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FINAL REPORT

Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study Influenza A Virus (H1N1)

Test Article
2018.01.02-Active
2018.01.02-Control

Author
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Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
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Laboratory Project Identification Number
956-102

Sponsor
Dongguan Yimao Filter Media Co., ltd.
2654 Weaver Way
Doraville, GA 30340 USA

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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.
- Two Final Reports were generated

The following technical personnel participated in this study:

Salimatu Lukula, Justice Frimpong, Semhar Fanuel, Alex Cironi

Study Director: Microbac

Salimatu Lukula 01-31-2018

Salimatu Lukula, M.S. Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study Influenza A Virus (H1N1)

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 956-102 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	01/16/18	01/16/18	01/16/18
In Process	01/16/18	01/16/18	01/16/18
Final Report	01/25/18	01/25/18	01/25/18

01-31-2018

Jeanne M. Anderegg RQAP-GLP Date
Quality Assurance Manager

TEST SUMMARY

TITLE: Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study Influenza A Virus (H1N1)

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. 2018.01.02-Active; received at Microbac 01/04/18, assigned DS No. I002
2. 2018.01.02-Control; received at Microbac 01/04/18, assigned DS No. I003

SPONSOR: Dongguan Yimao Filter Media Co., ltd.
2654 Weaver Way
Doraville, GA 30340 USA

TEST CONDITIONS

Challenge organisms:

Influenza A Virus (H1N1), A/PR/8/34; Charles River Laboratories

Host

MDCK cells, ATCC CCL-34

Organic load:

Not required

Active ingredient in test products:

Ag-Cu Zeolite

Neutralizer used:

1X Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum (FBS) + 1% $NaHCO_3$ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B + 1mM EDTA

Dilution medium:

1X MEM + 3.0 µg/mL Trypsin

Virus suspension medium:

0.1X MEM

Contact time:

5 minutes

Contact temperature:

Ambient temperature (19-20°C)

Application:

Virus inoculum was misted onto a 2 x 2 inch area of pre-cut (approximately 2.5 x 2.5 inch or 3 x 3 inch) test fabric, control fabric, and liquid control using a Nalgene Aerosol Spray Bottle (Fisher Cat. # 15-232-8; Nalgene Cat. # 2430-0200) from 3" – 6" for one pump, and one second per pump.

TEST CONDITIONS (continued)

Media and reagents:

1X Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum (FBS) + 1% $NaHCO_3$ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B + 1mM EDTA

1X MEM + 3.0 µg/mL Trypsin

0.1X MEM

Phosphate Buffered Saline

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac, 105 Carpenter Drive, Sterling, VA 20164. Testing was laboratory initiated on 01/16/18 and was concluded on 01/22/18. The study director signed the protocol on 01/16/18. 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 Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc. 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
$\sum p_i$ = 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 – 3.

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)

Conversion of Log_{10} reduction to percent reduction

Log_{10} reduction = A

% reduction = B

$$B = \left[1 - \frac{1}{10^{A}} \right] \times 100$$

Example:

A = 3.5 Log_{10} reduction
B = {1 – 1/ [power (10,3.5)]} x 100 = 99.97%

Note: you should use sufficient decimal places so that the % reduction becomes less than 100%.

RESULTS (continued)

Table 1
Titer Results

Sample	Contact Time	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Cell viability/media sterility control	NA	no virus detected, cells viable; media sterile		
Volume application evaluation		average volume of challenge per run: 0.38 mL		
Virus Stock Titer Control		7.00	-	-
Theoretical load [a]				6.58
Liquid (no fabric) Control (replicate 1)	5 Minutes	6.00	40	7.60
Liquid (no fabric) Control (replicate 2)		6.00	40	7.60
Liquid (no fabric) Control (replicate 3)		5.50	40	7.10
Liquid (no fabric) Control (average)				7.49
2018.01.02-Control (replicate 1)		5.00	40	6.60
2018.01.02-Control (replicate 2)		5.00	40	6.60
2018.01.02-Control (replicate 3)		4.75	40	6.35
2018.01.02-Control (average)				6.53
2018.01.02-Active (replicate 1)[b]		2.75	40	4.35
2018.01.02-Active (replicate 2)[b]		3.00	40	4.60
2018.01.02-Active (replicate 3)[b]		2.75	40	4.35

[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 the Undilute dilution.
NA = Not applicable

Table 2
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls

Dilution of the Neutralized Sample	Neutralizer Effectiveness/Viral Interference Control	Cytotoxicity Control
Undilute	Cytotoxicity observed	Cytotoxicity observed
10^{-1}	virus detected in 4 out of 4 wells	no cytotoxicity observed in 4 out of 4 wells
10^{-2}	virus detected in 4 out of 4 wells	no cytotoxicity observed in 4 out of 4 wells

RESULTS (continued)

Table 3
Viral Reduction - based on Liquid (no fabric) Control

Test Agent	Replicate Number	Initial Viral Load ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Reduction (%)
2018.01.02-Active	1	7.49	4.35	3.14	99.93
	2		4.60	2.89	99.87
	3		4.35	3.14	99.93
	Mean Reduction[a]			**3.07**	**99.91**

[a] Results represent the average of three replicates.

CONCLUSIONS

Dongguan Yimao Filter Media Co., ltd's 2018.01.02-Active fabric was evaluated for the ability to inactivate Influenza A Virus (H1N1). Microbac personnel performed the inactivation procedure using Influenza A Virus (H1N1) independently to spike the fabric material. Samples were titrated by the 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) endpoint assay using MDCK cells.

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