IMSL

INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: **Determination of the Antiviral Activity of Blank Plates against Human Coronavirus NL63 using ISO 21702 : 2019.**

CLIENT: **Hager Ltd**
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Telford
Shropshire
TF1 7FT

REPORT NO: **IMSL2021/05/040.1B**

DATED: **16th July 2021**

Study: Determination of the Antiviral Activity of Blank Plates against Human Coronavirus NL63 using ISO 21702.

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The above study was conducted by a third party laboratory approved by Industrial Microbiological Services Ltd, Pale Lane Hartley Wintney, Hants, RG27 8DH, UK.

This report represents a true and accurate account of the results obtained.

Start Date 02nd July 2021

Report Issued 16th July 2021

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1 Aim

To test the virucidal activity of Blank Plates against Human Coronavirus NL63 at a contact time of 24 hours using the method described in ISO 21702:2019 (Ref. 1).

2 Test Materials

Samples of Blank Plates were supplied by Hager Group. These were held in the dark at 20°C prior to use.

3 Methods

Antiviral activity against Human Coronavirus NL63 (see Table 1) was determined using ISO 21702:2019 (Ref 1).

Table 1: Test Strain

Virus	Strain Reference	Host
Human Coronavirus	NL63	Rhesus Monkey Kidney Epithelial Cells (LLC-MK2 Line)

3.1 Assay Validity Control Tests

For the assay to be valid, the material tested must have no cytotoxic activity on the mammalian cells used to quantify the virus, nor interfere with cell sensitivity to infection. The two tests of these criteria are described below.

3.1.1 Cytotoxicity control

Assay media was added to replicate (3) samples of the test formulation and the reference material (untreated polystyrene) and left in contact for 5 minutes and then transferred onto mono-layers of cells seeded into the wells of a 96-well plate. The plates were then incubated 10 days. After incubation the mono-layers were stained using crystal violet and then assessed for cell viability. Media that had not been in contact with either the test sample or the reference material was included as a control.

For the test to be valid, no cytotoxic effect should be observed compared with the media control.

3.1.2 Sensitivity Control

Assay media was added to individual replicates (3) samples of each of the paint formulations and the reference material and left in contact for 5 minutes. The eluate from each was then transferred to individual sterile tubes. An aliquot of a suspension containing 1×10^5 infectious units of virus were then added into each tube. After a contact interval of 30 minutes at 25°C the number of infectious virus units in each sample was quantified using a 50% tissue culture infectious dose ($TCID_{50}$) assay. Media that had not been in contact with either the coating, the ethanol treated polystyrene or the reference material was included as a control.

For the test to be valid, the eluate from samples of the test formulation and the reference material must not cause interference to the sensitivity of the host cell to infection.

3.2 Test Method

The surface of replicate (3) samples of the Blank Plates were inoculated with an aliquot (200 µL) of a suspension containing 1 x 10^5 infectious virus units of Human Coronavirus NL63 in the presence of a low level of protein soiling (0.3% bovine serum albumin).

The suspension was placed onto the surface of each sample and then covered with an inert film (polyethylene, 40 mm x 40 mm x 12.7 µm thick). The samples were then incubated for 24 hours at 25°C in a humidified chamber.

After contact intervals of 24 hours, the cover film was removed and the samples were washed with media to recover the virus as described in ISO 21702. The number of infectious virus units recovered from each sample was then quantified by TCID50 assay.

The surface of replicate (3) samples of the a reference material were also inoculated with an aliquot (200 µL) of a suspension containing 1 x 10^5 infectious virus units of Human Coronavirus NL63, but not incubated. Instead, the samples were immediately washed with media to recover the virus as described in ISO 21702. The number of infectious virus units recovered from each sample was then quantified by $TCID_{50}$ assay. This was used to quantify the starting amount of virus used in the test.

3.2.1 50% Tissue Culture Infectious Dose ($TCID_{50}$) Assay

A seven-point, ten-fold serial dilution from the virus-containing wash media from each test sample was assayed in quadruplicate using Rhesus Monkey Kidney Epithelial Cells (LLC-MK2 Line) cells in microtitre plates. After inoculation, the plates were incubated for10 days and then stained using crystal violet and inspected visually to determine cell viability across each dilution series.

The dilution at which 50% of cells were infected / killed ($TCID_{50}$) was calculated using a regression analysis.

3.3 Calculations and Data Handling

The data were transformed to $TCID_{50}$ cm^{-2}. The antiviral activity (R) was calculated as:

$R = Ut - At$

Where Ut is the average of the common logarithm of the number of infectious units recovered from the untreated test specimens at the end of the incubation time and At is the average of the common logarithm of the number of infectious units recovered from the treated test specimens at the end of the incubation time.

An R value of $\geq$ 1 indicates antiviral activity.

4 Results / Discussion

The results as average $TCID_{50}$ cm^{-2} are shown in Table 2.

Table 2: Activity Against Human Coronavirus NL63 - Average of 3 replicates ± Standard Error the Mean as $TCID_{50}$ cm^{-2}

Sample	Contact Time (Hours)		Reduction from Reference	
	0	24	$Log_{10}(R)$	%
Polyester (Reference)	$8.30 \times 10^4 \pm 4.24 \times 10^4$	$6.64 \times 10^4 \pm 1.37 \times 10^4$	-	-
Blank Plate	-	$1.26 \times 10^3 \pm 8.55 \times 10^2$	1.7	98.10

NB: An R value of $\geq$ 1 indicates antiviral activity.

Antiviral activity was observed against Human Coronavirus NL63 in the presence of 0.3 g L^{-1} BSA on the surfaces of Blank Plate after 24 hours contact.

None of the test materials displayed cytotoxicity towards the cells used to host the virus in this experiment and they did not interfere with the infectivity of them (see Appendix 1).

5 Raw Data

The raw data for this study will be held in file IMSL2021/05/040.1B in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 6 years from the date of this report unless other specific instructions are given.

6 References

1 ISO 21702:2019 - Measurement of antiviral activity on plastics and other non-porous surfaces

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Appendix 1 : Validation Data

Cytotoxicity

Sample	Cytotoxicity
Polyester (Reference)	Not cytotoxic
Blank Plate	Not cytotoxic
Media	Not cytotoxic

Sensitivity Control

Sample	Sensitivity control ($TCID_{50}$ cm^{-2})	$Log_{10}TCID_{50}$ cm^{-2}	$\Delta^{‡}$
Polyester (Reference)	$2.97 \times 10^4 \pm 9.50 \times 10^3$	4.47	0.34
Blank Plate	$2.97 \times 10^4 \pm 9.50 \times 10^3$	4.47	0.34
Media	$6.51 \times 10^4 \pm 1.92 \times 10^4$	4.81	-

‡ = Media - Sample