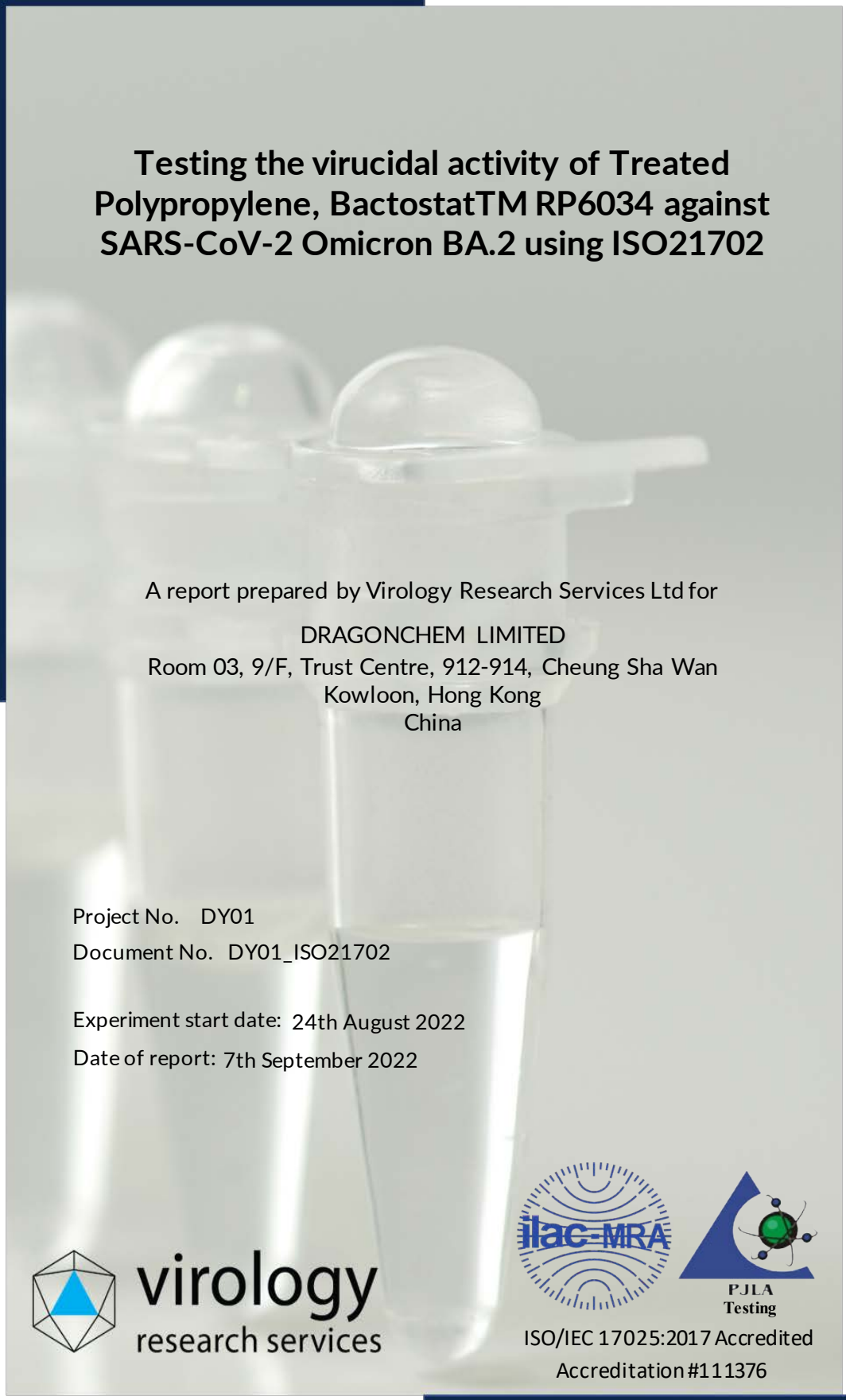
Testing the virucidal activity of Treated Polypropylene, BactostatTM RP6034 against SARS-CoV-2 Omicron BA.2 using ISO21702



A report prepared by Virology Research Services Ltd for

DRAGONCHEM LIMITED
Room 03, 9/F, Trust Centre, 912-914, Cheung Sha Wan
Kowloon, Hong Kong
China

Project No. DY01
Document No. DY01_ISO21702

Experiment start date: 24th August 2022
Date of report: 7th September 2022

virology research services

ISO/IEC 17025:2017 Accredited
Accreditation #111376

Virology Research Services Ltd
MRC LMCB, Gower Street, WC1E 6BT, London, UK

Contents

Summary

Aim
This study tests the antiviral activity of Treated Polypropylene, BactostatTM RP6034 against SARS-CoV-2 Omicron BA.2 at a contact time of 24h relative to a non-treated reference control.

Methods
ISO21702 is a standard protocol to quantify the antiviral properties of non-porous surfaces. In this protocol, a pre-determined concentration of virus was dispensed onto test and reference surfaces and incubated at room temperature for 24h in a humidified chamber.

Next, the samples were recovered by washing with media (neutraliser), and the amount of infectious virus in each suspension was quantified using a TCID50 assay. For the assay to be valid, the material tested must have no cytotoxic activity on the cells used to quantify the virus, nor interfere with cell sensitivity to infection.

Results
Virucidal activity against SARS-CoV-2 Omicron BA.2 was observed for the treated material relative to the reference control. The R value (antiviral activity) was 1.81.

The test material had cytotoxicity towards the cells used to quantify the virus; nevertheless, we were able to test the virucidal activity and report an R-value. No cytotoxic activity was detected for the reference control sample. No cytotoxic activity was detected for the media only sample.

Conclusion
Under the conditions tested, Treated Polypropylene, BactostatTM RP6034 displays virucidal activity against SARS-CoV-2 Omicron BA.2.

Aim

To test the virucidal activity of Treated Polypropylene, BactostatTM RP6034 against SARS-CoV-2 Omicron BA.2 at a contact time of 24h relative to a reference control, following ISO21702:2019.

Methods

Assay validity control tests

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

Cytotoxicity control: Is the tested material cytotoxic to the assay's host cells?

Assay media is added to treated material and reference control for 5 min, before being collected and added onto monolayers of cells seeded into the wells of a 96-well plate. Cells are then cultured, and after 10 days a viability assay (crystal violet staining) is used to determine cell viability. The test is carried out in triplicate for both the treated material and non-treated reference control.

Media that has been in contact with neither the treated material nor reference control is included as a reference. For the test to be valid, no cytotoxic effect should be observed compared to the media.

Sensitivity control: Do the tested materials affect the assay cells' sensitivity to the virus?

Assay media is added to treated material and reference control for 5 min, before being collected in tubes. Next, to test whether exposing the media to the materials affects the cells' sensitivity to infection, 5 x 10^5 infectious units of virus are added into each tube. After a 30-min incubation at room temperature, the amount of infectious virus in each sample is quantified (TCID50 assay). The 50% tissue culture infectious dose (TCID50) is the end-point virus dilution where 50% of the infected test cells die.

The tests are carried out in triplicate on treated and non-treated material. Media that has not been in contact with either material is also incubated with the virus.

When there is no cytotoxicity and the materials do not interfere with the host cell's sensitivity to infection, the assay is considered to meet the requirements for ISO 21702 and can be used to establish the antiviral activity of the test material.

Antiviral test procedure

Treated and non-treated materials were placed in individual discs in triplicate. A liquid volume (200 µl) of an appropriate concentration of virus (1 x 10^6 PFU/ml stock of SARS-CoV-2 Omicron BA.2) was added onto each surface and covered with an inert film.

A lid was placed over each disc, which was then incubated for 24h at room temperature in a humidified chamber. At the end of the incubation, the film was lifted, and the sample washed with media to recover the virus. The amount of infectious virus recovered from each sample was then quantified by TCID50.

As a further control, virus was added to three pieces of the reference control material and immediately recovered by washing (referred to as the 'virus recovery control' or 'back-titration'). This recovered virus is used to quantify the starting amount of virus.

TCID50 determination

A seven-point, ten-fold serial dilution from the virus-containing wash media was tested in quadruplicate for each sample on African Green Monkey Kidney Epithelial (Vero) cells. After 10 days, a viability crystal violet assay was carried out to determine cell viability across the dilution series. The dilution at which 50% of cells are infected/killed (TCID50) was calculated using the Reed and Muench method.

Quantification of antiviral activity

When the test is deemed valid, the antiviral activity (R) is calculated as follows:

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 ≥ 1 indicates antiviral activity.

Key test information

This page provides key additional information required when reporting the findings of an ISO 21702:2019 testing protocol:

Specimens

Test sample: Solid, Treated Polypropylene, BactostatTM, RP6034
Reference control: Solid, non-porous, Untreated polypropylene
Size, shape, and thickness: 50 × 50 mm squares, < 10 mm thick

Film

Type of polymer: polyethylene
Shape and thickness: 40 × 40 mm, approx. 12.7 µm thick

Virus/cells

Virus strain: SARS-CoV-2 Omicron BA.2
Host cells: African Green Monkey Kidney Epithelial (Vero)

Test inoculum

Volume: 200 µl
Virus titre: 5 × 10^6

Contact time

24h

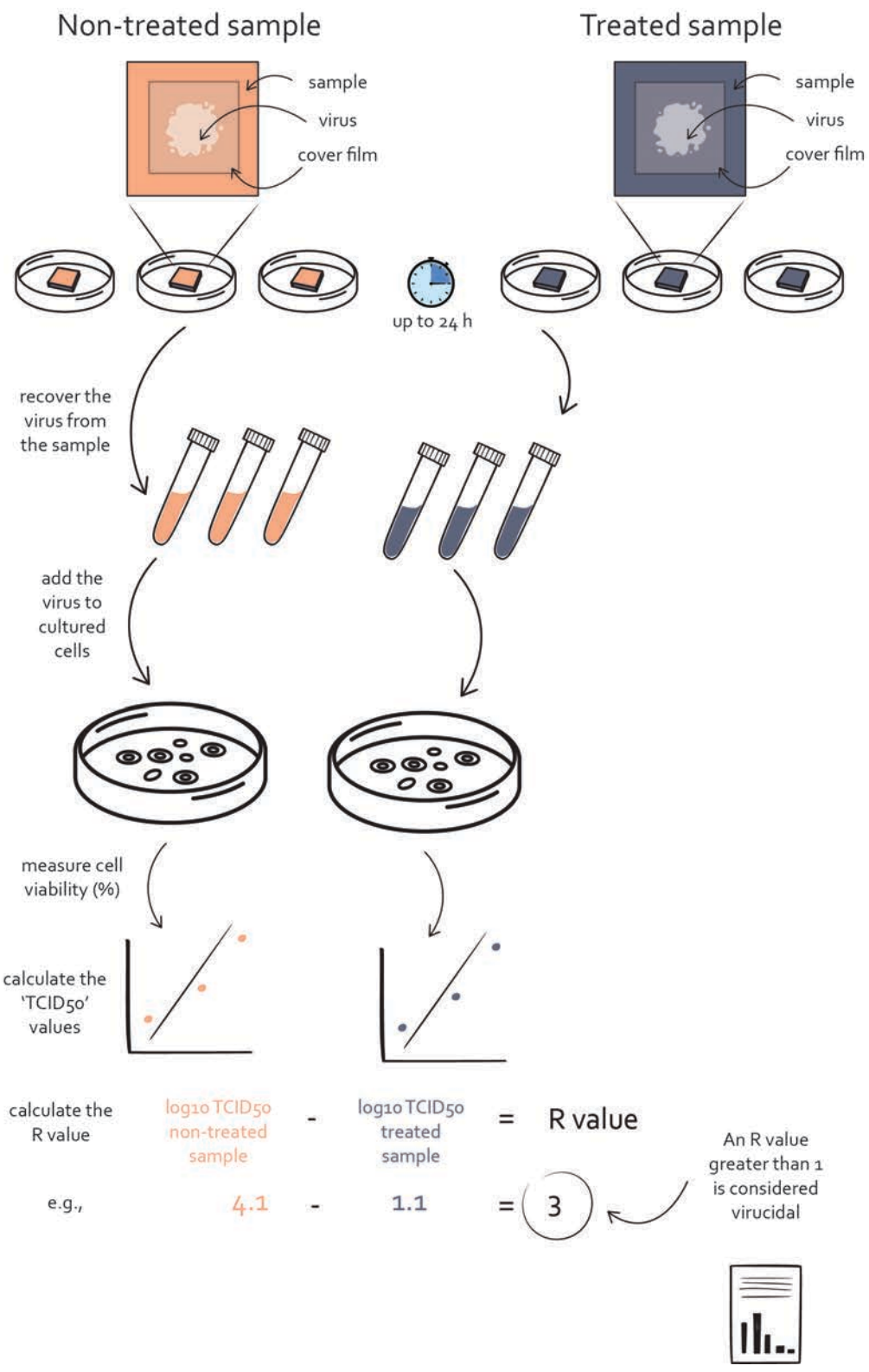
Note

Test laboratory

Virology Research Services Ltd, London

virology
research services

7th September 2022



Scheme 1. An outline of the method used to calculate the virucidal activity (R value) of treated samples relative to a non-treated control sample. Note that this scheme is not comprehensive and does not include all the various controls included in the ISO21702 protocol.

Results

Control tests

The control experiments are summarized in the Appendix.
The test material displays cytotoxicity towards the cells used to host the virus in this experiment. The test material does not interfere with the cells used to host the virus in this experiment.

Because of cytotoxicity, the first dilution of the recovered media (the undiluted sample) was excluded from the analyses. Like this, we were able to test the antiviral activity of the sample.

Antiviral tests

The results of the antiviral test for Treated Polypropylene, BactostatTM RP6034 are summarized in Table 1 and Figure 1. The treated material displays virucidal activity against SARS-CoV-2 Omicron BA.2 when using a contact time of 24h.

Treated Polypropylene, BactostatTM RP6034 displays antiviral activity against SARS-CoV-2 Omicron BA.2. The average recovered titre for the treated material was 8.01E+01 TCID50/cm² compared to the average recovered titre of 5.19E+03 TCID50/cm² for the non-treated reference control.

R (antiviral activity) = 1.81

Table 1. The average infectious units per cm² recovered from the test and reference control materials at a contact time of 24h with the virus.

Test Condition	Virus recovery control (TCID50/cm²)			Antiviral test (TCID50/cm²)		
Test	NA			8.01E+01	±	1.37E+01
Reference	4.24E+04	±	2.57E+04	5.19E+03	±	2.44E+03

Table 2. The average infectious units per cm² recovered from the test and reference control materials at a contact time of 24h with the virus.

Test Condition	TCID50 (log10)	R Value	% reduction
Test	1.90	1.81	90%
Reference	3.72		

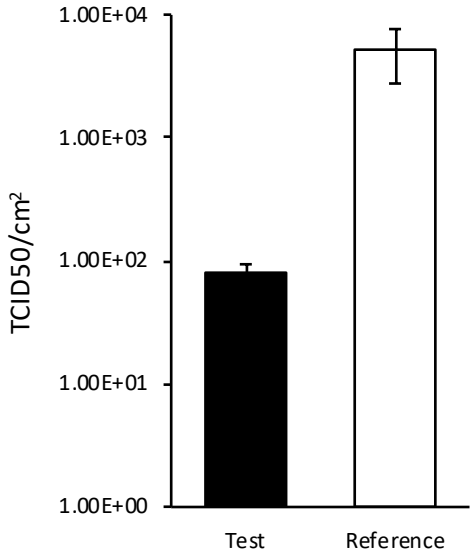


Figure 1. The mean TDIC50/cm² values for SARS-CoV-2 Omicron BA.2 following a contact time of 24h with test and reference control materials. Error bars are standard error of the mean.

Conclusion

Based on the findings reported here and following ISO 21702, the treated material displays virucidal activity against SARS-CoV-2 Omicron BA.2 after a contact time of 24h.

The results of control assays confirm that the tested material is not cytotoxic for the test cells (when excluding the first dilution - the undiluted sample - of the recovered media).

Also, the test material does not interfere with the cells' sensitivity to the virus. Thus, the experiment meets the requirements for a valid ISO 21702 test.

Appendix

Control tests

Cytotoxicity

Test Condition	Cytotoxicity
Test	Cytotoxic
Reference	Not cytotoxic
Media	Not cytotoxic

Cell viability (%) upon incubation with media recovered from reference and treated materials, relative to the fresh media control.

Sensitivity control

Test Condition	Sensitivity control (TCID50/cm^2)			Sensitivity control (Log10)	Media - material (Log10)
Test	8.31E+04	±	3.90E+04	4.92	-0.20
Reference	1.89E+05	±	3.90E+04	5.28	-0.55
Media	5.28E+04	±	1.69E+04	4.72	NA

Infectious TCID50/cm^2 recovered after 30 min incubation with 3 ml of media that has been in contact with the treated or untreated material. The difference between the common logarithm of the infectivity titre of virus recovered from the media only control and each specimen should be less than or equal to 0.5.

Final page

virology
research services

www.virologyresearchservices.com