A Copper-Iodine Complex-based wound irrigation solution with persistent and long-lasting activity against clinically relevant pathogens: an *in vitro* model

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Introduction

Clyra's "Bioclynse®" Wound Irrigation Solution (WIS) is an FDA 510(k) cleared medical device. The WIS is indicated in wound management, cleansing, irrigating, moisturizing, and debriding of acute and chronic dermal lesions that are partial or full thickness wounds. These indications include 1st and 2nd degree burns, stage I–IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, grafted and donor sites.

The WIS Preservative in Solution is based on a powerful Copper-Iodine Complex technology that releases a tailored amount of free iodine, I_2 (up to 250 ppm). This free iodine, acting in concert with copper ions, produces a synergistic effect that helps remove contamination within the solution and assists in the cleansing, irrigating, and debriding of wounds. The mechanism of action of the WIS is based on mechanical action of the pressurized fluid coming from the dispensing container or through a pulsed lavage system, which moves across the wound surface aiding in the removal of contamination or foreign objects such as dirt, debris or microorganisms.

This unique Copper-Iodine Complex of the WIS's preservative can neutralize a broad number of pathogens such as bacteria, viruses, yeast, and fungi without evoking bacterial resistance[1-4]. The WIS has been proven to be safe, non-cytotoxic, non-pyrogenic, non-irritating, and non-sensitizing to dermal tissue[5,6]. It can be applied directly to the wound bed and can be used with static or pulsed lavage, ultrasonic debridement and negative pressure wound therapy (instillation mode). Also, importantly, it does not need to be rinsed after treatment or application in either static or dynamic mode, potentially reducing procedure times.

The purpose of this study is to quantitatively evaluate the effect of the Wound Irrigation System (WIS) on bacteria, yeast, fungi, and SARS-CoV-2 virus in an *in vitro* model.

Materials and Methods

Trial #1 - Antimicrobial efficacy testing of WIS as a preservative in solution against five common organisms at 14 and 28 days, using the USP <51> test method.

Trial #2 - Time-Kill Data determination of WIS against 15 clinically relevant pathogens. Spiral and pour plate methods were used to examine antimicrobial activity of WIS. Tubes containing 10 mL of the test article were challenged with 0.10 mL test organism to yield ~10^6 CFU/mL. The test articles were mixed thoroughly.
At each time point, 1 mL aliquots of the test suspension were removed and added to 9 mL DEYE. Alternatively, 900 μl aliquots of the test suspension were removed and added to 110 μl 0.5M sodium thiosulfate, 9% saline solution. The tubes were mixed thoroughly. Samples were plates using a pour plate method. 1 mL aliquot was added to an empty petri dish. Molten agar (15 mL) was added and swirled to mix. Once agar had solidified, plates were incubated for 24-48 hours under organism appropriate conditions. Titer controls were also prepared.

The log reduction values were calculated using the following formula:

$$\text{Log reduction} = \log_{10} U - \log_{10} C$$

Where: U = Average titer control
C = Average recovered counts

The percent reduction values were calculated using the following formula:

$$\%\ \text{reduction} = (1 - 1/10^{(\log\ \text{reduction})}) \times 100\%$$

Trial #3 - Persistent antimicrobial efficacy determination of WIS against clinically relevant pathogens in the face of pathogen re-inoculation at 3 different time points. Test followed the same culture and plating procedure as Time-Kill test, with pathogen re-inoculations at 10 minutes, 4 hours, and 24 hours after initial inoculation.

Trial #4 - Validate efficacy of WIS against SARS-CoV-2. A virus stock concentration of $1x10^6$ median tissue culture infectious dose ($TCID_{50}$) per mL was used. Aliquots of stock virus (10 µl) were mixed with 90 µl of WIS or control solutions. Room temperature water was used as a negative control for virus inactivation, while boiling water (water pre-heated to 100°C) was used as a positive control. All mixtures were incubated for 30 seconds, 10 minutes, 30 minutes, or 60 minutes at room temperature, at which time 900 μl infection medium was added to neutralize antiviral activity. Subsequently, the SARS-CoV-2 viral titer ($TCID_{50}$/mL) for each test substance was determined. The experiment was conducted in triplicate.

Results

Antimicrobial Efficacy Test per USP <51> of WIS
Copper-Iodine Complex as Preservative in Solution
Results: WIS meets USP criteria for antimicrobial effectiveness for category 2

Organism	Log $_{10}$ reduction 14 days	Log $_{10}$ reduction 28 days
E. coli	>5.7	>5.7
P. aeruginosa	>5.3	>5.3
S. aureus	>5.4	>5.4
Candida	>5.3	>5.3
Aspergillus	>5.1	>5.1

***** Test performed by KML Laboratories, an FDA registered and ISO 17025 certified laboratory.

Time-Kill Data of WIS Copper-Iodine Complex against Clinically Relevant Pathogens

Microorganism	Exposure Time	Percent Reduction	Log_{10} Reduction
Staphylococcus epidermidis ATCC 35984	10 min	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Staphylococcus aureus ATCC 6538	10 min	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Streptococcus salivarius FUA3532	10 min	> 99.99991	>6.1
	24 hours	> 99.99991	>6.1
Streptococcus pyogenes ATCC 19615	10 min	> 99.99998	>7.0
	24 hours	> 99.99998	>7.0
Enterococcus faecalis ATCC 29212	10 min	~ 99.99981	~5.7
	24 hours	> 99.99998	>6.7
Cutibacterium acnes ATCC 11827	10 min	~ 99.9993	>6.2
	24 hours	> 99.99993	>6.2
Escherichia coli ATCC 25922	10 min	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9
Proteus mirabilis ATCC 29906	10 min	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9
Candida tropicalis ATCC 750	30 min	> 99.99915	>5.1
	24 hours	> 99.99915	>5.1
Candida albicans ATCC 10231	30 min	> 99.99952	>5.3
	24 hours	> 99.99952	>5.3
Enterococcus faecium ATCC 19434	10 min	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 4352	10 min	~ 99.9997	~5.8
	24 hours	> 99.99999	>6.8

Pseudomonas aeruginosa ATCC 10145	10 min	> 99.99997	>6.5
	24 hours	> 99.99997	>6.5
Klebsiella aerogenes ATCC 13048	10 min	> 99.99999	>7.0
	24 hours	> 99.99999	>7.0
Acinetobacter baumanii ATCC 19606	10 min	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9

* Tests performed by Nelson Labs, Biolargo Water and Keystone Labs.

Long-lasting WIS efficacy against ESKAPE pathogens and Candida albicans and Candida tropicalis has been demonstrated up to 3 days (Data on File).

Persistent Antimicrobial Efficacy of WIS against Clinically Relevant Pathogens.
(Sustained *in vitro* antimicrobial activity after pathogen re-inoculations over 24h)

Microorganism	Re-challenge interval	Percent Reduction	Log_{10} Reduction
Staphylococcus epidermidis ATCC 35984	10 min	> 99.99998	>6.7
	4 hours	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Staphylococcus aureus ATCC 6538	10 min	> 99.99998	>6.7
	4 hours	~ 99.9998	~5.7
	24 hours	~ 99.9998	~5.7
Streptococcus salivarius FUA3532	10 min	> 99.99991	>6.1
	4 hours	> 99.99991	>6.1
	24 hours	> 99.99991	>6.1
Streptococcus pyogenes ATCC 19615	10 min	> 99.99998	>7.0
	4 hours	> 99.99998	>7.0
	24 hours	> 99.99998	>7.0
Enterococcus faecalis ATCC 29212	10 min	> 99.99998	>6.7
	4 hours	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Cutibacterium acnes ATCC 11827	10 min	> 99.99993	~5.2
	4 hours	> 99.99993	>6.2
	24 hours	> 99.99993	>6.2
Escherichia coli ATCC 25922	10 min	> 99.99999	>6.9
	4 hours	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9
Proteus mirabilis ATCC 29906	10 min	> 99.99999	>6.9
	4 hours	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9
Candida tropicalis ATCC 750	30 min	> 99.99915	>5.1
	4 hours	> 99.99915	>5.1
	24 hours	> 99.99915	>5.1
Candida albicans	30 min	> 99.99952	>5.3
	4 hours	> 99.99952	>5.3

ATCC 10231	24 hours	> 99.99952	>5.3
Enterococcus faecium ATCC 19434	10 min	> 99.99998	>6.7
	4 hours	> 99.99998	>6.7
	24 hours	> 99.99998	>6.7
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 4352	10 min	> 99.99999	~5.8
	4 hours	~ 99.9999	>6.8
	24 hours	> 99.99999	>6.8
Pseudomonas aeruginosa ATCC 10145	10 min	> 99.99997	>6.5
	4 hours	> 99.99997	>6.5
	24 hours	~ 99.9997	~5.5
Klebsiella aerogenes ATCC 13048	10 min	> 99.99999	>7.0
	4 hours	> 99.99999	>7.0
	24 hours	> 99.99999	>7.0
Acinetobacter baumannii ATCC 19606	10 min	> 99.99999	>6.9
	4 hours	> 99.99999	>6.9
	24 hours	> 99.99999	>6.9

Tests performed by Keystone Labs and Biolargo Water.

Efficacy testing *in vitro* of WIS against SARS-CoV-2 virus.

Results: After incubation with WIS for 10 minutes, viral titers dropped by 2 logs (one tailed t-test p-value=0.0140). After incubation with undiluted WIS for either 30 minutes or 60 minutes, viral titers dropped below the limit of detection (< 75 $TCID_{50}$ per ml).

Conclusion

Clyra Wound Irrigation Solution has been shown to be effective with a very significant log reduction kill rate against multiple gram positive and gram-negative bacteria, yeast, and fungi, and against SARS-CoV-2 virus. Additionally, it is safe, non-toxic to healthy tissue, and does not require any rinse out. This yields potentially far reaching clinical application, especially considering the significant clinical and financial burden posed by infection in acute and chronic wound care, burns, and post-surgical management of complex wounds. Further studies will follow to support these findings.

References

1. GLP Time Kill Study 2016-2018. Nelson Laboratories
2. Antimicrobial Effectiveness Test (USP<51>) 2018. KLM Labs
3. Efficacy Test for SARS-CoV-2 Inactivation 2020. Galveston Nat'l Lab, Univ of Texas
4. Time Kill & Persistence Test 2023. Biolargo Water Laboratory, Univ. of Alberta
5. GLP Wound Healing Study (porcine wound model) 2019. Bridge PTS
6. GLP Cytotoxicity Test 2018, GLP Sensitization Test 2018, GLP Pyrogenicity Test 2018, GLP Skin Irritation Test 2019. Nelson Laboratories

- Additional information and further references available on request. -