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STUDY REPORT

Study Title

Non-GLP ISO 18184
Modified Test Method (Simulated Splash) for Determination of Antiviral Activity of Coated
Non-woven Fabric

Product(s) Identity

3 samples provided by the client:
Mask A – Grey coating
Mask B – Amber coating
Mask C – Untreated (control)

Test Microorganisms

- (i) Murine Coronavirus (MHV-A59)
- (ii) Human Coronavirus (229E)

Author

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Study Completion Date

10 May 2021

Client

Prof. Jas Pal Badyal, University of Durham

SUMMARY

General Study Information

Study Title Non-GLP ISO 18184 Modified Test Method (Simulated Splash) for Determination of Antiviral Activity of Coated Non-woven Fabric

Test System

Test Microorganisms (i) Murine Coronavirus (Murine Hepatitis Virus, strain MHV-A59)

(ii) Human Coronavirus (strain 229E)

Host Cells Murine fibroblast 17Cl-1 cells for MHV;
MRC5 lung fibroblasts for HCoV 229E.

Test Samples 3 x non-woven facemasks (outermost layer):
A – coated (grey)
B – coated (amber)
C – untreated (white; control sample)

Viral Titre Determination: TCID50 (using Reed & Muench calculations)

Test Parameters

Sample Preparation Fabric cut into 2 cm squares; sterilised by exposing each side of the fabric to UV irradiation for 15 minutes inside a Class II Microbiological Safety Cabinet prior to testing.

Total Organic Soil Load Viral inoculum was not supplemented with additional organic load.

Number of Replicates Per Sample Three

Contact Time 0 and 2 hours

Inoculum Volume per Sample 20 µl, dispensed as 5 x 4 µl droplets.

Incubation conditions Room temperature (21°C) and ambient humidity

Neutralisation Method Elution with 0.5 ml 1.5% (w/v) beef extract.

Study Dates 8 – 12 Feb for MHV test; 11 – 16 Apr for 229E test.

TEST PROCEDURE

Two different batches of samples were tested, the first with MHV, the second with HCoV 229E. Test procedures were essentially identical other than choice of cell line and culture medium employed to calculate the respective viral titres.

1. Aliquots of viral stocks were thawed on ice. MHV stock titre was approximately 10^9 infectious units per ml, and HCoV 229E approximately 5×10^6 infectious units per ml (titred when prepared).
2. Test materials were cut into 2 cm squares, sterilised by subjecting each surface to 15 min UV irradiation in a Class II MSC, and then placed into sterile plastic petri dishes.
3. $5 \times 4 \mu\text{l}$ aliquots of virus were inoculated onto the surface of each of the test materials, which were tested in triplicate.
4. Contact time began as soon as the inoculum was pipetted onto the surface of the material.
5. Test materials remained within petri dishes (without lids) inside a Class II Microbiological Safety Cabinet (MSC) at a stable temperature and humidity for the specified contact time (2 hours).
6. At T0 and T2 hours, the respective samples were submerged in 0.5 ml of 1.5% (w/v) beef extract in a 50 ml Greiner tube and vortexed vigorously for 10 seconds.
7. The resultant viral suspensions (eluates) were aseptically collected and $25 \mu\text{l}$ aliquots diluted by serial 10-fold dilutions in 2.5% FBS DMEM [low glucose, no glutamate] for MHV inoculated samples, or 5 % FBS Eagle's Minimum Essential Medium [with Earle's salts and non-essential amino acids] for HCoV 229E inoculated samples.
8. Non-inoculated samples were subject to the same elution and dilution procedures to assay for cytopathic effects associated with the test fabrics.
9. $50 \mu\text{l}$ aliquots of eluted and diluted viral suspensions were added to individual wells of 96-well culture plates containing monolayers of either 17Cl-1 or MRC5 cells cultured in $100 \mu\text{l}$ of the appropriate medium. Viral eluate from each sample was used to inoculate 4 wells of cells i.e. 12 wells in total for each dilution given triplicate samples. Dilutions ranged from neat eluate through to 10^{-6} dilution. The final row of wells/cells was inoculated with sterile culture medium.
10. Assay plates were incubated for up to 48 hours at 37°C in a 5% CO_2 atmosphere for MHV inoculated samples and up to 120 hours for HCoV 229E inoculated samples.
11. Plates were assessed and scored by microscopy at 24-hour intervals for the presence of cytopathic effects (CPE), as evidenced by the presence of gaps in cell confluence and/or detached cells. Wells in which $>50\%$ of the cells showed CPE were judged as being positive for TCID₅₀ purposes.
12. TCID₅₀ values were calculated via the Reed and Muench method.

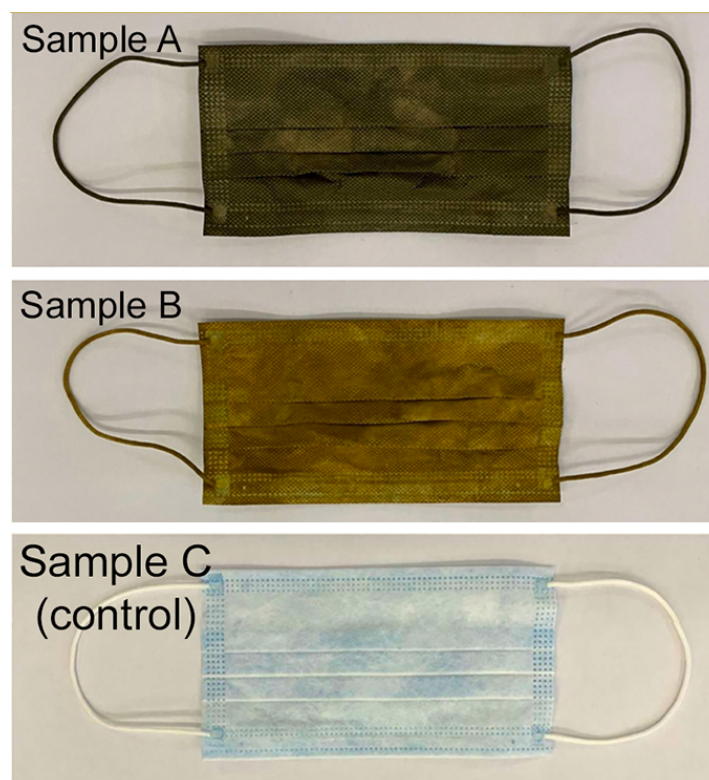


Figure - Test samples A and B, and control Sample C.

RESULTS

Test materials were subject to a modified ISO 18184 procedure - a so-called ‘splash test’ - which deviates from the Standard in terms of (i) volume of viral suspension used to inoculate test carriers (20 μ l instead of 200 μ l), (ii) viral species employed (MHV and HCoV 229E instead of Influenza A virus and/or Feline calicivirus), and (iii) application of the Reed and Muench method to calculate TCID₅₀ values instead of the Behrens and Karber method. Additionally, the 20 μ l inoculum was broken down further to 5 x 4 μ l droplets to promote drying of virus particles on the surface of the test materials, which more closely mimics the behaviour of aerosolised micro-droplets produced by sneezing, coughing etc.

The TCID₅₀ (median Tissue Culture Infectivity Dose) value represents the endpoint dilution where 50% of cell monolayers challenged by the eluted virus sample show observable cytopathic effects as a result of infection by the test virus. TCID₅₀ values are expressed as log₁₀ values, which – for ease of conceptualisation – can be converted to an approximate number of infectious units per ml recovered at each time point (using the formula $IU \cong 0.69 \times 1/TCID_{50}$). The development of CPE in this study was consistent across replicate samples, hence the reported TCID₅₀ and IU values were calculated from 12 inputs (wells) per dilution. Note - Cytotoxicity was not associated with medium eluates from the test or control fabrics, being observable only when samples were inoculated with viral suspensions.

TCID₅₀ and IU values associated with control and test fabrics at different contact times are detailed in Tables 1 and 2 below. Decreases in viral titre are expressed as percentage reductions in Table 3.

Table 1 – TCID₅₀ values

| | TCID ₅₀ values at designated contact time* | | | |
|--------------------|---|-------|-----------|-------|
| | MHV | | HCoV 229E | |
| | T0 hr | T2 hr | T0 hr | T2 hr |
| Sample A | -7.56 | -4.93 | -3.91 | -3.48 |
| Sample B | -7.63 | -5.78 | -4.00 | -4.08 |
| Sample C (control) | -7.28 | -7.37 | -3.97 | -4.03 |

* For example, TCID₅₀ for Sample A at T0 = 10^{-7.56}

Table 2 – Viral recovery expressed as Infectious Units per ml

| | Infectious Units per ml at designated contact time | | | |
|--------------------|--|----------|-----------|----------|
| | MHV | | HCoV 229E | |
| | T0 hr | T2 hr | T0 hr | T2 hr |
| Sample A | 2.52E+07 | 5.83E+04 | 5.58E+03 | 2.08E+03 |
| Sample B | 2.94E+07 | 4.13E+05 | 7.00E+03 | 8.37E+03 |
| Sample C (control) | 1.32E+07 | 1.61E+07 | 6.48E+03 | 7.47E+03 |

Table 3 – Log and percent reduction in viral titres after 2-hour contact time

| | Infectious Units per ml at designated contact time | | | |
|--------------------|--|-------|-----------|-------|
| | MHV | | HCoV 229E | |
| | Log | % | Log | % |
| Sample A | 2.64 | 99.77 | 0.43 | 62.81 |
| Sample B | 1.85 | 98.60 | – | – |
| Sample C (control) | – | – | – | – |

–, no reduction.

CONCLUSIONS

The purpose of this study was to determine the virucidal properties, if any, of two undefined coatings applied to non-woven fabric facemasks (Samples A and B). An uncoated facemask made of the same fabric was supplied as a negative control (Sample C). Virucidal efficacy was tested against two coronaviruses - the murine coronavirus MHV-A59 and human coronavirus 229E - which are commonly employed as surrogates for the causative agent of Covid-19 (SARS-CoV-2). Viral preparations in both cases were relatively crude (clarified infected cell culture supernatants), hence inocula were not supplemented with any additional organic load. Test conditions comprised 20 µl inocula, deposited as 5 x 4 µl drops, with a contact time of 2 hours at 21°C and ambient humidity. Results and observations from the study can be summarised thus:

1. The infectivity of recoverable MHV after a two-hour contact time on coated test fabrics A and B was observed to decrease (by >2 logs for Sample A and almost 2 logs for Sample B). In contrast, there was no decrease in MHV titre recovered from control fabric C after the same contact time. Error associated with the test technique employed is approximately 0.5 logs, hence these data are indicative of virucidal activity associated with coatings A and B. Note - a 3 log reduction is a common (if unofficial) benchmark for moderate virucidal activity with 5 logs being strongly anti-viral).
2. The infectivity of recoverable HCoV 229E after a two-hour contact time on test fabric A showed a near 0.5 log reduction. Neither Sample B nor control Sample C were associated with any reductions in 229E load after the same contact period (the observed increase in titre reflects experimental error). The 0.5 log reduction associated with Sample A is within the error associated with the method and is not considered significant in terms of virucidal activity.
3. Note that the initial MHV and 229E viral loads differed considerably, differing by approximately 3 orders of magnitude (relatively concentrated stock suspensions of MHV are much easier to prepare than 229E). Whether differences in viral load per droplet applied to test surfaces contributed to the differing outcomes is not clear but may well be a factor. Equally, it may be that 229E can remain infectious for longer periods than MHV when applied to the test (and potentially other) surfaces regardless of the number of viral particles deposited per unit volume.