Antibacterial and Antiviral Efficacy Testing of Micrillon® Glove

POINT OF CONTACT

Dr. Mina Izadjoo
Chief Science Officer Phone:
(301) 204-4201

Email: mizadjoo@ipharmaservices.com

BACKGROUND

Due to increase in emergence of hard-to-treat infections including drug resistant pathogens, there is a critical need for developing textiles with broad spectrum antimicrobial properties. However, very few scientists in the textile industry have attempted to develop textiles with antiviral properties. In addition, clear success in demonstrating antiviral properties of these candidate antiviral textiles has yet to be achieved.

In this study, the antimicrobial properties of Micrillon® Glove Fabric was studied. The antiviral efficacy testing was conducted based on ISO Standard 18184:2019- Determination of Antiviral Activity of Textile Products. This standard is a stringent methodology for demonstrating antiviral efficacy of textiles.

STUDY OBJECTIVES

The aim of this study was to evaluate antibacterial and antiviral properties of Micrillon® Glove's Fabric against Human Coronavirus, Human Influenza A H1N1 virus, *Staphylococcus aureus* (MRSA) and *E. coli* 0157:H7.

TECHNICAL APPROACH

I. Antiviral Efficacy Testing

The antiviral efficacy testing was conducted based on ISO Standard 18184:2019- Determination of Antiviral Activity of Textile Products. Results from antiviral tests of test sample is compared to results of control (untreated textile.

Test Principle- The viruses are deposited onto a specimen (textile or fabric). After specific contact time, the reduction rate is calculated between the antiviral product test specimen and the control specimen. The quantification of the virus titer will be conducted using Plaque Reduction Assay or $TCID_{50}$.

Integrated Pharma
SERVICES

Micrillon® Glove

Viruses and Host Cells Tested:

Virus	**Host Cells**
Human Influenza A Virus (H1N1)	MCDK Cell (Dog kidney cell origin)
Human Coronavirus OC-43	HCT-8 [HRT-18] (Human ileocecal colorectal adenocarcinoma-epithelial)

Cell culture Preparation

For antiviral testing, host cell for plaque assay titer determination was prepared in tissue culture 6-well plates and incubated for at 37 ˚C with 5% CO2 for up to 2 days. Host cell for TCID50 cytopathic effect assay titer determination was prepared in tissue culture 96-well plates and incubated for at 37 ˚C with 5% CO2 for up to 2 days. The host cell used for H1N1 virus is MDCK

Cell ATCC CL-34. The host cell HCT-8 [HRT-18] (Human ileocecal colorectal adenocarcinoma-epithelial) is the host cell for Human Coronavirus OC-43.

The host cells were sub-cultured from pre-cultured cells in 75 cm tissue cultured flask (T-75) to 80-90% confluency. The adherent cells were split by decanting growth media from T-75 flask. Trypsin was added to detach the cells. New growth media was added. Cells were observed and counted under a microscope. Based on the cell count, 6-well plates were prepared at 50,000-150,000 cells per well (2ml per well) for plaque assay and 96-well plates were prepared at 5,000-10,000 cells per well (200ul per well) for TCID50 assay. The cells in the 6-well plate and 96-well plate were ready for the test when they are at least 80-90% confluent after up to 2 days culturing.

Antiviral Testing Procedure

All test specimens were prepared in sterilized glass vial containers with caps. The samples used were 2cm x 2cm control and fabric. Exactly 0.2 ml of the virus suspension were deposited onto each activated fabric and caps were tightly closed. The test samples were incubated for 2 hours at room temperature. The control blank samples were immediately treated (T0) with 20 ml of wash solution, SCDLP (Caseine Peptone Lecithin Polysorbate Broth with Tween 80) medium. The specimens were treated with SCDLP medium after the contact time. The vials were closed and agitated by vortexing for 5 seconds and 5 times for washing out the virus.

Preparation of the series of dilutions for the virus suspension

The virus suspension was serially diluted with 1.8 ml of maintenance medium (Essential Minimum Essential Medium with 1% Penicillin/Streptomycin antibiotics) and 0.2 ml of wash-out virus suspension and vortexed. The procedure was repeated to prepare a series of dilutions for the virus suspension to 10^2.

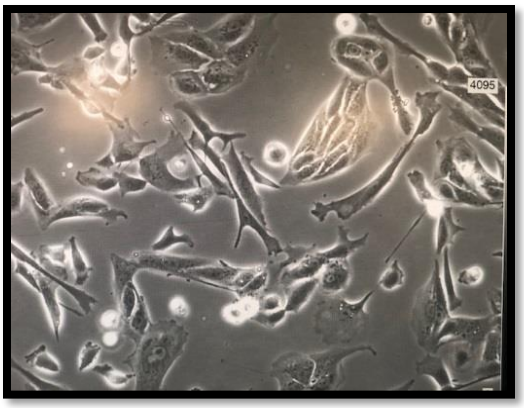
Plaque assay for infectious titer determination

Using a microscope, the confluency of cells in the 6-well plate was observed. When cells are 80-90% confluent, the growth medium is drained from the plate. The cells are washed and removed two times using 2 ml of maintenance medium added to each well. 0.1 ml of the washing out virus suspension dilutions ($10^5 – 10^2$) were inoculated onto 6-well plate (0.4 ml of maintenance media

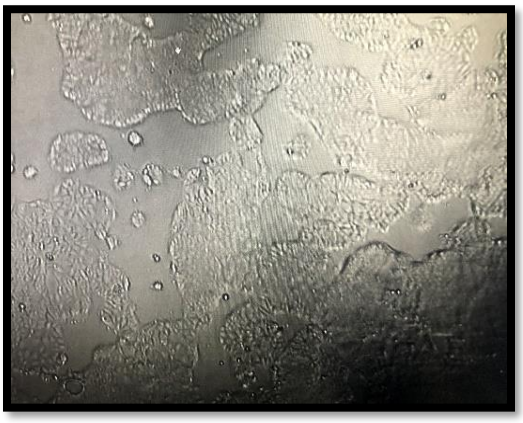
added immediately to keep cells from drying). The plates were placed in 35˚C with 5% CO_2 incubator for 1 hour, to let cells absorb the virus. The plates were tilted every 15 minutes to allow the entire area of the cells absorb the virus. After incubation, wells were washed and removed with 2 ml maintenance media again. A 2-ml of pre-made agar growth medium was added to each well and incubated at room temperature for 15 minutes to allow the agar medium to solidify. The plates were then incubated in 35 ˚C with 5% CO_2 for 2 days to 8 days to culture (depending on the virus strain). After incubation, the plates were removed from incubator and 2ml of formaldehyde solution added for cell fixation and incubated at room temperature for 1 hour. The formaldehyde was removed, and the agar medium drained and removed by rinsing wells with distilled water. Crystal violet solution was added and incubated at room temperature for 5 minutes to dye cells. The crystal violet solution was washed with distilled water. The plaques were counted and calculated to determine the titer.

TCID50 assay for infectious titer determination

Using a microscope, the confluency of cells in the 96-well plate was observed. When cells are 80-90% confluent, the growth medium is drained from the plate. The cells are washed and removed two times using multichannel pipette, 0.1 ml of maintenance medium added to each well. After wash, 0.1 ul of Maintenance Media added to all wells. 0.1 ml of media removed from Column 1 of 8 wells. 0.1 of the washing out virus suspension dilution (10^5) were inoculated onto the first 8 wells of 96-well plate and serially diluted in the plate. Wells were examined for Cytopathic Effect (CPE) after appropriate incubation period for each virus.

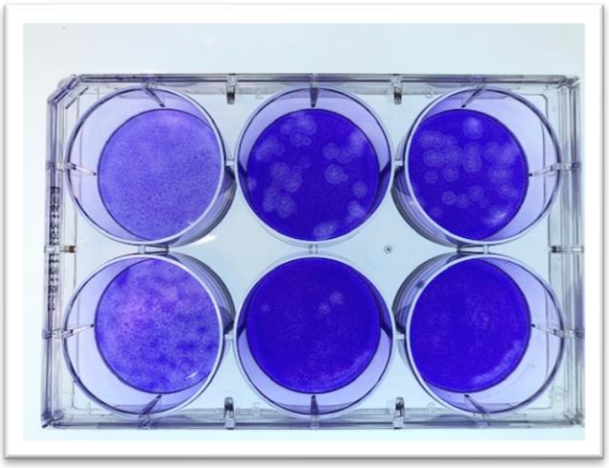


MCDK Cell (Dog kidney cell origin)

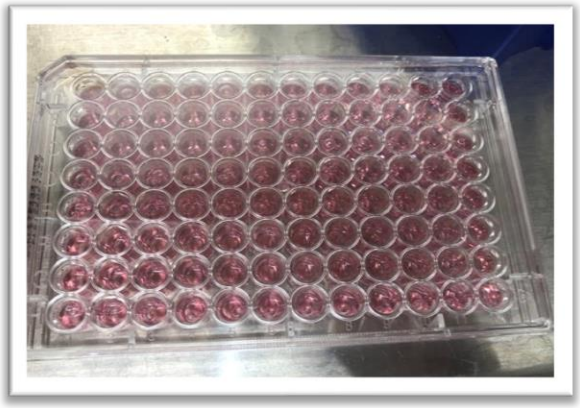


HCT-8 [HRT18] Cells

Human ileocecal colorectal adenocarcinoma-epithelial



Plaque Reduction Assay was conducted in 6-Well Plates



Cytopathic Effect (CPE) was conducted in 96-Well Plates

II. Antibacterial Efficacy Testing

Antibacterial efficacy testing of the test fabric will be conducted based on using AATCC Test Method 100 Standard. Three sample replicates were tested at 10 min, 30 min and 24 hrs against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* 0157:H7.

The recommended incubation period according to AATCC Test Method 100 Standard is 24h.

Time 0-Inoculation

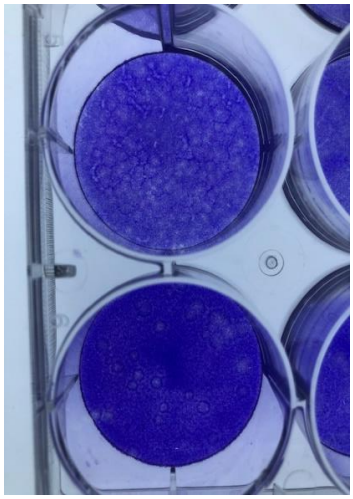
1) Inoculate control and test samples with 1 mL of the culture suspension (~5.0 x 10^6 CFU/ml)
2) Immediately remove the samples and transfer into 50 ml tube containing 10 mL of D/E broth
3) Vortex tube for 1 minute (1 mL aliquot of the D/E broth represents 10^{-1})
4) Add 0.1 mL of suspension to 0.9 mL D/E broth and vortex to mix
5) Serially dilute up to 10^{-4} and plate 0.1 mL of the serial dilutions to TSA plates in duplicate
6) After 24 hours incubation, count the plates to achieve final plate count

Time Points (10 min, 30 min, 24-hour) Testing

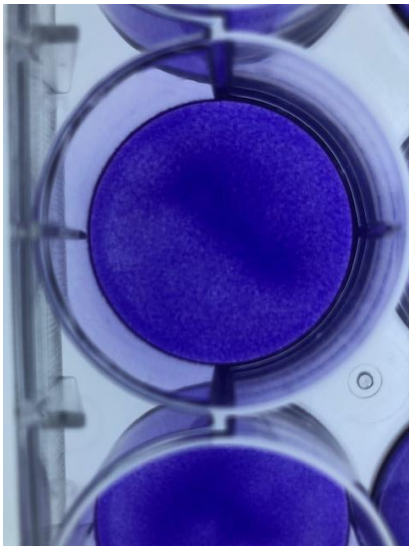
1) Inoculate control and test samples with 1 mL of the culture suspension (~5.0 x 10^6 CFU/ml)
2) Incubate at 37˚C for 24 hours
3) Remove the samples and transfer into 50 ml tube containing 10 mL of D/E broth
4) Vortex tube for 1 minute (1 mL aliquot of the D/E broth represents 10^{-1})
5) Add 0.1 mL of suspension to 0.9 mL D/E broth and vortex to mix
6) Serially dilute up to 10^{-4} and plate 0.1 mL of the serial dilutions to TSA plates in duplicate
7) After 24 hours incubation, count the plates to achieve final plate count.

RESULTS

The viral exposure was conducted in sterile glass jars.



Control Glove Showing Influenza A H1N1 Viral Plaques



Micrillon® Glove showing no Influenza A H1N1 Viral Plaques

Integrated Pharma
SERVICES

Antiviral Activity

The standard exposure period according to ISO Standard 18184:2019 is 2h.

Sample	Virus	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Coronavirus OC43	2h	2.25	99.44
Micrillon® Glove	Human Coronavirus OC43	2h	2.88	99.87
Micrillon® Glove	Human Coronavirus OC43	2h	2.37	99.57
Control Glove	Human Coronavirus OC43	2h	0.37	57.28

Sample	Virus	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Coronavirus OC43	30 min	1.82	98.48
Micrillon® Glove	Human Coronavirus OC43	30 min	1.82	98.48
Micrillon® Glove	Human Coronavirus OC43	30 min	1.69	97.95
Control Glove	Human Coronavirus OC43	30 min	0.29	39.91

Sample	Virus	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Coronavirus OC43	10 min	1.44	96.36
Micrillon® Glove	Human Coronavirus OC43	10 min	1.69	97.95
Micrillon® Glove	Human Coronavirus OC43	10 min	1.69	97.95
Control Glove	Human Coronavirus OC43	10 min	0.09	15.17

Sample	Exposure Time	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Influenza A H1N1	2h	3.27	99.90
Micrillon® Glove	Human Influenza A H1N1	2h	3.20	99.88
Micrillon® Glove	Human Influenza A H1N1	2h	3.04	99.83
Control Glove	Human Influenza A H1N1	2h	0.13	26.25

Sample	Exposure Time	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Influenza A H1N1	30 min	3.56	99.97
Micrillon® Glove	Human Influenza A H1N1	30 min	2.95	99.89
Micrillon® Glove	Human Influenza A H1N1	30 min	3.56	99.97
Control Glove	Human Influenza A H1N1	30 min	0.20	37.41

Sample	Exposure Time	Exposure Time	Log Reduction	% Reduction
Micrillon® Glove	Human Influenza A H1N1	10 min	3.56	99.97
Micrillon® Glove	Human Influenza A H1N1	10 min	3.56	99.97
Micrillon® Glove	Human Influenza A H1N1	10 min	3.56	99.97
Control Glove	Human Influenza A H1N1	10 min	0.13	26.47

Integrated Pharma SERVICES

Antibacterial Results

Antibacterial Efficacy Testing of Micrillon Glove Fabric against MRSA

Time Point	Sample	Final CFU/ml	Notes
T0	Control Glove	4.0×10^6	
	Micrillon® Glove	3.3×10^6	
T10 min	Control Glove	3.3×10^6	
	Micrillon® Glove	4.8×10^6	
T30 min	ControlGlove	4.9×10^6	
	Micrillon® Glove	4.8×10^6	
T24 h	Control Glove	3.3×10^6	
	Micrillon® Glove	$<0.9 \times 10^3$	100% kill

Antibacterial Efficacy Testing of Micrillon Glove Fabric against *E. coli* O157

Time Point	Sample	Final CFU/ml	Notes
T0	Control Glove	5.3×10^6	
	Micrillon® Glove	4.5×10^6	
T10 min	Control Glove	5.4×10^6	
	Micrillon® Glove	3.9×10^6	
T30 min	Control Glove	4.4×10^6	
	Micrillon® Glove	5.0×10^6	
T24 h	Control Glove	3.8×10^7	
	Micrillon® Glove	$<0.9 \times 10^3$	100% kill

CONCLUSIONS

- Micrillon® Glove fabric demonstrated significant antiviral properties against Human Coronavirus OC-43 and Human Influenza A H1N1.

- Micrillon® Glove fabric demonstrated significant antibacterial efficacy (100% kill) against MRSA and *E. coli* O157.

- The technology has significant potential for various applications including its use in Personal Protective Equipment (PPE).