

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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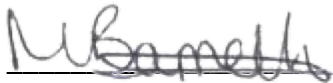
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PO/Quote number: Q003817

Report Date: 25/11/2020

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 log₁₀ (3 log₁₀ 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	Germbuster	/
Batch Number & Expiry Date	Bx 153820	
Date of Delivery	05/10/2020	
Period of Analysis	24/11/2020-25/11/2020	
Manufacturer / Supplier	Total Liquid Solutions Limited	
Storage Conditions	Ambient	
Appearance of the Product	Clear Liquid	
Neutraliser	N6	
Neutralisation Method	Dilution	
Product Diluent	Distilled 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:	<i>Pseudomonas aeruginosa</i> NCTC 13359 (ATCC 15442) <i>Staphylococcus aureus</i> NCTC 10788 (ATCC 6538) <i>Enterococcus hirae</i> NCTC 13383 (ATCC 10541) <i>Escherichia coli</i> NCTC 10418 (ATCC 10536) MRSA NCTC 12493	
Contact Times	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

<p>The test product received has achieved to achieve a >5 log reduction when tested under the condition stipulated in this report.</p> <p><i>See page 2 for acceptance criteria and raw data tables below for complete test results.</i></p>

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.	110		Vc1	Ps.	90		Vc1	Ps.	261		Vc1	Ps.	100	
	Sa.	131			Sa.	92			Sa.	161			Sa.	120	
	Ec.	91	Ps. 105		Ec.	49	Ps. 93		Ec.	124	Ps. 281		Ec.	91	Ps. 100
	Ent.	58	St. 129		Ent.	52	St. 91		Ent.	91	St. 158		Ent.	90	St. 120
Vc2	Ps.	100	Ec. 89	Vc2	Ps.	96	Ec. 48	Vc2	Ps.	300	Ec. 112	Vc2	Ps.	99	Ec. 91
	Sa.	127	Ent. 61		Sa.	89	Ent. 54		Sa.	155	Ent. 86		Sa.	119	Ent. 90
	Ec.	86			Ec.	46			Ec.	99			Ec.	91	
	Ent.	64			Ent.	55			Ent.	80			Ent.	89	
30 ≤ \bar{x} of Nv ₀ ≤ 160? Yes				\bar{x} of A ≥ 0.5 Nv ₀ Yes				\bar{x} of B ≥ 0.5 Nv ₀ Yes				\bar{x} of C ≥ 0.5 Nv ₀ Yes			

Test Results

MSL SOLUTION PROVIDERS		Test Procedure at concentrations % (V/V)			
Test Organism	Suspension N	Neat	50	0.1	
<i>Pseudomonas aeruginosa</i> ATCC 15442	10 ⁶ >330 ; >330	10 ⁰ 0 ; 0	10 ⁰ 0 ; 0	10 ⁴ >330 ; >330	
	10 ⁷ 46 ; 44	Na ; < 2.15	Na ; < 2.15	Na ; > 7.52	
	N ₀ : 7.65 Valid	R > 5.51	R > 5.51	R < 0.13	
<i>Escherichia coli</i> ATCC 10536	10 ⁶ >330 ; >330	10 ⁰ 0 ; 0	10 ⁰ 0 ; 0	10 ⁴ 140 ; 170	
	10 ⁷ 40 ; 41	Na ; < 2.15	Na ; < 2.15	Na ; > 7.19	
	N ₀ : 7.61 Valid	R > 5.46	R > 5.46	R > 0.42	
<i>Staphylococcus aureus</i> ATCC 6538	10 ⁶ >330 ; >330	10 ⁰ 0 ; 0	10 ⁰ 0 ; 0	10 ⁴ >330 ; >330	
	10 ⁷ 50 ; 49	Na ; < 2.15	Na ; < 2.15	Na ; > 7.52	
	N ₀ : 7.69 Valid	R > 5.55	R > 5.55	R < 0.18	
<i>Enterococcus hirae</i> ATCC 10541	10 ⁶ >330 ; >330	10 ⁰ 0 ; 0	10 ⁰ 0 ; 0	10 ⁴ 200 ; 170	
	10 ⁷ 46 ; 46	Na ; < 2.15	Na ; < 2.15	Na ; > 7.27	
	N ₀ : 7.66 Valid	R > 5.52	R > 5.52	R > 0.40	

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	MRSA	84	Vc1	MRSA	54	Vc1	MRSA	90	Vc1	MRSA	61
											Ps. 60
		MRSA 87			MRSA 55			MRSA 86			
Vc2	MRSA	89	Vc2	MRSA	56	Vc2	MRSA	82	Vc2	MRSA	59
30 ≤ \bar{x} of Nv ₀ ≤ 160?			\bar{x} of A ≥ 0.5 Nv ₀			\bar{x} of B ≥ 0.5 Nv ₀			\bar{x} of C ≥ 0.5 Nv ₀		
Yes			Yes			Yes			Yes		

Test Results

Test Organism	Suspension N	Test Procedure at concentrations % (V/V)			
		Neat	50	0.1	
MRSA	10 ⁶ 210 ; 216 10 ⁷ 41 ; 37	10 ⁰ 0 ; 0	10 ⁰ 0 ; 0	10 ⁴ 140 ; 130	Na ; < 7.13
NCTC 12493	N ₀ : 7.36 Valid	R > 5.21	R > 5.21	R	0.23

KEY

N_0	Log ₁₀ number of cfu/ml at the beginning of the contact time = $N/10$
N_{V0}	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
V_c	is the colony forming units counted per 1ml of sample
\bar{x}	is the average of V_{c1} & V_{c2}
\bar{x}_{wm}	is the weighted mean of N
N_a	Log ₁₀ number of surviving cfu/ml in the test mixture
R	($\lg N_0 - \lg N_a = \lg R$) is the calculation for reduction in viability
$>$	Greater than
\geq	Equal to or greater than
$<$	Less than
\leq	Equal to or less than