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)

Microbiological Solutions Limited (MSL)
Gollinrod, Walmersley, Bury, BL9 5NB, UK

Customer: Aktivora NI Ltd
Contact name: Mark Forsythe
Email: mark@aktivoraireland.com
Address: Aktivora NI LTD, 12 A Claredon Road, Belfast, BT1 3BG
PO/Quote number: Q005673
Report Date: 18/01/2023
Issue Number: 1

Megan Barrett
Laboratory Manager

Peter Thistlethwaite
Technical Projects Manager

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

A UKAS accredited testing laboratory No. 4045.

Test information		Deviation
Name of Product	Super Sanitiser for Hands & Surfaces	
Batch Number & Expiry Date	AK008	
Date of Delivery	13/01/2023	
Period of Analysis	18/01/2023	
Manufacturer / Supplier	Aktivora NI Ltd	
Storage Conditions	Ambient	
Active substance(s) and concentration (optional)	Quaternary Ammonium Compounds <1%	
Appearance of the Product	Clear Liquid	
Neutraliser	N6	
Neutralisation Method	Dilution	
Product Diluent	Synthetic hard water	
Test Concentrations	20%, 15%, 10%	
Experimental Conditions	Clean	
Interfering Substance	Clean 0.3g/l Bovine Albumin	
Test Temperature	4°C ± 1°C	1
Temperature of Incubation	Bacteria – 37°C ±1°C for 24hr to 48hrs	
Identification of the Bacterial Strains:	*Salmonella typhimurium* ATCC 13311	2
Contact Times	30 minutes ± 5s	
Stability and Appearance During Test	No Change Observed	

Deviations from Standard Method

1 – Testing was performed at 4°C.
2 – Testing was performed against *Salmonella typhimurium* only.

Test Result Summary

The test product received has achieved a >5 log reduction against *Salmonella typhimurium,* 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	**St.** 127	**St.** 117	**Vc1**	**St.** 96	**St.** 95	**Vc1**	**St.** 117	**St.** 93	**Vc1**	**St.** 67	**St.** 91
Vc2	**St.** 107		**Vc2**	**St.** 94		**Vc2**	**St.** 69		**Vc2**	**St.** 114	
$30 \leq \bar{x}$ of $Nv_0 \leq 160$? **Yes**			$\bar{x}$ of A ≥ 0.5 Nv0 **Yes**			$\bar{x}$ of B ≥ 0.5 Nv0 **Yes**			$\bar{x}$ of C ≥ 0.5 Nv0 **Yes**		

Test Results

		Test Procedure at concentrations % (V/V)		
Test Organism	**Suspension N**	**20%**	**15%**	**10%**
Salmonella	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^0 0 ; 0
typhimurium	10^7 47 ; 42	Na ; < 2.15	Na ; < 2.15	Na ; < 2.15
ATCC 15442	N_0 : 7.65 Valid	R > 5.50	R > 5.50	R > 5.50

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