Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Report on TEST A: SARS-CoV-2 Viricidal Effectiveness Testing with ViaClean Products

Study Identification Number:

100620A

Protocol Number:

100420A

Product Identity:

BioProtect DP RTU Lot No. 20191207DP

Product Shipping Date:

20200319, Ryerson University, Toronto

Test Microorganism:

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), patient isolate

(*Reference:* Caly L, Druce J, Roberts J, *et al*. 2020. Med J Aust 212:459-462).

The University of Melbourne acknowledges the support of Melbourne Health, through its Victorian Infectious Diseases Reference Laboratory at the Doherty Institute, in providing our laboratory with isolated SARS-CoV-2 material

Data Requirements:

U.S. EPA 40CFR § 160

Product Performance Guidelines OCSPP 810.2200

Testing Facility:

High Containment Laboratory (Physical Containment level 3 (PC3))

The Doherty Institute for Infection and Immunity at the University of Melbourne,

792 Elizabeth St, Melbourne VIC 3000, Australia

Relative temperature: 18-20°C; Relative Humidity: 40%

Recipient:

ViaClean Technologies LLC, Philadelphia, PA

Experimenter:

Dr. Julie McAuley

Results Verified by:

Prof. Damian Purcell

Date experiment performed:

24th April - 23rd May, 2020

Introduction: SARS-CoV-2 is an enveloped RNA virus. It was hypothesized that exposure of the virus to BioProtect will disrupt the virus envelope, thus rendering the virus non-infectious.

Aim: **Test A:** To dry SARS-CoV-2 on untreated stainless steel and fabric surface, then to investigate whether wet contact with Bioprotect reagent will reduce virus infectivity.

Protocols:

TEST A: Dried SARS-CoV-2 on untreated stainless steel, followed by wet contact with BioProtect reagent

Experiment date: 10th April, 2020.

1. Stainless steel disc (2cm round) was placed in a well of a sterile tissue culture plate. 50µL SARS-CoV-2 (=10^4 $TCID_{50}$) was added directly to the sample surface and allow to dry.
2. 100uL of 1:3, 1:64 dilutions of BioProtect liquid or water (n=2) was added directly on top of the dried virus inoculum. After a contact time of 10min, 1mL infection media (MEM containing 1mM HEPES, 50U/mL Penicillin, 50µg/mL Streptomycin (Gibco)) was added to each well. Surfaces were washed eight times with vigorous pipetting.
3. Eluate was collected and immediately serially diluted and assayed for quantitation via $TCID_{50}$.
    - TCID50 values were calculated using the method of Reed and Muench *(Reed LJ, Muench H. 1938. A simple method of estimating fifty per cent endpoints. The American Journal of Hygiene:3)*. Data is plotted mean ± standard deviation (Figure 1A). Raw Data is given in Appendix 1.
4. ***Quantitative Real-Time PCR (qRT-PCR):*** 140µL of sample was immediately added to AVL buffer for 10min, then 100% Ethanol to initiate RNA extraction for qPCR analysis. RNA purification was then completed using the QiaAmp Viral RNA mini kit, then samples dunked out of the PC3 facility and stored in our PC2 laboratory at -80°C until processed via RT-PCR. Standard curves were generated using known amounts of RNA extracted from SARS-CoV-2 stocks. Unknown sample concentrations were then interpolated from the standard curve and back calculated to give ng/µL present in the original sample. Data was plotted mean ± standard deviation (Figure 1 B). Raw Data is given in Appendix 3.
5. Left over sample aliquots were stored at-80°C.

Quantitation Protocol: 50% Tissue Culture Infectious Dose ($TCID_{50}$)

1. In the PC2 laboratory, plates to establish ~95% monolayers of Vero cells were seeded 24h prior to assay
2. After verification of quality/density of monolayer, the plates were washed using infection media (to remove any cell debris), then transferred into the PC3 laboratory
3. Samples were serially diluted and a known volume inoculated into each well (n=3 replicates/sample and up to 6 serial dilutions).
4. Plates were incubated for 45min to enable virus infection of monolayers, then 1mL MEM infection media +TPCK trypsin(1ug/mL, Trypsin Worthington) was added.
5. Plates were returned to incubator (37°C, 5%CO_2) and microscopically examined every 24 h (up to 72h) for cytopathic effect (CPE) on cells

6. The $TCID_{50}$ was then back calculated to determine the $TCID_{50}$/mL of infectious virus present in the original sample.

Quantitative Real-Time Polymerase Chain Reaction Assay (qRT-PCR)

Reaction solutions specific for detection of SARS-CoV-2 Envelope (E) gene containing known volumes of standards, samples and control will be set-up according to the following conditions:

MasterMix:	**Vol**	
H_2O (RNAse free)	2.6 µl	
2x Reaction mix*	12.5 µl	
$MgSO_4$(50mM)	0.4 µl	
BSA (1 mg/ml)**	1 µl	
Primer E_Sarbeco_F1 (10 µM stock solution)	1 µl	ACAGGTACGTTAATAGTTAATAGCGT
Primer E_Sarbeco_R2 (10 µM stock solution)	1 µl	ATATTGCAGCAGTACGCACACA
Probe E_Sarbeco_P1 (10 µM stock solution)	0.5 µl	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
SSIII/Taq EnzymeMix*	1 µl	
Total reaction mix	20 µl	
Template RNA, add	5 µl	
Total volume	25 µl	
* Thermo Fischer/Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase ** $MgSO_4$ (50 mM) [Sigma], This component is not provided with the OneStep RT-PCR kit		

RT-PCR temperature cycling conditions:

55°C 10’
94°C 3’
94°C 15’’ } 45x
58°C 30’’

Standard curves were then generated based on serial dilution of positive control samples and CT values. The amount of RNA present in the original sample was then determined by comparing CT value to standard curve and back-calculating. Data is plotted mean ± standard deviation. Raw Data is given in Appendix 2.

Results:

TEST A: Dried SARS-CoV-2 on untreated stainless steel, followed by wet contact with BioProtect reagent

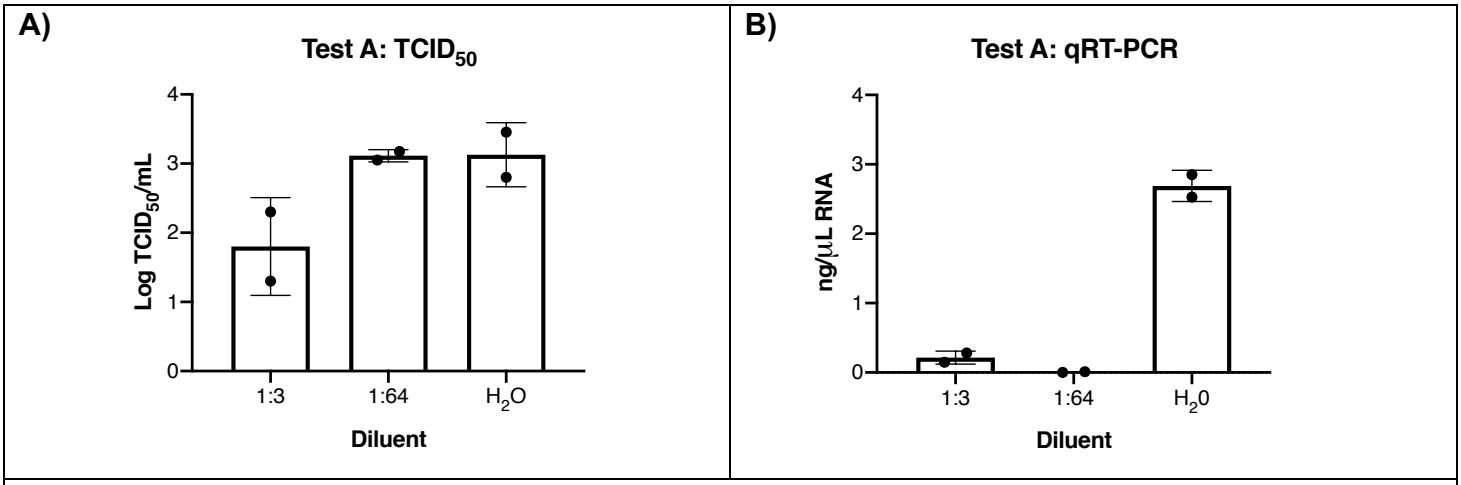


Figure 1: Samples (n=2) taken from stainless steel discs containing pre-dried SARS-CoV-2 (10^4 $TCID_{50}$) exposed to diluted BioProtect reagent or water revealed viricidal activity by **A)** $TCID_{50}$ **B)** qRT-PCR. Error bars = ± standard deviation.

Conclusion:

- Bioprotect reagent appears to have viricidal activity against SARS-CoV-2.
- Bioprotect reagent reduced the amount of viral genome present in the sample by 92% (for the 1:3 diluted product) and 99% (for the 1:64 diluted product) compared to the water control.

Appendix 1: Raw data for $TCID_{50}$ values

$TCID_{50}$ assay notes: Performed in 48 well plate with 50µL inoculation volume. To calculate $TCID_{50}$ remaining in the original 'dried' sample:

1) Calculate $TCID_{50}$/50µL based on method of Reed and Muench:

$$\text{proportional distance formula} = \frac{[(\%\text{ positive value} > 50\%) - 50\%]}{[(\%\text{ positive value} > 50\%) - (\%\text{ positive value} < 50\%)]}$$

- Knowing the proportional distance between dilutions, the 50%-endpoint can be calculated using the exact dilutions used:

Log tissue culture infectious dose 50 = (log dilution >50%) + (proportional distance X log

- The reciprocal of this number is used to express the titre in infectious units per unit volume.

2) $TCID_{50}$/50µL x 1000/50 = TCD_{50}/mL (eluted sample)

Detection of CPE from Quantitative $TCID_{50}$ Assay of samples

Dilution:	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}	
Sample:	1	2	1	2	1	2	1	2	1	2	1	2
1:3 BioProtect												
1:64 BioProtect												
H_2O												

CPE Detected | Moderate CPE detected (value given = 0.5) | Minor CPE Detected (value given = 0.25) | no CPE detected

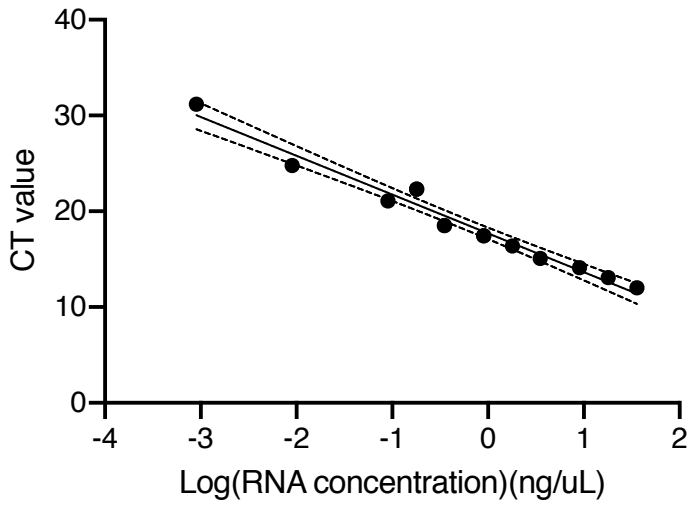
Log_{10} $TCID_{50}$/mL in eluted sample:

1:3	1:64	H_2O
2.30	3.17	3.46
1.30	3.05	2.80

Appendix 2: Raw data for qRT-PCR values

Standard Curve generation:

RNA concentration: Log_{10}(ng/µL)	Optical Density
1.56	12.03
1.26	13.09
0.95	14.10
0.54	15.08
0.26	16.39
-0.05	17.45
-0.46	18.51
-0.74	22.30
-0.05	17.45
-0.46	18.51
-0.74	22.30
-1.05	21.10
-2.05	24.78
-3.05	31.19



Sample	Optical Density	Interpolated Values	Sample Concentration (ng/mL)
1:3 BioProtect -1	19.97	-0.56	0.28
1:3 BioProtect -2	21.07	-0.83	0.15
1:64 BioProtect -1	26.92	-2.28	0.01
1:64 BioProtect -2	29.21	-2.84	0.00
H_2O- 1	19.97	0.46	2.85
H_2O- 2	21.07	0.40	2.53
Positive Control	9.02	2.14	139.09
Negative Control	30.3392	-3.12	0.00