CONFIDENTIAL FINAL REPORT

SPONSOR: VIROBLOCK SA

SPONSOR'S REPRESENTATIVE: Thierry Pelet, Ph.D.

STUDY TITLE: ASSESSMENT OF VIRUCIDAL EFFECTIVENESS OF TREATED FABRICS VIA DIRECT CONTACT- Influenza A Virus (H1N1) Misting Study

STUDY IDENTIFICATION: MicroBioTest Project No. 798-126 (refer to signed protocol)

TEST AGENT NAME	LOT NO.	DATE RECEIVED	DS NO.
FFP2 Respirator	31001	10/26/15	F872
FFP2 Respirator	31005	10/26/15	F873
FFP2 Respirator	31009	10/26/15	F874
FFP2 Respirator	31016	10/26/15	F875
FFP2 Respirator (Control Fabric)	VB-DEV-12-FEB-2013-NAT	10/26/15	F876

ACTIVE INGREDIENT(S): NPJ03

CHALLENGE ORGANISM Influenza A Virus, strain: A/PR/8/34 (H1N1); Charles River Laboratories

HOST CELL LINE: MDCK cells; ATCC CCL-34

DILUTION MEDIUM: 1X Minimum Essential Medium (MEM) + 3.0 µg/mL Trypsin

VIRUS SUSPENSION MEDIUM: 0.1X MEM

ORGANIC LOAD: Not required

NEUTRALIZER/EXTRACTION 1X MEM + 1% Fetal Bovine Serum (FBS) + 0.5% HEPES + 1% NaHCO3 + 1mM EDTA

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EXPOSURE (CONTACT) TIME(S): 10 minutes

EXPOSURE TEMPERATURE: Ambient temperature (20±1°C)

APPLICATION: As applicable, virus (or virus suspension medium) was misted through a 2 in x 2 in sterile barrier onto pre-cut test and control masks using Nalgene Aerosol Spray Bottle (Fisher Cat. # 15-232-8; Nalgene Cat. # 2430-0200) from a distance of 3 – 6" for two pumps, one second per pumps.

NUMBER OF REPLICATES: Four wells per dilution

INCUBATION TEMPERATURE: 36±2°C with 5±1% CO_2

INCUBATION TIME: 4 – 6 days (5 days actual)

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
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

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

RESULTS

Results are presented in Tables 1 – 6.

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} $TCID_{50}$)

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

Mean Viral Log_{10} Reduction = (LRF_1 + LRF_2 +……+LRF_n) / n

Note: The LRF's was anti-logged prior to performing calculations

RESULTS (Continued):

Table 1
Titer Results

Sample	Replicate	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Virus Stock Titer control		6.50	-	-
Volume application evaluation		average volume of challenge: 0.4 mL per run		
Cell viability/media sterility control		no virus detected, cells viable; media sterile		
Liquid (no fabric) Control	Rep. 1	6.25	40	7.85
	Rep. 2	6.25	40	7.85
	Average			7.85
FFP2 Respirator (Control Fabric) (Lot No.VB-DEV-12-FEB-2013-NAT)	Rep. 1	5.25	40	6.85
	Rep. 2	5.00	40	6.60
	Average			6.74
FFP2 Respirator (Lot No. 31001)[b]	Rep. 1	2.25	40	3.85
	Rep. 2	1.75	40	3.35
	Average			3.67
FFP2 Respirator (Lot No. 31005)[b]	Rep. 1	2.75	40	4.35
	Rep. 2	2.50	40	4.10
	Average			4.24
FFP2 Respirator (Lot No. 31009)[b]	Rep. 1	3.00	40	4.60
	Rep. 2	2.50	40	4.10
	Average			4.42
FFP2 Respirator (Lot No. 31016)[b]	Rep. 1	2.75	40	4.35
	Rep. 2	2.50	40	4.10
	Average			4.24

[b] Cytotoxicity observed at an undilute dilution.

RESULTS (Continued):

Table 2
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls – Lot No. 31001

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
Undilute	cytotoxicity observed; viral CPE could not be evaluated	cytotoxicity observed
10^-1	virus detected in all inoculated wells	no cytotoxicity observed
10^-2	virus detected in all inoculated wells	no cytotoxicity observed

Table 3
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls – Lot No. 31005

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
Undilute	cytotoxicity observed; viral CPE could not be evaluated	cytotoxicity observed
10^-1	virus detected in all inoculated wells	no cytotoxicity observed
10^-2	virus detected in all inoculated wells	no cytotoxicity observed

Table 4
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls – Lot No. 31009

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
Undilute	cytotoxicity observed; viral CPE could not be evaluated	cytotoxicity observed
10^-1	virus detected in all inoculated wells	no cytotoxicity observed
10^-2	virus detected in all inoculated wells	no cytotoxicity observed

Table 5
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls – Lot No. 31016

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
Undilute	cytotoxicity observed; viral CPE could not be evaluated	cytotoxicity observed
10^-1	virus detected in all inoculated wells	no cytotoxicity observed
10^-2	virus detected in all inoculated wells	no cytotoxicity observed

RESULTS (Continued):

Table 6
Viral Reduction Based on Liquid (no mask) Control

Test Article	Input Load* ($Log_{10}TCID_{50}$)	Output Load* ($Log_{10}TCID_{50}$)	Log_{10} Reduction
FFP2 Respirator (Lot No. 31001)	7.85	3.67	4.18
FFP2 Respirator (Lot No. 31005)		4.24	3.61
FFP2 Respirator (Lot No. 31009)		4.42	3.43
FFP2 Respirator (Lot No. 31016)		4.24	3.61
FFP2 Respirator (Control Fabric) (Lot No. VB-DEV-12-FEB-2013-NAT)		6.74	1.11

CONCLUSION:

VIROBLOCK SA' s FFP2 Respirator Lot No.'s 31001, 31005, 31009 and 31016 were evaluated for the ability to inactivate Influenza A virus (H1N1). MicroBioTest personnel performed the inactivation procedure using the challenge viruses to spike the test material. Samples were tested and titrated by 50% tissue culture infectious dose ($TCID_{50}$) endpoint assay using MDCK cells.

The log_{10} viral reductions for the test materials are presented in Tables 6. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

Study director: _____ 11/24/2015
S. Steve Zhou, Ph.D. Date