IN-VITRO VIRUCIDAL EFFECT OF A BENZALKONIUM CHLORIDE HAND SANITIZER ON CORONAVIRUS

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In-Vitro Virucidal Effect of a Benzalkonium Chloride Hand Sanitizer on Coronavirus

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Accepted for Publication in the Journal of the Mississippi State Medical Association

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Abstract

A commercially available hand sanitizer containing 0.12% benzalkonium chloride as the active ingredient was tested in the laboratory for its ability to inactivate Human Coronavirus Strain 229E (a surrogate for SARS-CoV-2) in an in-vitro cell culture test. The results showed a >99.9% inactivation of the test virus with 30 seconds, 60 seconds, and 120 seconds of exposure to the test product.

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Introduction

The World Health Emergency caused by the novel coronavirus (SARS-CoV-2) has as of 08 May 2020 resulted in 1,263,052 cases of infection (COVID-19) and 76,101 deaths in the United States of America. Trials are currently underway to evaluate medical treatments for this infection with several appearing promising to become recommended therapy. Vaccine development is also underway but availability is many months away.

For now prevention is the most effective way of attacking this epidemic. The World Health Organization (WHO) recommends hand washing with soap and water and use of alcohol based hand sanitizer if there is no visible soiling of the hands.[1] In the USA the FDA allows only three active ingredients for hand sanitizers, ethyl alcohol, isopropyl alcohol, and benzalkonium chloride. We evaluated the effect of a commercially available hand sanitizer (DAB, Three Kings Corp., Corinth, MS) using 0.12% benzalkonium chloride as its active ingredient on Human Coronavirus, strain 229E (American Type Cell Culture #VR-740). Strain 229E like other coronaviruses including SARS-CoV-2 is a large enveloped virus. Composition and structures of the envelope are not strain specific and sanitizers that are effective against one strain of an enveloped virus representing the virus family are likely to be effective against the whole family of viruses.[2] Strain 229E has been recommended by public health authorities as an appropriate surrogate virus for SARS-CoV-2.[3]

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Methods

The study evaluated the virucidal properties of the test product when challenged with Human Coronavirus based on ASTM E1052-11 *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension.* The percent and $\log_{10}$ reductions from the initial population of the viral strain were determined following exposure to the test product for 30 seconds, 60 seconds, and 120 seconds. The host cells were American Type Cell Culture Collection (ATCC) MRC-5 (CCL-171). The MRC-5 cell line was chosen because of its known susceptibility to infection by strain 229E.[4] Five parameters were evaluated, virucidal suspension test, virus control, cytotoxicity control, neutralization control, and cell culture control.

The host cell cultures were seeded onto multi-well cell culture treated plates. Cell monolayers were 80% to 90% confluent and less than 48 hours old before inoculation with the virus. The five parameters were evaluated using the following sequences:

Parameter	Sequence
Virucidal suspension test	virus+ test product – exposure – neutralization - dilution - plating
Virus control	virus + diluent – neutralization – dilution - plating
Cytotoxicity control	test product + diluent – neutralization – dilution - plating

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OF THE MISSISSIPPI STATE MEDICAL ASSOCIATION

Neutralization control	test product + diluent – neutralization – virus inoculation – dilution - plating
Cell culture control	maintenance medium

For the virucidal suspension test a 0.5 mL aliquot of test virus was added to a vial containing 4,5 mL of the undiluted test product to achieve a 90% (v/v) concentration of the test product. The test virus was then exposed to the test product for 30 seconds, 60 seconds, and 120 seconds. Immediately after each exposure the test virus / product suspension was neutralized in neutralizing broth, mixed thoroughly, and serially diluted in maintenance medium. Each dilution was plated in four replicates.

For the virus control a 0.5 mL aliquot of test virus was added to 4.5 mL of maintenance medium and exposed for 120 seconds at ambient temperature. The subsequent test virus dilution was made in maintenance medium and serially diluted in maintenance medium. Each dilution was plated in four replicates.

For the cytotoxicity control a 0.5 mL aliquot of maintenance medium was added to a vial containing 4.5 mL of the undiluted test product. The maintenance medium/product mixture was neutralized in neutralizing broth, mixed thoroughly and serially diluted in maintenance medium. Each dilution was plated in four replicates.

For the neutralization control a 0.5 mL aliquot of maintenance medium was added to a vial containing 4.5 mL of the undiluted test product. The maintenance medium/product mixture was diluted 1:10 in neutralizing broth. An aliquot of the virus was added to the

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neutralized product for 10 to 20 minutes. Additionally, the effect of the neutralizer on virus infectivity was assessed by adding virus to the neutralizer alone followed by exposure for 10 to 20 minutes. Subsequent 10-fold dilutions of neutralized test product/virus suspension were made in maintenance medium. Each dilution was plated in four replicates.

For the cell culture control the intact cell culture served as the control of cell culture viability. The growth medium was replaced by maintenance medium in all cell control wells.

The plates were incubated in a carbon dioxide incubator at temperature and duration times as recommended by ATCC. Cytopathic/cytotoxic effect was monitored using an Inverted Compound Microscope.

Viral and toxicity titers are expressed as $-\log_{10}$ of the 50% titration end point for infectivity. To quantitate the viral titer a 50% tissue culture infectious dose ($TCID_{50}$) calculation - the Quantal test (Spearman-Karber Method) was applied. To calculate the $\log_{10}$ of infectivity reduction the following formula was used:
Log_{10} Reduction = ($\log_{10}$ $TCID_{50}$ of the virus control) – ($\log_{10}$ $TCID_{50}$ of the virucidal suspension test)

The percent reduction was calculated as follows:

$$\% \text{ Reduction} = [\ 1\ -\ (TCID_{50}\ \textit{test}\ /\ TCID_{50}\ \textit{virus control}\ )\ ] \times 100$$

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Results

The results showed a >3.00 $\log_{10}$ reduction in the test product virucidal suspension parameter as compared to the virus control parameter showing significant inactivation (>99.90%) of the test coronavirus strain by the test product at 30 seconds exposure, 60 seconds exposure, and 120 seconds exposure. The cytotoxicity control and neutralization control showed no effect on the cell line. (See Table One)

Discussion

Although numerous studies on medical treatments for COVID-19 are underway and vaccine research is ongoing it may be some time before accepted treatments are in wide spread use. Prevention of person to person spread in health care facilities involves use of mask respirators and personal protective equipment along with adherence to hand hygiene protocols. For the general public the WHO has published several recommendations for protection, one of which is the use of alcohol based hand sanitizer when the hands are not visibly soiled.[1] Since there are situations where alcohol based hand sanitizers are not appropriate we tested a benzalkonium chloride (the only other FDA allowed active ingredient) hand sanitizer to see if it was effective against coronavirus. In a recent review article of disinfectants on surfaces and their effects on coronavirus Kampf found the data on benzalkonium chloride “conflicting” and also stated there was no study found on hands and coronavirus.[5] Our research showed that the test product we studied was very effective in inactivating coronavirus. Previous studies of this test product have shown that it has persistent antibacterial effects against

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Staphylococcus aureus for at least four hours.[6,7] Our study did not address any persistence of anti-viral effects. This question should be addressed in follow up studies.

Conclusions

A 0.12% benzalkonium chloride hand sanitizer was very effective in inactivating Human Coronavirus Strain 229E (ATCC- #VR-740) *in vitro*. In view of the known lack of persistence on the skin of alcohol based hand sanitizers (unless organic acids are added to the product) [8] and the previous research showing at least four hours of antibacterial persistence of this test product additional studies are recommended to see if benzalkonium chloride hand sanitizers have a persistent anti-viral effect.

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Table One

Test Formulation #1: DAB Protects BZK @ 0.12% (Lot #20027)

Virus: Coronavirus strain 229E (ATCC #VR-740)

Host Cell Line: MRC-5 (ATCC #CCL-171)

Volume Plated per Well: 1.0 mL

Dilution (- Log_{10})	**Virus Control**	**Test**			**NTC**	**NC**	**CTC**	**CC**
		30 seconds	**1 minute**	**2 minutes**				
								0000
-2	NT	CT	CT	CT	NT	NT	++++	
-3	++++	0000	0000	0000	++++	++++	0000	
-4	+0++	0000	0000	0000	+00+	0+++	0000	
-5	++++	0000	0000	0000	++0+	++0+	NT	
-6	00+0	0000	0000	0000	0000	0000	NT	N/A
-7	0000	0000	0000	0000	0000	0000	NT	
$TCID_{50}$ (log_{10})	5.50	≤2.50	≤2.50	≤2.50	4.75	5.00	2.50	
Log_{10} Reduction	N/A	**≥3.00**	**≥3.00**	**≥3.00**	N/A			
Percent Reduction		**≥99.90**	**≥99.90**	**≥99.90**				

\+ CPE (cytopathic/cytotoxic effect) present

0 CPE (cytopathic/cytotoxic effect) not detected

CC Cell Control

CTC Cytotoxicity Control

NC Neutralization Control

NTC Neutralizer Toxicity Control

NT Not tested

N/A Not applicable

CT Cytotoxicity

$TCID_{50}$ Tissue Culture Infective Dose – 50%

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Disclosures and Acknowledgements:

This study was funded by Three Kings Corporation, Corinth, Mississippi, who also furnished the test product for the study.

Sidney W. Bondurant, MD and Medical Spark Biologics, LLC are consultants to Three Kings Corporation.

Ronny Jackson, MD is a consultant to Three Kings Corporation.