**XtraSan Surface Coating – Solvent Free**
**Test Report**

**Testing carried out by:**
**Perfectus Biomed, Microbiological Service Provider.**

**Contents:**



## 1.0 Aim

To determine the anti-viral efficacy of three antimicrobial resin surfaces using a viral carrier method.

## 2.0 Materials and Methods

### 2.1 Test organisms

Cell types:

MRC-5 (ATCC® CCL-171™}

Virus:

Human coronavirus 229E (CoV 229E) (ATCC® VR-740™}

### 2.2 Test agents

Test agents to be used in this study are listed in Table 1.

| Test agent | Test agent format | Description |
|---|---|---|
| Antimicrobial resin 1 | Solid | Resin-coated glass surface |
| Antimicrobial resin 2 | Solid | Resin-coated glass surface |
| Antimicrobial resin 3 | Solid | Resin-coated glass surface |
| Untreated Control | Solid | Uncoated, glass surface |

**Table 1**. Test agents used throughout the study.

### 2.3 Equipment and media

Equipment:

Class II biosafety cabinet - BioMAT, ThermoFisher Scientific, UK
Vortex - Grant Instruments, UK
UKAS calibrated multichannel pipette (P300) - Gilson®, UK
UKAS calibrated pipettes (0.5 - 1000 µL range) - Proline® Plus, UK 96-well plates - ThermoFisher Scientific, UK
$CO_2$ Incubator - Thermo Scientific, UK
Tissue culture flasks - Nunc, ThermoFisher Scientific, UK
Inverted microscope - Olympus CK2, UK
Water bath - Lauda Aqualine, US

Media:

Phosphate buffered saline (PBS) - Gibco™, UK
Penicillin-streptomycin - ThermoFisher Scientific, UK
Eagle's Minimum Essential Medium (EMEM) - ATCC®, UK
Dulbecco's Phosphate buffered saline {DPBS} - Gibco™, UK Fetal Bovine Serum (FBS) - Gibco™, USA
Trypsin-EDTA - Gibco, UK
Trypan blue - Sigma-Aldrich, UK
Bovine serum albumin, Fraction V - Sigma-Aldrich, UK
Sterile defibrinated sheep blood - TCS Biosciences, UK
Deionised water - Purelab Flex, UK

### 2.4 Method

#### 2.4.1 Cell maintenance and assay set-up

MRC-5 cells were used as the host cell line for human coronavirus 229E (CoV 229E) propagation. MRC-5 cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 20% Foetal Bovine Serum (FBS) and 1% penicillin-streptomycin (complete culture medium) at 37 ± 2°C and 5% $CO_2$. In preparation for the cytotoxicity screening and viral carrier method test, MRC-5 cells were seeded into 96 well plates at 2.5 x $10^5$ cells/mL and incubated at 37 ± 2°C and 5% $CO_2$ for 24 hours, or until they reached approximately 95% confluency.

### 2.4.2 Cytotoxicity screen of test agents

One hundred microlitres of EMEM supplemented with 2% FBS and 1% penicillin-streptomycin (assay medium) was pipetted onto the resin coated glass slides and incubated at 20°C for 60 minutes. The slides were then rinsed with 900 µL of assay medium. Complete culture medium was aspirated from the wells of the cell plate and 100 µL of each test agent suspension was added to the corresponding test wells. Plates were incubated for 24 hours at 37 ± 2°C and 5% $CO_2$. Following incubation, visual scoring was performed on a scale of 0 to 4 according to ISO 10993-5 guidelines (Table 2). Cytotoxic effects were assessed based on a variety of morphological changes to the MRC-5 cells such as cell rounding, attachment and cell lysis.

| Visual Score | Cells with cytotoxic effects (%) | Reactivity classification |
|---|---|---|
| 0 | 0 | None |
| 1 | 0 - 20 | Slight |
| 2 | 20 - 50 | Mild |
| 3 | 50 - 70 | Moderate |
| 4 | 70 - 100 | Severe |

**Table 2.** Cytotoxicity visual scoring and reactivity classifications.

### 2.4.3 Carrier method test for anti-viral efficacy against human coronavirus 229E

The evaluation of virucidal activity was performed using the principles of BS EN 17111:2018. Five hundred microlitres of clean and dirty conditions (Appendix 1) as interfering substances were added separately to 4.5 mL of a 5.73 x $10^6$ $TCID_{50}$/mL human coronavirus 229E (CoV 229E) suspension and gently mixed. Following mixing, 100 µL of these suspensions were added to test surfaces and incubated at 20˚C for 60 minutes. Following incubation, test surfaces were placed in 900 µL of assay medium and rinsed to resuspend the remaining virus. Untreated controls (uncoated glass slides) were tested concurrently.

### 2.4.4 Viral infectivity quantification by $TCID_{50}$

Following preparation of the control and test suspensions as per section 2.4.3, a 10-fold serial dilution was performed in assay medium. Medium was aspirated from the wells of the cell plate and 100 µL of each dilution was added to the corresponding test wells. Test plates were incubated at 35 ± 2°C and 5% $CO_2$ for 7 days. There were six replicate wells for each test condition. After incubation, viral cytopathic effect (CPE) was determined using an Olympus CK2 inverted microscope. The viral titre was calculated using the Spearman-Kärber method.

## 3.0 Results

### 3.1 Phase 1: Cytotoxicity screen of nasal spray formulations

Following a 24-hour contact time, there was no observable cytotoxicity in MRC-5 cells exposed to test agent suspensions (Table 3).

| Treatment | Visual score | Reactivity classification |
|---|---|---|
| Antimicrobial resin 1 | 0 | No cytotoxicity |
| Antimicrobial resin 2 | 0 | No cytotoxicity |
| Antimicrobial resin 3 | 0 | No cytotoxicity |
| Untreated control | 0 | No cytotoxicity |

**Table 3.** Cytotoxicity of test agent formulations using visual scoring.

### 3.2 Viral infectivity quantification by TCID50

Following a 60 minute contact time under clean conditions, the untreated control resulted in 5.71 $Log_{10}TCID_{50}mL^{-1}$ viable CoV 229E. Treatment with antimicrobial resins 1, 2 and 3 resulted in a reduction of 2.18 $Log_{10}TCID_{50}mL^{-1}$, 3.26 $Log_{10}TCID_{50}mL^{-1}$ and 1.36 $Log_{10}TCID_{50}mL^{-1}$ viable CoV 229E respectively, when compared to the negative control (Table 4).

Following a 60 minute contact time under dirty conditions, the negative control resulted in 5.30 $Log_{10}TCID_{50}mL^{-1}$ viable CoV 229E. Treatment with antimicrobial resins 1, 2 and 3 resulted in a reduction of 1.77 $Log_{10}TCID_{50}mL^{-1}$, 2.04 $Log_{10}TCID_{50}mL^{-1}$ and 1.22 $Log_{10}TCID_{50}mL^{-1}$ viable CoV 229E respectively, when compared to the negative control (Table 4).

| **Test agent** | **Viable CoV 229E ($Log_{10}TCID_{50}mL^{-1}$)** | | **Log Reduction ($Log_{10}TCID_{50}mL^{-1}$)** | |
|---|---|---|---|---|
| | Clean conditions | Dirty conditions | Clean conditions | Dirty conditions |
| Antimicrobial resin 1 | 3.53 | 3.53 | 2.18 | 1.77 |
| Antimicrobial resin 2 | 2.45 | 3.26 | 3.26 | 2.04 |
| Antimicrobial resin 3 | 4.35 | 4.08 | 1.36 | 1.22 |
| Negative control | 5.71 | 5.30 | N/A | N/A |

**Table 4.** Log $TCID_{50}$ and Log reduction values for Human coronavirus 229E (CoV 229E) following contact with antimicrobial resins, under clean and dirty conditions. N/A = not applicable.

## 4.0 Discussion

The dissemination of potentially pathogenic viruses increases infection risk in both healthy and immunocompromised individuals. Coronaviruses are enveloped, single stranded RNA viruses responsible for a variety of upper-respiratory tract illnesses in humans. These illnesses range from mild conditions such as the common cold to severe acute respiratory syndrome as seen in the recent COVID-19 pandemic. Coronaviruses are thought to be predominantly transmitted through respiratory droplets with some evidence to suggest the virus can remain active on fomites for several days. Interventions, both preventative and curative, are essential to slowing and/or stopping the spread of coronaviruses.

Antimicrobial resins 1, 2 and 3 were assessed for their anti-viral efficacy against CoV 229E. When compared to the untreated control, all three antimicrobial resins showed reductions, with antimicrobial resin 2 showing the greatest Log reduction. During testing it was observed that the virus suspension dispersed evenly across microbial resin 2 allowing for the greatest amount of contact between the virus and the antimicrobial resin surface. When the viral suspension was added to antimicrobial resin 1 and 2 the viral suspension did not readily disperse and therefore covered a smaller surface area. This is likely a reflection of the hydrophobicity of the test surface.
Future work may investigate the anti-viral efficacy against other viral pathogens such as rhinoviruses or influenza viruses. The antibacterial properties of the resins may also be assessed.

## 5.0 Future work

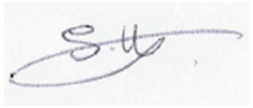
- Evaluation of antimicrobial resins on additional viruses, such as rhinovirus or influenza.
- Assessment of the antibacterial properties of antimicrobial resins.

**Project Start Date:** 06th April 2020

**Project Completion Date:** 1st May 2020

**Testing carried out by:** Perfectus Biomed; Microbiological Service Provider.

**CEO:** Samantha Westgate



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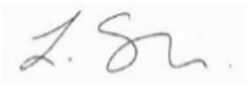

**APPENDIX 1**

**Interfering substances Clean conditions**

The composition of clean conditions is shown in Table A.

| Ingredient | Quantity |
|---|---|
| Bovine serum albumin | 3 g |
| Distilled water | 1 L |

**Table A.** Composition of clean conditions.

**Dirty conditions**

The composition of dirty conditions is shown in Table B.

| Ingredient | Quantity | | |
|---|---|---|---|
| Bovine serum albumin | 3 g | | |
| Sterile defibrinated sheep blood | 3 mL | Distilled water | 97 mL |

**Table B.** Composition of dirty conditions.

**End of report.**