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	GS24
Batch number	NA
Client	Goldshield Technologies
Project Code	BT-GLD-02
Date of Delivery	11-Jun-15
Storage conditions	Ambient temp, darkness
Active substances	6 months
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; Dulbecco's modified Eagles medium + 5.0% v/v foetal bovine serum at 4°C
Experimental Conditions	
Period of analysis	28-Jul-15 to 12-Aug-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)	5 minutes ± 10s
Test temperature	20°C ± 1°C
Interfering substance	0.3g/l V/V bovine albumin
Stability of mixture	6 months
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification of strains	**Respiratory syncytial virus ATCC VR-26 Long strain/Hep 2 cells**

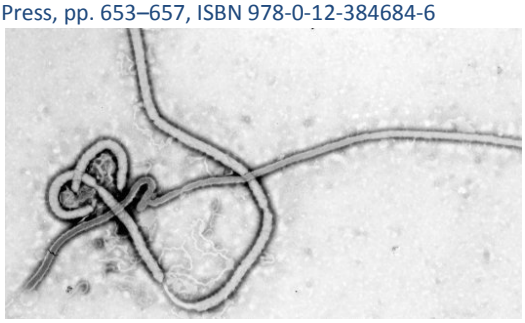
BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

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

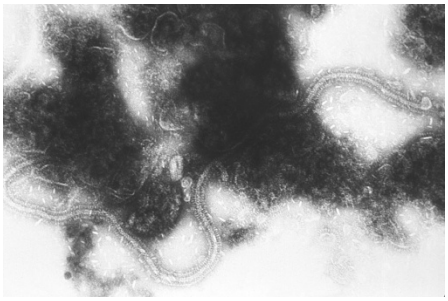
Source: International Committee on the Taxonomy of Viruses (2013 update).

A virus is a member of the order Mononegavirales if:
its genome is a linear, nonsegmented, 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

BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three concentrations of disinfectant and a 5 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 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 = 5 and at t =60. 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 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.

BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

Respiratory Syncytial Virus ATCC VR-26 Long strain. A surrogate for Ebola virus.

SOP 10000 V02 EN14476 Suspension test results for the efficacy of GS24, BT-GLD-02 from Goldshield Technologies against RSV

Exposure Time	Virus Recovery 0 min		Virus Recovery 5 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	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
t = 5	5.17	**4.68E+06**	5.17	**4.68E+06**	2.00	**3.16E+03**	3.33	**6.76E+04**	4.00	**3.16E+05**	1.00	**3.16E+02**	2.00	**3.16E+03**
		4.68E+06		**4.68E+06**		**3.16E+03**		**6.76E+04**		**3.16E+05**		**3.16E+02**		**3.16E+03**
log		**6.67**		**6.67**		**3.50**		**4.83**		**5.50**		**2.50**		**3.50**
log difference								**1.84**		**1.17**		**4.17**		**3.17**

Summary table of results of virucidal activity against RSV under clean conditions for GS24, BT-GLD-02 from Goldshield Technologies

Product: GS24	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$ 0 min	lg $TCID_{50}$ 5 min	lg $TCID_{50}$ Y min	lg $TCID_{50}$ Z min	lg $TCID_{50}$ 60 min	>4 lg reduction after .. Min
	0.3 g/l bovine albumin	80.00% (v/v)	3.50	6.67	3.50	n.a.	n.a.	n.a.	>5
		50.00% (v/v)	3.50	6.67	2.50	n.a.	n.a.	n.a.	<5
		5.00% (v/v)	3.50	6.67	5.50	n.a.	n.a.	n.a.	>5
	3.0g/l bovine albumin	n.a.	3.50	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		n.a.	3.50	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		n.a.	3.50	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Formaldehyde	PBS	0.7% (w/v)	1.50	6.67	5.50	3.83	2.83	2.50	60
Virus Control		n.a.	n.a.	6.67	6.67	n.a.	n.a.	6.67	n.a.

BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

Control Data

Control Data for:	BT-GLD-02

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	5.17	4.68E+06	5.17	4.68E+06	0.00	3.16E+01	4.00	3.16E+05	2.33	6.76E+03	1.33	6.76E+02	1.00	3.16E+02
		4.68E+06		4.68E+06		3.16E+01		3.16E+05		6.76E+03		6.76E+02		3.16E+02
log		6.67		6.67		1.50		5.50		3.83		2.83		2.50
log difference								1.17		2.84		3.84		4.17

No Column Control

Virus Recovery 30 min	
raw data	$TCID_{50}$/ml
5.00	3.16E+06
	3.16E+06
	6.50

PASS

Interference control

Virus dilution	Cytoxicity dilution				
	-1	-2	-3	Mock	
-4	0	3	3	3	
-5	0	3	3	3	
-6	0	0	0	0	

PASS

BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

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 X% 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, GS24 **POSSESSES VIRUCIDAL** activity at a concentration of **80.00 % V/V** of the working concentration as tested after **5 MINUTES** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin against **Respiratory Syncytial Virus ATCC VR-26 Long strain/Hep 2 cells, a surrogate for Ebola virus.**

Signed

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 18 August 2015

BLUTEST LABORATORIES LIMITED, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU.
Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597