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Report - Antiviral Activity of textile product HEFM47.CO.01

Tested by: Prof. Uwe Rösler and Dr. Anika Friese

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Tested textile product

- reference textile: Cotton (Co), untreated, 11.04.2020, lab number: C1
- antiviral textile: HEFM47.CO.01, 13.05.2020, LIVINGUARD, lab number: C2-neu

Method

- modified according to ISO 18184 (First edition 2014-09-01)
 - washing of all tested textiles with deionized water 10 times at **40°C**, drying
 - cutting pieces of approximately 20x20mm and making up a mass of 0,4g with several pieces
 - sterilization at 121°C for 15 min, drying
 - before starting the test conditioning the textiles overnight in a humid environment (incubator 37°C)
 - controls: verification of cytotoxic effect and cell sensitivity to virus/inactivation of antiviral activity

- test: inoculation of 0,4g textile with 1ml (or 2ml) virus suspension (at least 10^7 TCID₅₀/ml)
 - washing out using 19 ml (or 18 ml) cell culture medium (MEM Eagle EBSS + 10% fetal calf serum + 1% non-essential amino acids, +1 % penicillin (10.000 U/ml) / streptomycin (10 mg/ml)) after specific time points
 - titration of washing solution in 96-well plate and titer calculation with method of Spearman and Karber in TCID/ml
- comparison of titer from untreated textile (reference) and treated textile for the specific time points

Results

Code ITU	C1	C2-neu	C1	C2-neu	C1	C2-neu	C1	C2-neu	C1	C2-neu	C1	C2-neu
sequential nr.	1	1	2	2	3	3	4	4	5*	5*	6*	6*
textile after 30 min in log(10) TCID ₅₀ /ml	7,6	6,35	7,6	6,6	7,35	6,35	7,35	6,475	7,35	7,1	7,475	6,975
Antiviral Activity after 30min in log(10)		1,25		1		1		0,875		0,25		0,5
Reduction after 30min in %		94,38		90,00		90,00		86,66		43,77		68,38
textile after 1h in log(10) TCID ₅₀ /ml	7,1	5,975	7,1	6,1	7,35	5,475	7,35	5,85	-	-	-	-
Antiviral Activity after 1h in log(10)		1,125		1		1,875		1,5	-	-	-	-
reduction after 1h in %		92,50		90,00		98,67		96,84	-	-	-	-
textile after 2h in log(10) TCID ₅₀ /ml	7,1	5,225	7,1	5,225	6,6	5,35	6,975	5,35	7,725	6,475	7,35	5,6
Antiviral Activity after 2h in log(10)		1,875		1,875		1,25		1,625		1,25		1,75
reduction after 2h in %		98,67		98,67		94,38		97,63		94,38		98,22
textile after 6h in log(10) TCID ₅₀ /ml	6,975	3,85	6,85	4,475	6,475	4,1	6,725	4,35	6,85	4,1	7,1	3,975
Antiviral Activity after 6h in log(10)		3,125		2,375		2,375		2,375		2,75		3,125
reduction after 6h in %		99,93		99,58		99,58		99,58		99,82		99,93
textile after 12h in log(10) TCID ₅₀ /ml	6,475	< 3,1	6,225	3,35	6,475	3,35	5,975	3,1	-	-	-	-
Antiviral Activity after 12h in log(10)		>3,375		2,875		3,125		2,875	-	-	-	-
reduction after 12h in %		>99,9		99,87		99,93		99,87	-	-	-	-

*inoculation with 2ml virus suspension instead of 1ml

Conclusion

The treated textile sample "C2-neu" can efficiently inactivate SARS-CoV-2. The results show a reduction of infectious SARS-CoV-2 within 6 hours from 2.3 to 3.1 log-ranges in comparison to the untreated textile. This means a reduction rate up to 99.9% after 6 hours. As a result of the inoculation of 0.4 g textile with 1 ml virus suspension the whole textile was moistened. This allows the textile sample having the largest disinfectant effect, we assume. Therefore, especially the handling with moist masks seems to be safer in comparison to the untreated textile.

Berlin, 30.07.2020



Prof. Dr. Uwe Rösler



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

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Study Data

Table 1. Modified ISO 18184 Evaluation of Three Treated Fabrics 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 (Test Fabric)	2.25E+03	2.25E+03	3.35	2.30	99.5%
			2.25E+03				
			2.25E+03				
		FM-14 (Test Fabric)	4.00E+05	3.84E+05	5.58	0.06	13.8%
			4.00E+04				
			7.12E+05				
		FM-26 (Test Fabric)	1.26E+04	7.34E+03	3.87	1.78	98.4%
			7.12E+03				
			2.25E+03				

^aTCID₅₀: Tissue Culture Infectivity Dose at the 50% Endpoint.

^bLog₁₀ and Percent Reductions for Control Fabric at 2 hours calculated relative to Control Fabric immediately upon inoculation (Time Zero).

^cLog₁₀ 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 Treated Fabrics against Human Coronavirus 229E: Cytotoxicity 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 (Test Fabric)	2 Hours	1.26E+02	1.26E+02	2.10
			1.26E+02		
			1.26E+02		
	FM-14 (Test Fabric)	2 Hours	1.26E+03	1.26E+03	3.10
			1.26E+03		
			1.26E+03		
	FM-26 (Test Fabric)	2 Hours	1.26E+02	1.26E+02	2.10
			1.26E+02		
			1.26E+02		

^aCCD₅₀: Cell Cytotoxicity Dose at the 50% Endpoint.

^bControl Fabric, FM-7 and FM-26 Test Fabrics: cytotoxicity observed for MRC-5 cells in the 10⁰ dilution.

^cFM-14 Test Fabric: cytotoxicity observed for MRC-5 cells in the 10⁰ and 10⁻¹ dilutions.



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Table 3. ISO 18184 Evaluation of Three Treated Fabrics 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 (Test Fabric)	2 Hours	4.00E+03	3.30E+03	3.52	Yes
			2.25E+03			
			4.00E+03			
	FM-14 (Test Fabric)	2 Hours	4.00E+03	2.72E+03	3.44	Yes
			2.25E+03			
			2.25E+03			
	FM-26 (Test Fabric)	2 Hours	2.25E+03	2.25E+03	3.35	Yes
			2.25E+03			
			2.25E+03			

^aTCID₅₀: Tissue Culture Infectivity Dose at the 50% Endpoint.

^bNeutralization considered valid when mean viral titer on neutralized test fabrics differs by $\leq 0.5 \log_{10}$ relative to control fabric.



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Study Methods

Preparation of Control Fabric

1. An untreated, washed Control Fabric was provided by the Study Sponsor.
2. Pieces of the Control Fabric were 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 treated Test Fabrics (FM-7, FM-14, and FM-26) were provided by the Study Sponsor.
2. Pieces of the Test Fabric were cut to dimensions measuring 20 mm by 20 mm.
3. The Test Fabric pieces were loaded into a separate glass beaker, 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 0.2 mL of Human Coronavirus 229E viral stock (no soil load). 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 Lethen Broth. 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 Lethen Broth. 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 Lethen Broth as previously described to assess cytotoxicity and to validate neutralization as described in ISO 18184.



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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% CO₂ 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. Log₁₀ 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-Kärber TCID₅₀ Method.
6. Log₁₀ 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.

Study Conclusions

The FM-7 Test Fabric was the most efficacious against Human Coronavirus 229E, achieving a reduction of 99.5% (2.30 log₁₀) 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, toxicity was observed in the 10⁰ dilution of washes for the Control Fabric, FM-7, and FM-26 (Table 2). Toxic effects on host cell monolayers were greater by an order of magnitude for FM-14 (10⁰ and 10⁻¹ dilutions). Neutralization was validated for each of the Control and Test Fabrics beyond the level of cytotoxicity, with < 0.5 log₁₀ difference in viral titer observed between the Control and Test fabrics (Table 3).