Human Corona Virus Inactivation Test Report

Oxi-Tex
EPA Reg: 87358-1

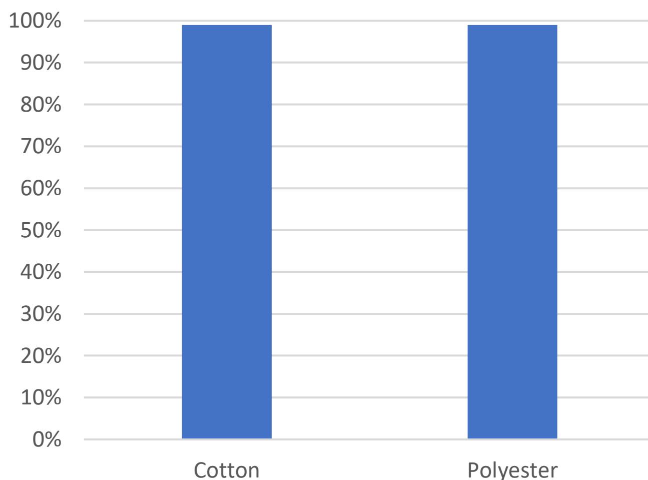
June 18, 2020

For Anti-Viral testing, Oxi-Tex was applied to two fabrics (a cotton and polyester) for initial assessment using ASTM 1053-97 test method. The polyester fabric is a commercially produced Skin Fold Management Textile that is FDA cleared (K121898) and marketed by a medical product distribution partner, while the cotton samples were treated at the lab for the study.

The testing was conducted at BCS Laboratories, Inc in Gainesville, FL per ASTM E 1053-97 against Human Coronavirus (HCoV) OC43 (ATCC #VR-1558). This testing follows procedures that EPA accepts for data package use when registering anti-microbials for inclusion in their Emerging Viral Pathogen List-N of products found effective against SARS-COV-2.

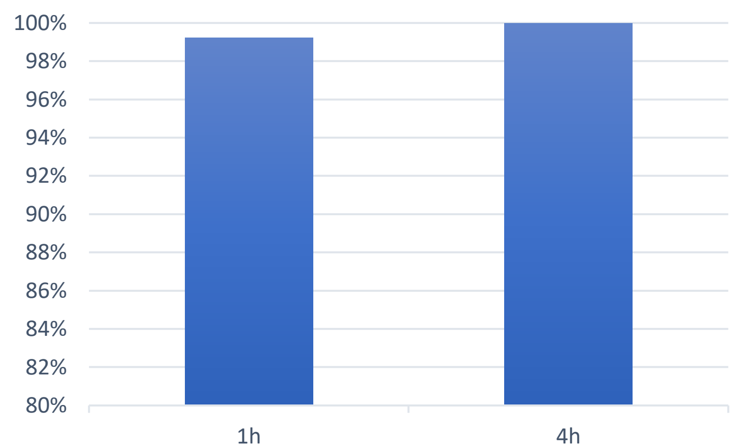
The initial overnight testing found that the virus was fully inactivated by the treated fabrics. Compared to the original amount of active virus applied to the surface, the inactivation percentage is >99.99%. However, the formal inactivation percentage is calculated as >99% compared to parallel controls due to the natural inactivation rate of the virus on the control surface.

The data comparing parallel controls overnight incubation time for virus inactivation is shown below as a chart.



Cotton samples were subsequently tested at shorter test times, specifically at 1h and 4h. The anti-viral efficacy compared to parallel controls is shown in the chart below. At 1 hour, the average inactivation compared to control was 99.2%, and at 4 hour this had increased to 99.99% inactivation.

The data comparing parallel controls for 1-hour and 4-hour incubation times needed for virus inactivation is shown below as a chart.



Anti – Viral Test Method

ASTM E 1053-97

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Inoculation Volume	Date Tested	Application Method
100 uL	5/26/2020	10 x 10 uL droplets and spread onto surface thoroughly

Viral Load added to each replicate of carrier: 1.7 x 10^5 Infectious Units

Enumerated titer of Stock Inoculum: 1.7 x 10^6 Infectious Units/mL

The protocol used is based on to ASTM E 1053-97 (Standard Test Method for Efficacy of Viricidal Agents Intended for Inanimate Surfaces). Briefly, the provided paper material was cut into 25 mm square sections. Two sections of each material (2each representing a replicate) and 2 sections of 100% cotton T-shirt material were each placed onto sterile stainless-steel grid suspended over a water saturated paper towel. The entire set-up was placed into a sealable plastic container. One hundred microliters of the virus stock amended with 2% Heat Inactivated Fetal Bovine Serum was evenly applied to the surface of each material section. The container was sealed and incubated for 17.5 hours at 20-22°C in a biological cabinet. Following, each of the inoculated sections were transferred to a sterile 50 ml conical bottom centrifuge tube (Corning, USA) containing 10 ml sterile D/E Neutralizing Broth. Tubes were placed onto an orbital shaker and agitated at low speed for 15 minutes. After agitation, ten-fold dilutions of the suspensions were performed in PBS. The number of viable (infectious) virus units in the undiluted and diluted samples was determined by the Most Probable Number (MPN) assay procedure. Coronavirus OC43 (ATCC VR-1558) virus was propagated and enumerated using ileocecal colorectal adenocarcinoma HCT-8 cell line (ATCC CCL-244) as the host. Cells were grown in T-25 cell culture plates. For enumeration, virus particles were enumerated as infectious units as per the assay methodology described in EPA/600/R-95/178. Briefly, aliquots of a sample containing the virus were inoculated on freshly prepared monolayers of HCT8 cells (approximately 90% confluence). Each sample volume was inoculated in replicates of 5 and this was conducted at 4 serial tenfold dilution. The cells were incubated in Dulbecco's Modified Eagle's medium (dMEM, Mediatech Inc, USA) media supplemented with 2% Fetal Bovine Serum (FBS, Mediatech, USA) at 35°C and 5% CO_2 for 8 days. Cells were microscopically monitored routinely for signs of degeneration. Cells in wells demonstrating signs of infectivity (Cytopathic effects; CPE) were recorded as positive (+) and those that did not demonstrate any CPE were recorded as negative (-). The most probable number of infectious virus in a sample was then calculated using MPNCALC software (version 0.0.0.23). For Challenge experiments, frozen viral stock (approximately 5 x 107 iu/ml) was thawed rapidly in a 35°C water bath. Positive control, negative control, cytotoxicity checks, and initial titer determination was performed and was in conformance with the anticipated result.