NON-GLP STUDY REPORT

STUDY TITLE

Test for Antiviral Activity and Efficacy – Modification of JIS Z 2801

Virus: Influenza A (H1N1) virus

PRODUCT IDENTITY

Avitex Anti Viruz
2020-09-22 Lab Batch

PROTOCOL NUMBER

PTA001092220.FLUA

AUTHOR

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STUDY COMPLETION DATE

May 19, 2021

PERFORMING LABORATORY

Analytical Lab Group-Midwest
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SPONSOR

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PROJECT NUMBER

A32769

This study was not performed under
EPA Good Laboratory Practice Regulations
(40 CFR Part 160)

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Test for Antiviral Activity and Efficacy – Modification of JIS Z 2801

Project Number: A32769

Protocol Number: PTA001092220.FLUA

TEST SUBSTANCE IDENTITY

Test Substance: Avitex Anti Viruz, 2020-09-22 Lab Batch

Control Substance: Paint X Blank

STUDY DATES

Date Sample Received: November 9, 2020
Study Initiation Date: April 19, 2021
Experimental Start Date: April 22, 2021
Experimental End Date: April 29, 2021
Study Completion Date: May 19, 2021

TEST PARAMETERS

Product Preparation: The test and control samples were ready to use. No preparation of carriers required.

Virus: Influenza A (H1N1) virus, ATCC VR-98, Strain A/Malaya/302/54

Exposure Time: 6 hours

Exposure Temperature: 25-30°C (27.0°C) in a humidified atmosphere of 55-60% relative humidity (55%)

Organic Soil Load: 1% fetal bovine serum

Test Medium: Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 2 µg/mL TPCK-trypsin, 100 units/mL penicillin, 10 µg/mL gentamicin, and 2.5 µg/mL amphotericin B

Indicator Cell Cultures: MDCK (canine kidney) cells

EXPERIMENTAL DESIGN

The test and control materials were approximately 50 mm x 50 mm (2 inch x 2 inch). A carrier film was prepared to fit over the test and control materials. The film was approximately 40 mm x 40 mm and was prepared from a sterile stomacher bag.

Test and Virus Control

One test carrier and one control carrier, contained in an individual sterile petri dishes, were each inoculated with a 200 µL aliquot of the test virus. The inoculum was covered with the carrier film and the film was pressed down so the test virus spread over the film, but did not spread past the edge of the film. The 6 hour exposure time began when each sample was inoculated. The samples were transferred to a controlled chamber set to 25-30°C (27.0°C) in a relative humidity of 55-60% (55%) for the duration of the Sponsor specified 6 hour exposure time.

Following the 6 hour exposure time, using sterile forceps, the film was lifted off and a 2.00 mL aliquot of test medium was pipetted individually onto each test and control carrier as well as the underside of the film used to cover each sample (side exposed to the test sample or control). The surface of each carrier was individually scraped with a sterile plastic cell scraper. The test medium was collected (10^{-1} dilution), mixed using a vortex type mixer, and serial 10-fold dilutions were prepared. The dilutions were assayed for infectivity and/or cytotoxicity.

Zero Time Virus Control

One control carrier, contained in an individual sterile petri dish, was inoculated with a 200 µL aliquot of the test virus. Immediately following inoculation, a 2.00 mL aliquot of test medium was pipetted onto the control carrier. The surface of the carrier was scraped with a sterile plastic cell scraper. The test medium was collected (10^{-1} dilution), mixed using a vortex type mixer and serial 10-fold dilutions were prepared. The dilutions were assayed for infectivity.

Cytotoxicity Control

One test carrier, contained in an individual sterile petri dish, was inoculated with a 200 µL aliquot of the test medium containing the Sponsor requested organic soil load (1% fetal bovine serum). The inoculum was covered with the carrier film and the film was pressed so that the test medium spread over the film, but did not spread past the edge of the film. The exposure time began when the carrier was inoculated. The carrier was transferred to a controlled chamber set to 25-30°C (27.0°C) in a relative humidity of 55-60% (55%) for the duration of the 6 hour exposure time.

Following the 6 hour exposure time, using sterile forceps the film was lifted off and a 2.00 mL aliquot of test medium was pipetted individually onto the cytotoxicity control carrier as well as the underside of the film used to cover the carrier (side exposed to the carrier). The surface of the carrier was scraped with a sterile plastic cell scraper. The test medium was collected (10^{-1} dilution), mixed using a vortex type mixer, and serial 10-fold dilutions were prepared. The dilutions were assayed for cytotoxicity.

An appropriate neutralization control was run concurrently.

Per Sponsor's direction, the study was not required to be conducted under US EPA 40 CFR Part 160 or US FDA 21 CFR Part 58.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, Avitex Anti Viruz, 2020-09-22 Lab Batch, demonstrated a 99.9% reduction in viral titer following a 6 hour exposure time at 25-30°C (27.0°C) in a relative humidity of 55-60% (55%) to Influenza A (H1N1) virus, as compared to the titer of the 6 hour exposure time virus control. The log reduction in viral titer was 3.00 $\log_{10}$, as compared to the titer of the 6 hour exposure time virus control. A 99.98% reduction in viral titer was demonstrated, as compared to the titer of the zero time virus control virus control. The log reduction in viral titer was 3.75 $\log_{10}$, as compared to the titer of the zero time virus control.

In the opinion of the Author, there were no circumstances that may have affected the quality or integrity of the data.

STUDY RESULTS

TABLE 1: Virus Control Results

Dilution	Input Virus Control	Zero Time Virus Control	6 Hour Virus Control
Cell Control	0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	+ + + +
10^{-2}	+ +	+ + + +	+ + + +
10^{-3}	+ +	+ + + +	+ + + +
10^{-4}	+ +	+ + + +	+ + + +
10^{-5}	+ +	+ + + +	+ + + +
10^{-6}	+ +	+ + + +	+ + + +
10^{-7}	+ +	0 + + +	0 0 0 0
10^{-8}	+ +	NT	NT
$TCID_{50}$/100 µL	$\geq 10^{8.50}$	$10^{7.25}$	$10^{6.50}$

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NT) = Not tested

TABLE 2: Test Results

Results of Avitex Anti Viruz (2020-09-22 Lab Batch) Exposed to Influenza A (H1N1) Virus Following a 6 Hour Exposure Time

Dilution	Test: Influenza A (H1N1) virus + Avitex Anti Viruz, 2020-09-22 Lab Batch
Cell Control	0 0 0 0
10^{-1}	+ + + +
10^{-2}	+ + + +
10^{-3}	+ + + +
10^{-4}	0 0 0 0
10^{-5}	0 0 0 0
10^{-6}	0 0 0 0
10^{-7}	0 0 0 0
$TCID_{50}$/100 µL	$10^{3.50}$
Percent Reduction (Based on the zero time virus control)	99.98%
Log_{10} Reduction (Based on the zero time virus control)	3.75 Log_{10}
Percent Reduction (Based on the 6 hour virus control)	99.9%
Log_{10} Reduction (Based on the 6 hour virus control)	3.00 Log_{10}

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

TABLE 3: Cytotoxicity Control and Neutralization Control

Dilution	Cytotoxicity Control Avitex Anti Viruz, 2020-09-22 Lab Batch	Neutralization Control Avitex Anti Viruz, 2020-09-22 Lab Batch
Cell Control	0 0	0 0
10^{-1}	0 0	+ +
10^{-2}	0 0	+ +
10^{-3}	0 0	+ +
10^{-4}	0 0	+ +
10^{-5}	0 0	+ +
10^{-6}	0 0	+ +
10^{-7}	0 0	+ +
TCD_{50}/100 µL	$\leq 10^{0.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control (neutralization control) indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of ≤0.50 $\log_{10}$.

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5-19-21
Date