Customer Report

Antiviral Testing

Project ID *0520-DKO-01-1*

Project Initiation Date 6/24/2020

prepared for:

SOLIANI EMC

Alessandro Soliani
Via Varesina 122
Lake Como CO, 22100
+39-3924032101
a.soliani@solianiemc.com

Anitmicrobials ○ Biodegradation ○ Toxicology ○ Analytics ○ Product Development

Table of Contents

Compliance Statement

Testing is conducted according to the required criteria established for ISO 17025 Accredited laboratories. The laboratory is independently audited, verifying this compliance.

This report is governed by and incorporates by reference, the conditions of testing as posted on the date of issuance, and is intended for the identified Project Owners exclusive use. This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted.

ISO 17025 Confidentiality

The lab shall be responsible through legally enforceable commitments for the management of all information obtained during the performance of lab activities.

The Project Owner will be contacted for approval in writing if the laboratory is requested to provide any details regarding the project, or project documentation.

Abstract

The **ISO 18184** test method is designed to measure the antiviral properties of textile samples incubated with the selected virus. The basis of the test method is the incubation of the virus inoculum in contact with the test sample for a duration of 2 hours without drying of the inoculum. Following this exposure, the inoculated virus are recovered, and the concentration of the viable virus is determined. The antiviral performance is determined by comparison of the recovered virus from the untreated material and treated material after the incubation period, which is also corrected for any overlapping effects of viral loss due to the sample recovery or effects of the required neutralizers.

The antiviral performance is reported as both the Log10 and % Reduction relative to the untreated control sample and other controls for the test method.

Results and Discussion

Results are provided in the Result Data Tables

Test Results are provided in the data tables section, followed by a detailed listing of raw data in the report addendum.

Report Result Tables

Sample List

Sample #	Method Name	Sample Name	Sample Notes
Project - Images			
1		80.10052-00 99% PURE SILVER FABRIC	
2		18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED	
ISO 18184 - Textiles - Determination of Antiviral Activity of Textile Products			
1		80.10052-00 99% PURE SILVER FABRIC	
2		18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED	
3		Untreated Control	

Test Method **ISO 18184 - Textiles - Determination of Antiviral Activity of Textile Products**

Sample # 1 **80.10052-00 99% PURE SILVER FABRIC**

	Interval	Result
Inoculum: *Influenza A virus (H1N1)*		
Notes Section		
percent reduction > = 99.99	**2** hr	**5.8** Mv (antiviral activity)
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section		
percent reduction = 99.99	**2** hr	**4** Mv (antiviral activity)

Sample # 2 **18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED**

	Interval	Result
Inoculum: *Influenza A virus (H1N1)*		
Notes Section		
percent reduction = 99.99	**2** hr	**4** Mv (antiviral activity)
percent reduction > = 99.99	**2** hr	**5.2** Mv (antiviral activity)
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section		
percent reduction = 99.99	**2** hr	**4** Mv (antiviral activity)

Sample # 3 **Untreated Control**

	Interval	Result
Inoculum: *Influenza A virus (H1N1)*		
Notes Section		
	0 hr	**6.3** TCID(50) viral count/ml
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section		
	0 hr	**5.5** TCID(50) viral count/ml

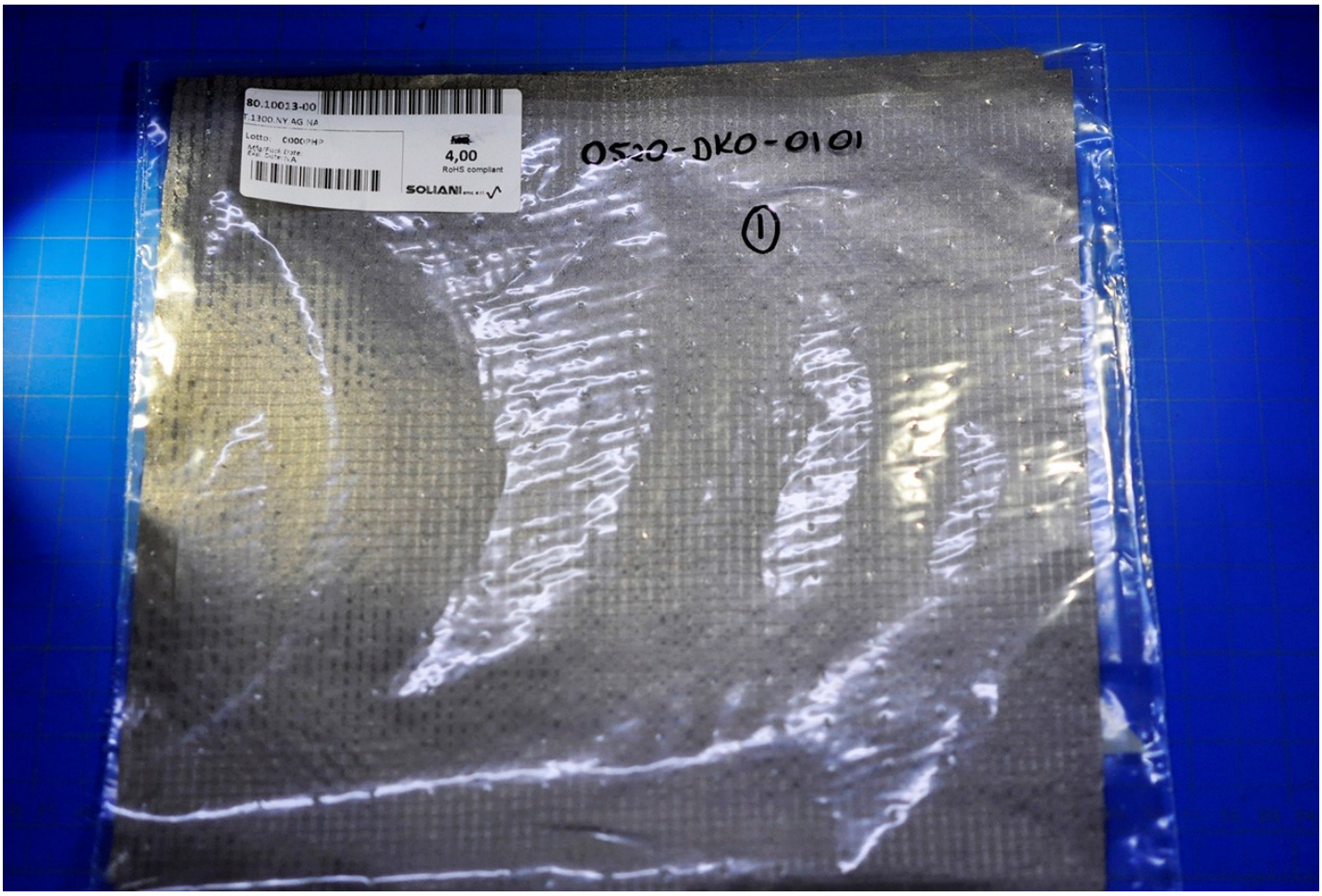
Report Image

Sample # 1 **80.10052-00 99% PURE SILVER FABRIC**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



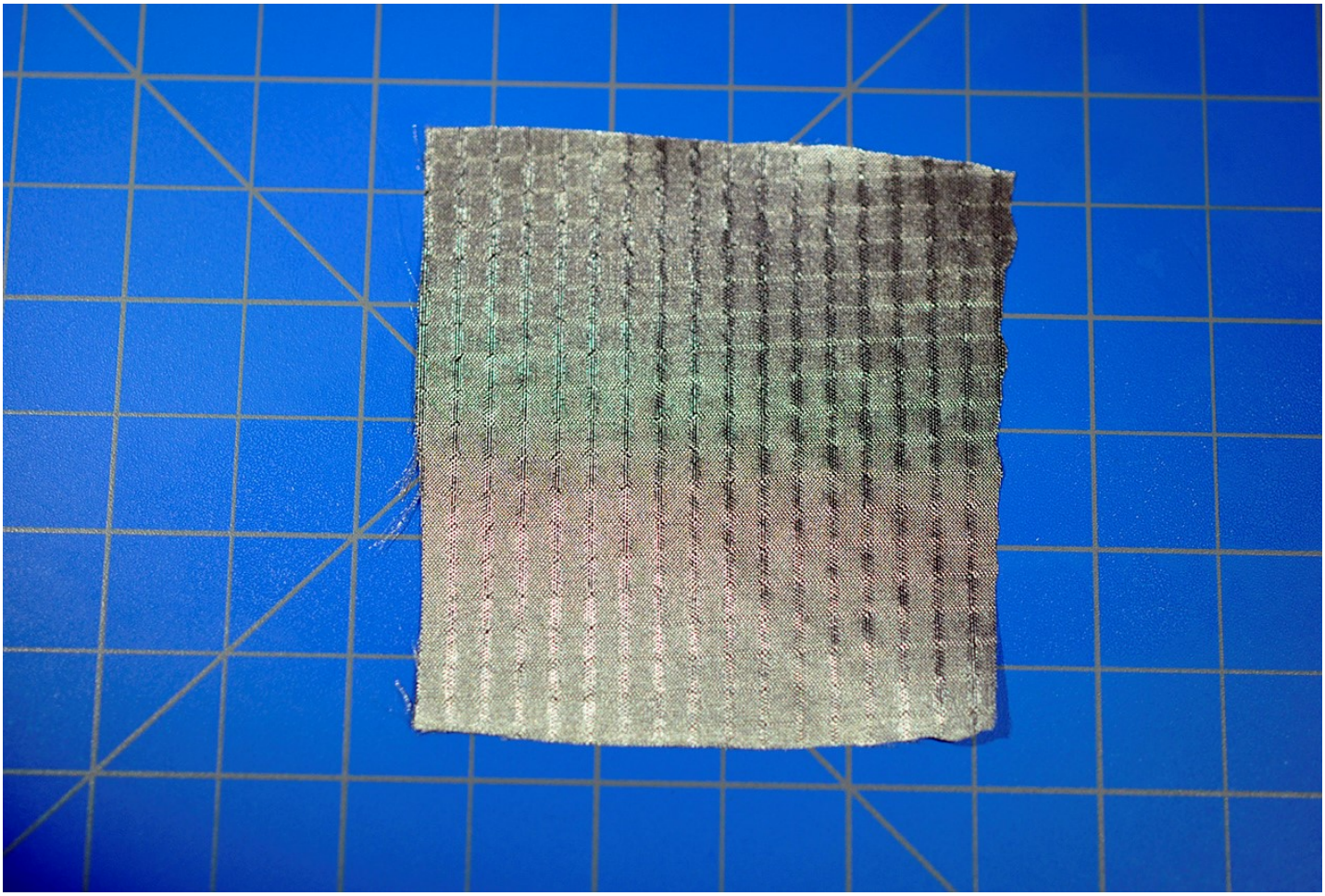
Report Image

Sample # 1 **80.10052-00 99% PURE SILVER FABRIC**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



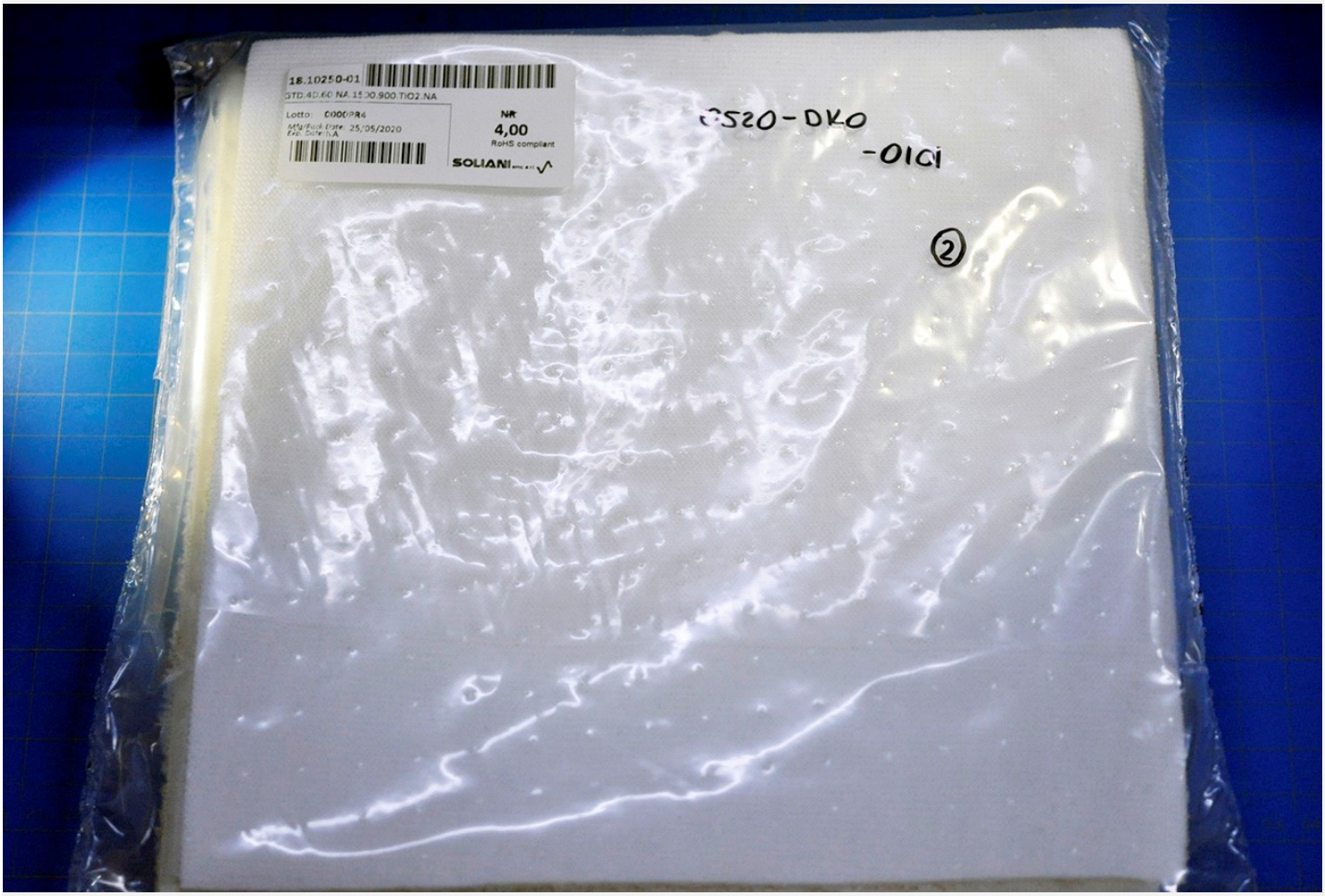
Report Image

Sample # 2 **18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



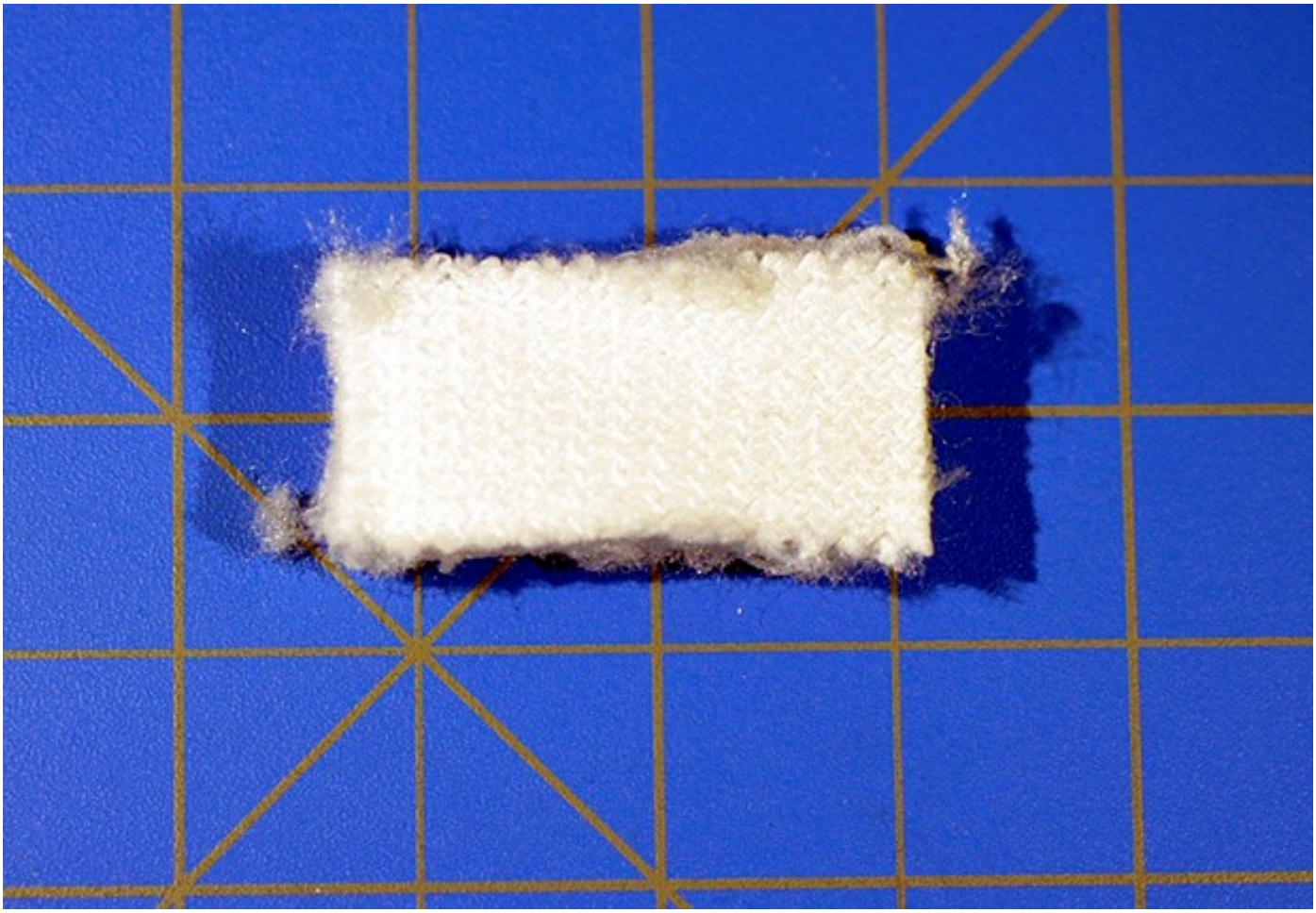
Report Image

Sample # 2 **18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED**

Test Method **Project - Images**

Inoculum ***None***

Image: **sample** ***Timepoint:*** **time - 0**



Introduction

ISO 18184 specifies a method of evaluating the antiviral activity of textile products. It is a method commonly used for evaluating the antimicrobial properties of many material types that can range from coated surfaces or those of monolithic composition.

Unless otherwise specified, secondary effects of antiviral treatments, the measured antiviral performance, or the durability of a measured activity are not covered by the standard. The standard is not intended to be used or referenced as a method to document or claim antiviral performance unless indicated by the test report. The determinations of product performance within a given environment can vary dramatically, and must be specifically documented and then determined within the context of a specific project plan.

Recommended Reading **Online Technical Resources**

Guidance on antimicrobial preservation http://www.situbiosciences.com/microbial-control-testing/

Antimicrobial testing with textiles http://www.situbiosciences.com/textile-testing-antimicrobials/

Method Summary https://www.situbiosciences.com/product/iso-18184-antiviral-activity-of-textiles/

Methods

Project - Images

Project images are provided for the submitted test samples. Images are taken for the samples as received to provide a reference for the materials submitted for testing. The provided images may or may not indicate other aspects of the sample condition but no analysis or inspection of the sample is conducted unless otherwise specifically noted in the project report.

ISO 18184 - Textiles - Determination of Antiviral Activity of Textile Products

The ISO 18184 method is used to evaluate the virucidal efficacy of a textile product. Testing can incorporate different exposure times, soiling, and virus types and other variables according to the test standard or specific needs of a product. The most common test conditions employ the standard method protocol requiring a 2-hour exposure to the test substance depending on the intended use of the product. The test virus is prepared in advance of the testing. A viral titer is determined, and the virus is then utilized as the inocula for the test material.

Sample List ***(test sample notes)***

1	80.10052-00 99% PURE SILVER FABRIC
2	18.10250-01 GTD.4D.60.NA.1500.900.TIO2.NA + UVC-LIGHT SUPPLIED
3	Untreated Control

Testing

Viral Inoculum Preparation

Host cells are grown from frozen stock or existing cultures with a pass number of less than 20. The cultures are grown in 70 or 150 sq cm size flasks, in a media generally defined as Dubeccor Minimum Essential Media with antibiotic and fetal bovine serum as needed for the specific cell line being cultured. Once the desired flask cell density is achieved, stock virus cultures are used to inoculate the cell flask. The virus infection is monitored by observable cytotoxic effects (CTE) and then harvested once the relative percent of cells affected by CTE is approximately 90% or higher typically 2 to 7 days. Once the virus culture is harvested, the virus infectivity titer is determined to establish the viral concentration.

Inoculation of test specimens

Test samples are prepared by cutting them into 0.4 g pieces (unless otherwise specified) and laying them out onto individual Petri dishes. 0.2 ml The surface to be tested is the exposed outer surface of the product. Unless otherwise specified, all test samples are autoclaved to sterilize them before testing. The test samples are inoculated with 0.2 ml of the viral inoculum, at several points on the sample surface, hydrophobic material may be buffed with the pipet tip to allow the inoculum to penetrate into the sample material

Incubation of the inoculated test specimens

The standard procedure for incubation of the inoculated specimens is to incubate the Petri dishes containing the inoculated test specimens at a temperature of 25 (+/- 1) C for 2 h (and not greater than 24 hrs), unless otherwise noted.

Recovery of virus from test specimens.

Two time points are created for each test item, a washout of the inoculated sample is collected immediately after inoculation by addition of the selected neutralizer solution by placing the sample into a vial and adding 20 ml of the neutralizer, followed by vortexing.

A second recovery is created following the intended incubation time (2 hr), after which the sample is placed into a vial with 20 ml of the neutralizing solution and vortexed.

Following the test sample neutralization, aliquotes of the sample are recovered and used to determine the infective titer following the respective incubation periods.

Reagents

Dulbecco's Modified Eagle Medium (DMEM; EM-1)
Soybean Casein Lecithin Polysorbate 80 Medium (SCDLP)
Phosphate Buffered Saline (PBS)
Formaldehyde solution (3.7%)

Crystal Violet (0.5%)
Fetal bovine serum
Viral Maintenance medium
Typsin
Ethylenediaminetetraacetic acid solution (EDTA)
Laboratory RO water, deionized

Equipment List

Thermo Orbital Shaker Incubator
Scales (Mettler H80, Mettler PM-11K, Mettler MS104S/03)
Nuaire BSL 2 cabinet
Nuaire water-jacketed incubator
Nikon inverted microscope
Vortex mixer
Centrifuge
Liquid Nitrogen Dewar
MarketForge Autoclave
Hach pH Meter / O2 measure / conductivity meter
Dwyer Hygrometer
Gilson Pipettes

Test Organisms (by Method)	***(Inventory ID / lot #)***
ISO 18184 - Textiles - Determination of Antiviral Activity of Textile Products	
Influenza A virus (H1N1)	VR-1469 70020665
Feline Calicivirus (F-9) *(CCL-94 Eu cell Host)*	VR-782 70027298

Sample Preparations

Each test substance was prepared according to the analytic method requirements.
Each test sample is prepared in triplicate for each time point.
As available, the samples are cut into a piece (0.4 g) approximately 50 x 50 mm. Sample variability is accommodated as needed for the standard test; notes regarding differences in the sample characteristic are recorded in the report summary.

Ideally, the test sample will be flat and non-hydrophobic and allow layering of the inoculum over the sample surface.

The test setup was modified for Sample 2 to all exposure of the sample to a supplied UV-LED light.

The UV light was attached to a plastic backboard, with a spacing of approximately 5 cm between each light strip.
The sample was exposed for the duration of testing at a distance of 5 cm from the face of the LED strips.

Sampling Procedure

Unless specified, samples will be cut from the provided pieces or used as received if precut.

Unless specified, the sample provided is treated as uniform, and monolithic for the purposes of testing, selective cutting, sidedness or other types of potential sample heterogeneity are disregarded unless instructions for sample handling are provided and noted in the test report.

Calculations

End-point dilutions are conducted with the recovered virus inocula using serial log10 dilution factors. TCID50 (Spearman-Karber; modified by M. A. Ramakrishnan) is used to determine the concentration of the inoculated virus based on the outcome of the end-point dilution resulting in the CTE of the host cells. It represents the end-point dilution (average) of the host cell monolayers exhibiting the CTE.

Log10 50% end-point Dilution = - [(total number of CTE wells / total number of dilution replicates) + 0.5] x log dilution factor

R = - [Total CTE / replicate count per dilution) + 0.5] x Log dilution factor

R = The log 50% end-point dilution

Total CTE - is the average of the common logarithm of the number of viable bacteria, in cells/cm2, recovered from the untreated test specimens immediately after inoculation;

Replicate count per dilution - the numbers of well replicates inoculated at each dilution

Log dilution factor - is the dilution factor used for each serial dilution (typically 10x or log10(10) = 1)

Antiviral Activity Value

Mv = lg(Va/Vc) = lg(Va) - lg(Vc)

Mv = the antiviral activity value
lg(Va) = the common logarithm average of 3 infectivity titer values immediately after inoculation of the control sample
lg(Vc) = the common logarithm average of 3 infectivity titer values after the contact time (2 hr) with the antiviral sample

Statistical Methods

Replicate data are utilized in the calculation by the Spearman-Karber method, no additional statistical analysis is conducted.

* This report is governed by and incorporates by reference the conditions of testing as posted on the date of issuance and is intended for the identified Project Owners exclusive use. Any copying or replication of this report to or for any other person or entity, or use of our company name or Service Mark is permitted only with our prior written consent. All images supplied as part of the report are provided as test result edification only and are the sole property of Situ Biosciences LLC and are copyright protected. Any exemption to the copyright of the report or images provided will be explicitly noted in this report.

This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted. Our report includes all tests requested and the results thereof based upon the information provided. Written notification within 60 days from the date of issuance of this report is required to address any material error or omission caused by the handling of the samples. Any such notification shall specifically address the issues related to the test samples supplied and testing conducted as provided in this report. A failure to raise such an issue within the prescribed time shall constitute the unqualified acceptance of the completeness of this report, the testing conducted, and the correctness of the report contents.

d.p. satchell, Ph.D.

Manager
Situ Biosciences LLC