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Mycobactericidal Activity of Clinell Formulations Using Modified European Standard Test Method BS EN 14348:2005.

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Project Report Prepared for Gama Healthcare LTD.

University of HUDDERSFIELD

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Mycobactericidal Activity of Clinell Formulations Using Modified European Standard Test Method BS EN 14348:2005.

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Tests Carried Out By:	**Hygiene and Disinfection Centre** School of Applied Sciences University of Huddersfield Queensgate, Huddersfield HD1 3DH
Test Method	British/European Standard BS EN 14348:2005. Dilution neutralisation approach
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Disinfectant	Clinell Universal Wipes formulation
Interfering Substance (Organic Challenge)	Simulated clean: 0.3 g l^{-1} bovine albumin (final concentration)
Temperature	20^{o}C
Contact Time Tested	2 and 5 minutes.
Test Organisms	*Mycobacterium bovis* var. BCG NCTC 5692
Culture Medium	Middlebrook 7H10 agar with OADC supplement, Difco Ltd.
Incubation	Plates were incubated at 37ºC with a 5% CO_2 atmosphere for 21 days.
Diluent	MRD, Lab M
Neutraliser	15g/l Saponin, 15g/l Tween 80 (Polysorbate 80), 10g/l granular Lecithin and 2.5g/l Sodium Dodecyl (Laurel) Sulphate

1 Introduction

Clinell Universal Wipes formulation was submitted for the following analysis:

- Mycobactericidal activity employing BS EN14348[1] under clean conditions at contact times of 2 and 5 minutes.
- *Mycobacteirum bovis* var. BCG (NCTC 5692) was used in place of the standard test organisms (*M. terrae, M.avium).*

1.1 Product

The product was used as provided.

2 Test Procedures

2.1 BS EN14348

The test was carried out as specified by BS EN14348[1] (Appendix 1). Briefly this involves the preparation of a suspension of test organisms containing 1.5 – 5.0 x 10^9 cells ml^{-1} of *Mycobacterium bovis* var. BCG*.* In order to carry out the test 1 ml of interfering substance (0.3 gl^{-1} Bovine Serum Albumin (BSA)) was pipetted into a Universal bottle, followed by 1 ml of the desired mycobacterial suspension. The mixture was mixed and left for 2 minutes at 20°C, after which 8 ml of product was added and mixed. The reaction mixture was then left for 2 minutes at 20°C, after this contact time a 1 ml sample was transferred to a tube containing 8 ml of neutraliser and 1 ml of water and left for a further 5 minutes at 20°C. A second aliquot was then taken from the reaction mixture after 5 minutes and treated in the same manner. The neutralisation mixture was then plated onto Middlebrook 7H10 agar with OADC supplement and incubated at 37ºC and a 5% CO_2 atmosphere for 21 days. For increased accuracy 10^{-2} dilutions of the neutralisation mixture were also plated onto the same media. Following incubation the fraction of surviving organisms was noted and a log reduction factor calculated. In addition to the test procedure outlined above a range of validations were performed to ensure the validity of the test (Appendix 1 and 2). In addition, a second test was carried out with a test suspension of 1.5 – 5.0 x 10^6 cells ml^{-1}, being the minimum load required to generate a 3 Log reduction.

2.1.1 Requirements of this standard

The product was tested to a modified version of the standard at the customer's request, and as such the requirements of the standard were provided by Gama Healthcare ltd. In which a > 3 Log reduction was required against the selected test strain within 2 minutes with either initial test inoculum under simulated clean conditions.

3 Results and Conclusions

The BS EN 14348 results (Tables 1 and 2, Appendix 2) show that Clinell Universal Wipes formulation was capable of generating a >3 Log reduction within 2 minutes

(Tables 1 & 2). When challenged with an initial mycobacterial load of 8 Log of organisms (Table 1) the product was also able to generate a >6 Log reduction in viable *M. bovis* BCG.

Product	Performance at 2 minutes	Performance at 5 minutes
Clinell Universal Wipes formulation	Pass >6Log^{Rdn}	Pass >6Log^{Rdn}
Log^{Rdn} - Log$_{10}$ reduction		

Table 1. BS EN14348 Results with an initial inoculum of 1.5-5x10^{9} cfu ml^{-1}

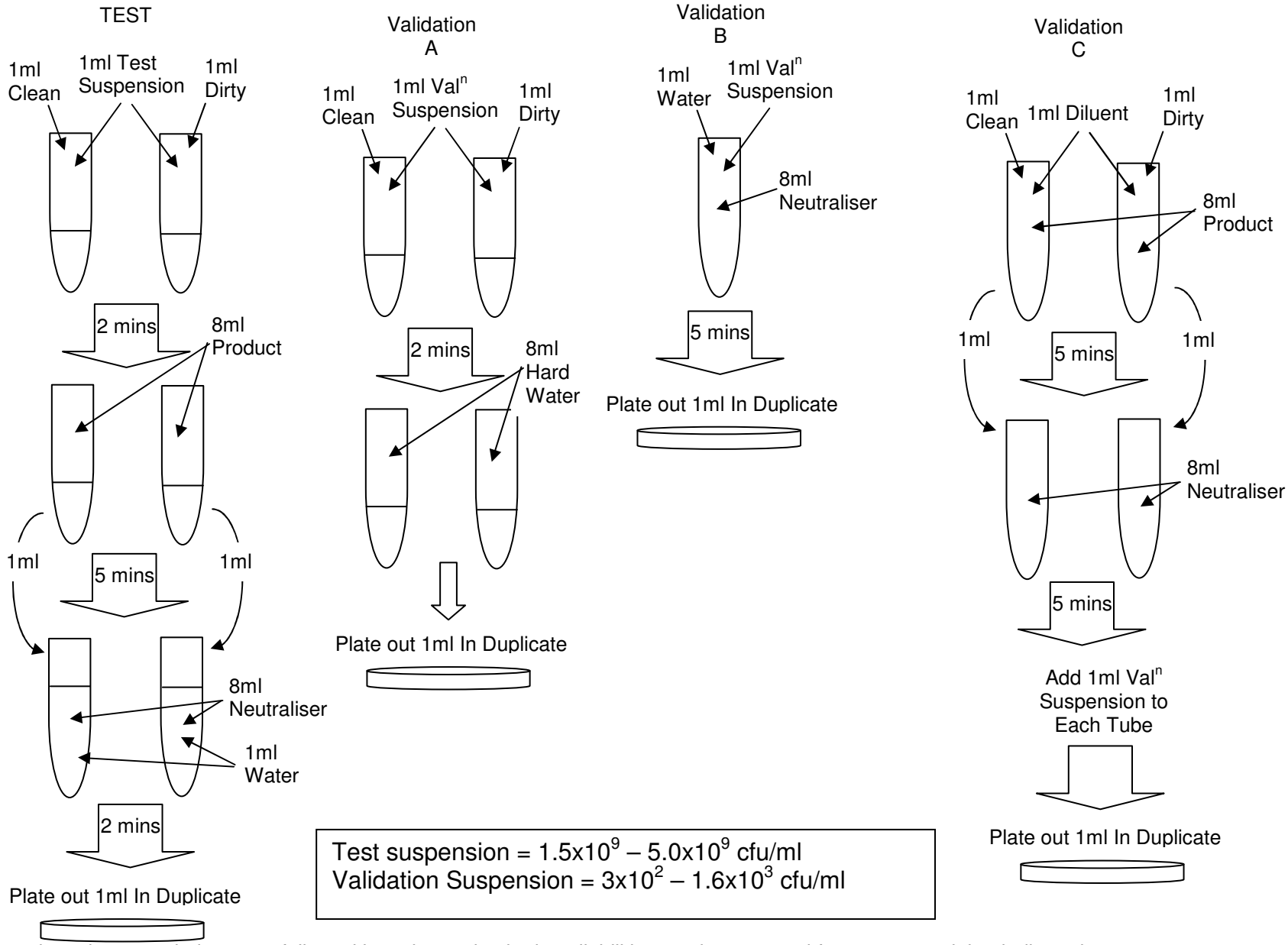
Product	Performance at 2 minutes	Performance at 5 minutes
Clinell Universal Wipes formulation	Pass >3Log^{Rdn}	Pass >3Log^{Rdn}
Log^{Rdn} - Log$_{10}$ reduction		

Table 2. BS EN14348 Results with an initial inoculum of 1.5-5x10^{6} cfu ml^{-1}

4 References

1. BSI (2005) *BSEN 14348:2005. Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants — Test method and requirements (phase 2, step 1).* British Standards Institute, London.

BSEN 14348 Flow Sheet.



Test suspension = $1.5x10^9 – 5.0x10^9$ cfu/ml
Validation Suspension = $3x10^2 – 1.6x10^3$ cfu/ml

Whilst these analyses have been carried out carefully and have been checked, no liabilities can be accepted for consequential or indirect damages.

Appendix 2 Results:

Test Organism	VALIDATIONS									Bacterial Test Suspension			10^9 test Procedure Results					
	Bacterial Suspension			Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control						2 minutes		5 minutes	
M.bovis		50	49	Vc	39	41	Vc	50	41	Vc	50	42	10-7	189	176	Vc <	1.40E+01 1.40E+01	Vc <	1.40E+01 1.40E+01
UK Universal													10-8	19	19	Na <	1.4E+02	Na <	1.4E+02
	Nv	5.0E+02		A	4.0E+01		B	4.6E+01		C	4.6E+01		N	1.8E+09		R >	1.3E+06	R >	1.3E+06

Verification of Methodology		Passed
N is between 1.5E+9 cfu/ml and 5E+9 cfu/ml, N =	1.8E+09	Yes
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	5.0E+02	Yes
CLEAN A ≥ 0.5 N_{vo}=	4.0E+01	Yes
B≥ 0.5 N_{vo}=	4.6E+01	Yes
CLEAN C ≥ 0.5 N_{vo}=	4.6E+01	Yes

Log10 Reductions/cfu/ml	2 min	5 min
10^9Load	6.116754	6.116754
10^6Load	3.116754	3.116754

10^6 test Procedure Results					
	2 minutes		5 minutes	
Vc <	1.40E+01 1.40E+01	Vc <	1.40E+01 1.40E+01		
Na <	1.4E+02	Na <	1.4E+02		
R >	1.3E+03	R >	1.3E+03		

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