NON-GLP STUDY REPORT

STUDY TITLE

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

Virus: Influenza A (H1N1) virus

PRODUCT IDENTITY

立邦健康卫士抗菌+净味全效 — Nippon Paint Health Guard Anti-Bacterial + Odour-Less All In One Interior Emulsion Paint
(also known as **立邦健康**卫盾抗菌+净味全效 — Nippon Paint Health Shield Anti-Bacterial + Odour-Less All In One Interior Emulsion Paint)

PROTOCOL NUMBER

NPC02030520.FLUA

AUTHOR

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Senior Virologist

STUDY COMPLETION DATE

April 28, 2020

PERFORMING LABORATORY

Analytical Lab Group-Midwest
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Nippon Paint (China) CO.,LTD.
NO.287 ChuangYe Road
South JinQiao Export Processing Zone
Pudong District
Shanghai, China

PROJECT NUMBER

A29363

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: A29363

Protocol Number: NPC02030520.FLUA

TEST SUBSTANCE IDENTITY

Test Substance: 立邦健康卫士抗菌+净味全效 — Nippon Paint Health Guard Anti-Bacterial+ Odour-Less All In One Interior Emulsion Paint (also known as 立邦健康卫盾抗菌+净味全效 — Nippon Paint Health Shield Anti-Bacterial+ Odour-Less All In One Interior Emulsion Paint)

Control Substance: Control (untreated)

ADDITIONAL PRODUCT IDENTITY

Nippon Paint (Singapore) Co. Pte Ltd
1 First Lok Yang Road
Jurong, Singapore 629728

Product Name: Nippon Paint VirusGuard Emulsion Paint

Nippon Paint (M) Sdn Bhd
Lot I, 17, Jalan SU 4
Taman Perindustrian Subang Utama
40300 Shah Alam
Selangor, Malaysia

Product Name: Nippon Paint VirusGuard Emulsion Paint

STUDY DATES

Date Sample Received: March 16, 2020
Study Initiation Date: March 23, 2020
Experimental Start Date: March 23, 2020
Experimental End Date: March 31, 2020
Study Completion Date: April 28, 2020

TEST PARAMETERS

Product Preparation: The test and control samples were wiped with ethanol and allowed to dry prior to use in testing.

Virus: Influenza A (H1N1) virus, ATCC VR-1469, Strain A/PR/8/34

Exposure Time: 12 hours and 24 hours

Exposure Temperature: 20°C (20.0°C) in a relative humidity of 50% (50%)

Organic Soil Load: 5% fetal bovine serum

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

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

For the test and control substance, four carriers, contained in individual sterile petri dishes, were each inoculated with a 100 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 exposure time began when each carrier was inoculated. The carriers were transferred to a controlled chamber set to 20°C (20.0°C) and a relative humidity of 50% (50%) for the duration of the Sponsor specified 12 and 24 hour exposure times.

Following each exposure time, the film was lifted off each carrier using sterile forceps and a 1.00 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 carrier (side exposed to the test or control carrier). 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. The 12 and 24 hour exposure control carriers are for informational purposes only.

Zero Time Virus Control

One control carrier, contained in an individual sterile petri dish, was inoculated with a 100 µL aliquot of the test virus. Immediately following inoculation, a 1.00 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 100 µL aliquot of the test medium containing the Sponsor requested organic soil load (5% 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 20°C (20.0°C) and a relative humidity of 50% (50%) for the duration of the 24 hour exposure time.

Following the 24 hour exposure time, using sterile forceps the film was lifted off and a 1.00 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 5% fetal bovine serum organic soil load, the treated test substance demonstrated a ≥99.4% reduction in viral titer following a 12 hour exposure time at 20°C (20.0°C) and a relative humidity of 50% (50%) to Influenza A (H1N1) virus, compared to the titer of the zero time virus control. The log reduction in viral titer was ≥2.25 $\log_{10}$, compared to the titer of the zero time virus control.

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, the treated test substance demonstrated a ≥99.97% reduction in viral titer following a 24 hour exposure time at 20°C (20.0°C) and a relative humidity of 50% (50%) to Influenza A (H1N1) virus, compared to the titer of the zero time virus control. The log reduction in viral titer was ≥3.50 $\log_{10}$, compared to the titer of the zero time virus control.

TABLE 1: Input Virus Control Results

Dilution	Input Virus Control March 23, 2020	Input Virus Control March 24, 2020
Cell Control	0 0	0 0
10^{-1}	+ +	+ +
10^{-2}	+ +	+ +
10^{-3}	+ +	+ +
10^{-4}	+ +	+ +
10^{-5}	+ +	+ +
10^{-6}	+ +	+ +
10^{-7}	+ +	+ +
10^{-8}	+ 0	+ +
$TCID_{50}$/100 µL	$10^{8.00}$	$\geq 10^{8.50}$

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

TABLE 2: Virus Control Results

Dilution	Virus Controls		
	Zero Time	12 hour exposure	24 hour exposure
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +	+ + + 0
10^{-5}	+ + + +	+ + + +	0 0 + 0
10^{-6}	+ + + +	0 0 0 +	0 0 0 0
10^{-7}	+ + + +	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$\geq 10^{7.50}$	$10^{5.75}$	$10^{4.50}$

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

TABLE 3: Test Results

Dilution	Test	
	12 hour exposure	24 hour exposure
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ 0 + +
10^{-4}	+ + + +	+ + 0 +
10^{-5}	0 0 + +	0 0 0 0
10^{-6}	+ 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{5.25}$	$10^{4.00}$
Percent Reduction*	≥99.4%	≥99.97%
Log_{10} Reduction*	≥2.25 log_{10}	≥3.50 log_{10}

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(*) = Reduction calculated using the zero time control

TABLE 4: Cytotoxicity Control and Neutralization Control

Dilution	Cytotoxicity Control Treated	Neutralization Control Treated
Cell Control	0 0	0 0
10^{-1}	0 0	+ +
10^{-2}	0 0	+ +
10^{-3}	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}.

PREPARED BY:

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4-28-2020
Date