TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

TEST REPORT NO: TS4-0259		**DATE OF ISSUE: 04/08/2020**
CUSTOMER DETAILS		
NAME	Cypress Bio-Tech Company Limited	
ADDRESS	Room 301-303/F, Block B, Shatin Industrial Centre, 5-7 Yuen Shun Circuit, SHATIN, NT, Hong Kong	
CONTACT	Michael Choi	
SAMPLE & TEST INFORMATION		
JOB NO.	TS4-0259	
DATE RECEIVED	16.06.2020	
TEST PERFORMED	20.07.2020	
ENVIRONMENTAL CONDITIONS	Ambient Temperature: Store below 30ºC Relative Humidity: NA	
TYPE OF SAMPLE	Cypress Bio-Tech Disinfectant Spray (Version B) Sample delivered in solution form in clear plastic bottle (100 ml) Active ingredients: Benzalkonium chloride Sample Color: Colorless	
SAMPLE ID.	Lot No: 2020060401 MGF Date: 20200604 EXP Date: 20220603	
TEST METHOD (TM) *Please tick (√) at least one TM*	√	EN14476

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

REPORT ON THE EFFICACY OF CYPRESS BIO-TECH DISINFECTANT SPRAY (VERSION B) AGAINST SARS-COV-2 (COVID-19) IN AN *IN-VITRO* SUSPENSION ASSAY ACCORDING TO EN14476 PROTOCOL

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

EXECUTIVE SUMMARY

Cypress Bio-Tech Disinfectant Spray (Version B), also known as Cypress Bio-Tech Hand Sanitizing Foam with QUAC2™ formula, was evaluated for its virucidal activity against the SARS-CoV-2, the virus that caused COVID-19 pandemic. The efficacy of Cypress Bio-Tech Disinfectant Spray (Version B) against SARS-CoV-2 was tested in a suspension assay in both clean and dirty conditions as per the European Standard EN14476. It was also tested for its lasting virucidal activity following spraying onto surfaces. All tests were performed in a Biocontainment Level III Facility of the Tropical Infectious Diseases Research and Education Center (TIDREC), University of Malaya, Malaysia. Cypress Bio-Tech Disinfectant Spray (Version B) when tested undiluted achieved >5 $\log_{10}$ reduction in virus titer for a 30s exposure in both clean and dirty conditions. The product when diluted 2-fold to obtain a half concentration demonstrated a 5 $\log_{10}$ virus titer reduction for a 30 second exposure for both clean and dirty conditions. The disinfectant spray when used to coat the inner wall of the test tubes, retained >5 $\log_{10}$ virus titer reduction compared to the controls even after 12 hours post-spraying. These findings suggest that the Cypress Bio-Tech Disinfectant Spray (VersionB), also known as Cypress Bio-Tech Hand Sanitizing Foam with QUAC2™ formula is effective against COVID-19, can kill 99.99% SARS-CoV-2 in 30 seconds and the virucidal activity of the product is retained up to 12 hours post-spraying.

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

EXPERIMENTAL CONDITION

Test period	20.07.2020
Test temperature	21.0°C ± 1°C
Product test concentrations	Concentrated & half of concentration
Contact times	Contact time: 30s, 3min & 10min; Coated surface: 0h, 2h, 4h, 8h, 12h (Post-spray)
Conditions	Clean conditions: 0.3 g/l BSA Dirty conditions: 3.0 g/l BSA + 3.0 ml/l human erythrocytes
Diluent for product test solution	Distilled water
Temperature of incubation	37°C ± 1°C, CO 2 incubator (5% CO2)
Virus	SARS-CoV-2
Virus: source	Tropical Infectious Disease Research and Education Center (TDREC), University of Malaya, Malaysia
Virus: number of passages	2
Cell line	Vero E6
Cell line: source	ATCC
Cell line: number of passages	Passages 29 to 33

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

MATERIAL AND METHODS

Cells and viruses

The SARS-CoV-2 used was isolated, propagated and maintained in Vero E6 cells at TIDREC. The Vero E6 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% FBS. The cells were maintained at 37°C with 5% CO2. Virus titers were determined by microtitration using the Vero E6 cells and expressed in $TCID_{50}$/mL. When cytopathic effects (CPE) were microscopically evident, supernatant was harvested, clarified by centrifugation and stored at -80ºC until needed.

Virucidal assay

The Cypress Bio-Tech Disinfectant Spray was tested against SARS-CoV-2 in accordance to the European Standard EN14476:2013/FprA1:2015 protocol. The product was tested undiluted and at a 2-fold dilution under 2 different conditions; dirty condition (3.0 g/l BSA + 3 ml/l erythrocytes interfering substance) and clean conditions (0.3 g/l BSA interfering substance) at 30s contact time. The test assay comprised of 100 µl of interfering substance, 100 µl of virus suspension at concentration of 5.42×10^5 $TCID_{50}$/mL and 800 µl of Cypress Disinfectant Spray (Version B). After the specified contact time (30s), virucidal activity of the product was suppressed by adding DMEM+ 2% FBS and then the mixture was diluted in 10-fold dilution in ice cold media (DMEM+ 2% FBS). This diluted virus media was added to the Vero E6 cells to determine the $TCID_{50}$/mL. Virus controls for this test was distilled water in place of the test product for both dirty and clean conditions. The cells were incubated for 72 hours until CPE developed. A mixture of paraformaldehyde and crystal violet were used to fix and stain the infected cells. The virus titers were determined using the Spearman-Karber method and expressed as tissue culture infectious dose 50% (TCID50/ml). The virucidal activity was determined by the difference of the logarithmic titer of the virus control minus the logarithmic titer of the test virus (Δ log10 TCID50/ml). A reduction in virus titer of 4 $\log_{10}$ (corresponding to an inactivation of ≥ 99.99%) was necessary for claiming virucidal activity of the test product.

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Retention of disinfectant spray virucidal effects: Testing for prolonged efficacy of the Cypress Disinfectant Spray (Version B) virucidal activity was done by spraying the inner wall of plastic micro-centrifuge tubes. Following the spraying, the tubes were left to dry in sterile condition. At 2, 4, 8 and 12 hours post-spraying, virus or test products were added to the tubes. After 3 mins contact time, test product activity was neutralized by immediate serial dilution in DMEM supplemented with 2% FBS. The mixture was then added to Vero E6 cells maintained in tissue culture plates and the formation of virus cytopathic effects were monitored daily.

RESULTS

Product Suppression Assay

The product suppression assay was performed to accurately determine the activity of the test product at the given contact time. The activity was suppressed by adding cold DMEM+2% FBS, followed by serially diluting it 10-fold in cell culture medium. The suppression of product activity was assayed at 30s exposure. Results from the suppression assay showed no differences in the viral titers compared to controls (Table 1). This suggested that the addition of cold media and the serial dilution effectively suppresses the product activity, resulting in no reduction of the viral titers.

Table 1: Suppression of product activity assay

*Contact time (sec)	Interfering substance	Viral Titer [Control] ($TCID_{50}$/ml)	Viral Titer [After product suppression] ($TCID_{50}$/ml)	Difference in Viral Titer ($TCID_{50}$/ml)
30	clean conditions	5.4×10^5	5.4×10^5	0.00
30	dirty conditions	5.4×10^5	5.4×10^5	0.00

*Undiluted mixture

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Virucidal activity of Cypress Bio-Tech Disinfectant Spray (Version B)

The Cypress Bio-Tech Disinfectant Spray (Diluted & Undiluted) was tested against SARS-CoV-2 in accordance to the European Standard EN14476:2013/FprA1:2015. Manifestation of virus cytopathic effects in cell culture was used as the end-point and it was determined following the crystal violet staining. Results were determined by comparing the study product-treated groups against that of the water-treated controls. The SARS-CoV-2 titer in the control-treated samples under clean and dirty conditions, respectively, were at 5.42 $x10^5$ $TCID_{50}$/ml. The Cypress Bio-Tech Disinfectant Spray (Version B) when tested neat or diluted half of the concentration achieved >5 log_{10} reduction in viral titers when exposed for 30s, 3min and 10min under both clean and dirty conditions (Table 2).

Table 2: Virucidal activity of Cypress Bio-Tech Disinfectant Spray (Version B) against SARS-CoV-2

Virus	Concentration	Log_{10} Reduction in viral titer compared to control					
		Clean Condition			Dirty Condition		
		30s	3min	10min	30s	3min	10min
SARS-CoV-2	Undiluted	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
	Diluted 1/2	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Retention of Cypress Bio-Tech Disinfectant Spray (Version B) virucidal activity

Microcentrifuge tubes inner wall were coated with the test product and left to dry. At different intervals post-spraying, virus inoculum or control were added and left for 3 mins. The mixtures were then titrated for virus reduction. Results obtained suggested that >5 $\log_{10}$ reduction of virus titers were observed in all the coated tubes treated for the different durations (Table 3). The findings suggested that the Cypress Bio-Tech Disinfectant Spray (Version B) retained its virucidal properties up to 12 hours post-application.

Table 3: Retention of Cypress Bio-Tech Disinfectant Spray (Version B) virucidal activity

Virus	$\log_{10}$ reduction in viral titer compared to control			
	Time Post-Spraying			
	2h	4h	8h	12h
SARS-CoV-2	>5.00	>5.00	>5.00	>5.00
	>5.00	>5.00	>5.00	>5.00

SUMMARY

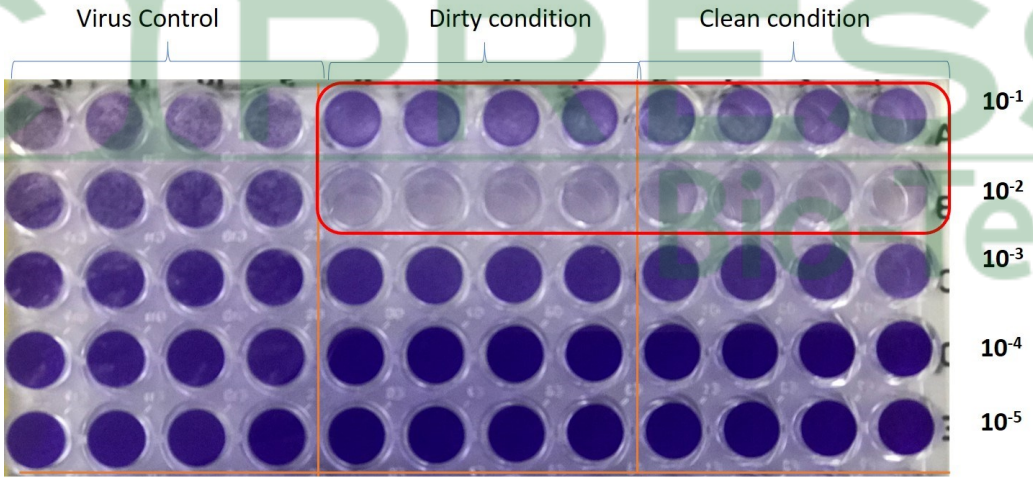
The virucidal efficacy of Cypress Bio-Tech Disinfectant Spray (Version B) was tested against SARS-CoV-2 in a suspension assay following the European Standard EN14476:2013/FprA1:2015 protocol. The Cypress Bio-Tech Disinfectant Spray (Version B), also known as Cypress Bio-Tech Hand Sanitizing Foam with QUAC2™ formula, when tested undiluted and at dilution of 1:2 demonstrated potent and rapid virucidal activity of ≥5 $\log_{10}$ reduction of SARS-CoV-2 viral titer in 30 seconds in both clean and dirty conditions. The Cypress Bio-Tech Disinfectant Spray (Version B), hence, is effective against COVID-19, can kill 99.99% SARS-CoV-2. The Cypress Bio-Tech Disinfectant Spray (Version B) retained its virucidal properties against SARS-CoV-2 up to 12 hours post-application.

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Figure 1: Virucidal activity of Cypress Bio-Tech Disinfectant Spray (Version B) using TCID50 assay. A) Virucidal activity at 30s in both dirty and clean conditions compared to virus control. No infectivity and detachment of cell monolayers were observed at dilutions of $1x10^{-1}$ and $1x10^{-2,}$ respectively. B) Virucidal activity at 12 hours post-application. Detachment of cell monolayers were observed at dilutions of $1x10^{-1}$. No virus CPE was observed at all other dilutions. Control with no virus added is labelled as Normal control. Pictures shown in A and B were from plates fixed and stained 3 days post-infection.

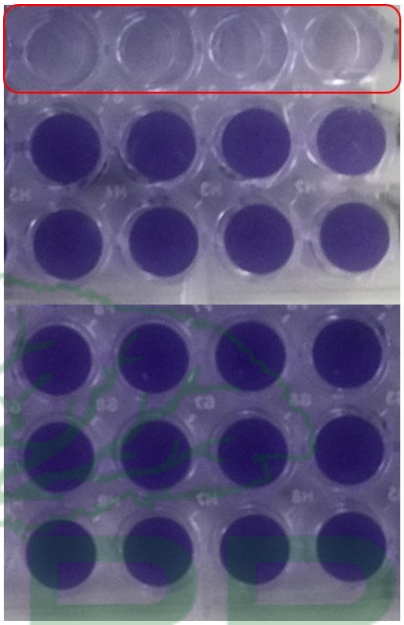
A)



TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

B)



PREPARED BY:	APPROVED BY:
Name: Pouya Hassandarvish Position: Postdoctoral Research Fellow	Approved Signatory (ies): Sazaly Abu Bakar, Ph.D., FASc ☑ Teoh Boon Teong, Ph.D. ☐ Juraina Abd Jamil, MMedSc ☐
Date: August 04, 2020	Date: August 04, 2020

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

About TIDREC

The Tropical Infectious Diseases Research and Education Center or TIDREC was established in 2008 to serve as a focal point for national and international collaborative research for academic institutions and research industries in Malaysia. The center was recognized as the Universiti Malaya Center of Excellence (UMCOE) in 2013 and in April 2019 was designated as the Ministry of Higher Education Higher Institution Center of Excellence (HICOE). The center houses the WHO Collaborating Centre for Arbovirus Reference & Research and the Tick Cells Biobank-Asia Outpost. The center is fully equipped with the facility to train and undertake research including those involving highly virulent pathogens. In addition to teaching and research, TIDREC also offers services such as reference laboratory diagnostics, drug screening, and validation tests for diagnostic kits. TIDREC is also one of the centers designated by the Ministry of Health of Malaysia to perform the COVID-19 laboratory screening tests. TIDREC is an ISO 9001 compliant organization of Universiti Malaya and subscribed to ISO 17025 for its testing services. TIDREC aspires to be an internationally recognized center of excellence in tropical infectious disease research and education that serves the health needs of the global communities.

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Appendix

Naming the coronavirus disease (COVID-19) and the virus that causes it

Official names have been announced for the virus responsible for COVID-19 (previously known as "2019 novel coronavirus") and the disease it causes. The official names are:

Disease

coronavirus disease
(COVID-19)

Virus

severe acute respiratory syndrome coronavirus 2
(SARS-CoV-2)

Source:
https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Appendix

Company: Cypress Bio-Tech Company Limited

Brand: Cypress Bio-Tech

Sample: Disinfectant Spray (Version B)

Product Name: (i) Disinfectant Spray
(ii) Hand Sanitizing Foam

Formula: QUAC2™

Composition / information on ingredients

Chemical name	INCI name	CAS number	Content (W/W)
Benzalkonium chloride	Benzalkonium chloride	8001-54-5	0.2%
Water	Water	/	97-99%
Non-hazardous base	/	/	up to 100%

TEST REPORT

LABORATORY ADDRESS:
Level 4, Block N & O, Faculty of Medicine, University of Malaya
50603 Kuala Lumpur.
Tel: +603-79676670 Email: tidrec@um.edu.my
Website: www.tidrec.com
HEAD OF LABORATORY: Sazaly Abu Bakar, Ph.D., FASc

Appendix

TIDREC Biosafety Level 3 Containment Facility (BSL3)