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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PO/Quote number: Q002820/3
Report Date: 30/06/2020
Issue Number: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	Amb Clean	
Batch Number & Expiry Date	WP:2012 UN:3082	
Date of Delivery	28/05/2020	
Period of Analysis	29/06/2020-30/06/2020	
Manufacturer / Supplier	Wessex Chemical Factors Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Yellow liquid	
Neutraliser	N6	
Neutralisation Method	Dilution	
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	
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	Bacteria – 5 minutes ± 10s	
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 >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.	125		Vc1	Ps.	124		Vc1	Ps.	125		Vc1	Ps.	119	
	Sa.	100			Sa.	112			Sa.	78			Sa.	85	
	Ec.	127	Ps. 120		Ec.	132	Ps. 123		Ec.	126	Ps. 122		Ec.	128	Ps. 119
	Ent.	119	St. 100		Ent.	121	St. 102		Ent.	126	St. 75		Ent.	119	St. 84
Vc2	Ps.	115	Ec. 126	Vc2	Ps.	122	Ec. 129	Vc2	Ps.	119	Ec. 126	Vc2	Ps.	119	Ec. 128
	Sa.	100	Ent. 118		Sa.	92	Ent. 118		Sa.	72	Ent. 121		Sa.	82	Ent. 120
	Ec.	124			Ec.	125			Ec.	125			Ec.	127	
	Ent.	117			Ent.	115			Ent.	115			Ent.	120	
$30 \leq \bar{x}$ of $Nv_0 \leq 160$?				$\bar{x}$ of A $\geq$ 0.5 Nv0				$\bar{x}$ of B $\geq$ 0.5 Nv0				$\bar{x}$ of C $\geq$ 0.5 Nv0			
Yes				**Yes**				**Yes**				**Yes**			

Test Results

		Test Procedure at concentrations % (V/V)		
Test Organism	**Suspension N**	**10%**	**5%**	**1%**
Pseudomonas	10^6 167 ; 175	10^0 0 ; 0	10^0 0 ; 0	10^0 0 ; 0
aeruginosa	10^7 23 ; 19	Na ; < 2.15	Na ; < 2.15	Na ; < 2.15
ATCC 15442	N_0 : 7.24 Valid	R > 5.10	R > 5.10	R > 5.10
Escherichia	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^0 0 ; 1
coli	10^7 47 ; 42	Na ; < 2.15	Na ; < 2.15	Na ; < 2.15
ATCC 10536	N_0 : 7.65 Valid	R > 5.50	R > 5.50	R > 5.50
Staphylococcus	10^6 178 ; 184	10^0 1 ; 0	10^0 0 ; 0	10^0 0 ; 0
aureus	10^7 17 ; 16	Na ; < 2.15	Na ; < 2.15	Na ; < 2.15
ATCC 6538	N_0 : 7.25 Valid	R > 5.11	R > 5.11	R > 5.11
Enterococcus	10^6 188 ; 174	10^0 0 ; 0	10^0 0 ; 0	10^0 0 ; 0
hirae	10^7 17 ; 15	Na ; < 2.15	Na ; < 2.15	Na ; < 2.15
ATCC 10541	N_0 : 7.25 Valid	R > 5.11	R > 5.11	R > 5.11

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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