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Microbiological Report

RM-6D523-A

06 May 2016

EVALUATION OF E-Klean50 HOCL SOLUTION IN ACCORDANCE WITH BS EN 1276:2009

CONTENTS

For:
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signed_____
on behalf of Donnington Laboratories Ltd

date _____

For:	Mark Staplehurst	Company:	ActivStorm Ltd
By:	John Reed	Date:	06/05/2016
Rept No:	**RM-6D523-A**	**EVALUATION OF E-Klean50 HOCL SOLUTION IN ACCORDANCE WITH BS EN 1276:2009**	

Sample details:

DLL ref	Description	Client Ref	Produced
M-6D523-1	hypochlorous acid		28/04/2106
Declared active system: hypochlorous acid			

Client: ActivStorm Ltd

Date received: 29/04/2016 **Date of test:** 29/04/2016 – 01/05/2016

Storage conditions: 20±2°C in dark

Test method: BS EN 1276: 2009 - *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants & antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase2/step1)*.

Test performed under conditions simulating light and heavy organic soil.

Test organism(s):

Pseudomonas aeruginosa	ATCC 15442	(PA)
Staphylococcus aureus	ATCC 6538	(SA)
Enterococcus hirae	ATCC 10541	(EH)
Escherichia coli	ATCC 10536	(EC)

plus

Salmonella typhimurium	ATCC 14028	(ST)
Escherichia coli 0157	ATCC 700723	(EC 0157)
Listeria monocytogenes	NCTC 7973	(LM)

Organisms derived from Selectrol discs and maintained on Tryptic Soy Agar slopes. Suspensions for experimental purposes prepared from 18h/37°C plate cultures on Tryptic Soy Agar (Neogen).

Suspending medium: Maximum Recovery Diluent (Oxoid)

Interfering substances: 0.3% and 3.0% bovine serum albumen Cohn Factor V

Test product concentration: Neat **Contact time(s):** 60±5s

Test temperature: 20 - 22°C

Neutralising diluent: D/E neutralizing broth (Neogen)

Bacterial enumeration: Tryptone Soy Agar without additional neutralizer(s). Pour plates (1ml) prepared in duplicate at each dilution. Plates incubated aerobically at 37±1°C for 24±2h and re-examined after a further 24±2h incubation at 37±1°C.

Validation: Performed on neat test solution in accordance with BS EN 1276: 2009 sections 5.5.2.3 - 5.5.2.6.

Test product performance:

[A] Bacterial test suspension - cell density (N):

Organism	Target cell density cfu/ml	Actual cell density cfu/ml (N)	Verification	Inoculum level cfu/ml (N_0)
PA	1.50 – 5.00x10e8	2.22x10e08	complies	2.22x10e07
SA		1.96x10e08	complies	1.96x10e07
EH		1.93x10e08	complies	1.93x10e07
EC		1.86x10e08	complies	1.86x10e07
ST		1.65x10e08	complies	1.65x10e07
EC 0157		1.93x10e08	complies	1.93x10e07
LM		2.72x10e08	complies	2.72x10e07

[B] Dilution : Neat - contact time 60±5s - 0.3% albumin:

Test Organism	Inoculum level cfu/ml	cfu/ml recovered after 60s	Log reduction factor	Log reduction factor ≥5.00	% Reduction
PA	2.22x10e7	<1.50x10e02	>5.17	Pass	>99.99
SA	1.96x10e7	<1.50x10e02	>5.12	Pass	>99.99
EH	1.93x10e7	<1.50x10e2	>5.11	Pass	>99.99
EC	1.86x10e07	<1.50x10e2	>5.09	Pass	>99.99
ST	1.65x10e07	<1.50x10e2	>5.04	Pass	>99.99
EC 0157	1.93x10e07	<1.50x10e2	>5.11	Pass	>99.99
LM	2.72x10e07	<1.50x10e2	>5.26	Pass	>99.99

[C] Dilution: Neat - contact time 60±5s – 3.0% albumin:

Test Organism	Inoculum level cfu/ml	cfu/ml recovered after 60s	Log reduction factor	Log reduction factor ≥5.00	% Reduction
PA	2.22x10e7	>3.00x10e05	<1.87	Fail	<99.87
SA	1.96x10e7	>3.00x10e05	<1.82	Fail	<99.85
EH	1.93x10e7	>3.00x10e05	<1.81	Fail	<99.85
EC	1.86x10e07	>3.00x10e05	<1.79	Fail	<99.84
ST	1.65x10e07	>3.00x10e05	<1.74	Fail	<99.82
EC 0157	1.93x10e07	>3.00x10e05	<1.81	Fail	<99.85
LM	2.72x10e07	>3.00x10e05	<1.96	Fail	<99.89

[D] Dilution : 50% in hard water - contact time 60±5s - 0.3% albumin:

Test Organism	Inoculum level cfu/ml	cfu/ml recovered after 60s	Log reduction factor	Log reduction factor ≥5.00	% Reduction
PA	2.22x10e7	<1.50x10e02	>5.17	Pass	>99.99
SA	1.96x10e7	9.95x10e02	4.29	Fail	>99.99
EH	1.93x10e7	1.60x10e2	5.08	Pass	>99.99
EC	1.86x10e07	<1.50x10e2	>5.09	Pass	>99.99
ST	1.65x10e07	1.80x10e2	4.96	Fail	>99.99
EC 0157	1.93x10e07	<1.50x10e2	>5.11	Pass	>99.99
LM	2.72x10e07	3.60x10e2	4.88	Fail	>99.99

[E] Dilution: 50% in hard water - contact time 60±5s – 3.0% albumin:

Test Organism	Inoculum level cfu/ml	cfu/ml recovered after 60s	Log reduction factor	Log reduction factor ≥5.00	% Reduction
PA	2.22x10e7	>3.00x10e05	<1.87	Fail	<99.87
SA	1.96x10e7	>3.00x10e05	<1.82	Fail	<99.85
EH	1.93x10e7	>3.00x10e05	<1.81	Fail	<99.85
EC	1.86x10e07	>3.00x10e05	<1.99	Fail	<99.84
ST	1.65x10e07	>3.00x10e05	<1.74	Fail	<99.82
EC 0157	1.93x10e07	>3.00x10e05	<1.81	Fail	<99.85
LM	2.72x10e07	>3.00x10e05	<1.96	Fail	<99.89

[F] Validation:

	Test strain	Validation suspension cfu/ml (Nv)	Experimental conditions control cfu/ml (A)	A ≥0.05Nv
Experimental Conditions	PA	2.22x10e03	2.19x10e02	complies
	SA	1.96x10e03	1.88x10e02	complies
	EH	1.93x10e03	2.00x10e02	complies
	EC	1.86x10e03	1.81x10e02	complies
	ST	1.65x10e03	1.47x10e02	complies
	EC 0157	1.93x10e03	1.93x10e02	complies
	LM	2.72x10e03	2.85x10e02	complies

	Test strain	Validation suspension cfu/ml (Nv)	Neutraliser toxicity control cfu/ml (B)	B ≥0.05Nv
Neutraliser toxicity	PA	2.22x10e03	1.95x10e02	complies
	SA	1.96x10e03	2.04x10e02	complies
	EH	1.93x10e03	1.99x10e02	complies
	EC	1.86x10e03	1.95x10e02	complies
	ST	1.65x10e03	1.61x10e02	complies
	EC 0157	1.93x10e03	2.00x10e02	complies
	LM	2.72x10e03	2.86x10e02	complies

	Test strain	Validation suspension cfu/ml (Nv)	Neutralisation-dilution control cfu/ml (C)	C ≥0.05Nv
Neutralisation-dilution	PA	2.22x10e03	2.12x10e02	complies
	SA	1.96x10e03	1.94x10e02	complies
	EH	1.93x10e03	1.86x10e02	complies
	EC	1.86x10e03	1.98x10e02	complies
	ST	1.65x10e03	1.57x10e02	complies
	EC 0157	1.93x10e03	2.03x10e02	complies
	LM	2.72x10e03	2.72x10e02	complies

Interpretation of results:

Pass: product achieves a reduction in viability of ≥1.0x10e5 (log reduction factor of ≥5.00) within 5m±5s at 20±1°C when the test organisms are Pseudomonas aeruginosa ATCC 15442, Escherichia coli ATCC 10536, Staphylococcus aureus ATCC 6538 and Enterococcus hirae ATCC 10541.

Fail: product fails to achieve a reduction in viability of ≥1.0x10e5 (log reduction factor of <5.00) within 5m±5s at 20±1°C when the test organisms are Pseudomonas aeruginosa ATCC 15442, Escherichia coli ATCC 10536 , Staphylococcus aureus ATCC 6538 and Enterococcus hirae ATCC 1054.

Summary for Activstorm E-Klean50 hypochlorous acid solution

Test strain	**BS EN 1276: 2009 **** Test method and requirements (phase2/step1)			
	R value – neat solution		R value – 50% dilution	
	BSA 0.3%	BSA 3.0%	BSA 0.3%	BSA 3.0%
Pseudomonas aeruginosa ATCC 15442	Passes	Fails	Passes	Fails
Staphylococcus aureus ATCC 6538	Passes	Fails	Fails	Fails
Enterococcus hirae ATCC 10541	Passes	Fails	Passes	Fails
Escherichia coli ATCC 10536	Passes	Fails	Passes	Fails
Salmonella typhimurium ATCC 14028	Passes	Fails	Fails	Fails
Escherichia coli 0157 ATCC 700723	Passes	Fails	Passes	Fails
Listeria monocytogenes NCTC 7973	Passes	Fails	Fails	Fails

****** BS EN 1276: 2009: *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants & antiseptics used in food, industrial, domestic and institutional areas*

Overall conclusion

When tested at neat concentration in the presence of 0.3% albumin, Activstorm E-Klean50 HOCL solution achieved the required 5 log reduction in the viability of all seven test organisms within 60±5s at 20 - 22°C. However, after dilution to 50% in hard water, the product failed to achieve the required reductions against Staphylococcus aureus, Listeria monocytogenes and Salmonella typhimurium.

At the higher albumin level, the necessary reductions in viability were not achieved in the case of all test organisms at both neat and 50% dilution.

It is well established that organic materials and food residues decrease the antibacterial effectiveness of chlorine and that, in order to achieve effective disinfection, chlorine-based sanitisers should be used on clean or previously cleaned surfaces only as the final stage of an appropriate cleaning and sanitisation regime.

The above results support the use of the neat hypochlorous acid solution as a terminal sanitiser for use in the catering/food environment as part of an appropriate cleaning regime.