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Final Report

Title: *In vitro* assay using A549 human lung indicator cells for the detection of cytopathic adventitious viruses

Sample identification:

Sample name:	VX1-A 15-06-2016
MicroSafe sample number:	69884
Sample name:	VX1-B 15-06-2016 for suitability test, 6ml
MicroSafe sample number:	69886

Administrative details:

Sponsor name and address: Sponsor representative: Sponsor code:	[redacted]
Testing facility:	[redacted]
Test schedule: Laboratory start date: Laboratory completion date:	 26 July 2016 26 August 2016
Raw data references:	00010584TR[A]: T16F405 LAB-VIRO-260-TR02: T16G438

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Study details:

Test method:	According to protocol TM-SV-227 revision 1.0 Part A and B with study specific supplement. On request of the sponsor A549 cells were used as indicator cells.
Sample preparation:	Part A (sample 69886): one vial was thawed at 35-37°C prior to use and diluted 1:3, 1:10, 1:30 and 1:100 in 5%FBS/DMEM/Gentamicin. 0.1 ml of every dilution was used as test article (quadruple testing). Part B (sample 69884): one vial was thawed at 35-37°C prior to use and diluted 1:10 in 5%FBS/DMEM/Gentamicin. 5 ml of this dilution is used as test article (quadruple testing).
Negative controls:	Non-infected A549 cells cultured in parallel
Positive control:	Encephalomyocarditis virus (EMCV)
Protocol amendments:	Not applicable
Deviations:	There was one deviation (OOO-300 2016-07-29-A): the A549 cells were not seeded one day before initiation of part B of the test, but they were seeded on the same day as the test was initiated. When the sample was inoculated the cells were attached and were suitable for use. Therefore this deviation has no impact on the test.

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Results

Part A – Cytotoxicity testing

Item			Result (3 dpi)
A549 cells	69886	1:3 dilution	Cells look swollen
		1:10 dilution	No cell damage
		1:30 dilution	No cell damage
		1:100 dilution	No cell damage
		Negative control	No cell damage
		Positive control	CPE

Table 1: Test for cytotoxicity of the sample

Part B - Test on A549 indicator cell line

Item				Requirement	Result
A549 cells	69884, 1:10 diluted	14 dpi	Negative control	No CPE	Complies
		14 dpi	Positive control	CPE	Complies
		14 dpi	Test article	No CPE	Complies
		28 dpi	Negative control	No CPE	Complies
		28 dpi	Positive control	CPE	Complies
		28 dpi	Test article	No CPE	Complies

Table 2: Test for cytopathic effect (CPE), visual inspection

Part B - Cytological staining

Item				Requirement	Result
A549 cells	69884, 1:10 diluted	14 dpi	Negative control	No CPE	Complies
		14 dpi	Positive control	CPE	Complies
		14 dpi	Test article	No CPE	Complies
		28 dpi	Negative control	No CPE	Complies
		28 dpi	Positive control	CPE	Complies
		28 dpi	Test article	No CPE	Complies

Table 3: Giemsa assay

Conclusions part A

1. The assay meets the criteria for a valid test.
2. The incubation of a 1:3 dilution of the test article on the indicator cells resulted in aberrant appearance of the cells. Therefore for part B a 1:10 dilution was chosen as test article.

Conclusions part B

1. The assay meets the criteria for a valid test.
2. The sample does not show indications of the presence of adventitious viruses.

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Quality Statement:

The study was performed in compliance with the agreed protocol and was executed in accordance with MicroSafe Laboratories Standard Operating Procedures except when clearly documented otherwise. The execution of the study conformed to the principles of Good Manufacturing Practices of the European Community.

Report prepared by:

[redacted] [redacted] 29aug16

Name Signature Date

Authorisation and approval (Quality Assurance):

10.2.e

[redacted] [redacted] 29 Aug 16

Name Signature Date

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