BluTest
GLOBAL MICROBIOLOGY EXPERTISE

Test Report: EN 14476 2005 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) Modification for surface testing and Respiratory Syncytial Virus a surrogate for Ebola virus.

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	**Cleenol Alcohol Free Hand sanitiser**
Batch number	Not specified
Client	Cleenol Group Ltd Neville House, Beaumont Road, Banbury OX15 6BL
Project Code	BT-CNL-01
Date of Delivery	22 Oct 2014
Storage conditions	Tightly sealed, original container. Well ventilated, cool place
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.
Neutralization	Dilution-neutralization/gel filtration; Dulbecco's modified Eagles medium + 5% v/v foetal bovine serum at 4°C
Experimental Conditions	
Period of analysis	31-Oct-14 to 25-Nov-14
Product diluents used	Sterile distilled water
Product test concentrations	1.0%V/V; 50.0% V/V; 80.0% V/V
Appearance product dilutions	Clear
Contact times (minutes)	1 ± 10s;
Test temperature	20°C ± 1°C
Interfering substances	0.3g/l bovine albumin
Stability of mixture	Stable under normal conditions
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification of virus	**Respiratory Syncytial virus/Hep2 cells**

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PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with one test per three concentrations of disinfectant and a 1 minute contact time. 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 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=1 and at t = 60. The virus titre after 1 minute is then compared to the recovery of disinfectant-treated virus to measure the log reduction in virus titre. The virus titre after 60 minutes is compared to the reference virus inactivation control.

Reference virus inactivation control
Virus is in contact with 0.7% 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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Surface test results for the efficacy of Cleenol Alcohol Free Hand sanitiser from Cleenol Group Ltd against Respiratory Syncytial virus under CLEAN CONDITIONS

Exposure Time	Virus Recovery 0 min		Virus Recovery 1 min		Cytotoxicity		Disinfectant Suppression		1.0% (v/v)		50.0% (v/v)		80.0% (v/v)	
	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
t = 1 min	5.33	**6.76E+06**	5.33	**6.76E+06**	1.00	**3.16E+02**	2.00	**3.16E+03**	2.50	**1.00E+04**	1.50	**1.00E+03**	2.00	**3.16E+03**
		6.76E+06		**6.76E+06**		**3.16E+02**		**3.16E+03**		**1.00E+04**		**1.00E+03**		**3.16E+03**
log		**6.83**		**6.83**		**2.50**		**3.50**		**4.00**		**3.00**		**3.50**
log difference								**3.33**		**2.83**		**3.83**		**3.33**

Table of results of virucidal activity against RSV under clean conditions for Cleenol Alcohol Free Hand sanitiser from Cleenol Group Ltd

Product: Alcohol Free Hand sanitiser	Interfering substance	Concentration	Level of cytotoxicity	lg TCID50					>4 lg reduction after .. Min
				0 min	1 m (prod) / 5 m (form)	15 min	30min	60 min	
	0.3g/l BSA	80.0% (v/v)	2.50	6.83	3.50	na	na	na	>1
		50.0% (v/v)	2.50	6.83	3.00	na	na	na	>1
		1.0% (v/v)	2.50	6.83	4.00	na	na	na	>1
Formaldehyde		0.07% (w/v)	1.50	6.83	5.17	4.17	3.17	2.00	30
Virus Control		n.a.	n.a.	6.83	6.83	n.a.	n.a.	7.17	n.a.

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Control Data

Control Data for: BT-CNL-01 RSV

Stock Virus ($TCID_{50}$)	7.33	**6.76E+08**

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	5.33	**6.76E+06**	5.67	**1.48E+07**	0.00	**3.16E+01**	3.67	**1.48E+05**	2.67	**1.48E+04**	1.67	**1.48E+03**	0.50	**1.00E+02**
		6.76E+06		**1.48E+07**		**3.16E+01**		**1.48E+05**		**1.48E+04**		**1.48E+03**		**1.00E+02**
log		**6.83**		**7.17**		**1.50**		**5.17**		**4.17**		**3.17**		**2.00**
log difference								**2.00**		**3.00**		**4.00**		**5.17**

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**
-5	C	C	3	3
-6	C	C	1	2
-7	C	C	0	1

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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 poliovirus.

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 probably indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% 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 2005, **Cleenol Alcohol Free Hand sanitiser POSSESSES VIRUCIDAL** activity at a concentration of **50.0 % V/V** of the working concentration as tested after **1 MINUTE** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) of >3.83 $\log_{10}$ **against Respiratory Syncytial virus, a surrogate for Ebola virus.**
The result at 80.0% V/V of the working concentration was not demonstrated because residual cytotoxicity of the product reduced the sensitivity of the assay.

Signed

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 10 December 2014

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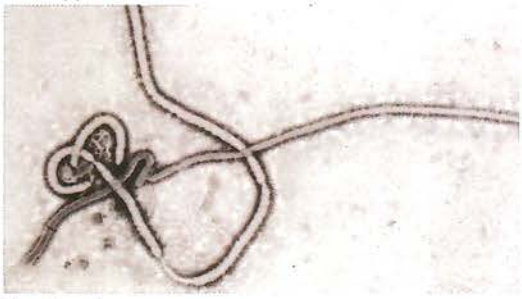
	Ebola virus	Respiratory syncytial virus
Order:	Mononegavirales	Mononegavirales
Family:	Filoviridae	Paramyxoviridae
Genus:	Ebolavirus	Pneumovirus
Species:	Ebola virus	Human respiratory syncytial virus

Source: International Committee on the Taxonomy of Viruses (2013 update).

A virus is a member of the order Mononegavirales if:
its genome is a linear, non-segmented, single-stranded, non-infectious RNA of negative polarity; possesses inverse-complementary 3' and 5' termini; is not covalently linked to a protein
its genome has the characteristic gene order 3'-UTR–core protein genes–envelope protein genes–RNA-dependent RNA polymerase gene–5'-UTR
it produces 5–10 distinct mRNAs from its genome via polar sequential transcription from a single promoter located at the 3' end of the genome; mRNAs are 5' capped and polyadenylated
it replicates by synthesizing complete antigenomes
it forms infectious helical ribonucleocapsids as the templates for the synthesis of mRNAs, antigenomes, and genomes
it encodes an RNA-dependent RNA polymerase (RdRp) that is highly homologous to those of other mononegaviruses
it forms enveloped virions with a molecular mass of 300–1,000×10^6; an S20W of 550–>1,045; and a buoyant density in CsCl of 1.18–1.22 g/cm^3

Easton, C. R.; Pringle (2011), "Order Mononegavirales", in King, Andrew M. Q.; Adams, Michael J.; Carstens, Eric B. et al., Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses, London, UK: Elsevier/Academic Press, pp. 653–657, ISBN 978-0-12-384684-6



Ebola virus

RSV

DISCLAIMER

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