

## ANTIBACTERIAL TEST REPORT

### PROCEDURE

#### Quantitative Antibacterial Assessment:

ISO 22196:2011 was used to quantitatively test the specimen for antibacterial activity. In brief:

1. The sample was placed into a container with a lid.
2. A 0.3 mL inoculum of *Klebsiella pneumoniae* (ATCC #4352) was placed, in microdroplets, on the surface of the samples.
3. The specimen was incubated 24 hours at 37C.
4. 20 mL of Lethen broth was added to the container and shook. The liquid was plated using dilution techniques.
5. The "Value of Antimicrobial Activity" was carried out using the formula  $R = [\log (B/C)]$

Where:

R= value of antimicrobial activity

B = Average of the number of viable cells of bacteria on the untreated test piece / inoculum control after 24 hours

C = Average of the number of viable cells of bacteria on the antimicrobial test piece after 24 hours

### RESULTS

Quantitative Assessment of Activity - ISO 22196:2011					
<i>K. pneumoniae</i>					
Concentration of starting inoculum			9.40 x 10 <sup>5</sup> CFU/mL		
Sample Description		No. Bacteria Recovered	Log Value	R = [log(B/C)]	% Reduction
1	Safe pad coating Standard, Coated plaques – Painted brown coating	3.37 x 10 <sup>5</sup>	5.5	---	---
2	Safe pad coating + AMC Additive, Coated plaques – Painted brown coating	<2.00 x 10 <sup>1</sup>	<1.3	>4.2	>99.9%
Inoculum Control		7.95 x 10 <sup>6</sup>	6.9	---	---

Note: The level of treatment stated above indicates theoretical levels only.

Revision 2: May 2020

**Study Title:**

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

**Scope**

The standard describes the method for measuring antiviral activity on plastics and other non-porous surfaces of antiviral-treated products against specified viruses.

**Outline of Test Method (Obligatory Test Conditions)**

A test suspension of is inoculated onto a test plastic surface and covered with a cover film. The surface is maintained at a specified temperature for a defined period. At the end of the contact time media is added to the surface of the plastic, and the surface is washed over to recover any remaining organism. The number of surviving organisms which can be recovered from the surface is determined quantitatively taking in to account the test surface size.

**Feline coronavirus comparison**

	<b>Feline coronavirus</b>	<b>COVID-19 (SARS-CoV2)</b>
<b>Realm</b>	Riboviria	Riboviria
<b>Order</b>	Nidovirales	Nidovirales
<b>Family</b>	Coronaviridae	Coronaviridae
<b>Genus</b>	Alphacoronavirus	Betacoronavirus
<b>Species</b>	Alphacoronavirus 1	COVID-19

The members of the family Coronaviridae are enveloped and have a positive sense RNA genome. Coronaviruses have a distinct morphology with an outer 'corona' of embedded envelope spikes. These viruses cause a broad spectrum of animal and human disease.

Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz 'Virus Taxonomy, Classification and Nomenclature of Viruses, Ninth Report of the International Committee on Taxonomy of Viruses' 2012 ISBN 9780123846846

Test information		Deviation
Name of Product	Safe pad coating STP-ANTIBACPAD	
Batch Number & Expiry Date	N/S	
Date of Delivery	20/03/2020	
Period of Analysis	27/03/2020-03/04/2020	
Manufacturer / Supplier	Security Trade Products Ltd	
Storage Conditions	Ambient	
Appearance of the Product	White paint	
Test Concentrations	As supplied	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Viral Strains:	Feline coronavirus, Strain Munich	1
Contact Times	2 hours	
Stability and Appearance During Test	No Change Observed	

#### Deviations from Standard Method

- 1 - The test surface was challenged against Feline coronavirus.  
2 – No control material was supplied so samples were compared to glass slides.

Bond 52 was added to the sample and mixed thoroughly before painting the product onto glass slides and allowing to dry before testing.

#### Calculation notes

All recovery and log reduction calculations were performed for TCID50 rather than plaque assays. Cytotoxicity of the test product was performed through adding 10ml of culture media and washing the surface, this solution was then added to cells in serial dilution and cytotoxicity calculated by TCID50. Log recovery per surface is calculated and an average reduction given.

#### Test Result Summary

The test product has shown a log reduction of 0.47 (66.12%) against Feline coronavirus.

*See page 2 for acceptance criteria and raw data tables below for complete test results.*

## Summary

Log recovery						
	1	2	3	Average	Log recovered per surface	
<b>Test 1</b>	4.08	3.83	4.04	3.99	<i>At</i>	5.99
<b>Control (<i>t</i>)</b>	4.54	4.5	4.33	4.46	<i>Ut</i>	6.46
<b>Control (<i>0</i>)</b>	5.17	5.29	5.29	5.25	<i>Uo</i>	7.25

Antiviral activity per surface ( <i>R</i> )
0.47
$R=(Ut-Uo)-(At-Uo)$

## Controls

<b>Cytotoxicity (Test 1)</b>	Negative
<b>Cytotoxicity (Test 2)</b>	Negative
<b>Cytotoxicity (Test 3)</b>	Negative
<b>Cytotoxicity (Control)</b>	Negative

Inactivation control				
		Log recovered	Difference	Valid
<b>Test</b>	<i>St</i>	4.29	0.29	Y
<b>Control (Untreated)</b>	<i>Su</i>	4.42	0.17	Y
<b>Negative control</b>	<i>Sn</i>	4.58	N/A	Y

Raw data

Test								% CPE	p(1-p)	1
Dilution	Counts									
-2	4	4	4	4	4	4	4	1	0	
-3	4	4	4	4	4	4	4	1	0	
-4	1	1	1	2	2	3	0.41666667	0.243056		
-5	1	1	2	0	0	0	0.16666667	0.138889		
-6	0	0	0	0	0	0	0	0	0	
-7	0	0	0	0	0	0	0	0	0	
-8	0	0	0	0	0	0	0	0	0	
-9	0	0	0	0	0	0	0	0	0	

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.58
n	8
SD50	-4.08
SE	0.23
xp	-3

Test								% CPE	p(1-p)	2
Dilution	Counts									
-2	4	4	4	4	4	4	4	1	0	
-3	4	4	4	4	4	4	4	1	0	
-4	1	1	1	2	1	0	0.25	0.1875		
-5	1	1	0	0	0	0	0.08333333	0.076389		
-6	0	0	0	0	0	0	0	0	0	
-7	0	0	0	0	0	0	0	0	0	
-8	0	0	0	0	0	0	0	0	0	
-9	0	0	0	0	0	0	0	0	0	

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.33
n	8
SD50	-3.83
SE	0.19
xp	-3

Test								% CPE	p(1-p)	3
Dilution	Counts									
-2	4	4	4	4	4	4	4	1	0	
-3	4	4	4	4	4	4	4	1	0	
-4	3	3	2	1	1	1	0.45833333	0.248264		
-5	1	1	0	0	0	0	0.08333333	0.076389		
-6	0	0	0	0	0	0	0	0	0	
-7	0	0	0	0	0	0	0	0	0	
-8	0	0	0	0	0	0	0	0	0	
-9	0	0	0	0	0	0	0	0	0	

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.54
n	8
SD50	-4.04
SE	0.22
xp	-3

**Raw data**

Control T								1
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	3	3	4	4	2	2	0.75	0.1875
-5	1	1	2	2	1	0	0.29166667	0.206597
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	2.04
n	8
SD50	-4.54
SE	0.24
xp	-3

Control T								2
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	3	3	3	4	1	1	0.625	0.234375
-5	1	1	2	2	2	0	0.33333333	0.222222
-6	1	0	0	0	0	0	0.04166667	0.039931
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	2.00
n	8
SD50	-4.50
SE	0.27
xp	-3

Control T								3
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	2	2	2	3	3	0.66666667	0.222222
-5	1	1	1	1	0	0	0.16666667	0.138889
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.83
n	8
SD50	-4.33
SE	0.23
xp	-3

Raw data

Control 0									1
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	4	1	0
-5	3	2	2	1	1	1	0.41666667	0.243056	
-6	2	2	1	1	0	0	0.25	0.1875	
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.67
n	8
SD50	-5.17
SE	0.25
xp	-4

Control 0									2
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	4	1	0
-5	3	3	3	2	2	1	0.58333333	0.243056	
-6	1	1	1	0	2	0	0.20833333	0.164931	
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.79
n	8
SD50	-5.29
SE	0.24
xp	-4

Control 0									3
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	4	1	0
-5	3	3	3	1	2	2	0.58333333	0.243056	
-6	1	1	1	1	1	0	0.20833333	0.164931	
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.79
n	8
SD50	-5.29
SE	0.24
xp	-4



Raw data

Inactivation control Test									1
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	3	3	3	2	4	1	0.66666667	0.222222	
-5	1	1	1	0	0	0	0.125	0.109375	
-6	0	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.79
n	8
SD50	-4.29
SE	0.22
xp	-3

Inactivation control Control									2
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	3	3	3	3	3	3	3	0.75	0.1875
-5	1	1	0	2	0	0	0.16666667	0.138889	
-6	0	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.92
n	8
SD50	-4.42
SE	0.22
xp	-3

Inactivation control Negative									3
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	4	1	0
-4	3	3	3	4	3	3	0.79166667	0.164931	
-5	1	1	2	3	0	0	0.29166667	0.206597	
-6	0	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	2.08
n	8
SD50	-4.58
SE	0.23
xp	-3

**KEY**

CPE	Cytopathic effect
Counts	0-4 indicating degree of cytopathic effect 0 = No effect, 1 = 25% CPE, 2 = 50% CPE, 3 = 75% CPE, 4 = 100% CPE
d	Dilution factor (log)
Sum px	Sum of % CPE from the highest dilution showing 100% CPE to the lowest dilution assessed.
n	Number of dilutions
SD50	Dilution showing 50% of the end point according to Spearman-Kärber method
SE	Standard error
xp	Lowest dilution showing 100% CPE
TCID50	Titre causing 50% of the end point according to Spearman-Kärber

Sn Log of negative control  
 Su log of untreated control  
 St Log of test control

At Log of treated test  
 Ut Log of untreated control at time  
 Uo Log of untreated control at time 0  
 R Log reduction