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	Z-71 Surface Sanitizer	
Batch Number & Expiry Date	Batch no. 365291 Expiry June 2024	
Date of Delivery	31/03/2021	
Period of Analysis	07/06/2021	
Manufacturer / Supplier	Zoono UK & Europe	
Storage Conditions	Ambient	
Appearance of the Product	Clear Liquid	
Neutralisation Method	Large volume plating	
Product Diluent	Distilled water	
Test Concentrations	Neat, Mid-range, Non active	
Experimental Conditions	Clean	
Interfering Substance	Clean - 0,3 g/l bovine serum albumin	
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	15 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	15 minute	7.00	N/A	Validated
Cytotoxicity (product)	N/A	N/A	< 1.50	N/A	Validated
Product supression control	0.001	N/A	6.83	0.17	Validated
Reference virus inactivation (Glutardialdehyde)	50ppm	5 minutes	4.04	2.96	Validated
Cytotoxicity (Glutardialdehyde)	50ppm	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	7.71	N/A	N/A
Interference control (treated)	0.10%	N/A	7.63	0.08	Validated

Test Results

Condition	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	0.1%	15 minute	6.67	0.33	Fail

Concentration	Neat
Contact time	15 minutes
n	*96*
np	3
D	1000
Vw	0.1
Infectious particles/ml	317.49
TCID50/ml	460.13
Log10 recovery	2.66
Log reduction	4.34

Concentration	50%
Contact time	15 minutes
n	*96*
np	7
D	1000
Vw	0.1
Infectious particles/ml	757.12
TCID50/ml	1097.27
Log10 recovery	3.04
Log reduction	3.96

Raw data

Virus control (water)				Contact time		15 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	2	2	2	2	0.5	0.25
-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	1.50
n	8
SD50	-7.00
SE	0.19
xp	-6

Cytotoxicity (product)				Product concentration			N/A	
Dilution	Counts						% CPE	p(1-p)
-2	0	0	0	0	0	0	0	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	< -1.50
SE	0.00
xp	-1

Product supression control				Product concentration			0.10%	
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	1	1	1	2	0	0.33333333	0.222222
-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	-6.83
SE	0.18
xp	-6

Interference control (untreated)				Product concentration			0.10%	
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	0	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	1.2083
n	10
SD50	-7.708
SE	0.1354
xp	-7

Raw data

Interference control (treated)				Product concentration			0.10%	
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	1	1	0	0	0	0.125	0.109375
-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	1.125
n	10
SD50	-7.625
SE	0.1102
xp	-7

Reference virus inactivation (Glutardialdehyde)					Contact time		5 min	
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	1	0	0	0.54166667	0.248264
-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	1.54
n	8
SD50	-4.04
SE	0.19
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

Test product		Product concentration			0.10%	Contact time		15 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	0	0	0	0	0.16666667	0.138889
-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	1.17
n	8
SD50	-6.67
SE	0.14
xp	-6

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.

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