

# Study Title: Quantitative suspension test for evaluation of virucidal activity in the medical area (Phase 2 Step1)

Microbiological Solutions Limited (MSL) Gollinrod, Walmersley, Bury, BL9 5NB, UK

Angela Davies, CEO

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### <u>Scope</u>

The standard method BS EN 14476 describes a test method and the minimum requirements for virucidal activity of a chemical disinfectant and antiseptic products that form a homogenous physically stable preparation when diluted with hard water – or in the case of ready to use products that are not diluted when applied, - with water. Products can only be tested at a concentration of 80% (97% with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substances. This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, instrument disinfection by immersion, surface disinfection by wiping, spraying, flooding or other means and textile disinfection.

This European standard applies to areas and situations where disinfection is medically indicated. Such indication occurs in patient care, for example: In hospitals, in community medical facilities and in dental institutions or in clinics of schools, of kindergartens and of nursing homes, and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for patients.

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

A sample of the test product is diluted in synthetic hard water in products diluted at point of use or water in the case of ready to use products is added to a test suspension of viruses in a solution of interfering substance. The mixture is maintained at one of the temperatures and contact times specified in the standard. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilutions of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Käber or by plaque counting. Reduction of virus infectivity is calculated from differences of Ig virus titres before (virus control) and after treatment with the product. The standard minimum spectrum of test organisms is Poliovirus, Adenovirus and Murine Norovirus.

### Acceptance Criteria

The product when tested as above shall demonstrate at least a 4  $log_{10}$  reduction against the test virus. The test is deemed valid where all control requirements are met.

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	Test information	Deviation
Name of Product	BioShield Guard 80	
Batch Number & Expiry Date	N/S	
Date of Delivery	01/05/2020	
Period of Analysis	07/05/2020-14/05/2020	
Manufacturer / Supplier	Sanglier Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Clear liquid	
Neutralisation Method	Dilution	
Product Diluent	Distilled water	
Test Concentrations	Neat (80%), Mid-range (50%) , Non active (0.1%)	
Experimental Conditions	Clean	
Interfering Substance	Clean 0.3g/l Bovine Albumin	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C for 72hrs	
Identification of the Bacterial Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-1508	1
	Feline Coronavirus, Strain Munich	
Contact Times	5 Minutes <u>+</u> 10 s	
Stability and Appearance During Test	No Change Observed (Homogenous)	

### **Deviations from Standard Method**

1 – The product was tested against non standard organism Feline coronavirus, therefore reference inactivation controls were not performed due to no acceptance criteria available.

### **Test Result Summary**

The test product received has achieved a 4-log reduction against Vaccina virus and Feline coronavirus when tested under the condition stipulated in this report.

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



# Summary Vaccinia virus

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Controls			-	-		
	MSL					
Conditions	SOLUTION PROVIDERS	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (water)		N/A	5 minutes	7.38	N/A	Validated
Cytotoxicity (product)		Neat	N/A	2.50	N/A	Validated
Product supression contro	I	Neat	Neat	7.13	0.25	Validated
Reference virus inactivation	on (formaldyehyde)	1.4%	5 minutes	4.92	2.46	Validated
Reference virus inactivation (formaldyehyde)		1.4%	15 minutes	4.21	3.17	Validated
Cytotoxicity (formaldehyd	e)	1.4%	N/A	2.50	N/A	Validated



Interference controls					
Condition	Concentration	Contact time	log TCID50	Log difference	Control validation
Interference control (untreated)	N/A	N/A	8.13	N/A	N/A
Interference control (treated)	Neat	N/A	8.08	0.04	Validated



Test Results	SOLUTION PROVIDERS					
Condition		Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product		Neat	5 minutes	3.38	4.00	Pass
Test product		50%	5 minutes	3.71	3.67	Fail
Test product		0.10%	5 minutes	7.29	0.08	Fail

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# Summary Feline coronavirus

Controls						
	MSL					
Conditions	SOLUTION PROVIDERS	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (water)		N/A	5 minutes	7.04	N/A	Validated
Cytotoxicity (product)		Neat	N/A	2.50	N/A	Validated
Product supression control		Neat	Neat	7.21	-0.17	Validated



Interference controls	JTION PROVIDERS					
Condition		Concentration	Contact time	log TCID50	Log difference	Control validation
Interference control (untreated)		Neat	N/A	8.08	N/A	N/A
Interference control (treated)		Neat	N/A	8.13	-0.04	Validated

Test Results	SOLUTION PROVIDERS				
Condition		Concentration	Contact time	log TCID50	log reduction
Test product		Neat	5 minutes	3.00	<mark>4.04</mark>
Test product		50%	5 minutes	3.79	<mark>3.25</mark>
Test product		0.10%	5 minutes	7.13	<mark>-0.08</mark>

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# Raw data

	lu							
Virus cont	trol (water)			Contact ti	me	5 minutes		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	(
-3	4	4	4	4	4	4	1	
-4	4	4	4	4	4	4	1	
-5	4	4	4	4	4	4	1	
-6	4	4	4	4	4	4	1	(
-7	2	3	3	3	4	1	0.66666667	0.22222
-8	1	1	1	2	0	0	0.20833333	0.16493
-9	0	0	0	0	0	0	0	

Organism	Vacciniavirus	
	ATTC VR-1508	3
d	1	
sum px	1.88	
n	8	
SD50	-7.38	
SE	0.24	
хр	-6	

Cytotoxic	ty (produc	t)		Product co	oncentratio	on	Neat	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-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	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.00	
n	8	
SD50	-2.50	
SE	0.00	
хр	-2	

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.63	
n	8	
SD50	-7.13	
SE	0.23	
хр	-6	

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.625	
n	10	
SD50	-8.125	
SE	0.1998	
хр	-7	

roduct su	pression c	ontrol		Product co	oncentratio	Neat		
ilution	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	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	3	3	1	1	0.5	0.25
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0
nterferen	ice control	(untreated	d)	Product co	oncentratio	on	Neat	
	•						- /	

Interferer	nce control	(untreated	d)	Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	2	2	3	3	1	1	0.5	0.25
-9	1	1	1	0	0	0	0.125	0.109375
-10	0	0	0	0	0	0	0	0

### Microbiological Solutions Ltd

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# BS EN 14476:2013+A2:2019

# Raw data

Interferer	nce control	(treated)		Product co	Product concentration			
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	2	2	2	2	2	2	0.5	0.25
-9	1	1	0	0	0	0	0.08333333	0.076389
-10	0	0	0	0	0	0	0	0

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.5833	
n	10	
SD50	-8.083	
SE	0.1904	
хр	-7	

Organism Vacciniavirus

d

n <mark>SD50</mark>

SE

n SD50

SE

хр

sum px

ATTC VR-1508

1 1.42

8

-4.92

0.21

Reference	e virus inac	tivation (fo	ormaldyeh	yde)	Contact ti	me	5 minutes	
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	4	4	4	4	4	1	0
-5	2	3	3	0	0	0	0.33333333	0.222222
-6	1	1	0	0	0	0	0.08333333	0.076389
-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

Reference	e virus inac	tivation (fo	ormaldyeh	yde)	Contact ti	me	15 minutes	
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	2	2	2	0.625	0.234375
-5	1	1	0	0	0	0	0.08333333	0.076389
-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

хр	-4	
Oversiens		
Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.71	

8

-4.21

0.21 -3

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.00	
n	8	
SD50	-2.50	
SE	0.00	
хр	-2	

# Cytotoxicity (formaldehyde) Dilution Counts -2 4 4 4 4

-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-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

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Gollinrod Walmersley Bury, BL9 5NB 4

% CPE

4

p(1-p)

0

1

# BS EN 14476:2013+A2:2019

MSL	
SOLUTION PROVIDERS	
Test identification Reference:	J001683

Test prod	uct	Product concentration		Neat	Contact time		5 minutes		
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	1	C	
-3	3	3	3	3	3	3	0.75	0.1875	
-4	1	1	1	0	0	0	0.125	0.109375	
-5	0	0	0	0	0	0	0	0	
-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	
Test prod	uct	Product co	oncentratio	on	50%	Contact time		5 minutes	
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	1	1	1	2	0	0	0.20833333	0.164931	
-5	0	0	0	0	0	0	0	0	
-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	
-9	0	0	0	0	0	0	0	0	
Test prod	uct	Product co	oncentratio	on	0.10%	Contact time		5 minutes	
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	4	4	4	4	4	1	0	
-5	4	4	4	4	4	4	1	0	
-6	4	4	4	4	4	4	1	0	

Organism	Vacciniavirus
	ATTC VR-1508
d	1
sum px	1.88
n	8
SD50	-3.38
SE	0.21
хр	-2

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.21	
n	8	
SD50	-3.71	
SE	0.15	
хр	-3	

Organism	Vacciniavirus	
	ATTC VR-1508	
d	1	
sum px	1.79	
n	8	
SD50	-7.29	
SE	0.22	
хр	-6	

0.666666667 0.222222

0

0.125 0.109375

0

2

0

0

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-7

-8

-9

3

1

0

3

1

0

3

1

0

3

0

0

2 0

0



# Raw data

Virus cont	trol (water)	(water) Contact time 5 minutes		5 minutes				
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	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	2	2	1	2	0.45833333	0.248264
-8	1	1	0	0	0	0	0.08333333	0.076389
-9	0	0	0	0	0	0	0	0

Organism	Drganism Feline Coronavirus						
Strain Munich							
d	d 1						
sum px	1.54						
n	8						
SD50	-7.04						
SE	0.22						
хр	-6						

Cytotoxici	Cytotoxicity (product)				Product concentration			
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-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

Product su	upression o	control		Product concentration			Neat	
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	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	2	3	3	3	0.625	0.234375
-8	1	1	0	0	0	0	0.08333333	0.076389
-9	0	0	0	0	0	0	0	0

Interferer	terference control (untreated)				Product concentration			
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	
-2	4	4	4	4	4	4	1	
-3	4	4	4	4	4	4	1	
-4	4	4	4	4	4	4	1	
-5	4	4	4	4	4	4	1	
-6	4	4	4	4	4	4	1	
-7	4	4	4	4	4	4	1	
-8	3	3	2	2	1	1	0.5	0.2
-9	1	1	0	0	0	0	0.08333333	0.07638
-10	0	0	0	0	0	0	0	

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Organism	Feline Coronavirus
	Strain Munich
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
хр	-2

Organism	Feline Coronavirus
-	Strain Munich
d	1
sum px	1.71
n	8
SD50	-7.21
SE	0.21
хр	-6

Organism Feline Coronavirus					
	Strain Munich				
d	1				
sum px	1.5833				
n	10				
SD50	-8.083				
SE	0.1904				
хр	-7				

# Raw data

Interferer	nce control	(treated)		Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	2	2	2	3	4	1	0.58333333	0.243056
-9	1	0	0	0	0	0	0.04166667	0.039931
-10	0	0	0	0	0	0	0	0

Organism	Feline Coronavirus						
Strain Munich							
d	1						
sum px	1.625						
n	10						
SD50	-8.125						
SE	0.1773						
хр	-7						

Test prod	uct	Product concentration			Neat Contact time			5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	2	2	2	2	2	2	0.5	0.25
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-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

Test prod	uct	Product concentration			50%	Contact time	5 minutes	
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	1	1	1	2	2	0	0.29166667	0.206597
-5	0	0	0	0	0	0	0	0
-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

Test prod	uct	Product concentration		0.10%	Contact time		5 minutes	
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	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	3	0.95833333	0.039931
-7	2	2	2	2	2	2	0.5	0.25
-8	1	1	2	0	0	0	0.16666667	0.138889
-9	0	0	0	0	0	0	0	0

rain Munich 1
1
4.50
1.50
8
-3.00
0.19
-2

Organism	Feline Coronavirus					
Strain Munich						
d	1					
sum px	1.29					
n	8					
SD50	-3.79					
SE	0.17					
хр	-3					

Organism	Organism Feline Coronavirus					
Strain Munich						
d	1					
sum px	2.63					
n	8					
SD50	-7.13					
SE	0.25					
хр	-5					

### Microbiological Solutions Ltd

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# BS EN 14476:2013+A2:2019



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# <u>KEY</u>

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								
хр	Lowest dilution showing 100% CPE								
TCID50	Titre causing 50% of the end point according to Spearman-Kärber								
PASS	= lg R greater than or equal to 4								
FAIL	=	lg R less than 4							
>	greater than		≥	equal to or greater than					
<	less than			equal to or less than					

#### Calculation notes

In cases where the highest dilution assessed has not shown 100% CPE, the value has been calculated assuming the dilution above this would give 100% CPE and the corresponding value has been assigned as <x.

The standard requires the product suppression control to show a <0.5 log reduction in viral titre. In cases where the product has failed to achieve the required 4 log reduction, but the product suppression control shows a >0.5 log reduction the result has been deemed as valid for fail as the consequence of inadequate suppression would be a partially extended contact time which would generate false positives, but not false negatives.

A similar approach has been taken in regards to the cytotoxicity controls. The standard requires a 4-log difference between the cytotoxicity level and the viral titre. In cases where this is not obtained, but the log reduction observed by the product is within the difference between the cytotoxicity levels and the viral titre the result is deemed acceptable for a fail as there will be no impact on the determination of efficacy.

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