Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas (Phase 2 Step 1)

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Scope

The standard method BS EN 1276:2019 describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have bactericidal activity in the fields described.
The test takes into account practical conditions of application of the product, including contact time, temperature, test organisms and interfering substance, i.e. conditions which may influence its action in practical situations.
The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to defined experimental conditions. However, for some applications, the recommendations of use of a product may differ and therefore additional test conditions may need to be used.

Outline of Test Method (Obligatory Test Conditions)

A sample of the test product is diluted in synthetic hard water for products diluted at point of use (or distilled water in the case of ready to use products). A test suspension of bacteria and interfering substance is then added to the dilutions and maintained at 20°C for 1-60 minutes (general purpose disinfection) or 30-60 seconds (hand hygiene products) At the end of the contact time an aliquot is taken, and the bacterial / bacteriostatic activity is immediately neutralised or suppressed by the validated method. The numbers of surviving bacteria in each sample are determined and the reduction is calculated.
The test is performed using *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae* as standard organisms.
Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.

Acceptance Criteria

The product when tested as above shall demonstrate at least a 5 $\log_{10}$ (3 $\log_{10}$ hand washes) reduction in viable bacterial counts. The test is deemed valid where all control requirements are met.

UKAS Accreditation

This method has been audited by UKAS to the ISO 17025 standard, for tests where no deviations from the standard method are stipulated.

Test information		Deviation
Name of Product	Clean Care Shield Concentrate All In One Multi-Purpose Cleaner and Sanitiser	
Batch Number & Expiry Date	N/S	
Date of Delivery	23/10/2020	
Period of Analysis	25/11/2020-26/11/2020	
Manufacturer / Supplier	APA Cleaning Solutions Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Yellow Liquid	
Neutraliser	N6	
Neutralisation Method	Dilution	
Product Diluent	Distilled water	
Test Concentrations	8%, 4%, Non active (0.4%)	
Experimental Conditions	Clean	
Interfering Substance	Clean 0.3g/l Bovine Albumin	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	Bacteria – 37°C ±1°C for 24hr to 48hrs	
Identification of the Bacterial Strains:	*Pseudomonas aeruginosa* NCTC 13359 (ATCC 15442) *Staphylococcus aureus* NCTC 10788 (ATCC 6538) *Enterococcus hirae* NCTC 13383 (ATCC 10541) *Escherichia coli* NCTC 10418 (ATCC 10536)	
Contact Times	30 Seconds and 60 Seconds ± 5s	
Stability and Appearance During Test	No Change Observed	

Deviations from Standard Method

1 - Testing showed failure to fully neutralize the product, to overcome this additional validation was performed. This showed that neutralizing using a 1:100 dilution of the product showed effective neutralization. In order to account for the addition serial dilution from 1:10 to 1:100, 10 plates per duplicate have been sampled for the -1 dilution. These were then summed to give the equivalent count at neat.

Test Result Summary

The test product received has achieved a >5 log reduction against all bacterial test isolates when tested under the condition stipulated in this report.

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

Validation and Controls

Validation suspension (Nv_0)				Experimental condition controls (A)				Neutraliser or Filtration Control (B)				Method Validation (C)			
			$\bar{x}$ =				$\bar{x}$ =				$\bar{x}$ =				$\bar{x}$ =
Vc1	**Ps.**	51		**Vc1**	**Ps.**	40		**Vc1**	**Ps.**	46		**Vc1**	**Ps.**	83	
	Sa.	56			**Sa.**	40			**Sa.**	30			**Sa.**	33	
	Ec.	59	**Ps.** 48		**Ec.**	69	**Ps.** 43		**Ec.**	55	**Ps.** 66		**Ec.**	84	**Ps.** 80
	Ent.	52	**St.** 48		**Ent.**	49	**St.** 43		**Ent.**	60	**St.** 34		**Ent.**	67	**St.** 36
Vc2	**Ps.**	45	**Ec.** 74	**Vc2**	**Ps.**	46	**Ec.** 72	**Vc2**	**Ps.**	86	**Ec.** 62	**Vc2**	**Ps.**	76	**Ec.** 87
	Sa.	39	**Ent.** 50		**Sa.**	46	**Ent.** 52		**Sa.**	37	**Ent.** 56		**Sa.**	39	**Ent.** 62
	Ec.	89			**Ec.**	74			**Ec.**	69			**Ec.**	90	
	Ent.	47			**Ent.**	54			**Ent.**	52			**Ent.**	56	
$30 \leq \bar{x}$ of $Nv_0 \leq 160$?				$\bar{x}$ of A ≥ 0.5 Nv0				$\bar{x}$ of B ≥ 0.5 Nv0				$\bar{x}$ of C ≥ 0.5 Nv0			
Yes				**Yes**				**Yes**				**Yes**			

Test Results

30 seconds

		Test Procedure at concentrations % (V/V)		
Test Organism	**Suspension N**	**8**	**4**	**0.4**
Pseudomonas	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^4 230 ; 200
aeruginosa	10^7 46 ; 50	Na ; < 2.15	Na ; < 2.15	Na ; 7.33
ATCC 15442	N_0 : 7.68 Valid	R > 5.54	R > 5.54	R 0.35
Escherichia	10^6 226 ; 212	10^0 0 ; 0	10^0 0 ; 0	10^4 150 ; 136
coli	10^7 21 ; 27	Na ; < 2.15	Na ; < 2.15	Na ; 7.16
ATCC 10536	N_0 : 7.34 Valid	R > 5.20	R > 5.20	R 0.19
Staphylococcus	10^6 186 ; 179	10^0 0 ; 0	10^0 0 ; 0	10^4 >330 ; >330
aureus	10^7 39 ; 44	Na ; < 2.15	Na ; < 2.15	Na ; > 7.52
ATCC 6538	N_0 : 7.31 Valid	R > 5.16	R > 5.16	R < -0.21
Enterococcus	10^6 285 ; 290	10^0 0 ; 0	10^0 0 ; 0	10^4 199 ; 200
hirae	10^7 30 ; 26	Na ; < 2.15	Na ; < 2.15	Na ; 7.30
ATCC 10541	N_0 : 7.46 Valid	R > 5.31	R > 5.31	R 0.16

60 seconds

		Test Procedure at concentrations % (V/V)		
Test Organism	**Suspension N**	**8**	**4**	**0.4**
Pseudomonas	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^4 139 ; 126
aeruginosa	10^7 46 ; 50	Na ; < 2.15	Na ; < 2.15	Na ; 7.12
ATCC 15442	N_0 : 7.68 Valid	R > 5.54	R > 5.54	R 0.56
Escherichia	10^6 226 ; 212	10^0 0 ; 0	10^0 0 ; 0	10^4 222 ; 218
coli	10^7 21 ; 27	Na ; < 2.15	Na ; < 2.15	Na ; 7.34
ATCC 10536	N_0 : 7.34 Valid	R > 5.20	R > 5.20	R 0.00
Staphylococcus	10^6 186 ; 179	10^0 0 ; 0	10^0 0 ; 0	10^4 186 ; 190
aureus	10^7 39 ; 44	Na ; < 2.15	Na ; < 2.15	Na ; 7.27
ATCC 6538	N_0 : 7.31 Valid	R > 5.16	R > 5.16	R 0.03
Enterococcus	10^6 285 ; 290	10^0 0 ; 0	10^0 0 ; 0	10^4 148 ; 141
hirae	10^7 30 ; 26	Na ; < 2.15	Na ; < 2.15	Na ; 7.16
ATCC 10541	N_0 : 7.46 Valid	R > 5.31	R > 5.31	R 0.30

KEY

N_0	Log_{10} number of cfu/ml at the beginning of the contact time = $N/10$
Nv_0	is the number of cfu/ml in the validation test suspension at the beginning of the contact time
A	is the verification of experimental conditions control
B	is the neutraliser toxicity control
C	is method validation
Vc	is the colony forming units counted per 1ml of sample
$\bar{x}$	is the average of Vc_1 & Vc_2
$\bar{x}$ wm	is the weighted mean of *N*
Na	Log_{10} number of surviving cfu/ml in the test mixture
R	($lg N_0 – lg Na = lg R$) is the calculation for reduction in viability
>	Greater than
≥	Equal to or greater than
<	Less than
≤	Equal to or less than

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