, and their training records are on-file in the Quality Assurance Unit at the Testing Facility.

STUDY DIRECTOR:

Volha Teagle [Dzyakanava], Ph.D.
Virologist

Kelly Burningham
Microbiologist

Marc Charnholm
Manager of Laboratory Support

Stephanie Cebulla
Laboratory Support Technician

Jennifer Robinson
Laboratory Support Technician

Michelle Chandler
Product Handler

21.0 QUALITY ASSURANCE AND QUALITY CONTROL PERSONNEL:

Danielle Goveia
Quality Assurance Specialist

Renee LaFond, M.S.
Quality Assurance Specialist

Jeremy Duley
QC/Maintenance Specialist

Kim Potter
Quality Assurance Associate

Amy L. Juhnke, RQAP-GLP
Director of Quality Assurance

Carl Schmidt
ISO Technical Manager (QC, Training, Safety)

22.0 DOCUMENTATION AND RECORD KEEPING:

All documentation and records were compiled, analyzed, and will be retained by BioScience Laboratories, Inc. at its facility in Bozeman, Montana. All raw data for this study, as well as the Final Report, will be retained in safe storage by the Testing Facility for a period of at least 5 years. BioScience Laboratories, Inc., will notify the Study Sponsor before any documents or records are destroyed.

23.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC. (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

President and CEO: Daryl S Paulson
Daryl S. Paulson, Ph.D.

12-12-16
Date

Study Director: V Teagle
Volha Teagle [Dzyakanava], Ph.D.

12-12-16
Date of Study Completion

QUALITY ASSURANCE STATEMENT:

This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

Phase Inspected	Audit Date	Date reported to Study Director	Date reported to Management
Product Testing	10/28/2016	10/31/2016	11/02/2016
Data Audit	12/10/2016	12/12/2016	12/12/2016
Final Report Review	12/10/2016	12/12/2016	12/12/2016

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (21 CFR Part 58), with the exception that the characterization of the identity, strength, purity, composition, stability, and solubility of the test product was not performed by BioScience Laboratories, Inc. A deviation to an applicable BioScience Laboratories, Inc., Standard Operating Procedure was documented appropriately. This statement also serves to confirm that the Final Report reflects the raw data.

Director Of Quality Assurance: Amy Juhnke
Amy L. Juhnke, RQAP-GLP

12/12/16
Date

INDEX OF ADDENDA

1 Protocol #1608407-402
Protocol and SOP Deviation Recording Form (Form No: 99-QA-004)

2 Product Information
- Product Receipt Log (Form No. 92-L-023)
- Sample Submission Form (Form No. 94-G-007)
- Product-Tracking Form (Form No. 93-L-029)

3 Time-Kill Evaluation
- Tissue Culture Subculture Data Sheets (Form No. 00-L-006)
- Virus Culture Subculture Data Sheet (Form No. 07-L-001)
- General Data Gathering Forms (Form No. 91-L-002)
- Virucidal Test Evaluation Forms (Form No. 03-L-017)
- Virucidal Test Tracking Forms (Form No. 07-L-002)
- Virology Media Production Data Sheet (Form No. 03-L-013)
- Virology Equipment and Supplies Tracking Forms (Form No. 07-L-011)
- CO_2 Incubator Log Forms (Form No. 01-L-004)