ATS LABS

**FINAL STUDY REPORT**

STUDY TITLE

**Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay**

**Virus:** Swine Influenza A (H1N1) virus

PRODUCT IDENTITY

BGP Liquid Complex
Batch 2123

AUTHOR

Karen M. Ramm, B.A.
Study Director

STUDY COMPLETION DATE

September 18, 2009

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Micro Quality Labs
3200 North San Fernando
Burbank, CA 91504

PROJECT NUMBER

A08179

## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

Submitter: MQ Labs — 9-18-09 (Date)

Sponsor: Allan Lord BGP Complex — 9.18.09 (Date)

Study Director: Karen M. Ramm — 9-18-09 (Date)
Karen M. Ramm, B.A.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice Regulations (21 CFR Part 58) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols 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.

| Phase Inspected | Date of Phase Inspection | Date Reported To Study Director | Date Reported To Management |
|---|---|---|---|
| Critical Phase | August 28, 2009 | August 28, 2009 | September 18, 2009 |
| Final Report | September 18, 2009 | September 18, 2009 | |

The findings of these inspections have been reported to Management and the Study Director.

Quality Assurance Auditor: Judy Heidemann Date: 9-18-09

## TABLE OF CONTENTS

## STUDY PERSONNEL

STUDY DIRECTOR: Karen M. Ramm, B.A.

Professional Personnel Involved:
Matthew Cantin, B.S. - Research Assistant II
Katherine A. Paulson, M.L.T. - Research Assistant II

## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

**Project Number:** A08179

**Protocol Number:** MQL01081209.SFLU

**Sponsor:** Micro Quality Labs
3200 North San Fernando
Burbank, CA 91504

**Testing Facility:** ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance:** BGP Liquid Complex

**Lot/Batch(s):** Batch 2123

**Test Substance Characterization**
Test substance characterization as to content, stability, solubility, storage, etc., (21 CFR, Part 58, Subpart [58.105]) is the responsibility of the Sponsor.

### STUDY DATES

**Date Sample Received:** August 18, 2009
**Study Initiation Date:** August 28, 2009
**Experimental Start Date:** August 28, 2009
**Experimental End Date:** September 4, 2009
**Study Completion Date:** September 18, 2009

### OBJECTIVE

The objective of this study was to evaluate the antiviral properties of a product against Swine Influenza A (H1N1) virus when exposed (in suspension) for the specified exposure time. The protocol is a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E 1052).

## SUMMARY OF RESULTS

| | |
|---|---|
| Test Substance: | BGP Liquid Complex, Batch 2123 |
| Dilution Tested: | Ready to use (RTU) |
| Virus: | Swine Influenza A (H1N1) virus, Strain A/Swine/Iowa/15/30, ATCC VR-333 |
| Exposure Time(s): | 30 Seconds |
| Exposure Temperature: | Room Temperature (25.0°C) |
| Organic Soil Load: | 1% fetal bovine serum |
| Efficacy Result: | Under these test conditions, BGP Liquid Complex, Batch 2123 demonstrated a ≥99.97% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reduction in viral titer was ≥3.5 $\log_{10}$. |

## TEST SYSTEM

1. Virus
The A/Swine/Iowa/15/30 strain of Swine Influenza A (H1N1) virus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-333). Stock virus was prepared by collecting the supernatant culture fluid from 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 (ATS Labs Lot SF-18) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Influenza virus on Rhesus monkey kidney cells.

2. Indicator Cell Cultures
Rhesus monkey kidney (RMK) cells were obtained from ViroMed Laboratories, Inc., Minneapolis, MN, Cell Culture Division. Cultures were maintained and used as monolayers in disposable tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. On the day of testing, 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
Test medium used in this study was Minimum Essential Medium (MEM) supplemented with 1% heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures used. See the report text for a more detailed explanation.

| PARAMETERS TESTED FOR VIRUCIDAL EFFICACY ASSAY | | | |
|---|---|---|---|
| **Test or Control Group** | **Dilutions Assayed ($log_{10}$)** | **Cultures per Dilution** | **Total Cultures** |
| Cell Control | N/A | 4 | 4/group |
| Virus Control | -2,-3,-4,-5,-6,-7 | 4 | 24 |
| Sample batch + virus | -2,-3,-4,-5,-6,-7 | 4 | 24 |
| Cytotoxicity Control | -2,-3,-4 | 4 | 12 |
| Neutralization Control | -2,-3,-4 | 4 | 12 |

## METHODS

1. Preparation of Test Substance
BGP Liquid Complex was used as it was received from the Sponsor. The test substance removed from the original container was in solution as determined by visual observation. The test substance was shaken prior to using in the test and was pre-equilibrated to the exposure temperature (25.0°C).

2. Treatment of Virus Suspension
A 1.80 mL aliquot of the test substance was dispensed into a sterile tube and mixed with a 0.2 mL aliquot of the stock virus suspension. The mixture was vortex mixed for 10 seconds and held for the remainder of the specified exposure time at room temperature (25.0°C). The exposure time assayed was 30 seconds. Following the exposure time, a 0.1 mL aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilution (0.1 mL + 0.9 mL test medium) and assayed for the presence of virus. Note: To decrease the test substance cytotoxicity, the first dilution was made in FBS with the remaining dilutions in test medium.

3. Treatment of Virus Control
A 0.2 mL aliquot of stock virus suspension was exposed to a 1.80 mL aliquot of test medium in lieu of test substance and treated as previously described. Following the exposure time, a 0.1 mL aliquot was removed from the tube and the mixture was immediately titered by 10-fold serial dilution (0.1 mL + 0.9 mL test medium) and assayed for the presence of virus. The control employed the FBS neutralizer as described in the Treatment of Virus Suspension section. The virus control titer was used as a baseline to compare the percent and log reduction following exposure to the test substance.

4. Cytotoxicity Control
A 1.80 mL aliquot of the test substance was mixed with a 0.2 mL aliquot of test medium containing the Sponsor requested organic soil load in lieu of virus and treated as previously described. The cytotoxicity of the cell cultures was scored at the same time as virus-test substance and virus control cultures. Cytotoxicity was graded on the basis of cell viability as determined microscopically. Cellular alterations due to toxicity were graded and reported as toxic (T) if greater than or equal to 50% of the monolayer was affected.

5. Neutralization Control
Each cytotoxicity control mixture (above) was challenged with 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 0.1 mL aliquot of each dilution in quadruplicate. A 0.1 mL aliquot of low titer stock virus was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

6. Infectivity Assay
The RMK cell line, which exhibits CPE in the presence of Swine Influenza A (H1N1) virus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

7. Statistical Methods: Not applicable

## PROTOCOL CHANGES

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

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

## DATA ANALYSIS

### Calculations

Viral and cytotoxicity titers are 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.

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

Calculation of Percent (%) Reduction

$$\text{\% Reduction} = 1 - \left[\frac{TCID_{50}\ \text{test}}{TCID_{50}\ \text{virus control}}\right] \times 100$$

Calculation of Log Reduction

$TCID_{50}$ of the virus control – $TCID_{50}$ of the test substance = Log reduction

## STUDY ACCEPTANCE CRITERIA

A valid test requires 1) that stock virus be recovered from the virus control, 2) that the cell controls be negative for virus, and 3) that negative cultures are viable.

## STUDY RETENTION

### Record Retention

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. 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.

### Test Substance Retention

The test substance will be returned following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

## REFERENCES

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1052-96 (Reapproved 2002).

2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482-04.

3. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A., and Lennette, E.T. editors. Seventh edition, 1995.

## STUDY RESULTS

The titer of the virus control was exposed for 30 seconds was 7.0 $\log_{10}$. Following the 30 second exposure time, test virus infectivity was not detected in the virus-test substance mixture in any dilution tested ($\leq$3.5 $\log_{10}$).

Test substance cytotoxicity was observed at 3.5 $\log_{10}$. The neutralization control demonstrated that the test substance was neutralized at $\leq$3.5 $\log_{10}$.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, BGP Liquid Complex (Batch 2123), ready to use, demonstrated a ≥99.97% reduction in viral titer following a 30 second exposure time to Swine Influenza A (H1N1) virus as compared to the titer of the corresponding virus control. The log reduction in viral titer was ≥3.5 $\log_{10}$.**

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

**TABLE 1: Virus Control and Test Results**

**Effects of BGP Liquid Complex (Batch 2123) Against Swine Influenza A (H1N1) virus in Suspension Following a 30 second Exposure Time**

| Dilution | Virus Control | Test: Swine Influenza A (H1N1) virus + BGP Liquid Complex |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | + + + + | T T T T |
| $10^{-3}$ | + + + + | T T T T |
| $10^{-4}$ | + + + + | 0 0 0 0 |
| $10^{-5}$ | + + + + | 0 0 0 0 |
| $10^{-6}$ | + + + + | 0 0 0 0 |
| $10^{-7}$ | 0 + + 0 | 0 0 0 0 |
| $TCID_{50}$/0.1 mL | $10^{7.0}$ | $\leq 10^{3.5}$ |
| Percent Reduction | NA | ≥99.97% |
| $Log_{10}$ Reduction | NA | ≥3.5 $log_{10}$ |

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present
(NA) = Not applicable

**TABLE 2: Cytotoxicity and Neutralization Control Results**

| Dilution | Cytotoxicity Control | Neutralization Control |
|---|---|---|
| | BGP Liquid Complex | Cytotoxicity Control + test virus |
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-2}$ | T T T T | T T T T |
| $10^{-3}$ | T T T T | T T T T |
| $10^{-4}$ | 0 0 0 0 | + + + + |
| $TCD_{50}$/0.1 mL | $10^{3.5}$ | Neutralized at a $TCID_{50}$/0.1 mL of $\leq 3.5$ $Log_{10}$ |

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

(For Laboratory Use Only)
ATS Labs Project # A 0 8 1 7 9
8/28/09

ATS LABS

**PROTOCOL**

# Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

Virus: Swine Influenza A (H1N1) virus

**PROTOCOL**

MQL01081209.SFLU

**PREPARED FOR**

Micro Quality Labs
3200 North San Fernando
Burbank, CA 91504

**PERFORMING LABORATORY**

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**PREPARED BY**

Mary J. Miller, M.T.
Research Scientist II

**DATE**

August 12, 2009

EXACT COPY
INITIALS KMR DATE 9-18-09





1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

## Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

**SPONSOR:** Micro Quality Labs
3200 North San Fernando
Burbank, CA 91504

**TEST FACILITY:** ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

**PURPOSE**
The purpose of this study is to evaluate the antiviral properties of a product against Swine Influenza A (H1N1) virus when exposed (in suspension) for a specified exposure period(s). This protocol is a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E 1052).

**TEST SUBSTANCE CHARACTERIZATION**
Test substance characterization as to content, stability, solubility, storage, etc., (21 CFR, Part 58, Subpart [58.105]) is the responsibility of the Sponsor. The test substance shall be characterized before the experimental start date of 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 ATS Labs.

**SCHEDULING AND DISCLAIMER OF WARRANTY**
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs 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 26, 2009. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of September 16, 2009. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current EPA and/or FDA guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency (EPA or FDA) before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

If a test must be repeated, or a portion of it, because of failure by ATS Labs 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 ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

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

**TEST SYSTEM JUSTIFICATION**
This *in-vitro* virucidal suspension assay is designed to evaluate the antiviral properties of a product against Swine Influenza A (H1N1) virus. The presence of virus (infectivity) is determined by monitoring the virus specific cytopathic effect (CPE) on the appropriate indicator cell line, Rhesus monkey kidney. The indicator cell line chosen is capable of supporting the growth of Swine Influenza A (H1N1) virus.



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

Protocol Number: MQL01081209.SFLU
Micro Quality Labs
Page 3 of 8

## EXPERIMENTAL DESIGN

**Protocol Summary**
A suspension of virus is exposed to the use dilution of the product. At each pre-determined exposure time an aliquot is removed, neutralized by serial dilution, and assayed for the presence of virus. The positive virus controls, cytotoxicity controls, and neutralization controls are assayed in parallel. Antiviral properties of the product will be evaluated and compared at the specified concentrations and time intervals.

**Test Parameters**
The following is a list of the test and control groups, usual dilutions and number of cultures to be assayed.

| | Dilutions to be assayed | Cultures/diln |
|---|---|---|
| Cell Control | | 4 |
| Virus Control (for each exposure time) | $10^{-2}$ to $10^{-7*}$ | 4 |
| Test (for each exposure time and/or product concentration) | $10^{-2}$ to $10^{-7*}$ | 4 |
| Cytotoxicity Control (for each product concentration) | $10^{-2}$ to $10^{-4*}$ | 4 |
| Neutralization Control (for each product concentration) | $10^{-2}$ to $10^{-4*}$ | 4 |

* Alternate dilutions may be assayed as determined by the stock virus titer.

## CULTURE MATERIALS

**Virus**
The A/Swine/Iowa/15/30 strain of Swine Influenza A (H1N1) virus to be used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-333). The 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 is stored at ≤-70°C until the day of use. On the day of use, the appropriate number of aliquots of virus 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**
Rhesus monkey kidney (RMK) cells are received from ViroMed Laboratories, Inc., Minneapolis, MN Cell Culture Division. Cultures are maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. RMK cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.
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 the virucidal assays is Minimum Essential Medium (MEM) supplemented with 1-10% (v/v) heat inactivated FBS. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. 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 final report.



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

## TEST METHOD

**Preparation of Test Substance**
The test substance(s) will be prepared according to the directions for the intended use and furnished by the Sponsor. The test substance(s) will be pre-equilibrated to the exposure temperature if applicable.

**Treatment of Virus Suspension**
A 1.8 mL aliquot of each concentration of each test substance is dispensed into separate tubes and each is mixed with a 0.2 mL aliquot of the stock virus suspension. The mixtures are vortex mixed for a minimum of 10 seconds and held for the remainder of the specified exposure times at the appropriate temperature. This is considered the $10^{-1}$ dilution of the virus. Immediately following each exposure time, a 0.1 mL aliquot is removed from each tube and the mixtures are titered by 10-fold serial dilutions (0.1 mL + 0.9 mL test medium) and assayed for the presence of virus. Note: To decrease the product cytotoxicity, the first dilution may be made in fetal bovine serum or other appropriate neutralizer with the remaining dilutions in test medium. Sterile glass beads may be added to aid in mixing of viscous products.

Alternate volumes of the virus and test substance may be utilized as long as the 1:10 virus to test substance ratio is maintained. All controls will be adjusted accordingly.

If excessive cytotoxicity to the indicator cell cultures is caused by the test substance or suspected, the affected dilution(s) may be passed through individual Sephadex gel filtration columns following titration to aid in reducing the toxicity. If this procedure is performed, the same dilutions of the controls must also be passed through individual columns.

**Treatment of Virus Control**
A 0.2 mL aliquot of the stock virus suspension is exposed to a 1.8 mL aliquot of test medium instead of test substance and treated as previously described in Treatment of Virus Suspension section. A virus control will be performed for each exposure time tested. All controls will employ the neutralizer utilized in the test. The virus control titer will be used as a baseline to compare the percent and log reduction of each test parameter following exposure to the test substance(s).

**Cytotoxicity Control**
A 1.8 mL aliquot of each concentration of each test substance is mixed with a 0.2 mL aliquot of test medium containing the Sponsor requested organic soil load (if applicable) in lieu of virus and treated as previously described. When multiple exposure times are requested, the cytotoxicity control will be performed at the longest requested exposure time. The cytotoxicity of the cell cultures is scored at the same time as virus-test substance and virus control cultures. Cytotoxicity is graded on the basis of cell viability as determined microscopically. Cellular alterations due to toxicity will be graded and reported as toxic (T) if greater than or equal to 50% of the monolayer is affected.

**Neutralization Control**
Each cytotoxicity control mixture (above) will be challenged with 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 each dilution in quadruplicate. A 0.1 mL aliquot of a 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.



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

**Infectivity Assay**
The RMK cell line, which exhibits cytopathic effect (CPE) in the presence of Swine Influenza A (H1N1) virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 0.1 mL of the dilutions prepared from test and control groups. 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. The cultures will be scored periodically for approximately seven days for the absence or presence of CPE, cytotoxicity and for viability.

**Test Criteria**
A valid test will require 1) that stock virus be recovered from the virus control, 2) that the cell controls be negative for virus; 3) that negative cultures be viable.

**Calculations**
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.

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

Calculation of Percent (%) Reduction

$$\text{\% Reduction} = 1 - \left[\frac{TCID_{50}\ \text{test}}{TCID_{50}\ \text{virus control}}\right] \times 100$$

Calculation of Log Reduction

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

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**
The specialized virucidal testing section of ATS Labs 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

**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, and a conclusion as it relates to the purpose of the test.



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

**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.

**PRODUCT DISPOSITION**
Test substance retention shall be the responsibility of the Sponsor. Unused test material 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 ATS Labs. 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 ATS Labs. 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. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1052-96 (Reapproved 2002).

2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482-04.

3. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E. H, Lennette, D.A., and Lennette, E.T. editors. Seventh edition, 1995.



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

## STUDY INFORMATION

*(All sections must be completed prior to submitting protocol)*

* Please shake before testing

**Sponsor (Date/Initial):** KA / A.L. 8/14/09

**Test Substance (Name and Batch Number - exactly as it should appear on final report):** BGP Liquid Complex batch 2123

**Expiration Date:** N/A

**Product Description**

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☐ Other: Proprietary Complex

**Test Substance Active Concentration (upon submission to ATS Labs):** N/A

**Storage Conditions**

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

**Hazards**

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

**Product Preparation**

- ☑ No dilution required, Use as received (RTU)
- ☐ *Dilutions/Concentrations to be tested _____
  - ☐ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☐ Other_____

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

**Test Virus:** Swine Influenza A (H1N1) virus **Indicator Cell Line:** RMK

**Exposure Time(s):** 1) 30 seconds 2) _____ 3) _____ per Sponsor e-mail dated 8/19/09. ln 8/29/09 wo 8/28/09 ln 28

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

**Organic Soil Load**

- ☑ 1% fetal bovine serum (lowest level that can be tested)
- ☐ 5% fetal bovine serum
- ☐ Other _____ N/A per Sponsor e-mail dated 8/19/09 ln 8/29/09 28 wo 8/28/09 ln



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

08/14/2009 09:55 818-845-0030 MICRO QUALITY LABS PAGE 03/07



**TEST SUBSTANCE SHIPMENT STATUS**

- ☐ Has been used in one or more previous studies at ATS Labs.
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs: 8/14/09 Sent via *overnight* delivery? ☑ Yes ☐ No
- ☑ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: 8/17/09
- ☐ Sender (if other than Sponsor): Micro Quality Labs.

**COMPLIANCE**

This study will be conducted in compliance with the FDA and/or EPA Good Laboratory Practices Regulations (21 CFR Part 58, 40 CFR Part 160 respectively) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

**PROTOCOL MODIFICATIONS**

- ☑ Approved without modification
- ☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

N/A

**APPROVAL SIGNATURES**

***SPONSOR:***

NAME: Allen Lord / Karine Aylazyan TITLE: 8-14-09

SIGNATURE: DATE: 8-14-09

PHONE: 816-845-0070;310-613-8697 (cell) FAX: 818-845-8036 EMAIL: adl911@mac.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

N/A

***ATS LABS:***

NAME: Karen Rymm
Study Director

SIGNATURE: Karen Rymm
Study Director

DATE: 8/28/09
WD 8/28/09



1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549