Hand Sanitizer Product Line

White Paper

Meditizer
Medical-Grade Hand Sanitizer

A product suite scientifically proven to kill dangerous bacteria and viruses for 4+ hours with one application.

- COVID-19
- C. difficile & C. difficle spores
- MRSA
- Other dangerous pathogens

02.01 - The Mission

Our Mission and Goals

Our Immediate and Future Mission

As the world is locked in a cycle of uncertainty due to the novel COVID-19 virus, personal preventative measures are key to returning our societies and economies to a greater degree of normalcy. While social distancing and face coverings are chief among these measures, unique products are needed to fill the gaps and help reduce the risk of transmission.

Though COVID-19 remains at the forefront of global concerns, the climbing rate of hospitalizations has brought to light the need to combat may other harmful and infectious pathogens. Bacteria such as Clostridium difficle (C. diff), E. Coli, Burkholderia cepacia, Methicillin-resistant Staphylococcus aureus (MRSA) and many other pathogens have proven to be formidable adversaries in their own right.

Our mission is to supply a suite of products that are clinically proven to kill these pathogens and help protect front line workers and essential personnel while they perform their jobs. The average citizen can also greatly benefit from having access to technology that can help them reduce the risk of incidental transmission. The need for these products extends far beyond the end of this current pandemic. They will always be needed to help mitigate the risk of infection and transmission.

Act 1 - COVID-19

A scientifically proven, continuous 4+ hour kill rate with a single application of both the Hand Sanitizer and Mask Spray Products gives us a powerful new weapon against the transmission of this novel virus.

Act 2 - C. DIFFICILE & SPORES

C. diff transmission and infection is a serious concern in the medical arena. This harmful bacteria and its spores are very difficult to kill. Our products are scientifically proven to kill both with a 5.2+ log reduction over 30 seconds.

Act 3 - OTHER PATHOGENS

Our products do not just kill COVID-19 and C. diff. They are effective in killing many other harmful bacteria and viruses that may plague us now or in the fututre.

03.01 - The Technology

Copper & Magnesium Microplatelets

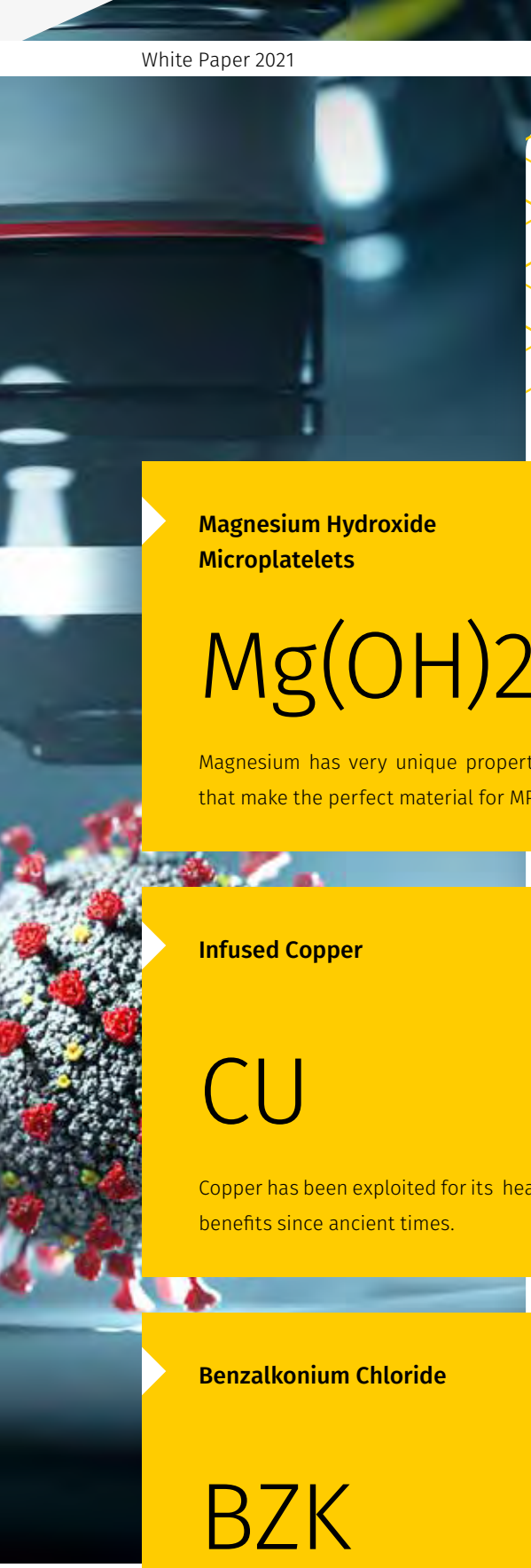
Copper infused Magnesium Hydroxide Microplatelet Technology.

This technology represents a novel material science comprising discs or wafers of Magnesium Hydroxide molecules arranged in ‘sheets’ or ‘layers’. This results in extremely large surface area with potentially reactive hydroxyl groups studding the surface.

Microplatelets (MP’s) require contact between themselves and the target microorganism or virus. MP action is focused and direct. Our typical MP configuration is a disc of 200 nm x 100 nm x 10 nm. For comparison is about one tenth of the length of an E.coli bacterium (1,000nm), and about 2/5 it’s width (500 nm).

For further reference, the COVID-19 Coronavirus is a sphere of about 125 nm in diameter. These size relations indicate that Microplatelets are in the size range of a number of pathogens and the intimate contact that occurs between the surface of MicroPlatelets and target microorganisms is key to MP antimicrobial potency.

Copper, long known for its anti-microbial properties, is then infused onto the surface of these Magnesium Hydroxide Microplatelets. This combined with the reactive hydroxyl effects of the platelet itself and aided by the addition of Benzalkonium Chloride work in unison to destroy the target micro-organisms.



Magnesium Hydroxide Microplatelets

Mg(OH)2

Magnesium has very unique properties that make the perfect material for MP's.

Infused Copper

CU

Copper has been exploited for its health benefits since ancient times.

Benzalkonium Chloride

BZK

Benzalkonium Chloride is recommended by the FDA for sanitizing purposes.

Patent Pending Technologies are the essence of these products.

Surface Area is the key

The key to our technological advantage is in the microplatelet itself. Our partner has developed a cutting edge material science manufacturing method that produces flat plates rather than nodules. Nodules, while possessing a large roughly spherical surface area, have the disadvantage of a very low potential contact area with regards to viruses, bacteria, and other pathogens. Since our kill methodology requires surface contact, it is essential that we have as large a surface area for the pathogens to interact with as possible.

Infused Copper adds to the kill rate

Copper has been exploited for health purposes since ancient times. The process involves the release of copper ions (electrically charged particles) when microbes, transferred by touching, sneezing or vomiting, land on the copper surface. The ions prevent cell respiration, punch holes in the bacterial cell membrane or disrupt the viral coat and destroy the DNA and RNA inside.

These technologies have U.S. patents and patents pending status which is shown in the Documentation section of this white paper.

Benzalkonium Chloride

- U.S. FDA recommends Benzalkonium Chloride as an effective sanitizer.
- Has recently shown a marked reduction in colony forming units over a several hour period after an extensive antibacterial study.
- Studied for virucidal properties against influenza, Newcastle disease, and avian infectious bronchitis.

The FDA has recently indicated support for one of our key ingredients, Benzalkonium Chloride (BZK). BZK is thought to work by cation (positive ion) donation or surfactant activity, either of which have the effect of disrupting the bacterial membrane or viral envelope. In recent clinical studies to demonstrate persistent antibacterial efficacy of a hand sanitizer, BZK produced a marked reduction in colony-forming units at each time points tested at one hour, two hours, and three hours of (3.75-4.16-log10 reductions).

This active ingredient also actively assists by disrupting the cell membranes of the target organisms and is active at relatively low concentrations (0.12%-0.13%). Benzalkonium chloride has also been studied for virucidal activity against influenza, Newcastle disease, and avian infectious bronchitis viruses.

03.04 - Killing Mechanism

Key Facts and Highlights.

Proven Results

ASU's BSL3 Lab from the Bio Design institute and the Southwest College of Naturopathic Medicine & Health Sciences conducte our SARS-CoV-2 test

Prolonged Kill

Test reults show a continuous kill rate of more than 4 hours on viruses and bacteria.

How It Works

Viruses and micro-organisms such as bacteria exist within a gel like capsid envelope which protects them from the normal environment. This biofilm surrounds the virus or bacteria and is largely responsible for keeping it viable between hosts. Bacteria within these biofilms are over 1,000 times more resistant to antibiotics. Essentially, the antibiotics can not penetrate the biofilm layer to work against the pathogen contained within.

MicroPlatelets kill from the outside. Unlike other approaches, MicroPlatelets are not ingested by the the bacteria or fungi. MP's are not taken up by cells. Their surface area render them too large for this concern. They interact with the biofilm directly.

Our MicroPlatelet technology is designed to destroy this biofilm by a chemical/mechanical means, destroying the capsid envelope and ultimately killing the virus, fungi or bacteria hidden inside. The MicroPlatelet is unaffected by this interaction and can survive the encounter to continue killing destroying any biofilm it comes in contact with. Thus, the prolonged and sustained killing effect is realized.

04.01 - Water Based Advantages

Moisturizing, non-alcohol based

4.1 Drawbacks of Alcohol Based Hand Sanitizers

Alcohol based hand sanitizers have several drawbacks versus water based sanitizers. Alcohol based gels or foaming sanitizers tend to dry out the hands by effectively flushing the natural oils from the skin. These oils act as both a skin moisturizer and as part of the body's anti-microbial defense system.

By flushing these oils from the skin, there is a greater chance of hands drying and cracking. Dry hands lead to tiny fissures in the skin that can run deep into the epidermis. These fissures allow additional entry points for harmful bacteria and viruses to enter the body. A moisturizing, water based sanitizer keeps the hands from drying out thereby reducing this risk.

Additionally, alcohol based sanitizers typically use either alcohol or isopropanol. Both are highly flammable substances. The FDA recommends concentrations between 60-90% for maximum efficiency in killing germs. At these high concentrations, these sanitizers become fire hazards.

Hospitals and other medical facilities are required to consult with local fire authorities and adhere to strict regulations and codes regarding flammable substances. This can result in the alcohol based sanitizer being placed in awkward and inefficient locations for routine staff access when placing dispenser stations or storing large quantities of the sanitizer.

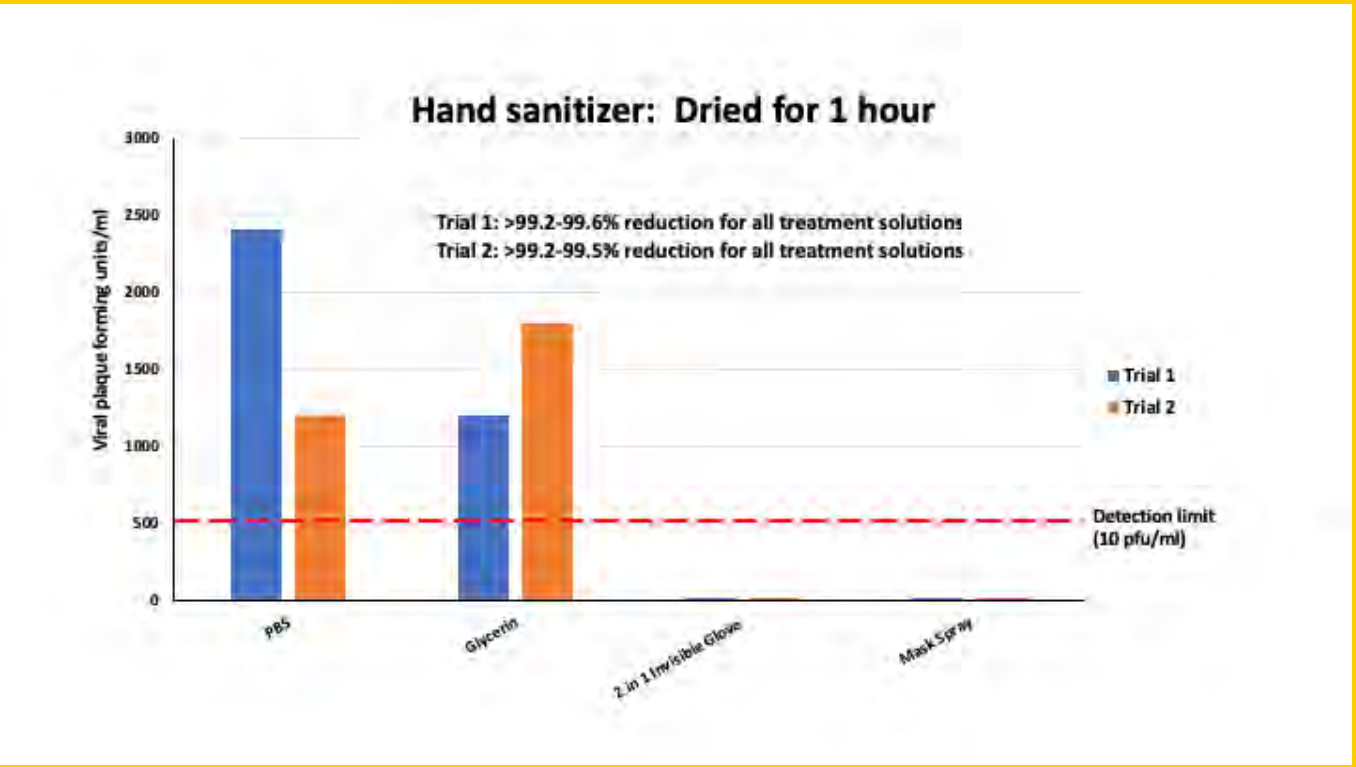
4.2 Benefits of Our Water Based Hand Sanitizer Solutions

- Moisturizes the skin while effectively killing 99.9% of harmful bacteria and viruses on contact.
- Continues to kill for a period of time longer than that of an alcohol based sanitizer after the solution has dried on the skin.
- Hypoallergenic Formulation for less skin irritation.
- Uses a Federally approved effectiveness protocol.
- Protects against germs and fungus.
- Works synergistically with the hands natural defenses.
- Painless application for those with cuts, scrapes, or other wounds on the hands. .
- Delivered as a pleasant lotion and drys within 30 seconds leaving the hands feeling soft and clean.
- Nontoxic formula is safer for children if accidentally ingested.
- Is non-flammable and will not stain surfaces.
- Will not dry out and crack the skin.
- Works synergistically with the hands natural defenses.

05.01 - COVID-19 Assay

Summary of SARS-Cov-2 Assay

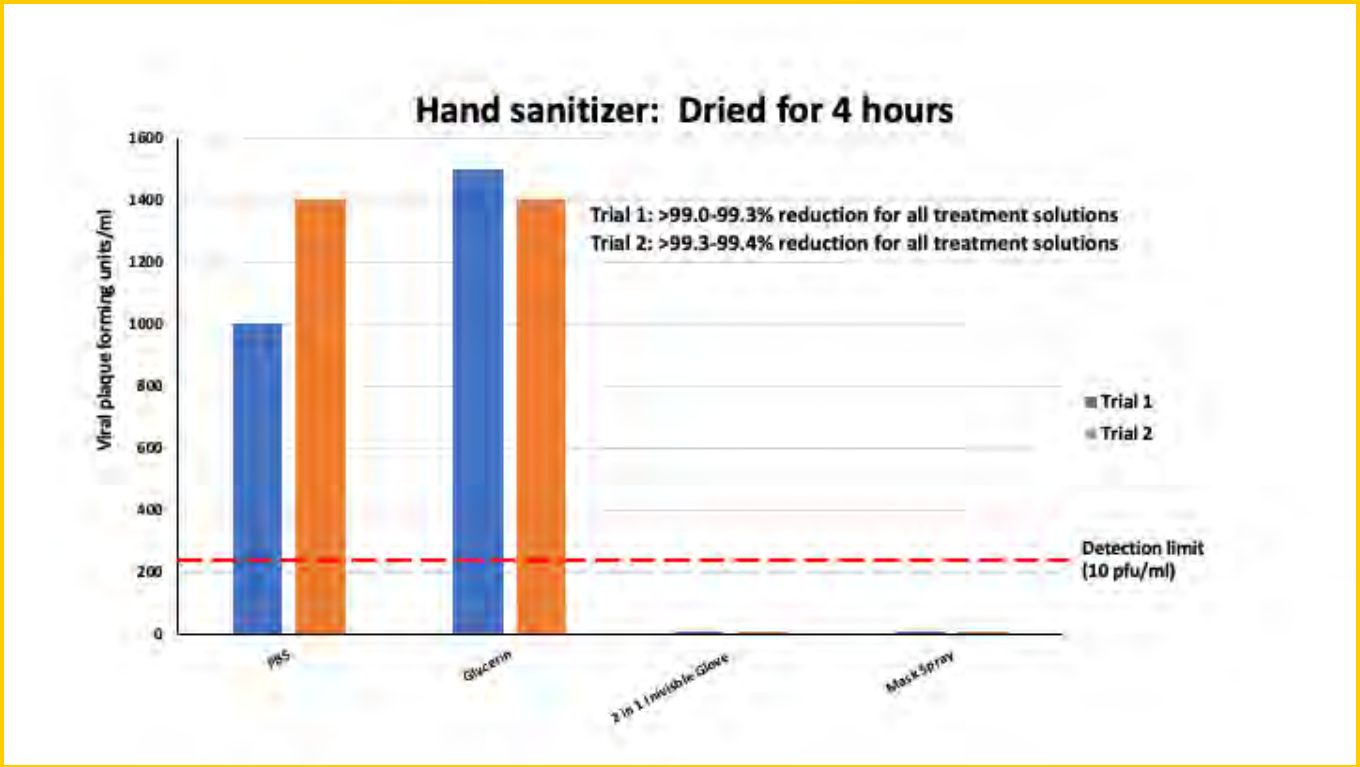
In February of 2021, the Arizona State University‘s Biodesign institute in conjunction with the Southwest College Of Naturopathic Medicine & Health Sciences conducted an assay to characterize the long term antimicrobial properties of our sanitizing products. The following are the results of that assay.



05.02 - Test Results

4+ Hour Kill Time Claim, Confirmed!

The results of this study, conducted in a certified Biosafety Level 3 facility, support that the 2 in 1 Invisible Glove and Mask Spray products are all able to kill the SARS-CoV-2 virus even after drying on a surface for 1 hour or 4 hours.



06.01 - Bacteria Kill Rate

Summary of Bacteria Kill Rate Test.

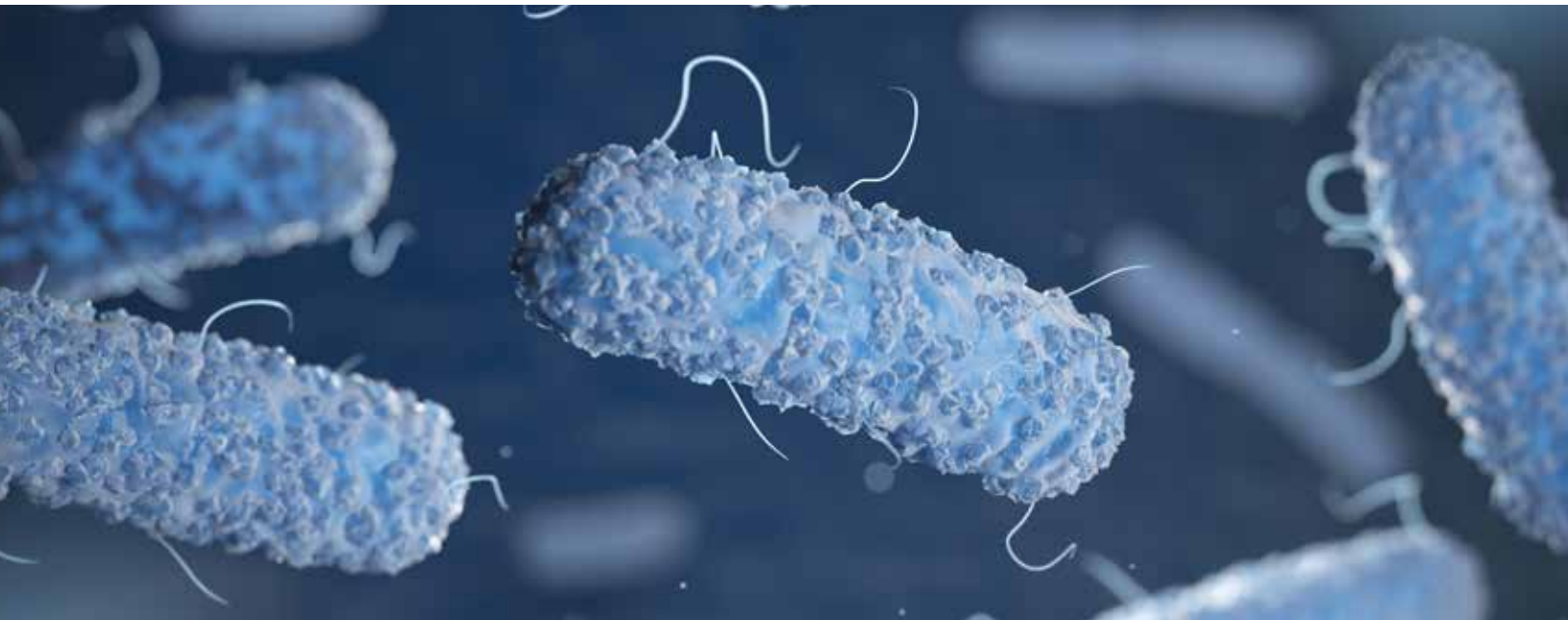
In a test conducted in September of 2020, by Microconsult Inc., a microbiological & analytical testing labratory, conducted a kill rate test. The following are the results of that test.

Bacteria	30 seconds	Log Reduction	60 seconds	Log Reduction
C. difficile	No Growth	5.38	No Growth	5.38
C. difficile (spore form)	No Growth	5.22	No Growth	5.22
MRSA	No Growth	5.88	No Growth	5.88
L. monocytogenes	No Growth	5.78	No Growth	5.78
E. Coli	No Growth	5.93	No Growth	5.93
P. aeruginosa	No Growth	5.75	No Growth	5.75
B. cepacia	3.10	3.30	No Growth	5.8
S. enterica	No Growth	5.77	No Growth	5.77
E. faecalis	No Growth	5.95	No Growth	5.95
K. pneumoniae	15	4.40	No Growth	5.58
S. pyogenes	No Growth	5.41	No Growth	5.41
C. jejuni	No Growth	5.38	No Growth	5.38

6.2 Explanation of Results

The 2 in 1 Invisible Glove was tested in a Kill Rate Study using eleven bacterial species by a leading microbiological testing facility. The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms from (> 105 cfu/mL) from nine of the species and greater that three log10 from the other two. The 60 second exposure killed all of the organisms from all eleven species.

A second Kill Rate Study was performed on the spore stage of C. difficile using the same exposure times.). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data show a very high degree of efficacy suggesting that this hand sanitizer could have a strong impact on bacterial transmission. The action against the spores of C. difficile is particularly remarkable.



07.01 - Manufacturing
Capabilities & Shipping

Packaging
A wide range of packaging are available. Typically, the 2 in 1 Invisible Glove is packaged in 1oz airless pumps, 4 oz squeeze bottles, and various automatic dispenser bladders. However, any size from 1/2 gallon to sachets can be accommodated.

Bulk Shipments
Bulk shipments of product can be be delivered in 5 gallon pales, 30 gallon drums, 55 gallon drums, or 250 gallon totes. These can be shipped domestically or internationally as needed.

Production Capacity
Our manufacturing partner has many production facilities in Tennessee, USA and abroad. As of this writing, the production capacity is over 20,000 gallons per day, per shift. New productions facilities have been purchased and are in the construction phase. Once complete, the production capacity will be doubled. Greater production capacity will be addressed as needed.

08.01 - Documentation

List of attached documents.

Product Sales Sheets
Product Descriptions

ASU BSL3 Lab Results
Dr. Jeff Langland

Kill Rate Results
Microconsult

Product Safety Report
Dr. John Harbell

Benzalkonium Chloride Study
Dr. John Harbell

Summary of Antimicrobial Effects
Dr. John Harbell

Platelet Technology White Paper
LifeHope Health LLC/Biocellerex

FDA NDC Listing
FDA Website Listing

Cost Comparison Analysis
LifeHope Health LLC

09.01 - Conclusions

White Paper Summary

Meditizer Hand Sanitizing Product Line Summary

Our revolutionary product line utilizes cutting-edge material science to achieve unparalleled killing power against a wide range of harmful microorganisms. The Patented and Patent **pending** microplatelet technology utilized, coupled with copper and benzalkonium chloride, provides for a highly effective product. In addition to being extremely efficacious, our water-based solution removes the harmful side effects of alcohol-based solutions and provides a much safer product.

Recent scientific studies conducted at highly reputable labs show that the products kill the novel SARS-CoV-2 (COVID-19) virus with a 99.99% continuous kill rate over more than 4 hours, while also killing c. difficile active cultures and spores as well as many other dangerous pathogens, all with a single application. These products are true game changers, adding an essential layer of protection in the fight against dangerous diseases.

We hope you now share our enthusiasm for these products and can help us set them to work in more common usage. Together, we can help make a positive change by helping to reduce the spread of dangerous pathogens and disease.

Hand Sanitizer Product Line

White Paper

2021

Meditize LLC

11 680 Great Oaks Way
Suite 160
Alpharetta, GA 30022

770-755-1733 corp office

Meditizer
Medical-Grade Hand Sanitizer

Re: A word on terminology used to describe the technology.

Monday, March 15, 2021

To Whom It May Concern,

The technologies referenced within this white paper are new and novel configurations in material science. They are measured on the nano scale. Therefore, early references to the technology highlighted the term “nano”. At the time, it wasn’t understood the cultural misrepresentations and science fiction connotations the use of that term carried.

Though some of the supporting material attached to this white paper reference “nano-platelets” and “nanotechnologies”, we wish to make it clear that these products have no relation to the science fiction representation of nanotechnology. There are not nano scale robots swimming within our solutions.

In fact, it is in hard, provable science that our products are firmly rooted. The proof of this science is contained within the attachments. Therefore, to reduce confusion, we have elected to alter the descriptions to the still accurate “micro” moniker.

You will notice reference of “microplatelets” in lieu of “nanoplatelets” used in this white paper and some attached documents. Please note that this is the same copper infused magnesium hydroxide platelet technology described and referenced throughout. We sincerely hope you understand the need for the change and can appreciate that we want this product to be judged on its quality and efficacy, not by poorly understood terminology.

08.02 - Product Sales Sheets

Hand Sanitizer
Medical-Grade Hand Sanitizer

Mask & Surface Spray
Medical-Grade Spray

Meditizer™
Medical-Grade Hand Sanitizer

The Problem

Bacteria and pathogens can survive on surfaces for extended periods of time and transfer through touch to others. The CDC recommends regular and thorough hand washing to help prevent the spread of disease -but hand washing only kills pathogens already on your hands. You can become infected immediately after washing.

- Kills COVID-19, C. Difficile Spores, MRSA, & E. Coli
- Kills 99.9% Germs & Bacteria
- 4+ Hours Protection
- Moisturises & prevents dryness Non-Flammable
- Non-Flammable
- Non-Toxic
- Alcohol Free
- MHRA Licensed & FDA Approved Facilities
- NHS Approved Supplier

The Solution

A revolutionary bacteria and pathogen defense developed by DGH Pharma, Incl exclusively for Meditizer sanitizes and provides anti-bacterial protection against pathogens in a one-step application that is alcohol-free.

Meditizer Hand Sanitizer kills COVID-19 on contact and uses a unique, proprietary and patented mode of action that penetrates the epidermis, to provide continuous protection. The copper and magnesium platelets utilized are safe for humans. These platelets disable the pathogens by destroying their capsid envelope and disrupting their genetic code.

U.S. Pat. No. 7,892,447 Other Patents Pending "Powered By Aqua"
NDC# 77238-231-24

www.meditize.us

Meditizer™
Medical-Grade Hand Sanitizer

Hand & Mask Spray

Meditizer™ Mask & Surface Spray kills bacteria and viruses. When applied to your mask and hands, it provides continuous viral and bacterial killing protection to the wearer for an extended time. While sanitizing and protecting, its clean, fresh fragrance refreshes and extends the life of your mask. It's safe for humans, but deadly for bacteria and viruses. The formula is natural, non-toxic, and doctor recommended.

- Kills Covid-19
- Kills C. Difficile Spores & Live Cultures
- Kills MRSA
- Kills E. Coli
- Kills 99.9% Germs & Bacteria
- 4+ Hours Protection
- Can be sprayed on Hands and Mask
- Moisturises & prevents dryness Non-Flammable
- Non-Flammable
- Non-Toxic
- Alcohol Free
- MHRA Licensed & FDA Approved Facilities
- NHS Approved Supplier

FDA APPROVED FDA CLEARED MHRA NHS Approved Supplier NT

U.S. Pat. No. 7,892,447 Other Patents Pending "Powered By Aqua"
NDC# 77238-231-24

www.meditize.us

08.03 - ASU BSL3 Lab Results

- Arizona State University Biodesign Institute
- Southwest College Of Naturopathic Medicine & Health Sciences
- DR. Jeffrey Langland Research Director
- February 23, 2021

Project Name	Anti-SARS-CoV-2 properties of novel hand sanitizer solutions
Project Description	Characterization of the long-term anti-SAR-CoV-2 properties of novel hand sanitizer solutions
Project Lead	Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research. Dr. Jeffrey Langland, Research Director
Start Date	February 2021
Summary Date	February 23, 2021

Purpose
Characterize the long-term antimicrobial properties of Meditizer^{tm} Hand Sanitizer & Mask Spray products against SARS-CoV-2. Herein referenced as 2 in 1 Hand & Mask Spray.

SARS-CoV2 Long-term killing assay:

Materials:

SARS-CoV-2 strain USA-WA1/2020 (BEI Resources)
Vero E6 cells (ATCC)
BSL3 facility (contract service with Arizona State University, Biodesign Institute)
D-MEM media with 10% fetal bovine serum (COMPLETE MEDIA)
PBS (phosphate buffered saline)

Stocks:

Vero cells were maintained in D-MEM media with 10% fetal bovine serum
All cells were used under limited passage conditions

SARS-CoV-2 virus stocks were grown in Vero cells under standard protocols. Viral titers were determined by plaque assay in Vero cells. Final stock titer was $3x10^7$ PFU/ml

Experimental procedure 1:

1. In the BSL2 tissue culture room, treat four 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution

c. 2 in 1 Invisible Glove
d. Mask Spray

2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 24 samples total (6 in duplicate at 1 hr, and 6 in duplicate at 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

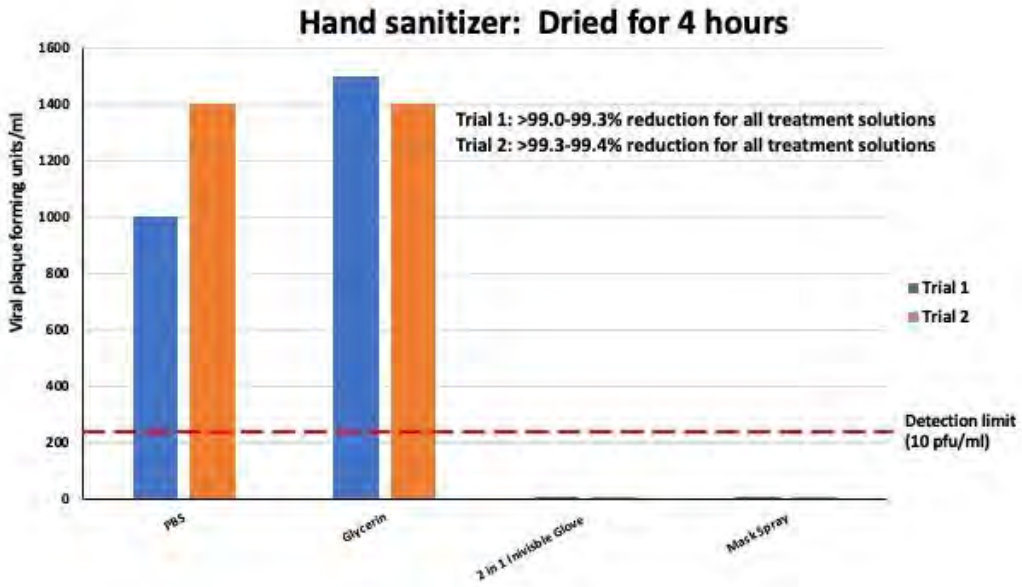
Experimental procedure 2:

1. In the BSL2 tissue culture room, treat two sets of 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution
    c. 2 in 1 Invisible Glove
    d. Mask spray
    e. 2 in 1 Invisible Glove (diluted 1:5 in PBS)
    f. Mask Spray (diluted 1:5 in PBS)

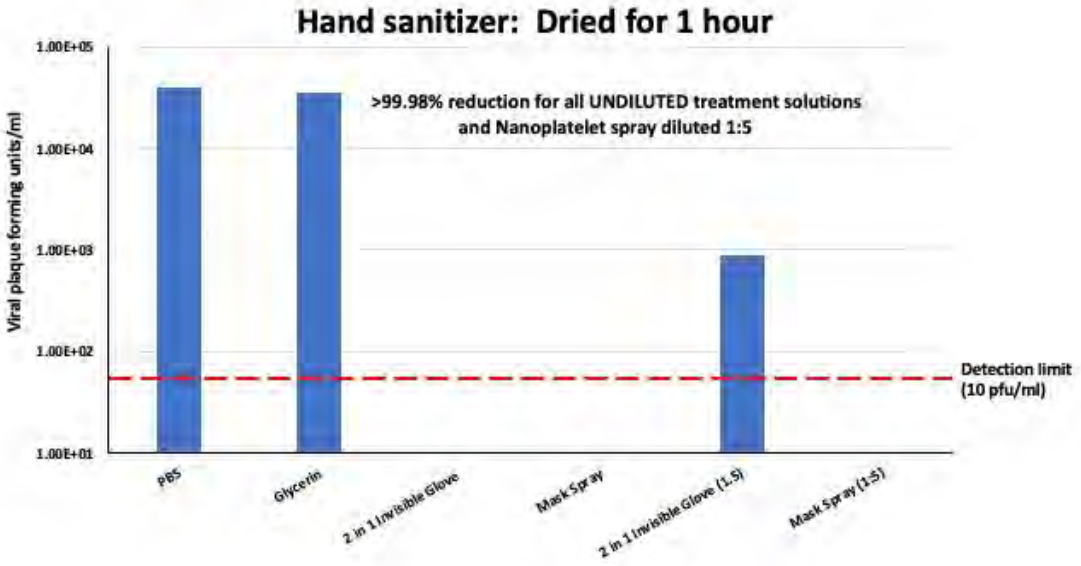
2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 20 samples total (10 for the 1 hr, and 10 for the 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

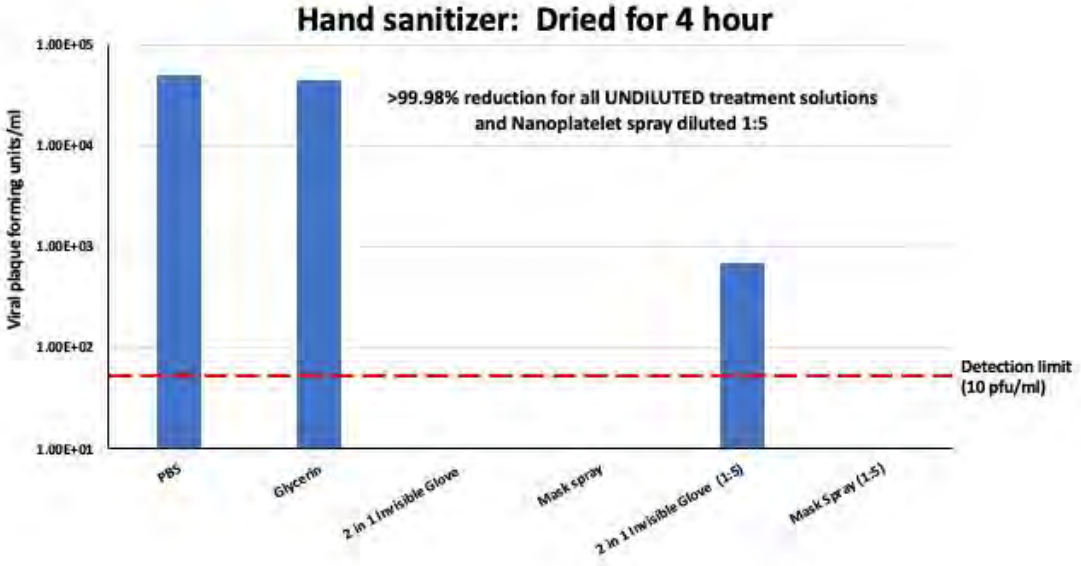
Project results:

Experimental study 1:



Experimental study 2:


Hand sanitizer: Dried for 1 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask Spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)


Hand sanitizer: Dried for 4 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)

Results summary:

The results of this study support that the hand sanitizer solutions containing 2 in 1 Hand and Mask Spray are all able to kill the SARS-CoV-2 virus even after drying on a surface for 1 hour or 4 hours.

In the first study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 1000-2500 virus/ml. When this amount of virus was applied to surfaces treated with any of the hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.0% virus killing response.

In the second study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 30,000 virus/ml. When this amount of virus was applied to surfaces treated with any of the undiluted hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.98% virus killing response.

In the second study, when the hand sanitizer solutions were diluted 1:5 in PBS and then applied to the surface, virus killing was approximately 90-95% for the hand sanitizer solution containing 2 in 1 Invisible Glove. The Mask Spray diluted 1:5 in PBS was still able to kill with over a 99.98% virus killing response.

Certification:

Experimental design and analysis were conducted at the Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research under the guidance and supervision of Dr. Jeffrey Langland, Research Director. Experimental procedures were performed at the Arizona State University Biodesign Institute, Biosafety Level 3 facility.

Results are certified as valid based on experimental procedures performed

Jeffrey Langland
Research Director
Southwest College of Naturopathic Medicine
Ric Scalzo Institute for Botanical Research

SCNM
SOUTHWEST COLLEGE OF NATUROPATHIC MEDICINE & HEALTH SCIENCES

08.04 - Microconsult Testing

- Microbiological & Analytical Testing Laboratory
- 2 in 1 Hand & Mask Spray Kill Rate Test for 11 Microorganisms
- Microconsult, Inc. Carrollton, Texas
- September 15, 2020

Kill Rate Test – Hand Sanitizer Lot # 1152

Summary:

Meditizer Hand sanitizer was tested in a Kill Rate Study using eleven bacterial species (Microconsult Report 1 September 2020). The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms (> 10^5 cfu/mL) from nine of the species and greater that three $\log_{10}$ from the other two. The 60 second exposure killed all of the organisms from all eleven species. A second Kill Rate Study was performed on the spore stage of C. difficile using the same exposure times (Microconsult Report 15 September 2020). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data show a very high degree of efficacy suggesting that this hand sanitizer could have a strong impact on bacterial transmission. The action against the spores of C. difficile is particularly remarkable.

This summary report was compiled from two Kill Rate Study reports issued by Microconsult, Inc. 1 September 2020 (11 organisms) and 15 September 2020 (C. difficile in the spore stage).

Objective:
To demonstrate the antibacterial properties of the test product against a selection of gram positive and gran negative bacteria.

References:

A. 21 CFR 333. Topical antibacterial products for over-the-counter human use.
B. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics: Topical antimicrobial drug products for over-the-counter human use; proposed amendment of the tentative final monograph. Section V. Comments on the Proposed Rule and FDA Response Subsection C. Comments on Effectiveness and FDA Response [list of test organisms for *in vitro* efficacy testing]
C. Microconsult, Inc. Test Method 011_00 Kill Rate Testing

Test Article: Labeled as Hand Sanitizer, lot # 1152 (Meditizer)

Test Organisms
The list of test organisms, their American Type Culture Collection (ATCC) numbers, source and short names (see Table 2) are provided in Table 1. Active cultures of the test organisms were maintained by the laboratory and renewed from the reference stock after five passages. *Campylobacter jejuni* and *Clostridium difficile* were maintained under anaerobic culture conditions. To kill the C. difficile vegetative organisms, the 24-hour growth plate was treated quickly with 70% isopropyl alcohol to yield the spore form cells for the second study.

Table 1 List of organisms tested

Organism	ATCC Number	Source	Short Name
Escherichia coli	8739	Microbiologics	*E. coli*
Methicillin-resistant (MRSA) Staphylococcus aureus	33591	Microbiologics	*MRSA*
Pseudomonas aeruginosa	24853	Microbiologics	*P. aeruginosa*
Burkholderia cepacia	25416	Microbiologics	*B. cepacia*
Salmonella enterica	14028	Microbiologics	*S. enterica*
Enterococcus faecalis	51575	Microbiologics	*E. faecalis*
Klebsiella pneumoniae	700603	Microbiologics	*K. pneumoniae*
Streptococcus pyogenes	19615	Microbiologics	*S. pyogenes*
Listeria monocytogenes	SLR2249	Microbiologics	*L. monocytogenes*
*Campylobacter jejuni**	49943	Microbiologics	*C. jejuni*
*Clostridium difficile**	9689	Microbiologics	*C. difficile*

*Anaerobes

Reagents:
Tryptic Soy Agar with Lecithin and Tween 80
Sterile Phosphate Buffered Saline (for diluting)
DE Neutralizing Broth: Dey-Engley Neutralizing Broth is intended to stop the action of the antimicrobial preparation at the end of the exposure period. It is formulated to neutralize several types of antibacterial active ingredients including benzalkonium chloride.

Procedure:

1. Prepare each bacterial culture, inoculate the growth medium (broth) with the actively growing bacteria and allow to grow at 30-35ºC for 24-48 hours. These suspension cultures will be used to determine the antibacterial activity of the test article against the specific bacterium. Eleven such suspension cultures were prepared, one for each organism. These cultures were incubated for 24 to 48 hours to obtain the desired bacterial titers. At this point the number of organisms per mL (cfu/mL) was determined and the same cultures were used to challenge the test article. It should be understood that the exact number of organisms in the inoculum will not be known until step 2 is completed.

2. To obtain the number of viable microorganisms (colony forming units per mL [cfu/mL]), a sample was removed and diluted in sterile phosphate buffered saline. Subsequent serial

dilutions were prepared from this sample to seed test plates with dilutions of 10^{-6} and 10^{-7} of the original suspension. Each test plate was then filled with 20 mL of 45°C Tryptic Soy Agar, swirled to mix and then allowed to harden. The plates were incubated for 24 – 48 hours to allow the viable bacteria to form colonies in the agar. The bacterial colonies were counted and the number of colony forming units per mL in the original inoculum determined. The number of cfu/mL in the inoculum was then calculated to determine the number in the test samples using the formula below:

$$\frac{\text{(cfu/mL inoculum)x(volume added to the test article sample)}}{\text{Weight of test article (g)}} = \text{cfu/g product}$$

$$\frac{\text{(cfu/mL inoculum)x(0.1 mL)}}{\text{9.9 (g)}} = \text{cfu/g of test article}$$

3. Samples of test article were prepared for inoculation with each bacterium. A volume of 9.9 mL was measured out into properly labeled test tube. These tubes were held at room temperature until the bacteria were added.
4. Inoculation of the test article with the bacterial inoculum was performed by adding 0.1 mL of the bacterial inoculum to the tube holding 9.9 mL of the test article. The tube was mixed and then allowed to stand for the time of the first incubation period (30 seconds). At that point one mL of the test article-inoculum mixture was removed a placed immediately into 9.0 mL of DE Neutralizing solution to stop the action of the test article. After the second incubation period (60 seconds) a second sample of one mL was taken and added to a second tube containing 9.0 mL of DE Neutralizing solution. This process was repeated for each of the bacteria tested.
5. Each suspension of bacteria in the DE Neutralizing solution was serially diluted (1:10) in duplicate in phosphate buffer to prepare dilutions of 10^{-1} to 10^{-5}.
6. One mL of each dilution was transferred to a prelabeled 100 x 15 mm petri plate.
7. Each plate was overlaid with 20 mL of melted (45°C) Tryptic Soy Agar and the plate gently swirled to mix the bacteria with the agar. The plates were then allowed to harden,
8. The inoculated plates were placed into an incubator at 30-35°C for 48 to 72 hours. Again, the *C. jejuni* and *C. difficile* plates were incubated under anaerobic conditions.
9. At the end of the incubation period, the number of colonies in each plate was counted. From the count value and the dilution of the original sample, the number of colony forming units remaining in the treated samples was calculated
10. The $\log_{10}$ reduction was calculated from ratio $\log_{10}$ of the inoculum to the log10 of the remaining colony forming units after treatment. For example:

For the E. coli sample treated for 30 seconds, the $\log_{10}$ inoculum of bacteria was 5.93/mL and the number of colony forming units after treatment was zero. The zero value is converted to one

which has a $\log_{10}$ of zero. The $\log_{10}$ reduction is 5.93-0 = 5.93. A second example shows the case where there was some survival at 30 seconds of exposure. *B. cepacia* had an initial inoculum of $6.24x10^5$ cfu/ml ($\log_{10}$ is 5.80). At 30 seconds of exposure, 310 cfu/mL ($\log_{10}$ 310 is 2.49) remained viable. The $\log_{10}$ reduction was 5.80-2.49 = 3.30.

The $\log_{10}$ reduction for each bacterium at each of the two exposure times is shown in Table 2.

Table 2 Log reduction of viable cfu/mL

Organism (Exposure Time)	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log_{10} Reduction
E. coli (30 seconds)	8.59×10^5	No Growth	5.93
E. coli (60 seconds)	8.59×10^5	No Growth	5.93
MRSA (30 seconds)	7.55×10^5	No Growth	5.88
MRSA (60 seconds)	7.55×10^5	No Growth	5.88
P. aeruginosa (30 seconds)	5.56×10^5	No Growth	5.75
P. aeruginosa (60 seconds)	5.56×10^5	No Growth	5.75
B. cepacia (30 seconds)	6.24×10^5	310	3.30
B. cepacia (60 seconds)	6.24×10^5	No Growth	5.8
S. enterica (30 seconds)	5.91×10^5	No Growth	5.77
S. enterica (60 seconds)	5.91×10^5	No Growth	5.77
E. faecalis (30 seconds)	8.84×10^5	No Growth	5.95
E. faecalis (60 seconds)	8.84×10^5	No Growth	5.95
K. pneumoniae (30 seconds)	3.81×10^5	15	4.40
K. pneumoniae (60 seconds)	3.81×10^5	No Growth	5.58
S. pyogenes (30 seconds)	2.25×10^5	No Growth	5.41
S. pyogenes (60 seconds)	2.25×10^5	No Growth	5.41
L. monocytogenes (30 seconds)	5.98×10^5	No Growth	5.78
L. monocytogenes (60 seconds)	5.98×10^5	No Growth	5.78
C. jejuni (30 seconds)	2.42×10^5	No Growth	5.38
C. jejuni (60 seconds)	2.42×10^5	No Growth	5.38
C. difficile (30 seconds)	2.40×10^5	No Growth	5.38
C. difficile (60 seconds)	2.40×10^5	No Growth	5.38
C. difficile (Spore form) (30 seconds)	1.67×10^5	No Growth	5.22
C. difficile (Spore form) (60 seconds)	1.67×10^5	No Growth	5.22

Discussion:

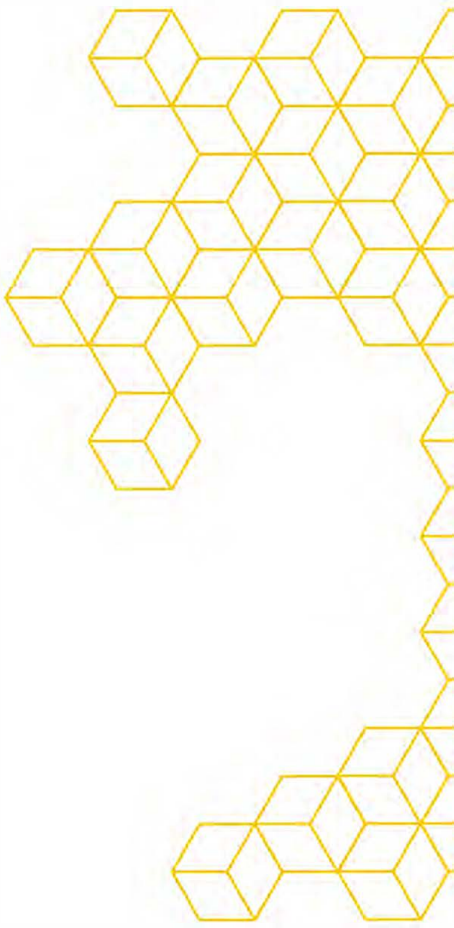
As shown in Table 2, most of the bacterial species tested were completely killed with the 30 second exposure and all were completely killed with a 60 second exposure. 21 CFR 333 Topical antibacterial products for over-the-counter human use calls for a two $\log_{10}$ reduction in viability for a product to be considered antibacterial. This regulation applied to topical antiseptics. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics calls for a three $\log_{10}$ reduction in viability for a hand rub (hand sanitizer) to be considered to have antibacterial efficacy. This hand sanitizer achieved a three $\log_{10}$ kill with a 30 second exposure and complete kill with a sixty second exposure for all eleven species tested. Of particular interest was the activity against C. difficile spores. Complete kill of the 1.67×10^5 cfu/mL inoculum was achieved with a 30 second exposure.

This study was performed at Microconsult, Inc. Carrollton, TX under the direction of Alix Paulson, Microbiology Technician II September 2020.

Summary prepared by John W. Harbell, Ph.D.

08.05 - Product Safety Report

- Hand Sanitizer
- Mask & Surface Spray
- March 29, 2020



Safety Data Sheet

1. Identification

Product Name: Meditizer Hand Sanitizer

Intended uses: Consumer product – use as labeled.

Company Identification

[redacted]

Customer Information Number: TBD

Emergency Telephone Number: TBD

For information regarding the use of this product by a consumer, please refer directly to the product label. This industrial SDS is provided for workplace employees, per US OSHA regulations. It contains recommendations for handling of this product in an occupational, or workplace, setting.

Any first aid or warnings that are applicable to consumer use are stated directly on the product label, in accordance with all applicable government regulations.

2. Hazards Identification

Physical Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Health Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Environmental Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
OSHA defined Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Label elements	
Hazard Symbol	None
Signal Word	None
Hazard statement	This mixture does not meet the criteria for classification – Not a dangerous substance or mixture.
Precautionary Statement	
Prevention	None required according to OSHA HazCom 2012.
Response	None required according to OSHA HazCom 2012.
Storage	None required according to OSHA HazCom 2012.
Disposal	None required according to OSHA HazCom 2012.
Hazard(s) not otherwise Classified (HNOC)	None Known
Supplemental Information	None

3. Composition/information on ingredients

Mixture

Chemical name	Common name and synonyms	CAS Number	%
Benzalkonium Chloride	None	63449-41-2	0.10
Water	None	7732-18-5	50 – 100
Magnesium Hydroxide	Milk of Magnesia	1309-42-8	0 - 10
Polyethylene Glycol	PEG-8, PEG-90	25322-68-3	0 - 10
Glycerin	None	56-81-5	0 - 10
Hydroxyethylcellulose	None	9004-62-0	0 - 10
Polysorbate 20	None	9005-64-5	0 - 10
Fragrance	None	Mixture	0 - 10

4. First Aid Measures

General Advice No hazards which require special first aid measures

Inhalation If symptoms develop move victim to fresh air. Get medical attention if symptoms persist.

Skin contact Harmful effects are not expected under normal usage. Wash off with water, if skin irritation or rash develop get medical attention/advice if persistent.

Eye contact Any material that contacts the eye should be washed out immediately with water. Remove contact lenses. Get medical attention if symptoms persist.

Ingestion Clean mouth with water and drink plenty of water. Never give anything by mouth to an unconscious person

Most important symptoms/effects, Acute and delayed No Data Available

Indication of immediate medical Attention and special treatment Needed Treat symptomatically

SECTION 5: Firefighting measures

Suitable extinguishing media: Non-flammable. Use an extinguishing agent suitable for the surrounding fire.

Unsuitable extinguishing media: None Known

Hazardous combustion products: Combustion products may include and are not limited to: Carbon Monoxide. Carbon Dioxide.

Special Protective equipment and precautions for firefighters

In the event of fire, wear self-contained breathing apparatus.

6: Accidental release measures

Personal precautions, protective equipment and emergency procedures: Keep unnecessary personnel away. Wear appropriate personal protective equipment. Do not touch damaged containers or spilled materials unless wearing appropriate protective equipment. For personal protection, see Section 8 of the SDS.

Environmental precautions: Avoid discharge into drains, water courses or onto the ground.

Methods and material for containment and cleaning up: Absorb spill with vermiculite or other inert material, then place in a sealed container for chemical waste.

Large Spills: Dike area to prevent spread and absorb spill with vermiculite or other inert material, then place in a sealed container for chemical waste.

Small Spills: Wipe up with absorbent material (e.g. Cloth, paper). Clean surface thoroughly with water and detergent to remove residual contamination.

SECTION 7: Handling and storage

Precautions for safe handling No special handling advice required. Follow label directions.

Conditions for safe storage, including any incompatibilities Keep containers closed when not in use. Follow label directions.

SECTION 8: Exposure controls/personal protection

Occupational exposure limits None

Exposure limits at intended use Follow labeled directions. Product is non-hazardous.

Appropriate engineering controls None normally required, Ensure adequate ventilation in confined spaces.

Personal protective equipment

Eye / Face protection	Not required for normal use. Wear safety glasses with side shields when exposure possible
Skin protection	Not required for normal use. Wear chemical-resistant gloves for prolonged or occupational exposures
Respiratory protection	Not required under normal conditions.
Thermal hazards	Not applicable when product used as intended

SECTION 9. Physical and chemical properties

Appearance

Physical State:	Suspension in thickened liquid
Color:	Not Available
Odor	Fragrant Orange Scent
Odor Threshold	Not Available
pH	9 – 11
Melting point/freezing point	Not Available
Initial boiling point/range	Not Available
Flash point (TCC)	>200F

Evaporation Rate	<1 (butyl acetate=1.0)
Flammability (solid,gas)	Not applicable
Flammable Limit – LEL	Not Available
Flammable Limit – UEL	Not Available
Vapor Pressure	<0.1 mmHg at 300C
Vapor Density	Not Available
Relative Density	1.0 - 1.1 g/cm3 at 20 C
Solubility in Water	Dispersible
Partition Coefficient (n-octanol/water)	Not Available
Autoignition Temperature	Not Available
Decomposition Temperature	Not Available
Viscosity	Not Available

SECTION 10: Stability and reactivity

Reactivity:	This product is stable and non-reactive under normal conditions of use, storage and transport.
Chemical stability:	Stable at normal conditions.
Possibility of hazardous reactions	Hazardous polymerization does not occur.
Conditions to avoid:	No Data Available
Incompatible materials:	Strong oxidizing agents. Acids. See also Section 7 (Handling and Storage)
Hazardous decomposition products:	Formed under fire conditions - Carbon monoxide and dioxide. See also Section 5 (Fire Fighting Measures).

SECTION 11: Toxicological information

Information on likely routes of exposure

Inhalation	Under normal conditions of intended use, this material is not expected to be an inhalation hazard.
Skin Contact	No adverse effects due to skin contact are expected.
Eye Contact	Direct contact with eyes may cause serious but temporary irritation.
Ingestion	No harmful effects expected in amounts likely to be ingested by accident.
Most important symptoms/ Effects, acute and delayed	Direct contact with eyes may cause temporary irritation.
Acute toxicity	No adverse effects are expected.
Acute oral toxicity	Very low toxicity if swallowed.
Acute dermal toxicity	Prolonged skin contact is unlikely to result in absorption of harmful amounts.
Acute inhalation toxicity	At room temperature, exposure to vapor is minimal due to low volatility; single exposure is not likely to be hazardous.

Skin corrosion/irritation	Contact is essentially non-irritating to skin.
Eye damage/irritation	May cause serious eye irritation
Sensitization	Based on available data, not a skin or respiratory sensitizer.
For respiratory sensitization	No relevant data found.

Specific Target Organ Systemic Toxicity (Single Exposure)
Evaluation of available data suggests that this material is not an STOT-SE toxicant.

Specific Target Organ Systemic Toxicity (Repeated Exposure)
Based on available data, repeated exposures are not anticipated to cause significant adverse effects.

Carcinogenicity
No known significant effects or critical hazards

Reproductive Toxicity
No relevant data found

Mutagenicity
In vitro genic toxicity studies were negative.

Aspiration Hazard
No information available

SECTION 12: Ecological information

Ecotoxicity	No known significant effects or critical hazards
Persistence and degradability	No known significant effects or critical hazards
Bio-accumulative potential	Not available
Mobility in soil / water	No known significant effects or critical hazards
Other adverse effects	No other adverse environmental effects (e.g. ozone depletion, photochemical ozone creation potential, endocrine disruption, global warming potential) are expected from this mixture.

SECTION 13: Disposal considerations

Do not dump into any sewers, on the ground, or into any body of water. All disposal practices must be in compliance with all Federal, State/Provincial and local laws and regulations. Regulations vary in different locations.

Disposal instructions	Material is non-hazardous. Absorb onto an inert substrate and discard as solid waste
Local disposal regulations	Dispose in accordance with all applicable regulations

SECTION 14: Transport information

DOT	Not regulated as dangerous goods
IATA	Not regulated as dangerous goods
IMDG	Not regulated as dangerous goods

This information is not intended to convey all specific regulatory or operational requirements/information relating to this product. Transportation classifications may vary by container volume and total amount being shipped.

SECTION 15: Regulatory information

OSHA Hazard Communication Standard
This product is not a “Hazardous Chemical” as defined by the OSHA Hazard Communication Standard, 29 CFR 1910.1200.

Superfund Amendments and Reauthorization Act of 1986 Title III (Emergency Planning and Community Right-to-Know Act of 1986) Sections 311 AND 312
This product is not a hazardous chemical under 29CFR 1910.1200, and therefore is not covered by Title III of SARA.

Superfund Amendments and Reauthorization Act of 1986 Title III (Emergency Planning and Community Right-to-Know Act of 1986) Section 313
This material does not contain any chemical components with known CAS numbers that exceed the threshold (de minimus) reporting levels established by SARA Title III, Section 313.

United States TSCA Inventory (TSCA)
All components of this product are in compliance with the inventory listing requirements of the U.S. Toxic Substances Control Act (TSCA) Chemical Substance Inventory.

SECTION 16: Other information

Legend:

ACGIH	American Conference of Governmental Industrial Hygienists
CA	California
CAS	Chemical Abstract Service
CEPA	Canadian Environmental Protection Act
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
CFR	Code of Federal Regulations
CPR	Controlled Products Regulation
CSA	Canadian Standards Association
DOT	Department of Transportation
DSL	Domestic Substances List
EPA	Environmental Protection Agency
HMIS	Hazardous Materials Identification System
HPA	Hazardous Products Act
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IATA	International Air Transport Association
IMDG	International Maritime Dangerous Goods
INH	Inhalation
LC / LD	Lethal Concentration / Lethal Dose
MA	Massachusetts
MN	Minnesota
NFPA	National Fire Protection Association
NIOSH	National Institute of Occupational Safety and Health
NJ	New Jersey
NOEC / NOEL	No observable effect concentration / no observable effect level
NTP	National Toxicology Program
OSHA:	Occupational Safety and Health Administration
PA	Pennsylvania
PEL	Permissible Exposure Limit
PPE	Personal Protective Equipment
RCRA	Resource Conservation and Recovery Act
RI	Rhode Island
RQ	Reportable Quantity
RTECS	Registry of Toxic Effects of Chemical Substances

SARA	Superfund Amendments and Reauthorization Act
SDA	Safety Data Sheet
STEL	Short Term Exposure Limit
TLV	Threshold Limit Values
TWA	Time Weighted Average
WEL	Workplace Exposure Limit
WHMIS	Workplace Hazardous Materials Identification System

References:

1. ACGIH, Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices for 2016
2. International Agency for Research on Cancer Monographs, Searched 2019
3. Material Safety Data Sheets from ingredient manufacturers
4. USEPA Title III List of Lists – 2018 version
5. California Proposition 65 List – 2018 version

Notice to reader:

To the best of our knowledge, the information contained herein is accurate. However, the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Safety Data Sheet

1. Identification

Product Name: Mask Shield Complete Mask Sanitizer

Intended uses: Consumer product – use as labelled.

Company Identification
Meditizer
1749 Florida Street
Memphis, TN 38109

Customer Information Number: TBD

Emergency Telephone Number: TBD

For information regarding the use of this product by a consumer, please refer directly to the product label. This industrial SDS is provided for workplace employees, per US OSHA regulations. It contains recommendations for handling of this product in an occupational, or workplace, setting.

Any first aid or warnings that are applicable to consumer use are stated directly on the product label, in accordance with all applicable government regulations.

2. Hazards Identification

Physical Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Health Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Environmental Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
OSHA defined Hazards	This material does not meet the classification criteria according to OSHA HazCom 2012
Label elements	
Hazard Symbol	None
Signal Word	None
Hazard statement	This mixture does not meet the criteria for classification – Not a dangerous substance or mixture.
Precautionary Statement	
Prevention	None required according to OSHA HazCom 2012.
Response	None required according to OSHA HazCom 2012.
Storage	None required according to OSHA HazCom 2012.
Disposal	None required according to OSHA HazCom 2012.
Hazard(s) not otherwise Classified (HNOC)	None Known
Supplemental Information	None

3. Composition/information on ingredients

Mixture

	Common name and synonyms	CAS Number	%
Benzalkonium Chloride	None	63449-41-2	0.10
Water	None	7732-18-5	50 – 100
Trisodium Citrate	Sodium Citrate	68-04-2	0 - 5
Magnesium Hydroxide	Milk of Magnesia	1309-42-8	0 - 5
Polyethylene Glycol	PEG-8, PEG-90	25322-68-3	0 - 5
Glycerin	None	56-81-5	0 - 5
Copper Chloride	Cupric Chloride	7447-39-4	0 - 5
Phenoxyethanol	None	122-99-6	0 - 5
Potassium Sorbate	None	24634-61-5	0 - 5
Polysorbate 20	None	9005-64-5	0 - 5
Limonene	Orange Oil	5989-27-5	0 – 0.5

4. First Aid Measures

General Advice No hazards which require special first aid measures

Inhalation If symptoms develop move victim to fresh air. Get medical attention if symptoms persist.

Skin contact Harmful effects are not expected under normal usage. Wash off with water, if skin irritation or rash develop get medical attention/advice if persistent.

Eye contact Any material that contacts the eye should be washed out immediately with water. Remove contact lenses. Get medical attention if symptoms persist.

Ingestion Clean mouth with water and drink plenty of water. Never give anything by mouth to an unconscious person

Most important symptoms/effects, Acute and delayed No Data Available

Indication of immediate medical Attention and special treatment Needed Treat symptomatically

SECTION 5: Firefighting measures

Suitable extinguishing media: Non-flammable. Use an extinguishing agent suitable for the surrounding fire.

Unsuitable extinguishing media: None Known

Hazardous combustion products: Combustion products may include and are not limited to: Carbon Monoxide. Carbon Dioxide.

Special Protective equipment and precautions for firefighters

In the event of fire, wear self-contained breathing apparatus.

6: Accidental release measures

Personal precautions, protective equipment and emergency procedures: Keep unnecessary personnel away. Wear appropriate personal protective equipment. Do not touch damaged containers or spilled materials unless wearing appropriate protective equipment. For personal protection, see Section 8 of the SDS.

Environmental precautions: Avoid discharge into drains, water courses or onto the ground.

Methods and material for containment and cleaning up: Absorb spill with vermiculite or other inert material, then place in a sealed container for chemical waste.

Large Spills: Dike area to prevent spread and absorb spill with vermiculite or other inert material, then place in a sealed container for chemical waste.

Small Spills: Wipe up with absorbent material (e.g. Cloth, paper). Clean surface thoroughly with water and detergent to remove residual contamination.

SECTION 7: Handling and storage

Precautions for safe handling No special handling advice required. Follow label directions.

Conditions for safe storage, including any incompatibilities Keep containers closed when not in use. Follow label directions.

SECTION 8: Exposure controls/personal protection

Occupational exposure limits None

Exposure limits at intended use Follow labeled directions. Product is non-hazardous.

Appropriate engineering controls None normally required, Ensure adequate ventilation in confined spaces.

Personal protective equipment

Eye / Face protection	Not required for normal use. Wear safety glasses with side shields when exposure possible
Skin protection	Not required for normal use. Wear chemical-resistant gloves for prolonged or occupational exposures
Respiratory protection	Not required under normal conditions.
Thermal hazards	Not applicable when product used as intended

SECTION 9. Physical and chemical properties

Appearance

Physical State:	Water-thin liquid
Color:	Blue
Odor	Fragrant Orange Scent
Odor Threshold	Not Available
pH	4 - 6
Melting point/freezing point	Not Available
Initial boiling point/range	Not Available
Flash point (TCC)	>200F – not flammable

Evaporation Rate	<1 (butyl acetate=1.0)
Flammability (solid,gas)	Not applicable
Flammable Limit – LEL	Not Available
Flammable Limit – UEL	Not Available
Vapor Pressure	<0.1 mmHg at 300C
Vapor Density	Not Available
Relative Density	1.0 - 1.1 g/cm3 at 20 C
Solubility in Water	Dispersible
Partition Coefficient (n-octanol/water)	Not Available
Autoignition Temperature	Not Available
Decomposition Temperature	Not Available
Viscosity	Not Available

SECTION 10: Stability and reactivity

Reactivity:	This product is stable and non-reactive under normal conditions of use, storage and transport.
Chemical stability:	Stable at normal conditions.
Possibility of hazardous reactions	Hazardous polymerization does not occur.
Conditions to avoid:	No Data Available
Incompatible materials:	Strong oxidizing agents. Acids. See also Section 7 (Handling and Storage)
Hazardous decomposition products:	Formed under fire conditions - Carbon monoxide and dioxide. See also Section 5 (Fire Fighting Measures).

SECTION 11: Toxicological information

Information on likely routes of exposure

Inhalation	Under normal conditions of intended use, this material is not expected to be an inhalation hazard.
Skin Contact	No adverse effects due to skin contact are expected.
Eye Contact	Direct contact with eyes may cause serious but temporary irritation.
Ingestion	No harmful effects expected in amounts likely to be ingested by accident.
Most important symptoms/ Effects, acute and delayed	Direct contact with eyes may cause temporary irritation.
Acute toxicity	No adverse effects are expected.
Acute oral toxicity	Very low toxicity if swallowed.
Acute dermal toxicity	Prolonged skin contact is unlikely to result in absorption of harmful amounts.
Acute inhalation toxicity	At room temperature, exposure to vapor is minimal due to low volatility; single exposure is not likely to be hazardous.

Skin corrosion/irritation	Contact is essentially non-irritating to skin.
Eye damage/irritation	May cause serious eye irritation
Sensitization	Based on available data, not a skin or respiratory sensitizer.
For respiratory sensitization	No relevant data found.

Specific Target Organ Systemic Toxicity (Single Exposure)
Evaluation of available data suggests that this material is not an STOT-SE toxicant.

Specific Target Organ Systemic Toxicity (Repeated Exposure)
Based on available data, repeated exposures are not anticipated to cause significant adverse effects.

Carcinogenicity
No known significant effects or critical hazards

Reproductive Toxicity
No relevant data found

Mutagenicity
In vitro genic toxicity studies were negative.

Aspiration Hazard
No information available

SECTION 12: Ecological information

Ecotoxicity	No known significant effects or critical hazards
Persistence and degradability	No known significant effects or critical hazards
Bio-accumulative potential	Not available
Mobility in soil / water	No known significant effects or critical hazards
Other adverse effects	No other adverse environmental effects (e.g. ozone depletion, photochemical ozone creation potential, endocrine disruption, global warming potential) are expected from this mixture.

SECTION 13: Disposal considerations

Do not dump into any sewers, on the ground, or into any body of water. All disposal practices must be in compliance with all Federal, State/Provincial and local laws and regulations. Regulations vary in different locations.

Disposal instructions	Material is non-hazardous. Absorb onto an inert substrate and discard as solid waste
Local disposal regulations	Dispose in accordance with all applicable regulations

SECTION 14: Transport information

DOT	Not regulated as dangerous goods
IATA	Not regulated as dangerous goods
IMDG	Not regulated as dangerous goods

This information is not intended to convey all specific regulatory or operational requirements/information relating to this product. Transportation classifications may vary by container volume and total amount being shipped.

SECTION 15: Regulatory information

OSHA Hazard Communication Standard
This product is not a “Hazardous Chemical” as defined by the OSHA Hazard Communication Standard, 29 CFR 1910.1200.

Superfund Amendments and Reauthorization Act of 1986 Title III (Emergency Planning and Community Right-to-Know Act of 1986) Sections 311 AND 312
This product is not a hazardous chemical under 29CFR 1910.1200, and therefore is not covered by Title III of SARA.

Superfund Amendments and Reauthorization Act of 1986 Title III (Emergency Planning and Community Right-to-Know Act of 1986) Section 313
This material does not contain any chemical components with known CAS numbers that exceed the threshold (de minimus) reporting levels established by SARA Title III, Section 313.

United States TSCA Inventory (TSCA)
All components of this product are in compliance with the inventory listing requirements of the U.S. Toxic Substances Control Act (TSCA) Chemical Substance Inventory.

SECTION 16: Other information

Legend:

ACGIH	American Conference of Governmental Industrial Hygienists
CA	California
CAS	Chemical Abstract Service
CEPA	Canadian Environmental Protection Act
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
CFR	Code of Federal Regulations
CPR	Controlled Products Regulation
CSA	Canadian Standards Association
DOT	Department of Transportation
DSL	Domestic Substances List
EPA	Environmental Protection Agency
HMIS	Hazardous Materials Identification System
HPA	Hazardous Products Act
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IATA	International Air Transport Association
IMDG	International Maritime Dangerous Goods
INH	Inhalation
LC / LD	Lethal Concentration / Lethal Dose
MA	Massachusetts
MN	Minnesota
NFPA	National Fire Protection Association
NIOSH	National Institute of Occupational Safety and Health
NJ	New Jersey
NOEC / NOEL	No observable effect concentration / no observable effect level
NTP	National Toxicology Program
OSHA:	Occupational Safety and Health Administration
PA	Pennsylvania
PEL	Permissible Exposure Limit
PPE	Personal Protective Equipment
RCRA	Resource Conservation and Recovery Act
RI	Rhode Island
RQ	Reportable Quantity
RTECS	Registry of Toxic Effects of Chemical Substances

SARA	Superfund Amendments and Reauthorization Act
SDA	Safety Data Sheet
STEL	Short Term Exposure Limit
TLV	Threshold Limit Values
TWA	Time Weighted Average
WEL	Workplace Exposure Limit
WHMIS	Workplace Hazardous Materials Identification System

References:

1. ACGIH, Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices for 2016
2. International Agency for Research on Cancer Monographs, Searched 2019
3. Material Safety Data Sheets from ingredient manufacturers
4. USEPA Title III List of Lists – 2018 version
5. California Proposition 65 List – 2018 version

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08.06 - Benzalkonium Chloride

- American Journal of Infection Control
- Benzalkonium Chloride Persistent Efficacy after 1, 2, & 4 hours.
- DR. John W. Harbell

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Major Article

Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application

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Key Words:
Antibacterial
Persistence
Ethanol
Staphylococcus aureus
ASTM E2752-10
Nosocomial infection

Background: Use of hand sanitizers has become a cornerstone in clinical practice for the prevention of disease transmission between practitioners and patients. Traditionally, these preparations have relied on ethanol (60%-70%) for bactericidal action.

Methods: This study was conducted to measure the persistence of antibacterial activity of 2 preparations. One was a non-alcohol-based formulation using benzalkonium chloride (BK) (0.12%) and the other was an ethanol-based formulation (63%) (comparator product). The persistence of antibacterial activity was measured against *Staphylococcus aureus* using a technique modification prescribed in American Society for Testing and Materials protocol E2752-10 at up to 4 hours after application.

Results: The test product (BK) produced a marked reduction in colony-forming units at each of the 3 time points tested (3.75-4.16-$\log_{10}$ reductions), whereas the comparator produced less than 1-$\log_{10}$ reduction over the same time. The differences were highly significant.

Discussion: In the course of patient care or examination, there are instances where opportunities exist for the practitioner's hands to become contaminated (eg, key boards and tables). Persistent antibacterial activity would reduce the chances of transfer to the patient.

Conclusions: These results show a major improvement in persistent antibacterial activity for the BK formulation compared to the comparator ethanol-based formulation.

The prevention of nosocomial infections has been a goal for the medical community since the elucidation of the germ theory of disease. Modern approaches include extensive facilities sanitation programs and multiple personal hygiene practices.[1] Of the latter, regular hand washing and the use of hand sanitizer products are now routine.[2] Hand sanitizer formulations have traditionally contained ethanol or other short-chained alcohols (60%-70%) as the active ingredient responsible for the antibacterial action. Ethanol provides its antimicrobial action through desiccation of the target organisms. Applied to the skin, the ethanol-based sanitizers are effective in reducing the bioburden of many types of microbes.[3] However, alcohols are volatile and can evaporate from the skin's surface, so the residual antibacterial activity may be limited.[4] The importance of persistent antimicrobial activity has been increasingly recognized in the medical/surgical setting.[2,5] Recent reports have also shown that certain pathogen populations are becoming more tolerant to ethanol exposure.[6] These data suggest that the use of alternative antibacterial actives might be a benefit in the clinical setting.

Alcohol-free formulations have been developed, with the surfactant benzalkonium chloride (BK) as the active antibacterial agent. This active ingredient acts by disrupting the cell membranes of the target organisms and is active at relatively low concentrations (0.12%-0.13%).[7] Since this surfactant is not volatile, it is expected to remain on the skin as the product dries. Although this report focuses only on the antibacterial action of BK against *Staphylococcus aureus*, this surfactant has also been studied for virucidal activity against influenza, Newcastle disease, and avian infectious bronchitis viruses.[8]

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This study was performed to measure the residual antibacterial activity of 2 hand sanitizer products using the standard method prescribed in the American Society for Testing and Materials protocol E2752-10.[9] The test product was a surfactant-based product using BK (0.12%) as its active antibacterial agent, and the second product was a standard commercial ethanol-based formulation (with 63% ethanol but no other antibacterial actives), which served as the comparator product. The comparator product's ethanol concentration falls within the recognized effective concentration range for effective immediate contact antimicrobial activity.[3] Persistence of antibacterial activity was measured as a function of $\log_{10}$ kill of reference bacteria versus time after application of the hand sanitizer. The antibacterial activity was measured from 1-4 hours after application of the products. The test product was evaluated at 1, 2, and 4 hours after application, whereas the comparator product was evaluated at 1 and 4 hours after application.

METHODS

For this study of residual antibacterial activity on the skin, 2 products were compared. The commercial brand DAB hand sanitizer (active ingredient 0.12% BK) and a comparator hand sanitizer, containing 63% ethyl alcohol), were provided by Best Sanitizers (Walton, KY) to the testing laboratory, Biosciences Laboratories, Inc. (Bozeman, MN).[10] The DAB brand is produced by Best Sanitizer under contract to Three Kings Inc. (Corinth, MS). The study was conducted in compliance with good laboratory practices for nonclinical studies (21CFR58). As stated in the study protocol, "The purpose of this study was to evaluate the residual antibacterial efficacy of 1 test product verses a comparator ethanol-based product, as determined by the difference between the number of challenge bacteria species recovered following exposure to the test materials and the number recovered from the untreated (negative control) test sites."

Panelists and skin preparation

The study was performed on 24 subjects (19-63 years old) with healthy skin (16 men and 8 women). The study protocol and informed consent form were approved by the Gallatin Internal Review Board. The volar forearms were used, and the test sites were marked for the test product, comparator product, and negative control. The volar forearm was chosen to provide multiple replicate test sites on each arm, which would not be possible using the hands. The sites and arms were randomized among the treatment groups to prevent anatomical bias. The arms were washed with nonmedicated soap to remove surface dirt and oil, dried, and finally decontaminated with 70% isopropyl alcohol and allowed to air dry. The test sites and control sites were marked with a surgical marker as rectangles (2 × 6 inch [5.08 × 15.24 cm]) for the test product on 1 arm and as rectangles (2 × 4 inch [5.08 × 10.16 cm]) for the comparator product on the other arm. An area for the untreated control skin (no further treatment) was also marked. The areas for the test and comparator products were randomized between arms across the test panel. Within the test sites, 3 circles (2 cm in diameter) were marked with a surgical marker. Only 2 circles were marked in the 2 × 4-inch box for the comparator product, as only 2 time points were to be assessed. These were the sites to which the bacteria were to be applied.

Challenge bacteria

The challenge bacterial strain for this study was *S aureus* (ATCC 6538). *S aureus* is a common skin contaminant and therefore provides an appropriate test organism.[11] Fresh, active stocks were prepared in broth medium daily. The day before testing, a sample of the broth culture was applied to and spread over the surface of a tryptic soy agar plate and incubated for 24 hours. Just before beginning the study, a portion of the bacteria on the surface of the agar plate was transferred to phosphate buffered saline. After mixing the bacteria into the saline to form a uniform suspension, the turbidity of the suspension was measured and the sample diluted to approximately 1.0×10^8 colony-forming units (CFU) per mL of suspension. Ten microliters of this suspension (approximately 10^6 CFU) were applied to and spread over the 2-cm circles at the appropriate times.

Product neutralizer

It is essential that once the bacteria are removed from the treated skin that residual skin sanitizer not continue to act on the bacteria as they are being prepared (diluted and plated). To this end, a product neutralizer was prepared and added to the dilution liquids. For this study, the same product neutralizer was selected for both the test and comparator products. Before the study began, the effectiveness of the product neutralizer was confirmed using American Society for Testing and Materials E1054 (2013), Standard Test Method for Evaluation of Inactivators of Antibacterial Agents.[12] Four replicate samples for each of the 2 exposure periods (1 and 30 minutes) were tested for each treatment condition: untreated control, test product, comparator product, Butterfield's Phosphate Buffer (BPB++), and Stripping Suspension Fluid (SSF++). The "++" refers to the presence of the product neutralizer. In addition, the antibacterial efficacy of the test and comparator products without neutralization were verified.

Evaluation of antibacterial efficacy

Application of the test and comparator products

Each product was applied to the skin at a rate of 0.25 mL per square inch (0.039 mL/cm^2) (3 mL for the 2 × 6-inch test rectangle and 2 mL for the 2 × 4-inch comparator product rectangle). In both cases, the liquid was applied in stages, spread over the whole area, and allowed to dry for 1-2 minutes between each application. Once all of the applications were made, the subjects were sequestered and monitored at the test facility to ensure test site integrity.

The persistent efficacy of the test product was evaluated at 1, 2, and 4 hours after application of the product to the skin. The comparator product was evaluated at only 1 and 4 hours after application. At each time point, 10 μL of the bacterial suspension were applied to 1 of the 2-cm circles in the test product treatment area and spread over the surface with a sterile glass rod. The procedure was repeated on the comparator product treatment area (except for the 2-hour time point) and on the negative control area. Each inoculation was allowed to dry in place for at least 20 but not for more than 25 minutes. At the end of this exposure period, a 2-step procedure known as the cup scrub technique was used to remove the bacteria for determination of viability. A sterile stainless steel cylinder with an interior area of 3.46 cm^2 was held against the skin within the 2-cm circle. A volume of 2.5 mL of sterile SSF was dispensed into the cylinder. The fluid contained the specific product neutralizer (SFF++) to stop the action of the test and comparator products. A sterile rod was used to massage the skin for 1 minute to lift the bacteria from the skin into the fluid. This fluid was transferred to a sterile tube, and a second 2.5 mL volume of SSF++ was dispensed into the cylinder. Again, the skin was massaged for 1 minute, and the second fluid sample was combined with the first. This process was repeated for each exposure condition at that time point. For example, at the 1-hour postexposure time point, 3 bacterial suspensions were collected from each of the 24 subjects; 1 from the test product-treated skin, 1 from the comparator product-treated skin, and 1 from the negative control-treated skin. To determine the number of viable bacteria (number of CFU) in each sample, serial 10-fold dilutions of each bacterial suspension sample were prepared in BPB solution again containing the product neutralizer (BPB++). Samples from each dilution were spread onto 2

Table 1
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 1 hour following application of the test product or comparator product

		Test product 1 h after application		Comparator product 1 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.23	0.86	4.22	4.81	0.51
Mean	5.20	1.08	4.12	4.50	0.70
SD	0.189	0.395	0.359	0.727	0.703
		P value (1 tailed)	*P* <.001		

individual mannitol salt agar plates, which were incubated at 35±2°C for 48 hours. On mannitol salt agar, *S aureus* produce golden-yellow colonies, and only those colonies were counted.

Calculation of the recovery of viable CFU of bacteria

By definition, a CFU is 1 bacterium that is capable of continued replication to produce a large number of bacteria to form a colony. Each inoculum to the skin contained approximately 10^6 CFU. Each sample from the skin was serially diluted and samples plated. Knowing the area of the skin sampled (3.46 cm^2), the volume of SSF (5 mL), the dilution of the sample producing the counted plate, and volume of the sample added to the plate, the number of CFU per unit area on the skin could be calculated.

The number of CFU from each site at each postapplication time was converted to a log_{10} value. The residual antibacterial activity was calculated by comparing the log_{10} value from the negative control site (time matched) to the log_{10} value from the test and comparator product-treated sites to determine the log_{10} difference (antibacterial effectiveness) for each treatment. The relative values were internally controlled for each subject. For the 1- and 4-hour postexposure times, the statistical significance between the log_{10} difference for the test and comparator values for the 24 subjects was evaluated using a paired Student t test (Excel).

RESULTS

The results of the product neutralizer testing showed the efficacy of the neutralization formulation. In all cases, there was no significant difference between the mean untreated control log_{10} colony counts (n = 4) and the mean treated log_{10} colony counts (n = 4), indicating that there was no significant residual antibacterial activity.

The results of the study are expressed as log_{10} mean recovery of CFU of *S aureus* from the untreated control site, the test product, and the comparator product sites for each postapplication time point. The mean values from the individual postapplication time point values for the test and the comparator products are provided (Tables 1-3).

DISCUSSION

This study was performed to measure the antibacterial efficacy of a benzalkonium-based test product in comparison with a comparator product containing 63% ethanol as a function of time after application of the individual products to human skin. *S aureus* was used as the test organism since it is a known skin pathogen.[11] The test and comparator products were applied to defined areas of opposing forearms at 0.039 mL/cm^2. Within those areas, 2-cm diameter circles were marked, to which the bacterial suspension would be applied at the specific times after application of the products. For the test product treatment, bacteria were applied at 1, 2, and 4 hours after product application and for the comparator product treatment, bacteria were applied at 1 and 4 hours after product application. Bacteria were applied to untreated skin at each time point to provide the baseline bacterial recovery. The difference in the recovery between the test and comparator products was striking. Although the test product reduced bacterial viability by 3-4 log_{10} at each time point, the comparator product did not reduce bacterial viability by even 1 log_{10}. The differences in efficacy were statistically significant at $P < .001$. These data suggest that the active ingredient BK (0.12%) can provide a marked improvement in persistent antibacterial activity over the 63% ethanol-based product.

The effectiveness of BK as an antibacterial agent on skin has been evaluated in the past. Dyer et al (1998) compared the efficacy of 3 hand sanitizer preparations containing either ethanol (63% or 70%) or BK (0.13%) against *Serratia marcescens* applied to the hands.[7] In this study, the hands were contaminated with 5 mL of *S marcescens*, spread over the hands, and allowed to dry for 45 seconds. Five grams of test product were used to "wash" the hands, and then the remaining bacteria were recovered using the "glove juice sampling method." Polyethylene gloves with 50 mL of recovery fluid were placed, and the hands and the fluid massaged for 1 minute to recover the bacteria. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. This process was

Table 2
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 2 hours following application of the test product

Sample	Sample size	Mean (log_{10})	SD
Untreated log_{10} microbial recovery (2 h)	23*	5.17	0.20
Treated log_{10} microbial recovery (2 h)	24	1.01	0.37
Log_{10} difference (2 h)	23	4.16	0.35

*One untreated control sample lost.

Table 3
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 4 hours following application of the test product or the comparator product

		Test product 4 h after application		Comparator product 4 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.08	0.86	3.96	4.58	0.17
Mean	4.92	1.17	3.75	4.59	0.32
SD	0.420	0.503	0.602	0.649	0.597
		P value (1-tailed)	*P* <.001		

repeated 10 times for each treatment condition, and the reduction factors were calculated. The process took approximately 10 minutes per cycle. Only the BK formulation produced a progressive increase in effectiveness (increased reduction factor) over the 10 cycles. The ethanol formulations showed declines in effectiveness relative to the first cycle for each.

The concentration of ethanol in the hand sanitizer formulation can have a marked impact on antibacterial activity. Kampf (2008) compared 4 ethanol-based formulations (85%, 62%, 61%, and 60%) and 2 application volumes of 2.4 and 3.6 mL (total both hands) were evaluated.[13] Again, *S marcescens* was used as the test bacterium. Approximately 5 mL of bacterial suspension were rubbed over the hands and allowed to dry. The viable bacteria were recovered using the glove juice sampling method described in the preceding text. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. The untreated recovery values were compared to the treated conditions where either 2.4 or 3.6 mL were provided to rub over the hands (covering all skin). Both volumes were sufficient to cover the hands of most of the 16 subjects in each test group. The mean $\log_{10}$ reductions for each treatment were statistically compared by an analysis of variance analysis. Although all of the preparations reduced the number of viable bacteria, the larger volume was more effective at all ethanol concentrations and the 85% ethanol formulation was statistically more effective than the other 3 concentrations. For the 3.6 mL application volume, the mean $\log_{10}$ reduction for the treatment groups were 3.04 ± 0.81 (85%), 2.85 ± 0.51 (62%), 2.63 ± 0.59 (61%), and 2.53 ± 0.60 (60%). However, 85% ethanol is much higher than what is normally contained in current commercial hand sanitizer formulations.

Although *S aureus* accounts for a large fraction of the hospital-acquired infections, other bacteria are a concern. *Enterococcus faecium* is a gram-positive bacterium, which has become a leading antibiotic-resistant pathogen (bloodstream, urinary tract, and surgical wounds).[14] Hospital strains can be resistant to multiple antibiotics, which make them particularly difficult to treat once the infection is established.[15] The rise in incidents of nosocomial infections has raised concerns that preventive measures, such as the use of ethanol-based hand sanitizers, have applied selection pressure on the populations to select for more tolerant strains. Pidot et al (2018) have examined the resistance to isopropyl alcohol in 139 strains of hospital-associated *E faecium* isolated from 2 major Australian hospitals over 17 years.[6] These hospitals have active hand sanitation programs based on alcohol-based hand disinfectants. To measure resistance, bacterial suspensions were exposed to 23% isopropyl alcohol for 5 minutes and the number of remaining CFU determined. The concentration of isopropanol and time of exposure were selected to maximize resolution among the strains. Breaking the isolates into groups by date of isolation (1997-2003, 2004-2009, and 2010-2015), there was a high statistically significant decrease in mean sensitivity (based on mean $\log_{10}$ reduction) for the 2010-2015 isolates compared to the 1997-2003 and to the 2004-2009 isolates. These data suggest that there has been a population selection, which has reduced the overall sensitivity to the alcohol-based infection control measures.

Selection for increased tolerance to other disinfectants as a function of repeated use/exposure has been examined under various environmental exposure conditions. Holah et al (2002)[16] compared *Listeria monocytogenes* and *Escherichia coli* populations found in cannery processing lines where quaternary anomia disinfectants were routinely used. These isolates were compared to isolates from sites not routinely subjected to disinfectant use. They concluded that the persistent populations on the cannery lines were not inherently more tolerant to the disinfectant but that other factors (ie, surface attachment, biofilm formation, and growth rate) were likely responsible for their ability to persist in the disinfectant-treated environment. Kim et al (2018)[17] examined the impact of continuous exposure to BK on bacterial populations isolated from contaminated river sludge. The sediment samples were maintained for extended periods (3 years) in bioreactors containing nutrient medium and increasing concentrations of BK or nutrient medium alone. Changes in benzalkonium tolerance were measured using the minimal inhibitory concentration assay on nutrient agar. Certain species (ie, *Pseudomonas aeruginosa*) showed increased tolerance to BK (200 vs 50 mg/L), whereas others did not (ie, *Klebsiella michiganensis*). The basis for the difference in the selected strains with increased tolerance was a small change in the antibiotic efflux gene sequence.

It is not surprising that disinfectants can provide some selective pressure on bacterial populations. This pressure is most effective at sublethal concentrations of the disinfectant, which allow the more tolerant subpopulations to thrive and predominate. Lethal concentrations are less likely to select for tolerant clones where the surviving fraction of the population is very low.[18,19] The current study was not designed to measure selection pressure on the *S aureus* population. It was designed to measure persistence of antibacterial efficacy. The persistence of high antibacterial efficacy from the BK-containing test product may reduce the chances for selection of more tolerant clones.

Normal clinical infection control protocols specify use of hand sanitizers between patients to prevent patient-to-patient microbial transfer. That is not expected to change with the use of a persistent antimicrobial agent. However, in the course of patient care or examination, there are instances where there are opportunities for the practitioner's hands to become contaminated. Various surfaces such as key boards, tables, chairs, bed frames and other fixtures will need to be touched or handled. Use of a persistent antimicrobial hand sanitizer would be expected to reduce the opportunity for microbial transfer to the patient.

This study was undertaken to measure the absolute and relative persistence of antibacterial activity under very controlled test conditions. Having demonstrated persistent activity, the logical next step would be a clinical use study. As a first evaluation, a study is planned that will compare a 70% ethanol product and the test product from this study. Subjects will be medical clinic personnel, who will use both products in a cross-over study design.

In the United States, hand sanitizers (both medical professional and consumer) fall under the purview of the U.S. Food and Drug Administration, the 1994 tentative final monograph or proposed rule (the 1994 TFM) for over-the-counter antiseptic drug products (Federal Register of June 17, 1994 [59 FR 31402]). These rules are in the process of being revised to separate the professional and consumer products, and the agency is seeking additional data on active ingredients, including ethanol and BK. One factor to consider is the persistence of the antibacterial activity on the skin. This study provides quantitative data on the persistence of BK-induced antibacterial action, which could be a marked benefit in the prevention of nosocomial infections.

CONCLUSIONS

These results show a major improvement in persistent antibacterial activity for the BK formulation compared to the comparator ethanol-based formulation. Persistent antibacterial activity may be beneficial in the patient care setting to reduce the chances of incidental contamination of the hands and subsequent transfer to the patient.

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08.07 - Antimicrobial Summary

- Explanation of Kill Rate Testing Results
- Ingredient Safety Assessment
- DR. John W. Harbell

JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry
John W. Harbell, Ph.D.
President
Direct: [redacted]

December 1, 2020

RE: Antimicrobial efficacy data and ingredient safety assessment on Meditize ™ formula (32015.0)

To Whom It May Concern:

The following provides a summary of the antimicrobial efficacy study performed by Microconsult, Inc. and a summary of the ingredient safety assessment of the individual ingredients for this formulation.

Antimicrobial Study:

Summary:

Meditizer Hand sanitizer was tested in a Kill Rate Study using eleven bacterial species (Microconsult Report 1 September 2020). The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms (> 10^5 cfu/mL) from nine of the species and greater that three $\log_{10}$ from the other two. The 60 second exposure killed all of the organisms from all eleven species. A second Kill Rate Study was performed on the spore stage of C. difficile using the same exposure times (Microconsult Report 15 September 2020). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data suggest that this hand sanitizer could have a strong impact on bacterial transmission.

This summary report was compiled from two Kill Rate Study reports issued by Microconsult, Inc. 1 September 2020 (11 organisms) and 15 September 2020 (C. difficile in the spore stage).

Objective:
To demonstrate the antibacterial properties of the test product against a selection of gram positive and gran negative bacteria.

References:

A. 21 CFR 333. Topical antibacterial products for over-the-counter human use.

B. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics: Topical antimicrobial drug products for over-the-counter human use; proposed amendment of the tentative final monograph. Section V. Comments on the Proposed Rule and FDA Response Subsection C. Comments on Effectiveness and FDA Response [list of test organisms for *in vitro* efficacy testing]
C. Microconsult, Inc. Test Method 011_00 Kill Rate Testing

Test Article: Labeled as Hand Sanitizer, lot # 1152 (Meditizer Pharma)

Test Organisms
The list of test organisms, their American Type Culture Collection (ATCC) numbers, source and short names (see Table 2) are provided in Table 1. Active cultures of the test organisms were maintained by the laboratory and renewed from the reference stock after five passages. *Campylobacter jejuni* and *Clostridium difficile* were maintained under anaerobic culture conditions. To kill the C. difficile vegetative organisms, the 24-hour growth plate was treated quickly with 70% isopropyl alcohol to yield the spore form cells for the second study.

Table 1 List of organisms tested

Organism	ATCC Number	Source	Short Name
Escherichia coli	8739	Microbiologics	*E. coli*
Methicillin-resistant (MRSA) Staphylococcus aureus	33591	Microbiologics	*MRSA*
Pseudomonas aeruginosa	24853	Microbiologics	*P. aeruginosa*
Burkholderia cepacia	25416	Microbiologics	*B. cepacia*
Salmonella enterica	14028	Microbiologics	*S. enterica*
Enterococcus faecalis	51575	Microbiologics	*E. faecalis*
Klebsiella pneumoniae	700603	Microbiologics	*K. pneumoniae*
Streptococcus pyogenes	19615	Microbiologics	*S. pyogenes*
Listeria monocytogenes	SLR2249	Microbiologics	*L. monocytogenes*
*Campylobacter jejuni**	49943	Microbiologics	*C. jejuni*
*Clostridium difficile**	9689	Microbiologics	*C. difficile*

*Anaerobes

Reagents:
Tryptic Soy Agar with Lecithin and Tween 80

Sterile Phosphate Buffered Saline (for diluting)
DE Neutralizing Broth: Dey-Engley Neutralizing Broth is intended to stop the action of the antimicrobial preparation at the end of the exposure period. It is formulated to neutralize several types of antibacterial active ingredients including benzalkonium chloride.

Procedure:

1. Prepare each bacterial culture, inoculate the growth medium (broth) with the actively growing bacteria and allow to grow at 30-35^{o}C for 24-48 hours. These suspension cultures will be used to determine the antibacterial activity of the test article against the specific bacterium. Eleven such suspension cultures were prepared, one for each organism. These cultures were incubated for 24 to 48 hours to obtain the desired bacterial titers. At this point the number of organisms per mL (cfu/mL) was determined and the same cultures were used to challenge the test article. It should be understood that the exact number of organisms in the inoculum will not be known until step 2 is completed.

2. To obtain the number of viable microorganisms (colony forming units per mL [cfu/mL]), a sample was removed and diluted in sterile phosphate buffered saline. Subsequent serial dilutions were prepared from this sample to seed test plates with dilutions of 10^{-6} and 10^{-7} of the original suspension. Each test plate was then filled with 20 mL of 45^{o}C Tryptic Soy Agar, swirled to mix and then allowed to harden. The plates were incubated for 24 – 48 hours to allow the viable bacteria to form colonies in the agar. The bacterial colonies were counted and the number of colony forming units per mL in the original inoculum determined. The number of cfu/mL in the inoculum was then calculated to determine the number in the test samples using the formula below:

$$\frac{\text{(cfu/mL inoculum)x(volume added to the test article sample)}}{\text{Weight of test article (g)}} = \text{cfu/g product}$$

$$\frac{\text{(cfu/mL inoculum)x(0.1 mL)}}{\text{9.9 (g)}} = \text{cfu/g of test article}$$

3. Samples of test article were prepared for inoculation with each bacterium. A volume of 9.9 mL was measured out into properly labeled test tube. These tubes were held at room temperature until the bacteria were added.
4. Inoculation of the test article with the bacterial inoculum was performed by adding 0.1 mL of the bacterial inoculum to the tube holding 9.9 mL of the test article. The tube was mixed and then allowed to stand for the time of the first incubation period (30 seconds). At that point one mL of the test article-inoculum mixture was removed a placed immediately into 9.0 mL of DE Neutralizing solution to stop the action of the test article.

After the second incubation period (60 seconds) a second sample of one mL was taken and added to a second tube containing 9.0 mL of DE Neutralizing solution. This process was repeated for each of the bacteria tested.

5. Each suspension of bacteria in the DE Neutralizing solution was serially diluted (1:10) in duplicate in phosphate buffer to prepare dilutions of 10^{-1} to 10^{-5}.
6. One mL of each dilution was transferred to a prelabeled 100 x 15 mm petri plate.
7. Each plate was overlaid with 20 mL of melted (45^{o}C) Tryptic Soy Agar and the plate gently swirled to mix the bacteria with the agar. The plates were then allowed to harden,
8. The inoculated plates were placed into an incubator at 30-35^{o}C for 48 to 72 hours. Again, the *C. jejuni* and *C. difficile* plates were incubated under anaerobic conditions.
9. At the end of the incubation period, the number of colonies in each plate was counted. From the count value and the dilution of the original sample, the number of colony forming units remaining in the treated samples was calculated
10. The $\log_{10}$ reduction was calculated from ratio $\log_{10}$ of the inoculum to the log10 of the remaining colony forming units after treatment. For example:

For the E. coli sample treated for 30 seconds, the $\log_{10}$ inoculum of bacteria was 5.93/mL and the number of colony forming units after treatment was zero. The zero value is converted to one which has a $\log_{10}$ of zero. The $\log_{10}$ reduction is 5.93-0 = 5.93. A second example shows the case where there was some survival at 30 seconds of exposure. ***B. cepacia*** had an initial inoculum of 6.24×10^5 cfu/ml ($\log_{10}$ is 5.80). At 30 seconds of exposure, 310 cfu/mL ($\log_{10}$ 310 is 2.49) remained viable. The $\log_{10}$ reduction was 5.80-2.49 = 3.30.

The $\log_{10}$ reduction for each bacterium at each of the two exposure times is shown in Table 2.

Table 2 Log reduction of viable cfu/mL

Organism (Exposure Time)	**Inoculum Level (cfu/mL)**	**Growth Average (cfu/g)**	**$\log_{10}$ Reduction**
E. coli (30 seconds)	8.59×10^5	No Growth	5.93
E. coli (60 seconds)	8.59×10^5	No Growth	5.93
MRSA (30 seconds)	7.55×10^5	No Growth	5.88
MRSA (60 seconds)	7.55×10^5	No Growth	5.88
P. aeruginosa (30 seconds)	5.56×10^5	No Growth	5.75
P. aeruginosa (60 seconds)	5.56×10^5	No Growth	5.75
B. cepacia (30 seconds)	6.24×10^5	310	3.30
B. cepacia (60 seconds)	6.24×10^5	No Growth	5.8
S. enterica (30	5.91×10^5	No Growth	5.77

Organism (Exposure Time)	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log_{10} Reduction
seconds)			
S. enterica (60 seconds)	5.91×10^5	No Growth	5.77
E. faecalis (30 seconds)	8.84×10^5	No Growth	5.95
E. faecalis (60 seconds)	8.84×10^5	No Growth	5.95
K. pneumoniae (30 seconds)	3.81×10^5	15	4.40
K. pneumoniae (60 seconds)	3.81×10^5	No Growth	5.58
S. pyogenes (30 seconds)	2.25×10^5	No Growth	5.41
S. pyogenes (60 seconds)	2.25×10^5	No Growth	5.41
L. monocytogenes (30 seconds)	5.98×10^5	No Growth	5.78
L. monocytogenes (60 seconds)	5.98×10^5	No Growth	5.78
C. jejuni (30 seconds)	2.42×10^5	No Growth	5.38
C. jejuni (60 seconds)	2.42×10^5	No Growth	5.38
C. difficile (30 seconds)	2.40×10^5	No Growth	5.38
C. difficile (60 seconds)	2.40×10^5	No Growth	5.38
C. difficile (Spore form) (30 seconds)	1.67×10^5	No Growth	5.22
C. difficile (Spore form) (60 seconds)	1.67×10^5	No Growth	5.22

Discussion:

As shown in Table 2, most of the bacterial species tested were completely killed with the 30 second exposure and all were completely killed with a 60 second exposure. 21 CFR 333 Topical antibacterial products for over-the-counter human use calls for a two log_{10} reduction in viability for a product to be considered antibacterial. This regulation applied to topical antiseptics. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics calls for a three log_{10} reduction in viability for a hand rub (hand sanitizer) to be considered to have antibacterial efficacy. This hand sanitizer achieved a three log_{10} kill with a 30 second exposure and complete kill with a sixty second exposure for all eleven species tested. Of particular interest was the activity against C. difficile spores. Complete kill of the 1.67×10^5 cfu/mL inoculum was achieved with a 30 second exposure.

This study was performed at Microconsult, Inc. Carrollton, TX under the direction of Alix Paulson, Microbiology Technician II September 2020.

Ingredient safety assessment:

The first step in assessing the potential toxicity of a formulation is a complete review of the toxicological hazard of each of the ingredients. This review is based accepted measures of potential toxicity by oral ingestion, absorption through the skin, irritation to the skin and eyes, sensitization of the skin (delayed contact hypersensitivity), genetic toxicity, phototoxicity (enhancing sunburn potential) and, where appropriate, developmental toxicity and carcinogenesis potential. This review includes the assessment of hazard (independent of the concentration used in the formulation) as well as the risk from the ingredient at the concentration employed in the formulation and the amount applied to the skin on a daily basis.

The first issue is oral toxicity. We use this as the basic measure of toxicity of the formulation and it is assessed in two ways. Even though this product is going onto the skin, we use oral toxicity to model the maximum exposure and toxicity. First what is the toxicity of a onetime exposure and second what is the toxicity of repeated exposure over months. The first is measured by the "Acute Toxicity Classification for Mixtures" proposed by the Globally Harmonized System (GHS) for toxicological assessment (https://www.chemsafetypro.com/Topics/GHS/GHS_classification_mixture.html). While this is more of an EPA program, the results can be instructive. The GHS has five classes of acute oral toxicity with Category 5 being the least toxic. **The Meditizer tm formula is projected to be even less toxic than a Category 5 by these calculations!** The second consideration is the repeat systemic exposure over weeks and months of using the product. For this measure, we calculate a Margin of Safety for each ingredient [1]. The Margin of Safety compares the maximum potential systemic exposure (if any) from using the product with the published no effect exposures from 3 month studies. Here we are looking to see how much less our potential exposure is compared to the published data for no effect. A good figure is 100 fold less. **Our values are 5,000 or more less so our Margins of Safety are excellent.** The full spreadsheet of the calculations is available as client confidential data since it contains the detailed formula.

The absence of skin irritation is important for any product used on a daily basis. At the concentrations used, none of our ingredients are expected to show any skin irritation potential. A review of the formulation (Table 1) shows that in fact many of the ingredients would also be found in cosmetic formulation to provide esthetics for the product.

The lack of skin sensitization potential is also important. Skin sensitization is an immune-mediated action and a minimum dose to the skin is required to begin the process. The weaker the sensitization potential, the more that is required. For example, d-limonene is listed as a sensitizer

by some but in fact, the amount of d-limonene required to produce this action is far greater than could be achieved with this formulation[2]. Thus, skin sensitization is not an issue with this formulation,

Even though Viraxshield is intended to be applied to the hands and not the face, it is important that the formulation not be an eye irritant just in case of accidental eye exposure. At the concentrations employed none of the ingredients are eye irritants and so we do not expect that the formulation will have any eye irritation potential.

Genetic toxicity is damage to the genetic material (DNA) of the cell and is something one wishes to avoid completely. All the ingredients have been tested in one or more assays and found not to induce genetic damage. Depending on the ingredient, genetic toxicity was assessed using the bacterial reverse mutation assay (with and without S9 metabolic activation), in vitro chromosome aberration assay (with and without S9 metabolic activation), and in vivo mouse micronucleus assay.

Phototoxicity can be induced when a chemical absorbs ultraviolet light and releases that energy in a way that activates surrounding chemicals that can act to damage the surrounding cells. Certain drugs and some natural products are known to cause this problem. If the ingredient absorbs UV light, then it should be tested. All of the ingredients in this formulation do not absorb UV light or have been tested and found negative for phototoxic activity.

Developmental toxicity and carcinogenesis: Many of the ingredients in this formulation are so nontoxic they have no potential to cause these issues. Others have been used extensively in cosmetic, drugs and other products so that testing has been performed. In all cases, they were not toxic.

This document is just a summary of the review of the ingredients. Ultimately, the final formulation will be subjected to confirmatory tests in both the laboratory and clinic for final mildness assessment.

Table 1 Meditizer tm (35015.0)

Number	Description	CAS#
	Active ingredient	
1	Benzalkonium Chloride	8001-54-5
	Inactive Ingredients	
1	Purified Water	7732-18-5
2	Polyethylene Glycol 4000	none
3	Polyethylene Glycol 400	5117-19-1
4	Glycerin, 99.5%	56-81-5
5	Hydroxyethylcellulose	9004-62-0
6	Trisodium Citrate	68-04-2
7	Polysorbate 20	9005-64-5
8	Phenoxyethanol	122-99-6
9	Potassium sorbate	24634-61-5
10	Copper (II) Chloride, Dihydrate	10125-13-0
11	d-Limonene	5989-27-5
12	Magnesium Hydroxide	1309-42-8

Prepared by:
John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]

References:

1. SCCS, *The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 10th Revision, SCCS/1602/18.* 2018.
2. Basketter, D., et al., *Categorization of chemicals according to their relative human skin sensitization potential.* Dermatitis, 2014. **25**(1): p. 11-21.

08.08 - Platelet Technology

- Breakthrough Material Science
- Benefits of Platelets over spheres and other shapes

PROPRIETARY INFORMATION

White Paper
An Innovative Platform of Technology

Biocellerex focuses on nanotechnology research and development to develop technology which is safe, highly efficacious and patentable while being affordable for practical use in products referred to as nanoparticles. Simply stated – the technology is produced in nano and micron forms providing formulas configurations along an x,y,z axis. This configuration results in exceptional nanoparticle efficacy and safety.

Biocellerex has successfully conducted studies in independent labs to validate safety and proof of concept for a variety of uses with its proprietary disc wafer like nanoparticles. The objectives of this White Paper are to describe key points about our new nanoparticles structure, efficacy against target microbes, and safety to non target organisms.

Structure:

Recent manufacturing and structural breakthroughs enable new shapes and sizes that have surpassed performance and dispelled many of the inherent concerns attributed to use of previous nanotechnologies. Unlike any known previous nanotechnology, the disc wafer like nanoparticles have been shown to be highly effective for control of prokaryotes and viruses. At sizes in the nano range (10^{-9}m, one billionth of a meter), metal compounds exhibit properties not observed for larger particles of the same chemical composition. Notable is the ability to kill a broad range of bacteria, fungi and viruses. Almost all previous nanoparticles exist as some form of spheres, rods, belts and other variations of shapes and sizes. Many of which are metal salts and are toxic upon prolonged exposure (Lewinski, et al 2008).

Biocellerex has primarily concentrated on is nano copper and magnesium hydroxide and/or a magnesium oxide lattice of unique disc wafer like morphology. This arrangement is like the micelle structure of clay particles in that both contain a prodigious surface/volume ratio. Magnesium hydroxide ($Mg(OH)_2$) occurs naturally as the mineral brucite. Magnesium oxide (MgO) is the oxide salt of magnesium.

Biocellerex's research team and partner companies have developed an electrolytic process that uses $MgCl_2$ to gradually deliver Mg2+ ions from one side of a conduction cell and –OH ions from the other, as shown by the reaction equation [Mg2+ + 2 –OH $Mg(OH)_2$]. This reaction proceeds with a slow, gradual growth of crystals that arrange into platelets only a few molecular layers thick. These $Mg(OH)_2$ nanotechnology are synthesized to great specificity to achieve a consistent size and shape uniformity with mass production cost efficiencies.

Anhydrous nanoparticles of $Mg(OH)_2$ and MgO are individual crystallites, approximately 200–200 nm in a highly specific, narrow width range (Figure 1 photo). This dimensional feature of the nanoparticles has a distinct advantage over traditional nanoparticles where most of the molecules are embedded within the nanoparticle rather than on the surface (Pal, et al. 2007, Ruparelia, et al. 2008).

Particles this thin appear to interact as 2 dimensional structures on the nano scale. However, these nanoparticles are edged with free $Mg(OH)_2$ groups available for attachment to a broad range of ester , acetal , and ether forming reagents. This disc wafer like structure enables functional substituents at the edge surfaces to act as connectors to modify the physicochemical properties of the nanoparticles. When the nanoparticles are integrated into a polymeric material, then further significant modifications can occur (Makhluf, et al. 2005, Thill, et al. 2006).

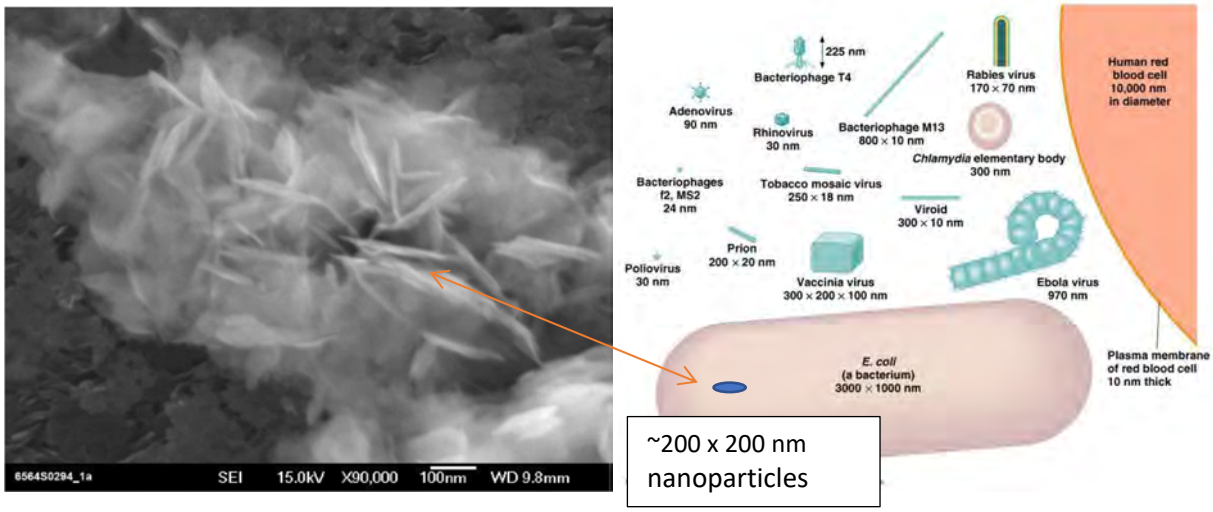


Figure 1.
Left: Magnesium Hydroxide Nanoparticles.
Right: A diagram showing approximate size ranges of cells, viruses and other infective particles relative to ARC's approx. 200 x 200 nanometer wafer like disc nanotechnology.

The nanoparticle production process conducted is entirely safe, green, and efficient with byproducts of chlorine and hydrogen that can be recycled for use in other industries (Gao, et al. 2009).

Efficacy:

The antimicrobial properties of nanoparticle technology have been tested and proven to be effective for use in medical facilities for control of human pathogens including against Multiple Drug Resistance Organisms (MDRO's). Nanoparticle technologies has been used to effectively mitigate infections from wounds and burns. The Technology is also very effective on surfaces to sanitize. (Adams, et al. 2004, Auffan, et al. 2008, Morones, et al. 2005, Stoimenov, et al. 2002, Thill, et al. 2006, Zhang, et al. 2007)

Independent tests of nanoparticle technologies were done against challenging human pathogens such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and Carbapenem Resistant *Enterobacteriacea E. coli* (CRE). Our nanoparticles demonstrated sustained efficacy with no pathogen rebounding (i.e., no resurgence) of pathogens after treatment even from spore forming pathogens (Fabrega, et al. 2009, Li, et al. 2005, Lok, et al. 2007).

Biocellerex research team along with our research partner companies also developed micron sized agglomerates composed of safe, effective nanotechnology intercalated with MgO, termed Nanoparticle

Antimicrobial Spheres (NAS). NAS exhibit extremely strong biocidal activity against a broad range of bacteria species that are resistant to current antibiotics and generally recalcitrant to other biocides. Independent lab assay tests, demonstrated MgO comprised NAS has high efficacy against Gram positive and Gram negative pathogens, including spore formers. Tests have shown effectiveness against: *Bacillus anthracis (Ames), Bacillus anthracis (Sterne), Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa, Salmonella typhimurium*, *Staphylococcus aureus*, and industrial isolates of Microbacteriaceae, Propionibacteria species and several environmental Fungal isolates. A subset example of results, taken from lab assays, is summarized in Table 1. This illustrates log kill efficacy of NAS against both ATCC and clinical isolates.

This lab test demonstrated that our NAS MgO material at various levels of concentration provided complete kill of this MRSA strain. Concentrations of 5mg/mL and 10mg/mL provided complete kill within 3 hr, while 1mg/mL achieved complete kill after 6 hr. Levoflaxin provided a reduction of MRSA population but did not achieve complete kill after 24 hr. Note that there is little to no difference in the concentration levels of 5mg/mL and 10mg/mL, with the lower concentration of 1mg/mL still achieving the same efficacy but at a slower rate to register log 7 kill. In all NAS MgO treatment cases, zero growth was observed at 24hr. By contrast the antibiotic Levoflaxin, administered at a current clinical dose rate, reduced the population by 4 logs, thereafter its activity plateaus – to leave log 2 cfu/mL at 24hr.

SUSTAINED EFFICACY – Kill with No Rebounding

Bacterial populations can exhibit resistance when continually challenged by antimicrobial agents. Selection pressures for survival lead to development of subsequent generations of 'Persisters', also called small colony variants (SCVs) and spore formation. These subpopulations of SCVs are genetically identical to the parent population but have altered metabolism that allows them to combat or evade the effects of antibiotics/biocides (Cohen, et al 2013). While the majority of the vegetative bacterial population may be killed by a biocide, the few SCVs that persist gradually multiply when conditions permit and return population levels to previous, or even greater numbers (e.g. *Colistin* rebound in *Figure 3 below*). The consequence of this 'rebound' is that patients who appeared clear of infection and in recovery, are afflicted a second time when SCVs that were not cleared from their system, resurge once the course of antibiotics is concluded.

NAS doses provided identical "Time Kill Kinetics". The Colistin antibiotic applied at a clinical dose rate provided apparent kill at 3 hours. However, the Colistin treatment resulted in incomplete sustained killing, then 'rebounding' occurred when the assay was extended to 24hr. This rebound CRE population resurgence over time elevated to levels that exceeded the initial inoculum starting point. By contrast NAS MgO clearly mitigated the CRE bacteria, as the bacteria were completely killed with no observed rebound effects.

Multiple Modes-of-Action

NPs kill pathogenic microbes by several mechanisms, thus resistance is less likely to arise in bacterial populations. Mechanisms of cytotoxicity that have been described include production of reactive oxygen species (ROS), penetration of cells via ionic uptake, and influence on electrostatic charges of cell membranes. Physical contact between a nanoparticle and a bacterial cell has been shown to be necessary to cause mortality (Kang, et al 2007, Thill, et al 2006, Stoimenov, et al 2002, Zhang, et al 2007, ARC unpublished results). The physical interaction appears to damage the membrane (Gorgoi, et al 2006; Makhluf, et al 2005, Stoimenov, et al 2002). Smaller nanoparticles appear to be more cytotoxic

than larger particles (Lok, et al 2007; Zhang, et al 2007;) and in a mixture, the small sized nanoparticles may be responsible for most of the toxicity (ARC unpublished results).

NPs trigger initiation of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals that are responsible for membrane damage (Zhang, et al 2007; Dawson, et al 2009). This claim is given support by correlations between degree of ROS production and degree of membrane damage measured by bacterial mortality.

UNIVERSALLY SAFE, NON-TOXIC BIOMEDICAL DECONTAMINATION TECHNOLOGY

Biocellerex has developed two biological decontamination formulations based on magnesium hydroxide nanoplatelet (NP) and NP antimicrobial spheres (NAS) technology

Universally safe and non toxic for people, food, water and the environment

Deployable in wet or dry formats

Effective against spore forming and non spore forming bacteria, mold/fungi

Dramatically outperforms frontline antibiotics

Efficacy verified and documented by independent labs

Multiple kill mechanisms confer broad based target efficacy, restricting microbial response

Kills antibiotic resistant bacteria (MRSA, CRE, etc)

Sustained efficacy; NP/NAS capable of multiple cycles of killing, protects against re emergence

Long shelf life (maintains potency over 5 years and counting)

Heat resistant (185^{o}C/365^{o}F), cold resistant (85^{o}C/ 121^{o}F)

Functional at temperature extremes (i.e., should be effective against extremophile pathogens)

- Nanoplatelet Technology vs Frontline Antibiotics

In every head to head lab test, Nanoplatelet Antibacterial Spheres (NAS) dramatically outperformed frontline antibiotics (see Table 1) [Tests performed by Micromyx, Kalamazoo, MI, except *B. anthracis* Ames which was performed by MRI Global, Kansas City, MO]

No internal testing in humans or animals have yet been conducted

Table 1. NAS vs Antibiotic Resistant Pathogens, Compared with Efficacy of Frontline Antibiotics

Independent Lab Tested	Pathogen	NAS			Antibiotic Parallel Tests		
		Agent Used	Log Kill	Time to Zero	**Antibiotic Used**	Log Kill	Time to Zero
Yes	MRSA (S.*aureus*, MMX 5999)	**NAS**	Log 7	3 hr	Levoflaxin	Log 4.5	Not Reached
Yes	CRE (K. pneumoniae, MMX 4691, CDC)	**NAS**	Log 8	3 hr	Tigecycline	Log 8	24 hr
Yes	CRE (E.coli MMX 5980) (CRE, NDM-1; ATCC 14579)	**NAS**	Log 8	3 hr	Colistine	See Note	Not Maintained
Yes	MRSA (S.*aureus,* MMX 2123) (V1SA)	**NAS**	Log 7	3 hr	Linezolid	Log 0.5	Not Reached
Yes	B. *cereus* MMX 2006 (ATCC 14579)	**NAS**	Log 8	1 hr	Ciprofloxacine	Log 0.5	Not Reached
Yes	B. *anthracis* (Ames) (B.E.I strain NR-411)	**NAS**	Log 6	6 hr	Not Tested	-	-

Note: Colistine appeared to achieve Log 8 kill in 3 hours, then pathogen rebounded back to Log 9

- Safety: Magnesium hydroxide affirmed as "Generally Recognized As Safe (GRAS)"

Title 21, Food and Drug Admin, Part 184, Direct Food Substances Affirmed As "Generally Recognized As Safe" (GRAS) Sec. 184.1428 Magnesium hydroxide

"(c) IAW 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice."

"(d) Prior sanctions for this ingredient different from the uses established in the section do not exist or have been waived."

Bocellerex's patented magnesium hydroxide NP is commercially used in meat processing industry to increase shelf life and lower/prevent bacterial growth

- Technology Readiness Level (TRL) by Pathogen Types

Table 2. TRL Status of R&D					
Tasks	**Pathogen Type**	**Examples**	**Deployment Type**	**Lab Status**	Next Step
RD 1	Bacteria (Non Sporulating)	E.coli, MRSA, Plague	Wet or Dry Kill	Formulation and kill testing complete	
RD 2	Bacteria (Sporulating)	Anthrax, C.diff, Bacillus thuringiensis	Wet Kill	Formulation and kill testing complete	
			Dry Kill	Formulation 50% complete (additional R&D)	
RD 3	Fungi/Mold	T. rubrum, Candida albins, rust, blight	Wet Kill	Formulation and kill testing complete	
			Dry Kill	Formulation 50% complete (additional R&D)	
RD 4	Viral	COVID 19, Smallpox, Ebola, Marburg, Lassa	Wet Kill	Formulation and kill testing complete	Other viral tests complete, COVID 19 complete by 3 22 20

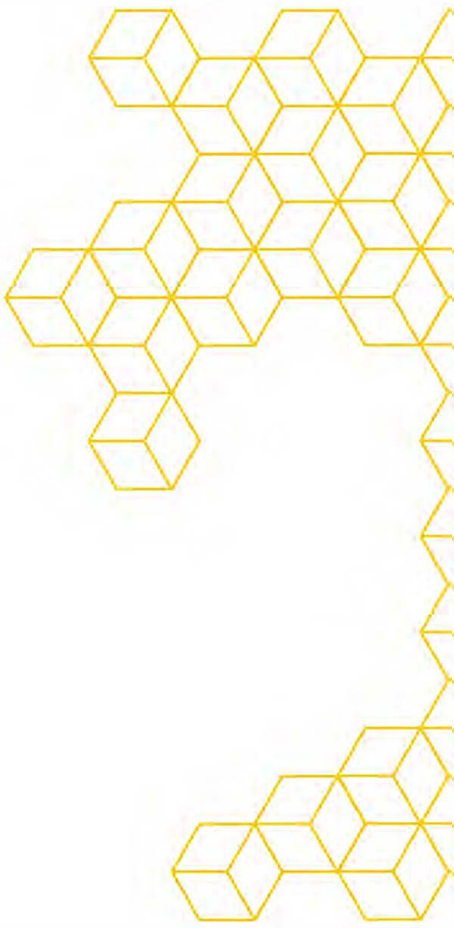
All diseases/threats in table are Category A (Centers for Disease Control and Prevention)

Wet or dry formulation is for spaces without sensitive equipment issues (office spaces, public venues, public transportation), though dry is preferred option

Dry formulation is for spaces *with* sensitive equipment issues (e.g., aircraft, ships, spacecraft)

08.09 - FDA NDC Listing

- FDA Labels Website Listing for Meditizer
- NDC 77238-231-24 Hand Sanitizer
- NDC 77238-241-21 Mask & Surface Spray



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Proprietary Name	NDC	Company Name	Application Number or Regulatory Citation	Product Type	Marketing Category
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Hand Sanitizer	77238-241-21	Medtize LLC	part333A	HUMAN OTC DRUG	OTC monograph not final

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