REGA INSTITUTE
DEPARTEMENT MICROBIOLOGY, IMMUNOLOGY AND TRANSPLANTION
GROUP VIROLOGY EN CHEMOTHERAPY
HERESTRAAT 49 POSTBUS1043
3000 LEUVEN, BELGIUM

Evaluation of ViaClean disinfectants against SARS-CoV-2 in vitro

STUDY TITLE

Evaluation of ViaClean Self-Sanitizing Dry Coating against SARS-CoV-2 in vitro

Product Identity

BioProtect DP RTU

Lot No. 20191206DP

Carrier Identity

2 cm SS304-2B Testing Disc EN13697-2001, Pegen Industries Inc, Ottawa

DP RTU application date: 20200319, Ryerson University, Toronto

Test Microorganism

SARS-Related Coronavirus 2 Isolate CHINA-WUHAN 1/2020, ATCC No. non-clinical isolate

Study Completion Date

08 MAY 2020

Testing Facility

Rega Institute KULEUVEN

Herestraat 49 3000 Leuven BELGIUM

Study Sponsor

ViaClean Technologies LLC, Philadelphia, PA

Study Objective

The objective of the study was to confirm the self-sanitizing residual virucidal efficacy of BioProtect DP RTU against SARS-Related Coronavirus 2 Isolate CHINA-WUHAN 1/2020, ATCC No. non-clinical isolate at a dry contact time of 10 minutes (±10 seconds).

Study Conclusion in Brief

BioProtect DP RTU (Lot No. 20191206DP) coating of Stainless Steel carriers demonstrated a 2.5 log_{10} out of 6.9 log_{10} inactivation representative of a 99.53% reduction of SARS-Related Coronavirus 2 Isolate CHINA-WUHAN 1/2020, ATCC No. non-clinical isolate at a 10-minute dry contact time at room temperature versus untreated control carriers.

FINAL STUDY REPORT

Important Dates

Study Initiation Date:	04 MAY 2020
Experimental Start Date:	04 MAY 2020 (10:30 A.M.)
Experimental End Date:	08 MAY 2020 (11:15 A.M.)

Test Substance Information

Name:	BioProtect DP RTU
Date Treated:	19 MARCH 2020
Date Received:	23 MARCH 2020
Lot Number(s):	20191206DP
Active Ingredient(s):	(1) TMSiQAC [0.13% LCL] (3-(Trihydroxysilyl)propyl dimethyl octadecyl ammonium chloride) (2) ADBAC (n-Alkyl (C14 50%, C12 10%, C16 40%) Dimethyl Benzyl Ammonium Chloride) [0.024% LCL] (3) Octyl decyl dimethyl ammonium chloride [0.018% LCL] (4) Dioctyl dimethyl ammonium chloride [0.008% LCL] (5) DDAC (Didecyl dimethyl ammonium chloride) [0.071% LCL] (6) Total Quat [0.19%]
Expiration Date(s):	06 MAR 2021
Form:	Ready-to-Use
Storage Conditions:	Room Temperature, Under Fluorescent Lighting

Test Parameters

Microorganism:	SARS-Related Coronavirus 2 Isolate CHINA-WUHAN 1/2020, ATCC No. non-clinical isolate Stock Lot Number: SARS-CoV-2-P7
Host Cell Line:	VERO E6 (ATCC® CRL-1586™) Subculture Passage No. 42
Propagation Growth Medium	DMEM (Gibco cat no 41965-039) with 10% v/v heat-inactivated FCS ( ) and 5 mL sodium bicarbonate 7.5% (Gibco 25080-060).
Virus Isolation:	Freeze-thaw and centrifugation
Number of Test Carriers:	3 (three dried virus films per test substance lot) 3 three dried virus films per control carrier
Test Assay Medium:	50 uL per carrier of DMEM (Gibco cat no 41965-039) with 2% v/v heat inactivated FCS and 5 mL sodium bicarbonate 7.5% (Gibco 25080-060).
Organic Soil Load:	3 ± 0.1% (Fetal Bovine Serum) in test assay medium, 5% FBS total.
Neutralizer:	1 mL of 3% BSA supplemented with test assay medium
Carrier Type:	Stainless Steele Disks (2 cm diameter, 2B)
Carrier Dry Time:	51 Minutes

Carrier Dry Temperature:	22.5 °C
Carrier Dry Humidity:	39% Relative Humidity Test
Contact Time (dry):	10 minutes ± 10 Seconds
Test Temperature:	Room Temperature
Exposure Method:	Application by pipet
Assay Temperature:	37 ± 1.0 °C
Assay Period:	3 days

Test Method

The test was conducted according to the attached protocol based on the US EPA copper method and the prEN16777/ASTM 2197 surface methodology adapted for the SARS-CoV-2 virus.

Test Method Purpose

To evaluates the residual sanitizing efficacy (RSS) of the BioProtect antimicrobial coating after application to inanimate, nonporous, and non-food contact hard surfaces against viruses.

PROTOCOL CHANGES

Protocol Amendments

The following protocol amendments were initiated during the course of this study to evaluate a non-copper coating and to accommodate handling a BSL virus for which no known regulatory protocol guidance is available to date.

The cleaning with alcohol, mechanical abrasion, repeated chemical exposure to simulate disinfection steps and re-inoculations per the EPA copper method were not possible due to the safety guidelines set by the Rega Institute for working with a BSL-3 virus.

In retrospect the applicant wishes to qualify the effectiveness of the technology to maintaining the integrity of the antimicrobial surface coating per the care and use instructions on the label. For example:

“This coated surface when properly applied and dried kills at least 99.9% of SARS-CoV-2 after a 10 minute dry contact time when the integrity of the surface* is maintained in accordance with the product care and use directions.”

*Coated surfaces should not be destroyed with abrasive cleaners, mechanically abraded, waxed, painted, lacquered, varnished, or otherwise coated. Follow the post cleaning protocol on the label.

The contact time for drying 50 uL of the SARS-CoV-2 was optimized at 51 minutes and a further 10 minute dry contact time was used for the ability of the coated test surface to kill > 99.5% of viruses within this contact time once dry. A longer exposure from previous virucidal test was shown to result in

increased virucidal activity.

Protocol Deviations

Test Assay Propagation Growth Medium: Dulbecco's Minimal Essential Medium with 10% (v/v) heat inactivated Fetal Bovine Serum plus 7.5% Sodium Bicarbonate (Gibco 25080-060) was used without antibiotics because of removing the antibiotics makes the cells less stressed.

Test Assay medium: DMEM (Gibco cat no 41965-039) supplemented with 2% v/v heat-inactivated FCS and 5 mL sodium bicarbonate 7.5% (Gibco 25080-060) was used instead of DMEM 10% FCS in PBS supplemented with antibiotics because of a better growth of the host cells and to avoid cell over growth.

Carrier Type: BioProtect coated Stainless Steele disks (2B, 2cm) were used instead of copper because copper is inherently antimicrobial.

Replicate carriers: The number of replicate test surfaces and replicate control surfaces for this test was n = 3 because of the limitations of multiple sample processing by one analyst inside a BSL3 laboratory.

Carrier volume: 50 uL of dried virus was optimized to allow for the appropriate drying time under one hour and reduce recovery error from transfer from the 2 cm carriers.

Soil Load: 5% FBS supplemented was used without 0.01% Triton X to prevent inoculum spreading and spilling over from the 2 cm disk and to avoid inactivation of the test pathogen.

Recovery: Vigorous pipetting technique was used instead of vortex mixing or scraping because of BSL-3 safety guidelines. This technique was validated for full recovery of the virus from the carrier recovery controls.

CONTROL PERFORMANCE RESULTS

Virus Stock Titer Control

The designated host cell line, Vero E6 cells, demonstrated susceptibility to the challenge virus, SARS-CoV-2. The resulting stock titer was 6.9 ± 0.1 $\log_{10}$ $TCID_{50}$ per 1 ml.

Plate Recovery Control

The carrier designated as "Plate Recovery Control" during the course of testing yielded a viral titer of 6.9 ± 0.1 $\log_{10}$ $TCID_{50}$ per ml.

Cytotoxicity Control

Cytotoxic effects were observed in the 10^{-1} dilution of Vero E6 host cell monolayers for the evaluated test substance lot (BioProtect DP RTU Lot No. 20191206DP). No complete cytotoxicity was observed in dilutions.

Neutralization Effectiveness Control

Minor cytotoxic effects were observed in the lowest dilutions of Vero E6 host cell monolayers for the evaluated test substance lot (BioProtect DP RTU Lot No. 20191206DP). Viral cytopathic effects (CPE) were observed of Vero E6 host cells infected by low titers of SARS-CoV-2 (dilutions shown in table).

Cell Viability Controls

No microbial contamination of host cell cultures (BSL3) was observed during the course of the study.

STUDY ACCEPTANCE CRITERIA

The validity and acceptability of virucidal efficacy data are based on the following standards:

1. A minimum of 4.00 log10 infectious viruses (TCID50) is recovered from the plate recovery control film.

2. Quantification of the plate recovery control titer, the virus titer following test substance exposure, cytotoxicity levels, and neutralization effectiveness controls is conducted at a minimum of four determinations per dilution for each assay system.

3. The use of special methods used to increase the viral titer and further neutralize the test substance is disclosed.

4. Viral cytopathic effects are to be distinguishable from cytotoxic effects caused by test substance exposure. If cytotoxicity is observed, a 3.00-log10 reduction in titer is confirmed past the level of cytotoxicity.

5. In the absence of cytotoxicity, the product demonstrates complete inactivation of virus at all dilutions.

6. The TCLD50 values are calculated and provided for each test assay.

7. The test results are reported as the reduction of the virus titer due to the activity of the test substance [TCID50 of the plate recovery control less the TCLD50 of the virus test carrier(s)] expressed as the logarithm to the base of 10 (log10) and calculated by a statistical method (e.g. Spearman-Karber) and compared to the Reed-Muench method.

8. Assay wells designated as "negative" controls be absent of infectivity, contamination, and cytotoxicity.

CALCULATIONS AND STATISTICAL ANALYSIS

Determination of Viral and Cytotoxicity Titers

Viral and cytotoxicity titers (TCID50/TCLD50 and TCCD50, respectively) were determined according to the method developed by Spearman-Karber:

-Log of 1st dilution inoculated – [(Sum of % mortality at each dilution / 100) – 0.5 x (logarithm of dilution)]

this was also done with the method developed by Reed-Muench

$\log_{10}$ 50% end point dilution = $\log_{10}$ of dilution showing a mortality next above 50% - (difference of logarithms × logarithm of dilution factor).

Calculation of Virus Inactivation Due to Test Substance Exposure

Log10 Reduction of Virus Due to Inactivation by Test Substance = A – B, where:
A = Plate Recovery Control Film TCID50
B = Average Virus-Substance Test Film TCLD50

Calculation of Percent Reduction

$$P = (1 - 10^{-L}) \, x \, 100$$

Where:

P is the percent reduction

L is the log reduction

Statistical Analysis

Students paired T-test was performed during the course of this study.

STUDY RECORD AND TEST SUBSTANCE RETENTION

Test Substance Retention

The test substance may be returned to the Study Sponsor at Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request the sample, it may be destroyed 30 days after study completion.

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RESULTS

TEST	TCID50 (REED-MUENCH)	TCID50 (SPEARMAN-KARBER)	LOGDIL TO REACH 50%CPE (REED-MUENCH)	LOGDIL TO REACH 50%CPE (SPEARMAN-KÄRBER)	MEAN TCID50 (REED-MUENCH)	MEAN TCID50 (SPEARMAN-KARBER)	MEAN (REED-MUENCH)	MEAN (SPEARMAN-KÄRBER
PRETREATED1	7,962	7,962	3.901	3.967				
PRETREATED2	20,000	19,999	4.301	3.467	40,264	40,2140	4.390±0.539	5.467±0.500
PRETREATED3	92,832	92,683	4.967	4.967				
CONTROL1	7,962,143	92,682,980	6.901	7.967				
CONTROL2	11,246,827	136,144,470	7.051	8.134	8,511,175	97,356,210	6.918±0.126	7.967±0.167
CONTROL3	6,324,555	63,241,190	6.801	7.801				

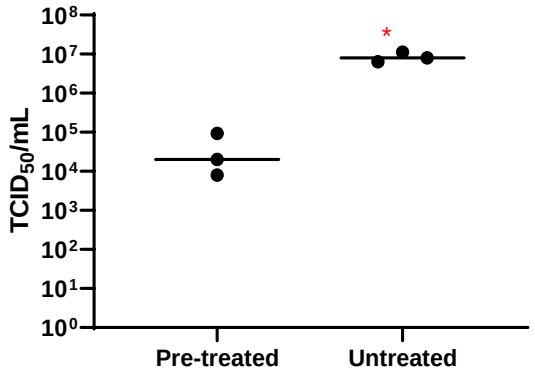
TCID50: 50% cell culture infective dose

ORIGINAL STOCK	TCID50 (REED-MUENCH)	TCID50 (SPEARMAN-KARBER)	LOGDIL TO REACH 50%CPE (REED-MUENCH)	LOGDIL TO REACH 50%CPE (SPEARMAN-KÄRBER)	MEAN TCID50 (REED-MUENCH)	MEAN TCID50 (SPEARMAN-KARBER)	MEAN (REED-MUENCH)	MEAN (SPEARMAN-KÄRBER
VIRUS TITRE 1	11,246,827	136,144,470	7.051	8.134	8,785,690	99,692,830	6.918	7.967
VIRUS TITRE 2	6,324,555	63,241,190	6.801	7.801				

TCID50: 50% cell culture infective dose

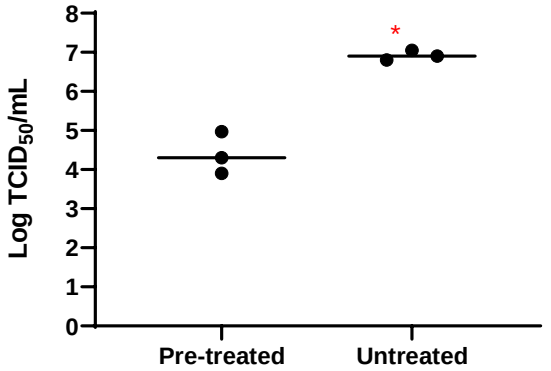
TEST	TCID50 REDUCTION (REED-MUENCH)	TCID50 REDUCTION (SPEARMAN-KÄRBER)	LOG REDUCTION (REED-MUENCH)	LOG REDUCTION (SPEARMAN-KÄRBER)	PERCENTAGE REDUCTION (REED-MUENCH)	PERCENTAGE REDUCTION (SPEARMAN-KÄRBER)
PRETREATED1	7,953,632	92,603,370	3.017	3.000	99.90	99.90
PRETREATED2	11,226,051	135,944,480	2.617	2.500	99.76	99.68
PRETREATED3	6,231,436	62,314,360	1.951	2.000	98.88	99.00
PRETREATED AVG	8,470,372.85	96,954,070	2.528±0.539	2.500±0.500	99.70±0.55	99.68±0.47

Dried virus (+10min contact time)
on pre-treated and untreated stainless steel discs



* p=0.0279 Pre-treated versus Untreated, Student's Unpaired T-test

Dried virus (+10min contact time)
on pre-treated and untreated stainless steel discs



* p=0.0115 Pre-treated versus Untreated, Student's Unpaired T-test

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TOXICITY PLATE 1

Dilution	Result	% positive
1	232 232	33.3
2	322 222	16.7
3	221 221	0
4	111 111	0
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead		

TOXICITY PLATE 2

Dilution	Result	% positive
1	322 223	33.3
2	222 322	16.7
3	212 221	0
4	111 111	0
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

TOXICITY PLATE 3

Dilution	Result	% positive
1	223 322	33.3
2	223 222	16.7
3	222 212	0
4	111 111	0
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

TEST 1

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	151 111	16.7
4	111 111	0
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

TEST 2

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	241 234	50
4	131 111	16.7
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

TEST 3

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	324 334	83.3
4	213 223	33.3
5	000 000	0
6	000 000	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

RECOVERY/CONTROL TEST 1

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	555 555	100
4	555 555	100
5	555 555	100
6	123 221	16.7
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

RECOVERY/CONTROL TEST 2

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	555 555	100
4	555 555	100
5	555 555	100
6	123 321	33.3
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

RECOVERY/CONTROL TEST 3

Dilution	Result	% positive
1	555 555	100
2	555 555	100
3	555 555	100
4	555 555	100
5	555 555	100
6	121 112	0
7	000 000	0
8	000 000	0
9	000 000	0
0= No dead cells, 1= ±20% dead cells, 2= ±40% dead cells, 3= ±60% dead cells, 4= ±80% dead cells, 5= all cells are dead.		

TOXICITY PLATE 1

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
2	2	1	1	0	0	0	0	0	0
3	2	2	1	0	0	0	0	0	0
2	2	2	1	0	0	0	0	0	0
2	2	1	1	0	0	0	0	0	0
3	2	2	1	0	0	0	0	0	0
2	3	2	1	0	0	0	0	0	0

TOXICITY PLATE 2

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
3	2	1	1	0	0	0	0	0	0
2	2	2	1	0	0	0	0	0	0
2	3	2	1	0	0	0	0	0	0
2	2	2	1	0	0	0	0	0	0
2	2	1	1	0	0	0	0	0	0
3	2	2	1	0	0	0	0	0	0

TOXICITY PLATE 3

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
2	2	2	1	0	0	0	0	0	0
2	2	1	1	0	0	0	0	0	0
3	2	2	1	0	0	0	0	0	0
3	3	2	1	0	0	0	0	0	0
2	2	2	1	0	0	0	0	0	0
2	2	2	1	0	0	0	0	0	0

TEST 1

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	1	1	0	0	0	0	0	0
5	5	1	1	0	0	0	0	0	0
5	5	1	1	0	0	0	0	0	0
5	5	1	1	0	0	0	0	0	0
5	5	5	1	0	0	0	0	0	0
5	5	1	1	0	0	0	0	0	0

TEST 2

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	4	1	0	0	0	0	0	0
5	5	3	1	0	0	0	0	0	0
5	5	2	1	0	0	0	0	0	0
5	5	1	1	0	0	0	0	0	0
5	5	4	3	0	0	0	0	0	0
5	5	2	1	0	0	0	0	0	0

TEST 3

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	4	3	0	0	0	0	0	0
5	5	3	2	0	0	0	0	0	0
5	5	3	2	0	0	0	0	0	0
5	5	4	3	0	0	0	0	0	0
5	5	2	1	0	0	0	0	0	0
5	5	3	2	0	0	0	0	0	0

RECOVERY/CONTROL TEST 1

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	5	5	5	1	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	3	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	1	0	0	0	0

RECOVERY/CONTROL TEST 2

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	5	5	5	1	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	3	0	0	0	0
5	5	5	5	5	3	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	1	0	0	0	0

RECOVERY/CONTROL TEST 3

Dil 1	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7	Dil 8	Dil 9	CC
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	1	0	0	0	0
5	5	5	5	5	1	0	0	0	0
5	5	5	5	5	1	0	0	0	0
5	5	5	5	5	2	0	0	0	0
5	5	5	5	5	1	0	0	0	0

KEY (5= all cells are death; 4= ±80% of cells are death; 3= ±60% of cells are death; 2= ±40% of cells are death; 1= ±20% of cells are death; 0= cells are normal) (dilution in plates is 1/10)

SUMMARY OF RESULTS

For the pretreated condition with a contact time of 10 minutes, a toxicity assay was carried out with **PBS** as a substitute for the virus. In none of the first 4 dilutions more than 40% of cell death was noted.

The pretreated discs (Adjusted EPA copper protocol) with a contact time of 10 minutes showed on average a logarithmic (log_{10}) reduction of 2.528 (Reed-Muench) and 2.500 (Spearman-Kärber) representative of a 99.70% ±0.55 (Reed-Muench) and 99.68±0.47 (Spearman-Kärber) inactivation of the SARS-CoV-2 virus on BioProtect pre-coated (46 days old coating) Stainless Steel carriers.

29/05/2020

X

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X

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Prof. Dr.

Jasper Rymenants

Dr. Johan Neyts