Test Report: BS EN 14476:2013 + A2:2019 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area- Test method and requirements (Phase 2/Step 1)

Test Laboratory	**BluTest Laboratories Ltd** 5 Robroyston Oval, Nova Business Park, Glasgow, G33 1AP

Identification of sample

Name of the product	COV-RID High-Level Disinfectant
Batch number	102414 DOM 17/12/2020
Client	Knights Security Group Limited
Client Address	Nene House, 4 Rushmills, Northampton, NN4 7YB
Project Code	BT-KSG-01
Date of Delivery	04 January 2021
Storage conditions	Ambient
Active substances	ADBAC & DDAC
Appearance	Liquid
Condition upon receipt	Undamaged

Test Method and its validation

Method	1 part interfering substance + 1 part virus suspension + 8 parts biocide were mixed and incubated at the indicated contact temperature for the indicated contact times. Assays were validated by a cytotoxicity control, interference control, neutralisation control and a formaldehyde internal standard.
Neutralisation	Dilution-neutralisation/gel filtration Eagles Minimum Essential Medium + 5.0% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	22 January 2021 to 27 January 2021
Product diluents used	Sterile distilled water
Product test concentrations	10.0% v/v; 50.0% v/v; 80.0% v/v
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	Sedimentation and turbidity observed at all concentrations
Contact times (minutes)	5 ± 10s
Test temperature	20°C ± 1°C
Interfering substances	0.3g/l bovine albumin
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification and passage (P) of virus	***Vaccinia virus* VR-1549 Elstree strain (P 09)**
Identification and passage (P) of cells	Vero Cells (P 49)

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PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of test product solution and a 5-minute contact time. Virus is exposed to disinfectant in 24-well plates, then neutralised, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose$_{50}$ ($TCID_{50}$) of surviving virus. *Vaccinia virus* VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. $TCID_{50}$ is determined by the method of Karber[1].

Cytotoxicity control
The test product solution is measured for its effects on the host cells used to propagate the virus, to determine the sensitivity of the assay.

Interference control
The effect of the cells after treatment of the test product solution are verified to ensure the cells can show susceptibility for virus infection. This is compared against cells that have not been treated with test product.

Disinfectant suppression control VS1
Virus is added to the highest concentration of test product solution and then the mixture immediately removed and neutralised. The neutralised virus titre is then determined to assess the efficiency of the neutralisation procedure.

Disinfectant suppression control VS2
Internal control which adds virus to neutralised test product solution to assess the efficiency of the neutralisation procedure.

No column Control
Internal control on the highest contact time to assess any impact of the Microspin™ S 400 HR columns.

Virus recovery control
Virus titre is determined for virus in contact with sterile distilled water at t=0, t = 5 and at t =15. The virus titre after 5 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 15 minutes is compared to the reference virus inactivation control.

Reference virus inactivation control
Virus is exposed to 0.7% W/V formaldehyde and the recovery of virus determined by $TCID_{50}$ after 5 and 15 minutes, in order to assess that the test virus has retained reproducible biocide resistance. In addition, the formaldehyde cytotoxicity of neutralised formaldehyde is determined, to measure assay sensitivity.

1Kärber, G.: Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmak. 162 (1931): 480-487.

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Vaccinia virus (VR-1549) Elstree strain Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of COV-RID High Level Disinfectant, Batch 102414 DOM. 17/12/20, BT-KSG-01-01 from Knights Security Group Ltd. against Vaccinia virus VR-1549 under clean conditions						
Test Results						
Concentration	**10%**		**50%**		**80%**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 5 mins	3.67	**1.47E+05**	0.33	**6.81E+01**	0.00	**3.16E+01**
Raw Data	666400	**1.47E+05**	200000	**6.81E+01**	000000	**3.16E+01**
log		**5.17**		**1.83**		**1.50**
log difference		**1.33**		**4.67**		**5.00**

EN14476:2013 + A2:2019 Suspension test for the efficacy of COV-RID High Level Disinfectant, Batch 102414 DOM. 17/12/20, BT-KSG-01-01 from Knights Security Group Ltd. against Vaccinia virus VR-1549 under clean conditions									
Summary Table									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after 'X' Min
				0 min	5 min	15 min	30 min	60 min	
COV-RID High Level Disinfectant	3.0g/l BSA + 3.0ml/l erythrocytes	80%	1.50	3.17	1.50	n.a.	n.a.	n.a.	<5 mins
		50%	1.50	n.a.	1.83	n.a.	n.a.	n.a.	<5 mins
		10%	1.50	n.a.	5.17	n.a.	n.a.	n.a.	>5 mins
Virus Control	Clean			6.50	6.50	6.50	n.a.	n.a.	n.a.
							5 min	15 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				5.00	3.50	>15 mins

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Vaccinia virus (VR-1549) Elstree strain Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of COV-RID High Level Disinfectant, Batch 102414 DOM. 17/12/20, BT-KSG-01-01 from Knights Security Group Ltd. against Vaccinia virus VR-1549 under clean conditions

Controls

Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 15 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2	
raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
5.00	3.16E+06	5.00	3.16E+06	5.00	3.16E+06	0.00	3.16E+01	1.67	1.47E+03	5.00	3.16E+06
666651	3.16E+06	666660	3.16E+06	666651	3.16E+06	000000	3.16E+01	640000	1.47E+03	666660	3.16E+06
	6.50		6.50		6.50		1.50		3.17		6.50
									3.33		0.00

Formaldehyde reference inactivation controls

Cytotoxicity		Exposure time	0.7% Formaldehyde			
			5 mins		15 mins	
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
1.00	3.16E+02		3.50	1.00E+05	2.00	3.16E+03
600000	3.16E+02		666300	1.00E+05	660000	3.16E+03
	2.50	log		5.00		3.50
		log difference		1.50		3.00

No column Control

5 mins	
raw data	$TCID_{50}$/ml
5.33	6.81E+06
666662	6.81E+06
	6.83

Interference control	Virus dilution					
	-3	-4	-5	-6	-7	-8
PBS Control	1	1	1	1	0.33	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	6.76E+01	3.16E+01
	2.50	2.50	2.50	2.50	1.83	1.50
Raw Data	6	6	6	6	2	0
Product	1	1	1	1	0.17	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01
	2.50	2.50	2.50	2.50	1.67	1.50
Raw Data	6	6	6	6	1	0
Log Difference	0.00	0.00	0.00	0.00	0.16	0.00
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
6.50
1.00E+08
6666663000

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CONCLUSION

Verification of the methodology

A test is only valid if the following criteria are fulfilled:

a) The titre of the test suspension of at least 10^8 TCID50 /ml is sufficiently high to at least enable a titre reduction of 4 lg to verify the method.

b) Detectable titre reduction is at least 4 $\log_{10}$.

c) Difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test is between:
   - Between 0.75 and 3.5 after 5 min and between 2.0 and 4.0 after 15 min for Vaccinia virus

d) Cytotoxicity of the product solution does not affect cell morphology and growth or susceptibility for the test virus in the dilutions of the test mixtures which are necessary to demonstrate a 4 $\log_{10}$ reduction of the virus.

e) The interference control result does not show a difference of > 1.0 $\log_{10}$ of virus titre for test product treated cells in comparison to the non-treated cells.

f) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The disinfectant was neutralised by column chromatography through an Illustra Microspin S-400 HR column to achieve the best possible neutralisation available for this test. The difference for virus is greater than 0.5 $\log_{10}$ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v for VS1. This neutralisation validation has been verified by VS2, which shows the product has been successfully neutralised.

According to EN 14476:2013 + A2:2019, **COV-RID High-Level Disinfectant POSSESSES VIRUCIDAL** activity at a concentration of **50.0% v/v and 80.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) against *Vaccinia virus* VR-1549 Elstree strain / Vero cells.

<u>This product therefore is effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019 Annex A*. This therefore includes all coronaviruses and SARS-CoV-2.</u>

Authorised signatory

BluTest Laboratories Ltd Glasgow UK SC364409

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Date: 08 FEBRUARY 2021

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*EN 14476 2013 + A2 2019 Annex A (informative – Enveloped viruses)

Poxviridae
Herpesviridae
Filoviridae (e.g. Ebola, Marburg)
Flavivirus
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Influenza Virus
Paramyxoviridae
Rubella Virus
Measles Virus
Rabies Virus
Coronavirus (e.g. SARS, MERS)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Hepatitis B virus (HBV)

Reference: Van Regenmortel MHV et al.,Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000

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K122160-1 12th December 2020

LABORATORY REPORT

SOURCE: Knights Security Group Ltd

ITEMS: Cov-Rid High Level Disinfectant
Batch: 100212, DOM: 23/10/2020

TESTS: BSEN1650:2019
Concentration: Neat (Ready to use)
Temperature: 20°C
Contact time: 15 minutes
Interfering substance: Bovine Albumin 3.0g/l (dirty)
Storage conditions: Room temperature, out of direct sunlight
Active substances: Not given
Test Date: 8th December 2020

Recovery: Dilution neutralisation, using:-
Tryptone Soya Broth containing Tween 80 100ml/l, Lecithin 30g/l, Sodium thiosulphate 5g/l, L-histidine 1g/l, L-cystine 1g/l

Test organism: Candida albicans ATCC 10231

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Date: 31/8/21

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SUMMARY & CONCLUSIONS:

K122160-1

Organism/Time	Control	Cov-Rid High Level Disinfectant	Log Reduction
Candida albicans ATCC 10231	2.22x10 E6	<10 (<140)	>5.35 (>4.20)

All test results below 140 (1.4x10 E2) are required to be reported as <140.

The sample complies with the criteria of BSEN1650:2019 (log 4 reduction) after 15 minutes contact against Candida albicans, under the test conditions stated.

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Detailed Results K122160-1 Cov-Rid High Level Disinfectant

Candida albicans ATCC 10231

Test Suspension (N + No)

N	Vc1	Vc2		
10^{-5}	208	232	Weighted Mean = 2.22x10 E7	log = 7.35
10^{-6}	22	26	No = N/10 = 2.22x10 E6	log = 6.35

Test (Na)

Vc1	Vc2	mean		
<1	<1	<1	Na = mean x 10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>5.35 (>4.20)

Validation & Controls

Validation Suspension (Nvo)

Vc1	Vc2	mean
54	58	56

Experimental Conditions Control (A)

Vc1	Vc2	mean
54	56	55

Neutraliser Toxicity Control (B)

Vc1	Vc2	mean
50	55	52.5

Dilution Neutralisation Control (C)

Vc1	Vc2	mean
48	58	53

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K123714-5

24th August 2021

LABORATORY REPORT

SOURCE: Knights Security Group Ltd

ITEMS: Cov-Rid High Level Disinfectant

TESTS: BSEN1276:2019
Concentration: Neat (Ready to use)
Temperature: 20°C
Contact time: 5 minutes
Interfering substance: Bovine Albumin 3.0g/l (dirty)
Storage conditions: Room temperature, out of direct sunlight
Active substances: Not Given
Test Date: 21st August 2021

Recovery: Dilution neutralisation, using:-
Tryptone Soya Broth containing Tween 80 100ml/l, Lecithin 30g/l, Sodium thiosulphate 5g/l, L-histidine 1g/l, L-cystine 1g/l

Test organisms:		
	Staphylococcus aureus	ATCC 6538
	Escherichia coli	ATCC 10536
	Pseudomonas aeruginosa	ATCC 15442
	Enterococcus hirae	ATCC 10541

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SUMMARY & CONCLUSIONS:

K123714-5

Organism	Control	Cov-Rid High Level Disinfectant	Log Reduction
Staphylococcus aureus ATCC 6538	2.72x10 E7	<10 (<140)	>6.43 (>5.28)
Escherichia coli ATCC 10536	2.45x10 E7	<10 (<140)	>6.39 (>5.24)
Enterococcus hirae ATCC 10541	2.24x10 E7	<10 (<140)	>6.35 (>5.20)
Pseudomonas aeruginosa ATCC 15442	2.95x10 E7	6.0x10 E1 (<140)	5.69 (>5.32)

All test results below 140 (1.4x10 E2) are required to be reported as <140

The sample complies with the criteria of BSEN1276:2019 (log 5 reduction) after 5 minutes contact, against all four organisms, under the test conditions stated.

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Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Staphylococcus aureus ATCC 6538

Test Suspension (N + No)

N	V_{C1}	V_{C2}		
10^{-6}	266	274	Weighted Mean = 2.72x10 E8	log = 8.43
10^{-7}	27	32	No = N/10 = 2.72x10 E7	log = 7.43

Test (Na)

V_{C1}	V_{C2}	mean		
<1	<1	<1	Na = mean x10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>6.43 (>5.28)

Validation & Controls

Validation Suspension (Nvo)

V_{C1}	V_{C2}	mean
67	74	70.5

Experimental Conditions Control (A)

V_{C1}	V_{C2}	mean
65	70	67.5

Neutraliser Toxicity Control (B)

V_{C1}	V_{C2}	mean
64	74	69

Dilution Neutralisation Control (C)

V_{C1}	V_{C2}	mean
66	71	68.5

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Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Escherichia coli ATCC 10536

Test Suspension (N + No)

N	V_{C1}	V_{C2}		
10^{-6}	236	250	Weighted Mean = 2.45x10 E8	log = 8.39
10^{-7}	25	27	No = N/10 = 2.45x10 E7	log = 7.39

Test (Na)

V_{C1}	V_{C2}	mean		
<1	<1	<1	Na = mean x10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>6.39 (>5.24)

Validation & Controls

Validation Suspension (Nvo)

V_{C1}	V_{C2}	mean
62	58	60

Experimental Conditions Control (A)

V_{C1}	V_{C2}	mean
61	66	63.5

Neutraliser Toxicity Control (B)

V_{C1}	V_{C2}	mean
59	64	61.5

Dilution Neutralisation Control (C)

V_{C1}	V_{C2}	mean
58	66	62

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Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Enterococcus hirae ATCC 10541

Test Suspension (N + No)

N	V_{C1}	V_{C2}		
10^{-6}	218	230	Weighted Mean = 2.24x10 E8	log = 8.35
10^{-7}	20	25	No = N/10 = 2.24x10 E7	log = 7.35

Test (Na)

V_{C1}	V_{C2}	mean		
<1	<1	<1	Na = mean x10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>6.35 (>5.20)

Validation & Controls

Validation Suspension (Nvo)

V_{C1}	V_{C2}	mean
52	60	56

Experimental Conditions Control (A)

V_{C1}	V_{C2}	mean
55	61	58

Neutraliser Toxicity Control (B)

V_{C1}	V_{C2}	mean
52	56	54

Dilution Neutralisation Control (C)

V_{C1}	V_{C2}	mean
51	60	55.5

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Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Pseudomonas aeruginosa ATCC 15442

Test Suspension (N + No)

N	V_{C1}	V_{C2}		
10^{-6}	284	302	Weighted Mean = 2.95x10 E8	log = 8.47
10^{-7}	30	34	No = N/10 = 2.95x10 E7	log = 7.47

Test (Na)

V_{C1}	V_{C2}	mean		
8	4	6	Na = mean x 10 = 6.0x10 E1 (<140)	log = 1.78 (<2.15)

Log Reduction

5.69 (>5.32)

Validation & Controls

Validation Suspension (Nvo)

V_{C1}	V_{C2}	mean
72	78	75

Experimental Conditions Control (A)

V_{C1}	V_{C2}	mean
70	74	72

Neutraliser Toxicity Control (B)

V_{C1}	V_{C2}	mean
69	76	72.5

Dilution Neutralisation Control (C)

V_{C1}	V_{C2}	mean
70	76	73

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K123716-7

26th August 2021

LABORATORY REPORT

SOURCE: Knights Security Group Ltd

ITEMS: Cov-Rid High Level Disinfectant

TESTS: BSEN1650:2019
Concentration: Neat (Ready to use)
Temperature: 20°C
Contact time: 15 minutes
Interfering substance: Bovine Albumin 3.0g/l (dirty)
Storage conditions: Room temperature, out of direct sunlight
Active substances: Not given
Test Date: 21st August 2021

Recovery: Dilution neutralisation, using:-
Tryptone Soya Broth containing Tween 80 100ml/l, Lecithin 30g/l, Sodium thiosulphate 5g/l, L-histidine 1g/l, L-cystine 1g/l

Test organism: Aspergillus brasiliensis (Niger) ATCC 16404

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Date: 31/8/21

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SUMMARY & CONCLUSIONS:

K123716-7

Organism/Time	Control	Cov-Rid High Level Disinfectant	Log Reduction
Aspergillus brasiliensis (Niger) ATCC 16404	1.63x10 E6	5.0x10 E1 (<140)	4.51 (>4.06)

All test results below 140 (1.4x10 E2) are required to be reported as <140.

The sample complies with the criteria of BSEN1650:2019 (log 4 reduction) after 15 minutes contact against Aspergillus brasiliensis (Niger), under the test conditions stated.

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Detailed Results K123716-7 Cov-Rid High Level Disinfectant

Aspergillus brasiliensis (Niger) ATCC 16404

Test Suspension (N + No)

N	V_{C1}	V_{C2}		
10^{-5}	158	164	Weighted Mean = 1.63x10 E7	log = 7.21
10^{-6}	16	20	No = N/10 = 1.63x10 E6	log = 6.21

Test (Na)

V_{C1}	V_{C2}	mean		
4	6	5	Na = mean x 10 = 5.0x10 E1 (<140)	log = 1.70 (<2.15)

Log Reduction

4.51 (>4.06)

Validation & Controls

Validation Suspension (Nvo)

V_{C1}	V_{C2}	mean
42	40	41

Experimental Conditions Control (A)

V_{C1}	V_{C2}	mean
43	38	40.5

Neutraliser Toxicity Control (B)

V_{C1}	V_{C2}	mean
46	39	42.5

Dilution Neutralisation Control (C)

V_{C1}	V_{C2}	mean
36	41	38.5

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K123718-9

24th August 2021

LABORATORY REPORT

SOURCE: Knights Security Group Ltd

ITEMS: Cov-Rid High Level Disinfectant

TESTS: BSEN13697:2015
Concentration: Ready to use
Temperature: 20°C
Contact time: 5 minutes
Interfering substance: Bovine Albumin 3.0g/l (dirty)
Storage conditions: Room temperature, out of direct sunlight
Active substances: Not given
Test Date: 21st August 2021

Recovery: Dilution neutralisation, using:-
Tryptone Soya Broth containing Tween 80 100ml/l, Lecithin 30g/l, Sodium thiosulphate 5g/l, L-histidine 1g/l, L-cystine 1g/l

Test organisms:		
	Staphylococcus aureus	ATCC 6538
	Pseudomonas aeruginosa	ATCC 15442
	Escherichia coli	ATCC 10536
	Enterococcus hirae	ATCC 10541

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Stewton, Louth
Lincolnshire
LN11 8SD

Date: 31/8/21

Signed:

SUMMARY & CONCLUSIONS:

K123718-9

Cov-Rid High Level Disinfectant

Organism	Test Suspension (N)	Water Control (Nc)	Test Result	Log Reduction
S.aureus	6.75x10 E6	1.98x10 E6	<10 (<140)	>5.30
Ps.aeruginosa	7.64x10 E6	2.47x10 E6	1.85x10 E2	4.12
E.coli	6.17x10 E6	1.68x10 E6	<10 (<140)	>5.23
E.hirae	5.83x10 E6	1.60x10 E6	<10 (<140)	>5.20

All test results below 140 (1.4x10 E2) are required to be reported as <140.

The sample complies with the criteria of BSEN13697:2015 (Bacteria log 4 reduction in 5 minutes) against all four organisms, under the test conditions stated.

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KMSelf

K.M.Self, M.B.I.C.Sc.,M.R.S.P.H,.A.M.S.B.

Validation Results

K123718-9

Organism	NC (Neutraliser only)	NT (Neutraliser + Cov-Rid High Level Disinfectant)
Staphylococcus aureus ATCC 6538	1.93x10 E6	1.78x10 E6
Pseudomonas aeruginosa ATCC 15442	2.36x10 E6	2.23x10 E6
Escherichia coli ATCC 10536	1.54x10 E6	1.62x10 E6
Enterococcus hirae ATCC 10541	1.75x10 E6	1.68x10 E6

Criteria

N – Nc = < 2 log

N - NC = < 2 log

NC – NT = < +/- 0.3 log

Pass: Bacteria log 4 in 5 minutes.

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