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	DuoMax General Purpose Cleaner
Batch number	Not supplied
Client	DuoTech Holdings
Client Address	Feeder Road, Bristol, BS2 0TH
Project Code	BT-DUO-22
Date of Delivery	27 January 2020
Storage conditions	Ambient
Appearance	Clear 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 Dulbecco's modified Eagles medium + 5% v/v or 10.0% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	18 February 2020 to 25 February 2020
Product diluents used	Sterile distilled water
Product test concentrations	80.0%(v/v); 50.0%(v/v); 10.0%(v/v) (*Poliovirus and Adenovirus*) 10.0%(v/v); 5.0%(v/v); 1.0%(v/v) (*Murine norovirus*)
Appearance product dilutions	Turbidity observed at 10.0% v/v and 5.0% v/v (*Murine norovirus*) Turbidity observed at 10.0% v/v (*Poliovirus and Adenovirus*)
Appearance in test mixture	Turbidity and sedimentation observed at all concentrations
Contact times (minutes)	5 ± 10s
Test temperature	20°C ± 1°C
Interfering substances	3.0 g/l bovine albumin + 3.0 ml/l erythrocytes
Temperature of incubation	37°C ± 1°C + 5% CO_2
Identification and passage (P) of virus	***Poliovirus type* 1 (LSc-2ab/NIBSC Code 01/528) (P 7);** ***Adenovirus type* 5 (ATCC VR-5) (P 10);** ***Murine norovirus* (s99) (P 7)**
Identification and passage (P) of cells	HeLa cells (P 18) (*Poliovirus type* 1); HeLa cells (P 18) (*Adenovirus type* 5); RAW 264.7 cells (P 23) (*Murine norovirus*)

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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 dose$_{50}$ ($TCID_{50}$) of surviving virus. *Poliovirus type* 1 (LSc-2ab/NIBSC Code 01/528)/HeLa cells, *Adenovirus type* 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 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 =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.

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Adenovirus type 5 (ATCC VR-5) Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Adenovirus type 5 ATCC VR-5 under DIRTY conditions						
Test Results						
Concentration	**10.0% (v/v)**		**50.0% (v/v)**		**80.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 5 minutes	1.17	**4.68E+02**	2.00	**3.16E+03**	3.00	**3.16E+04**
Raw Data	610000	**4.68E+02**	660000	**3.16E+03**	666000	**3.16E+04**
log		**2.67**		**3.50**		**4.50**
log difference		**4.16**		**3.33**		**2.33**

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Adenovirus type 5 ATCC VR-5 under DIRTY conditions									
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	
DuoMax General Purpose Cleaner	3.0g/l BSA + 3.0ml/l erythrocytes	80.0% (v/v)	4.50	4.50	4.50	n.a.	n.a.	n.a.	>5 mins
		50.0% (v/v)	4.50	n.a.	3.50	n.a.	n.a.	n.a.	>5 mins
		10.0% (v/v)	4.50	n.a.	2.67	n.a.	n.a.	n.a.	<5 mins
DuoMax General Purpose Cleaner	3.0g/l BSA	80.0% (v/v)	4.50	n.a.	4.50	n.a.	n.a.	n.a.	>5 mins
		50.0% (v/v)	4.50	n.a.	3.50	n.a.	n.a.	n.a.	>5 mins
		10.0% (v/v)	4.50	n.a.	2.83	n.a.	n.a.	n.a.	<5 mins
Virus Control	DIRTY			6.83	6.83	n.a.	n.a.	6.83	n.a.
Virus Control	CLEAN			7.17	6.83	n.a.	n.a.	6.83	n.a.
							30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				3.50	2.50	>60 mins

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Adenovirus type 5 (ATCC VR-5) Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Adenovirus type 5 ATCC VR-5 under DIRTY conditions

Controls

Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2	
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**	5.33	**6.76E+06**	5.33	**6.76E+06**	3.00	**3.16E+04**	3.00	**3.16E+04**	5.33	**6.76E+06**
666662	**6.76E+06**	666662	**6.76E+06**	666662	**6.76E+06**	666000	**3.16E+04**	666000	**3.16E+04**	666662	**6.76E+06**
	6.83		**6.83**		**6.83**		**4.50**		**4.50**		**6.83**
									2.33		**0.00**

Formaldehyde reference inactivation controls

Cytotoxicity		Exposure time	0.7% Formaldehyde			
			30		60	
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
1.00	**3.16E+02**		2.00	**3.16E+03**	1.00	**3.16E+02**
600000	**3.16E+02**		660000	**3.16E+03**	600000	**3.16E+02**
	2.50	log		**3.50**		**2.50**
		log difference		**3.33**		**4.33**

No column Control	
5 mins	
raw data	$TCID_{50}$/ml
5.67	**1.48E+07**
666664	**1.48E+07**
	7.17

Interference control	Virus dilution					
	-3	-4	-5	-6	-7	-8
PBS Control	1	1	1	1	0.5	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.00**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**3**	**0**
Product	1	1	1	1	0.5	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.00E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.00**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**3**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Product Cyt Dilution	-4	-4	-4	-4	-4	-4
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
6.67
1.48E+08
6666664000

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Adenovirus type 5 (ATCC VR-5) Control Data

Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Concentration	10.0% (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	**Exposure Time**	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
5.67	**1.48E+07**	5.33	**6.76E+06**	5.33	**6.76E+06**	**t = 5 minutes**	1.33	**6.76E+02**	2.00	**3.16E+03**	3.00	**3.16E+04**
666664	**1.48E+07**	666662	**6.76E+06**	666662	**6.76E+06**	Raw data	620000	**6.76E+02**	660000	**3.16E+03**	666000	**3.16E+04**
	7.17		**6.83**		**6.83**	log		**2.83**		**3.50**		**4.50**
						log difference		**4.00**		**3.33**		**2.33**

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

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) under DIRTY conditions						
Test Results						
Concentration	**10.0% (v/v)**		**50.0% (v/v)**		**80.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 5 minutes	2.50	**1.00E+04**	2.00	**3.16E+03**	3.00	**3.16E+04**
Raw Data	663000	**1.00E+04**	660000	**3.16E+03**	666000	**3.16E+04**
log		**4.00**		**3.50**		**4.50**
log difference		**3.50**		**4.00**		**3.00**

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) under DIRTY conditions									
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	
DuoMax General Purpose Cleaner	3.0g/l BSA + 3.0ml/l erythrocytes	80.0% (v/v)	4.50	3.50	4.50	n.a.	n.a.	n.a.	>5 mins
		50.0% (v/v)	4.50	n.a.	3.50	n.a.	n.a.	n.a.	5 mins
		10.0% (v/v)	4.50	n.a.	4.00	n.a.	n.a.	n.a.	>5 mins
DuoMax General Purpose Cleaner	3.0g/l BSA	80.0% (v/v)	4.50	n.a.	4.50	n.a.	n.a.	n.a.	<5 mins
		50.0% (v/v)	4.50	n.a.	3.50	n.a.	n.a.	n.a.	5 mins
		10.0% (v/v)	4.50	n.a.	1.50	n.a.	n.a.	n.a.	<5 mins
Virus Control	DIRTY			7.50	7.50	n.a.	n.a.	7.50	n.a.
Virus Control	CLEAN			7.50	7.50	n.a.	n.a.	7.50	n.a.
							30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				5.50	5.00	>60 mins

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

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) under DIRTY conditions

Controls

Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2	
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
6.00	**3.16E+07**	6.00	**3.16E+07**	6.00	**3.16E+07**	3.00	**3.16E+04**	2.00	**3.16E+03**	6.00	**3.16E+07**
666666	**3.16E+07**	666666	**3.16E+07**	666666	**3.16E+07**	666000	**3.16E+04**	660000	**3.16E+03**	666666	**3.16E+07**
	7.50		**7.50**		**7.50**		**4.50**		**3.50**		**7.50**
									4.00		**0.00**

Formaldehyde reference inactivation controls

Cytotoxicity		Exposure time	0.7% Formaldehyde			
			30		60	
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
1.00	**3.16E+02**		4.00	**3.16E+05**	3.50	**1.00E+05**
600000	**3.16E+02**		666600	**3.16E+05**	666300	**1.00E+05**
	2.50	log		**5.50**		**5.00**
		log difference		**2.00**		**2.50**

No column Control

5 mins

raw data	$TCID_{50}$/ml
6.00	**3.16E+07**
666666	**3.16E+07**
	7.50

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	1	1	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	3.16E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.50**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**6**	**0**
Product	1	1	1	1	0.67	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.48E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.17**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**4**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.33**	**0.00**
Product Cyt Dilution	-4	-4	-4	-4	-4	-4
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
7.00
3.16E+08
6666666000

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

Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Concentration	10.0% (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	**Exposure Time**	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
6.00	**3.16E+07**	6.00	**3.16E+07**	6.00	**3.16E+07**	**t = 5 minutes**	0.00	**3.16E+01**	2.00	**3.16E+03**	3.00	**3.16E+04**
666666	**3.16E+07**	666666	**3.16E+07**	666666	**3.16E+07**	Raw data	000000	**3.16E+01**	660000	**3.16E+03**	666000	**3.16E+04**
	7.50		**7.50**		**7.50**	log		**1.50**		**3.50**		**4.50**
						log difference		**6.00**		**4.00**		**3.00**

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Murine norovirus (s99) Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Murine norovirus s99 under DIRTY conditions						
Test Results						
Concentration	**5.0% (v/v)**		**10.0% (v/v)**		**50.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 5 minutes	2.00	**3.16E+03**	1.33	**6.76E+02**	1.00	**3.16E+02**
Raw Data	660000	**3.16E+03**	620000	**6.76E+02**	600000	**3.16E+02**
log		**3.50**		**2.83**		**2.50**
log difference		**3.00**		**3.67**		**4.00**

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Murine norovirus s99 under DIRTY conditions									
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	
DuoMax General Purpose Cleaner	3.0g/l BSA + 3.0ml/l erythrocytes	50.0% (v/v)	2.50	3.50	2.50	n.a.	n.a.	n.a.	5 min
		10.0% (v/v)	2.50	n.a.	2.83	n.a.	n.a.	n.a.	>5 min
		5.0% (v/v)	2.50	n.a.	3.50	n.a.	n.a.	n.a.	>5 min
DuoMax General Purpose Cleaner	3.0g/l BSA	50.0% (v/v)	2.50	n.a.	2.50	n.a.	n.a.	n.a.	<5 min
		10.0% (v/v)	2.50	n.a.	3.00	n.a.	n.a.	n.a.	>5 min
		5.0% (v/v)	2.50	n.a.	4.00	n.a.	n.a.	n.a.	>5 min
Virus Control	DIRTY			6.50	6.50	n.a.	n.a.	6.50	n.a.
Virus Control	CLEAN			6.67	6.67	n.a.	n.a.	6.50	n.a.
							30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	3.50				4.00	3.50	>60 mins

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Murine norovirus (s99) Control Data

EN14476:2013 + A2:2019 Suspension test for the efficacy of DuoMax General Purpose Cleaner, BT-DUO-22 from DuoTech Holdings against Murine norovirus s99 under DIRTY conditions

Controls

Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Cytotoxicity		Disinfectant Suppression VS		Disinfectant Suppression VS2	
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
5.00	**3.16E+06**	5.00	**3.16E+06**	5.00	**3.16E+06**	1.00	**3.16E+02**	2.00	**3.16E+03**	4.67	**1.48E+06**
666660	**3.16E+06**	666660	**3.16E+06**	666660	**3.16E+06**	600000	**3.16E+02**	660000	**3.16E+03**	666641	**1.48E+06**
	6.50		**6.50**		**6.50**		**2.50**		**3.50**		**6.17**
									3.00		**0.33**

Formaldehyde reference inactivation controls

Cytotoxicity		Exposure time	0.7% Formaldehyde			
			30		60	
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
2.00	**3.16E+03**		2.50	**1.00E+04**	2.00	**3.16E+03**
660000	**3.16E+03**		663000	**1.00E+04**	660000	**3.16E+03**
	3.50	log		**4.00**		**3.50**
		log difference		**2.50**		**3.00**

No column Control 5 mins	
raw data	$TCID_{50}$/ml
5.33	**6.76E+06**
666662	**6.76E+06**
	6.83

Interference control	Virus dilution					
	-3	**-4**	**-5**	**-6**	**-7**	**-8**
PBS Control	1	1	1	1	0.33	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	6.76E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**1.83**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**2**	**0**
Product	1	1	1	1	0.17	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	4.68E+01	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**1.67**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**1**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.16**	**0.00**
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
6.50
1.00E+08
6666663000

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Murine norovirus (s99) Control Data

Parallel Control Test												
Controls						Test Results						
Virus Recovery 0 min		Virus Recovery 5 min		Virus Recovery 60 min		Concentration	5.0% (v/v)		10.0% (v/v)		50.0% (v/v)	
raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
5.17	**4.68E+06**	5.17	**4.68E+06**	5.00	**3.16E+06**	**t = 5 minutes**	2.50	**1.00E+04**	1.50	**1.00E+03**	1.00	**3.16E+02**
666661	**4.68E+06**	666661	**4.68E+06**	666660	**3.16E+06**	Raw data	663000	**1.00E+04**	621000	**1.00E+03**	600000	**3.16E+02**
	6.67		**6.67**		**6.50**	log		**4.00**		**3.00**		**2.50**
						log difference		**2.67**		**3.67**		**4.17**

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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 $\log_{10}$.
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:
    - Between 0.5 and 2.5 after 30 min and between 2.0 and 4.5 after 60 min for poliovirus
    - Between 3.0 and 5.0 after 30 min and between 3.5 and 5.5 after 60 min for adenovirus
    - Between 1.0 and 3.0 after 30 min and between 2.0 and 4.0 after 60 min for murine norovirus
    - Between 0.0 and 2.0 after 30 min and between 0.5 and 2.5 after 60 min for parvovirus
    - 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 $\log_{10}$ reduction of the virus.
e) The interference control result does not show a difference of < 1.0 $\log_{10}$ of virus titre for test product treated cells in comparison to the non-treated cells.
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 greater than 0.5 $\log_{10}$ indicating rapid irreversible virucidal activity of the disinfectant by dilution at a concentration of 80.0% v/v/ 50.0% 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, **DuoMax General Purpose Cleaner POSSESSES VIRUCIDAL** activity at a concentration of **10.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Adenovirus type* 5 (ATCC VR-5)/HeLa cells.

The cytotoxicity of the product prevented a 4-log reduction being observed at 50.0% v/v and 80.0% v/v against *Adenovirus type* 5 (ATCC VR-5).

According to EN 14476:2013 + A2:2019, **DuoMax General Purpose Cleaner POSSESSES VIRUCIDAL** activity at a concentration of **50.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Poliovirus type* 1 (LSc-2ab/NIBSC Code 01/528)/HeLa cells.

The cytotoxicity of the product prevented a 4-log reduction being observed at 80.0% v/v against *Poliovirus type* 1 (LSc-2ab/NIBSC Code 01/528).

According to EN 14476:2013 + A2:2019, **DuoMax General Purpose Cleaner POSSESSES VIRUCIDAL** activity at a concentration of **50.0% v/v** of the working concentration as tested after **5 MINUTES** at **20°C** under **DIRTY** conditions (3.0 g/l bovine albumin + 3.0 ml/l erythrocytes) against *Murine norovirus* (s99)/RAW 264.7 cells.

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Authorised signatory



Dr Chris Woodall, Director
BluTest Laboratories Ltd
Glasgow, UK
Date: 26 March 2020

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Expanded Uncertainty of Measurement U = ± 0.047

DISCLAIMER

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