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LABORATORY SERVICES

MicroBioTest
A Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

# FINAL REPORT

# EVALUATION OF FILTRATION EFFICIENCY OF TREATED FACE MASKS AGAINST AEROSOLIZED VIRUS - 2013 INFLUENZA A (H7N9) VIRUS

**Test Agents**

FFP2

FFP2 CTL

**Author**

Salimatu Lukula, M.S.

**Performing Laboratory**

MicroBioTest
A Division of Microbac Laboratories Inc.
105 Carpenter Drive
Sterling, Virginia 20164

**Laboratory Project Identification Number**

798-114

**Sponsor**

VIROBLOCK SA
18, chemin des Aulx
CH-1228 Plan-les-Ouates
Switzerland



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www.microbac.com

## TABLE OF CONTENTS

## COMPLIANCE STATEMENT

This study meets the requirements for 21 CFR § 58 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Salimatu L. Lukula, Michael Parker

Study Director: MicroBioTest

Salimatu Lukula 7/19/13

Salimatu Lukula, M.S. Date

## QUALITY ASSURANCE UNIT STATEMENT

Title of Study: EVALUATION OF FILTRATION EFFICIENCY OF TREATED FACE MASKS AGAINST AEROSOLIZED VIRUS - 2013 INFLUENZA A (H7N9) VIRUS

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 798-114 in compliance with current Good Laboratory Practice regulations, (21 CFR § 58).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

| PHASE INSPECTED | DATE OF INSPECTION | DATE REPORTED TO STUDY DIRECTOR | DATE REPORTED TO MANAGEMENT |
|---|---|---|---|
| Protocol | 06/26/13 | 07/01/13 | 07/01/13 |
| In Process | 06/26/13 | 07/01/13 | 07/01/13 |
| Final Report | 07/16/13 | 07/16/13 | 0716/13 |

Jeanne M. Anderegg 7-18-13

Jeanne M. Anderegg Date
Manager, Quality Assurance

## TEST SUMMARY

**TITLE:** EVALUATION OF FILTRATION EFFICIENCY OF TREATED FACE MASKS AGAINST AEROSOLIZED VIRUS - 2013 INFLUENZA A (H7N9) VIRUS

**STUDY DESIGN:** This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (See Appendix).

**TEST MATERIALS SUPPLIED BY THE SPONSOR OF THE STUDY:**

1. FFP2, Lot No. 310001, received at MicroBioTest on 05/10/13, assigned DS No. D287

2. FFP2 CTL, Lot No. VB-DEV-7-FEB-2013-NAT, received at MicroBioTest on 05/10/13, assigned DS No. D289

**SPONSOR:** VIROBLOCK SA
18, chemin des Aulx
CH-1228 Plan-les-Ouates
Switzerland

## TEST CONDITIONS

Challenge virus:

2013 Influenza A (H7N9) Virus, A/Shanghai/1/2013, U.S. Centers for Disease Control and Prevention (CDC) CCID/NCIRD/ID/MVVB

Host:

Madin-Darby canine kidney (MDCK) cells, ATCC CCL-34

Active ingredient in test products:

NPJ03 (FFP2)

Cell culture medium:

1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS)

Dilution medium:

1X MEM + 3.0 µg/mL Trypsin

Flush medium:

1X MEM + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

Collection (semi solid) medium:

1X MEM + 5% Gelatin + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

Aerosol medium:

0.1X MEM

Incubation time:

4 – 6 days (Actual: 5 days)

## TEST CONDITIONS (continued)

Incubation temperature:

36±2C with 5±1% $CO_2$

Aerosol challenge:

20 minutes virus aerosol followed by 3 minutes regular (medium) aerosol then 1 minute additional vacuum at a continuous air flow rate of 28.3 L/min

Media and reagents:

1X Minimum Essential Medium (MEM) + 3.0 µg/mL Trypsin
1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS)
1X MEM + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B
1X MEM + 5% Gelatin + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B
0.1X MEM
Phosphate Buffered Saline
0.1N NaOH
Sterile Deionized Water
70% Isopropanol
Cavicide

## STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164. Testing was laboratory initiated on 06/26/2013 and was concluded on 07/01/2013. The study director signed the protocol on 06/25/2013. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

## RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

## CALCULATION OF TITER

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where:

m = the logarithm of the titer relative to the test volume
$x_k$ = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
$p_i$ = the proportion of positive results at dilution i

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

## RESULTS

Results are presented in Tables 1-2.

The Theoretical load was determined in the following manner:

Theoretical Load ($Log_{10}$ $TCID_{50}$) = $Log_{10}$ [Virus Stock Titer ($Log_{10}$ $TCID_{50}$/mL) x Average Volume challenge per run (mL)]

The Viral load was determined in the following manner:

Viral Load ($Log_{10}$ $TCID_{50}$) = Titer ($Log_{10}$ $TCID_{50}$/mL) + $Log_{10}$[Volume (mL)]

The $log_{10}$ Reduction Factor (LRF) was calculated in the following manner:

$Log_{10}$ Reduction Factor = Initial viral load ($Log_{10}$ $TCID_{50}$) – Output viral load ($Log_{10}$ $TCID_{50}$)

## RESULTS (continued)

The Mean Viral $Log_{10}$ Reduction from n replicates was determined as follows:

$$\text{Mean Viral } Log_{10} \text{ Reduction} = \frac{LRF_1 + LRF_2 + \ldots\ldots + LRF_n}{n}$$

The 95% Confidence interval (CI)* of the average viral $Log_{10}$ reduction was determined as follows:

$$95\% \text{ Confidence interval} = \frac{1.96 \times \sqrt{\frac{\sum (x - \bar{x})^2}{(n-1)}}}{\sqrt{n}}$$

* equivalent to an alpha value of 0.05

where:

x = the individual sample value
$\bar{x}$ = the sample mean value
n = the sample size

## RESULTS (continued)

### Table 1
### Titer Results

| Sample | Titer ($Log_{10}TCID_{50}/mL$) | Volume (mL) | Viral Load ($Log_{10}TCID_{50}$) |
|---|---|---|---|
| Cell viability/media sterility control | no virus detected, cells viable; media sterile | | |
| Volume application evaluation | average volume of challenge per run: 6.1mL | | |
| Virus Stock Titer Control | 8.00 | - | - |
| Theoretical load [a] | | | 8.79 |
| Virus Input (no mask) Control (replicate 1) | 7.25 | 10 | 8.25 |
| Virus Input (no mask) Control (replicate 2) | 7.25 | 10 | 8.25 |
| Virus Input (no mask) Control (replicate 3) | 6.75 | 10 | 7.75 |
| Virus Input (no mask) Control (average) | | | 8.14 |
| FFP2 (replicate 1)[b] | 3.00 | 10 | 4.00 |
| FFP2 (replicate 2)[b] | 2.75 | 10 | 3.75 |
| FFP2 (replicate 3)[b] | 3.00 | 10 | 4.00 |
| FFP2 CTL (replicate 1) | 5.50 | 10 | 6.50 |
| FFP2 CTL (replicate 2) | 5.25 | 10 | 6.25 |
| FFP2 CTL (replicate 3) | 5.00 | 10 | 6.00 |

[a] The theoretical load is determined based on the Virus Stock Titer control and average volume of virus challenged per run.

[b] Cytotoxicity observed at undilute dilution.

## RESULTS (continued)

**Table 2 - Viral Reduction**
**Virus Filtration Reduction - based on Virus Input Control (no mask)**

| Test Agent(s) | Replicate Number | Initial Viral Load* ($Log_{10}TCID_{50}$) | Output Viral Load ($Log_{10}TCID_{50}$) | $Log_{10}$ Reduction |
|---|---|---|---|---|
| FFP2 | 1 | 8.14 | 4.00 | 4.14 |
| | 2 | | 3.75 | 4.39 |
| | 3 | | 4.00 | 4.14 |
| | **Mean Reduction ± 95% Confidence Interval** | | | **4.24 ± 0.16** |
| FFP2 CTL | 1 | 8.14 | 6.50 | 1.64 |
| | 2 | | 6.25 | 1.89 |
| | 3 | | 6.00 | 2.14 |
| | **Mean Reduction ± 95% Confidence Interval** | | | **1.93 ± 0.28** |

* Results represent the average of three replicates.

## CONCLUSIONS

The viral reduction for the test materials are presented in Table 2. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

# APPENDIX

MICROBIOTEST
A Division of Microbac Laboratories, Inc.
105-B Carpenter Drive
Sterling, VA 20164

# MicroBioTest PROTOCOL

# EVALUATION OF FILTRATION EFFICIENCY OF TREATED FACE MASKS AGAINST AEROSOLIZED VIRUS – 2013 INFLUENZA A (H7N9) VIRUS

Testing Facility
MicroBioTest
A Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
VIROBLOCK SA
18, chemin des Aulx
CH-1228 Plan-les-Ouates
Switzerland

June 14, 2013



MicroBioTest Protocol: 798.1.06.14.13

MicroBioTest Project: 798-114

T.P.

## OBJECTIVE:

This test is designed to evaluate virus filtration efficiency of treated face mask materials against Influenza A (H7N9) virus using a two-chamber system and aerosolized virus. This test is based on the ASTM Method F 2101 entitled "Standard Test Methods for Evaluating the Bacterial Filtration Efficiency of Medical Face Mask Materials, Using a Biological Aerosol of *Staphylococcus aureus*", with modifications and customization for virus testing.

## OVERVIEW OF TESTING CONDITIONS / EXPERIMENTAL DESIGN:

Face mask material to be evaluated will be secured between two air chambers. Aerosolized Influenza A (H7N9) virus will be introduced into the upstream chamber and pulled through the test mask at a defined rate of air flow created by upstream high-pressure air and a downstream vacuum. The pass-through aerosol in the downstream chamber will be drawn into a one-stage Anderson Sampler that contains a Petri dish with semi-solid media to collect virus particles from the pass-through aerosol. Additionally, the stage surface of the Anderson Sampler, which may retain residual pass-through virus, will be flushed with media. The flush media and the media in the collection dish will be combined to form the "pass-through" sample, which will be liquefied and assayed for the amount of infectious virus by a Tissue Culture Infectious Dose 50% ($TCID_{50}$) infectivity assay to determine the viral filtration efficiency of the face mask.

One type of treated test mask and one type of control mask (see Table 1 for details) will be tested, each in triplicates.

Note: Virus inactivation via direct contact kill will not be evaluated in this study.

## MATERIALS

A. Test materials will be supplied by the sponsor: refer to "Miscellaneous Information" section.

All operations performed on the test agent such as specialized conditioning or storage conditions must by specified by the sponsor prior to the initiation of testing and should be detailed on the "Miscellaneous Information" section.



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The sponsor assures MicroBioTest testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MicroBioTest will retain all unused test agents for a period of three months after completion of the test, and then discard them in a manner that meets the approval of the safety officer. Alternatively, the test agent will be returned to the sponsor upon request.

B. Materials supplied by MicroBioTest, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study): 2013 Influenza A (H7N9) Virus, A/Anhui/1/2013, Centers for Disease Control & Prevention CCID/NCIRD/ID/MVVB

2. Host: Madin-Darby canine kidney (MDCK) cells, ATCC CCL-34

3. Media and reagents:

   a. Cell culture medium: 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS)

   b. Aerosol medium: 0.1X MEM

   c. Semi-solid collection medium: 1X MEM + 5% Gelatin + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% NaHCO3 +1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

   d. Flush medium: 1X MEM + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% NaHCO3 +1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

   e. Sample dilution medium: 1X MEM + 3.0 ug/mL Trypsin

   Media and reagents relevant to the test system will be documented in the first project sheet and data pack.



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4. Laboratory equipment and supplies:

   a. Biohazard hood
   b. One-stage Anderson sampler (Anderson Impactor)
   c. Two-chamber test rig
   d. Humidity Incubator
   e. Compressed air tank (≥15 PSIG)
   f. Cell Incubator
   g. Six-jet Collison Nebulizer (target Mean Particle Size: 1.8 – 2.5 μm)
   h. Vacuum pump
   i. Pressure gauges (35 kPa, ±1 kPa accuracy)
   j. Flowmeter (able to measure 28.3 L/min)

   Laboratory equipment and supplies relevant to the test system will be documented in the first project sheet and data pack.

**TEST SYSTEM IDENTIFICATION:**

All inoculated cells will be individually numbered and identified within the data package. All dilution tubes and assay dishes, etc. will be labeled with the challenge organism, test start date, and project number.



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**STERILITY:**

In order to prevent microbial or other viral contamination (other than the test virus) during the test, the following general measures will be followed:

1. The experiment will be conducted under a biohazard hood, which will be disinfected with Cavicide and 70% Alcohol followed by UV radiation prior to introduction of the aerosol challenge apparatus (see Fig. 1 below) and prior to commencement of the experiment;

2. The buffers and media used in the study will be sterile;

3. The one-stage Anderson sampler, nebulizer, applicable plastic ware (tubing, collection dishes, microtubes, etc), scissors and forceps, will be sterilized;

4. The technicians performing the tests will be wearing sterile latex gloves during the whole process;

5. The handling of the test items, the test virus, media, dilutions, and the infection of the cells will be conducted under the biohazard hood;

6. The test apparatus will be decontaminated with an aerosol run of 0.1N NaOH followed by 70% Alcohol and then sterile deionized water runs (at least 5 minutes per run) prior to use.

In order to verify the sterility of the process, a cell viability/media sterility control will be performed as outlined in Experimental Design, Section E1, below.

**EXPERIMENTAL DESIGN:**

Procedures involved in the performance of virucidal studies are described in a series of SOPs and logs that are maintained at MicroBioTest. The procedures used in different phases of the study will be documented in the data pack.



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**A. Inoculum preparation:**

Viral stocks are acquired from reputable sources that identify them by scientifically accepted methods. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at -60C to -90C.

Frozen viral stocks will be thawed on the day of the test. The original viral stock will be reconstituted or diluted in 0.1 X Media (e.g., 1:10 dilution of MEM in sterile deionized water) to a concentration of not less than $10^{6.5}$ $TCID_{50}$/mL. The total virus units delivered per run should be no less than $10^{7.0}$ $TCID_{50}$.

**B. Test mask material preparation and conditioning:**

The exact types of test and control face masks as specified by the Sponsor are listed in Table 1. No pre-conditioning will be performed prior to testing.

Note: Any additional physical, thermal, and chemical stressors which could compromise the virus filtration efficiency of the face masks under real-use situations must be specified by the Sponsor. These stressors include, for example, laundering (for reusable products), extreme environmental conditions, wetting with contaminants such as alcohol, sweat or other body fluids, and effects such as abrasion or flexing. Any extraneous stressor and its method will be provided in detail by the sponsor. The extraneous stressor may be performed at additional cost to the sponsor. Without this instruction, the masks will be used without any stressing or pre-conditioning.



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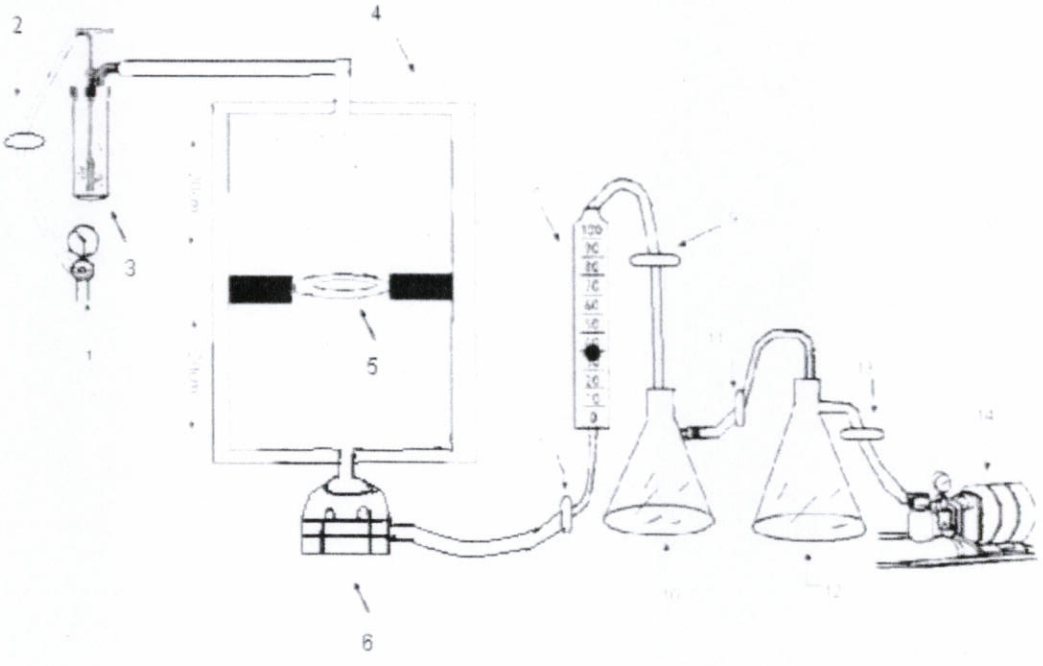
## C. Test:

The aerosol challenge apparatus is illustrated in Figure 1.

**Figure 1**




Key

1. High pressure air source
2. Filter #1
3. Nebulizer
4. Mask chamber
5. Test material location
6. Anderson Impactor
7. Filter #2
8. Calibrated Flowmeter, L/min
9. Filter #3
10. 4L Vacuum flask #1
11. Filter #4
12. 4L Vaccum flask #2
13. Filter #5
14. Vacuum pump

Figure 1: Mask Chamber



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## (I) Test and control mask runs

1. Three replicate virus-challenge runs will be performed for each type of test and control masks. Each replicate and each mask type will be randomized or alternated to avoid the effect of the change in virus titer over the course of the test on test results.

2. For each run, the mask material will be placed between the upstream and downstream chambers, covering the 7cm-diameter circular opening in the center, and secured with autoclave tape. The adjunction between the two chambers will be closed and sealed so that no air is leaked. The external side of the mask should face the upstream chamber, from which the virus aerosol will enter.

3. The virus inoculum will be delivered to the upstream air chamber using a nebulizer and high-pressure air. The delivery will be set up so that a consistent challenge volume will be delivered throughout the testing interval.

4. The aerosol challenge will be initiated by powering on the high pressure air source connected to the nebulizer containing the challenging virus.

    a. The aerosolized **virus** will be delivered to the upstream chamber for **20 minutes** using high pressure air along with a downstream vacuum at a constant air flow rate of 28.3 L/min (i.e. 1 cubic foot per min).

    b. After the virus delivery, the air pressure and vacuum will be turned off and immediately, the nebulizer bottle will be switched to another bottle that contains only the 0.1X MEM **aerosol medium,** without virus. The high pressure air along with the downstream vacuum pump will be turned on for **3 minutes** to allow the aerosol medium to flow through the mask to flush the chambers, at a constant air flow rate of 28.3 L/min.

    c. Upon conclusion of the aerosol medium delivery, the high pressure air source will be turned off. The **vacuum pump** will be left on for an additional **1 minute** to draw residual aerosol from the chambers into the Anderson Sampler.



T.P.

d. After the last vacuum period is finished, the test rig will be opened and the mask material will be aseptically removed and discarded.

e. The collection dish will be removed from the Anderson Sampler.

f. The stage surface of the Anderson sampler, which may contain some pass-through virus, will be flushed with 5 mL flush media.

g. The flush media (5 mL) and the semi-solid collection media (5 mL) will be combined to form the "pass-through" sample (10 mL). This sample will be liquefied at 36±2C and assayed for the amount of infectious virus (see Section D).

5. To start the next aerosol challenge run, a new collection dish will be placed into the Anderson Sampler and a new piece of mask will be orientated and placed into the test system.

6. The aerosol challenge against the new test material will be initiated as described in Steps 1-4. The flush media and the liquefied collection media from each dish will be combined ("Pass-through" sample) and assayed (see Section D).

**(II) Virus input control (no mask) runs**

1. A virus aerosol challenge run without any mask material will be performed following the above procedure to serve as the virus input (baseline) control. Three replicate runs will be performed for this control.

2. Post virus aerosol challenge, the "collection dish" and "stage" samples will be combined and assayed for infectious virus. The total number of infectious viral units challenged per run will be determined from this control run.

**D. Infectivity assay:**

The residual infectious virus in both test and controls will be detected by viral-induced cytopathic effect (CPE). Selected dilutions of the recovery solution ("pass-through" samples) will be added to cultured host cells (see Test section above) and incubated at 36±2°C with 5±1% $CO_2$ for a period of 4-6 days. The

host cell cultures will be observed and refed, as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will be examined for presence of infectious virus. The resulting virus-specific cytopathic effects and test article-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

## E. Controls:

1. Cell viability/media sterility control:

   At least four wells of host cells will be inoculated with an appropriate medium during the incubation phase of the study. This control will demonstrate that the cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the media employed throughout the assay period.

2. Virus input control (no mask):

   This control will be performed in the absence of mask material. The combined "collection dish" and "stage" samples will be assayed for infectious virus as described in Section D.

3. Virus Stock Titer control (VST)

   An aliquot of the virus inoculum used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

4. Volume application evaluation

   The volume of virus delivered per run will be evaluated by measuring the starting and ending total virus inoculum volumes, and calculating the volume per run by dividing the total volume used by the total number of runs performed.



T. P.

**F. Calculation:**

1. The 50% Tissue Culture Infective Dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G., Arch. Exp. Pathol. Pharmakol. 1931, 162: 480-483) or other appropriate methods such as Reed and Muench (Am. J. of Hyg. 1938, 27:493). In the case where a sample contains no detectable virus, a statistical analysis may be performed based on Poisson distribution (International Conference on Harmonization, Topic Q5A, 1999: 24-25) to determine the theoretical maximum possible titer for that sample. These analyses will be described in detail in the final report. The test results will be reported as reduction of the virus titer post treatment with the test article expressed as $log_{10}$.

## TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The cell viability/media sterility control must exhibit viable cells, absence of virus and free of contamination at test conclusion.
- The average virus units from the Virus Input Control runs must be at least $10^{7.0}$ $TCID_{50}$.

## PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164.



T.P.

## REPORT FORMAT:

MicroBioTest employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification and test agent identification
- Type of test and project number
- Interpretation of results and conclusions
- Test results
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

## RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately. The date the study director signs the protocol will be the study initiation date. All project sheets will be forwarded to the study sponsor. All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.



T.P.

**Table 1**
**Summary of samples to be assayed**

| Sample # | Mask type | Description |
|---|---|---|
| 1 | NA | Virus Stock Titer control |
| 2 | NA | Cell Viability Control / Media Sterility Control |
| 3 | Virus input control (no mask) – replicate # 1 | Virus input control |
| 4 | Virus input control (no mask) – replicate # 2 | Virus input control |
| 5 | Virus input control (no mask) – replicate # 3 | Virus input control |
| 6 | Test Mask – replicate # 1 | Pass-through |
| 7 | Test Mask – replicate # 2 | Pass-through |
| 8 | Test Mask – replicate # 3 | Pass-through |
| 9 | Control Mask – replicate # 1 | Pass-through |
| 10 | Control Mask – replicate # 2 | Pass-through |
| 11 | Control Mask – replicate # 3 | Pass-through |



T.P.

**MISCELLANEOUS INFORMATION:**

The following information is to be completed by sponsor before initiation of study:

A. Name and address: VIROBLOCK SA
18, chemin des Aulx
CH-1228 Plan-les-Ouates
Switzerland

B. Test Mask: FFP2
Active ingredient(s): NPJ03
Lot No.: 310001 (received at MicrobioTest on 5/10/13)

Control Mask : FFP2 CTL
Lot No.: VB-DEV-7-FEB-2013-NAT
(received at MicrobioTest on 5/10/13)

C. MSDS or certificate of analysis: ☐ provided ☒ not provided

**REPORT HANDLING:**

The sponsor intends to submit this information to: ■ other: EU Notified Body

**STUDY CONDUCT:** ■ GLP

**PROTOCOL APPROVAL:**

Sponsor Signature: T. Pelet Date: 21/06/2013
Thierry Pelet, Ph.D.

Study Director Signature: Salimatu Lukula Date: 6/25/2013
Salimatu Lukula, M.S.

| Date Issued: 06/25/2013 Project Sheet No. 1 | Page No. 1 Laboratory Project Identification No. 798-114 | | |
|---|---|---|---|
| **STUDY TITLE:** Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Virus – 2013 Influenza A (H7N9) Virus | **STUDY DIRECTOR:** Salimatu Lukula, M.S.<br>Salimatu Lukula 6/25/2013<br>Signature Date | | |
| **TEST MATERIAL(S):** | **LOT NO.** | **DATE RECEIVED:** | **DS NO.** |
| FFP2 | 310001 | 05/10/13 | D287 |
| FFP2 CTL | VB-DEV-7-FEB-2013-NAT | 05/10/13 | D289 |
| **PERFORMING DEPARTMENT(S):**<br>**Virology and Molecular Biology** | **STORAGE CONDITIONS:** Location: H2<br>■ Dark ■Ambient Room Temperature<br>☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other: | | |
| **PROTECTIVE PRECAUTION REQUIRED:** MSDS ☐Yes / ■ No | | | |
| **PHYSICAL DESCRIPTION:** ☐ Solid ☐ Liquid ☐ Aerosol ■ Other: Fabric | | | |
| **PURPOSE:** See attached protocol. **AUTHORIZATION:** See client signature. | | | |
| **PROPOSED EXPERIMENTAL START DATE:** 06/26/2013 **TERMINATION DATE:** 07/02/2013 | | | |
| **CONDUCT OF STUDY:** ☐ FDA ☐ EPA ☐ R&D ■GLP ☐ GCP ■Other: EU Notified Body | | | |
| **SPONSOR:** VIROBLOCK SA<br>18, Chemin des Aulx<br>CH-1228 Plan-les-Ouates<br>Switzerland | **CONTACT PERSON: Thierry Pelet, Ph.D.**<br>Telephone No. +41 22 884 83 44 | | |

**TEST CONDITIONS**

**Challenge organism:** 2013 Influenza A (H7N9) Virus, A/Shanghai/1/2013, U.S. Centers for Disease Control and Prevention (CDC) CCID/NCIRD/ID/MVVB

**Host:** Madin-Darby canine kidney (MDCK) cells, ATCC CCL-34

**Active ingredient:** NPJ03 (FFP2)

**Cell culture medium:** 1X Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS)

**Dilution medium:** 1X MEM + 3.0 µg/mL Trypsin

**Flush medium:** 1X MEM + 1% FBS + 1% HEPES + 10 µg/mL Gentamycin +1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

**Semi-solid collection medium:** 1X MEM + 5% Gelatin + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

Continued on page 2

**TEST CONDITIONS (continued)**

**Aerosol medium:** 0.1X MEM

**Aerosol challenge**: 20 minutes of virus aerosol challenge delivered with high pressure air accompanied by a downstream vacuum at a constant air flow rate of 28.3 L/min followed by 3 minutes of aerosol medium delivered at an air flow rate of 28.3 L/min along with a vacuum, then 1 minute vacuum without pressured air.

**Incubation time:** 4 - 6 days

**Incubation temperature:** 36±2C with 5±1 % CO2

**AMENDMENT(S):**

1. Protocol, Page 3 states the challenge virus strain as "A/Anhui/1/2013". The correct challenge virus strain is "A/Shanghai/1/2013". This amendment serves to address the typographical error in the Protocol.