Customer Report

Non-Porous Surface Cleaning Following Virus Inoculation

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prepared for:

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Anitmicrobials ○ Biodegradation ○ Toxicology ○ Analytics ○ Product Development

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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 test method was provided to test the viral removal from inoculated ceramic tile surfaces. Glazed, ceramic tile was used as the substrate for the test surfaces. Glass slides were used for virus titer controls. Testing was conducted with three samples, two coatings (which were respectively used for coating the ceramic tile), and one cleaning product. Tile coating and cleaning were conducted following the manufacturer's instructions.

Two test procedures were followed.
For uncoated ceramic tile surfaces, the tiles were inoculated with virus, followed by the cleaning cycle. Following cleaning, the surface was swabbed for recovery of virus.

For coated surfaces, the virus was inoculated on the surface and allowed to dwell for 2 hours (without drying). Then cleaned with the cleaning product, and then the surface was swabbed for recovery of virus.

The manufacturer provided the cleaning test procedure as a component of the project, and the ASTM E1053 was followed for culturing of the virus before and after recovery from the test surfaces.

The change is infective-virus counts is reported as both the Log10 and % Reduction relative to the infective-virus counts recovered from the untreated control tile substrate.

Introduction
Cleaning product testing was conducted to measure the recovered residual virus from a test surface following two cleaning schemes.

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 Study Sponsor will be contact for approval in writing if the laboratory intends to make publicly available any information about an assignment.

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.

Standard Practice to Assess Viral titer following a cleaning proceedure of a non-porous test surface.

The method is used to evaluate the recovered viral titer following the cleaning of non-porous surfaces. Testing can incorporate different exposure times, soiling, and virus types and other variables according to the specific needs of a product. The most common test conditions employ exposure of the virus to the test surface, followed by the application of a cleaning product and then recovery of the virus by neutralization of the surface and swabbing. The procedures for the cleaning process are provided for a given product and conducted according to the manufacturers' instructions. Recovery of the virus, determination of titer, and culturing of the required cell lines follow established microbiological procedures. Removal of virus is determined by the measurement of control (uncleaned surfaces) and is taken as the difference between the titer determined for surface that have been cleaned and those that have not been cleaned.

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

1	6600 Multi-Surface Ceramic Coating + Multi Surface Clean Degreaser Sanitizer	coat 2x and allow to dry and cure for 24 hrs
2	7700 High Shine Ceramic Floor Coating + Multi Surface Clean Degreaser Sanitizer	coat 4x and allow to cure for 24 hrs
3	Multi Surface Clean Degreaser Sanitizer	after inoculation, spray and wipe, then spray and wait 10 min then wipe, recover from this surface (according to instructions)
4	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 Dulbecco's Modified Eagle Medium 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% typically 2 to 7 days. Once the virus culture is harvested, the virus infectivity titer is determined.

Inoculation of test specimens

The test samples are inoculated with 0.2 ml of the viral inoculum on the sample surface, in an area of approximately 1 sq inch. This inoculum was then swabbed out to cover the surface using a small sterile cell harvester.

Incubation of the inoculated test specimens

The procedure for incubation of the inoculated specimens is to incubate the Petri dishes containing the inoculated test specimens at ambient laboratory conditions.

Recovery of virus from test specimens.

Following the designated time point, the samples were wash according to the manufacturers instructions as follows:

Uncoated Surfaces

Spray the cleaning product (Sample 3) on to the tile surface to fully wet-out and then wipe with a microfiber cloth using a side-to-side and then up-down motion. A second spray with the cleaning solution was applied and allowed to dwell for 10 minutes, followed by the same wiping action using a clean cloth.

The surface of the test substrate was then neutralized by swabbing with a neutralizer soaked cotton swab (3 times) with purging of the swab into 5 ml of neutralizer solution. The 5 ml of neutralizer solution constituted the recovered virus.

Coated Surfaces

Ceramic tile was coated with Sample 1 and Sample 2 (respectively).

The products were applied to a microfiber cloth (provided) and coated with a circular wiping action with visual confirmation that the coating was evenly applied.

Sample 1, the coating was applied 2 times with drying between each and then followed by a 24 h final drying step.

Sample 2, the coating was applied 4 times with drying between each and then followed by a 24 h final drying step.

Before inoculation, spray cleaning with Sample 3 was conducted on to the coated surfaces in a manner to thoroughly wet the surface and then wipe with a microfiber cloth as described and then allowed to dry.

The surface was then inoculated and allowed to sit for 2 hrs, followed by an application of the spray cleaner, a 1 min dwell, and then a final cleaning with the spray cleaner.

The virus was then recovered, as indicated previously by swabbing with the cotton swab.

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 #)***

Standard Practice to Assess Viral titer following a cleaning proceedure of a non-porous test surface.

Feline infectious peritonitis virus FIPV-1146 , P20 (CCL-94 Eu cell Host)	VR-2126 214999
Feline Calicivirus (F-9) (CCL-94 Eu cell Host)	VR-782 70027298

Sample Preparations

Each sample was provided as a concentrate.
Samples 1 and 2 were mixed 1 part with 4 parts water.
Sample 3, used the provided small scoop and was mixed by adding 2 small scoops (approximately 20 g) into 1 gallon of hot water.

Each sample was mixed until clear and homogenous and then used.

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)

Changes to the recovered viral titer following cleaning

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

Mv = the change in recovered viral titer
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 indicated contact time and or cleaning proceedure.

Statistical Methods

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

Results and Discussion

Results are provided in the Result Data Tables

The data summary provides the test result for the test samples and control sample treated equivalently but without a coating or cleaning procedure.

The cleaning method evaluated the recovered infective virus from tile surfaces (test and control). For the control surface, the cleaning method applied to the control surface was conducted in an identical manner with the exception that no coating, wiping, or cleaning product was applied to the surface following inoculation of the virus.

From the cleaning method conducted, the recovered viral counts following cleaning are recorded as the reduction of recovered infective-viral counts relative to the Control Surface (Sample 4) recovered infective-viral counts:

Sample 1 following cleaning:
FIPV-1146, P20 (VR-2126) (coronavirus) = 99.79%
Feline Calicivirus (F-9) (norovirus surrogate) = 99.54%

Sample 2 following cleaning:
FIPV-1146, P20 (VR-2126) (coronavirus) = 99.53%
Feline Calicivirus (F-9) (norovirus surrogate) = 99.0%

Sample 3 following cleaning:
FIPV-1146, P20 (VR-2126) (coronavirus) = 99.94%
Feline Calicivirus (F-9) (norovirus surrogate) = 99.74%

rev. 1; revised data tables and result summary

Report Result Tables

Sample List

Sample # / Method Name	Sample Name	Sample Notes
Project - Images		
1	6600 Multi-Surface Ceramic Coating + Multi Surface Clean Degreaser Sanitizer	
2	7700 High Shine Ceramic Floor Coating + Multi Surface Clean Degreaser Sanitizer	
3	Multi Surface Clean Degreaser Sanitizer	
Standard Practice to Assess Viral titer following a cleaning proceedure of a non-porous test surface.		
1	6600 Multi-Surface Ceramic Coating + Multi Surface Clean Degreaser Sanitizer	coat 2x and allow to dry and cure for 24 hrs
2	7700 High Shine Ceramic Floor Coating + Multi Surface Clean Degreaser Sanitizer	coat 4x and allow to cure for 24 hrs
3	Multi Surface Clean Degreaser Sanitizer	after inoculation, spray and wipe, then spray and wait 10 min then wipe, recover from this surface (according to instructions)
4	Untreated Control	

Test Method **Standard Practice to Assess Viral titer following a cleaning proceedure of a non-porous test surface.**

Sample # 1 **6600 Multi-Surface Ceramic Coating + Multi Surface Clean Degreaser Sanitizer**

	Interval	Result
Inoculum: *FIPV-1146, P20 (VR-2126)*		
Notes Section (coronavirus); Percent Reduction in Viral Count = 99.79	**2** hrs (with 2 cleaning cycles)	**2.7** Log Reduction in Viral Count
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section (norovirus surrogate); Percent Reduction in Viral Count = 99.54	**2** hrs (with 2 cleaning cycles)	**2.3** Log Reduction in Viral Count

Sample # 2 **7700 High Shine Ceramic Floor Coating + Multi Surface Clean Degreaser Sanitizer**

	Interval	Result
Inoculum: *FIPV-1146, P20 (VR-2126)*		
Notes Section (coronavirus); Percent Reduction in Viral Count = 99.53	**2** hrs (with 2 cleaning cycles)	**2.3** Log Reduction in Viral Count
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section (norovirus surrogate); Percent Reduction in Viral Count = 99.0	**2** hrs (with 2 cleaning cycles)	**2** Log Reduction in Viral Count

Sample # 3 **Multi Surface Clean Degreaser Sanitizer**

	Interval	Result
Inoculum: *FIPV-1146, P20 (VR-2126)*		
Notes Section (coronavirus); Percent Reduction in Viral Count = 99.94	**10** m (with 2 cleaning cycles)	**3.3** Log Reduction in Viral Count
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section (norovirus surrogate); Percent Reduction in Viral Count = 99.74	**10** m (with 2 cleaning cycles)	**2.6** Log Reduction in Viral Count

Sample # 4 **Untreated Control**

	Interval	Result
Inoculum: *FIPV-1146, P20 (VR-2126)*		
Notes Section (coronavirus)		**32000** TCID50 / ml
Inoculum: *Feline Calicivirus (F-9)*		
Notes Section (norovirus surrogate)		**1500000** TCID50 / ml

Report Image

Sample # 1 **6600 Multi-Surface Ceramic Coating + Multi Surface Clean Degreaser Sanitizer**

Test Method **Project - Images**

Inoculum ***None***

Image: **Sample** ***Timepoint:*** **time - 0**

Report Image

Sample # 2 **7700 High Shine Ceramic Floor Coating + Multi Surface Clean Degreaser Sanitizer**

Test Method **Project - Images**

Inoculum ***None***

Image: **Sample** ***Timepoint:*** **time - 0**

Report Image

Sample # 3 **Multi Surface Clean Degreaser Sanitizer**

Test Method **Project - Images**

Inoculum ***None***

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

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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)	Sample (Tx)	Cytotoxicity	Neutralization
FIPV VR-1146	replicate count	6	3	3	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	5	5	5	5	5
Sample						
1	dilution factor (df)	***(+)****	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	3	0	0	0
	10^-3	5	3	0	0	0
	10^-4	1	2	0	0	0
	10^-5	0	0	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	**Log10(TCID50/0.1 ml)**	**Log10(TCID50/ml)**	**Alog(TCID50/ml)**	**TCID50 / vial**
Virus	4.0	**4.5**	3.2E+04	1.6E+05
Control (T0)	2.7	**3.2**	1.5E+03	7.3E+03
Sample	0.0	**0.5**	3.2E+00	1.6E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	1.6E+01
Neutralization	0.0	**0.5**	3.2E+00	1.6E+01

	Log10 Reduction	**Percent Reduction**
Recovered viral counts (to Control T0)	**2.7**	**99.785**
Recovered viral counts (to inoculum)	**4.0**	**99.990**

Legend

(+) = **CTE measured (active virus)** **T =** **non-viral CTE**

0 = **no CTE measured** **(Tx) =** **see data table for sample time point**

Cytotoxicity measure of CTE due to the sample extracted without virus

Neutralization measure of the CTE due to the neutralizer used

Spearman-Karber Method (Modified, Ramakrishnan MA)

	Titer	Virus	Control (T0)	Sample (Tx)	Cytotoxicity	Neutralization
FC-F9	replicate count	6	3	3	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	5	5	5	5	5
Sample						
1	dilution factor (df)	***(+)****	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	3	0	0	0
	10^-3	6	3	0	0	0
	10^-4	6	1	0	0	0
	10^-5	4	0	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	**Log10(TCID50/0.1 ml)**	**Log10(TCID50/ml)**	**Alog(TCID50/ml)**	**TCID50 / vial**
Virus	5.7	**6.2**	1.5E+06	7.3E+06
Control (T0)	2.3	**2.8**	6.8E+02	3.4E+03
Sample	0.0	**0.5**	3.2E+00	1.6E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	1.6E+01
Neutralization	0.0	**0.5**	3.2E+00	1.6E+01

	Log10 Reduction	**Percent Reduction**
Recovered viral counts (to Control T0)	**2.3**	**99.536**
Recovered viral counts (to inoculum)	**5.7**	**100.000**

Legend

(+) = **CTE measured (active virus)** **T =** **non-viral CTE**

0 = **no CTE measured** **(Tx) =** **see data table for sample time point**

Cytotoxicity measure of CTE due to the sample extracted without virus
Neutralization measure of the CTE due to the neutralizer used

Spearman-Karber Method (Modified, Ramakrishnan MA)

	Titer	Virus	Control (T0)	Sample (Tx)	Cytotoxicity	Neutralization
FIPV VR-1146	replicate count	6	3	3	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	5	5	5	5	5
Sample 2	dilution factor (df)	***(+)***	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	3	0	0	0
	10^-3	6	3	0	0	0
	10^-4	4	1	0	0	0
	10^-5	2	0	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	**Log10(TCID50/0.1 ml)**	**Log10(TCID50/ml)**	**Alog(TCID50/ml)**	**TCID50 / vial**
Virus	5.0	**5.5**	3.2E+05	1.6E+06
Control (T0)	2.3	**2.8**	6.8E+02	3.4E+03
Sample	0.0	**0.5**	3.2E+00	1.6E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	1.6E+01
Neutralization	0.0	**0.5**	3.2E+00	1.6E+01

	Log10 Reduction	**Percent Reduction**
Recovered viral counts (to Control T0)	**2.3**	**99.536**
Recovered viral counts (to inoculum)	**5.0**	**99.999**

Legend

(+) = **CTE measured (active virus)** **T =** **non-viral CTE**

0 = **no CTE measured** **(Tx) =** **see data table for sample time point**

Cytotoxicity measure of CTE due to the sample extracted without virus
Neutralization measure of the CTE due to the neutralizer used

Spearman-Karber Method (Modified, Ramakrishnan MA)

	Titer	Virus	Control (T0)	Sample (Tx)	Cytotoxicity	Neutralization
FC-F9	replicate count	6	3	3	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	5	5	5	5	5
Sample 2	dilution factor (df)	***(+)***	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	3	0	0	0
	10^-3	6	3	0	0	0
	10^-4	4	0	0	0	0
	10^-5	2	0	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	**Log10(TCID50/0.1 ml)**	**Log10(TCID50/ml)**	**Alog(TCID50/ml)**	**TCID50 / vial**
Virus	5.0	**5.5**	3.2E+05	1.6E+06
Control (T0)	2.0	**2.5**	3.2E+02	1.6E+03
Sample	0.0	**0.5**	3.2E+00	1.6E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	1.6E+01
Neutralization	0.0	**0.5**	3.2E+00	1.6E+01

	Log10 Reduction	**Percent Reduction**
Recovered viral counts (to Control T0)	**2.0**	**99.000**
Recovered viral counts (to inoculum)	**5.0**	**99.999**

Legend

(+) = **CTE measured (active virus)** **T =** **non-viral CTE**

0 = **no CTE measured** **(Tx) =** **see data table for sample time point**

Cytotoxicity measure of CTE due to the sample extracted without virus
Neutralization measure of the CTE due to the neutralizer used

Spearman-Karber Method (Modified, Ramakrishnan MA)

Titer		Virus	Control (T0)	Sample (Tx)	Cytotoxicity	Neutralization
FIPV VR-1146	replicate count	6	12	12	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	20	20	20	20	20
Sample						
3	dilution factor (df)	***(+)****	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	12	0	0	0
	10^-3	5	12	0	0	0
	10^-4	1	7	0	0	0
	10^-5	0	0	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	Log10(TCID50/0.1 ml)	Log10(TCID50/ml)	Alog(TCID50/ml)	TCID50 / vial
Virus	4.0	**4.5**	3.2E+04	6.3E+05
Control (T0)	2.6	**3.1**	1.2E+03	2.4E+04
Sample	0.0	**0.5**	3.2E+00	6.3E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	6.3E+01
Neutralization	0.0	**0.5**	3.2E+00	6.3E+01

	Log10 Reduction	Percent Reduction
Recovered viral counts (to Control T0)	**2.6**	**99.739**
Recovered viral counts (to inoculum)	**4.0**	**99.990**

Legend

(+) = CTE measured (active virus) **T =** non-viral CTE

0 = no CTE measured **(Tx) =** see data table for sample time point

Cytotoxicity measure of CTE due to the sample extracted without virus

Neutralization measure of the CTE due to the neutralizer used

Spearman-Karber Method (Modified, Ramakrishnan MA)

	Titer	Virus	Control (T0)	Sample (Tx)	Cytotoxicity	Neutralization
FC-F9	replicate count	6	12	12	3	6
	dilution factor	10	10	10	10	10
	washout volume (ml)	20	20	20	20	20
Sample						
3	dilution factor (df)	***(+)****	***(+)***	***(+)***	***(+)***	***(+)***
	10^0	6	T	T	T	T
	10^-1	6	T	T	T	T
	10^-2	6	12	0	0	0
	10^-3	6	12	0	0	0
	10^-4	6	12	0	0	0
	10^-5	4	3	0	0	0
	10^-6	0	0	0	0	0
	10^-7	0	0	0	0	0
	Cell Blank	0	0	0	0	0

Titer Values	**Log10(TCID50/0.1 ml)**	**Log10(TCID50/ml)**	**Alog(TCID50/ml)**	**TCID50 / vial**
Virus	5.7	**6.2**	1.5E+06	2.9E+07
Control (T0)	3.3	**3.8**	5.6E+03	1.1E+05
Sample	0.0	**0.5**	3.2E+00	6.3E+01
Cytotoxicity	0.0	**0.5**	3.2E+00	6.3E+01
Neutralization	0.0	**0.5**	3.2E+00	6.3E+01

	Log10 Reduction	**Percent Reduction**
Recovered viral counts (to Control T0)	**3.3**	**99.944**
Recovered viral counts (to inoculum)	**5.7**	**100.000**

Legend

(+) = CTE measured (active virus) **T =** non-viral CTE

0 = no CTE measured **(Tx) =** see data table for sample time point

Cytotoxicity measure of CTE due to the sample extracted without virus

Neutralization measure of the CTE due to the neutralizer used