



CONFIDENTIAL FINAL REPORT

SPONSOR: Hypoallergenic Air, LLC

SPONSOR'S REPRESENTATIVE: W. David Milburn

STUDY TITLE: Virucidal Efficacy Hard-Surface Test for a Device using Glass Carriers - per ASTM E1053 - Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY IDENTIFICATION: Microbac Project No. 1040-101 (refer to signed protocol)

TEST AGENT NAME	LOT NO.	DATE RECEIVED	DS NO.
MG-100	N/A	06/29/20	K899

CHALLENGE ORGANISM: Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281

HOST CELL LINE: Vero E6 cells, ATCC CRL-1586

DILUTION MEDIUM: Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

RECOVERY MEDIUM: MEM + 10% NCS

CONTACT TIME: 1 hour

CONTACT TEMPERATURE: Ambient Temperature (Actual: 21°C)

INCUBATION TEMPERATURE: 36±2°C with 5±3% CO₂

INCUBATION TIME: 4-9 days (7 days actual)

ORGANIC LOAD: 5% serum in viral inoculum

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 dilution at which half the wells are infected 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 0.05 mL.

RESULTS:

Results are presented in Tables 1-5.

The Viral Load was determined in the following manner:

Viral Load (Log₁₀ TCID₅₀) = Titer (Log₁₀ TCID₅₀/mL) + Log₁₀ [Volume (mL) per sample]

Note: The volume (mL) of the Undiluted (10⁰) sample was used in the above equation.

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

LRF = Initial Viral Load (Log₁₀ TCID₅₀) – Output Viral Load (Log₁₀ TCID₅₀)

The percentage of virus inactivation was calculated in the following manner:

$[1 - 10^{\text{Output Viral Load} / 10^{\text{Initial Viral Load}}}] \times 100 = (1 - 1/10^{(\text{Log}_{10} \text{Reduction Factor})}) \times 100$

RESULTS (continued)

Table 1
Plate Recovery Control

Dilution*	PRC	
	T = 0	T = 1 hour
10 ⁻²	8/8	8/8
10 ⁻³	8/8	8/8
10 ⁻⁴	7/8	5/8
10 ⁻⁵	0/8	0/8
10 ⁻⁶	0/8	0/8
10 ⁻⁷	0/8	0/8
Titer (Log ₁₀ TCID ₅₀ /mL)	5.68	5.43
Load (Log ₁₀ TCID ₅₀)**	4.98	4.73

*Dilution refers to the fold of dilution from the virus inoculum.

**Per carrier (0.2 mL of Undilute [10⁰])

Table 2
Test Material

Dilution*	MG-100
10 ⁻¹	8/8
10 ⁻²	8/8
10 ⁻³	5/8
10 ⁻⁴	0/8
10 ⁻⁵	0/8
10 ⁻⁶	0/8
Titer (Log ₁₀ TCID ₅₀ /mL)	4.43
Load (Log ₁₀ TCID ₅₀)**	3.73
Log ₁₀ Reduction***	1.25
Percent Reduction	94.38

*Dilution refers to the fold of dilution from the virus inoculum.

**Per carrier (0.2 mL of Undilute [10⁰])

***Per assayed volume and per carrier

RESULTS (continued)

Table 3
Cytotoxicity Controls (CT)

Dilution*	CT
10^{-1}	0/8
10^{-2}	0/8
10^{-3}	0/8

*Dilution refers to the fold of dilution from the mock inoculum.

Table 4
Cell Viability Control (CVC)

CVC
0/8
Cells were viable; media was sterile

Table 5
Virus Stock Titer (VST)


Dilution*	VST
10^{-3}	8/8
10^{-4}	8/8
10^{-5}	2/8
10^{-6}	0/8
10^{-7}	0/8
10^{-8}	0/8
Titer (Log_{10} TCID ₅₀ /mL)	6.05

*Dilution refers to the fold of dilution from the virus inoculum.

CONCLUSION:

Hypoallergenic Air LLC's MG100 was evaluated for its ability to inactivate Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus). The results are presented in Tables 1 – 5.

All of the controls met the criteria for a valid test. These conclusions are based on observed data.

Study Director: 
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11/18/2020
Date