Study Title:

Quantitative suspension test for evaluation of bactericidal efficacy of Chemical disinfectants and antiseptics used in the veterinary area (Phase 2 Step1)

BS EN 1656:2019

Job reference – J001243

Lab Ref/Report No.	**J001243**
Testing Laboratory **Site** **Company owner**	**Microbiological Solutions Limited** **Gollinrod, Walmersley, Bury, BL9 5NB** **Angela Davies, Managing Director**
Report Date	**10/01/2020**
Period of Analysis	**06/01/2020**

Customer	**Zoono UK & Europe**
Contact Name	**James Milnes**
Address	**Unit 15, Bunting Road, Bury St Edmunds IP32 7BX**
Email	**james.milnes@zoono.com**
PO Number/Quote Ref	**Q001926**

Name of product	Microbe Shield
Batch number	8318 Exp:11.2022
Manufacturer / Supplier	Zoono UK & Europe
Storage Conditions	Ambient
Appearance of the Product	Colourless liquid
Preservatives/Actives & Conc.%	Quaternary ammonium compound CAS 68424-85-1
Standard Method	BS EN 1656:2019
Neutraliser/Inactivator	N6
Product diluent	Distilled Water
Test Concentrations *	Neta, 50%, 0.1%
Experimental Conditions	Clean
Interfering substances	Clean 3g/l Bovine Albumin
Test Temperature	10°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) *Proteus vulgaris* ATCC 13315
Contact times	5 minutes ± 10s (surface)

* Products supplied as “neat” can only be tested at a concentration of 80% or less, as some dilution is always produced by adding test organisms and interfering substance.

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Introduction

The standard method BS EN 1656 describes a suspension test method and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or – in the case of ready-to-use products – with water. 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.

This European Standard applies to products that are used in the veterinary area – e.g. in the breeding, husbandry, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

Outline of Test Method (Obligatory test conditions)

A sample of the test product is diluted in synthetic hard water in products diluted at point of use or deionized 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 10°C for 30 minutes (general disinfection products) or at 30°C for 5 minutes (teat disinfection 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. For general disinfection the test is performed using *Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus* and *Enterococcus hirae* as standard organisms. For teat disinfection the test is performed using *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis* as standard organisms.

Deviations from Standard Method

The product was tested with a contact time of 5 minutes.
Initial testing showed failure to fully neutralize the product. Neutralization was therefore performed with a 1:100 dilution of the product which showed effective neutralization, shown in the C validation. 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.

Acceptance Criteria

The product when tested as above shall demonstrate at least a 5 $\log_{10}$ reduction in viable bacterial counts.

Conclusion

The product **Microbe Shield** has **passed** the test according to the acceptance criteria as outlined in the standard for **general disinfection**, when tested under **clean** conditions with a contact time of **5 minutes** at a minimum concentration of **50%.**

See raw data tables below for test results.

The sample will be retained for 1 month unless otherwise requested.

Laboratory Manager
Megan Barrett

Technical Project Manager
Peter Thistlethwaite

Job reference – J001243
Validation and controls
General disinfection

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.	74			Vc1	Ps.	110			Vc1	Ps.	85			Vc1	Ps.	63		
	Pv.	101				Pv.	115				Pv.	120				Pv.	90		
	St	112	Ps.	84		St.	78	Ps.	110		St.	85	Ps.	74		St.	87	Ps.	61
	Ent.	39	Pv.	104		Ent.	27	Pv.	112		Ent.	21	Pv.	123		Ent.	40	Pv.	90
Vc2	Ps.	94	St.	112	Vc2	Ps.	109	St.	88	Vc2	Ps.	63	St.	84	Vc2	Ps.	59	St.	85
	Pv.	107	Ent.	38		Pv.	108	Ent.	26		Pv.	126	Ent.	30		Pv.	90	Ent.	45
	St.	111				St.	98				St.	82				St.	82		
	Ent.	36				Ent.	24				Ent.	38				Ent.	49		
	$30 \leq \bar{x}$ 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			

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Test results

		Test concentration		
Test Organism	Suspension N	Neat	50%	0.10%
Pseudomonas	10^6 237 ; 293	10^0 0 ; 0	10^0 0 ; 0	10^4 205 ; 203
aeruginosa	10^7 32 ; 36	Na ; < 2.15	Na ; < 2.15	Na ; 7.31
ATCC 15442	N_0 : 7.43 Valid	R > 5	R > 5	R 0.12
Proteus	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^4 >330 ; >330
vulgaris	10^7 45 ; 41	Na ; < 2.15	Na ; < 2.15	Na ; > 7.52
ATCC 13315	N_0 : 7.63 Valid	R > 5	R > 5	R < 0.11
Staphylococcus	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^4 206 ; 198
aureus	10^7 48 ; 50	Na ; < 2.15	Na ; < 2.15	Na ; 7.31
ATCC 6538	N_0 : 7.69 Valid	R > 5	R > 5	R 0.38
Enterococcus	10^6 >330 ; >330	10^0 0 ; 0	10^0 0 ; 0	10^4 199 ; 198
hirae	10^7 28 ; 31	Na ; < 2.15	Na ; < 2.15	Na ; 7.30
ATCC 10541	N_0 : 7.47 Valid	R > 5	R > 5	R 0.17

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KEY

No	Log_{10} number of cfu/ml at the beginning of the contact time = N/10		
Nvo	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	($lg N_O - lg Na = lg R$) is the calculation for reduction in viability		
PASS	= lg R greater than or equal to 5		
FAIL	= lg R less than 5		
>	greater than		
≥	equal to or greater than		
<	less than		
≤	equal to or less than		