

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)

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 dose₅₀ (TCID₅₀) of surviving virus. *Vaccinia virus* VR-1549 Elstree strain / Vero cells are assayed in parallel in each test. TCID₅₀ is determined by the method of Karber¹.

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 TCID₅₀ 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.

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

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%	
	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml	data	TCID ₅₀ /ml
Exposure Time 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

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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.	n.a.	<5 mins
		50%	1.50	n.a.	1.83	n.a.	n.a.	n.a.	n.a.	<5 mins
		10%	1.50	n.a.	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.	n.a.
Formaldehyde	PBS	0.7% (w/v)	2.50				5 min	15 min		
							5.00	3.50		>15 mins

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

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CONCLUSION

Verification of the methodology

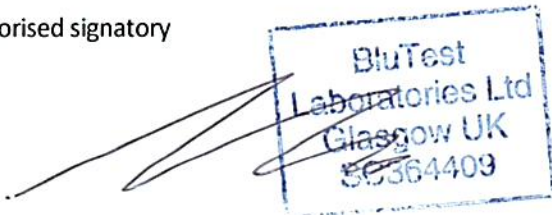
A test is only valid if the following criteria are fulfilled:

- 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 log₁₀.
- 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 log₁₀ reduction of the virus.
- e) The interference control result does not show a difference of > 1.0 log₁₀ 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 log₁₀ 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.

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.

Authorised signatory



Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 08 FEBRUARY 2021

DISCLAIMER

The results in this test report only pertain to the sample supplied.

BluTest (BT) has performed the testing detailed in this report using reasonable skill and care and has used reasonable endeavours to carry out the testing in accordance with an EN 14476 protocol. All forecasts, recommendations and results contained in this report are submitted in good faith. However, other than as expressly set out in this report, no warranty is given (i) in relation to the testing or the use(s) to which any results or deliverables produced in the course of the testing are or may be put by the Client or their fitness or suitability for any particular purpose or under any special conditions notwithstanding that any such purpose or conditions may have been made known to BT or (ii) that the intended results or deliverables from the testing can be achieved or (iii) that the Client can freely make use of the results or the deliverables without infringing any third party intellectual property rights and the Client will be deemed to have satisfied itself in this regard. BT shall have no liability (which is hereby excluded to the fullest extent permissible by law) in respect of any loss, liability or damage, including without limitation any indirect and/or consequential loss such as loss of profit or loss of business, market or goodwill, that the Client may suffer directly or indirectly as a result of or in connection with: (i) the performance of the testing; (ii) the use of any materials, samples or other information provided by the Client for use in the testing; and (iii) the Client's reliance upon or use of any results or deliverables provided as part of the testing.

***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

Scientific Services

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Animal feed Chemists**

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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

Scientific Services

Willow Farm
Stewton, Louth
Lincolnshire
LN11 8SD

Date: 31/8/21

Signed: 

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.

Scientific Services

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Date: 31/8/21

Signed: 

KMSelf

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

Proprietor: K M Self, M.R.S.P.H., M.B.I.C.Sc., A.M.S.B., Member of the Society for General Microbiology,
Participating in the National Agricultural Check Sample Service

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)

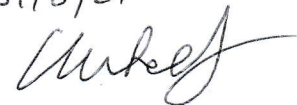
Vc1	Vc2	mean
48	58	53

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Date: 31/8/21

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Scientific Services

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**Consultant Microbiologists
Animal feed Chemists**

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

Scientific Services

Willow Farm
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Lincolnshire
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Date: 31/8/21

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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 (1.4×10^2) 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.

Scientific Services

Willow Farm
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Date: 31/8/21

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KMSelf

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

Proprietor: K M Self, M.R.S.P.H., M.B.I.C.Sc., A.M.S.B., Member of the Society for General Microbiology,
Participating in the National Agricultural Check Sample Service

Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Staphylococcus aureus ATCC 6538

Test Suspension (N + No)

N	Vc1	Vc2		
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)

Vc1	Vc2	mean		
<1	<1	<1	Na = mean x10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>6.43 (>5.28)

Validation & Controls

Validation Suspension (Nvo)

Vc1	Vc2	mean
67	74	70.5

Experimental Conditions Control (A)

Vc1	Vc2	mean
65	70	67.5

Neutraliser Toxicity Control (B)

Vc1	Vc2	mean
64	74	69

Dilution Neutralisation Control (C)

Vc1	Vc2	mean
66	71	68.5

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Date: 31/8/21

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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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Date: 31/8/21

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Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Enterococcus hirae ATCC 10541

Test Suspension (N + No)

N	Vc1	Vc2		
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)

Vc1	Vc2	mean		
<1	<1	<1	Na = mean x10 = <10 (<140)	log = <1 (<2.15)

Log Reduction

>6.35 (>5.20)

Validation & Controls

Validation Suspension (Nvo)

Vc1	Vc2	mean
52	60	56

Experimental Conditions Control (A)

Vc1	Vc2	mean
55	61	58

Neutraliser Toxicity Control (B)

Vc1	Vc2	mean
52	56	54

Dilution Neutralisation Control (C)

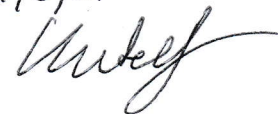
Vc1	Vc2	mean
51	60	55.5

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Date: 31/8/21

Signed:



Detailed Results K123714-5 Cov-Rid High Level Disinfectant

Pseudomonas aeruginosa ATCC 15442

Test Suspension (N + No)

N	Vc1	Vc2		
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)

Vc1	Vc2	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)

Vc1	Vc2	mean
72	78	75

Experimental Conditions Control (A)

Vc1	Vc2	mean
70	74	72

Neutraliser Toxicity Control (B)

Vc1	Vc2	mean
69	76	72.5

Dilution Neutralisation Control (C)

Vc1	Vc2	mean
70	76	73

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Date: 31/8/21

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Scientific Services

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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

Scientific Services

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Stewton, Louth
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LN11 8SD

Date: 31/8/21

Signed: 

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.

Scientific Services

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Date: 31/8/21

Signed: 

KM Self

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

Proprietor: K M Self, M.R.S.P.H.,M.B.I.C.Sc.,A.M.S.B., Member of the Society for General Microbiology,
Participating in the National Agricultural Check Sample Service

Detailed Results K123716-7 Cov-Rid High Level Disinfectant

Aspergillus brasiliensis (Niger) ATCC 16404

Test Suspension (N + No)

N	Vc1	Vc2		
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)

Vc1	Vc2	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)

Vc1	Vc2	mean
42	40	41

Experimental Conditions Control (A)

Vc1	Vc2	mean
43	38	40.5

Neutraliser Toxicity Control (B)

Vc1	Vc2	mean
46	39	42.5

Dilution Neutralisation Control (C)

Vc1	Vc2	mean
36	41	38.5

Scientific Services

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Date: 31/8/21

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**Consultant Microbiologists
Animal feed Chemists**

K123718-9

24th August 2021

LABORATORY REPORT

SOURCE: Knights Security Group Ltd

ITEMS: Cov-Rid High Level Disinfectant

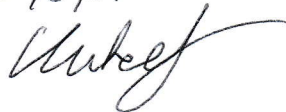
TESTS: BSEN13697:2015
Concentration: Ready to use
Temperature: 20°C
Contact time: 5 minutes
Interfering substance: Bovine Albumin 3.0g/l (dirty)
Storage conditions: Room temperature, out of direct sunlight
Active substances: Not given
Test Date: 21st August 2021

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

Scientific Services

Willow Farm
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.

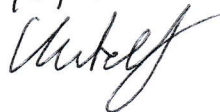
The sample complies with the criteria of BSEN13697:2015 (Bacteria log 4 reduction in 5 minutes) against all four organisms, under the test conditions stated.

Scientific Services

Willow Farm
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Date: 30/6/21

Signed:



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

$N - N_c = < 2 \log$

$N - NC = < 2 \log$

$NC - NT = < +/- 0.3 \log$

Pass: Bacteria log 4 in 5 minutes.

Scientific Services

Willow Farm
Stewton, Louth
Lincolnshire
LN11 8SD

Date: 31/8/21

Signed: 