Study Title:
Chemical disinfectants and antiseptics – Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area -Test method and requirements (phase 2/step 2)

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Scope

The standard method BS EN 16777 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 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 for disinfection of non-porous surfaces including surfaces surfaces of medical devices without medical action.

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;
- 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 test suspension of viruses in a solution of interfering substances is inoculated onto a test surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The test surface is maintained at a specified temperature for a defined period. The test surface is transferred to cell maintenance medium so that the action of the disinfectant is immediately neutralized. The titre of the virus recovered from the test surface is determined. The titre of the inoculum on a test surface treated with hard water in place of the disinfectant is also determined and the reduction in virus titre attributed to the product is calculated by difference.

The standard minimum spectrum of test organisms is Adenovirus and Murine Norovirus. For activity against enveloped viruses Vaccinia virus is tested.

Acceptance Criteria

The product shall be deemed to have passed the test if it demonstrates a 4 lg or more reduction in titre for adenovirus and murine norovirus at the specific contact time chosen at between 18°C ± 1°C and 25°C ± 1°C, with the chosen interfering substance under the conditions defined by the test.

Test information		Deviation
Name of Product	Spectricept F-474	
Batch Number & Expiry Date	Lot LO72020-3-1	
Date of Delivery	24/07/2020	
Period of Analysis	13/08/2020-17/08/2020	
Manufacturer / Supplier	N/S	
Storage Conditions	Ambient	
Appearance of the Product	Clear liquid	
Neutraliser	Dilution	
Neutralisation Method	Dilution	
Product Diluent	Distilled water	
Test Concentrations	Neat, Mid-range (50%), Non active (0.1%)	
Experimental Conditions	Clean & Dirty	
Interfering Substance	Clean - 0,3 g/l bovine serum albumin Dirty - 3,0 g/l bovine serum albumin plus 3,0 ml erythrocytes	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Bacterial Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-1508	
Contact Times	1 minute ± 5s (clean only) 20 minutes ± 10s (dirty only)	
Stability and Appearance During Test	No Change Observed	

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

Controls					
Conditions	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (Water) Clean	N/A	1 minute	7.38	N/A	Validated
Cytotoxicity (product)	Neat	N/A	< 1.75	N/A	Validated
Product supression control	Neat	Neat	7.13	0.25	Validated
Reference virus inactivation (Glutardialdehyde)	Neat	5 minutes	4.50	2.88	Validated
Cytotoxicity (Glutardialdehyde)	Neat	N/A	2.50	N/A	Validated

Controls					
Conditions	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (Water) Dirty	N/A	20 minutes	6.54	N/A	Validated

Interference controls					
Condition	Concentration	Contact time	log TCID50	Log difference	Control validation
Interference control (untreated)	Neat	N/A	8.04	N/A	N/A
Interference control (treated)	Neat	N/A	7.92	0.12	Validated

Test Results					
Condition: Clean	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	Neat	1 minute	2.50	>4	Pass
Test product	50%	1 minute	2.75	>4	Pass
Test product	0.1%	1 minute	7.13	0.25	Fail

Test Results					
Condition Dirty	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	Neat	20 minutes	2.50	>4	Pass
Test product	50%	20 minutes	3.13	3.42	Fail
Test product	0.1%	20 minutes	6.50	0.04	Fail

Raw data

Virus control (water)				Contact time		1 minute		
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	3	3	3	3	3	3	0.75	0.1875
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.88
n	8
SD50	-7.38
SE	0.21
xp	-6

Cytotoxicity (product)				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-2	1	1	1	1	1	1	0.25	0.1875
-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	1.25
n	8
SD50	< -1.75
SE	0.16
xp	-1

Product supression 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	3	4	0.95833333	0.039931
-7	2	2	2	2	1	3	0.5	0.25
-8	1	1	1	1	0	0	0.16666667	0.138889
-9	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	2.63
n	8
SD50	-7.13
SE	0.25
xp	-5

Interference control (untreated)				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	1	2	2	2	2	2	0.45833333	0.248264
-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	1.5417
n	10
SD50	-8.042
SE	0.1899
xp	-7

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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	1	1	1	2	0.375	0.234375
-9	1	0	0	0	0	0	0.04166667	0.039931
-10	0	0	0	0	0	0	0	0

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.4167
n	10
SD50	-7.917
SE	0.1746
xp	-7

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	3	3	3	0.75	0.1875
-5	1	1	1	1	1	1	0.25	0.1875
-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

	ATTC VR-1508
d	1
sum px	2.00
n	8
SD50	-4.50
SE	0.23
xp	-3

Cytotoxicity (Glutardialdehyde)								
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			Neat	Contact time		1 minute
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

Test product		Product concentration			50%	Contact time		1 minute
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	2	2	1	0	0	0.25	0.1875
-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	1.25
n	8
SD50	-2.75
SE	0.16
xp	-2

Test product		Product concentration			0.1%	Contact time		1 minute
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	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

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

Raw data

Virus control (water)				Contact time		20 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	3	3	3	3	3	3	0.75	0.1875
-7	2	2	1	1	1	0	0.29166667	0.206597
-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	2.04
n	8
SD50	-6.54
SE	0.24
xp	-5

Test product		Product concentration			Neat	Contact time		20 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	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Test product		Product concentration			50%	Contact time		20 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	1	1	1	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

Organism	*Vacciniavirus* ATTC VR-1508
d	1
sum px	1.63
n	8
SD50	-3.13
SE	0.23
xp	-2

Test product		Product concentration			0.1%	Contact time		20 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	2	3	3	3	0.79166667	0.164931
-7	1	1	1	1	0	1	0.20833333	0.164931
-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	2.00
n	8
SD50	-6.50
SE	0.22
xp	-5

KEY

KEY

CPE	Cytopathic effect		
Counts	0-4 indicating degree of cytopathic effection 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.