Test Report: EN 14476 2013 Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2/step 1)

Test Laboratory	**BluTest Laboratories Ltd** Robertson Incubator (Level 4) Robertson Building 56 Dumbarton Road Glasgow UK - G11 6NU
Identification of sample	
Name of the product	Clinell Universal Wipes
Batch number	20190515
Client	Gama Healthcare
Project Code	BT-GAM-10-02
Date of Delivery	15-Apr-15
Storage conditions	Room Temperature
Active substances	Not specified
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, neutralization control and a formaldehyde internal standard.
Neutralizer	Dilution-neutralization/gel filtration; MEM + 10% v/v foetal bovine serum at 4°C
Experimental Conditions	
Period of analysis	03-Oct-15 to 05-Oct-15
Product diluent used	Sterile water
Product test concentrations	5.00%(v/v) 50.00%(v/v) 80.00%(v/v)
Appearance product dilutions	N/A
Contact time (mins)	1 minutes ± 10s; 2 minutes ± 10s;
Test temperature	20°C ± 1°C
Interfering substance	3.0g/l bovine albumin + 3ml/l sheep erythrocytes
Stability of mixture	6 months
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification of strains	Bovine viral diarrhoea virus ATCC VR-1422/BT Cells Hepatitis C virus surrogate

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PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of disinfectant and 1, 2 and 60 minute contact times. Virus is exposed to disinfectant in 24-well plates, then neutralized, serially diluted and virus titred in 96-well tissue culture plates to determine the tissue culture infectious dose$_{50}$ ($TCID_{50}$) of surviving virus. $TCID_{50}$ is determined by the method of Karber[1].

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

Interference control
The end point titration of the virus is exposed to three different sub-lethal concentrations of neutralized disinfectant to measure the effect of sub-lethal concentrations of disinfectant on virus infectivity in relation to the titre achieved on untreated cells.

Disinfectant suppression control
Virus is added to the highest concentration of disinfectant and then the mixture immediately removed and neutralized. The neutralized virus titre is then determined to assess the efficiency of the neutralization procedure.

Virus recovery control
Virus titre is determined for virus in contact with sterile hard water at t=0, t = 2 and at t =60. The virus titre after 2 minutes is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre at 60 minutes is compared to the reference virus inactivation control.

Reference virus inactivation control
Virus is exposed to 0.07% W/V formaldehyde and the recovery of virus determined by $TCID_{50}$ after 5, 15, 30 and 60 minutes, in order to assess that the test virus has retained reproducible biocide resistance. In addition, the formaldehyde cytotoxicity of neutralized 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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Bovine viral diarrhoea virus ATCC VR-1422/BT Cells

SOP 10000 V02 EN14476 Suspension test results for the efficacy of Clinell Universal Wipes, Batch 20190515, BT-GAM-10-02 from Gama Healthcare against BVDV

Exposure Time	Virus Recovery 0 min		Virus Recovery 2 min		Cytotoxicity		Disinfectant Suppression		5.00% (v/v)		50.00% (v/v)		80.00% (v/v)	
	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml						
	5.50	**1.00E+07**	5.67	**1.48E+07**	0.50	**1.00E+02**	4.17	**4.68E+05**						
		1.00E+07		**1.48E+07**		**1.00E+02**		**4.68E+05**						
log		**7.00**		**7.17**		**2.00**		**5.67**						
log difference								**1.50**						

	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
t = 2	5.50	**1.00E+07**	5.67	**1.48E+07**					2.67	**1.48E+04**	0.00	**3.16E+01**	0.00	**3.16E+01**
		1.00E+07		**1.48E+07**						**1.48E+04**		**3.16E+01**		**3.16E+01**
log		**7.00**		**7.17**						**4.17**		**1.50**		**1.50**
log difference										**3.00**		**5.67**		**5.67**

	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
t = 1	5.50	**1.00E+07**	5.67	**1.48E+07**					4.33	**6.76E+05**	0.00	**3.16E+01**	0.00	**3.16E+01**
		1.00E+07		**1.48E+07**						**6.76E+05**		**3.16E+01**		**3.16E+01**
log		**7.00**		**7.17**						**5.83**		**1.50**		**1.50**
log difference										**1.34**		**5.67**		**5.67**

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Summary table of results of virucidal activity against BVDV under dirty conditions for Clinell Universal Wipes, Batch 20190515, BT-GAM-10-02 from Gama Healthcare

Product: Clinell Universal Wipes	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after .. Min
				0 min	n.a.	1 min	2 min	60 min	
	3.0g/l BSA + 3.0ml/l erythrocytes	80.00% (v/v)	2.00	7.00	n.a.	1.50	1.50	n.a.	<1
		50.00% (v/v)	2.00	7.00	n.a.	1.50	1.50	n.a.	<1
		5.00% (v/v)	2.00	7.00	n.a.	5.83	4.17	n.a.	>2
	3.0g/l BSA	80.00% (v/v)	2.00	7.00	n.a.	1.50	1.50	n.a.	<1
		50.00% (v/v)	2.00	7.00	n.a.	1.50	1.50	n.a.	<1
		5.00% (v/v)	2.00	7.00	n.a.	6.00	4.00	n.a.	>2
Virus Control	BSA + erythrocytes	n.a.	n.a.	7.00	n.a.	n.a.	n.a.	7.00	n.a.
Virus Control	BSA	n.a.	n.a.	7.00	n.a.	n.a.	n.a.	7.00	n.a.

Product: Clinell Universal Wipes	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after .. Min
				0 min	5 min	15 min	30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	3.50	7.00	6.50	4.83	3.83	3.50	>60

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Control Data for:	BT-GAM-10-02									
Parallel control test										

Exposure Time	**Virus Recovery 0 min**		**Virus Recovery 2 min**		**5.00% (v/v)**		**50.00% (v/v)**		**80.00% (v/v)**	
	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml						
	5.50	**1.00E+07**	5.67	**1.48E+07**						
		1.00E+07		**1.48E+07**						
log		**7.00**		**7.17**						
log difference										

	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
t = 2	5.50	**1.00E+07**	5.67	**1.48E+07**	2.50	**1.00E+04**	0.00	**3.16E+01**	0.00	**3.16E+01**
		1.00E+07		**1.48E+07**		**1.00E+04**		**3.16E+01**		**3.16E+01**
log		**7.00**		**7.17**		**4.00**		**1.50**		**1.50**
log difference						**3.17**		**5.67**		**5.67**

	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
t = 1	5.50	**1.00E+07**	5.67	**1.48E+07**	4.50	**1.00E+06**	0.00	**3.16E+01**	0.00	**3.16E+01**
		1.00E+07		**1.48E+07**		**1.00E+06**		**3.16E+01**		**3.16E+01**
log		**7.00**		**7.17**		**6.00**		**1.50**		**1.50**
log difference						**1.17**		**5.67**		**5.67**

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Stock Virus ($TCID_{50}$)	6.33	**6.76E+07**

Formaldehyde reference inactivation control

Exposure time	Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		0.07% Formaldehyde							
							5		15		30		60	
	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	raw data	$TCID_{50}$/ml
60 min	5.50	**1.00E+07**	5.50	**1.00E+07**	2.00	**3.16E+03**	5.00	**3.16E+06**	3.33	**6.76E+04**	2.33	**6.76E+03**	2.00	**3.16E+03**
		1.00E+07		**1.00E+07**		**3.16E+03**		**3.16E+06**		**6.76E+04**		**6.76E+03**		**3.16E+03**
log		**7.00**		**7.00**		**3.50**		**6.50**		**4.83**		**3.83**		**3.50**
log difference								**0.50**		**2.17**		**3.17**		**3.50**

No Column Control

Virus Recovery	
30	**min**
raw data	$TCID_{50}$/ml
5.50	**1.00E+07**
	1.00E+07
	7.00

Interference control

Virus dilution	Cytoxicity dilution			
	-1	**-2**	**-3**	**Mock**
-4	1	3	3	3
-5	1	3	3	3
-6	1	3	3	3

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CONCLUSION

Verification of the methodology

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

a) Test virus suspension has at least a concentration which allows the determination of a 4 $\log_{10}$ reduction of the virus titre.
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 – 0.5 and – 2.5 after 30 min and between – 2 and – 4.5 after 60 min for 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 reduction of the virus.
e) The interference control result does not show a difference of < 1.0 $\log_{10}$ of virus titre in comparison to the virus recovery control; dilutions of disinfectant to sub-acute levels did not interfere in the generation of viral cytopathic effect.
e) Neutralisation validation. This is called the disinfectant suppression test in this protocol. The difference for virus is slightly elevated indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.00% v/v.
f) A difference of <0.5 $\log_{10}$ is not observed between virus recovered directly from the virus recovery control at 60 minutes and virus from the same control recovered through an Illustra Microspin S-400 HR column.

According to EN 14476 2013, **Clinell Universal Wipes POSSESSES VIRUCIDAL** activity at a concentration of **50.00 % V/V** of the working concentration as tested after **1 MINUTE** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against Bovine viral diarrhoea virus ATCC VR-1422/BT Cells a surrogate for Hepatitis C virus.

Signed

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Date: 07 October 2015

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