

Company Name: Sanzonate

Contact Name: John Little

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Purchase Order No: TBC

Report Date: 08/12/2022

Melbec Ref Number: 48443

Name of Test Product: Sanzonate Ozone

Batch Number: NA

Sample Details:

Manufacture / Supplier:..... Sanzionate
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 CO₂

The test product for testing when received.

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

Experimental Conditions:

Interfering substance: Adapted clean conditions (No interfering substance)
Test temperature: 20 +/- 1 °C
Contact time: 5 minutes
Test organisms: *Vaccinia virus VR-1508 (Modified Vaccinia Ankara)*
Cell line identification: BHK-21 Clone 13
Cell culture media: Dulbecco'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
No interfering substance at the clients 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.

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 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: 08/12/2022

All samples are tested as received and the condition on receipt is deemed to be satisfactory for testing unless client is informed otherwise. If an unsatisfactory sample is received and tested on instruction from the client comments are included on the report detailing this information. Results given for this may be invalid. Results detailed above relate only to the samples tested. Sample description and batch references stated are as provided by the customer. This test report shall not be reproduced except in full without the approval of Melbec Microbiology Ltd.

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 (TCID₅₀) 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 its 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 its 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.

***Vaccinia virus VR-1508 (Modified
 Vaccinia Ankara)***

Test Results				
Contact time	5 minutes	Raw data	log TCID ₅₀ /ml	Log reduction
Product (RTU)		T0000000	3.50	4.00
Virus Test Suspension		Start 66666000	7.50	
		Finish 66666000		

Inactivation control (0.7% Formaldehyde)			
Contact time	Raw data	log TCID ₅₀ /ml	Log reduction
5 mins	66660000	6.50	1.00
15 mins	66660000	5.50	2.00

Formaldehyde cytotoxicity	
Raw data	00000000
Level of cytotoxicity	2.50

Product neutralisation		
Raw data	log TCID ₅₀ /ml	Log reduction
06666400	7.17	0.33
Product cytotoxicity		
Raw data	Level of cytotoxicity	
0T000000	3.50	

Product interference			
	Raw data	log TCID ₅₀ /ml	Log reduction
PBS	06666400	7.17	0.33
Test product	06666300	7.00	
Difference		0.17	

Note: "T" = Cytotoxicity

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)	3.50		4.00	Log Reduction \geq 4 Log	Met
Reference Test for Inactivation (Formaldehyde) 5 mins	6.50		1.00	between 0.75 and 3.5	Met
Reference Test for Inactivation (Formaldehyde) 15 mins	5.50		2.00	between 2.0 and 4.0	Met
Efficiency of Suppression	7.17		0.33	\leq 0.5 log of Average	Met
Inactivation Control (Product)	7.00		0.50	\leq 1.0 log of Average	Met
Inactivation Control (PBS)	7.17		0.33	\leq 1.0 log of Average	N/A
Product Cytotoxicity	3.50				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 0.75 and 3.5 after 5 mins and between 2.0 and 4.0 after 15 mins for Vaccinia virus VR-1508 (Modified Vaccinia Ankara).
- 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 <1 lg.
- 5) Control of efficiency for suppression of product activity (the difference to the test suspension shall be $\leq 0,5$ 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.