RESEARCH, DISCOVERY & INNOVATION

Water & Energy Sustainable Technology Center

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

## Company: Livinguard


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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 | | | | |

[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 Treated Fabrics 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 (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 | | |

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

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

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

**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 | | | |

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

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

## 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 Letheen 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 Letheen 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 Letheen Broth 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.

## 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, toxicity was observed in the $10^0$ 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 ($10^0$ and $10^{-1}$ dilutions). 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).