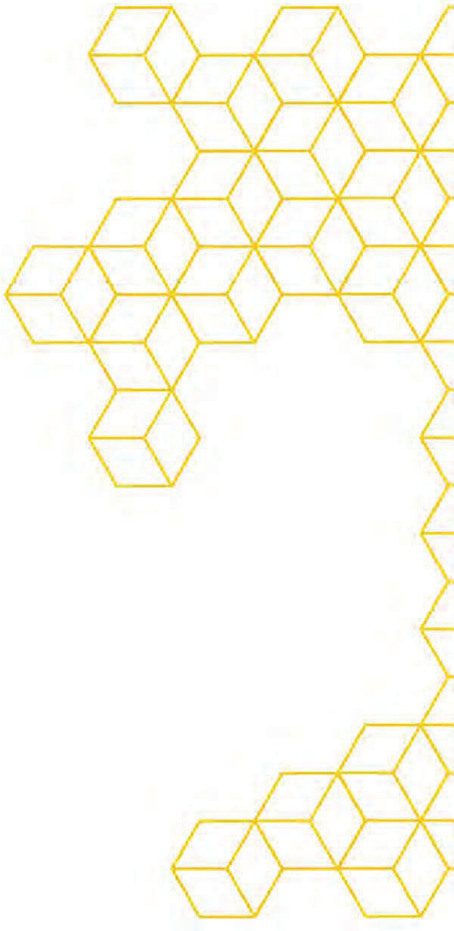
08.07 - Antimicrobial Summary

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



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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:
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[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.