



Campus-UNICAMP, April 17th, 2020

**Company:**

Hunter Douglas Europe B.V.  
Bosscheweg 79  
5735 GT Aarle-Rixtel  
The Netherlands

Phone: +31 492 386 500

Cell: +49 (0)172 247 3371

[www.hunterdouglasfabrics.com](http://www.hunterdouglasfabrics.com)

To: Andre Weiss

Email: [a.weiss@artex.hde.nl](mailto:a.weiss@artex.hde.nl)

**Report # 82/2021: Antiviral Activity of Textile Products**

See below the report of the virucidal test carried out in this laboratory.

**1. Product:**

GREENSCREEN SEA-TEX (Textile)

Date of arrival: February 09th, 2021

- 2. Virus:** Coronavirus strain MHV-3 genus *Betacoronavirus* (same family and genus as SARS-CoV-1, SARS-CoV-2/COVID19, MERS and others).

Virus	Cell line
Coronavirus MHV-3	Cell: L929 -NCTC clone 929 [L cell, L-929, derivative of Strain L] (ATCC® CCL-1™)

**3. Experimental procedure:**

- a) The tests were performed in laboratory NB-2 (Biosafety Level 2) following the Recommendations of ANVISA Art. 1 and Art. 3 of IN 04/13 and IN 12/16 and methodologies described in the standards (ISO 18184/ 2019-06-25: "Textiles — Determination of antiviral activity of textile products, and the Robert Koch Institute - RKI). Dulbecco Minimal Essential Medium (DMEM) containing 2% to 10% fetal bovine serum was used as culture medium for virus and cell line.
- b) The titration of the Coronavirus (MHV) was carried out according to the DICT<sub>50</sub> method (Median Tissue Culture Infectious Dose). Sequential dilutions in base 10 were placed in a 96-well sterile microplate. Cell line L929 was added at a concentration of  $2 \times 10^5$  cells/mL per well. After 48 hours the Cytopathic Effect (CPE) of the viral infection is verified and compared with cell and virus control. The DICT<sub>50</sub>/mL was calculated based on the Reed-Muench method (1938).



Campus-UNICAMP, April 17th, 2020

- a) The fabrics were treated individually according to the methodology and steps indicated in ISO 18148/2019.  
In summary: Fabric sample "GREENSCREEN SEA-TEX Textile" was sterilized by autoclave, cut to 5 cm<sup>2</sup>, each sample received 100DICT<sub>50</sub> of virus and left for different times at room temperature. Then DMEM medium was added in tubes and shaken with the Vortex mixer to remove the virus from the tissue. The suspension was used in the virucidal / antiviral test.
- b) After the 2-hour, samples were collected and treated:
- d.1) All suspension of textile fabrics samples was tested in the cell lines for the "Determination of Maximum Non-Toxic Dose (DMTD)", to define the concentration that does not cause toxicity to the cells.
- d.2) The suspension of the fabric samples plus virus (MHV-3) were pipetted, individually and four repetition, 100µL into 96-well sterile Microplates with different times (until 2 hours) and added 100µL/well with L929 cells (with previously formed monolayer). Then they were incubated at 37°C with 5% CO<sub>2</sub> for 48 hours.
- c) After 48 hours of incubation, the microplates were read through an Inverted Microscope to searching for characteristic Cytopathic Effect of both viruses and the titers were calculated based on the method of Reed and Muench, 1938.  
The results are expressed as a percentage of viral inactivation (Table 1) in compared to untreated viral control (virus titer).

**Summary/Controls:**

- Negative: cell control (2x10<sup>5</sup> cell/mL) in Dulbecco minimal essential medium (DMEM), without virus and fabric samples;
- Virus control: Virus titration (10<sup>1</sup> to 10<sup>12</sup>) and cell culture in Dulbecco minimal essential medium (DMEM) containing 10% fetal bovine serum.
- Positive test: suspension of textile fabrics with presence of virus and cell lines.

**Table 1** - Results are expressed as a percentage of viral inactivation (≥ 99.99%) compared to the untreated viral control:

Log Reduction	Reduction Factor	Percent reduction/virus inactivation
1	10	90%
2	100	99%
3	1000	99.9%
4	10.000	99.99%
5	100.000	99.999%
6	1.000,000	99.9999%

<https://microchemlab.com/information/log-and-percent-reductions-microbiology-and-antimicrobial-testing>

Campus-UNICAMP, April 17th, 2020

#### 4. Results:

4.1. Titer of Coronavírus (MHV-3):  $10^{8,25}$  DICT<sub>50</sub>/mL.

**Tabela 2** - Results of assays with Coronavirus MHV-3 strain in textile with antiviral products and times.

Product	Times	Percent of virus inhibition results (Table 1) against Coronavírus (MHV)	Cytotoxicity Test <i>in vitro</i> results (L929 cell line)
GREENSCREEN SEA-TEX	1 minute	99% inhibition	No toxicity
	5 minutes	99.9% inhibition	No toxicity
	15 minutes	99.9% inhibition	No toxicity
	2 hours	99.9% inhibition	No toxicity

#### 5. Conclusions:

- The product “GREENSCREEN SEA-TEX” inhibited between 99% and 99.9% of the virus tested, therefore, it can be concluded that it is effective for the Coronavirus group.
- The product “GREENSCREEN SEA-TEX” in contact with the cells did not show cytotoxic activity.



Prof. Dr. Clarice Weis-Arns  
Responsible for the Report



Campus-UNICAMP, April 17th, 2020

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