


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

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**Scope**

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 lg 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<sub>10</sub> reduction against the test virus. The test is deemed valid where all control requirements are met.

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 Feline Coronavirus, Strain Munich	1
Contact Times	5 Minutes ± 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


Controls					
					
Conditions	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 control	Neat	Neat	7.13	0.25	Validated
Reference virus inactivation (formaldehyde)	1.4%	5 minutes	4.92	2.46	Validated
Reference virus inactivation (formaldehyde)	1.4%	15 minutes	4.21	3.17	Validated
Cytotoxicity (formaldehyde)	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					
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

Summary Feline coronavirus

Controls					
					
Conditions	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 suppression control	Neat	Neat	7.21	-0.17	Validated

Interference controls					
					
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				
				
Condition	Concentration	Contact time	log TCID50	log reduction
Test product	Neat	5 minutes	3.00	4.04
Test product	50%	5 minutes	3.79	3.25
Test product	0.10%	5 minutes	7.13	-0.08

Raw data

Virus control (water)				Contact time			5 minutes		% CPE	p(1-p)
Dilution	Counts									
-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	4	4	4	4	4	4	4	1	0	
-6	4	4	4	4	4	4	4	1	0	
-7	2	3	3	3	4	1	0.66666667	0.222222		
-8	1	1	1	2	0	0	0.20833333	0.164931		
-9	0	0	0	0	0	0	0	0	0	

Organism <i>Vacciniavirus</i> ATTC VR-1508	
d	1
sum px	1.88
n	8
SD50	-7.38
SE	0.24
xp	-6

Cytotoxicity (product)				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-2	4	4	4	4	4	4	4	1	0	
-3	0	0	0	0	0	0	0	0	0	
-4	0	0	0	0	0	0	0	0	0	
-5	0	0	0	0	0	0	0	0	0	
-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>Vacciniavirus</i> ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Product supression control				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-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	4	4	4	4	4	4	4	1	0	
-6	4	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	0	

Organism <i>Vacciniavirus</i> ATTC VR-1508	
d	1
sum px	1.63
n	8
SD50	-7.13
SE	0.23
xp	-6

Interference control (untreated)				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-1	4	4	4	4	4	4	4	1	0	
-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	4	4	4	4	4	4	4	1	0	
-6	4	4	4	4	4	4	4	1	0	
-7	4	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	0	

Organism <i>Vacciniavirus</i> ATTC VR-1508	
d	1
sum px	1.625
n	10
SD50	-8.125
SE	0.1998
xp	-7

Raw data

Interference control (treated)				Product concentration				Neat	
Dilution	Counts						% CPE	p(1-p)	
-1	4	4	4	4	4	4	4	1	0
-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	4	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	4	1	0
-8	2	2	2	2	2	2	2	0.5	0.25
-9	1	1	0	0	0	0	0	0.08333333	0.076389
-10	0	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
xp	-7

Reference virus inactivation (formaldehyde)				Contact time				5 minutes	
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	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	0
-8	0	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0	0

Organism	Vacciniavirus ATTC VR-1508
d	1
sum px	1.42
n	8
SD50	-4.92
SE	0.21
xp	-4

Reference virus inactivation (formaldehyde)				Contact time				15 minutes	
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	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	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	Vacciniavirus ATTC VR-1508
d	1
sum px	1.71
n	8
SD50	-4.21
SE	0.21
xp	-3

Cytotoxicity (formaldehyde)									
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0	0
-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	Vacciniavirus ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Raw data

Test product		Product concentration				Neat	Contact time		5 minutes	
Dilution	Counts						% CPE	p(1-p)		
-2	4	4	4	4	4	4	4	1	0	
-3	3	3	3	3	3	3	3	0.75	0.1875	
-4	1	1	1	0	0	0	0	0.125	0.109375	
-5	0	0	0	0	0	0	0	0	0	
-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	
Vacciniavirus	
ATTC VR-1508	
d	1
sum px	1.88
n	8
SD50	-3.38
SE	0.21
xp	-2

Test product		Product concentration				50%	Contact time		5 minutes	
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	1	1	1	2	0	0	0.20833333	0.164931		
-5	0	0	0	0	0	0	0	0	0	
-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	
Vacciniavirus	
ATTC VR-1508	
d	1
sum px	1.21
n	8
SD50	-3.71
SE	0.15
xp	-3

Test product		Product concentration				0.10%	Contact time		5 minutes	
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	4	4	4	4	4	4	4	1	0	
-6	4	4	4	4	4	4	4	1	0	
-7	3	3	3	3	2	2	0.66666667	0.222222		
-8	1	1	1	0	0	0	0.125	0.109375		
-9	0	0	0	0	0	0	0	0	0	

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



Raw data

Virus control (water)				Contact time			5 minutes		% CPE	p(1-p)
Dilution	Counts									
-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	4	4	4	4	4	4	4	1	0	
-6	4	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 <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.54
n	8
SD50	-7.04
SE	0.22
xp	-6

Cytotoxicity (product)				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-2	4	4	4	4	4	4	4	1	0	
-3	0	0	0	0	0	0	0	0	0	
-4	0	0	0	0	0	0	0	0	0	
-5	0	0	0	0	0	0	0	0	0	
-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	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Product supression control				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-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	4	4	4	4	4	4	4	1	0	
-6	4	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		

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.71
n	8
SD50	-7.21
SE	0.21
xp	-6

Interference control (untreated)				Product concentration			Neat		% CPE	p(1-p)
Dilution	Counts									
-1	4	4	4	4	4	4	4	1	0	
-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	4	4	4	4	4	4	4	1	0	
-6	4	4	4	4	4	4	4	1	0	
-7	4	4	4	4	4	4	4	1	0	
-8	3	3	2	2	1	1	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 <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.5833
n	10
SD50	-8.083
SE	0.1904
xp	-7

Raw data

Interference 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 <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	1.625
n	10
SD50	-8.125
SE	0.1773
xp	-7

Test product		Product concentration				Neat		Contact time		5 minutes	
Dilution	Counts							% CPE	p(1-p)		
-2	4	4	4	4	4	4	4	1	0		
-3	2	2	2	2	2	2	2	0.5	0.25		
-4	0	0	0	0	0	0	0	0	0		
-5	0	0	0	0	0	0	0	0	0		
-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	1.50
n	8
SD50	-3.00
SE	0.19
xp	-2

Test product		Product concentration				50%		Contact time		5 minutes	
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	1	1	1	2	2	0	0.29166667	0.206597			
-5	0	0	0	0	0	0	0	0	0		
-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	1.29
n	8
SD50	-3.79
SE	0.17
xp	-3

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

Organism <i>Feline Coronavirus</i> Strain Munich	
d	1
sum px	2.63
n	8
SD50	-7.13
SE	0.25
xp	-5





