RESEARCH, DISCOVERY & INNOVATION

Water & Energy Sustainable Technology Center

Modified ISO 18184: Determination of Antiviral Activity of Textile Products to Evaluate Three Distinct Treated Fabric Formulations against Human Coronavirus 229E

Company: Livinguard

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Study Data

Table 1. Modified ISO 18184 Evaluation of Three Distinct Treated Fabric Formulations against Human Coronavirus 229E at One Contact Time[a,b,c]

Test Virus	Contact Time	Sample ID	Virus Titer ($TCID_{50}$ per Carrier)	Mean Virus Titer ($TCID_{50}$ per Carrier)	Mean Log_{10} Virus Titer ($TCID_{50}$ per Carrier)	Log_{10} Reduction	Percent Reduction
Human Coronavirus 229E (ATCC VR-740)	Time Zero	Control Fabric	2.25E+06	3.54E+06	6.55	N.A.	N.A.
			7.12E+06				
			1.26E+06				
	2 Hours	Control Fabric	7.12E+05	4.46E+05	5.65	0.90	87%
			4.00E+05				
			2.25E+05				
	2 Hours	FM-7 (Fabric Formulation 1)	2.25E+03	2.25E+03	3.35	2.30	99.5%
			2.25E+03				
			2.25E+03				
		FM-14 (Fabric Formulation 2)	4.00E+05	3.84E+05	5.58	0.06	13.8%
			4.00E+04				
			7.12E+05				
		FM-26 (Fabric Formulation 3)	1.26E+04	7.34E+03	3.87	1.78	98.4%
			7.12E+03				
			2.25E+03				

[a] $TCID_{50}$: Tissue Culture Infectivity Dose at the 50% Endpoint.

[b] Log_{10} and Percent Reductions for Control Fabric at 2 hours calculated relative to Contol Fabric immediately upon innoculation (Time Zero).

[c] Log_{10} and Percent Reductions for the three Test Fabrics at 2 hours calculated relative to Control Fabric mean viral titer at 2 hours.

Table 2. ISO 18184 Evaluation of Three Distinct Treated Fabric Formulations against Human Coronavirus 229E: Cytoxicity Controls[a,b,c]

Test Virus	Sample ID	Contact Time	Toxicity Titer (CCD_{50} per Replicate)	Mean Toxicity Titer (CCD_{50} per Replicate)	Mean Log_{10} Toxicity Titer (CCD_{50} per Carrier)
Human Coronavirus 229E (ATCC VR-740)	Control Fabric	2 Hours	1.26E+02	1.26E+02	2.10
			1.26E+02		
			1.26E+02		
	FM-7 (Fabric Formulation 1)	2 Hours	1.26E+02	1.26E+02	2.10
			1.26E+02		
			1.26E+02		
	FM-14 (Fabric Formulation 2)	2 Hours	1.26E+03	1.26E+03	3.10
			1.26E+03		
			1.26E+03		
	FM-26 (Fabric Formulation 3)	2 Hours	1.26E+02	1.26E+02	2.10
			1.26E+02		
			1.26E+02		

[a]CCD_{50}: Cell Cytotoxicity Dose at the 50% Endpoint.

[b]Control Fabric, FM-7 and FM-26 Test Fabric Formulations: cytoxicity observed for MRC-5 cells in the 10^0 dilution.

[c]FM-14 Test Fabric Formulation: cytotoxicty observed for MRC-5 cells in the 10^0 and 10^{-1} dilutions.

Table 3. ISO 18184 Evaluation of Three Distinct Treated Fabric Formulations against Human Coronavirus 229E: Neutralization Validation (NV) Controls[a,b]

Test Virus	Sample ID	Contact Time	NV Ctrl Titer ($TCID_{50}$ per Carrier)	Mean NV Ctrl Titer ($TCID_{50}$ per Carrier)	Mean Log_{10} Virus Titer ($TCID_{50}$ per Carrier)	Neutralization Validated?
Human Coronavirus 229E (ATCC VR-740)	Control Fabric	2 Hours	2.25E+03	2.72E+03	3.44	N.A.
			2.25E+03			
			4.00E+03			
	FM-7 (Fabric Formulation 1)	2 Hours	4.00E+03	3.30E+03	3.52	Yes
			2.25E+03			
			4.00E+03			
	FM-14 (Fabric Formulation 2)	2 Hours	4.00E+03	2.72E+03	3.44	Yes
			2.25E+03			
			2.25E+03			
	FM-26 (Fabric Formulation 3)	2 Hours	2.25E+03	2.25E+03	3.35	Yes
			2.25E+03			
			2.25E+03			

[a] $TCID_{50}$: Tissue Culture Infectivity Dose at the 50% Endpoint.

[b] Neutralization considered valid when mean viral titer on neutralized test fabric formulations differs by ≤ 0.5 log_{10} relative to control fabric.

Summary of the Study Methods

The testing was conducted according to ISO 18184 (Determination of Antiviral Activity of Textile Products), with modifications. A listing of all modifications that were made to the method are listed below in the subsection titled "*Modifications to the Study Method ISO 18184*".

Preparation of Control Fabric

1. An untreated Control Fabric was washed 10 times with water, and provided by the Study Sponsor.
2. Once received, the Control Fabric was cut to dimensions measuring 20 mm by 20 mm.
3. The Control Fabric cuttings were loaded into a glass beaker, covered with foil, and autoclaved for 20 minutes at 121 °C (103 kPa).

Preparation of Test Fabric

1. The three distinct Test Fabric formulations (FM-7, FM-14, and FM-26) were washed 10 times with water, and provided individually packaged by the Study Sponsor.
2. Once received, the Test Fabrics were cut to dimensions measuring 20 mm by 20 mm.
3. The cut pieced of the three Test Fabric formulations were loaded into three separate glass beakers, covered with foil, and autoclaved for 20 minutes at 121 °C (103 kPa).

Test Procedure

1. On the day of testing, five to six 20 mm x 20 mm pieces each of the Control and Test Fabrics were aseptically transferred to and stacked within sterile Petri dishes. The final mass per container for the six swatches (Control or Test) was 0.40 ± 0.05 g. Nine Petri containers were prepared for the Control Fabrics, and six were prepared for each of the Test Fabric.
2. Six Control Fabric stacks and three Test Fabric stacks (per formulation) were each inoculated drop-by-drop with a total of 0.2 mL of Human Coronavirus 229E viral stock (no soil load). Each individual swatch per stack was inoculated with 0.033 mL of the test virus (6 swatches per stack = 0.2 mL total inoculum volume). A sterile pipette tip was used to press each stack and ensure that the inoculum was evenly spread through each piece of fabric.
3. Three Control Fabric and three Test Fabric stacks were parafilmed and incubated at 20 °C in a humidified chamber for the 2-hour study contact time.
4. Three Control Fabric stacks were immediately neutralized to assess the viral titer upon inoculation (i.e. Time Zero) by transfer into conical tubes containing 4 mL of Letheen Broth Base. The tubes were then vortexed five times for five seconds each to wash out the viruses from the fabric pieces.
5. At the close of the 2-hour contact time, the triplicate Control and Test Fabric stacks were neutralized by transfer into conical tubes containing 4 mL of Letheen Broth Base. The tubes were vortexed five times for five seconds each to wash out the viruses from the fabric pieces.

6. The additional triplicate Control and Test Fabric stacks (containing no viral inoculum) were incubated concurrently for 2-hours and harvested in Letheen Broth Base as previously described to assess cytotoxicity and to validate neutralization as described in ISO 18184.

Cell Culture Infectivity Assay

1. Control and test suspensions were diluted ten-fold in 0% FBS MEM.
2. Each dilution was plated in quadruplicate (0.1 mL per well) onto MRC-5 host cell monolayers in 24-well trays prepared to a confluency range of 70% to 80%.
3. Following an adsorption period of 30 minutes to facilitate virus-host cell interaction, 1 mL of 2% FBS MEM was added to each well.
4. The 24-well trays were incubated at 35 °C in a humidified chamber with an atmosphere of 5% CO_2 for 10 days. Assay trays were monitored regularly for changes to host cell monolayers indicative of cytotoxicity or viral cytopathogenic effects (CPE).
5. Assay trays were formally scored on Day 10 of incubation. Log_{10} and percent reductions of the mean viral inoculum on the Test Fabrics at 2 hours were calculated relative to the mean viral titer of the Control Fabric at 2 hours using the Spearman-Karber $TCID_{50}$ Method.
6. Log_{10} and percent reductions were also determined for the Control Fabric at 2 hours relative to the titer yielded from Control Fabrics at Time Zero to ascertain stability of the viral inoculum over the course of testing.

Modifications to the Study Method ISO 18184

1. Modification No.1: Letheen Broth Base was employed as the neutralizing medium in place of SCDLP Medium. The modification was made for two reasons:
    - SCDLP Medium, when prepared according to ISO 18184, call for the use of a nonionic surfactant which can be physically disruptive to the lipid-based envelope encasing the nucleocapsid of the test virus - a coronavirus - as previously documented (1). Damage to the viral envelope would have potentially resulted in loss of infectivity. This has been demonstrated more recently for several enveloped viruses following exposure to nonionic surfactants (2,3).
    - Letheen Broth Base contains key neutralizing ingredients for quaternary ammonium compounds and other disinfectant actives including lecithin, beef extract, and enzymatic digest of animal tissue, but does not employ Polysorbate 80, a nonionic surfactant, for neutralization as SCDLP Medium does. Letheen Broth Base was also non-toxic when applied directly (non-diluted) to MRC-5 host cell monolayers.
2. Modification No. 2: Neutralization of the control and test textiles was performed using 4 mL of Letheen Broth Base rather than 20 mL. This modification was made to alleviate dilution effects of the test virus that would have required the assay of greater replicate volumes to effectively compute log10 reductions and limits of detection – the latter were not found to be applicable in the current study upon final scoring of the assay trays as the test virus was detected on all fabric formulations following the 2-hour contact time.

Study Conclusions

The FM-7 Test Fabric was the most efficacious against Human Coronavirus 229E, achieving a reduction of 99.5% (2.30 $\log_{10}$) given two hours of exposure relative to the Control Fabric (Table 1). Test Fabric FM-26 reduced levels of the test virus by 98.4%, while reductions were minimal for Test Fabric FM-14 at 13.8%.

With regard to cytotoxicity effects on the MRC-5 cell line, the level of toxicity observed for test fabrics FM-7 and FM-26 was similar to that of the Control Fabric (Table 2). Toxicity levels on MRC-5 host cell monolayers were slightly greater for the FM-14 test fabric. However, the sensitivity of MRC-5's, an internal human lung fibroblast cell line, should not necessarily be extrapolated assume any effects on the protective and resilient cells comprising the external cell layers of human skin.

Neutralization was validated for each of the Control and Test Fabrics beyond the level of cytotoxicity, with < 0.5 $\log_{10}$ difference in viral titer observed between the Control and Test fabrics (Table 3).

References

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3. Colavita, F., Quartu, S., Lalle, E., Bordi, L., Lapa, D., Meschi, S., Vulcano, A., Toffoletti, A., Bordi, E., Paglia, M.G. and Di Caro, A., 2017. Evaluation of the inactivation effect of Triton X-100 on Ebola virus infectivity. *Journal of Clinical Virology*, *86*, pp.27-30.