Customer Report

Antiviral Testing

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prepared for:

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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.

Abstract

The ISO 21702 provides a test method for the quantitative evaluation of virucidal activity on plastics and other non-porous surfaces. Products tested are intended to be treated antiviral products, that are tested against the specified virus.
The basis of the test method is the incubation of the viral inoculum in contact with the test sample for a duration of 24 hours without drying of the inoculum. Following this exposure, the inoculated virus is recovered, and the concentration of the infective virus is determined. The antiviral performance is determined by a comparison of the recovered virus from the untreated material and treated material after the 24-hour incubation.

The antimicrobial performance is reported as both the Log10 and % Reduction relative to the untreated control sample.

Results and Discussion

Results are provided in the Result Data Tables and Addendum

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		WITH PERMABLOK3	
ISO 21702 - Measurement of antiviral activity on plastics and other non-porous surfaces			
1		WITH PERMABLOK3	
2		SBSC Untreated Control	

Test Method **ISO 21702 - Measurement of antiviral activity on plastics and other non-porous surfaces**

Sample # 1 **WITH PERMABLOK3**

	Interval	Result
Inoculum: *Feline Coronavirus*		
Notes Section		
percent reduction = 99.97; cytotoxic effect noted at T0	**24** hr	**3.5**

Sample # 2 **SBSC Untreated Control**

	Interval	Result
Inoculum: *Feline Coronavirus*		
Notes Section		
	24 hr	**195000** TCID50 / sq cm
	0 hr	**6150000** TCID50 / sq cm

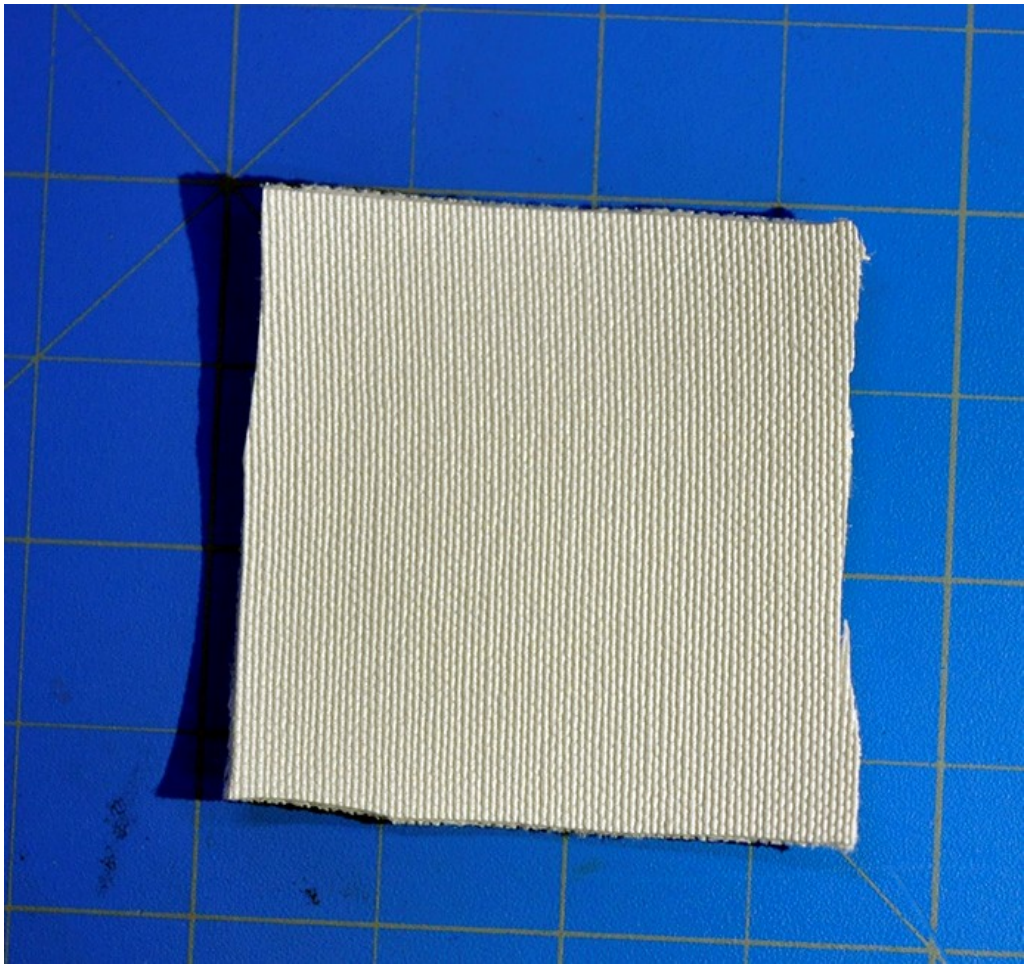
Report Image

Sample # 1 WITH PERMABLOK3

Test Method **Project - Images**

Inoculum ***None***

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



Introduction

ISO 21702 specifies a method of evaluating virucidal activity of non-porous surfaces.

Each product was tested using clean test condition (no additional soil) .

Unless otherwise specified, secondary effects of antibacterial treatments, the measured antimicrobial 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 antimicrbial 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/

Method Summary https://www.situbiosciences.com/product/iso-21702-antiviral-activity-on-plastics-and-other-non-porous-surfaces/

Sample Appearance

Each test sample was submitted as an approximately 2x2" flat surface. Each sample was covered with a paraffin film 2.5x2.5 cm square.

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 21702 - Measurement of antiviral activity on plastics and other non-porous surfaces

The ISO 21702 method is used to evaluate the virucidal efficacy of a non-porous 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 24-hour exposure to the test material depending on the intended use of the product. Test virus are prepared in advance of the testing followed by a determination of viral titer. The inocula created is then utilized as the inocula for the exposure of the test material to the virus.

Project List	*(notes)*
1	WITH PERMABLOK3
2	SBSC Untreated Control

Testing

Inoculum Preparation

A known viral titer suspension is prepared to a concentration of at least 1E6 TCID50 / ml. Passaged of the virus are not used beyond ten passes from the original seed culture.

Experimental Conditions

Following inoculation, the samples are incubated at 25 C +/- 1C (unless otherwise specified). The incubation is conducted to prevent the inoculum from drying while in contact with the test surface. Following the incubation period, the virus is recovered in neutralizing media and then diluted for culturing.

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 10 ml of the neutralizer, followed by vortexing.

A second recovery is created following the intended incubation time (24 hr), after which the sample is placed into a vial with 10 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 21702 - Measurement of antiviral activity on plastics and other non-porous surfaces

Feline Coronavirus, strain Munich (CCL-94 Eu cell Host)	FIPV

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 approximately 50x50 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.

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

R= U(t24)-C(t24)

R = the antiviral activity value
C(t24) = the common logarithm average of 3 infectivity titer values after 24 hours from the untreated material
U(t24) = the common logarithm average of 3 infectivity titer values after the contact time (24 hr) with the treated (test) sample

Statistical Methods

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

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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

			Spearman-Karber Method (Modified, Ramakrishnan MA)			
	Titer	**Virus**	**Control (T0)**	**Control (Tx)**	**Sample (T0)**	**Sample (Tx)**
	replicate count	6	6	6	6	6
	dilution factor [D]	10	10	10	10	10
	washout volume (ml) [V]	20	20	20	20	20
	sample area (sq cm) [A]	6.5	6.5	6.5	6.5	6.5
	inoculation volume (ml)	0.2	0.2	0.2	0.2	0.2
	dilution factor (df)	***(+)***	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	T	T	T	T	T
	10^-1	6	6	6	T	0
	10^-2	6	6	6	0	0
	10^-3	6	6	4	0	0
	10^-4	6	6	4	0	0
	10^-5	4	6	1	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0
Infectivity Titer (N)	**Log10(TCID50/ well)**	**Alog(TCID50/well) [C]**	**TCID50/sq cm [10*C*D*V]/A**			
Virus	4.7	4.6E+04	**2.86E+06**			
Control (T0)	5.0	1.0E+05	**6.15E+06**			
Control (Tx)	3.5	3.2E+03	**1.95E+05**			
Sample (T0)	0.0	1.0E+00	**6.15E+01**			
Sample (Tx)	0.0	1.0E+00	**6.15E+01**			
Result		**Log10**	**Percent**			
Reduction	***R= U(t24)-C(t24)***	**3.5**	**99.968**			
Control (T0) variability	**(Lmax - Lmin)/Lmean**	**0.20**				