

Study Title:

Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas (Phase 2, step 2)

BS EN 13697:2019

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Lab Ref/Report No.	J001337
Testing Laboratory Site	Microbiological Solutions Limited Gollinrod, Bury, BL9 5NB
Company owner	Angela Davies, Managing director
Report Date	17/02/2020
Period of Analysis	10/02/2020-12/02/2020

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	Q002020/1

Name of product	Microbe Shield
Batch number	8318
Manufacturer / Supplier	Zoono UK & Europe
Storage Conditions	Ambient
Appearance of the Product	Clear Liquid
Preservatives/Actives & Conc. %	Quaternary Ammonium Compound CAS 68424-85-1 <1%
Method	BS EN 13697:2019
Neutraliser/Inactivator	N6
Product diluent	Distilled Water
Test Concentrations	100%, 50%, 0.1%
Experimental Conditions	Clean
Interfering substances	Clean - Bovine Albumin 0.3 g/l
Test Temperature	20°C ± 1°C
Temperature of Incubation	Bacteria - 37°C ± 1°C for 24hr to 48hrs Fungal - 30°C ± 1°C for 24hr to 48hrs
Identification of the reference strains	<i>Pseudomonas aeruginosa</i> ATCC 15442 <i>Staphylococcus aureus</i> ATCC 6538 <i>Enterococcus hirae</i> ATCC 10541 <i>Escherichia coli</i> ATCC 10536 <i>Aspergillus brasiliensis</i> ATCC 16404 <i>Candida albicans</i> ATCC 10231
Contact times	Bacteria – 5 min ± 10s Fungi - 15min ± 10s

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Introduction

The standard method BS EN 13697 describes a surface test method for establishing whether a product proposed as a disinfectant in the specified fields has or does not have bactericidal and/or fungal activity on non-porous surfaces.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, organisms etc) reflect parameters which are found in practical situations including conditions which may influence the action of disinfectants. Each use concentration found from this test corresponds to defined experimental conditions.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types.

However, for some applications the recommendations of use of a product can differ and therefore additional test conditions may need to be used.

Outline of method (Obligatory test conditions)

A test suspension of bacteria or fungi in a solution of interfering substances is inoculated onto a test stainless steel surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The surface is maintained at a specified temperature for a defined period of time (5 minutes for bacteria, 15 minutes for fungi). The surface is transferred to a previously validated neutralization medium so that the action of the disinfectant is immediately neutralized. The number of surviving organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria or fungi on a surface treated with hard water in place of the disinfectant is also determined and the reduction in viable counts attributed to the product is the calculated difference. This test used *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus hirae* as standard organisms for bactericidal activity, *Candida albicans* for yeasticidal activity and *Candida albicans* and *Aspergillus brasiliensis* for fungicidal activity

Deviation from Standard Method

None

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Acceptance Criteria

The product when tested according to the standard shall demonstrate at least a 4 log₁₀ reduction in viable bacterial counts; and at least a 3 log₁₀ reduction in viable fungal counts.

Conclusion

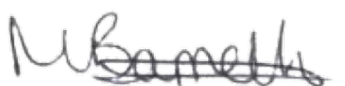
The product **Microbe Shield** has **passed** the test according to the acceptance criteria as outlined in the standard for **bacterial isolates**, when tested under **clean** conditions with a contact time of **5 minutes** at a concentration of **neat**.

The product **Microbe Shield** has **achieved a >3 log reduction** against **C.albicans**, when tested under **clean** conditions with a contact time of **15 minutes** at concentrations of **neat** and **50%**.

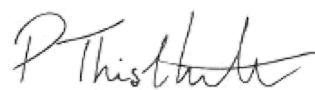
The product **Microbe Shield** has **failed** to achieve a 3-log reduction against, when tested under **clean** conditions with a contact time of **15 minutes** at concentration of **neat**.

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

The test results on this report refer only to the items tested as supplied by the customer. This report shall not be reproduced except in full and with written approval of Microbiological Solutions Ltd. All reports are archived for a minimum of 2 years.

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Bacterial Test Isolates

Test Organism	Validation test			Water control	Test Procedure at Concentrations (V/V)		
	Suspension: N	NT	NC		Neat	50%	0.10%
<i>Pseudomonas aeruginosa</i> ATCC 15442	10 ⁷ : >330 ; >330 10 ⁸ : 40 ; 59 N : 8.09 Validated? Yes	10 ⁴ : 61 ; 43 NT : 6.72 Validated? Yes	10 ⁴ : 20 ; 21 NC : 6.31 Validated? Yes	10 ⁴ : 35 ; 30 Nc : 6.51 Nts : 7 Validated? Yes	10 ⁰ : 9 ; 8 Nd : < 2.15 Nts : 0 ME : > 4	10 ¹ : 90 ; 76 Nd : 3.92 Nts : 0 ME : 2.59	10 ³ : 216 ; 230 Nd : 6.35 Nts : 29 ME : 0.16
<i>Escherichia coli</i> ATCC 10536	10 ⁶ : 223 ; 200 10 ⁷ : 21 ; 15 N : 6.72 Validated? Yes	10 ⁴ : 52 ; 42 NT : 6.67 Validated? Yes	10 ⁴ : 52 ; 42 NC : 6.67 Validated? Yes	10 ⁴ : 46 ; 35 Nc : 6.61 Nts : 330 Validated? Yes	10 ⁰ : 0 ; 0 Nd : < 2.15 Nts : 0 ME : > 4	10 ⁰ : 0 ; 0 Nd : < 2.15 Nts : 0 ME : > 4	10 ⁴ : 39 ; 34 Nd : 6.56 Nts : 330 ME : 0.05
<i>Staphylococcus aureus</i> ATCC 6538	10 ⁶ : 294 ; 281 10 ⁷ : 25 ; 23 N : 6.85 Validated? Yes	10 ⁴ : 88 ; 69 NT : 6.89 Validated? Yes	10 ⁴ : 75 ; 73 NC : 6.87 Validated? Yes	10 ⁴ : 84 ; 62 Nc : 6.86 Nts : 330 Validated? Yes	10 ⁰ : 3 ; 0 Nd : < 2.15 Nts : 0 ME : > 4	10 ⁰ : 10 ; 7 Nd : < 2.15 Nts : 0 ME : > 4	10 ⁴ : 62 ; 51 Nd : 6.75 Nts : 330 ME : 0.11
<i>Enterococcus hirae</i> ATCC 10541	10 ⁶ : >330 ; >330 10 ⁷ : 36 ; 33 N : 6.94 Validated? Yes	10 ⁴ : 49 ; 43 NT : 6.66 Validated? Yes	10 ⁴ : 109 ; 102 NC : 7.02 Validated? Yes	10 ⁴ : 80 ; 72 Nc : 6.88 Nts : 330 Validated? Yes	10 ⁰ : 0 ; 0 Nd : < 2.15 Nts : 0 ME : > 4	10 ⁰ : 7 ; 2 Nd : < 2.15 Nts : 0 ME : > 4	10 ³ : 52 ; 35 Nd : 5.64 Nts : 92 ME : 1.24

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Fungal Test Isolates

Test Organism	Suspension: N	Validation test		Water control	Test Procedure at Concentrations % (V/V)		
		NT	NC		Neat	50%	0.1%
<i>Candidia albicans</i> NCPF 03179	10 ⁶ : >330 ; >330	10 ⁴ : 120 ; 119	10 ⁴ : 96 ; 93	10 ⁴ : 101 ; 97	10 ⁰ : 0 ; 0	10 ⁰ : 1 ; 0	10 ³ : 52 ; 35
	10 ⁷ : 31 ; 26			Nc : 7.00	Nd : < 2.15	Nd : < 2.15	Nd : 5.64
	N : 6.85	NT : 7.08	NC : 6.98	Nts : 19	Nts : 0	Nts : 0	Nts : 29
	Yes	Validated? Yes	Validated? Yes	Validated? Yes	ME : > 3	ME : > 3	ME : 1.36
<i>Aspergillus brasiliensis</i> ATCC 16404	10 ⁵ : 198 ; 192	10 ³ : 126 ; 116	10 ³ : 115 ; 110	10 ³ : 113 ; 113	10 ¹ : 36 ; 26	10 ¹ : 32 ; 30	10 ³ : 116 ; 115
	10 ⁶ : 18 ; 17			Nc : 6.05	Nd : 3.49	Nd : 3.49	Nd : 6.06
	N : 5.68	NT : 6.08	NC : 6.05	Nts : 113	Nts : 4	Nts : 6	Nts : 165
	Yes	Validated? Yes	Validated? Yes	Validated? Yes	ME : 2.56	ME : 2.56	ME : -0.01

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KeyN : Log₁₀ number of cfu of spike inoculum.NT : Log₁₀ number of recovered cfu per test surface of neutralisation test.NC : Log₁₀ number of recovered cfu per test surface of neutralisation control.Nc : Log₁₀ number of recovered cfu per test surface of the water control.

Nts : Number of cfu remaining on the test surface.

Nd : Log₁₀ number of recovered cfu per test surface from test samples.10^x: Dilution Factor.

ME : Microbicidal Effect (Nc - Nd).

V/V – Volume/volume dilution.

Green highlight – Concentration showed target log reduction or greater.

Red highlight – Concentration showed lower than target log reduction.