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Assessment of Antiviral Fabric Material against SARS-CoV-2

Final Report

MRIGlobal Project No. 311743.01.001

March 30, 2021

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Preface

This report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311743.01.001, "Assessment of Antiviral Fabric Material against SARS-CoV-2."

The experimental phase of this task was initiated by MRIGlobal on March 5, 2021 and ended on March 9, 2021.

The test was performed by Kristy Solocinski, Ph.D. She was assisted by Jacob Wilkinson. The project was managed by William Sosna.

The study was not performed in compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58). This study is based off a modified version of ISO 18184. All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal, and any deviations were documented.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL

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Staff Scientist
Life Sciences Division

Approved:

Claire R. Croutch, Ph.D.
Portfolio Director,
Medical Research

March 30, 2021

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Executive Summary

Objective:

The objective of this project was to determine if treated, non-woven fabric [YMNT-K (YMDS70K)], hereafter referred to as "test fabric," has the ability to decrease viral infectivity of SARS-CoV-2 *in vitro* after exposure.

Study Design:

This study is based off a modified version of ISO 18184. Control and test samples were inoculated with 200 µL virus stock (1.47E7 $TCID_{50}$/ml) by pipetting 25 µl from 4 tips of a multichannel pipette twice, for a total of 8 spots inoculated per coupon. Control coupons were tested at 0 and 120 minutes while test coupons were tested for 0, 5, 30 and 120 minutes. After the contact time was reached, coupons were placed in conical tubes with 20 ml DMEM/F12 and vortexed on high for approximately 30 seconds each. Samples were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero cells with media removed. After approximately 35 minutes, DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next 4 days. The inoculated plates were incubated for 3 days and then read for cytopathic effects (CPE).

Results and Conclusions:

Based on these experiments, we conclude that the test fabric is effective at reducing SARS-CoV-2 infection of Vero E6 cells *in vitro*. After immediate exposure, viral infectivity was reduced 3.5 log (99.97%) compared to control samples.

Section 1.
Objective

The objective of this project was to determine if treated, non-woven fabric [YMNT-K (YMDS70K)], "test fabric," has the ability to decrease viral infectivity of SARS-CoV-2 *in vitro* after exposure.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

2.2 Testing Laboratories

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2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

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2.3.2 Analyst – MRIGlobal

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Section 3.
Test Conditions

3.1 Test Product

3.1.1 Control Fabric

YMD75GL
Untreated Non-Woven Fabric

3.1.2 Test Fabric

YMNT-K (YMDS70K)
Treated Non-Woven Fabric

3.2 Test Components

3.2.1 Cell Media

DMEM/F12 (Serum-free media)
Vendor: Gibco
Lot No.: 2235277
Expiration date: 7/21

Growth Media – 5% FBS (fetal bovine serum)
Lot No.: 202010217JW
Expiration date: 8/21

3.2.2 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2)
Strain: USA-WA1/2020
Vendor: BEI Resources
Lot: 202010212KS-B CON
Passage: 9

3.2.3 Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage No.: 37

Section 4.
Test System

MRIGlobal utilized the USA-WA1/2020 strain of the virus, acquired from BEI Resources (NR-52281). This was propagated in Vero E6 cells (ATCC CRL-1586); these cells were also used for the neutralization assay. Vero E6 cells were cultured in growth media consisting of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) supplemented with 5% FBS (Fetal Bovine Serum), and PSN (penicillin, streptomycin, and neomycin).

Section 5.
Study Design

The Vero E6 cells were plated on 96-well plates the day before the assay and were allowed to grow to ~ 60%-70% confluence. Control (YMD75GL) and test fabric was cut to 2×2 inch pieces and placed in individual petri dishes. Fabric was exposed to UV light for 5 minutes on each side to sterilize the fabric. Fabric was then cut in half and the two pieces were stacked in one petri dish prior to inoculation. This was done to minimize virus leaking through the fabric and contacting the petri dish. Samples were inoculated with 200 µL virus stock (1.47E7 $TCID_{50}$/ml) by pipetting 25 µl from 4 tips of a multichannel pipette twice, for a total of 8 spots inoculated per coupon. Control coupons were tested at 0 and 120 minutes while test coupons were tested for 0, 5, 30 and 120 minutes. Time 0 coupons were inoculated and immediately added to conicals with DMEM/F12. Lids were kept on the petri dishes during incubation times.

After the contact time was reached, coupons were placed in conical tubes with 20 ml DMEM/F12 and vortexed on high for approximately 30 seconds each. It was noted that immediately upon addition of test fabric, DMEM/F12 changes from a red to yellow color, indicating acidification of the liquid. Samples were diluted 1:10 down a 96 deep well plate in DMEM/F12. These dilutions were transferred to a plate of Vero cells with media removed. After approximately 35 minutes, DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next 4 days. This incubation period allowed the virus to adsorb to cells without interference from FBS.

After the incubation time, cells were examined for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination is done using a microscope (10x objective to view the entire well at once) and observing the morphology of the cells. Healthy Vero E6 cells are semitransparent with a fusiform appearance (pinched or narrowing ends and more round in the middle) in a monolayer of cells with little to no space between cells. Dead cells displaying CPE are often detached from the plate, round, less transparent, and much smaller than living cells. Furthermore, the healthy Vero E6 cells cover much of the surface of the well but wells containing cells with CPE have areas of the well where no cells are adherent, described as empty space. Any well displaying CPE is marked as positive whether the whole well is affected or only a small patch as both are indicative of the presence of viable virus.

Section 6.
Statistical Analysis of Data

The number of positive and negative wells were entered into a modified Excel spreadsheet that was published as part of Lindenbach BD. Measuring HCV infectivity produced in cell culture and *in vivo*. Methods Mol Biol. 2009;510:329-336. doi:10.1007/978-1-59745-394-3_24. The $TCID_{50}$/ml is calculated using the below equations, all using Microsoft Excel.

$$\text{Proportionate Distance (PD)} = \frac{\%\text{CPE at dilution above 50\%} - 50\%}{\%\text{ CPE at next dilution above 50} - \%\text{ CPE at next dilution below 50}}$$

$$\text{TCID50} = 10^{\text{log of dilution above 50\% CPE}} - \text{PD}$$

$$\text{TCID50/ml} = \frac{1}{\text{volume used per well}} x \frac{1}{\text{TCID50}}$$

The log10 of the three technical replicates was averaged for control and treatment samples. This number for the treatment is subtracted from the number for the control and is reported as "log reduction." This log reduction is converted into a percent log reduction via the following equation.

$$\%\text{ Log Reduction} = 1 - 10^{-\text{log reduction}}$$

Section 7.
Results

Plates were read 4 days after the initiation of the assay. For all contact times, test fabric solution reduced viral infectivity by 3.5 log (99.97%) compared to controls. The test $TCID_{50}$/ml values are the same as the test fabric cytotoxicity levels. This indicates that the test wells displaying cytotoxicity may not have had virus in them, it is just not possible to determine in this assay. Thus, the actual log reductions for test fabric compared to control fabric may be greater than reported here. Table 1 summarizes these findings.

Table 1. $TCID_{50}$/ml Calculations of Control and Test Fabric Inoculated with SARS-CoV-2

Sample Name	Test Description	Contact Time (min)	Replicate	$TCID_{50}$/mL	Log10 $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log10 $TCID_{50}$/mL	Log Reduction	Percent Log Reduction
T0-1	Test	0	1	3.16E+03	3.50	3.16E+03	3.50	3.52	99.97%
T0-2			2	3.16E+03	3.50				
T0-3			3	3.16E+03	3.50				
T5-1	Test	5	1	3.16E+03	3.50	3.16E+03	3.50	3.52	99.97%
T5-2			2	3.16E+03	3.50				
T5-3			3	3.16E+03	3.50				
T30-1	Test	30	1	3.16E+03	3.50	3.16E+03	3.50	3.52	99.97%
T30-2			2	3.16E+03	3.50				
T30-3			3	3.16E+03	3.50				
T120-1	Test	120	1	3.16E+03	3.50	3.16E+03	3.50	3.52	99.97%
T120-2			2	3.16E+03	3.50				
T120-3			3	3.16E+03	3.50				
C0-1	Control	0	1	1.47E+07	7.17	1.31E+07	7.02	N/A	N/A
C0-2			2	6.81E+06	6.83				
C0-3			3	3.16E+07	7.50				
C120-1	Control	120	1	1.47E+07	7.17			N/A	N/A
C120-2			2	6.81E+06	6.83				
C120-3			3	4.22E+06	6.63				
Control cytotox	cytotoxicity	N/A	1	3.16E+02	2.50	N/A			
Test cytotox	cytotoxicity		1	3.16E+03	3.50				
20210212KS-B CON	backtiter		1	1.47E+07	7.17				

Section 8.
Conclusions

Based on these experiments, we conclude that the test fabric is effective at reducing SARS-CoV-2 infection of Vero E6 cells *in vitro*. After immediate exposure, viral infectivity was reduced 3.5 log (99.97%) compared to control samples.