


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

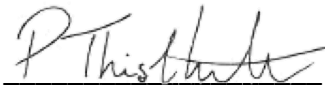
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**Scope**

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	Amb Clean	/
Batch Number & Expiry Date	WP:2012 UN:3082	
Date of Delivery	28/05/2020	
Period of Analysis	18/06/2020-22/06/2020	
Manufacturer / Supplier	Wessex Chemical Factors Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Yellow liquid	
Neutralisation Method	N6	
Product Diluent	Synthetic hard water	
Test Concentrations	Neat (80%), Mid-range (50%), Non active (0.1%)	
Experimental Conditions	Dirty	
Interfering Substance	Dirty 3.0g/l Bovine Albumin + 3m/l erythrocytes	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C for 72hrs	
Identification of the Viral Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-	
Contact Times	5 minutes ± 10s	
Stability and Appearance During Test	No Change Observed (Homogenous)	

**Deviations from Standard Method**


There were no deviations from the standard method


**Test Result Summary**


The test product received has achieved a 4-log reduction against Vaccinia virus 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	7.00	N/A	Validated
Cytotoxicity (product)	10.00%	N/A	2.50	N/A	Validated
Product supression control	10.00%	Neat	6.88	0.13	Validated
Reference virus inactivation (formaldehyde)	1.4%	5 minutes	4.83	2.17	Validated
Reference virus inactivation (formaldehyde)	1.4%	15 minutes	3.83	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	7.29	N/A	N/A
Interference control (treated)	10%	N/A	7.25	0.04	Validated

					
Test Results					
Condition	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	10%	5 minutes	2.50	>4	Pass
Test product	2%	5 minutes	2.83	>4	Pass
Test product	1%	5 minutes	2.92	>4	Pass

**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	3	3	4	4	3	3	0.83333333	0.138889		
-7	2	2	2	2	2	2	0.5	0.25		
-8	1	1	1	1	0	0	0.16666667	0.138889		
-9	0	0	0	0	0	0	0	0	0	

Organism	Vacciniavirus ATTC VR-1508
d	1
sum px	2.50
n	8
SD50	-7.00
SE	0.27
xp	-5

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	Vacciniavirus 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	2	2	4	4	4	4	0.83333333	0.138889		
-7	1	1	2	2	2	2	0.41666667	0.243056		
-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	2.38
n	8
SD50	-6.88
SE	0.26
xp	-5

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	3	3	3	2	2	2	0.625	0.234375		
-8	1	1	1	1	0	0	0.16666667	0.138889		
-9	0	0	0	0	0	0	0	0	0	
-10	0	0	0	0	0	0	0	0	0	

Organism	Vacciniavirus ATTC VR-1508
d	1
sum px	1.7917
n	10
SD50	-7.292
SE	0.2037
xp	-6

**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	2	2	2	3	3	1	0.54166667	0.248264
-8	1	1	1	2	0	0	0.20833333	0.164931
-9	0	0	0	0	0	0	0	0
-10	0	0	0	0	0	0	0	0

Organism	Vacciniavirus ATTC VR-1508
d	1
sum px	1.75
n	10
SD50	-7.25
SE	0.2143
xp	-6

Reference virus inactivation (formaldehyde)				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	2	2	2	1	1	0	0.33333333	0.222222
-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	1.33
n	8
SD50	-4.83
SE	0.18
xp	-4

Reference virus inactivation (formaldehyde)				Contact time			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	2	2	2	1	1	0	0.33333333	0.222222
-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	1.33
n	8
SD50	-3.83
SE	0.18
xp	-3

Cytotoxicity (formaldehyde)								
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
xp	-2

**Raw data**

Test product		Product concentration				10%	Contact time	5 minutes	
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	<i>Vacciniavirus</i> ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Test product		Product concentration				2%	Contact time	5 minutes	
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	1	0	
-3	3	1	1	1	1	1	0.33333333	0.222222	
-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	<i>Vacciniavirus</i> ATTC VR-1508
d	1
sum px	1.33
n	8
SD50	-2.83
SE	0.18
xp	-2

Test product		Product concentration				1.00%	Contact time	5 minutes	
Dilution	Counts						% CPE	p(1-p)	
-2	4	4	4	4	4	4	1	0	
-3	2	2	1	1	3	1	0.41666667	0.243056	
-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	<i>Vacciniavirus</i> ATTC VR-1508
d	1
sum px	1.42
n	8
SD50	-2.92
SE	0.19
xp	-2

**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		
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.