

# Testing the virucidal activity of AGS-WB against SARS-CoV-2 Omicron BA.2 using ISO21702

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# Summary

## **Aim**

This study tests the antiviral activity of AGS-WB against SARS-CoV-2 Omicron BA.2 at a contact time of 24 hours 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 24 hours 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 3.36.

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.

## **Conclusion**

Under the conditions tested, AGS-WB displays virucidal activity against SARS-CoV-2 Omicron BA.2.

# Aim

To test the virucidal activity of AGS-WB against SARS-CoV-2 Omicron BA.2 at a contact time of 24 hours 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,  $4.1 \times 10^4$  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 (TCID<sub>50</sub> assay). The 50% tissue culture infectious dose (TCID<sub>50</sub>) 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 ( $4.1 \times 10^5$  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 24 hours 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 (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 a regression analysis.

### Quantification of antiviral activity

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

$$R = U_t - A_t$$

where  $U_t$  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  $A_t$  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.

## Key test information

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

### Specimens

Test sample: AGS-WB paper/card

Reference control: VRS plastic

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 (Vero) cells

### Test inoculum

Volume: 200 µl

Virus titre:  $6 \times 10^6$

### Contact time

24 hours

### Deviations from the standard protocol

None

### Notes

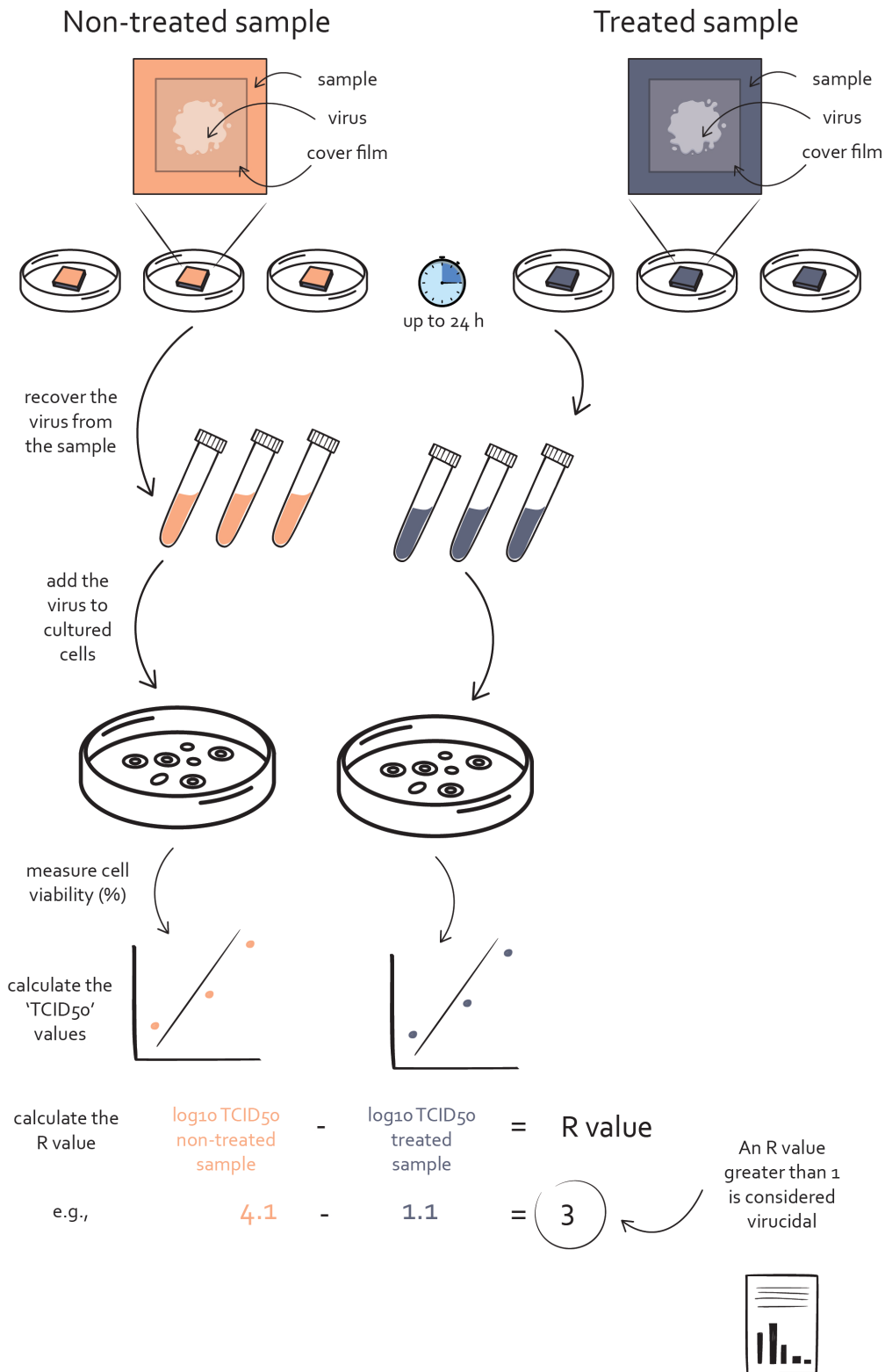
The virus was added on the sample 'top' surface (rougher side) which has the antiviral coating. Once the contact time had passed the test material (cardboard) was soaked in infection media (5ml) for 50 mins until it became soft and could be mashed to elute the virus from inside.

### Test laboratory

Virology Research Services Ltd, London



24th January 2023



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 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

The results of the antiviral test for AGS-WB 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 24 hours.

AGS-WB displays antiviral activity against SARS-CoV-2 Omicron BA.2. The average recovered titre for the treated material was 2.29E+00 TCID<sub>50</sub>/cm<sup>2</sup> compared to the average recovered titre of 5.19E+03 TCID<sub>50</sub>/cm<sup>2</sup> for the non-treated reference control.

$$R(\text{antiviral activity}) = 3.36$$

Table 1. The average infectious units per cm<sup>2</sup> recovered from the test and reference control materials at a contact time of 24 hours with the virus.

Test Condition	Virus recovery control (TCID <sub>50</sub> /cm <sup>2</sup> )		Antiviral test (TCID <sub>50</sub> /cm <sup>2</sup> )		
Test	NA		2.29E+00	±	6.77E-01
Reference	6.64E+03	± 1.37E+03	5.19E+03	±	2.44E+03

Table 2. The average infectious units per cm<sup>2</sup> recovered from the test and reference control materials at a contact time of 24 hours with the virus.

Test Condition	TCID <sub>50</sub> (log10)	R Value	% reduction
Test	0.36	3.36	99.9%
Reference	3.72		

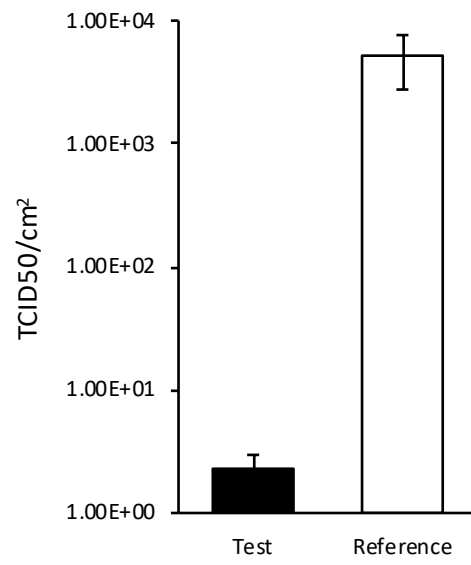


Figure 1. The mean TDIC50/cm<sup>2</sup> values for SARS-CoV-2 Omicron BA.2 following a contact time of 24 hours with test and reference control materials. Error bars are standard error of the mean.

# 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 24 hours.**

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

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	Not cytotoxic
Reference	Not cytotoxic
Media	Not cytotoxic

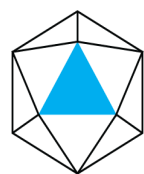
Cell viability (%) upon incubation with media recovered from reference and treated materials, relative to the fresh media control.

### Sensitivity control

Test Condition	Sensitivity control (TCID <sub>50</sub> /cm <sup>2</sup> )		Sensitivity control (Log <sub>10</sub> )	Media - material (Log <sub>10</sub> )
Test	4.98E+04	± 2.54E+04	4.70	0.32
Reference	1.26E+05	± 2.60E+04	5.10	-0.09
Media	1.03E+05	± 4.23E+04	5.01	NA

Infectious TCID<sub>50</sub>/cm<sup>2</sup> 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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