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	**Velox Oxy ETA**
Batch number	181220_96
Client	Medisept Sp. Z.o.o
Client address	Konopnica 159C, 21-030 Konopnica
Project code	BT-MDS-02
Date of delivery	29 January 2021
Storage conditions	Ambient
Active substances	Not indicated
Appearance	Liquid
Condition upon receipt	Undamaged

Test Method and Neutralisation

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.
Neutraliser	Dilution-neutralization/gel filtration/ enhanced neutralisation on highest dilution; Dulbecco's modified Eagles medium + 5% v/v foetal bovine serum at 4°C

Experimental Conditions

Period of analysis	15 April 2021 to 28 April 2021
Product diluent used	Sterile distilled water
Product test concentrations	**60.0% v/v; 70.0% v/v; 80.0% v/v (*poliovirus* only)** **1.0% v/v; 10.0% v/v; 25.0% v/v; 50.0% v/v (*adenovirus* only)**
Appearance product dilutions	No changes noted- stable
Appearance in test mixture	Turbidity and sedimentation observed at 70.0% v/v; 80.0% v/v (*poliovirus* only)
Contact time	t = 15 seconds; 30 seconds; 1 mins ± 5 s
Test temperature	20°C ± 1°C
Interfering substance	3.0g/l bovine albumin + 3.0g/l sheep erythrocytes
Temperature of incubation	37°C ± 1°C + 5% CO2

Test Organism(s)

Identification and passage (P) of virus	Adenovirus-5 ATCC VR-5 (P 08) Poliovirus-1 LSc-2ab (NIBSC Code 01/528) (P 07)

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Identification and passage (P) of cells	HeLa cells (*Poliovirus type* 1) (P 34) HeLa cells (*Adenovirus type* 5) (P 35)

PROTOCOL SUMMARY

The basic virucidal efficacy test is set up with three/four concentrations of test product solution and a 15 second, 30 second- and 1-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 and *Adenovirus type* 5 (ATCC VR-5)/HeLa 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 hard water/ sterile, synthetic 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 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 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): 480-483.

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Adenovirus type 5 (ATCC VR-5) Test Results

EN14476:2013 + A2:2019 Suspension test for the efficacy of Velox Oxy ETA, Batch 181220_96, BT-MDS-02 from Medisept Sp. Z.o.o against Adenovirus-5 ATCC VR-5 under DIRTY conditions

Test Results

Concentration	1.0% (v/v)		10.0% (v/v)		25.0% (v/v)		50.0% (v/v)	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 15 seconds	4.67	**1.47E+06**	2.67	**1.47E+04**	2.33	**6.81E+03**	1.17	**4.64E+02**
Raw Data	666640	**1.47E+06**	663100	**1.47E+04**	662000	**6.81E+03**	610000	**4.64E+02**
log		**6.17**		**4.17**		**3.83**		**2.67**
log difference		**0.33**		**2.33**		**2.67**		**3.83**
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 30 seconds	3.83	**2.15E+05**	3.00	**3.16E+04**	2.33	**6.81E+03**	1.00	**3.16E+02**
Raw Data	666500	**2.15E+05**	666000	**3.16E+04**	662000	**6.81E+03**	600000	**3.16E+02**
log		**5.33**		**4.50**		**3.83**		**2.50**
log difference		**1.17**		**2.00**		**2.67**		**4.00**
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 1 minute	3.83	**2.15E+05**	2.50	**1.00E+04**	2.17	**4.64E+03**	1.00	**3.16E+02**
Raw Data	666500	**2.15E+05**	663000	**1.00E+04**	661000	**4.64E+03**	600000	**3.16E+02**
log		**5.33**		**4.00**		**3.67**		**2.50**
log difference		**1.17**		**2.50**		**2.83**		**4.00**

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Adenovirus type 5 (ATCC VR-5) Control Data

Adenovirus-5 ATCC VR-5 Controls											
Virus Recovery 0 min		Virus Recovery 1 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**	1.50	**1.00E+03**	5.33	**6.81E+06**
666660	**3.16E+06**	666660	**3.16E+06**	666660	**3.16E+06**	600000	**3.16E+02**	630000	**1.00E+03**	666662	**6.81E+06**
	6.50		**6.50**		**6.50**		**2.50**		**3.00**		**6.83**
									3.50		**-0.33**

Formaldehyde reference inactivation controls							No column Control	
Cytotoxicity		Exposure time	0.7% Formaldehyde					
			30 mins		60 mins			
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
1.00	**3.16E+02**		1.00	**3.16E+02**	1.00	**3.16E+02**	5.33	**6.81E+06**
600000	**3.16E+02**		600000	**3.16E+02**	600000	**3.16E+02**	666662	**6.81E+06**
	2.50	log		**2.50**		**2.50**		**6.83**
		log difference		**4.00**		**4.00**		

Interference control	Virus dilution						Stock Virus ($TCID_{50}$)
	-3	**-4**	**-5**	**-6**	**-7**	**-8**	6.50
PBS Control	1	1	1	1	0.33	0.17	**1.00E+08**
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	6.76E+01	4.68E+01	6666663
	2.50	**2.50**	**2.50**	**2.50**	**1.83**	**1.67**	
Raw Data	**6**	**6**	**6**	**6**	**2**	**1**	
Product	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**	
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.17**	
Product Cyt Dilution	-2	-2	-2	-2	-2	-2	
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat	

SOP 11000
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Adenovirus type 5 (ATCC VR-5) Summary Table

Adenovirus-5 ATCC VR-5 Summary Table									
Product:	**Interfering substance**	**Concentration**	**Level of cytotoxicity**	**lg $TCID_{50}$**					**>4 lg reduction after 'X' Min**
				0 min	**15 secs**	**30 secs**	**1 min**	**60 min**	
Velox Oxy ETA	3.0g/l BSA + 3.0ml/l erythrocytes	50.0% (v/v)	2.50	3.00	2.67	2.50	2.50	n.a.	30 secs
		25.0% (v/v)	2.50	n.a.	3.83	3.83	3.67	n.a.	>1 min
		10.0% (v/v)	2.50	n.a.	4.17	4.50	4.00	n.a.	>1 min
		1.0% (v/v)	2.50	n.a.	6.17	5.33	5.33	n.a.	>1 min
Velox Oxy ETA	3.0g/l BSA	50.0% (v/v)	2.50	n.a.	2.67	2.50	2.50	n.a.	15 secs
		25.0% (v/v)	2.50	n.a.	4.00	3.83	3.50	n.a.	>1 min
		10.0% (v/v)	2.50	n.a.	6.00	6.00	5.50	n.a.	>1 min
		1.0% (v/v)	2.50	n.a.	6.33	5.50	5.50	n.a.	>1 min
Virus Control	DIRTY			6.50	n.a.	n.a.	6.50	6.50	n.a.
Virus Control	3.0g/l BSA			6.50	n.a.	n.a.	6.50	6.67	n.a.
							30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				2.50	2.50	>60 mins

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Adenovirus type 5 (ATCC VR-5) Control Data

Adenovirus-5 ATCC VR-5 Parallel Control Test															
Controls						Test Results									
Virus Recovery 0 min		Virus Recovery 1 min		Virus Recovery 60 min		Concentration	1.0% (v/v)		10.0% (v/v)		25.0% (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	data	$TCID_{50}$/ml
5.00	**3.16E+06**	5.00	**3.16E+06**	5.17	**4.64E+06**	**t = 15 seconds**	4.83	**2.15E+06**	4.50	**1.00E+06**	2.50	**1.00E+04**	1.17	**4.64E+02**
666651	**3.16E+06**	666660	**3.16E+06**	666652	**4.64E+06**	Raw data	666641	**2.15E+06**	666630	**1.00E+06**	663000	**1.00E+04**	610000	**4.64E+02**
	6.50		**6.50**		**6.67**	log		**6.33**		**6.00**		**4.00**		**2.67**
						log difference		**0.17**		**0.50**		**2.50**		**4.00**
						Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
						t = 30 seconds	4.00	**3.16E+05**	4.50	**1.00E+06**	2.33	**6.81E+03**	1.00	**3.16E+02**
						Raw data	666600	**3.16E+05**	666630	**1.00E+06**	662000	**6.81E+03**	600000	**3.16E+02**
						log		**5.50**		**6.00**		**3.83**		**2.50**
						log difference		**1.00**		**0.50**		**2.67**		**4.17**
						Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
						t = 1 minute	4.00	**3.16E+05**	4.00	**3.16E+05**	2.00	**3.16E+03**	1.00	**3.16E+02**
						Raw data	666600	**3.16E+05**	666600	**3.16E+05**	660000	**3.16E+03**	600000	**3.16E+02**
						log		**5.50**		**5.50**		**3.50**		**2.50**
						log difference		**1.00**		**1.00**		**3.00**		**4.17**

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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 Velox Oxy ETA, Batch 181220_96, BT-MDS-02 from Medisept Sp. Z.o.o against Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) under DIRTY conditions						
Test Results						
Concentration	**60.0% (v/v)**		**70.0% (v/v)**		**80.0% (v/v)**	
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 15 seconds	3.00	**3.16E+04**	2.00	**3.16E+03**	1.00	**3.16E+02**
Raw Data	666000	**3.16E+04**	660000	**3.16E+03**	600000	**3.16E+02**
log		**4.50**		**3.50**		**2.50**
log difference		**2.50**		**3.50**		**4.50**
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 30 seconds	2.33	**6.81E+03**	2.67	**1.47E+04**	1.00	**3.16E+02**
Raw Data	661100	**6.81E+03**	664000	**1.47E+04**	600000	**3.16E+02**
log		**3.83**		**4.17**		**2.50**
log difference		**3.17**		**2.83**		**4.50**
Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
t = 1 minute	2.00	**3.16E+03**	3.00	**3.16E+04**	1.00	**3.16E+02**
Raw Data	660000	**3.16E+03**	666000	**3.16E+04**	600000	**3.16E+02**
log		**3.50**		**4.50**		**2.50**
log difference		**3.50**		**2.50**		**4.50**

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

Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) Controls											
Virus Recovery 0 min		Virus Recovery 1 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
5.50	**1.00E+07**	5.50	**1.00E+07**	5.50	**1.00E+07**	0.00	**3.16E+01**	3.17	**4.64E+04**	5.83	**2.15E+07**
666663	**1.00E+07**	666663	**1.00E+07**	666663	**1.00E+07**	000000	**3.16E+01**	666100	**4.64E+04**	666665	**2.15E+07**
	7.00		**7.00**		**7.00**		**1.50**		**4.67**		**7.33**
									2.33		**-0.33**

Formaldehyde reference inactivation controls						
Cytotoxicity		Exposure time	0.7% Formaldehyde			
			30 mins		60 mins	
raw data	$TCID_{50}$/ml		raw data	$TCID_{50}$/ml	raw data	$TCID_{50}$/ml
1.00	**3.16E+02**		3.50	**1.00E+05**	2.83	**2.15E+04**
600000	**3.16E+02**		666300	**1.00E+05**	665000	**2.15E+04**
	2.50	log		**5.00**		**4.33**
		log difference		**2.00**		**2.67**

No column Control	
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	0.67	0.17
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	1.48E+02	4.68E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.17**	**1.67**
Raw Data	**6**	**6**	**6**	**6**	**4**	**1**
Product	1	1	1	1	0.83	0
	3.16E+02	3.16E+02	3.16E+02	3.16E+02	2.14E+02	3.16E+01
	2.50	**2.50**	**2.50**	**2.50**	**2.33**	**1.50**
Raw Data	**6**	**6**	**6**	**6**	**5**	**0**
Log Difference	**0.00**	**0.00**	**0.00**	**0.00**	**-0.16**	**0.17**
Product Cyt Dilution	-1	-1	-1	-1	-1	-1
PBS Dilution	Neat	Neat	Neat	Neat	Neat	Neat

Stock Virus ($TCID_{50}$)
7.17
4.68E+08
6666666100

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

Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) Summary Table									
Product:	**Interfering substance**	**Concentration**	**Level of cytotoxicity**	**lg $TCID_{50}$**					**>4 lg reduction after 'X' Min**
				0 min	**15 sec**	**30 sec**	**1 min**	**60 min**	
Velox Oxy ETA	3.0g/l BSA + 3.0ml/l erythrocytes	80.0% (v/v)	1.50	4.67	2.50	2.50	2.50	n.a.	<15 sec
		70.0% (v/v)	1.50	n.a.	3.50	4.17	4.50	n.a.	>1 min
		60.0% (v/v)	1.50	n.a.	4.50	3.83	3.50	n.a.	>1 min
Velox Oxy ETA	3.0g/l BSA	80.0% (v/v)	1.50	n.a.	4.50	2.50	2.50	n.a.	<30 sec
		70.0% (v/v)	1.50	n.a.	5.00	3.50	4.50	n.a.	>1 min
		60.0% (v/v)	1.50	n.a.	5.00	3.50	4.50	n.a.	>1 min
Virus Control	DIRTY			7.00	n.a.	n.a.	7.00	7.00	n.a.
Virus Control	3.0g/l BSA			7.17	n.a.	n.a.	7.00	7.17	n.a.
							30 min	60 min	
Formaldehyde	PBS	0.7% (w/v)	2.50				5.00	4.33	>60 mins

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

Poliovirus type 1 LSc-2ab (NIBSC Code 01/528) Parallel Control Test													
Controls						Test Results							
Virus Recovery 0 min		Virus Recovery 1 min		Virus Recovery 60 min		Concentration	60.0% (v/v)		70.0% (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	**1.47E+07**	5.50	**1.00E+07**	5.67	**1.47E+07**	**t = 15 seconds**	3.50	**1.00E+05**	3.50	**1.00E+05**	3.00	**3.16E+04**
666664	**1.47E+07**	666663	**1.00E+07**	666664	**1.47E+07**	Raw Data	666300	**1.00E+05**	666210	**1.00E+05**	666000	**3.16E+04**
	7.17		**7.00**		**7.17**	log		**5.00**		**5.00**		**4.50**
						log difference		**2.00**		**2.00**		**2.50**
						Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
						t = 30 seconds	2.00	**3.16E+03**	2.00	**3.16E+03**	1.00	**3.16E+02**
						Raw Data	660000	**3.16E+03**	660000	**3.16E+03**	600000	**3.16E+02**
						log		**3.50**		**3.50**		**2.50**
						log difference		**3.50**		**3.50**		**4.50**
						Exposure Time	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml	data	$TCID_{50}$/ml
						t = 1 minute	3.00	**3.16E+04**	3.00	**3.16E+04**	1.00	**3.16E+02**
						Raw Data	666000	**3.16E+04**	666000	**3.16E+04**	600000	**3.16E+02**
						log		**4.50**		**4.50**		**2.50**
						log difference		**2.50**		**2.50**		**4.50**

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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.
f) 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, **Velox Oxy ETA POSSESSES VIRUCIDAL** activity at a concentration of **50.0% v/v** of the working concentration as tested after **30 SECONDS** 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.

According to EN 14476:2013 + A2:2019, **Velox Oxy ETA POSSESSES VIRUCIDAL** activity at a concentration of **80.0% v/v** of the working concentration as tested after **15 SECONDS** 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.

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Date: 05 MAY 2021

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Expanded Uncertainty of Measurement U = ± 0.055

DISCLAIMER

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