Test Report for Virology
BS EN 14476:2013+A2:2019

Company Name:	**Sanzonate**
Contact Name:	John Little
Contact Email:	John.Little@sanzonate.co.uk
Purchase Order No:	TBC
Report Date:	25/11/2022
Melbec Ref Number:	48495
Name of Test Product:	**Sanzonate Ozone**
Batch Number:	**NA**

Melbec Microbiology Ltd, Imperial House, Kingsway Haslingden BB4 4QJ. Tel: 01706 214492
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Sample Details:

Manufacture / Supplier:.....	Sanzonate
Product storage conditions:.....	NA
Product appearance:.....	Clear colourless Liquid
Active substance and concentration:.....	Ozone only
Product dilution preparation:.....	NA
Product dilutions/concentrations:.....	Ready to Use (RTU)
Diluent used to dilute product:.....	Sterile Deionised Water
Cytotoxicity Reduction method:.....	MicroSpin S 400 HR columns and Large volume plating
Incubation temperature:.....	37°C +/- 1°C CO_2

The test product for testing when received.

Date product received: 17/11/22 Test Date: 17/11/22

Experimental Conditions:

Interfering substance:	Adapted Clean no interfering substance
Test temperature:	20 +/- 1 °C
Contact time:	5 minutes
Test organisms:	*Poliovirus type 1 LSc2ab*
Cell line identification:	HeLa
Cell culture media:	Eagle's Minimum Essential Medium + 2.0% v/v Foetal Bovine Serum

Deviations:

The sample was tested at a single concentration at the client's request.

Requirements of the Standard:

The test product shall demonstrate at least a 4 decimal logarithm (lg) reduction when tested in accordance with this standard under simulated clean or dirty conditions.

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Conclusion:

For the product Sanzonate Ozone , [Batch code: NA] the log reduction requirements as specified in BS EN 14476:2013+A2:2019 (4 lg within the relevant contact time) were not met in Adapted Clean no interfering substance conditions with a contact time of 5 minutes.

Report authorised by:

Name: Liam Stephens
Position: Technical Manager
Date: 25/11/2022

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Method

Test procedure

To determine the virucidal activity of the product, test virus is exposed to product dilutions for the required contact time and subsequently, the product is neutralised. The solution is then serially diluted and titrated on cell monolayers. The surviving virus tissue culture infective dose ($TCID_{50}$) is determined by the appearance of cytopathic effect (CPE) on the cells and is calculated using the Spearman-Kärber calculation.

Several controls are run alongside each test to validate the assay.

Titration of Virus control: The titration of the virus test suspension is determined at the start of the test and at the end of the test to determine its infectivity.

Reference for Virus Inactivation control: Formaldehyde is used instead of the test product, at 2 time points to demonstrate that the virus remains resistant to biocidal action at known concentrations.

Efficiency of Suppression: The test product is neutralised during the test, prior to the addition of test virus. Recovery of the test virus at it's original titre demonstrates effective product neutralisation.

Interference control: Cell are incubated with the test product for 1 hour and subsequently the test virus is added. Recovery of the test virus at it's original titre demonstrates that the presence of the product does not prevent infection of the cells by the test virus, and thus does not interfere with quantification of virucidal activity.

Cytotoxicity: Both the product and formaldehyde are incubated with cells, without the addition of test virus, to determine if any morphological changes occur that may mirror CPE normally caused by virus. This ensures any CPE seen is a result of residual virus and not the product.

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Poliovirus type 1 LSc2ab

Test Results				
Contact time	5 minutes	**Raw data**	**log $TCID_{50}$/ml**	**Log reduction**
Product (RTU)		63000000	4.00	3.50
Virus Test Suspension	Start	66666000	**7.50**	
	Finish	66666000		

Inactivation control (0.7% Formaldehyde)			
Contact time	**Raw data**	**log $TCID_{50}$/ml**	**Log reduction**
30 mins	66640000	6.17	1.33
60 mins	66440000	5.17	2.33

Formaldehyde cytotoxicity	
Raw data	00000000
Level of cytotoxicity	2.50

Product neutralisation		
Raw data	**log $TCID_{50}$/ml**	Log reduction
66666400	7.17	0.33

Product cytotoxicity	
Raw data	**Level of cytotoxicity**
00000000	2.50

Product interference			
	Raw data	**log $TCID_{50}$/ml**	Log reduction
PBS	66666500	7.33	0.17
Test product	66666300	7.00	
Difference		**0.33**	

Note: "T" = Cytotoxicity

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Verification of the methodology

Result Summary	Log of TCID50	Average	Log Reduction	Criteria	met/not met
Titration of Virus Control (Start)	7.50	7.50			
Titration of Virus Control (End)	7.50				
Product (RTU)	4.00		3.50	Log Reduction >= 4 Log	**Not Met**
Reference Test for Inactivation (Formaldehyde) 30 mins	6.17		1.33	between 0.5 and 2.5	**Met**
Reference Test for Inactivation (Formaldehyde) 60 mins	5.17		2.33	between 2.0 and 4.5	**Met**
Efficiency of Suppression	7.17		0.33	<=0.5 log of Average	**Met**
Inactivation Control (Product)	7.00		0.50	<=1.0 log of Average	**Met**
Inactivation Control (PBS)	7.33		0.17	<=1.0 log of Average	**Met**
Product Cytotoxicity	2.50				**N/A**

1) The titre of the test suspension is at least 10^8 TCID50 /ml or is sufficiently high to at least enable a titre reduction of 4 lg to verify the method: detectable titre reduction shall be at least 4 lg.

2) The difference between the logarithmic titre of the virus control and the logarithmic titre of the test organism in the reference inactivation test should be between 0.5 and 2.5 after 30 mins and between 2.0 and 4.5 after 60 mins for Poliovirus type 1 LSc2ab.

3) Cytotoxicity of the product test solution should not affect cell morphology and growth or susceptibility for the test organism in the dilutions of the test mixtures which are necessary to demonstrate a 4 lg reduction of the virus.

4) The product should not interfere with susceptibility of the cells to the test organism, the difference in the titre of the test suspension and the recovered titre of the interference control should be <1lg.

5) Control of efficiency for suppression of product activity (the difference to the test suspension shall be ≤ 0,5 lg).

6) At least one concentration per test shall demonstrate a 4 lg or more reduction and at least one concentration shall demonstrate a lg reduction of less than 4.

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