Final Report

Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium

Mycobacterium terrae

Test Materials

FFP2

FFP2 CTL

Author

Shirshendu Saha

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

Laboratory Project Identification Number
798-116

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

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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:

Shirshendu Saha, Angela L. Hollingsworth, Tatiana Ballreich

Study Director: Shirshendu Saha Date 11/29/13
Name of Laboratory: MicroBioTest Division Microbac Laboratories Inc.

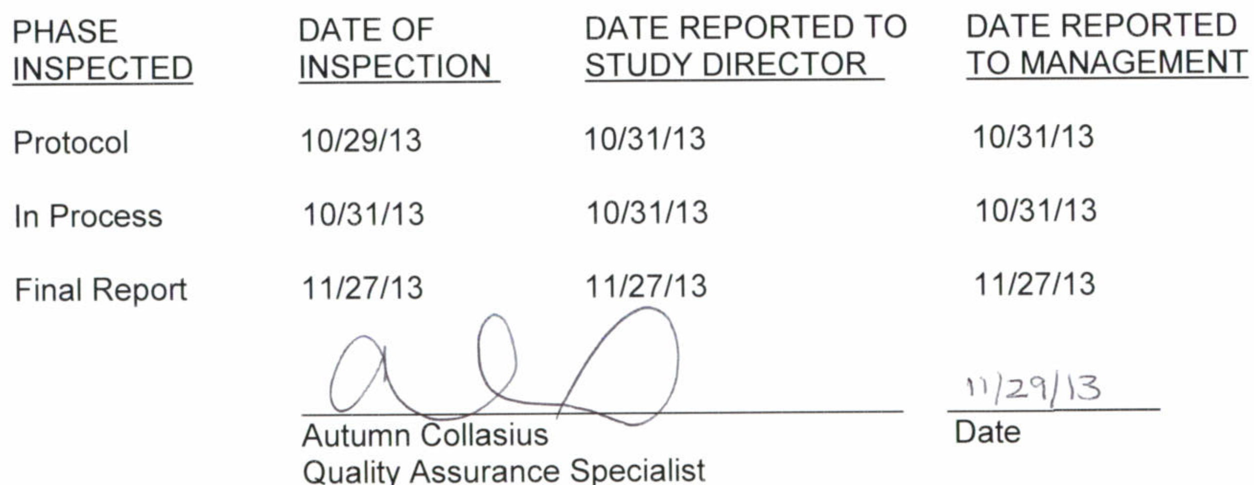
QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium – *Mycobacterium terrae*

The Quality Assurance Unit of MicroBioTest has inspected Project Number 798-116 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	10/29/13	10/31/13	10/31/13
In Process	10/31/13	10/31/13	10/31/13
Final Report	11/27/13	11/27/13	11/27/13



Autumn Collasius
Quality Assurance Specialist

Date 11/29/13

TEST SUMMARY

TITLE: Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium – *Mycobacterium terrae*

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 microorganism:

Mycobacterium terrae, ATCC 15755

Active ingredient in test product:

NPJ03 (FFP2)

Neutralizer/Flush medium:

Middlebrook 7H9 Broth

Semi-solid collection medium:

Middlebrook 7H9 Broth containing 5% Gelatin

Dilution medium/Aerosol medium:

Phosphate buffer saline solution (PBS)

Aerosol challenge:

The inoculum aerosol challenge was delivered for 20 minutes with high pressure air at 20 psi accompanied by a downstream vacuum at a constant air flow rate of 28.3 L/min, followed by 3 minutes of delivery of aerosol medium with high pressure air at 20 psi along with a vacuum flow rate of 28.3 L/min, followed by 1 minute of vacuum without pressured air.

Media and reagents:

Phosphate Buffered Saline
0.1N NaOH
Sterile Deionized Water
Middlebrook 7H9 Broth (7H9 Broth)
Middlebrook 7H9 Broth containing 5% Gelatin
Middlebrook 7H11 Agar
70% Isopropanol
Acid Fast Stain reagents

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164. Testing was initiated in the laboratory on 10/30/13 and was concluded on 11/18/13. The study director signed the protocol on 10/29/13. 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.

RESULTS

Results are presented in Tables 1- 2. The challenge microorganism was confirmed by colony morphology and Acid-fast stain to be consistent with *Mycobacterium terrae*. The average volume of stock titer delivered per run was 9.1 mL. The following calculations were used for reporting purposes:

The Mycobacterium load was determined in the following manner:
Mycobacterial Load (CFU) = Stock Titer (Log_{10} CFU/mL) + Log_{10}[Volume (mL)]

The Log_{10} Reduction Factor (LRF) was calculated in the following manner:
Log_{10} Reduction Factor = Initial mycobacterium load (Log_{10} CFU) – Output mycobacterium load (Log_{10} CFU)

The Mean Mycobacterium Log_{10} Reduction from *n* replicates was determined as follows:

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

where: n = the sample size

Note: CFU = Colony-forming units

RESULTS (continued)

Table 1
Test and Control Results

Sample	Replicate	Titer		Volume – 15 mL (Log_{10})	Mycobacterium Load (Log_{10})
		CFU/mL	Log_{10} CFU/mL		
Stock Titer Control	1	1.3×10^8	8.11	Not applicable	Not applicable
Mycobacterium Input Control (no mask)	1	3.9×10^6	6.59	1.17	7.76
	2	3.7×10^6	6.57	1.17	7.74
	3	3.7×10^6	6.57	1.17	7.74
				Average	7.75
FFP2	1	4.9×10^4	4.69	1.17	5.86
	2	3.7×10^4	4.57	1.17	5.74
	3	3.4×10^4	4.53	1.17	5.70
FFP2 CTL	1	3.7×10^6	6.57	1.17	7.74
	2	1.2×10^6	6.08	1.17	7.25
	3	2.1×10^6	6.32	1.17	7.49

Table 2 - Mycobacterium Reduction
Mycobacterium Filtration Reduction (based on Mycobacterium Input Control (no mask))

Test Agent	Initial Mycobacterium Load* (Log_{10})	Replicate	Output Mycobacterium Load (Log_{10})	Log_{10} Reduction	Mean Log_{10} Reduction
FFP2	7.75	1	5.86	1.89	1.98
		2	5.74	2.01	
		3	5.70	2.05	
FFP2 CTL	7.75	1	7.74	0.01	0.26
		2	7.25	0.50	
		3	7.49	0.26	

*Average of three replicates

CONCLUSIONS

The mycobacterium 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

Microbac
LABORATORY SERVICES

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

MicroBioTest Protocol

Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium *Mycobacterium terrae*

<u>Testing Facility</u>
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

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

October 1, 2013

MicroBioTest Protocol: 798.1.10.01.13

MicroBioTest Project: 798-116

OBJECTIVE:

This test is designed to evaluate mycobacterium filtration efficiency of treated face mask materials against *Mycobacterium terrae* using a two-chamber system and aerosolized inoculum. 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 mycobacterium testing.

OVERVIEW OF TESTING CONDITIONS / EXPERIMENTAL DESIGN:

Face mask material to be evaluated will be secured between two air chambers. Aerosolized challenge mycobacterium 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 mycobacterium particles from the pass-through aerosol. Additionally, the stage surface of the Anderson Sampler, which may retain residual pass-through mycobacterium, 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 mycobacterium by membrane filtration. After incubation, the $\log_{10}$ reductions will be calculated to determine 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: Only the mycobacterium filtration efficiency will be evaluated. The mycobacterium inactivation via direct contact will *not* be evaluated in this study.

MATERIALS

A. Test materials will be supplied by the sponsor:

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.

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 microorganism required by the sponsor of the study: *Mycobacterium terrae*, ATCC 15755

2. Media and reagents:
   a. 0.85% NaCl containing 0.1% Polysorbate 80 (SS+)
   b Middlebrook 7 H 9 Broth
   c. Middlebrook 7H11 agar
   d. Phosphate buffer saline solution (PBS)
   e. Modified Proskauer-Beck Medium (MPB)
   f. Neutralizer: FBS or other suitable solution
   g. Saline solution

   Any additional media and reagents relevant to the test system will be documented in the first project sheet and data pack and reported.

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. 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 tube supports, baskets, or other culture-containing devices will be labeled with the following information: microorganism, test agent and project number.

STERILITY:

In order to prevent microbial or other viral contamination (other than the test Mycobacterium) 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 prior to introduction of the aerosol challenge apparatus (see Fig. 1 below) and prior to commencement of the experiment;

2. The media and reagents 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 gloves during the whole process;

5. The handling of the test items, the test Mycobacterium, media, dilutions, and plating 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 media sterility control will be performed as outlined in Experimental Design, Section E1, below.

EXPERIMENTAL DESIGN:

A. Inoculum preparation(Test suspension):

One-hundred mL of sterile 7H9 broth will be transferred to two culture flasks (each will contain 100 mL). Each flask will be inoculated with 1 mL of thawed stock culture and incubated for 21±1 days at 36±1C in a stationary state.

Following 21±1 days incubation, the culture will be used to prepare the test suspension.

Twenty-five mL portions of broth culture will be placed into each of two 50 mL conical screw cap tubes. The tubes will be centrifuged at 10,000 rpm for 20±5 minutes and the pellet will be resuspended in 25 mL of sterile deionized water.

The tubes will be centrifuged at 10,000 rpm for 20±5 minutes a second time ~~at 10,000 rpm for 20±5 minutes.~~ After centrifugation the pellet will be re-suspended in 5 mL of sterile deionized water (1/10 of the starting volume).

The suspensions from both tubes will be pooled and placed in a bijou bottle with 10 glass beads. The bijou bottle will be vortex for five minutes. The suspension will be standardized as needed to achieve approximately $10^{6.5}$-10^{7} colony-forming units per mL (CFU/mL).

On the day of test, the prepared test suspension will be vortex-mixed for 10-30 seconds to evenly distribute the cells.

B. Test mask material preparation and conditioning:

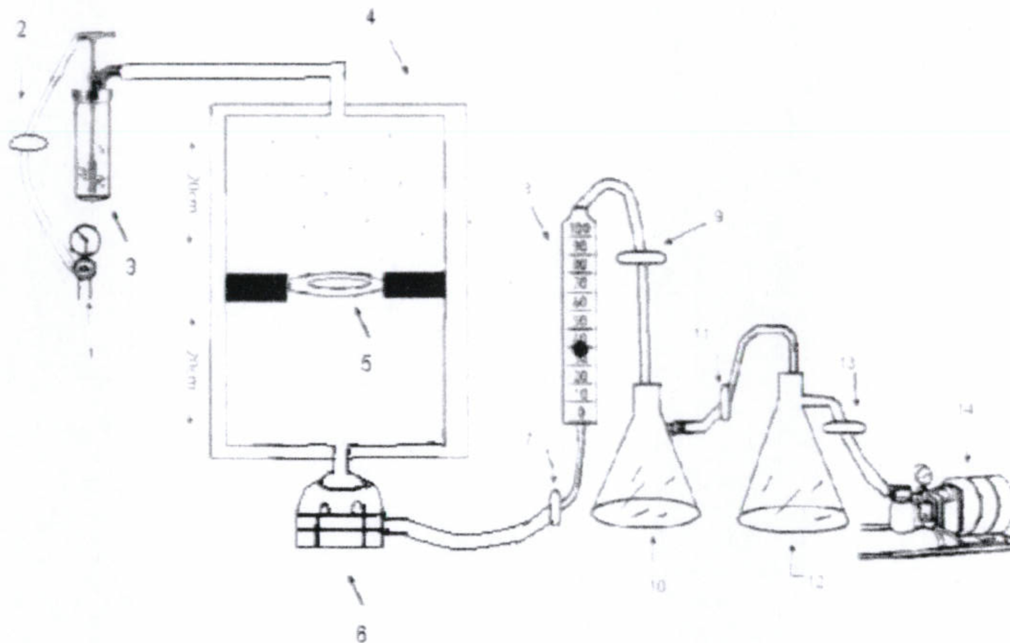
The exact types of test and control face masks as specified by the Sponsor are listed in Table 1. <u>No pre-conditioning will be performed prior to testing</u>.

Note: Any additional physical, thermal, and chemical stressors which could compromise the mycobacterium 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.

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

(I) **Test and control mask runs**

1. Three replicate mycobacterium-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 mycobacterium 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 mycobacterium aerosol will enter.

3. The prepared 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 mycobacterium.

   a. The aerosolized **inoculum** 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 inoculum 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 PBS (or equivalent) **aerosol medium,** without mycobacterium. 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.

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 mycobacterium, 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±1C and assayed for the amount of mycobacterium (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) Mycobacterium input control (no mask) runs

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

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

D. Culturing:

For the test agent:

- A single ten-fold dilution will be performed to represent the 10^{-1} dilution. Using a membrane filtration, all dilutions generated from the vials (including the carrier count controls (see Section F, part 1)), will be cultured based on the following procedures:
- Prior to activating the vacuum of the manifold, approximately 10 mL of PBS will be added to the membrane filter holder.
- The entire contents of each dilution tube will be filtered using independent filter holders.
- The sides of the membrane filter holder will be rinsed with approximately 10 mL of PBS and the membrane filter will then be aseptically transferred to a 7H11 agar plate.

For the 10^{0} (the contents of each vial) will be cultured based on the following procedures.

- Prior to activating the vacuum of the manifold, approximately 10 mL of PBS will be added to the membrane filter holder.
- The vials will be vortex-mixed for 5 seconds. The contents of the vial will be poured into a membrane filter holder and the vacuum will be activated.
- The vial containing the carrier will then be rinsed with 20 mL of PBS, vortex-mixed, and the rinse will be filtered in the same manner as the initial eluate. This rinsing procedure will be repeated one additional time for a total of two rinses.
- The sides of the membrane filter holder will be rinsed with at least 40 mL of PBS and the membrane filter will then be aseptically transferred to a 7H11 plate.

Incubation:

All plates will be incubated initially for 14 – 28 days at 36±1C the average CFU calculated.

E. Controls:

1. Media sterility control:

   Aliquots of neutralizer and PBS will be plated using standard filtration membrane technique (one mL aliquot per neutralizer and diluent in singlet onto agar plates. These plates, along with a single 7H11 plate will be incubated with the test.

2. Mycobacterium 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 mycobacterium as described in Section D.

3. Mycobacterium Stock Titer control:
   Serial ten-fold dilutions of the inoculum suspension will be performed in PBS blanks. Duplicate 0.1 mL aliquots from selected dilutions will be spread plated using 7H11 agar plates. All plates will be incubated with the test plates at 36C± 1C and the average CFU/mL determined.

4. Volume application evaluation

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

PRODUCT EVALUATION CRITERIA:

There are no specified product evaluation criteria for this test. The $\log_{10}$ reduction of the test mask (average of three replicate runs) from the control mask (average of three replicate runs) will be calculated to determine efficiency of the test face mask.

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 viable mycobacteria recovered from the aerosol stabilization control must be $\geq 1 \times 10^7$ CFU.
- The neutralizer is effective and non-toxic.

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.

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.

Table 1
Summary of samples to be assayed

Sample #	Mask type	Description
1	NA	Mycobacterium Stock Titer control
2	NA	Cell Viability Control / Media Sterility Control
3	Mycobacterium input control (no mask) – replicate # 1	Mycobacterium input control
4	Mycobacterium input control (no mask) – replicate # 2	Mycobacterium input control
5	Mycobacterium input control (no mask) – replicate # 3	Mycobacterium 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

TP

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

Control Mask: FFP2 CTL
Lot No.: VB-DEV-7-FEB-2013-NAT

C. MSDS or certificate of analysis: ■ not provided

REPORT HANDLING:

The sponsor intends to submit this information to: ☒ Other: EU Notified Body

STUDY CONDUCT: ☒ GLP

PROTOCOL APPROVAL:

Sponsor Signature: ______________________ Date: 09/10/2013
Thierry Pelet, Ph.D.

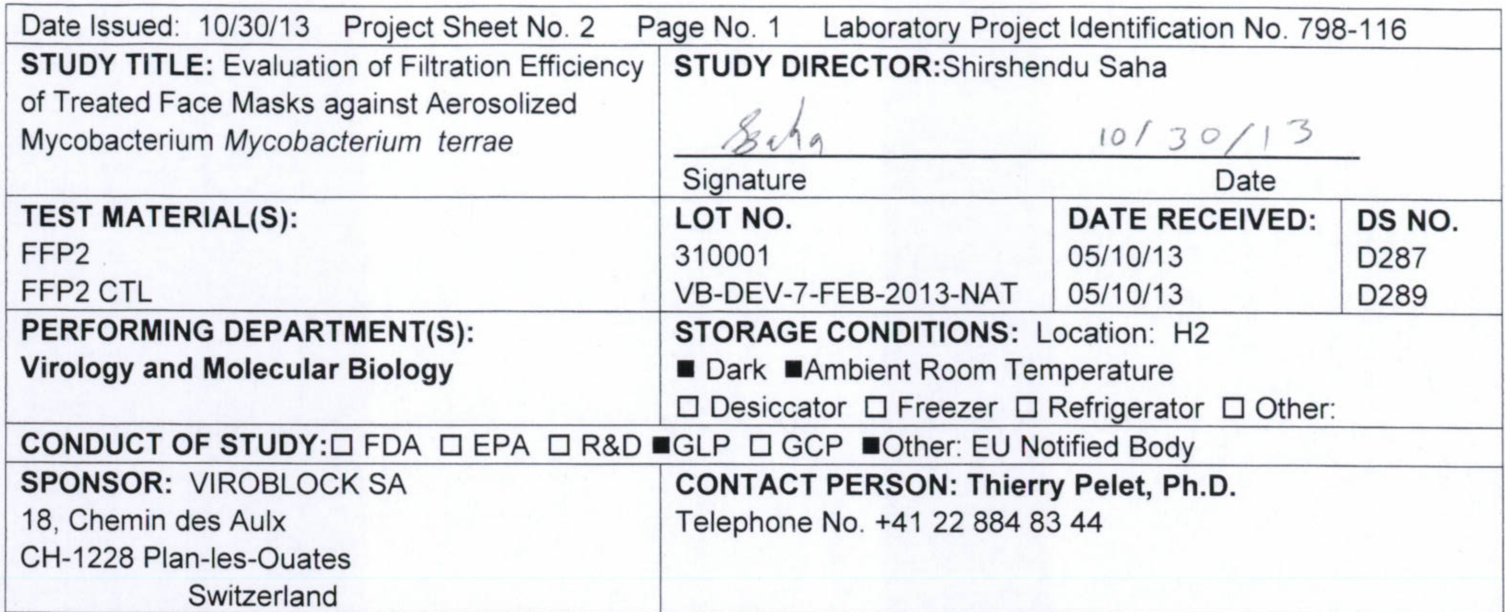
VIROBLOCK SA
Chemin des Aulx 18
1228 Plan-les-Ouates
Tel. +41 22 884 83 44

Study Director Signature: ______________________ Date: 10/29/13
Shirshendu Saha (Shawn)

<table>
<tr><td colspan="4">Date Issued: 10/30/2013 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 798-116</td></tr>
<tr><td>STUDY TITLE: Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium Mycobacterium terrae</td><td colspan="3">STUDY DIRECTOR: Shirshendu Saha
Saha 10/30/13
Signature Date</td></tr>
<tr><td>TEST MATERIAL(S):
FFP2
FFP2 CTL</td><td>LOT NO.
310001
VB-DEV-7-FEB-2013-NAT</td><td>DATE RECEIVED:
05/10/13
05/10/13</td><td>DS NO.
D287
D289</td></tr>
<tr><td>PERFORMING DEPARTMENT(S):
Virology and Molecular Biology</td><td colspan="3">STORAGE CONDITIONS: Location: H2
■ Dark ■Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:</td></tr>
<tr><td colspan="4">PROTECTIVE PRECAUTION REQUIRED: MSDS ☐Yes / ■ No</td></tr>
<tr><td colspan="4">PHYSICAL DESCRIPTION: ☐ Solid ☐ Liquid ☐ Aerosol ■ Other: Fabric</td></tr>
<tr><td colspan="4">PURPOSE: See attached protocol. AUTHORIZATION: See client signature.</td></tr>
<tr><td colspan="4">PROPOSED EXPERIMENTAL START DATE: 10/30/2013 TERMINATION DATE: 11/27/2013</td></tr>
<tr><td colspan="4">CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■GLP ☐ GCP ■Other: EU Notified Body</td></tr>
<tr><td>SPONSOR: VIROBLOCK SA
18, Chemin des Aulx
CH-1228 Plan-les-Ouates
Switzerland</td><td colspan="3">CONTACT PERSON: Thierry Pelet, Ph.D.
Telephone No. +41 22 884 83 44</td></tr>
</table>

TEST CONDITIONS

Challenge organism:	*Mycobacterium terrae*, ATCC 15755
Active ingredient:	NPJ03 (FFP2)
Neutralizer/ Flush medium:	7H9 Broth
Aerosol medium :	Phosphate buffer saline solution (PBS)
Semi-solid collection medium:	7H9 Broth containing 5% Gelatin
Incubation Time(s):	14 – 28 days
Incubation Temperature(s):	36±1C
Comment(s):	



Date Issued: 10/30/13 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 798-116

STUDY TITLE: Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium *Mycobacterium terrae*	**STUDY DIRECTOR:** Shirshendu Saha Saha 10/30/13 Signature Date		
TEST MATERIAL(S): FFP2 FFP2 CTL	**LOT NO.** 310001 VB-DEV-7-FEB-2013-NAT	**DATE RECEIVED:** 05/10/13 05/10/13	**DS NO.** D287 D289
PERFORMING DEPARTMENT(S): **Virology and Molecular Biology**	**STORAGE CONDITIONS:** Location: H2 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: EU Notified Body			
SPONSOR: VIROBLOCK SA 18, Chemin des Aulx CH-1228 Plan-les-Ouates Switzerland	**CONTACT PERSON: Thierry Pelet, Ph.D.** Telephone No. +41 22 884 83 44		

This project sheet was issued to document the following:

EXPLANATION:

Protocol Amendment(s):

1. In reference to the Inoculum preparation (Test Suspension) section of the protocol. There is a typographical error in reference to the secondary centrifugation procedure. The reference to 10,000 rpm for 20±5 minutes is inadvertently listed twice. The sentence should have read "The tubes will be centrifuged at 10,000 rpm for 20±5 minutes a second time.

2. In reference to the Test Section, Part I (4)(g); the volume of the semi-solid collection media will be changed from 5 mL to 10 mL each for a final volume of 15 mL. This modification is required when using bacteria.

3. In reference to the Culturing section of the protocol:

    a. The procedure indicates that a single dilution will be made from the test (or control) sample. In order to evaluate a broader range of detection, multiple serial ten-fold dilutions will be performed (PBS will be used).
    b. There is also an inadvertent reference to membrane filtration from the vials (including the carrier count control procedures (see Section F, part 1) – this statement should be disregarded as it does not apply to this method. All remaining bullet points, providing details for membrane filtration plating should also be disregarded.
    c. Duplicate 0.1 mL aliquots from selected dilutions of the test (or control) samples will be spread plated using 7H11 agar plates.

This amendment serves to correct the culturing procedures.

Continued on next page.

Date Issued: 10/30/13 Project Sheet No. 2 Page No. 2 Laboratory Project Identification No. 798-116

Protocol Amendment(s):

4. In reference to Table 1 on Page 13 of the protocol; the 2nd sample (Cell Viability Control/Media Sterility Control). The description should not have included Cell Viability Control, only the Media Sterility Control applied to this sample. Any additional references to cell viability should be disregarded.

5. The protocol did include a statement regarding the proposed statistical methods. For this test there are no proposed statistical methods.

Date Issued: 11/29/13 Project Sheet No. 3	Page No. 1 Laboratory Project Identification No. 798-116		
STUDY TITLE: Evaluation of Filtration Efficiency of Treated Face Masks against Aerosolized Mycobacterium *Mycobacterium terrae*	**STUDY DIRECTOR:** Shirshendu Saha [signature] 11/29/13 Signature Date		
TEST MATERIAL(S): FFP2 FFP2 CTL	**LOT NO.** 310001 VB-DEV-7-FEB-2013-NAT	**DATE RECEIVED:** 05/10/13 05/10/13	**DS NO.** D287 D289
PERFORMING DEPARTMENT(S): **Virology and Molecular Biology**	**STORAGE CONDITIONS:** Location: H2 ■ Dark ■Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■GLP ☐ GCP ■Other: EU Notified Body			
SPONSOR: VIROBLOCK SA 18, Chemin des Aulx CH-1228 Plan-les-Ouates Switzerland	**CONTACT PERSON: Thierry Pelet, Ph.D.** Telephone No. +41 22 884 83 44		

This project sheet was issued to document the following:

EXPLANATION:

Protocol Amendment(s):

6. The protocol did not include any microorganism confirmation procedures. A representative colony from the mycobacterium stock titer control was acid-fast stained along with a detected representative colony from a test plate (FFP2 or FFP2 CTL). The colony morphology was documented as well. This amendment serves to define the procedures used to confirm the challenge microorganism.

7. In reference to the Test Acceptance Criteria section of the protocol. The secondary bullet point regarding the neutralizer should be disregarded as it does not apply to this test methodology. For the initial bullet point, regarding the viable mycobacterium recovered. To clarify, the aerosol stabilization control is referring to the Mycobacterium Input Control (no mask). In addition, the minimum recovery should be at least ≥ 1.0 x 10^7 colony forming units (CFU) or 7.00 Log_{10}. This amendment serves to correct and clarify the Test Acceptance Criteria section of the protocol.

Protocol Deviations(s):

1. In reference to the Inoculum preparation and Incubation section on Page 10 of the protocol regarding the incubation temperature range (36±1C), during the incubation phase of the test culture, the temperature deviated outside this range on two occasions (recorded at 34.6C and 34.5C). In addition, during the incubation of test plates, the temperature deviated outside the range to 34.9C. Since the deviations were limited in duration in respect to the total incubation period, and the temperature discrepancy was minimal, it was determined by the Study Director to have no significant impact on the study. All controls met the criteria for a valid test.

2. The sterility control results were inadvertently not documented. Since no contamination was detected and all remaining controls met the criteria for a valid test, it was determined by the Study Director to have no significant impact on the test.