mgsLABORATORIES Microbiological Services and Consultancy						Doc No.	ENR-2020-257-01	UKAS TESTING 4393
Title	**Microbiological Analysis Based on EN 1500 (2013) Chemical disinfectants and antiseptics – Hygienic handrub (Phase 2 / Step 2)**							
Product	**Antibacterial Hand Gel FO 28-00004**	**MGS No**	**S34010**	**SO No**	**8351**			

a) **Identification of test laboratory:**	
Test laboratory	MGS Laboratories Ltd Unit 2, Merlin Park Airport Service Road Portsmouth Hampshire PO3 5FU
b) **Identification of the Customer:**	
Customer Name	Zidac Laboratories Ltd
Customer Address	Unit 5 Merlin Park Airport Service Road Portsmouth PO3 5FU
c) **Identification of the sample:**	
Name of product	Antibacterial Hand Gel FO 28-00004
Batch number (and expiry date if available)	Not stated
Manufacturer (or supplier)	Zidac Laboratories Ltd
Date of delivery	24 AUG 20
Storage conditions	Room temperature and darkness
Product diluent recommended by the manufacturer for use	N/A
Active substance(s) and their concentration(s) (optional)	Not stated
Appearance of the product	Clear colourless gel
d) **Test method and its validation:**	
MGS procedure reference	WIN-1000.018-09
Method	Dilution neutralisation
Neutraliser	30g/l Tryptone soya broth, Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine1g/l, saponin 30g/l, phosphate buffer powder 0.35g/l
Details of validation of the neutraliser	Neutraliser validation performed according to EN 1276 (2019) and EN 1500 (2013) 5.5.2
e) **Experimental conditions:**	
Date of Test	08 SEP 20

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Diluent used for the product test solution	N/A
Product test concentrations	Ready to Use (RTU)
Appearance of product dilutions	Clear colourless gel
Volume of product applied	6ml
Contact time	60 seconds ± 10s
Frequency of application	Rub-in 3ml for 30s repeat once
Stability and appearance of the mixture	Precipitate absent throughout test
Temperature of incubation	36°C ± 2°C
Identification of the bacterial strain used	*Escherichia coli* K12 NCTC 10538
f) **Results:**	
Test results	See tables: 1-6
g) **Conclusion:**	Based on EN 1500 (2013), the batch supplied of the product Antibacterial Hand Gel FO 28-00004, when tested using 3ml, in 30 seconds with one reapplication, the referenced strain of *E. coli* is not significantly less effective than the reference product; and therefore does conform to the requirements of EN 1500 (2013).
h) **Deviations:**	None
i) Comments:	None

Prepared by:	**Approved by:**
Name: Ruth Robinson	**Name:** Kim Morwood
Position: Customer Services Coordinator	**Position:** Technical Director
Date: 17SEP20	**Date:** 17SEP20
Locality: Hampshire, UK	**Locality: Hampshire, UK**

The MGS procedure WIN-1000.018 is a laboratory method based on the EN 1500 (2013) standard; the minor deviations from the standard, which do not affect the overall results, are detailed below:

- EN 1500 states an allowed tolerance of 36°C ±1°C, MGS laboratories equipment is validated to ±2°C therefore MGS procedures state ±2°C.
- MGS validated the preparation of the Soft hand soap, only the identity, purity and pH tests are performed for every batch.

Preparation: Propan-2-ol
Application: Rub-in 3ml/30s, repeat once
Date of experiment: 08 SEP 20
Test organism: *E. coli* K12 NCTC 10538
Suspension: 2.77 x 10^8 cfu/ml

Table 1: Reference handrub procedure – experimental results

Subject No	Hand	Number of cfu per plate from dilution 10^x						
		Pre values			Post values			
		10^{-3}	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}	10^{-3}
1	L	>330	102	<14	>330	117	<14	<14
	R	>330	167	21	14	<14	<14	<14
2	L	>330	130	14	14	<14	<14	<14
	R	>330	104	<14	36	<14	<14	<14
3	L	125	<14	<14	10	<14	<14	<14
	R	130	14	<14	65	<14	<14	<14
4	L	>330	172	<14	3	<14	<14	<14
	R	>330	151	23	82	<14	<14	<14
5	L	>330	136	<14	70	<14	<14	<14
	R	>330	191	17	>330	28	<14	<14
6	L	>330	96	<14	>330	50	<14	<14
	R	>330	108	<14	>330	134	16	<14
7	L	>330	149	16	>330	102	<14	<14
	R	>330	90	16	>330	108	<14	<14
8	L	>330	>330	37	>330	59	<14	<14
	R	>330	>330	31	>330	306	15	<14
9	L	>330	53	<14	>330	>330	33	<14
	R	>330	113	<14	>330	>330	74	<14
10	L	>330	185	29	3	<14	<14	<14
	R	>330	153	<14	14	<14	<14	<14
11	L	>330	122	16	97	16	<14	<14
	R	>330	133	<14	166	<14	<14	<14
12	L	>330	180	14	>330	>330	37	<14
	R	>330	124	<14	317	36	<14	<14
13	L	>330	>330	38	>330	>330	75	<14
	R	>330	>330	37	>330	242	27	<14
14	L	>330	>330	38	>330	117	<14	<14
	R	>330	>330	37	>330	108	<14	<14

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

15	L	>330	112	18	>330	>330	39	<14
	R	>330	100	<14	>330	225	23	<14
16	L	>330	232	27	>330	>330	317	35
	R	>330	196	20	>330	303	34	<14
17	L	>330	97	15	>330	36	<14	<14
	R	>330	173	17	>330	65	<14	<14
18	L	>330	>330	35	>330	90	<14	<14
	R	>330	>330	60	>330	>330	60	<14

Figures between 14-330cfu are used for calculations. However, for Post value counts where the 10^0 dilution has counts <14, the actual figure is used.

Preparation: Antibacterial Hand Gel FO 28-00004
Application: Rub-in 3ml/30s, repeat once
Date of experiment: 08 SEP 20
Test organism: *E. coli* K12 NCTC 10538
Suspension: 2.77 x 10^8 cfu/ml

Table 2: Handrub procedure with test product – experimental results

Subject No	Hand	Number of cfu per plate from dilution 10^x						
		Pre values			Post values			
		10^{-3}	10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}	10^{-3}
1	L	>330	68	<14	>330	58	<14	<14
	R	>330	150	15	106	<14	<14	<14
2	L	>330	154	17	14	<14	<14	<14
	R	>330	129	15	14	<14	<14	<14
3	L	>330	60	<14	12	<14	<14	<14
	R	148	14	<14	15	<14	<14	<14
4	L	>330	203	23	39	<14	<14	<14
	R	>330	240	24	17	<14	<14	<14
5	L	>330	166	15	18	<14	<14	<14
	R	>330	175	16	154	26	<14	<14
6	L	256	26	<14	>330	192	20	<14
	R	267	14	<14	>330	86	<14	<14
7	L	>330	129	<14	>330	180	<14	<14
	R	>330	151	18	>330	>330	170	15
8	L	>330	152	18	294	29	<14	<14
	R	>330	209	18	>330	104	<14	<14
9	L	>330	219	20	>330	>330	252	34
	R	>330	214	21	>330	>330	81	<14
10	L	>330	103	<14	1	<14	<14	<14
	R	>330	128	<14	5	<14	<14	<14
11	L	>330	282	23	>330	137	<14	<14
	R	>330	148	15	>330	189	<14	<14
12	L	>330	105	19	>330	>330	207	21
	R	>330	76	<14	256	17	<14	<14

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

13	L	>330	157	17	18	<14	<14	<14
	R	>330	226	22	>330	172	<14	<14
14	L	134	<14	<14	>330	>330	116	<14
	R	133	<14	<14	>330	73	<14	<14
15	L	>330	247	29	>330	90	17	<14
	R	>330	160	14	>330	81	<14	<14
16	L	>330	231	26	>330	>330	318	31
	R	>330	45	<14	200	20	<14	<14
17	L	>330	206	21	10	<14	<14	<14
	R	>330	255	24	38	<14	<14	<14
18	L	>330	205	24	>330	>330	84	<14
	R	>330	>330	37	>330	>330	46	<14

Figures between 14-330cfu are used for calculations. However, for Post value counts where the 10^0 dilution has counts <14, the actual figure is used.

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

Table 3: List of computed log values (means of left and right hand) and log reduction factors of the experimental results (tables 1 and 2)

Subject No	Group[1]	Reference			Test Product		
		Log Pre Value	Log Post Value	Log Reduction Factor	Log Pre Value	Log Post Value	Log Reduction Factor
1	1	6.14	2.08	4.06	6.05	2.17	3.87
2	2	6.07	1.40	4.67	6.16	1.15	5.01
3	1	5.39	1.58	3.81	5.27	1.15	4.12
4	2	6.46	1.63	4.83	6.35	1.45	4.90
5	2	6.21	1.95	4.27	6.23	1.97	4.25
6	1	6.01	2.98	3.03	5.41	3.15	2.26
7	2	6.09	3.02	3.07	6.15	4.14	2.01
8	2	6.53	3.26	3.27	6.26	2.55	3.70
9	1	5.92	3.73	2.19	6.33	4.24	2.09
10	2	6.24	0.95	5.29	6.06	0.48	5.59
11	1	6.11	2.12	3.99	6.33	3.21	3.12
12	2	6.18	2.82	3.36	5.98	3.31	2.67
13	2	6.58	3.46	3.12	6.28	2.24	4.05
14	1	6.58	3.05	3.53	5.13	3.24	1.89
15	1	6.04	3.38	2.66	6.31	2.95	3.36
16	2	6.33	4.26	2.07	6.16	3.49	2.67
17	1	6.14	2.71	3.43	6.36	1.38	4.98
18	2	6.68	3.13	3.55	6.35	3.92	2.42
Mean		6.21	2.64	3.57	6.06	2.57	3.50
Standard deviation (s)		0.30	0.90	0.86	0.39	1.13	1.17
Total number of values (N)		18	18	18	18	18	18
Mean (Group 1)		6.04	2.70	3.34	5.90	2.69	3.21
s (Group 1)		0.33	0.73	0.66	0.54	1.04	1.09
N (Group 1)		8	8	8	8	8	8
Mean (Group 2)		6.34	2.59	3.75	6.20	2.47	3.73
s (Group 2)		0.21	1.05	0.99	0.12	1.24	1.24
N (Group 2)		10	10	10	10	10	10

[1] Group one used the Reference Product then the Test Product; Group 2 used the Test product then the Reference Product

NOTE 1: The results relate only to the sample which was tested and cannot be guaranteed to represent the batch from which it was taken.

The absolute difference of mean differences between log reductions of Reference Product and Test Product for Group 1 and Group 2 are less than 2.00: YES

Group 1 mean Reference Product – mean Test Product: 3.34 – 3.21 = 0.13

Group 2 mean Reference Product – mean Test Product: 3.75 – 3.73 = 0.02

Absolute difference of differences: 0.13 – 0.02= 0.11

Results are available from at least 18 volunteers,
The mean log pre-value for each of the Reference and Test products is ≥5.00
No more than three log reduction factors for the Reference product are <3.00,
Therefore, the test is valid.

Table 4: Statistical comparison of values as obtained with the reference and test products (WILCOXON's matched-pairs signed-ranks test)

Subject No	Log reduction factor derived from:		Difference Reference product - Test product
	Reference product	Test product	
1	4.06	3.87	0.19
2	4.67	5.01	-0.34
3	3.81	4.12	-0.31
4	4.83	4.90	-0.08
5	4.27	4.25	0.01
6	3.03	2.26	0.77
7	3.07	2.01	1.06
8	3.27	3.70	-0.43
9	2.19	2.09	0.10
10	5.29	5.59	-0.30
11	3.99	3.12	0.87
12	3.36	2.67	0.69
13	3.12	4.05	-0.92
14	3.53	1.89	1.64
15	2.66	3.36	-0.69
16	2.07	2.67	-0.60
17	3.43	4.98	-1.55
18	3.55	2.42	1.13

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Table 5: Sorting of individual differences and computation of Hodges-Lehmann 97.5% upper confidence limits

Sorted Differences		0	0	0	0	0	0	0	0	0	0	0
1	**1.64**	1.64										
2	**1.13**	0.57	1.13									
3	**1.06**	0.53	0.53	1.06								
4	**0.87**	0.44	0.44	0.44	0.87							
5	**0.77**	0.39	0.39	0.39	0.385	0.77						
6	**0.69**	0.35	0.35	0.35	0.345	0.35	0.69					
7	**0.19**	0.1	0.1	0.1	0.095	0.1	0.1	0.19				
8	**0.1**	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.01			
9	**0.01**	0.01	0.01	0.01	0.005	0.01	0.01	0.01	0.01	-0.1		
10	**-0.08**	-0	-0	-0	-0.04	-0	-0	-0	-0	-0	-0.3	
11	**-0.3**	-0.2	-0.2	-0.2	-0.15	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.3
12	**-0.31**	-0.2	-0.2	-0.2	-0.155	-0.2	-0.2	-0.2	-0.2	-0.2	-0.3	
13	**-0.34**	-0.2	-0.2	-0.2	-0.17	-0.2	-0.2	-0.2	-0.2	-0.3		
14	**-0.43**	-0.2	-0.2	-0.2	-0.215	-0.2	-0.2	-0.2	-0.4			
15	**-0.6**	-0.3	-0.3	-0.3	-0.3	-0.3	-0.3	-0.6				
16	**-0.69**	-0.3	-0.3	-0.3	-0.345	-0.3	-0.7					
17	**-0.92**	-0.5	-0.5	-0.5	-0.46	-0.9						
18	**-1.55**	-0.8	-0.8	-0.8	-1.55							

The differences of the individual log reductions of Reference Product minus Test Product from table 4 are sorted in the second column and in the headline according to their size in descending order

The median is between the 9th and 10th value. The superscript represents the ranks.

The mean pairwise differences that do not exceed the median (9) are computed. From table 6 of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for N=18 and a one-sided 0.025 level of significance, the critical value of 40 is found. Hence c=40+1 = 41. The pairwise differences are sorted in descending order. The 41st value is 0.01. Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in log reductions between the Reference Product and Test Product is 0.01, which is less than the agreed inferiority margin of 0.6. Therefore, the hypothesis of inferiority of Antibacterial Hand Gel FO 28-00004 is rejected and it can be concluded that the test preparation Antibacterial Hand Gel FO 28-00004 is not inferior to the Reference Product.

Table 6: Wilcoxon's matched pairs signed-ranks test

n Number of pairs	**Level of Significance**		
	0.05	**0.025**	**0.01**
18	47	**40**	32
19	53	**46**	37
20	60	**52**	43
21	68	**59**	49
22	**75**	**66**	56

Critical values of the lower of both sums of ranks with (+) or (-) sign at different significance levels used for calculation of the Hodges-Lehmann confidence limits. In this case zero differences are included.

All test results have an associated uncertainty of measurement; for this test the expanded uncertainty is based on the estimated uncertainty multiplied by a coverage factor K=2 providing a level of confidence of approximately 95%. The uncertainty evaluation has been assessed in accordance with MGS laboratories' UKAS Accreditation and is available on request.

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