Test Report: EN 1276:2019

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1)

Identification of the test laboratory: Abbott Analytical Ltd
Unit 2, Hickmans Road, Birkenhead, CH41 1JH, United Kingdom

Identification of the client: Centrego Ltd
The Coach House, Newbury, Frome, BA11 3RG, United Kingdom

Identification of the sample: 20B/170

Name of the product:	Toucan ECA Solutions
Batch number/reference and expiry date (if available):	N/A
Date of delivery:	11 February 2020
Storage conditions:	Room temperature in darkness
Product diluent recommended by the manufacturer for use:	Not disclosed
Active substance(s) and their concentrations (s) (optional):	A solution of Hypochlorous acid generated using a single activation of Centrego's Toucan Electrochemical Activation (ECA) system. Active substances: HOCl and NaOCl.
Appearance of the product:	Clear colourless liquid

Notes:

1) The test results in this report relate only to the sample(s) tested.
2) This test report may not be reproduced except in full, adapted, altered or used to create a derivative work, without written approval from Abbott Analytical Ltd.
3) Re-issue of report CEG.20B170.IB, dated 06 March 2020.
Reason: Addition of active substances and their method of production.

Test method and its validation:

Method:	Dilution-neutralisation
Neutraliser:	30.0 g/l Polysorbate 80 + 5.0 g/l Lecithin + 1.0 g/l L-histidine + 1.0 g/l L-cysteine (Neutraliser A)
Neutraliser validation:	Validated in accordance with EN 1276:2019 (5.5.2)

Experimental conditions:

Period of analysis:	03 March 2020 to 05 March 2020
Product test concentration(s):	Neat
Diluent used for product test solution(s):	N/A
Contact time(s):	1 min ± 10 s
Test temperature(s):	20°C ± 1°C
Interfering substance:	0.3 g/l bovine albumin (clean conditions)
Temperature of incubation:	36°C ± 1°C
Identification of the bacterial strain(s) used:	*Pseudomonas aeruginosa* (NCIMB 10421) *Escherichia coli* (NCTC 10418) *Staphylococcus aureus* (NCTC 10788) *Enterococcus hirae* (NCIMB 8192)

Deviations: None

Remarks:

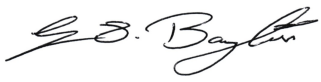
1) All test conditions are as requested by the client, irrespective of whether these are in accordance with EN 1276:2019 (5.4.2) or EN 1276:2019 (5.5.1.1).
2) Products can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

Requirements:

The product shall demonstrate at least a 5 decimal log (lg) reduction against every test organism.

Conclusion:

According to EN 1276:2019, this sample of Toucan ECA Solutions possesses bactericidal activity against all of the referenced strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*, when tested neat with a contact time of 1 minute at 20°C under clean conditions.

Report re-issued by:		**Approved by:**	
Signed:		Signed:	
Name:	Tony Watson	Name:	Gareth Bayliss
Position:	General Manager	Position:	Laboratory Manager
Date:	13 March 2020	Date:	13 March 2020

Results: EN 1276:2019

RST 002 (Issue 4)

Test organism:	*Pseudomonas aeruginosa*		(NCIMB 10421)
Date of test:	03 March 2020		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	A	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *Neat*		
*Vc*1	*87*	$\overline{n}$ =	*Vc*1	*90*	$\overline{n}$ =	*Vc*1	*91*	$\overline{n}$ =	*Vc*1	*101*	$\overline{n}$ =
*Vc*2	*94*	90.5	*Vc*2	*87*	88.5	*Vc*2	*93*	92	*Vc*2	*94*	97.5
$30 \le \overline{n}$ of $Nv_o \le 160$?			$\overline{n}$ of $A \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $B \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $C \ge 0.5 \times \overline{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*>330*	*>330*	$\overline{n}$ wm = 4.15×10^8 ;	lg *N* =	8.62
10^{-7}	*39*	*44*	$N_o = N / 10$;	lg N_o =	7.62
			$7.17 \le \lg N_o \le 7.70$?	☒ yes	☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\overline{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
Neat	*1 min*	*0*	*0*	<140	<2.15	>5.47

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Results: EN 1276:2019

RST 002 (Issue 4)

Test organism:	*Escherichia coli*		(NCTC 10418)
Date of test:	03 March 2020		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	A	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *Neat*		
*Vc*1	*109*	$\bar{n}$ =	*Vc*1	*104*	$\bar{n}$ =	*Vc*1	*120*	$\bar{n}$ =	*Vc*1	*113*	$\bar{n}$ =
*Vc*2	*117*	113	*Vc*2	*111*	107.5	*Vc*2	*110*	115	*Vc*2	*107*	110
$30 \le \bar{n}$ of $Nv_o \le 160$?			$\bar{n}$ of $A \ge 0.5 \times \bar{n}$ of Nv_o ?			$\bar{n}$ of $B \ge 0.5 \times \bar{n}$ of Nv_o ?			$\bar{n}$ of $C \ge 0.5 \times \bar{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*276*	*274*	$\bar{n}$ wm = 2.74×10^8 ;	lg *N* =	8.44
10^{-7}	*27*	*25*	$N_o = N / 10$;	lg N_o =	7.44
			$7.17 \le \lg N_o \le 7.70$?	☒ yes	☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\bar{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
Neat	*1 min*	*0*	*0*	<140	<2.15	>5.29

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Results: EN 1276:2019

RST 002 (Issue 4)

Test organism:	*Staphylococcus aureus*		(NCTC 10788)
Date of test:	03 March 2020		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	A	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *Neat*		
*Vc*1	*54*	$\bar{n}$ =	*Vc*1	*61*	$\bar{n}$ =	*Vc*1	*47*	$\bar{n}$ =	*Vc*1	*51*	$\bar{n}$ =
*Vc*2	*50*	52	*Vc*2	*50*	55.5	*Vc*2	*53*	50	*Vc*2	*50*	50.5
$30 \le \bar{n}$ of $Nv_o \le 160$?			$\bar{n}$ of $A \ge 0.5 \times \bar{n}$ of Nv_o ?			$\bar{n}$ of $B \ge 0.5 \times \bar{n}$ of Nv_o ?			$\bar{n}$ of $C \ge 0.5 \times \bar{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{n}$ wm = 3.60×10^8 ; lg *N* = 8.56
10^{-6}	*>330*	*>330*	$N_o = N / 10$; $\lg N_o$ = 7.56
10^{-7}	*38*	*34*	$7.17 \le \lg N_o \le 7.70$? ☒ yes ☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\bar{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
Neat	*1 min*	*0*	*0*	<140	<2.15	>5.41

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Results: EN 1276:2019

RST 002 (Issue 4)

Test organism:	*Enterococcus hirae*		(NCIMB 8192)
Date of test:	03 March 2020		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	A	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *Neat*		
*Vc*1	*107*	$\overline{n}$ =	*Vc*1	*109*	$\overline{n}$ =	*Vc*1	*104*	$\overline{n}$ =	*Vc*1	*103*	$\overline{n}$ =
*Vc*2	*113*	110	*Vc*2	*117*	113	*Vc*2	*98*	101	*Vc*2	*102*	102.5
$30 \leq \overline{n}$ of $Nv_o \leq 160$?			$\overline{n}$ of $A \geq 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $B \geq 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $C \geq 0.5 \times \overline{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*>330*	*>330*	$\overline{n}$ wm = 4.75×10^{8} ;	lg *N* =	8.68
10^{-7}	*46*	*49*	$N_o = N / 10$;	lg N_o =	7.68
			$7.17 \leq \lg N_o \leq 7.70$?	☒ yes	☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\overline{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
Neat	*1 min*	*0*	*0*	<140	<2.15	>5.53

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Explanations:

Vc	count per ml (one plate or more)
$\overline{n}$	average of *Vc*1 and *Vc*2 (1 + 2 duplicate)
$\overline{n}$ wm	weighted mean of $\overline{n}$
N	number of cells per ml in the test suspension
N_o	number of cells in the test mixture at the beginning of the contact time ($N_o = N / 10$)
Na	number of survivors per ml in the test mixture at the end of the contact time (before neutralisation or filtration)
R	reduction ($\lg R = \lg N_o - \lg Na$)
Nv	number of cells per ml in the validation suspension
Nv_o	number of cells in the validation mixtures at the beginning of the contact time ($Nv_o = Nv / 10$)
A	number of survivors per ml in the experimental conditions control mixture
B	number of survivors per ml in the neutraliser or filtration control mixture
C	number of survivors per ml in the method validation mixture

All test results have an associated uncertainty of measurement, details of which are available on request.

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Test Report

Product name:	Toucan Eco		
Batch or ref no:			
Manufacturer or supplier:	Centrego Ltd The Coach House, Newbury, Frome, BA11 3RG		
Sample ref:	18A/071	**Date received:**	23 January 2018
Date tested:	24 January 2018	**Certificate date:**	26 January 2018
Certificate no:	18A.071SB.CEG	**Page:**	1 of 7
Analysis required:	EN 13697:2015, 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 - Test method and requirements without mechanical action (phase 2, step 2)		
Storage conditions:	Room temperature in darkness		
Appearance of product (solution):	Clear colourless liquid		
Active substance(s) and their concentration(s):	A solution of Hypochlorous acid generated using a single activation of Centrego's Toucan Electrochemical Activation (ECA) system		

Notes

The test results in this report relate only to the sample(s) tested.
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Experimental conditions

Concentration(s) of product tested:	Neat as received
Product diluent:	N/A
Test organism(s):	*Pseudomonas aeruginosa* (DSM 939) *Escherichia coli* (NCTC 10418) *Staphylococcus aureus* (NCTC 10788) *Enterococcus hirae* (DSM 3320)
Contact time(s):	1 min ± 5 s
Test temperature:	Between 18 °C ± 1 °C and 25 °C ± 1 °C (room temperature)
Test conditions:	Clean
Interfering substance:	0.3 g/l bovine albumin
Method:	Dilution-neutralisation
Neutralising solution:	30 g/l Polysorbate 80 + 3g /l Lecithin + 1 g/l L-histidine + 1 g/l L-cysteine
Incubation temperature:	36 °C ± 1 °C

Remarks

The standard EN 13697:2015 method requires that clean conditions of 8.5 g/l reconstituted milk should be used for *Pseudomonas aeruginosa*, with 0.3 g/l bovine albumin for the other three obligatory organisms.

However, in line with current ECHA guidance and the requirements of EN 14885:2015, 0.3 g/l bovine albumin was used for all four organisms in this test.

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Conclusion

When tested neat this sample of Toucan Eco meets the requirements of EN 13697:2015 for bactericidal activity in 1 minute at 20 °C, under clean conditions, against the referenced strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus hirae*.

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Results: *Pseudomonas aeruginosa* (DSM 939)

Validation and controls:

Bacterial test suspension (*N*)			Neutralizer toxicity control (*NC*)			Method validation (*NT*)		
	Vc1	*Vc2*		*Vc1*	*Vc2*		*Vc1*	*Vc2*
10^{-6}	*232*	*228*	10^{-3}	*201*	*199*	10^{-3}	*207*	*204*
10^{-7}	*27*	*24*	10^{-4}	*30*	*27*	10^{-4}	*26*	*29*
			10^{-5}	*2*	*1*	10^{-5}	*2*	*2*
$\bar{\kappa}$(wm) = 5.81 x10^6 lg = 6.76			$\bar{\kappa}$(wm) = 2.08 x10^6 lg = 6.32			$\bar{\kappa}$(wm) = 2.12 x10^6 lg = 6.33		
6.57 ≤ lg *N* ≤ 7.10 ? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NC)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NT)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*		
Control of weighted mean counts (*N*)			Quotient = 9.02 Between 5 and 15 ? ☒ *yes* ☐ *no*					

Water control:

Nc	*Vc1*	*Vc2*	$\bar{\kappa}$(wm) = 2.07 x10^6 lg *Nc* = 6.32 lg *Nc* ≥ 6.27 ? ☒ *yes* ☐ *no*
10^{-3}	*197*	*202*	
10^{-4}	*26*	*31*	
10^{-5}	*1*	*4*	
Ntw	*>100*		

Test:

Product test conc.	Contact time	Diln. step	*Vc1*	*Vc2*	*Nd* = $\bar{\kappa}$(wm) x10 lg *Nd* =	lg *R* = (lg *Nc* − lg *Nd*)	Status
Neat	*1 min*	10^{0}	*0*	*0*	< 2.15	> 4.17	*PASS*
		10^{-1}	*0*	*0*			
		10^{-2}	*0*	*0*			
		Nts	*0*				

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Results: *Escherichia coli* (NCTC 10418)

Validation and controls:

Bacterial test suspension (*N*)			Neutralizer toxicity control (*NC*)			Method validation (*NT*)		
	Vc1	*Vc2*		*Vc1*	*Vc2*		*Vc1*	*Vc2*
10^{-6}	*184*	*196*	10^{-3}	*186*	*222*	10^{-3}	*190*	*181*
10^{-7}	*20*	*22*	10^{-4}	*23*	*19*	10^{-4}	*21*	*20*
			10^{-5}	*1*	*2*	10^{-5}	*0*	*3*
$\bar{\kappa}$(wm) = 4.80 x10^6 lg = 6.68			$\bar{\kappa}$(wm) = 2.05 x10^6 lg = 6.31			$\bar{\kappa}$(wm) = 1.87 x10^6 lg = 6.27		
6.57 ≤ lg *N* ≤ 7.10 ? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NC)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NT)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*		
Control of weighted mean counts (*N*)			Quotient = 9.05 Between 5 and 15 ? ☒ *yes* ☐ *no*					

Water control:

Nc	*Vc1*	*Vc2*
10^{-3}	*190*	*179*
10^{-4}	*20*	*22*
10^{-5}	*3*	*3*
Ntw	*>100*	

$\bar{\kappa}$(wm) = 1.87 x10^6
lg *Nc* = 6.27
lg *Nc* ≥ 6.27 ? ☒ *yes* ☐ *no*

Test:

Product test conc.	Contact time	Diln. step	*Vc1*	*Vc2*	*Nd* = $\bar{\kappa}$(wm) x10 lg *Nd* =	lg *R* = (lg *Nc* − lg *Nd*)	Status
Neat	*1 min*	10^{0}	*0*	*0*	< 2.15	> 4.12	*PASS*
		10^{-1}	*0*	*0*			
		10^{-2}	*0*	*0*			
		Nts	*0*				

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Results: *Staphylococcus aureus* (NCTC 10788)

Validation and controls:

Bacterial test suspension (*N*)			Neutralizer toxicity control (*NC*)			Method validation (*NT*)		
	Vc1	*Vc2*		*Vc1*	*Vc2*		*Vc1*	*Vc2*
10^{-6}	*256*	*218*	10^{-3}	*230*	*229*	10^{-3}	*231*	*238*
10^{-7}	*28*	*32*	10^{-4}	*28*	*31*	10^{-4}	*23*	*30*
			10^{-5}	*4*	7	10^{-5}	*2*	*5*
$\bar{\kappa}$(wm) = 6.07 x10^6 lg = 6.78			$\bar{\kappa}$(wm) = 2.35 x10^6 lg = 6.37			$\bar{\kappa}$(wm) = 2.37 x10^6 lg = 6.38		
6.57 ≤ lg *N* ≤ 7.10 ? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NC)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*			$\bar{\kappa}$ *(NT)* ≥ 0.5 x $\bar{\kappa}$ *(Nc)*? ☒ *yes* ☐ *no*		
Control of weighted mean counts (*N*)			Quotient = 7.90 Between 5 and 15 ? ☒ *yes* ☐ *no*					

Water control:

Nc	*Vc1*	*Vc2*
10^{-3}	*240*	*224*
10^{-4}	*28*	*26*
10^{-5}	*2*	*4*
Ntw	*>100*	

$\bar{\kappa}$(wm) = 2.35 x10^6
lg *Nc* = 6.37
lg *Nc* ≥ 6.27 ? ☒ *yes* ☐ *no*

Test:

Product test conc.	Contact time	Diln. step	*Vc1*	*Vc2*	*Nd* = $\bar{\kappa}$(wm) x10 lg *Nd* =	lg *R* = (lg *Nc* − lg *Nd*)	Status
Neat	*1 min*	10^{0}	*18*	*20*	2.28	4.09	*PASS*
		10^{-1}	*2*	*4*			
		10^{-2}	*0*	*0*			
		Nts	*2*				

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Results: *Enterococcus hirae* (DSM 3320)

Validation and controls:

Bacterial test suspension (*N*)			Neutralizer toxicity control (*NC*)			Method validation (*NT*)		
	Vc1	*Vc2*		*Vc1*	*Vc2*		*Vc1*	*Vc2*
10^{-6}	*280*	*252*	10^{-3}	*253*	*269*	10^{-3}	*266*	*259*
10^{-7}	*26*	*24*	10^{-4}	*30*	*27*	10^{-4}	*28*	*31*
			10^{-5}	*3*	*6*	10^{-5}	*3*	*3*
$\bar{\kappa}$(wm) = 6.61 x10^6 lg = 6.82			$\bar{\kappa}$(wm) = 2.63 x10^6 lg = 6.42			$\bar{\kappa}$(wm) = 2.65 x10^6 lg = 6.42		
6.57 ≤ lg *N* ≤ 7.10 ? ☒ *yes* ☐ *no*			$\bar{\kappa}$ (*NC*) ≥ 0.5 x $\bar{\kappa}$ (*Nc*)? ☒ *yes* ☐ *no*			$\bar{\kappa}$ (*NT*) ≥ 0.5 x $\bar{\kappa}$ (*Nc*)? ☒ *yes* ☐ *no*		
Control of weighted mean counts (*N*)			Quotient = 10.64 Between 5 and 15 ? ☒ *yes* ☐ *no*					

Water control:

Nc	*Vc1*	*Vc2*
10^{-3}	*272*	*248*
10^{-4}	*32*	*29*
10^{-5}	*2*	*5*
Ntw	*>100*	

$\bar{\kappa}$(wm) = 2.64 x10^6
lg *Nc* = 6.42
lg *Nc* ≥ 6.27 ? ☒ *yes* ☐ *no*

Test:

Product test conc.	Contact time	Diln. step	*Vc1*	*Vc2*	*Nd* = $\bar{\kappa}$(wm) x10 lg *Nd* =	lg *R* = (lg *Nc* − lg *Nd*)	Status
Neat	*1 min*	10^{0}	*27*	*21*	2.38	4.04	*PASS*
		10^{-1}	*4*	*3*			
		10^{-2}	*1*	*0*			
		Nts	*2*				

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Study Title:
Quantitative suspension test for evaluation of virucidal activity in the medical area (Phase 2 Step1)

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

Angela Davies, CEO

Customer: Centrego Ltd
Contact name: Robin Turner
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Address: Unit N4, Marshall Way, Commerce Park, Frome
PO/Quote number: Q003263
Report date: 07/08/2020
Issue number: 1

Megan Barrett
Laboratory Manager

Peter Thistlethwaite
Technical Projects Manager

Scope

The standard method BS EN 14476 describes a test method and the minimum requirements for virucidal activity of a chemical disinfectant and antiseptic products that form a homogenous physically stable preparation when diluted with hard water – or in the case of ready to use products that are not diluted when applied, - with water. Products can only be tested at a concentration of 80% (97% with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substances. This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, instrument disinfection by immersion, surface disinfection by wiping, spraying, flooding or other means and textile disinfection.

This European standard applies to areas and situations where disinfection is medically indicated. Such indication occurs in patient care, for example: In hospitals, in community medical facilities and in dental institutions or in clinics of schools, of kindergartens and of nursing homes, and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for patients.

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 water in the case of ready to use products is added to a test suspension of viruses in a solution of interfering substance. The mixture is maintained at one of the temperatures and contact times specified in the standard. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilutions of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Käber or by plaque counting. Reduction of virus infectivity is calculated from differences of lg virus titres before (virus control) and after treatment with the product. The standard minimum spectrum of test organisms is Poliovirus, Adenovirus and Murine Norovirus.

Acceptance Criteria

The product when tested as above shall demonstrate at least a 4 $\log_{10}$ reduction against the test virus. The test is deemed valid where all control requirements are met.

Test information		Deviation
Name of Product	Toucan Solution Hypochlorous Acid & Sodium Hypochlorite mix	
Batch Number & Expiry Date	N/S	
Date of Delivery	16/07/2020	
Period of Analysis	24/07/2020-29/07/2020	
Manufacturer / Supplier	Centrego Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Clear liquid	
Neutralisation Method	Dilution/Filtration	
Product Diluent	Distilled water	
Test Concentrations	500ppm (as calibrated), 200ppm, 100ppm	
Experimental Conditions	Clean	
Interfering Substance	Clean 0.3g/l Bovine Albumin	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Viral Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-1508	
Contact Times	1 minute $\pm$ 5s and 5 minutes $\pm$ 10s	
Stability and Appearance During Test	No Change Observed	

Deviations from Standard Method

There were no deviations from the standard method

Test Result Summary

The test product received has achieved a 4-log reduction against Vaccinia virus, when tested under the condition stipulated in this report.

See page 2 for acceptance criteria and raw data tables below for complete test results.

Microbiological Solutions Ltd
Gollinrod
Walmersley
Bury, BL9 5NB

Tel: 0844 824 6003
Email: info@mls.io
Web: www.msl.io
Company number: 4218514

Summary

Controls					
Conditions	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Control validation**
Virus control (water)	N/A	5 minutes	7.17	N/A	Validated
Cytotoxicity (product)	500ppm	N/A	2.50	N/A	Validated
Product supression control	500ppm	500ppm	7.00	0.17	Validated
Reference virus inactivation (formaldyehyde)	1.4%	5 minutes	4.67	2.50	Validated
Reference virus inactivation (formaldyehyde)	1.4%	15 minutes	4.00	3.17	Validated
Cytotoxicity (formaldehyde)	1.4%	N/A	2.50	N/A	Validated

Controls					
Conditions	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Control validation**
Virus control (water)	N/A	1 minutes	7.21	N/A	Validated

Interference controls					
Condition	**Concentration**	**Contact time**	**log TCID50**	**Log difference**	**Control validation**
Interference control (untreated)	N/A	N/A	7.92	N/A	N/A
Interference control (treated)	500ppm	N/A	7.83	0.08	Validated

Test Results					
Condition	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Pass/Fail**
Test product	500ppm	5 minutes	2.67	>4	Pass
Test product	200ppm	5 minutes	2.71	>4	Pass
Test product	100ppm	5 minutes	2.88	>4	Pass

Test Results					
Condition	**Concentration**	**Contact time**	**log TCID50**	**log reduction**	**Pass/Fail**
Test product	500ppm	1 minutes	2.63	>4	Pass
Test product	200ppm	1 minutes	2.88	>4	Pass
Test product	100ppm	1 minutes	3.25	3.96	Fail

Raw data

Virus control (water)				Contact time		5 minutes		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	2	2	2	2	0.5	0.25
-8	1	1	1	1	0	0	0.16666667	0.138889
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.67
n	8
SD50	-7.17
SE	0.24
xp	-6

Cytotoxicity (product)				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Product supression control				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	1	1	1	2	2	0.375	0.234375
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.50
n	8
SD50	-7.00
SE	0.22
xp	-6

Interference control (untreated)				Product concentration			Neat	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	2	2	2	1	0	1	0.33333333	0.222222
-9	1	1	0	0	0	0	0.08333333	0.076389
-10	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.4167
n	10
SD50	-7.917
SE	0.1822
xp	-7

Raw data

Interference control (treated)				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	4	4	4	4	1	0
-8	2	2	1	1	0	0	0.25	0.1875
-9	1	1	0	0	0	0	0.08333333	0.076389
-10	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.3333
n	10
SD50	-7.833
SE	0.1712
xp	-7

Reference virus inactivation (formaldyehyde)					Contact time		5 minutes	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	3	3	3	3	4	4	0.83333333	0.138889
-5	2	2	2	1	1	0	0.33333333	0.222222
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	2.17
n	8
SD50	-4.67
SE	0.23
xp	-3

Reference virus inactivation (formaldyehyde)					Contact time		15 minutes	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	2	2	2	1	1	2	0.41666667	0.243056
-5	1	1	0	0	0	0	0.08333333	0.076389
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.50
n	8
SD50	-4.00
SE	0.21
xp	-3

Cytotoxicity (formaldehyde)								
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Raw data

Test product		Product concentration			500ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	1	1	1	0	0	0.16666667	0.138889
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.17
n	8
SD50	-2.67
SE	0.14
xp	-2

Test product		Product concentration			200ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	1	2	1	0	0	0.20833333	0.164931
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.21
n	8
SD50	-2.71
SE	0.15
xp	-2

Test product		Product concentration			100ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	1	1	0	0	0	0	0.08333333	0.076389
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.08
n	8
SD50	-3.58
SE	0.10
xp	-3

Raw data

Virus control (water)				Contact time		1 minutes		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	3	2	2	1	2	2	0.5	0.25
-8	2	1	1	1	0	0	0.20833333	0.164931
-9	0	0	0	0	0	0	0	0

Organism *Feline Coronavirus* Strain Munich	
d	1
sum px	1.71
n	8
SD50	-7.21
SE	0.24
xp	-6

Test product		Product concentration			500ppm	Contact time		1 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	1	1	0	0	0	0.125	0.109375
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Feline Coronavirus* Strain Munich	
d	1
sum px	1.13
n	8
SD50	-2.63
SE	0.13
xp	-2

Test product		Product concentration			200ppm	Contact time		1 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	2	2	2	2	0.66666667	0.222222
-4	1	1	0	0	0	0	0.08333333	0.076389
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Feline Coronavirus* Strain Munich	
d	1
sum px	1.75
n	8
SD50	-3.25
SE	0.21
xp	-2

Test product		Product concentration			100ppm	Contact time		1 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	2	1	1	1	0	0	0.20833333	0.164931
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Feline Coronavirus* Strain Munich	
d	1
sum px	1.21
n	8
SD50	-3.71
SE	0.15
xp	-3

KEY

CPE	Cytopathic effect
Counts	0-4 indicating degree of cytopathic effect
	0 = No effect, 1 = 25% CPE, 2 = 50% CPE, 3 = 75% CPE, 4 = 100% CPE
d	Dilution factor (log)
Sum px	Sum of % CPE from the highest dilution showing 100% CPE to the lowest dilution assessed.
n	Number of dilutions
SD50	Dilution showing 50% of the end point according to Spearman-Kärber method
SE	Standard error
xp	Lowest dilution showing 100% CPE
TCID50	Titre causing 50% of the end point according to Spearman-Kärber
PASS	= lg R greater than or equal to 4
FAIL	= lg R less than 4

>	greater than	≥	equal to or greater than
<	less than	≤	equal to or less than

Calculation notes
In cases where the highest dilution assessed has not shown 100% CPE, the value has been calculated assuming the dilution above this would give 100% CPE and the corresponding value has been assigned as <x.

The standard requires the product suppression control to show a <0.5 log reduction in viral titre. In cases where the product has failed to achieve the required 4 log reduction, but the product suppression control shows a >0.5 log reduction the result has been deemed as valid for fail as the consequence of inadequate suppression would be a partially extended contact time which would generate false positives, but not false negatives.
A similar approach has been taken in regards to the cytotoxicity controls. The standard requires a 4-log difference between the cytotoxicity level and the viral titre. In cases where this is not obtained, but the log reduction observed by the product is within the difference between the cytotoxicity levels and the viral titre the result is deemed acceptable for a fail as there will be no impact on the determination of efficacy.

Study Title:
Chemical disinfectants and antiseptics – Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area -Test method and requirements (phase 2/step 2)

Microbiological Solutions Limited (MSL)
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Angela Davies, CEO

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PO/Quote number: Q003263
Report date: 07/08/2020
Issue number: 1

Megan Barrett
Laboratory Manager

Peter Thistlethwaite
Technical Projects Manager

Scope

The standard method BS EN 16777 describes a test method and the minimum requirements for virucidal activity of a chemical disinfectant and antiseptic products that form a homogenous physically stable preparation when diluted with hard water – or in the case of ready to use products that are not diluted when applied, - with water as some dilution is always produced by adding the test organisms and interfering substances.
This European Standard applies to products that are used in the medical area for disinfection of non-porous surfaces including surfaces surfaces of medical devices without medical action.

This European standard applies to areas and situations where disinfection is medically indicated. Such indication occurs in patient care, for example:

- In hospitals, in community medical facilities and in dental institutions;
- In clinics of schools, of kindergartens and of nursing homes.

and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for patients.

Outline of Test Method (Obligatory Test Conditions)

A test suspension of viruses in a solution of interfering substances is inoculated onto a test surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The test surface is maintained at a specified temperature for a defined period. The test surface is transferred to cell maintenance medium so that the action of the disinfectant is immediately neutralized. The titre of the virus recovered from the test surface is determined. The titre of the inoculum on a test surface treated with hard water in place of the disinfectant is also determined and the reduction in virus titre attributed to the product is calculated by difference.

The standard minimum spectrum of test organisms is Adenovirus and Murine Norovirus. For activity against enveloped viruses Vaccinia virus is tested.

Acceptance Criteria
The product shall be deemed to have passed the test if it demonstrates a 4 lg or more reduction in titre for adenovirus and murine norovirus at the specific contact time chosen at between 18°C ± 1°C and 25°C ± 1°C, with the chosen interfering substance under the conditions defined by the test.

Test information		Deviation
Name of Product	Toucan Solutions Hypochlorous Acid & Sodium Hypochlorite	
Batch Number & Expiry Date	N/S	
Date of Delivery	16/07/2020	
Period of Analysis	23/07/2020-29/07/2020	
Manufacturer / Supplier	Centrego Ltd	
Storage Conditions	Ambient	
Appearance of the Product	Clear liquid	
Neutraliser	Dilution	
Neutralisation Method	Dilution	
Product Diluent	Distilled water	
Test Concentrations	500ppm (as calibrated), 200ppm, 100ppm	
Experimental Conditions	Clean	
Interfering Substance	Clean - 0,3 g/l bovine serum albumin	
Test Temperature	20°C ± 1°C	
Temperature of Incubation	37°C ±1°C	
Identification of the Bacterial Strains:	Modified vaccinia virus Ankara (MVA), ATCC VR-1508	
Contact Times	5 minutes ± 10s	
Stability and Appearance During Test	No Change Observed	

Deviations from Standard Method

There were no deviations from the standard method

Test Result Summary

The test product received has achieved a 4-log reduction against Vaccinia virus, when tested under the condition stipulated in this report.

See page 2 for acceptance criteria and raw data tables below for complete test results.

Microbiological Solutions Ltd
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Web: www.msl.io
Company number: 4218514

Summary

Controls					
Conditions	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (water)	N/A	5 minutes	6.92	N/A	Validated
Cytotoxicity (product)	N/A	N/A	2.50	N/A	Validated
Product supression control	500ppm	500ppm	6.96	-0.04	Validated
Reference virus inactivation (Glutardialdehyde)	500ppm	5 minutes	4.21	2.71	Validated
Cytotoxicity (Glutardialdehyde)	500ppm	N/A	2.50	N/A	Validated

Controls					
Conditions	Concentration	Contact time	log TCID50	log reduction	Control validation
Virus control (water)	N/A	1 minute	7.13	N/A	Validated

Interference controls					
Condition	Concentration	Contact time	log TCID50	Log difference	Control validation
Interference control (untreated)	N/A	N/A	7.79	N/A	N/A
Interference control (treated)	500ppm	N/A	7.58	0.21	Validated

Test Results					
Condition	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	500ppm	5 minutes	2.50	>4	Pass
Test product	200ppm	5 minutes	2.88	>4	Pass
Test product	100ppm	5 minutes	2.84	>4	Pass

Test Results					
Condition	Concentration	Contact time	log TCID50	log reduction	Pass/Fail
Test product	500ppm	1 minute	2.63	>4	Pass
Test product	200ppm	1 minute	2.88	>4	Pass
Test product	100ppm	1 minute	3.23	3.98	Fail

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Company number: 4218514

Raw data

Virus control (water)				Contact time		5 minutes		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	1	1	2	1	0.375	0.234375
-8	1	0	0	0	0	0	0.04166667	0.039931
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.42
n	8
SD50	-6.92
SE	0.20
xp	-6

Cytotoxicity (product)				Product concentration			N/A	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Product supression control				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	1	1	2	0	0.33333333	0.222222
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.46
n	8
SD50	-6.96
SE	0.22
xp	-6

Interference control (untreated)				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	3	3	4	4	4	4	0.91666667	0.076389
-8	2	2	1	0	1	1	0.29166667	0.206597
-9	1	1	0	0	0	0	0.08333333	0.076389
-10	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	2.2917
n	10
SD50	-7.792
SE	0.1998
xp	-6

Raw data

Interference control (treated)				Product concentration			500ppm	
Dilution	Counts						% CPE	p(1-p)
-1	4	4	4	4	4	4	1	0
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	4	4	2	2	2	2	0.66666667	0.222222
-8	2	2	2	1	1	0	0.33333333	0.222222
-9	1	1	0	0	0	0	0.08333333	0.076389
-10	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	2.0833
n	10
SD50	-7.583
SE	0.2406
xp	-6

Reference virus inactivation (Glutardialdehyde)				Contact time			5 min	
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	3	3	3	2	3	1	0.625	0.234375
-5	1	1	0	0	0	0	0.08333333	0.076389
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.71
n	8
SD50	-4.21
SE	0.21
xp	-3

Cytotoxicity (Glutardialdehyde)								
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

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Raw data

Test product		Product concentration			500ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	0	0	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.00
n	8
SD50	-2.50
SE	0.00
xp	-2

Test product		Product concentration			200ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	1	2	2	1	1	0.33333333	0.222222
-4	1	0	0	0	0	0	0.04166667	0.039931
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.38
n	8
SD50	-2.88
SE	0.19
xp	-2

Test product		Product concentration			100ppm	Contact time		5 minutes
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	3	3	3	3	2	2	0.66666667	0.222222
-4	1	1	0	0	0	0	0.08333333	0.076389
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.75
n	8
SD50	-3.25
SE	0.21
xp	-2

Raw data

Virus control (water)				Contact time		1 minute		
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	4	4	4	4	4	4	1	0
-5	4	4	4	4	4	4	1	0
-6	4	4	4	4	4	4	1	0
-7	2	2	2	2	2	2	0.5	0.25
-8	1	1	1	0	0	0	0.125	0.109375
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.63
n	8
SD50	-7.13
SE	0.23
xp	-6

Test product		Product concentration			500ppm	Contact time		1 minute
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	1	1	1	0	0	0	0.125	0.109375
-4	0	0	0	0	0	0	0	0
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.13
n	8
SD50	-2.63
SE	0.13
xp	-2

Test product		Product concentration			200ppm	Contact time		1 minute
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	2	4	4	2	2	4	0.75	0.1875
-4	1	1	0	0	0	0	0.08333333	0.076389
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.83
n	8
SD50	-3.33
SE	0.19
xp	-2

Test product		Product concentration			100ppm	Contact time		1 minute
Dilution	Counts						% CPE	p(1-p)
-2	4	4	4	4	4	4	1	0
-3	4	4	4	4	4	4	1	0
-4	2	2	1	1	0	1	0.29166667	0.206597
-5	0	0	0	0	0	0	0	0
-6	0	0	0	0	0	0	0	0
-7	0	0	0	0	0	0	0	0
-8	0	0	0	0	0	0	0	0
-9	0	0	0	0	0	0	0	0

Organism *Vacciniavirus* ATTC VR-1508	
d	1
sum px	1.29
n	8
SD50	-3.79
SE	0.17
xp	-3

KEY

KEY

CPE	Cytopathic effect		
Counts	0-4 indicating degree of cytopathic effection 0 = No effect, 1 = 25% CPE, 2 = 50% CPE, 3 = 75% CPE, 4 = 100% CPE		
d	Dilution factor (log)		
Sum px	Sum of % CPE from the highest dilution showing 100% CPE to the lowest dilution assessed.		
n	Number of dilutions		
SD50	Dilution showing 50% of the end point according to Spearman-Kärber method		
SE	Standard error		
xp	Lowest dilution showing 100% CPE		
TCID50	Titre causing 50% of the end point according to Spearman-Kärber		
PASS	= lg R greater than or equal to 4		
FAIL	= lg R less than 4		
>	greater than	≥	equal to or greater than
<	less than	≤	equal to or less than

Calculation notes

In cases where the highest dilution assessed has not shown 100% CPE, the value has been calculated assuming the dilution above this would give 100% CPE and the corresponding value has been assigned as <x.

The standard requires the product suppression control to show a <0.5 log reduction in viral titre. In cases where the product has failed to achieve the required 4 log reduction, but the product suppression control shows a >0.5 log reduction the result has been deemed as valid for fail as the consequence of inadequate suppression would be a partially extended contact time which would generate false positives, but not false negatives.

A similar approach has been taken in regards to the cytotoxicity controls. The standard requires a 4-log difference between the cytotoxicity level and the viral titre. In cases where this is not obtained, but the log reduction observed by the product is within the difference between the cytotoxicity levels and the viral titre the result is deemed acceptable for a fail as there will be no impact on the determination of efficacy.

Testing of an Air Treatment Machine based on the methodology of NF T 72-281

Client Details:	Eco-Mist Biotechnics Ltd, Unit A2, Mainline Ind Est Milnthorpe
Client Contact Name:	Eric Warren
Client Email:	Eric.warren@eco-mist-biotechnics.com
Date Of Report:	14/10/18
MelBec Reference Number:	7784
No. of samples:	1

Sample Details:

Name of Product:	Toucan
Batch Number:	-
Manufacturer / Supplier:	Eco-mist
Product Storage conditions:	Ambient in the dark
Appearance of the Product (as supplied):	-
Active Substance and concentration:	-
Product Dilutions/Concentrations and Diluent:	Ready to Use

Date Product Received: 10/10/18 **Date Tested: 10/10/18**

Experimental Conditions:

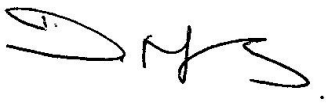
Interfering Substance:	Not applicable
Test Temperature:	20˚C
Contact Time:	One cycle of the machine
Test Organisms:	*Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 *Bacillus subtilis* spores NCTC 10400
Incubation Temperature:	36˚C
Neutraliser:	N1 Broth (5ml)

Conclusion:

There was no recovery of the vegetative cells from the discs after one cycle of the machine with the test product.

The spores gave a mean reduction of 4 lg after one cycle of the machine with the test product.

Testing carried out by:	Report authorised by:
Danika Weatherburn	Dawn Mellors
Laboratory Manager	Technical Director



Test Results

Organism	N_c (Control) cfu/test surface	N_d (Test) cfu / test surface
Ps. aeruginosa		
Replicate 1	0,0 neat	46,37 -5
Replicate 2	0,0 neat	27,35 -5
Replicate 3	0,0 neat	217, 206 -4
Mean	< 5.00×10^0 < 0.7 lg	1.56×10^7 7.19 lg
St. aureus		
Replicate 1	0,0 neat	29, 44 -6
Replicate 2	0,0 neat	27, 26 -6
Replicate 3	0,0 neat	42, 33 -6
Mean	< 5.00×10^0 < 0.7 lg	1.68×10^8 8.23 lg
E.coli		
Replicate 1	0,0 neat	45, 36 -5
Replicate 2	0,0 neat	48, 51 -5
Replicate 3	0,0 neat	43, 31 -5
Mean	< 5.00×10^0 < 0.7 lg	2.12×10^7 7.33 lg
Enterococcus hirae		
Replicate 1	0,0 neat	160, 166 -5
Replicate 2	0,0 neat	201, 201 -5
Replicate 3	0,0 neat	246, 274 -5
Mean	< 5.00×10^0 < 0.7 lg	1.04×10^8 8.02 lg
Bacillus subtilis *spores*		
Replicate 1	71, 63 neat	41,76 -4
Replicate 2	103, 97 neat	59,43 -4
Replicate 3	1, 7 neat	63,60 -4
Mean	2.85×10^2 2.45 lg	2.85×10^6 6.45 lg

Organism	Mean lgNc	Mean lgNd	Log Reduction
Ps. aeruginosa	7.19	<0.7	>6.49
St. aureus	8.23	<0.7	>7.53
E.coli	7.33	<0.7	>6.63
Enterococcus hirae	8.02	<0.7	>7.32
Bacillus subtilis spores	6.45	2.45	4.00

	Validation cfu/test surface	
	Control	**Test**
Ps. aeruginosa		
Replicate 1	1.30×10^7	2.15×10^7
Replicate 2	2.01×10^7	2.60×10^7
St. aureus		
Replicate 1	1.56×10^6	1.39×10^6
Replicate 2	1.63×10^6	1.51×10^6
E.coli		
Replicate 1	7.65×10^5	7.80×10^5
Replicate 2	8.65×10^5	8.65×10^5
Enterococcus hirae		
Replicate 1	6.2×10^5	8.25×10^5
Replicate 2	7.95×10^5	6.45×10^5
Bacillus subtilis spores		
Replicate 1	1.35×10^6	1.45×10^6
Replicate 2	2.02×10^6	1.78×10^6