TEST REPORT

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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: TS4-0388		**DATE OF ISSUE: 20/09/2021**
CUSTOMER DETAILS		
NAME	Zebra Biotech Enterprise	
ADDRESS	B2-2-3 Solaris Dutamas 1, Jalan Dutamas 50480 KL	
CONTACT	Dr. Loshiniy Vijayan (loshiniy86@gmail.com)	
SAMPLE & TEST INFORMATION		
JOB NO.	TS4-0388	
DATE RECEIVED	12.05.2021	
TEST PERFORMED	24.05.2021	
ENVIRONMENTAL CONDITIONS	Ambient Temperature: Store below 30°C Relative Humidity: NA	
TYPE OF SAMPLE	Zyvex Hypertonic Spray (30 mL) & Zyver Advanced Oral Spray (30 mL)	
SAMPLE ID.	Zyvex Hypertonic Spray Batch: KLB 004 Mfc: 04/2020 Exp: 03/2024 LOT: ZYVA 004	Zyver Advance Oral Spray KLB 004 10/2020 09/2023 ZOS 002
TEST METHOD (TM) *Please tick (√) at least one TM*	√	EN14476

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REPORT ON THE EFFICACY OF ZYVEX HYPERTONIC SPRAY AND ZYVER ADVANCE ORAL SPRAY AGAINST SARS-COV-2 (COVID-19) AN IN-VITRO SUSPENSION ASSAY ACCORDING TO EN14476 PROTOCOL

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EXECUTIVE SUMMARY

The Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were evaluated for its virucidal activity against the SARS-CoV-2, the virus that caused COVID-19 pandemic. The efficacy of Zyvex Hypertonic Spray & Zyver Advanced Oral Spray against SARS-CoV-2 were tested in a suspension assay in both clean and dirty conditions as per the European Standard EN14476. All tests were performed in a Biocontainment Level III Facility of the Tropical Infectious Diseases Research and Education Center (TIDREC), University of Malaya, Malaysia. All procedures strictly adhered to biosafety procedures and approved protocols. The Zyvex Hypertonic Spray when tested achieved >2.55 $\log_{10}$ reduction in virus titer for a 60s exposure in clean condition and >2 $\log_{10}$ reduction in dirty conditions. The Zyver Advanced Oral Spray when tested achieved >4 $\log_{10}$ reduction in virus titer for a 30s exposure in both clean and dirty conditions. These findings suggest that the Zyvex Hypertonic Spray, can kill 99.9% SARS-CoV-2 in 60 seconds in clean condition, however, Zyvex Hypertonic Spray can only kill 99% SARS-CoV-2 in 60 seconds in dirty condition. On the other hand, the Zyver Advanced Oral Spray, kill 99.99% SARS-CoV-2 in 30 seconds.

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EXPERIMENTAL CONDITION

Test period	24.05.2021 – 28.05.2021
Test temperature	21.0°C ± 1°C
Product composition	**Zyvex Advance Oral Spray:** Purified water, Saccarose, Methylsalicilate, cetylpiridine, Citric Acid, Polysorbate 80, Eucalyptol, Lysozyme, colouring Agent, Aroma, Quercetin, Xylitol. **Zyvex Hypertonic Nasal Spray:** Aqua, Lysozyme, Hydroxyethylcellulose, Sodium chloride, Sodium Benzoate, Sodium Citrate, Glycerol, Eucalyptol.
Contact times	Contact 30s, 60s
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 (MY.TIDREC/6-3/Vero/2020) (Wuhan Variant)
Virus: source	Tropical Infectious Disease Research & Education Centre (TIDREC), University of Malaya, Malaysia
Virus: number of passages	2
Cell line	Vero E6
Cell line: source	ATCC
Cell line: number of passages	Passages 30

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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 evident under the microscope, the supernatant was harvested, clarified by centrifugation and stored at -80°C until needed.

Viral kill time assay

The Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were tested against SARS-CoV-2 in accordance to the European Standard EN14476:2013/FprA1:2015 protocol. The product was tested undiluted 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 and 60s contact time. The test assay comprised of 100 µl of interfering substance, 100 µl of virus suspension at concentration of 5×10^5 $TCID_{50}$/mL & 800 µl of products. After the specified contact time (30s and 60s), 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 $TCID_{50}$/mL. Virus controls for this test was distilled water in place of test product for both dirty and clean conditions. The cells were incubated for 96 hours till the 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 $\geq$ 99.99%) was necessary for claiming virucidal activity of the product.

qRTPCR study

The qRTPCR method was optimized and established at WHO Collaborating Center, Tropical Infectious Diseases Research & Education Centre to quantify SARS-CoV-2 RNA copy. Supernatant from TCID50 experiment for The Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were collected in a

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tubes and Qiagen Viral RNA extraction kit was used to extract virus RNA. The extract virus RNA was quantified using Taqman PCR kit by plotting standard curve.

RESULTS

Cytotoxicity Assay of Zyvex Hypertonic Spray & Zyver Advanced Oral Spray

The Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were tested for its cytotoxicity effects on Vero cells using the crystal violet dye staining assay. Briefly, the product was diluted 10-fold in 2% DMEM and added to the Vero cells. The plates were incubated in CO_2 incubator at 37° C for 96 hours. Following after the cells were fixed using 4% paraformaldehyde and crystal violet dye to check cells viability. No cytotoxicity of Zyvex Hypertonic Spray & Zyver Advanced Oral Spray was observed at the concentrations used for virucidal activity treatment.

Product Suppression Assay

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

Table 1: Suppression of product activity

*Contact time (sec)	Interfering substance	Viral Titer [Control] ($TCID_{50}$/ml)	Viral Titer [After product suppression] ($TCID_{50}$/ml)	Difference in Viral Titre ($TCID_{50}$/ml)
60	clean conditions	5×10^5	No inhibition	0.00
60	dirty conditions	5×10^5	No inhibition	0.00

*Undiluted mixture

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Virucidal activity of Zyvex Hypertonic Spray & Zyver Advanced Oral Spray

The Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were tested against SARS-CoV-2 in accordance to the European Standard EN14476:2013/FprA1:2015. Manifestation of virus cytopathic effects in cell culture was 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 x 10^5 $TCID_{50}$/ml. The Zyvex Hypertonic Spray when tested achieved >2 log_{10} reduction in virus titer for a 60s exposure and >2.55 log_{10} reduction achieved in clean condition and >2 log_{10} reduction achieved in dirty conditions. The Zyver Advanced Oral Spray when tested achieved >4 log_{10} reduction in virus titer for a 30s exposure in both clean and dirty conditions. (Table 2).

Table 2: Virucidal activity of Different Zyver Advanced Oral Spray and Zyvex Hypertonic Spray against SARS-CoV-2

Product	Trade name	Log10 Reduction in viral titers compared to control			
		Clean condition		Dirty condition	
		30 sec	60 sec	30 sec	60 sec
A	Zyver Advanced Oral Spray	>4.00	>4.00	>4.00	>4.00
B	Zyvex Hypertonic Spray	>2.00	>2.55	>1.75	>2.00

Evaluation of Zyvex Hypertonic Spray and Zyver Oral Spray using qRTPCR Method

The qRTPCR method was used to quantify the virus RNA copy number in the treatment with Zyvex Hypertonic Spray & Zyver Advanced Oral Spray compared to the untreated virus control. Both products exposed for 60s contact time were chosen for qRTPCR study. The results were talied against the TCID50 assay. Zyver Advanced Oral Spray reduced the virus titre by 100% at all the dilution compared to Zyvex Hypertonic Spray which showed 100% reduction of SARS-CoV-2 RNA copies only at the lower dilution (Figure 1). The efficacy of Zyvex Hypertonic Spray eventually dropped to 95%, 80%, 35% and 11% in the rest of virus dilutions.

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qRTPCR study of The Zyver Advance Oral Spray & Zyvex Hypertonic Spray

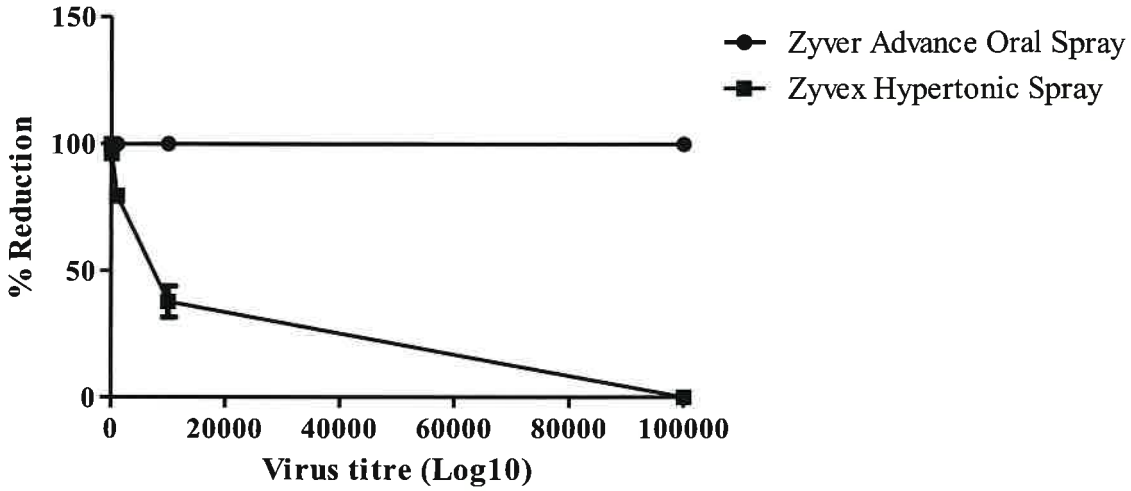

Figure 1: qRTPCR evaluation of Zyver Advance Oral Spray and Zyvex Hypertonic Spray. The graph shows Zyver Advance Oral Spray 100% reduction of SARS-CoV-2 RNA in all the virus dilutions. However, Zyvex Hypertonic Spray didn't show as effective as Zyver Advance Oral spray.

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SUMMARY

The virucidal efficacy of the Zyvex Hypertonic Spray & Zyver Advanced Oral Spray were tested against SARS-CoV-2 in a suspension assay following the European Standard EN144762013/FprA1:2015 protocol. The Zyvex Hypertonic Spray when tested achieved >2.55 $\log_{10}$ reduction in virus titer for a 60s exposure in clean condition and >2 $\log_{10}$ reduction in dirty conditions. The Zyver Advanced Oral Spray when tested achieved >4 $\log_{10}$ reduction in virus titer for a 30s exposure in both clean and dirty conditions. These findings suggest that the Zyvex Hypertonic Spray, can kill 99.9% SARS-CoV-2 in 60 seconds in clean condition, however, Zyvex Hypertonic Spray can only kill 99% SARS-CoV-2 in 60 seconds in dirty condition. On the other hand, the Zyver Advanced Oral Spray, kill 99.99% SARS-CoV-2 in 30 seconds.

PREPARED BY:	APPROVED BY:
Name: Pouya Hassandarvish (Ph.D) **Position: Senior Lecturer** Pouya Hassandarvish, PhD Senior Lecturer Tropical Infectious Diseases Research & Education Centre (TIDREC) Universiti Malaya, 50603 Kuala Lumpur	**Approved Signatory(ies):** **Sazaly Abu Bakar, Ph.D., FASc** ☑ **Teoh Boon Teong, Ph.D.** ☐ **Juraina Abd Jamil, MMedSc** ☐ Sazaly Abu Bakar, PhD, FASc Professor & Director Tropical Infectious Diseases Research & Education Centre (TIDREC) Higher Institution Centre of Excellence (HICoE) University of Malaya
Date: September 20, 2021	**Date: September 20, 2021**

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About TIDREC

The Tropical Infectious Diseases Research and Education Center or TIDREC was established in 2008 to serves 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 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 ISO9001 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 global communities.

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Appendix

World Health Organization

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

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Appendix

TIDREC Biosafety Level 3 laboratory (BSL3)