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REPORT ON THE ASSESSMENT OF THE ABILITY OF BIODETECTOR BD-500 TO DETECT AIRBORNE VIRUSES

Prepared by Perfectus Biomed Ltd for: IKO Science OÜ

Date: 14th April 2021

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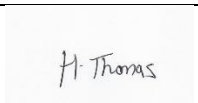
Approved by	Hannah Thomas		14th April 2021

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About Perfectus Biomed Limited

Perfectus Biomed Ltd specialise in developing 'fit for purpose' experiments that truly mimic 'real life' scenarios, along with regular testing according to ISO, EN, ASTM and AATCC standards. Perfectus Biomed Ltd is a quality driven laboratory, with ISO 9001 certification by BSI for the development and provision of standardised and customised biological test services. Further accreditations include a range of ISO 17025 test methodologies (UKAS Laboratory Number: 9192) and Good Laboratory Practice compliant as audited by MHRA.

Detailed list of accreditations:

- ISO 9001:2015 certified by BSI for the development and provision of standardised and customised microbiological services, biological evaluation, materials testing and site audits.
- ISO 17025:2017 accredited for a range of test methodologies to include,
    - SOP 536: Minimum Biofilm Eradication Concentration (MBEC) assay – *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
    - SOP 537: CDC biofilm reactor assay – *S. aureus* and *P. aeruginosa*.
    - SOP 538: Drip flow biofilm reactor assay – *S. aureus* and *P. aeruginosa*.
    - SOP 555: CDC biofilm reactor assay – *Candida albicans*
    - SOP 556: An *ex vivo* lung model to study bronchioles infected with *P. aeruginosa* biofilm.
    - SOP 575**:** Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm – *S. aureus* and *P. aeruginosa*.
    - SOP 582: Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm – *C. albicans.*
    - SOP 576: Suspension Test Method – *S. aureus*, *P. aeruginosa*, *Enterococcus hirae* and *Escherichia coli.*
- Good Laboratory Practice (GLP) compliant.

Report (IKO Science OÜ 001)

Test Facility Location:

Techspace One SciTech Daresbury, Keckwick Lane, Cheshire, WA4 4AB, UK.

1.0 Aim

To assess the ability of Biodetector BD – 500 manufactured by IKO Science OÜ, address Laki str 26, 12915, Tallinn, Estonia) to detect airborne viruses.

2.0 Materials and Methods

2.1 Test organisms

Cell types:

RAW 264.7 (ATCC® TIB-71™) – Passage number: 32
H1HeLa (ATCC® CRL-1958™) - Passage number: 34

Viruses:
Murine norovirus (STAMM S99) – Amplification number: 1
Human rhinovirus 16 (ATCC® VR-283™) – Amplification number: 1

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2.2 Test item

Test item used in the study is listed in Table 1.

Test item	Test item format
Biodetector BD - 500	Detection device

Table 1. Test item 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 multichannel pipette (P20) – Gilson®, UK
UKAS calibrated pipettes (0.5 - 1000 µL range) – Proline® Plus, UK
96-well plates – ThermoFisher Scientific, UK
CO_2 Incubator BB-15 – Thermo Scientific, UK
Tissue culture flasks – Nunc, ThermoFisher Scientific, UK
Olympus CK2 Inverted microscope – KeyMed, UK
VWB2 Water bath – VWR, UK
Vacuboy Aspirator – INTEGRA, UK
NE-C28P Compressor Nebuliser – Omron, UK

Media:

L-15 media - Gibco™, UK
Phosphate buffered saline (PBS) – Gibco™, UK
Penicillin-streptomycin – ThermoFisher Scientific, UK
Dulbecco's Minimum Essential Medium (DMEM) – ATCC®, UK
Eagles 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

2.4 Method

2.4.1 Cell maintenance and assay set-up

RAW 264.7 and H1HeLa cells were used as the host cell line for Murine norovirus and Human rhinovirus propagation. RAW 264.7 cells were maintained in Dulbecco's Minimum Essential Medium (DMEM) and H1HeLa cells were maintained in L-15 Medium, both supplemented with 10% Foetal Bovine Serum (FBS) and 1% penicillin-streptomycin (complete culture medium) at 37 ± 2 °C CO_2. In preparation for the assays, cells were seeded into 96 well plates and incubated at 37 ± 2 °C and 5% CO_2 for 24 hours, or until they reached 80-90% confluency.

2.4.2 Phase 1: Assessment of the ability of Biodetector BD – 500 to detect airborne viruses

The Biodetector BD – 500 inlet was connected to a silicon tube that was held at a height of 12 cm and located approximately 4-8 cm away from a compressor nebulizer. One millilitre of EMEM supplemented with 2% FBS and 1% penicillin-streptomycin (assay medium) or virus was added to the nebulizer and nebulized for 30 seconds. After the 5 minute measurement cycle, the resulting number was then recorded before waiting a further 5 minutes before switching the nebulizer on again. After the experiment, a swab was taken from the silicon tube that was attached to the Biodetector BD – 500 to recover any remaining residue or virus, before being mixed with 900 µL of assay media to resuspend any remaining virus. Testing was carried out in triplicate. Viral titre was determined by $TCID_{50}$ and calculated using the Spearman-Kärber method.

3.0 Results

3.1 Assessment of the ability of Biodetector BD – 500 to detect airborne viruses

3.1.1 Murine norovirus

The titre of the Murine norovirus stock was 6.96 ± 0.38 $Log_{10}TCID_{50}$ mL^{-1}. It was demonstrated during the study that virus could be successfully sampled using the swabs and Murine norovirus was detected inside the device tube. This demonstrated successful nebulisation of the virus and capture by the device (Table 2). A greater number of particles were detected when exposed to the Murine norovirus compared to the media only sample (Table 3).

Test item	Viral titre ($Log_{10}TCID_{50}mL^{-1}$)			Average Viral titre ± SD ($Log_{10}TCID_{50}mL^{-1}$)
	N=1	N=2	N=3	
Murine norovirus stock titre	6.88	7.38	6.63	6.96 ± 0.38
Murine norovirus swab only	5.13	4.88	1.50	3.83 ± 2.02
Murine norovirus tube swab	2.63	2.50	3.50	2.88 ± 0.54

Table 2. $Log_{10}TCID_{50}$ mL^{-1} recovery results for Murine norovirus.

Test item	Number of particles per cu m			Average number of particles per cu m
	Run 1	Run 2	Run 3	
Media only	1560	200	6080	2613
Murine norovirus	217320	303160	277640	266040

Table 3. Particle detection results for Murine norovirus following treatment with Biodetector BD – 500.

3.1.2 Human rhinovirus

The titre of the Human rhinovirus stock was 6.75 ± 0.57 $Log_{10}TCID_{50}$ mL^{-1}. It was demonstrated during the study that virus could be successfully sampled using the swabs and Human rhinovirus was detected inside the device tube. This demonstrated successful nebulisation of the virus and capture by the device (Table 4). A greater number of particles were detected when exposed to the Human rhinovirus compared to the media only sample (Table 5).

Test item	Viral titre ($Log_{10}TCID_{50}mL^{-1}$)			Average Viral titre ± SD ($Log_{10}TCID_{50}mL^{-1}$)
	N=1	N=2	N=3	
Human rhinovirus stock titre	7.38	6.23	6.25	6.75 ± 0.57
Human rhinovirus swab only	5.88	5.38	5.50	5.58 ± 0.26
Human rhinovirus tube swab	2.75	2.13	1.63	2.17 ± 0.56

Table 4. $Log_{10}TCID_{50}$ mL^{-1} recovery results for Human rhinovirus.

Test item	Number of particles per cu m			Average number of particles per cu m
	Run 1	Run 2	Run 3	
Media run	1040	320	1286	882
Human rhinovirus run	677000	445440	261080	461173

Table 5. Particle detection results for Human rhinovirus following treatment with Biodetector BD – 500.

4.0 Conclusion

Following nebulization of Murine norovirus during measurement cycles of Biodectector BD – 500, the average number of particles detected was 266040 cu m, this was greater than the average number of media only particles that was observed at 882 cu m. Following nebulization of Human rhinovirus during measurement cycles of Biodectector BD – 500, the average number of particles detected was 461173 cu m, this was greater than the average number of media only particles that was observed at 2613.33 cu m. These results suggest that the Biodectector BD – 500 is able to detect airborne Murine norovirus and Human rhinovirus.

Project Start Date: 18th January 2021

Project Completion Date: 25th March 2021

End of report.