



FINAL REPORT

Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study SARS-Associated Coronavirus

Test Article
2018.01.02-Active
2018.01.02-Control

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Laboratory Project Identification Number
956-101

Sponsor
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COMPLIANCE STATEMENT

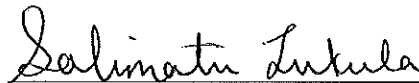
This study meets the requirements for 21 CFR § 58 with the following exceptions:

- 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, Zheng Chen

Study Director: Microbac



Salimatu Lukula, M.S.

01-31-2018

Date

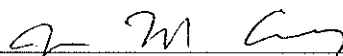
QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study SARS-Associated Coronavirus

The Quality Assurance Unit of Microbac has inspected the Project Number 956-101 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.

<u>PHASE INSPECTED</u>	<u>DATE OF INSPECTION</u>	<u>DATE REPORTED TO STUDY DIRECTOR</u>	<u>DATE REPORTED TO MANAGEMENT</u>
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



Jeanne M. Anderegg RQAP-GLP
Quality Assurance Manage

01-31-18
Date

TEST SUMMARY

TITLE: Assessment of Virucidal Effectiveness of Treated Fabric Material Via Direct Contact – Misting study SARS-Associated Coronavirus

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.
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TEST CONDITIONS

Challenge organisms:

SARS-Associated Coronavirus (SARS-CoV), Strain: CDC Strain 200300592,
ZeptoMetrix

Host

Vero-E6, ATCC CRL-1586

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%
NaHCO₃ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B +
1mM EDTA

Dilution medium:

1X MEM + 2% FBS

Virus suspension medium:

0.1X MEM

Contact time:

5 minutes

Contact temperature:

Ambient temperature (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%
NaHCO₃ + 1% HEPES + 10 µg/mL Gentamicin + 2.5 µg/mL Amphotericin B +
1mM EDTA
1X MEM + 2% FBS
0.1X MEM

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 01/23/18. The study director signed the protocol on 01/15/18. 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 (TCID₅₀/mL) was determined using the Spearman-Kärber 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
- ∑p_i = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to TCID₅₀/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:

$$\text{Viral Load (Log}_{10} \text{ TCID}_{50}) = \text{Titer (Log}_{10} \text{ TCID}_{50}/\text{mL}) + \text{Log}_{10} [\text{Volume (mL)}]$$

The Log₁₀ Reduction Factor (LRF) was calculated in the following manner:

$$\text{Log}_{10} \text{ Reduction Factor} = \text{Initial Viral Load (Log}_{10} \text{ TCID}_{50}) - \text{Output Viral Load (Log}_{10} \text{ TCID}_{50})$$

The Mean Viral Log₁₀ Reduction from n replicates was determined as follows:

$$\text{Mean Viral Log}_{10} \text{ Reduction} = (\text{LRF}_1 + \text{LRF}_2 + \dots + \text{LRF}_n) / n$$

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

RESULTS (continued)

Conversion of Log₁₀ reduction to percent reduction

Log₁₀ reduction = A

% reduction = B

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

Example:

A = 3.5 Log₁₀ 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: 0.40 mL		
Virus Stock Titer Control		7.50	-	-
Theoretical load ^a		7.10		
Liquid (no fabric) Control (replicate 1)	5 Minutes	5.50	40	7.10
Liquid (no fabric) Control (replicate 2)		4.75	40	6.35
Liquid (no fabric) Control (replicate 3)		5.00	40	6.60
Liquid (no fabric) Control (average)		6.80		
2018.01.02-Control (replicate 1)		4.50	40	6.10
2018.01.02-Control (replicate 2)		4.25	40	5.85
2018.01.02-Control (replicate 3)		4.50	40	6.10
2018.01.02-Control (average)		6.03		
2018.01.02-Active (replicate 1)		2.75	40	4.35
2018.01.02-Active (replicate 2)		2.75	40	4.35
2018.01.02-Active (replicate 3)		3.00	40	4.60

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

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	virus detected in 4 out of 4 wells	no cytotoxicity observed in 4 out of 4 wells
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	6.80	4.35	2.45	99.64
	2		4.35	2.45	99.64
	3		4.60	2.20	99.37
	Mean Reduction^a			2.38	99.58

^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 SARS-Associated Coronavirus. Microbac personnel performed the inactivation procedure using SARS-Associated Coronavirus independently to spike the fabric material. Samples were titrated by the 50% tissue culture infectious dose per mL (TCID₅₀/mL) endpoint assay using Vero-E6 cells.

The viral reduction for the test fabric material are presented in Table 3 above. All of the controls met the criteria for a valid test. These conclusions are based on observed data.