Instituto de Medicina Molecular João Lobo Antunes

Determination of antiviral activity of textile products against SARS-CoV-2

General protocol

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PRINCIPLE

Textile samples are assayed for their ability to inactivate SARS-CoV-2 at room temperature, according to ISO 18184. Samples are inoculated with 200 µL of SARS-CoV-2 (from a virus suspension at least with 10^6 PFU/mL) and after a specific contact time, the remaining infectious viruses are recovered and quantified by plaque assay using Vero CCL-81 cells. The antiviral activity is calculated by the comparison between the antiviral textile sample and the control textile sample. Tests are run in triplicate and control conditions are included. All experiments are performed in a certified BSL3 laboratory, following international and iMM safety guidelines.

TESTING METHOD

Virus and host cells

SARS-CoV-2 (isolate 606; BioProject PRJEB38351; READS ENA ACCESSION ERR4157960) used to assay textile samples, was isolated from a nasopharyngeal and oropharyngeal swab that had tested positive by RT-PCR at iMM.

Reagents and media

Cell growth media: Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % HI-FBS (10500-064 Gibco), penicillin 50 U/mL, streptomycin 50 µg/mL (15070-063 Gibco) and 2 mM glutamine (25030-024 Gibco)

Cell maintenance media: Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2.5 % HI-FBS (10500-064 Gibco), penicillin 50 U/mL, streptomycin 50 µg/mL (15070-063 Gibco) and 2 mM glutamine (25030-024 Gibco)

Washing solution: Tryptic Soy Broth (TSB) with 0.07 % lecithin and 0.5 % Polysorbate 80 (301122SA VWR)

Preparation of textile samples

Square-shaped textile samples are cut with approximately 20 mm × 20 mm and several pieces are combined in 50 mL falcon tubes to make a total mass of 0.40 ± 0.05 g. Falcon tubes containing samples and respective caps are sterilized separately by autoclaving. After sterilization, tubes are placed inside a safety cabinet for cooling down and then closed with caps.

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Control test

Textile samples (0.40 ± 0.05 g) are washed with 20 mL of washing solution in 50 mL falcon tubes, by vortexing 5 times for 5 seconds. Then, 5 mL are transferred to a new falcon tube and 50 µL of the virus stock diluted 1:10 were added. After 30 min incubation at room temperature, PFU are quantified by plaque assay.

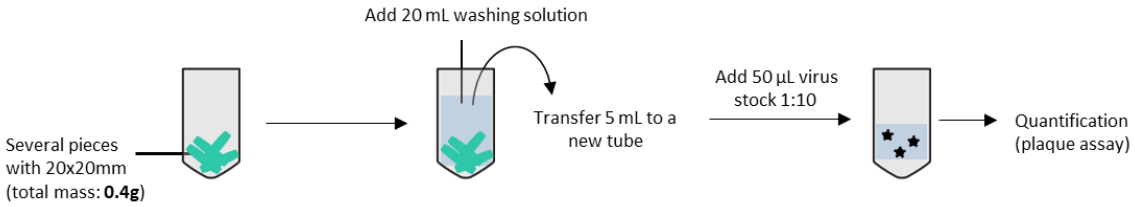

Figure 1 - *Schematic description of the control test.*

Antiviral test

Textile samples (0.40 ± 0.05 g) are exposed to 200 µL of SARS-CoV-2 (from a virus suspension at least with 10^6 PFU/mL) and incubated for specific exposure times, at room temperature. Following each exposure time, samples are washed with 20 mL of washing solution by vortexing 5 times for 5 seconds. 200 µL of washed solution are transferred to a tube containing 1.8 mL of maintenance medium (10^{-1} dilution). This procedure was repeated to prepare 10-fold serial dilutions (10^{-2} and 10^{-3}) and PFU are quantified by plaque assay.

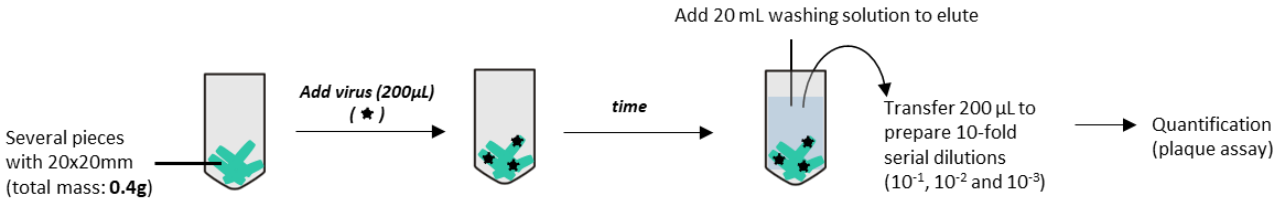

Figure 2 *– Schematic description of the method used to test textile samples.*

PFU quantification by plaque assay

One day before plaque assay, Vero cells are seeded in 6 well-plates at a density of $8x10^5$ cells/well. On the day of plaque assay, growth media is removed and cell monolayers are inoculated in duplicate with 500 µL of serial 10-fold dilutions, and incubated for 1 hour in a CO_2 incubator at 37 °C, with gently rocking every 15 min. Viral inoculum is removed and 2 mL of overlay media (maintenance media with 1.25 % carboxymethyl cellulose) are added to cell monolayers. After four days of incubation, overlay media is removed and cells are fixed with 4 % formaldehyde in PBS (1 hour) and stained with 0.1 % toluidine blue (30 min) to visualize viral plaques. The viral titer is calculated as plaque forming units/mL (PFU/mL), according to:

$$PFU/mL = \text{average number of plaques} \times 1/\text{dilution} \times 1/\text{inoculum}$$

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Limit of detection: under these experimental conditions, the limit of detection is considered to be 1 PFU at the lowest dilution tested (10^{-1}) and determined as 20 PFU/mL (or 400 PFU per 0.4 g of textile sample)

Antiviral activity

Antiviral activity (M_v) is calculated from the relation between one sample at a given time and the immediate recovery (t= 0 min) from the control sample, according to:

$$M_v = \lg(V_{a)} - \lg(V_c)$$

where,

$\lg(V_a)$: common logarithm average of 3 viral titer value immediate after exposure to the control sample (t= 0 min);

$\lg(V_c)$: common logarithm average of 3 viral titer value after the exposure time with the antiviral sample, at a given time.

The percentage of reduction ($R_{\%}$) is calculated by the following equation:

$$R_{\%} = \left(1 - \frac{1}{10^{Mv}}\right) \times 100$$

where,

M_v is the antiviral activity value.

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