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Assessment of OraCare 2-Part Rinse Against SARS-CoV-2 *in vitro*

Final Report

FOR

PRO BREATH MD, LLC

d/b/a OraCare Products
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MRIGlobal Project No. 311710.01.001

December 9, 2020

Preface

This report was prepared at MRIGlobal for the work performed under MRIGlobal Task No. 311710.01.001, "Assessment of OraCare 2-Part Rinse Against SARS-CoV-2 *in vitro*."

The experimental phase of this task was initiated by MRIGlobal on November 04, 2020 and ended on November 10, 2020.

The test was managed and performed by Kristy Solocinski, Ph.D. She was assisted by Sam Humphrey.

The study was not performed in compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58). All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal, and any deviations were documented.

All study records are stored at MRIGlobal.

Sincerely,

MRIGLOBAL

Kristy Solocinski, Ph.D.
Staff Scientist
Life Sciences Division

Approved:

Claire Croutch, Ph.D.
Portfolio Director,
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December 9, 2020

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Executive Summary

Objective:

The objective of this project was to determine if OraCare 2-Part Rinse has the ability to limit the replication of SARS-CoV-2 *in vitro*.

Study Design:

The two components of the OraCare 2-Part Rinse was added with cell media and allowed to mix for the proscribed amount of time. Virus was added to the test mixture, allowed to incubate briefly, and then serially diluted 1:10 down the rows of the plate. The dilutions were then transferred to Vero cells. The inoculated cells were incubated for 6 days and then read for cytopathic effect (CPE).

Results and Conclusions:

During the first test, OraCare exposure reduced SARS-CoV-2 infection by greater than two logs (99%) under all conditions tested, with the highest reduction (3.44 logs, 99.964%) seen with the 30 or 60 seconds mixing time and 30 seconds of exposure to the virus. In the second test, greater than four logs reduction in infection (> 99.99%) was seen at intermediate mixing times (4, 8, or 20 minutes), with the other mixing times demonstrating greater than three logs reduction in infection (> 99.9%). There was cytotoxicity from the test article so the actual reduction in SARS-CoV-2 infectivity may be greater than what can be measured in this assay. Based on these experiments, we conclude that the OraCare 2-Part Rinse does reduce SARS-CoV-2 infection in Vero cells.

Section 1.
Objective

The objective of this project was to determine if OraCare 2-Part Rinse has the ability to limit the replication of SARS-CoV-2 *in vitro*.

Section 2.
Sponsor, Testing Laboratory, and Personnel Responsibilities

2.1 Sponsor's Representative

Richard Downs DDS
OraCare

2.2 Testing Laboratories

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2.3 Personnel Responsibilities

2.3.1 Study Director—MRIGlobal

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2.3.2 Analyst – MRIGlobal

Sam Humphrey
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Section 3.
Test Conditions

3.1 Test Material

3.1.1 OraCare

Lot No.: 200310
Expiration date: 7/22

3.1.2 Cell Media

DMEM/F12 (Serum-free media)
Vendor: Gibco
Lot No.: 2186786
Expiration date: 5/21

Growth Media – 5% FBS (fetal bovine serum)
Lot No.: 20200918KS
Expiration date: 11/20

3.1.3 Challenge Virus

Severe Acute Respiratory Syndrome-related Coronavirus-2 (SARS-CoV-2) (COVID-19 Virus)
Strain: USA-WA1/2020
Vendor: BEI Resources

Passage number in assay: 9

3.1.4 Host

Vero E6 Cells
Vendor: ATCC
Cat: CRL 1586
Passage Number in Assay: 22

Section 4.
Test System

MRIGlobal utilized the USA-WA1/2020 strain of the virus, acquired from BEI Resources (NR-52281). This was propagated in Vero E6 cells (ATCC CRL-1586). Vero E6 cells were cultured in growth media consisting of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) supplemented with 5% FBS (Fetal Bovine Serum), and PSN (penicillin, streptomycin, and neomycin).

Section 5.
Study Design

The Vero E6 cells were plated on 96-well plates one to three days before the assay and were allowed to grow to ~ 60%-70% confluence. On the day of the assay, we added 630 uL of DMEM/F12 to each well in rows B-H of a 96 deep-well plate. We added 900ul of OraCare (450 ul pt l + 450ul pt 2 or 900ul of mixed for test 2) or PBS (for control) to Row A. After the prescribed amount of time (30 or 60 seconds for Test 1; 0.5, 2, 4, 8, 20, and 60 minutes for Test 2), we added 0.1 ml of SARS-CoV-2 strain USA-WA1/2020 and incubated for 30 or 60 seconds for Test 1 and 30 seconds for Test 2. Cytotoxicity controls of OraCare with 100 ul of PBS added intead of virus were also performed, plated 0.5, 20, and 60 minutes after mixing. After the designated time, 70 uL from Row A was transferred to Row B and pipeted at least 3 times to mix. 70 uL from Row B was transferred to Row C and pipeted at least 3 times to mix. This was repeated through Row H. Tips were changed between rows. These dilutions were then transferred to a plate of Vero E6 cells with media removed. After at least 15 minutes, DMEM/F12 supplemented with5% FBS was added to cells to feed them for the next 5 days. This incubation period is to allow the virus to adsorb to cells without interference from FBS. The assay was executed in three technical and five pipetting replicates for each condition.

After 6 days, cells were examined for the presence of cytopathic effect (CPE) associated with viral presence and replication. Examination is performed using a microscope (10x objective to view the entire well at once) and observing the morphology of the cells. Healthy Vero E6 cells are semi-transparent with a fusiform appearance (pinched or narrowing ends and more round in the middle) in a monolayer of cells with little to no space between cells. Dead cells displaying CPE are often detached from the plate, round, less transparent, and much smaller than living cells. Furthermore, the healthy Vero E6 cells cover much of the surface of the well but wells containing cells with CPE have areas of the well where no cells are adherent, described as empty space. Any well displaying CPE is marked as positive whether the whole well is affected or only a small patch as both are indicative of the presence of viable virus.

Section 6.
Statistical Analysis of Data

The number of positive and negative wells were entered into a modified Excel spreadsheet that was published as part of Lindenbach BD. *Measuring HCV infectivity produced in cell culture and in vivo*. Methods Mol Biol. 2009;510:329-336. doi:10.1007/978-1-59745-394-3_24. The $TCID_{50}$/ml is calculated using the below equations, all using Microsoft Excel.

$$\text{Proportionate Distance (PD)} = \frac{\%\text{CPE at dilution above } 50\% - 50\%}{\%\text{ CPE at next dilution above } 50 - \%\text{ CPE at next dilution below } 50}$$

$$\text{TCID50} = 10^{\log \text{ of dilution above } 50\% \text{ CPE}} - \text{PD}$$

$$\text{TCID50/ml} = \frac{1}{\text{volume used per well}} x \frac{1}{\text{TCID50}}$$

The log10 of the three technical replicates was averaged for control and treatment samples. This number for the treatment is subtracted from the number for the control and is reported as "log reduction." This log reduction is converted into a percent log reduction via the following equation.

$$\%\text{ Log Reduction} = 1 - 10^{-\log \text{reduction}}$$

Section 7.
Results - Efficacy of DiaSorin Buffer Against SARS-CoV-2

The plates were read 6 days after the initiation of the assay. In the first test, all conditions demonstrated at least a 2-log reduction (> 99%) in infection, with conditions exposing the virus to the rinse for 30 seconds demonstrating greater effectiveness than those where the virus was exposed to the solution for a minute. In the second test, conditions where the OraCare components were allowed to mix for between 4 and 20 minutes demonstrated the greatest reduction in infection (> 4 logs, > 99.99%). There was cytotoxicity observed from the test article so the true reduction in SARS-CoV-2 infectivity may be greater than reported here. However, since the test article killed the cells, it is impossible to say in this assay. Table 1 summarizes these findings.

Table 1. Results of *in vitro* Neutralization of SARS-CoV-2 with OraCare

Sample Name	Sample Type	Time after Oracare Mixing (min)	Time of Viral Exposure (min)	Replicate No.	TCID /mL	Log10 TCID /mL	Average TCID /mL	Average Log10 TCID /mL	Log Reduction	Percent Log Reduction
Test 1 3030-1	Test	0.5	0.5	1	3.16E+03	3.50	3.16E+03	3.50	3.44	99.964%
Test 1 3030-2				2	3.16E+03	3.50				
Test 1 3030-3				3	3.16E+03	3.50				
Test 1 3060-1	Test	0.5	1	1	3.16E+04	4.50	1.99E+05	4.58	2.36	99.565%
Test 1 3060-2				2	3.16E+03	3.50				
Test 1 3060-3				3	5.62E+05	5.75				
Test 1 6030-1	Test	1	0.5	1	3.16E+03	3.50	3.16E+03	3.50	3.44	99.964%
Test 1 6030-2				2	3.16E+03	3.50				
Test 1 6030-3				3	3.16E+03	3.50				
Test 1 6060-1	Test	1	1	1	3.16E+03	3.50	2.48E+04	3.94	3.00	99.900%
Test 1 6060-2				2	6.81E+04	4.83				
Test 1 6060-3				3	3.16E+03	3.50				
Test 1 Con-1	Control	N/A	1	1	6.81E+06	6.83	9.43E+06	6.94	N/A	N/A
Test 1 Con-2				2	1.47E+07	7.17				
Test 1 Con-3				3	6.81E+06	6.83				
Test 2 30-1	Test	0.5	0.5	1	3.16E+03	3.50	3.16E+03	3.50	3.32	99.953%
Test 2 30-2				2	3.16E+03	3.50				
Test 2 30-3				3	3.16E+03	3.50				
Test 2 2-1	Test	2	0.5	1	3.16E+03	3.50	1.26E+03	2.83	3.99	99.990%
Test 2 2-2				2	3.16E+02	2.50				
Test 2 2-3				3	3.16E+02	2.50				
Test 2 4-1	Test	4	0.5	1	3.16E+02	2.50	3.16E+02	2.50	4.32	99.995%
Test 2 4-2				2	3.16E+02	2.50				
Test 2 4-3				3	3.16E+02	2.50				
Test 2 8-1	Test	8	0.5	1	4.22E+02	2.63	3.51E+02	2.54	4.28	99.995%
Test 2 8-2				2	3.16E+02	2.50				
Test 2 8-3				3	3.16E+02	2.50				
Test 2 20-1	Test	20	0.5	1	3.16E+02	2.50	7.00E+02	2.72	4.10	99.992%
Test 2 20-2				2	3.16E+02	2.50				
Test 2 20-3				3	1.47E+03	3.17				
Test 2 60-1	Test	60	0.5	1	3.16E+03	3.50	3.16E+03	3.50	3.32	99.953%
Test 2 60-2				2	3.16E+03	3.50				
Test 2 60-3				3	3.16E+03	3.50				
Test 2 Con-1	Control	N/A	0.5	1	4.81E+06	6.68	7.90E+06	6.82	N/A	N/A
Test 2 Con-2				2	4.22E+06	6.63				
Test 2 Con-2				3	1.47E+07	7.17				
Test 2 cyto	N/A	0.5	N/A	1	3.16E+03	3.50	N/A	N/A	N/A	N/A
Test 2 cyto		20		2	1.00E+03	3.00				
Test 2 cyto		60		3	1.00E+03	3.00				

Section 8.
Conclusions

Based on this experiment, we conclude that OraCare 2-Part Rinse does reduce SARS-CoV-2 infection in Vero cells. Based on the company's instructions for use of Oracare rinse that states the user should mix the 2 part rinse for 30 seconds and rinse for 30 seconds, this study found a 3.44 average log (2,754 fold) reduction of SARS-CoV2 infection in Vero cells using those mixing and viral exposure times in this in vitro study.