

**Report:** SSL.22B059.RR

**Issued:** 14 March 2022

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**Test Report:**

**EN 1500:2013**

Chemical disinfectants and antiseptics – Hygienic handrub –  
Test method and requirements (phase 2, step 2)

**Identification of the test laboratory:**

Abbott Analytical Ltd  
Unit 2, Hickmans Road, Birkenhead, CH41 1JH, Great Britain

**Identification of the client:**

Safe Solutions (Safe4) Ltd  
Wharton Green, Bostock Road, Winsford, CW7 3BD, Great Britain

**Identification of the sample:**

22B/059

Name of the product:

Safe4 Hand Sanitiser

Batch number/reference and  
expiry date (if available):

516

Date of delivery:

08 February 2022

Storage conditions:

Room temperature in darkness

Product diluent recommended by  
the manufacturer for use:

Not disclosed

Active substance(s) and their  
concentrations (s) (optional):

Not disclosed

Appearance of the product:

White foam

**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.

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**Test method and its validation:**

Method: Dilution-neutralisation

Neutraliser: 100.0 g/l Polysorbate 80 + 30.0 g/l Lecithin +  
30.0 g/l Tryptone Soya Broth + 5.0 g/l Sodium thiosulphate +  
1.0 g/l L-histidine (Neutraliser B)

Neutraliser validation: Validated in accordance with EN 1500:2013 (5.5.2)

**Experimental conditions:**

Period of analysis: 11 March 2022 to 14 March 2022

Product test concentration: Neat

Diluent used for product test solution: N/A

Product test volume: 2 pumps of supplied dispenser (1.8 g ± 0.2 g)

Contact time: 60 s ± 5 s

Validation temperature: 20°C ± 1°C

Temperature of incubation: 36°C ± 1°C

Identification of the bacterial strain used: *Escherichia coli* K12 (NCTC 10538)

**Deviations:** None

**Remarks:**

- 1) All test conditions are as requested by the client, irrespective of whether these are in accordance with EN 1500:2013 (5.5.1).
- 2) Test performed with 20 volunteers. One set of results of the RP → PP sequence was invalid and therefore excluded from the calculations.

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**Requirements:**

The mean reduction of the test organism, *Escherichia coli* K12, achieved by product under test shall be at least non-inferior to that achieved by a specified reference hygienic handrub (60% v/v propan-2-ol).

**Conclusion:**

According to EN 1500:2013, it is concluded that this sample of Safe4 Hand Sanitiser is non-inferior to the reference product.

**Approved by:**

Signed:



Name: Tony Watson

Position: General Manager

Date: 14 March 2022

Experimental results - Reference handrub (60% propan-2-ol)						
Volunteer number	Hand left/right	Prevalues			Postvalues	
		10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
1	L	>330	112	264	34	2
	R	>330	72	240	29	4
2	L	>330	91	>330	49	3
	R	>330	86	>330	93	7
3	L	>330	103	>330	47	1
	R	>330	109	>330	60	3
4	L	>330	58	6	0	0
	R	>330	63	4	0	0
5	L	>330	83	5	0	0
	R	>330	46	3	0	0
6	L	>330	72	9	1	0
	R	>330	69	8	1	0
7	L	>330	60	2	0	0
	R	>330	92	0	0	0
8	L	>330	80	3	0	0
	R	>330	160	1	0	0
9	L	>330	80	>330	100	11
	R	>330	80	>330	100	8
10	L	>330	88	>330	53	8
	R	>330	56	>330	66	4
11	L	>330	88	>330	41	1
	R	>330	149	>330	38	2
12	L	>330	94	3	0	0
	R	>330	88	14	1	0
13	L	>330	72	7	1	0
	R	>330	136	17	2	0
14	L	>330	116	4	0	0
	R	>330	62	32	4	0
15	L	>330	58	144	10	0
	R	>330	61	264	30	0
16	L	>330	70	156	19	1
	R	>330	65	232	16	2
17	L	>330	52	108	17	0
	R	>330	45	152	21	0
18	L	>330	70	152	8	0
	R	>330	93	280	23	2
19	L	>330	74	216	15	0
	R	>330	120	240	22	2

Experimental results - Product under test						
Volunteer number	Hand left/right	Prevalues		Postvalues		
		10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>0</sup>	10 <sup>-1</sup>	10 <sup>-2</sup>
1	L	>330	47	1	0	0
	R	>330	36	1	0	0
2	L	>330	43	0	0	0
	R	>330	64	0	0	0
3	L	>330	56	1	0	0
	R	>330	61	1	0	0
4	L	>330	49	0	0	0
	R	>330	53	0	0	0
5	L	>330	100	0	0	0
	R	>330	65	0	0	0
6	L	>330	43	0	0	0
	R	>330	72	0	0	0
7	L	19	3	>330	>330	98
	R	6	0	>330	>330	174
8	L	10	0	>330	>330	164
	R	3	1	>330	>330	280
9	L	>330	48	9	2	0
	R	>330	51	0	0	0
10	L	>330	60	7	1	0
	R	>330	49	0	0	0
11	L	>330	83	6	1	0
	R	>330	40	0	0	0
12	L	>330	44	0	0	0
	R	>330	46	>330	128	8
13	L	>330	36	0	0	0
	R	>330	59	>330	88	18
14	L	>330	59	0	0	0
	R	>330	67	>330	120	12
15	L	>330	73	111	17	1
	R	>330	50	160	23	2
16	L	>330	82	1	0	0
	R	>330	83	216	27	2
17	L	>330	64	108	11	0
	R	>330	97	168	20	3
18	L	>330	76	5	1	0
	R	>330	69	>330	43	4
19	L	>330	69	4	0	0
	R	>330	55	>330	49	2

List of computed lg values (means of left and right hands) and lg reductions							
Volunteer number	Chronological sequence	Reference handrub (RP)			Product under test (PP)		
		lg prevalues	lg postvalues	lg reduction (R)	lg prevalues	lg postvalues	lg reduction (R)
1	RP → PP	7.95	3.41	4.54	7.61	1.00	6.61
2	RP → PP	7.95	3.83	4.12	7.72	1.00	6.72
3	RP → PP	8.03	3.73	4.30	7.77	1.00	6.77
4	RP → PP	7.78	1.69	6.09	7.71	1.00	6.71
5	RP → PP	7.79	1.59	6.20	7.91	1.00	6.91
6	RP → PP	7.85	1.93	5.92	7.75	1.00	6.75
7	RP → PP	7.87	1.15	6.72	6.03	5.12	0.91
8	RP → PP	8.05	1.24	6.81	5.74	5.33	0.41
9	RP → PP	7.90	4.00	3.90	7.69	1.48	6.21
10	PP → RP	7.85	3.77	4.08	7.73	1.42	6.31
11	PP → RP	8.06	3.60	4.46	7.76	1.39	6.37
12	PP → RP	7.96	1.81	6.15	7.65	2.55	5.10
13	PP → RP	8.00	2.04	5.96	7.66	2.49	5.17
14	PP → RP	7.93	2.05	5.88	7.80	2.54	5.26
15	PP → RP	7.77	3.29	4.48	7.78	3.14	4.64
16	PP → RP	7.83	3.28	4.55	7.92	2.17	5.75
17	PP → RP	7.68	3.13	4.55	7.90	3.13	4.77
18	PP → RP	7.91	3.31	4.60	7.86	2.67	5.19
19	PP → RP	7.97	3.35	4.62	7.79	2.65	5.14
$\bar{x}$	Overall	7.90	2.75	5.15	7.57	2.21	5.35
s		0.10	0.98	0.97	0.60	1.32	1.82
n		19	19	19	19	19	19
$\bar{x}$	RP → PP	7.91	2.51	5.40	7.32	1.99	5.33
s		0.10	1.20	1.17	0.82	1.84	2.66
n		9	9	9	9	9	9
$\bar{x}$	PP → RP	7.90	2.96	4.93	7.79	2.42	5.37
s		0.11	0.71	0.75	0.09	0.61	0.59
n		10	10	10	10	10	10
$\bar{x}$	= Mean						
s	= Standard deviation						
n	= Number of values (volunteers)						
				RP → PP	= Sequence: first RP, second PP		
				PP → RP	= Sequence: first PP, second RP		
Difference of mean lg reductions (RP → PP) :      5.40 - 5.33 = 0.07							
Difference of mean lg reductions (PP → RP) :      4.93 - 5.37 = -0.44							
Absolute difference of differences:        (0.07) - (-0.44)   = 0.5 < 2.00 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no							

<b>Computation of individual differences of lg reductions</b>			
<b>Volunteer</b>	<b>Lg reduction (R)</b>		<b>Difference (RP - PP)</b>
	<b>Reference procedure (RP)</b>	<b>Product procedure (PP)</b>	
1	4.54	6.61	-2.07
2	4.12	6.72	-2.60
3	4.30	6.77	-2.47
4	6.09	6.71	-0.62
5	6.20	6.91	-0.71
6	5.92	6.75	-0.83
7	6.72	0.91	5.81
8	6.81	0.41	6.40
9	3.90	6.21	-2.31
10	4.08	6.31	-2.23
11	4.46	6.37	-1.91
12	6.15	5.10	1.05
13	5.96	5.17	0.79
14	5.88	5.26	0.62
15	4.48	4.64	-0.16
16	4.55	5.75	-1.20
17	4.55	4.77	-0.22
18	4.60	5.19	-0.59
19	4.62	5.14	-0.52

Sorting of individual differences and computation for Hodges-Lehmann 97.5% upper confidence limits											
Mean pairwise differences $(d_i + d_{ii} / 2)$											
	6.40	5.81	1.05	0.79	0.62	-0.16	-0.22	-0.52	-0.59	-0.62	-0.71
6.40	6.40										
5.81	6.11	5.81									
1.05	3.73	3.43	1.05								
0.79	3.60	3.30	0.92	0.79							
0.62	3.51	3.22	0.84	0.71	0.62						
-0.16	3.12	2.83	0.44	0.31	0.23	-0.16					
-0.22	3.09	2.80	0.42	0.29	0.20	-0.19	-0.22				
-0.52	2.94	2.65	0.26	0.13	0.05	-0.34	-0.37	-0.52			
-0.59	2.91	2.61	0.23	0.10	0.01	-0.38	-0.41	-0.56	-0.59		
-0.62	2.89	2.60	0.22	0.09	0.00	-0.39	-0.42	-0.57	-0.61	-0.62	
-0.71	2.85	2.55	0.17	0.04	-0.04	-0.44	-0.47	-0.62			
-0.83	2.79	2.49	0.11	-0.02	-0.11	-0.50	-0.53				
-1.20	2.60	2.31	-0.07	-0.21	-0.29						
-1.91	2.25	1.95	-0.43	-0.56							
-2.07	2.17	1.87	-0.51								
-2.23	2.09	1.79	-0.59								
-2.31	2.05	1.75									
-2.47	1.97	1.67									
-2.60	1.90	1.61									
Ranked mean pairwise differences											
1	6.40	11	3.09	21	2.60	31	1.95	41	0.79		
2	6.11	12	2.94	22	2.60	32	1.90	42	0.71		
3	5.81	13	2.91	23	2.55	33	1.87	43	0.62		
4	3.73	14	2.89	24	2.49	34	1.79	44	0.44		
5	3.60	15	2.85	25	2.31	35	1.75	45	0.42		
6	3.51	16	2.83	26	2.25	36	1.67	46	0.31		
7	3.43	17	2.80	27	2.17	37	1.61	47	0.29		
8	3.30	18	2.79	28	2.09	38	1.05				
9	3.22	19	2.65	29	2.05	39	0.92				
10	3.12	20	2.61	30	1.97	40	0.84				

From the table of critical values for Wilcoxon's matched-pairs signed-ranks test, the entry for  $n = 19$  and a one-sided 0.025 level of significance, the critical value of 46 is found. Hence  $c = 46 + 1 = 47$ .

The pairwise differences are sorted in descending order.

The 47th value is 0.29, hence the Hodges Lehmann upper one-sided 97.5% confidence limit for the difference in lg reductions between RP and PP is 0.29, which is less than the agreed inferiority margin of 0.60.

Therefore the hypothesis of the inferiority of PP is rejected and it can be concluded that the test product PP is non-inferior to the reference product RP.



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Results: EN 1500:2013

RST 036a (Issue 2)

Test organism:	<i>Escherichia coli</i> K12	(NCTC 10538)
Date of test:	11 March 2022	Validation temperature: 20°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates: 1 / ml
Neutraliser:	B	Incubation temperature: 36°C ± 1°C

**Validation and controls:**

Validation suspension ( $N_{V_0}$ )			Validation suspension ( $N_{V_B}$ )			Neutraliser or filtration control ( $B$ )			Method validation ( $C$ ) Product conc.:		
Vc1	50	$\bar{x} =$	Vc1	56	$\bar{x} =$	Vc1	44	$\bar{x} =$	Vc1	50	$\bar{x} =$
Vc2	46	48	Vc2	46	51	Vc2	48	46	Vc2	45	47.5
30 ≤ $\bar{x}$ of $N_{V_0}$ ≤ 160 ?			30 ≤ $\bar{x}$ of $N_{V_B} / 1000$ ≤ 160 ?			$\bar{x}$ of $B$ ≥ 0.5 x $\bar{x}$ of $N_{V_0}$ ? (or $N_{V_B} / 1000$ ) ?			$\bar{x}$ of $C$ ≥ 0.5 x $\bar{x}$ of $N_{V_0}$ ?		
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Test suspension ( $N$ ):**

$N$	Vc1	Vc2	$\bar{x}$ wm = 2.83 x 10 <sup>8</sup> ;    lg $N$ = 8.45
10 <sup>-6</sup>	312	264	
10 <sup>-7</sup>	24	23	8.17 ≤ lg $N$ ≤ 8.70 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Explanations:**

$V_c$	count per ml (one plate or more)
$\bar{x}$	average of $V_{c1}$ and $V_{c2}$ (1 + 2 duplicate)
$\bar{x}_{wm}$	weighted mean of $\bar{x}$
$N$	number of cells per ml in the test suspension
$N_0$	number of cells in the test mixture at the beginning of the contact time ( $N_0 = N / 10$ )
$N_a$	number of survivors per ml in the test mixture at the end of the contact time (before neutralisation or filtration)
$R$	reduction ( $\lg R = \lg N_0 - \lg N_a$ )
$N_v$	number of cells per ml in the validation suspension
$N_{v_0}$	number of cells in the validation mixtures at the beginning of the contact time ( $N_{v_0} = N_v / 10$ )
$A$	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.