FINAL REPORT

STUDY TITLE

VIRUCIDE TEST OF ZEROMOLD PLUS

SwRI® STUDY NUMBER
GLP-SP-219, Project 01.19946.01.001

TEST GUIDELINE
OCSPP 810.2200

TESTING FACILITY
ATS Labs, Inc. (ATS)
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121
Phone: 651-379-5526
Fax: 651-379-5549

Southwest Research Institute® (SwRI®)
Microencapsulation and Nanomaterials Department
6220 Culebra Road
San Antonio, Texas 78238-5166, USA
Tel: 210-522-6354, Fax: 210-522-4565

SPONSOR
BIOSENTA Inc.
1120 Finch Avenue West, Suite 503
Toronto, Ontario, M3J 3H7 CANADA, Tel: 416-410-2019

TEST ORGANISM
Poliovirus type 1; Swine Influenza (H1N1); Herpes Simplex virus type 1; 2009-H1N1 Influenza A virus; Hepatitis A virus; Respiratory Syncytial Virus

TEST PRODUCT IDENTITY
ZeroMold Plus™ (48528, BI#0017-4, Lot #1)
ZeroMold Plus™ (48534, BI# 0018-4, Lot #2)
ZeroMold Plus™ (48542, BI# 0020-4, Lot #4)

STUDY DATES
Date Sample Received at SwRI: 02/17/2014
Date Sample at LCL Shipped to ATS: 03/20/2014 and 04/15/2014
Study Initiation Date: 03/19/2014
Experimental Start Date: 04/11/2014
Final Experimental End Date: 05/13/2014
Study End Date: 05/23/2014

GLP COMPLIANCE STATEMENT

This study was declared to be compliant with GLP standards, according to 40 CFR 160: U.S. EPA GLP standards.

Study Director:

05/23/2014

XingGuo Cheng, Ph.D. (Date)
Senior Research Scientist
Southwest Research Institute

APPROVED:

5/23/14

Joe A. McDonough, Ph.D. (Date)
Director, Microencapsulation and Nanomaterials Department
Southwest Research Institute

END SPONSOR:

Signature: _____ Date: MAY 23RD/2014

Sponsor Representative: Chris Bilz
President and CEO
BIOSENTA Inc.
Tel: (Office) 416-410-2019
(Mobile) 416-938-7300

Quality Assurance Unit Statement of Compliance

It is the intent of Southwest Research Institute ® (SwRI®) that all studies conducted by our facility shall be of the highest quality and meet or exceed the criteria promulgated by the EPA in accordance with 40 CFR 160 FIFRA Good Laboratory Practices to assure the quality and integrity of the data generated. Testing performed by ATS Labs added to the SwRI Approved Supplier List through a physical audit by the Study Director and Quality Assurance Manager. Final Report 01.19946.01.001 Virucide Test of ZeroMold Plus was inspected by the Southwest Research Institute Division Quality Assurance Unit and the findings submitted to the Study Director and Management on the following dates:

Inspection Type	Inspection Date	Submitted to Study Director & Management
GLP protocol/training	03-14-14	03-21-14
Data review of ATS	05-22-23-14	05-23-14
Final Report Review	05-23-14	05-23-14

SwRI's Quality Assurance Unit audited the raw data, all records, any study deviations, and the report. Documentation and verification of these inspections have been archived. The report was found to be an accurate reflection of the study and the data generated. All raw data will be maintained in the quality system archives.

Jo Ann Boyd

5/23/14

Jo Ann Boyd, Manager
Quality Assurance Unit
Southwest Research Institute

(Date)

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_____ _____

Jo Ann Boyd, Manager (Date)
Quality Assurance Unit
Southwest Research Institute

Table of Contents

I. INTRODUCTION

BIOSENTA Inc. is interested in assessing its ZeroMold Plus Product as an intermediate and/or low-level disinfectant that kills pathogenic and potentially pathogenic microorganisms on hard non-porous inanimate surfaces or inanimate objects, when used according to labeling. This project provided testing to perform "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces". Testing was performed at ATS Labs at the facility addresses provided in the cover. This lab was previously audited for the Approved Supplier List and has completed the annual qualification review. The project performance is in compliance with EPA's current FIFRA Good Laboratory Practices Standard (40 CFR 160).

II. TEST SUBSTANCE

Test substance information is shown below and active ingredient concentrations provided in **Table 1**. All ZeroMold Plus product tested was previously titrated and diluted according to the manufacturer's instructions to ensure compliance with EPA guidance on lower certified limit (LCL) (SwRI GLP-SP-210 study).

Product Name: ZeroMold Plus
Active Ingredient: NaClO
Lower Certified Limit (LCL): 0.43%
Acceptable Active Ingredient Concentration for Efficacy Testing: 0.43%-0.44% (2% above LCL)
Storage Conditions: Ambient Temperature, cap closed (all lots tested)
Test Substance Spray Conditions (as follows):

- Distance = 6 - 8"
- Number of pumps = 4
- Contact Time = 10 minutes
- Exposure Temperature = 18–25 °C (room temperature)
- Organic Soil Load = 5 % Fetal Bovine Serum (FBS)

Table 1. Test substance active ingredient concentration (LCL to up to 2% above LCL).

ZeroMold Plus Test batch/lot information	Active ingredient (NaClO) concentration	Concentration/Dilution Tested
48528, Lot #1 (B1#0017-4), >60 days	0.43%	Ready-to-use, Trigger Spray
48534,Lot #2 (B1#0018-4), >60 days	0.43%	
48542, Lot #4 (B1#0020-4), fresh lot	0.43%	

III. STUDY PERSONNEL

XingGuo Cheng, Study Director at SwRI
Shanen Conway, Study Director at ATS Labs, MN (ATS will provide their personnel details)

All study personnel have received training in Good Laboratory Practices standards and are experienced in their area of responsibility. Resumes and training records for these personnel are maintained with SwRI's and ATS's Quality Assurance Unit.

IV. EXPERIMENTAL METHODS AND RESULTS

The general experimental method for virucide testing is summarized in Figure 1 below. Briefly, virus films were prepared and sprayed with ZeroMold Plus using 4 sprays at a distance of 6-8 inches, at a 45 degree angle and held covered for 10 min. The viruses (live or dead) were separated from the test substance and the absence or presence of infectivity using cell culture was examined.

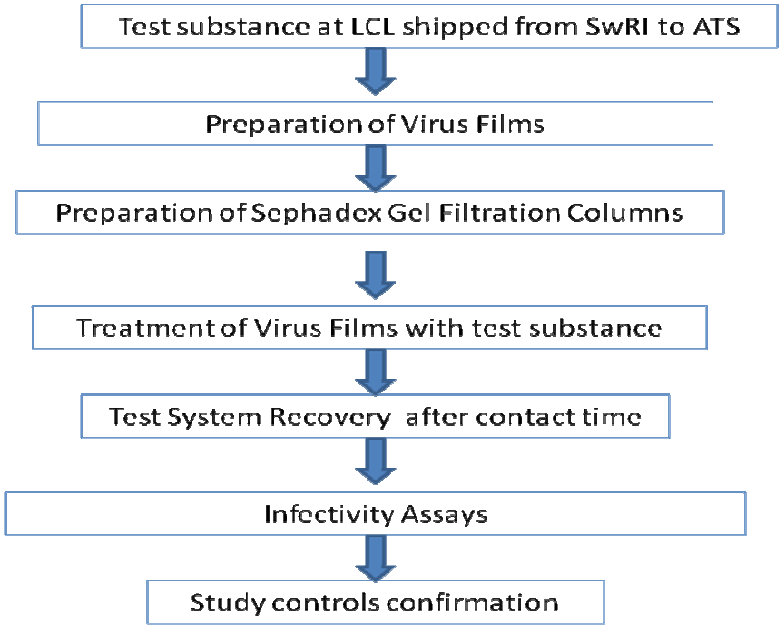

Figure 1 General test procedure for virucidal efficacy test of ZeroMold Plus disinfectant

Poliovirus Type 1 (ATCC VR-1562)

The controls and test substance assay results are summarized in Tables 2. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.POL) study acceptance criteria. Under the conditions of this investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus , ready to use as a trigger spray, demonstrated complete inactivation of Poliovirus type 1 following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 2 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 1**)

ZeroMold Plus	Dried Virus Control	Cell Control (Vero cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
All 3 lots	$10^{5.64}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

Swine Influenza A (H1N1) Virus (ATCC VR-333)

The controls and test substance assay results are summarized in Tables 3. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.SFLU) study acceptance criteria. Under the conditions of this investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus, ready to use as a trigger spray, demonstrated complete inactivation of Swine Influenza A (H1N1) Virus following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 3 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 2**)

ZeroMold Plus	Dried Virus Control	Cell Control (Rhesus monkey kidney cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
(48528, BI#0017-4, Lot #1) And (48534, BI# 0018-4, Lot #2)	$10^{5.5}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

Herpes Simplex Virus Type 1 (ATCC VR-733)

The controls and test substance assay results are summarized in Table 4. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.HSV1) study acceptance criteria. Under the conditions of this investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus, ready to use as a trigger spray, demonstrated complete inactivation of Herpes Simplex Virus Type 1 following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 4 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 3**)

ZeroMold Plus	Dried Virus Control	Cell Control (Rabbit kidney cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
(48528, BI#0017-4, Lot #1) And (48534, BI# 0018-4, Lot #2)	$10^{6.5}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

2009-H1N1 Influenza A virus (CDC#2009712192)

The controls and test substance assay results are summarized in Table 5. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.FLUA) study acceptance criteria. Under the conditions of this investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus, ready to use as a trigger spray, demonstrated complete inactivation of 2009-H1N1 Influenza A virus following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 5 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 4**)

ZeroMold Plus	Dried Virus Control	Cell Control (Cannine kidney cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
(48528, BI#0017-4, Lot #1) And (48534, BI# 0018-4, Lot #2)	$10^{5.25}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

Hepatitis A virus (HM-175, AppTec Lab Services)

The controls and test substance assay results are summarized in Table 5. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.HAV) study acceptance criteria. Under the conditions of this

investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus, ready to use as a trigger spray, demonstrated complete inactivation of Hepatitis A virus following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 6 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 5**)

ZeroMold Plus	Dried Virus Control	Cell Control (fetal Rhesus monkey kidney cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
(48528, BI#0017-4, Lot #1) And (48534, BI# 0018-4, Lot #2)	$10^{6.0}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

Respiratory Syncytial Virus (ATCC VR-26)

The controls and test substance assay results are summarized in Table 7. Controls and neutralization results are in compliance with the ATS test protocol (SWT01031414.RSV) study acceptance criteria. Under the conditions of this investigation and in the presence of 5% fetal bovine serum organic soil load, ZeroMold Plus, ready to use as a trigger spray, demonstrated complete inactivation of Respiratory Syncytial Virus following 10 minute exposure at room temperature as required by the U.S. EPA and Health Canada.

Table 7 Controls and Test Substance Assay Results (detailed results are shown in **Appendix 6**)

ZeroMold Plus	Dried Virus Control	Cell Control (fetal Rhesus monkey kidney cells)	Neutralization Control	Test Substance Cytotoxicity Control	Test Substance Assay Results
(48528, BI#0017-4, Lot #1) And (48534, BI# 0018-4, Lot #2)	$10^{4.5}$	No virus infectivity and/or no cytotoxicity present	Positive for the presence test virus after lower titer stock virus added	No cytotoxicity present	No virus recovered

V. PROCEDURAL MODIFICATIONS/PROTOCOL AMENDMENTS

One protocol amendment was written during the course of this study at SwRI which consisted of extending the completion date. Protocol amendments at ATS Labs were detailed in Appendix 1-6.

VI. STUDY CONCLUSIONS

Under the condition of this study and in the presence of 5% organic soil load, samples of ZeroMold Plus demonstrated complete inactivation of all the viruses tested following a 10 minute exposure at room temperature as required by U.S. EPA and Health Canada. ZeroMold Plus is qualified as a one-step disinfectant for the above viruses.

All original records (or exact copies of originals) of final reports will be kept at SwRI.

VII. RECORD & SAMPLE RETENTION

In accordance with SwRI TAP 01-0103-020*, Conduct of a GLP Study*, copies of the protocol, appendices, amendments, and the analytical method shall be available to the analysts, technicians and chemists involved in the study at SwRI. The analysts at SwRI shall maintain laboratory notebooks or equivalent documents in which they

will record all procedures, weighing, observations, etc., relevant to the experimental work. Chromatograms, computer printouts, etc., will be clearly labeled and notebooks will remain in the analyst's possession throughout the study. Records shall be archived as written in SwRI TAP 01-0103-023*, Project Setup and Record Archival for GLP.*

At the completion of the analytical portion of the study, a report shall be prepared as specified in SwRI TAP 01-0103-022*, Final Report for GLP Studies,* and delivered to the sponsor for final approval. After completion of the final report for the study, all methodology, raw data sheets, and original chromatograms will be inspected by the QAU at SwRI. At the sponsor's discretion, all original records will be sent to sponsor for archiving in accordance with 40 CFR 160.

The study for a research or marketing permit approved by EPA shall be maintained for the period during which the sponsor holds that research or marketing permit to which the study is pertinent unless all originals are provided to the client. For samples remaining at the conclusion of the study, SwRI will either have samples returned to the sponsor or disposed after five years from market permit submission to EPA. Materials that degrade will not be maintained.

VIII. REFERENCES

CAN/CGSB-2.161-97 “Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surface and Medical Devices”

http://www.epa.gov/oppad001/dis_tss_docs/dis-01.htm “Disinfectants for Use on Hard Surfaces”

“Environmental Protection Agency.” Product Performance Test Guidelines. OCSPP 810.2200: Disinfectants for Use on Hard Surfaces - Efficacy Data Recommendations.

IX. APPENDIX

The GLP final study reports provided by ATS are provided in the following appendix.
Appendix 1: Poliovirus Type 1 (ATS project number A16536)
Appendix 2: Swine Influenza A (H1N1) (ATS project number A16537)
Appendix 3: Herpes Simplex virus type 1 (ATS project number A16538)
Appendix 4: 2009-H1N1 Influenza A virus (ATS project number A16539)
Appendix 5: Hepatitis A virus (ATS project number A16540)
Appendix 6: Respiratory Syncytial Virus (ATS project number A16541)