Test Report: EN 14476:2013 + A1:2015 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	Hydrus 75
Batch number	E1651
Client	Hydrus Hygiene
Client Address	Hydrus House, Dromintree, Hilltop Industrial Estate, Coalville, Leicester, LE67 1TX
Project Code	BT-HDU-01
Date of Delivery	05 July 2019
Storage conditions	2.0°C to 8.0°C
Active substances	Sodium Hypochlorite

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 Dulbecco's modified Eagles medium + 5%/ 10% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	16 July 2019 to 23 July 2019
Product diluents used	Sterile, synthetic hard 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	Turbidity and Sedimentation observed at 10.0% v/v- Murine norovirus only
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 of virus	**Poliovirus 1 (LSc-2ab/NIBSC Code 01/528)/HeLa cells;** **Adenovirus 5 (ATCC VR-5)/HeLa cells;** **Murine norovirus (s99)/RAW 264.7 cells**

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Telephone: +44 (0)141 558 2782. 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 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. Poliovirus 1 (LSc-2ab/NIBSC Code 01/528)/HeLa cells, Adenovirus 5 (ATCC VR-5)/HeLa cells and Murine norovirus (s99)/RAW 264.7 cells are assayed in parallel in each test. $TCID_{50}$ is determined by the method of Karber[1].

Cytotoxicity control
The neutralised 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 neutralised 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 neutralised. The neutralised virus titre is then determined to assess the efficiency of the neutralisation 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 0.7% 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 neutralised 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, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Telephone: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

Adenovirus-5 (ATCC VR-5)

SOP 10000 V02 EN14476 Suspension test results for the efficacy of Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene against ADV-5														
Virus Recovery 0 min		**Virus Recovery 5 MINS**		**Cytotoxicity**		**Disinfectant Suppression**		**Exposure Time**	**10% (v/v)**		**50% (v/v)**		**80% (v/v)**	
ra w 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
5.33	**6.76E+06**	5.17	**4.68E+06**	0.00	**3.16E+01**	0.00	**3.16E+01**	**t =5 mins**	0.00	**3.16E+01**	0.00	**3.16E+01**	0.00	**3.16E+01**
	6.76E+06		**4.68E+06**		**3.16E+01**		**3.16E+01**			**3.16E+01**		**3.16E+01**		**3.16E+01**
	6.83		**6.67**		**1.50**		**1.50**	log		**1.50**		**1.50**		**1.50**
							5.17	log difference		**5.17**		**5.17**		**5.17**

Summary table of results of virucidal activity against ADV-5 under CLEAN conditions for Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene									
Product:	Interfering substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after 'X' Mi n
				0 min	5 min	15 min	30 min	60 min	
Hydrus 75	0.3g/l BSA	80% (v/v)	1.50	n.a .	1.50	n.a .	n.a.	n.a .	<5 min
		50% (v/v)	1.50	n.a .	1.50	n.a .	n.a.	n.a .	<5 min
		10% (v/v)	1.50	n.a .	1.50	n.a .	n.a.	n.a .	<5 min
Forma ldehyde	PBS	0.7% (w/v)	4.50	n.a.	4.83	4.50	3.83	3.50	>60 min
Virus Control	CLEAN	n.a.	n.a.	6.83	6.67	n.a.	n.a.	6.67	n.a.

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Control Data

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

Formaldehyde reference inactivation control

Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		Exposure time	0.7% Formaldehyde							
							5		15		30		60	
ra w 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
5.33	**6.76E+06**	5.17	**4.68E+06**	3.00	**3.16E+04**	**60 min**	3.33	**6.76E+04**	3.00	**3.16E+04**	2.33	**6.76E+03**	2.00	**3.16E+03**
	6.76E+06		**4.68E+06**		**3.16E+04**			**6.76E+04**		**3.16E+04**		**6.76E+03**		**3.16E+03**
	6.83		**6.67**		**4.50**	log		**4.83**		**4.50**		**3.83**		**3.50**
						log difference		**1.84**		**2.17**		**2.84**		**3.17**

No Column Control

Virus Recovery	
60	**min**
ra w data	$TCID_{50}$/ml
5.33	**6.76E+06**
	6.76E+06
	6.83

Interference control

Virus dilution	Cytoxicity dilution			
	-1	**-2**	**-3**	**Mock**
-5	3	3	3	3
-6	2	3	3	3
-7	2	2	3	1

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Poliovirus-1 (LSc-2ab/NIBSC Code 01/528)

SOP 10000 V02 EN14476 Suspension test results for the efficacy of Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene against PV-1														
Virus Recovery 0 min		**Virus Recovery 5 MINS**		**Cytotoxicity**		**Disinfectant Suppression**		**Exposure Time**	**10% (v/v)**		**50% (v/v)**		**80% (v/v)**	
ra w 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
5.67	**1.48E+07**	5.33	**6.76E+06**	0.00	**3.16E+01**	0.00	**3.16E+01**	**t =5 mins**	0.00	**3.16E+01**	0.00	**3.16E+01**	0.00	**3.16E+01**
	1.48E+07		**6.76E+06**		**3.16E+01**		**3.16E+01**			**3.16E+01**		**3.16E+01**		**3.16E+01**
	7.17		**6.83**		**1.50**		**1.50**	log		**1.50**		**1.50**		**1.50**
							5.33	log difference		**5.33**		**5.33**		**5.33**

Summary table of results of virucidal activity against PV-1 under CLEAN conditions for Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene									
Product:	Interferi ng substance	Concentration	Level of cytotoxicity	lg $TCID_{50}$					>4 lg reduction after 'X' Mi n
				0 min	5 min	15 min	30 min	60 min	
Hydrus 75	0.3g/l BSA	80% (v/v)	1.50	n.a .	1.50	n.a .	n.a .	n.a .	<5 min
		50% (v/v)	1.50	n.a .	1.50	n.a .	n.a .	n.a .	<5 min
		10% (v/v)	1.50	n.a .	1.50	n.a .	n.a .	n.a .	<5 min
Formaldehyde	PBS	0.7% (w/v)	3.50	n.a.	7.00	5.83	5.00	3.50	>60 min
Virus Control	CLEAN	n.a.	n.a.	7.17	6.83	n.a.	n.a.	7.33	n.a.

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Control Data

Stock Virus ($TCID_{50}$)	6.67	1.48E+08

Formaldehyde reference inactivation control

Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		Exposure time	0.7% Formaldehyde							
							5		15		30		60	
ra w 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
5.67	**1.48E+07**	5.83	**2.14E+07**	2.00	**3.16E+03**	**60 min**	5.50	**1.00E+07**	4.33	**6.76E+05**	3.50	**1.00E+05**	2.00	**3.16E+03**
	1.48E+07		**2.14E+07**		**3.16E+03**			**1.00E+07**		**6.76E+05**		**1.00E+05**		**3.16E+03**
	7.17		**7.33**		**3.50**	log		**7.00**		**5.83**		**5.00**		**3.50**
						log difference		**0.33**		**1.50**		**2.33**		**3.83**

No Column Control

Virus Recovery	
60	**min**
ra w data	$TCID_{50}$/ml
5.83	**2.14E+07**
	2.14E+07
	7.33

Interference control

Virus dilution	Cytoxicity dilution			
	-1	**-2**	**-3**	**Mock**
-5	3	3	3	3
-6	3	3	3	3
-7	2	2	3	1

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Murine norovirus (s99)

SOP 10000 V02 EN14476 Suspension test results for the efficacy of Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene against MNV														
Virus Recovery 0 min		**Virus Recovery 5 MINS**		**Cytotoxicity**		**Disinfectant Suppression**		**Exposure Time**	**10% (v/v)**		**50% (v/v)**		**80% (v/v)**	
ra w 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
5.17	**4.68E+06**	5.17	**4.68E+06**	0.00	**3.16E+01**	2.00	**3.16E+03**	**t =5 mins**	2.00	**3.16E+03**	1.00	**3.16E+02**	1.00	**3.16E+02**
	4.68E+06		**4.68E+06**		**3.16E+01**		**3.16E+03**			**3.16E+03**		**3.16E+02**		**3.16E+02**
	6.67		**6.67**		**1.50**		**3.50**	log		**3.50**		**2.50**		**2.50**
							3.17	log difference		**3.17**		**4.17**		**4.17**

Summary table of results of virucidal activity against MNV under CLEAN conditions for Hydrus 75 , Batch E1651, BT-HDU-01 from Hydrus Hygiene									
Product:	Interfering substance	Concentration	Le vel of cytotoxi city	lg $TCID_{50}$					>4 lg reduction after 'X' Min
				0 min	5 min	15 min	30 min	60 min	
Hydrus 75	0.3g/l BSA	80% (v/v)	1.50	n.a .	2.50	n.a.	n.a.	n.a.	<5 mins
		50% (v/v)	1.50	n.a .	2.50	n.a.	n.a.	n.a.	<5 mins
		10% (v/v)	1.50	n.a .	3.50	n.a.	n.a.	n.a.	>5mi ns
Formaldehyde	PBS	0.7% (w/v)	3.50	n.a .	6.00	4.50	3.50	3.50	>60 mins
Virus Control	CLEAN	n.a .	n.a .	6.67	6.67	n.a.	n.a.	6.67	n.a.

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 4597

Control Data

Stock Virus ($TCID_{50}$)	6.67	1.48E+08

Formaldehyde reference inactivation control

Virus recovery 0 min		Virus recovery 60 min		Cytotoxicity		Exposure time	0.7% Formaldehyde							
							5		15		30		60	
ra w 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
5.17	**4.68E+06**	5.17	**4.68E+06**	2.00	**3.16E+03**	**60 min**	4.50	**1.00E+06**	3.00	**3.16E+04**	2.00	**3.16E+03**	2.00	**3.16E+03**
	4.68E+06		**4.68E+06**		**3.16E+03**			**1.00E+06**		**3.16E+04**		**3.16E+03**		**3.16E+03**
	6.67		**6.67**		**3.50**	log		**6.00**		**4.50**		**3.50**		**3.50**
						log difference		**0.67**		**2.17**		**3.17**		**3.17**

No Column Control

Virus Recovery	
60	**min**
ra w data	$TCID_{50}$/ml
5.67	**1.48E+07**
	1.48E+07
	7.17

Interference control

Virus dilution	Cytoxicity dilution			
	-1	**-2**	**-3**	**Mock**
-5	3	3	3	3
-6	3	3	3	3
-7	2	2	2	2

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Tel: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com. Company No.: SC364409. VAT No.: GB 979 1131 96. UKAS No.: 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 Poliovirus
3.0 and 5.0 after 30 min and between 3.5 and 5.5 after 60 min for Adenovirus
0.0 and 2.0 after 30 min and between 0.5 and 2.5 after 60 min for Parvovirus
0.75 and 3.5 after 5 min and between 2.0 and ≥4.0 after 15 min for Vacciniavirus
NOTE: Specifications not met under the Adenovirus testing. This has had no effect on the outcome
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 thevirus.
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 does not interfere in the generation of viral cytopathic effect.
e) 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 slightly elevated indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v.
f) A difference of <0.5 $\log_{10}$ should be observed between virus recovered directly from the virus recovery control at 30 minutes and virus from the same control recovered through an Illustra Microspin S-400 HR column.

According to EN 14476:2013 + A1:2015, **Hydrus 75 POSSESSES VIRUCIDAL** activity at concentrations of **80.0% v/v, 50.0% and 10.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **CLEAN** conditions (0.3 g/l bovine albumin) against Poliovirus 1 (LSc-2ab/NIBSC Code 01/528)/HeLa cells, Adenovirus 5 (ATCC VR- 5)/HeLa cells and Murine norovirus (s99)/RAW 264.7 cells

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Telephone: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597

Signed

Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK.
Date: 05 August 2019

4597

Expanded Uncertainty of Measurement U = ± 0.086

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.

BLUTEST LABORATORIES LIMITED, 5 Robroyston Oval, Nova Business Park, Glasgow, UK, G33 1AP
Telephone: +44 (0)141 558 2782. Email: info@blutest.com. Web site: www.blutest.com.
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96 UKAS Number: 4597