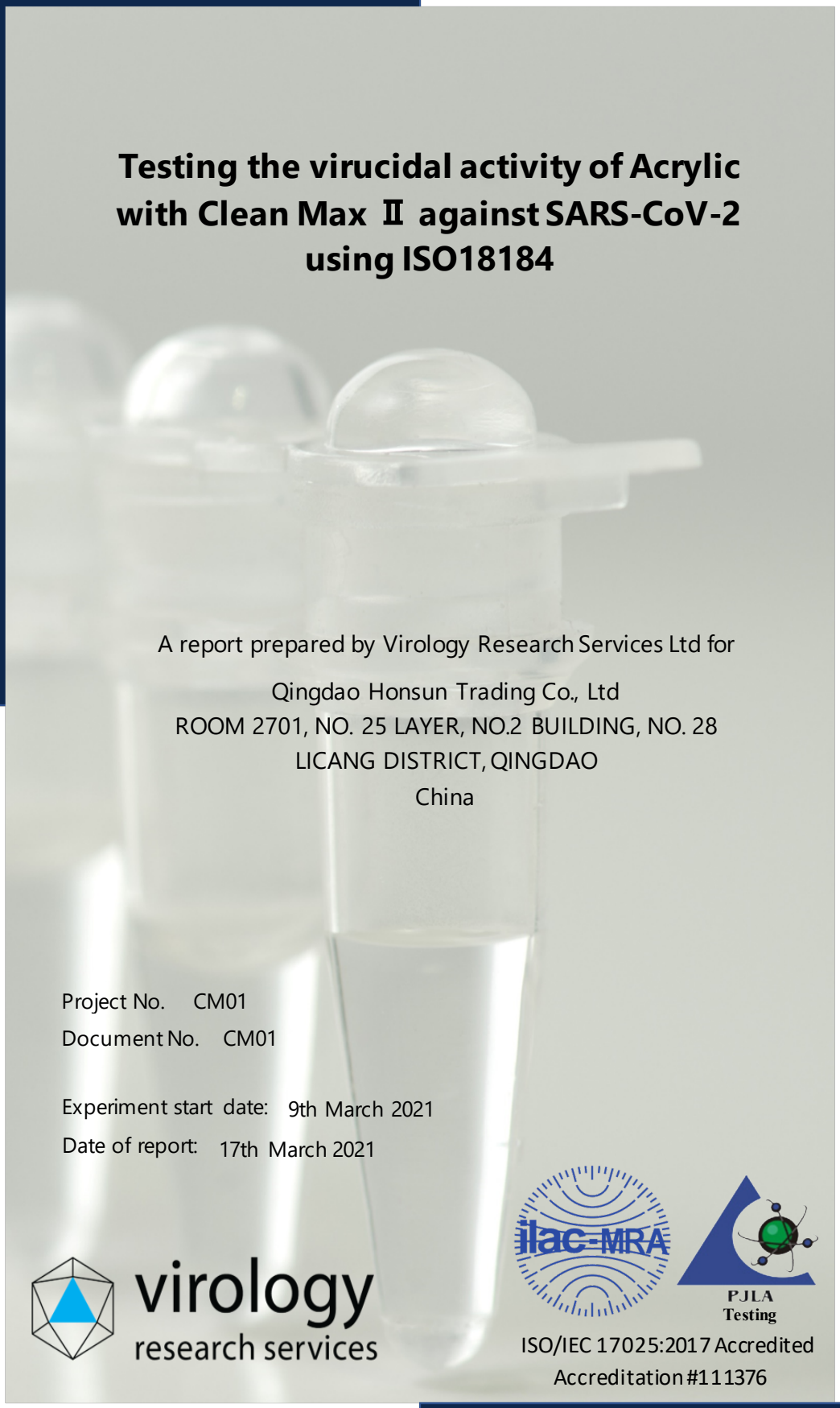
Testing the virucidal activity of Acrylic with Clean Max Ⅱ against SARS-CoV-2 using ISO18184



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Summary

Aim

This study tests the antiviral activity of Acrylic with Clean Max II against SARS-CoV-2 at a contact time of 2 hours relative to a non-treated reference control.

Methods

ISO18184 is a standard protocol to quantify the antiviral properties of textile materials. In this protocol, a pre-determined concentration of virus was dispensed onto test and reference material and incubated at room temperature for 2 hours.

Next, the samples were recovered by washing with media, 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

The test material had no cytotoxic activity towards the cells used to quantify the virus. No cytotoxic activity was detected for the reference control sample. No cytotoxic activity was detected for the media only sample.

Aim

To test the virucidal activity of Acrylic with Clean Max II against SARS-CoV-2 at a contact time of 2 hours relative to a reference control, following ISO18184:2019.

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 test 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 test material and non-treated reference control.

Media that has been in contact with neither the test 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 test 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, 0.5 x 10^6 infectious units (IU) 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 tested and reference 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 ISO18184 and can be used to establish the antiviral activity of the test material.

Antiviral test procedure

Test and control fabrics were placed in individual tubes in triplicate. 200 µl of virus (of concentration 1 x 10^7 IU/ ml) were added on top of each fabric so that the material was completely imbibed with the virus solution. A lid was placed over each tube, which was then incubated for the indicated contact time at room temperature. At the end of the incubation, the samples were washed with media several times 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 samples of the reference control material and immediately recovered by washing (referred to as the 'virus recovery control'). This recovered virus is used to quantify the starting amount of virus. The difference between the virus recovered from the reference immediately after inoculation and after the contact time must be less than 1 log (see Appendix Table 5).

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). After 3 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 (Mv) is calculated as follows:

$Mv = Log(Va) - Log(Vc)$

where Log(Va) is the average of the common logarithm of the number of infectious units recovered from the reference specimens immediately after inoculation;

and Log(Vc) is the average of the common logarithm of the number of infectious units recovered from the antiviral test specimens at the end of the incubation time.

A value of $2.0 > Mv \geq 1.0$ indicates mild antiviral effect.
A value of $3.0 > Mv \geq 2.0$ indicates good antiviral effect.
A value of $Mv \geq 3.0$ indicates excellent antiviral effect.

Key test information

This page provides key additional information required when reporting the findings of an ISO18184:2019 testing protocol:

Specimens

Test sample: Acrylic fiber with CLEAN MAX II

Reference control: Polyester

Size, shape, and thickness: 20 × 20 mm squares, 0.4 g

Virus/cells

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

Test inoculum

Volume: 200 µl
Virus titre: 1 × 10^7

Contact time

2 hours

Deviations from the standard protocol

None

Test laboratory

Virology Research Services Ltd, London

17th March 2021

Results

Control tests

The control experiments are summarized in the Appendix.
The test material does not display 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.

Antiviral tests

Test Condition	Virus recovery control (TCID50/sample)			Antiviral test (TCID50/sample)		
Test	NA			1.71E+03	±	1.37E+03
Reference	2.78E+06	±	8.90E+05	2.14E+03	±	3.65E+02

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

Test Condition	TCID50 (log10)	Mv Value	% reduction
Test Control	3.23 6.44	3.21	99.9%

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

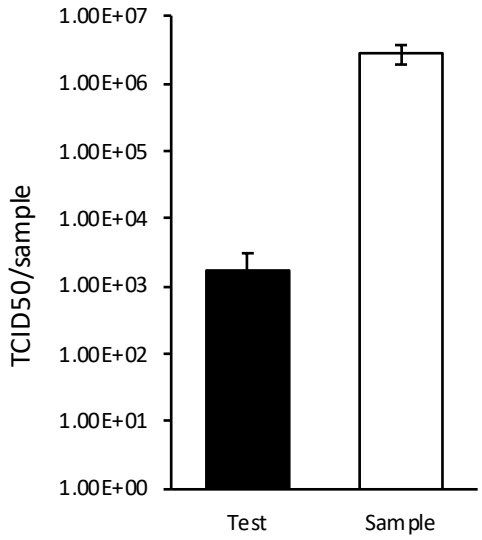

Figure 1. The mean TDIC50/sample values for SARS-CoV-2 following a contact time of 2 hours with the test sample and immediately harvested from the control material. Error bars are standard error of the mean.

Conclusion

The results of control assays confirm that the tested material is not cytotoxic for the test cells.

Appendix

Control tests

Cytotoxicity

Table 3

Test Condition	Cytotoxicity
Test	Not 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

Table 4

Test Condition	Sensitivity control (TCID50/sample)			Sensitivity control (Log10)	Media - material (Log10)
Test	6.75E+05	±	1.15E+05	5.83	-0.25
Reference	5.60E+05	±	1.15E+05	5.75	-0.17
Media	3.80E+05	±	6.49E+04	5.58	NA

Infectious TCID50/sample recovered after 30 min incubation with 5 ml of media that has been in contact with the treated or untreated material. The difference between the natural 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

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