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Assessment of Enhanced Filter Media in the Removal of SARS-CoV-2

Final Report

FOR

Purafil - Filtration Group Corporation

Jay Joshi, Ph.D.
2654 Weaver Way
Doraville, Georgia 30340

MRIGlobal Project No. 311686.01.001

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Preface

This report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311686.01.001, “Assessment of Enhanced Filter Media in the Removal of SARS-Cov-2.”

The experimental phase of this task was initiated by MRIGlobal on September 11, 2020 and ended on October 26, 2020.

The test was managed and performed by Kristy Solocinski, Ph.D. She was assisted by Sam Humphrey.

The study was not performed in compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58). 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

Kristy Solocinski, Ph.D.
Staff Scientist
Life Sciences Division

Approved:

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

December 7, 2020

Contents

Tables

Executive Summary

Objective:

The objective of this project was to determine if PuraShield Media, a filter material made of Puraward nonwoven fibers and Purafil SP/Molecular Filtration Media, has the ability to limit the replication of SARS-CoV-2 *in vitro*.

Study Design:

This study was based on the *ISO* 18184 standard method. Coupons of PuraShield Media were cut to 2×2 inches. 200 µl of SARS-CoV-2 strain USA-WA1/2020 was added to the coupon and incubated in a biosafety cabinet in a petri dish with the lid on for 120 minutes. A control test using untreated filter was also conducted. In addition, a cytotoxicity control was performed with 200 µl of DMEM/F12 rather than virus. After the contact time was over, samples were diluted 1:10 down an empty 96 deep well plate and added to Vero E6 cells. Plates were examined at 4 days post-infection for cytopathic effect (CPE).

Results and Conclusions:

PuraShield Media reduced SARS-CoV-2 by 99.46% after 120 minutes of contact time compared to untreated filter. The cell death seen with PuraShield Media samples was comparable to cytotoxicity observed without virus, so it is possible that the actual reduction of SARS-CoV-2 is greater than the reported value. Based on this experiment, we conclude that PuraShield Media is capable of reducing the amount of infectious SARS-CoV-2 by at least 99.46%.

Section 1.
Objective

The objective of this project was to determine if PuraShield Media, comprised of both Puraward nonwoven fibers and Purafil SP/Molecular Filtration Media, has the ability to limit the replication of SARS-CoV-2 *in vitro*. SARS-CoV-2 has been identified as the virus responsible for causing COVID-19 worldwide pandemic.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

Jay Joshi, Ph.D.
Purafil - Filtration Group Corporation

2.2 Testing Laboratories

MRIGlobal
425 Volker Boulevard
Kansas City, Missouri 64110
Telephone: (816) 753-7600
Fax: (816) 753-8823

2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

Kristy Solocinski, Ph.D.
Telephone: (816) 753-7600, ext. 5280
Email: ksolocinski@mriglobal.org

2.3.2 Analyst – MRIGlobal

Sam Humphrey
Telephone: (816) 753-7600, ext. 5027
Email: shumphrey@mriglobal.org

Section 3.
Test Conditions

3.1 Test Material

3.1.1 Control Filter

The control filter utilized was a flat, unpleated, and non-treated filter piece comprised of polyethylene terephthalate (PET) fibers. The non-scrim side of the control filter was utilized for testing.

3.1.2 PuraShield Media

The PuraShield Media tested was comprised of two of the four critical components within PuraShield's multilayered filtration system - Puraward fibers and Purafil SP/Molecular Filtration Media. The PuraShield Media was tested as a flat filter piece, where Purafil Molecular Media was embedded onto the filter with Puraward fibers on the non-scrim side of the filter.

3.1.3 Cell Media

DMEM/F12 (Serum-free media)
Vendor: Gibco
Lot No.: 2186976
Expiration date: 01/22

Growth Media – 5% FBS (fetal bovine serum)
Lot No.: 20200918
Expiration date: 11/20

3.1.4 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2) (COVID-19 Virus)
Strain: USA-WA1/2020
Vendor: BEI Resources

Passage number in assay: 8

3.1.5 Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage Number in Assay: 18

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 Dulbeco'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 three days before the assay and were allowed to grow to ~ 80% confluence. On the day of the assay, coupons were cut to 2×2 inches and placed in petri dishes. Coupons were exposed to UV light for 5 minutes on each side to sterilize them. 200 µl of SARS-CoV-2 strain USA-WA1/2020 was added to the coupons and rubbed with the pipette tip to increase surface area contact as well as absorption. The coupons were then allowed to sit in the biosafety cabinet for 120 minutes covered by the petri dish lid. There was also a time zero (T_0) control done to compare virus loss over 120 minutes of contact time. These samples were resuspended immediately after addition to control filter. After the designated time, virus was recovered from samples by placing coupons in a 50 ml conical and adding 20 ml of DMEM/F12. Samples were vortexed for approximately 30 seconds each. The PuraShield Media samples turned the liquid a dark purple color. Sodium thiosulfate (0.5%) was added to all samples to neutralize the permanganate. While sodium thiosulfate has been previously demonstrated by MRI not to interefere with viral infection or cause cytotoxicity at the 0.5% concentration, it was added to control samples to account for any variability in this specific test. The resulting precipitate was centrifuged for 50 seconds at 50 rcf to allow pipetting of the supernatant. The samples were added to an empty 96 deep well plate and diluted 1:10 down the plate in DMEM/F12. These dilutions were then transferred to a plate of Vero E6 cells with media removed. After approximately 1 hour, DMEM/F12 supplemented with 5% fetal bovine serum (FBS) was added to cells to feed them for the next 4 days. This incubation period without FBS is to allow the virus to adsorb to cells without interference from FBS. The assay was executed in three technical and five pipetting replicates for each condition. The samples are summarized in the following table (Table 1).

Table 1. Sample characteristic description.

Sample Name	Description
PuraShield Media	Test media comprised of Purafil's Puraward fibers and Purafil SP/Molecular Filtration Media
T_0 Control	Control coupon with viral extraction performed immediately at time (t) = 0 min
Control	Control coupons comprised of untreated PET fibers
PuraShield Media Cytotoxicity	Cytotoxicity testing of PuraShield Media not exposed to SARS-CoV-2 virus and evaluated to screen for potential test interference.

After 4 days, 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.

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

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

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

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.

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

Section 7.
Results - Efficacy of PuraShield Media Against SARS-CoV-2

The plates were read 4 days after infection. This showed a 99.46% reduction in virus infection after 120 minutes of contact with PuraShield Media compared to controls. There was no difference between the T_0 control and the control that was incubated for 120 minutes (0.05% reduction). Cytotoxicity was observed at the first row of dilution of PuraShield Media. This is calculated as $TCID_{50}$ even though no virus was present in the cytotoxicity wells in order to compare cytotoxicity with test $TCID_{50}$. Cytoxicity and PuraShield Media test samples gave the same value. Any cell death at the same level as the cytotoxicity indicates that it is possible that no virus was present in those wells marked as positive. However, since the cells died, there is no way to determine viral presence in this assay. Thus, while the reported log reduction is accurate, it may reflect an underestimate due to the interference of the cytotoxicity. All uninfected controls remained healthy at the end of the observation period. Table 2 summarizes these findings.

Table 2. Results of *in vitro* Neutralization of SARS-CoV-2 with PuraShield Media

Sample Name	Sample Type	SARS-CoV-2 Added	Test Duration (min)	Replicate	$TCID_{50}$/mL	Log10 $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log10 $TCID_{50}$/mL	Log Reduction	Percent Log Reduction
PuraShield Media - 1	Test	yes	120	1	3.16E+03	3.50	3.16E+03	3.50	2.26	99.46%
PuraShield Media - 2		yes		2	3.16E+03	3.50				
PuraShield Media - 3		yes		3	3.16E+03	3.50				
T_0 control-1	Time 0 Control	yes	0	1	6.81E+05	5.83	5.95E+05	5.76	0.00	0.05%
T_0 control-2		yes		2	4.22E+05	5.63				
T_0 control-3		yes		3	6.81E+05	5.83				
Control-1	Control	yes	120	1	4.22E+05	5.63	7.36E+05	5.76	N/A	N/A
Control-2		yes		2	1.47E+06	6.17				
Control-3		yes		3	3.16E+05	5.50				
PuraShield Media Cytotoxicity	Cytotoxicity	no	0	N/A	3.16E+03	3.50	N/A	N/A	N/A	N/A

Section 8.
Conclusions

Based on this experiment, we conclude that PuraShield Media is effective at reducing SARS-CoV-2 infection of Vero E6 cells by at least 99.46%. It is important to note that any cytotoxicity observed limits the enumeration of virus and, therefore, it cannot be said whether virus was present in those wells which showed cytotoxicity in the absence of CPE. Thus, actual viral reduction is potentially greater than reported for those samples.