

Klearsen Corporation

Peaceful Mountain Throat Rescue

Testing
Summary Report
10/15/02
srf

Objective:

The testing reported herein was intended to evaluate the effectiveness of the Klearsen Corp., Peaceful Mountain Throat Rescue product against Streptococcus A. Hemolytic bacteria. Quantifying the minimum bacteriacidal concentration (MBC), comparison to other available throat spray products and a correlation of the in vitro test to an in vivo model were the main thrusts of the testing.

In Vitro Test Method:

Since the Strep. A. Hemolytic bacteria would die when exposed to the ionically deprived environment of the Throat Spray, a special assay needed to be developed. The Throat Spray product is essentially ionic silver in a colloidal aqueous suspension. The tremendous difference in ionic balance (anions and cations) between the Throat Spray and the intracellular fluid of the bacteria causes a large osmotic pressure gradient which will ultimately cause the cell wall to burst.

Balancing the ionic gradient between the interior of the cell and the test solution was done with a buffered beef peptone solution. This protein and salt mixture is a standard for buffering bacteria in solution as it contains nutrients and the essential ionic balance of salts. This however, when added to the Throat Rescue product causes a problem. The free silver ions of the Throat Spray bind with the anions of the beef peptone solution and precipitate out of suspension. This compounding and precipitation of the silver renders the Throat Spray product useless in 30 minutes.

Because of this interaction, the standard MIC and MBC testing protocols could not be used to evaluate the effectiveness of a colloidal silver product.

The technique that was developed allowed the control groups of bacteria in distilled water and peptone solution to survive with very little attenuation in numbers. This allowed the calculation of a meaningful kill ratio.

Samples of the Throat Spray, competitive products, distilled water control and in some cases pharmaceutical comparison products were mixed with enough beef peptone solution to bring the salt concentration to just below normal. Then, the prepared microbes were added at a known concentration. The microbial challenges were typically in the 10^6 to 10^7 range.

The samples were incubated at 37 deg. C for a period of 30 minutes. After this time, 0.1ml and 0.5ml samples were drawn from each tube and were plated on sheep blood agar. These agar plates were then incubated for 24 hours. At the end of this time, they were counted.

From the counts, CFU/ml and a kill ratio were calculated by comparison to the survivors in the control plates.

The tests were repeated a number of times in a comparison the Peaceful Mountain Throat Rescue product to a pure silver colloid, a competitive silver colloidal throat spray and two popular antibiotics. Although initial testing included a 250ppm mild silver protein, this comparison was dropped as the mild silver protein

was so notably ineffective. When compared to the antibiotics, the antibiotic concentrations were chosen to represent tissue concentration levels at or above that which is suggested based on MBC testing for that product. This was generally in the 10 to 30 microgram/ml range.

Comparison Test Results:

Product	Survival/ml	Log₁₀Kill/ml
30 mcg/ml Penicillin VK	2.9x10 ⁵	1.1
10 mcg/ml Erythromycin	2.6x10 ⁶	0.1
Peaceful Mountain Throat Rescue	<1	6.5
10 ppm Competitive Spray	2.4x10 ⁶	0.1
DI/Buffered Peptone	3.4x10 ⁶	N/A
Direct Challenge (10 ⁻⁶ of suspension)	3.4x10 ⁸	N/A

In Vivo Correlation Method:

In order to verify that the in vitro test was indeed a reasonable analog to the natural in vivo environment, a simple in vivo test was performed. Since it would be impractical to seed a population of volunteers with Strep. A Hemolytic due to the obvious health consequences, the in vivo test was performed on the natural fauna of bacteria found in the throats of our four volunteers.

The four volunteers were had throats swabbed prior to any product administration. This would serve as a baseline. Then each of the volunteers received a single treatment of their designated product. Ten minutes later, the throats were swabbed, then 10 minutes later, they were swabbed again.

The samples were cultured on sheep's blood agar for 24 hours. These were then counted and identified.

Quantification

Subject	Initial	10 minutes	20 minutes	Product
1	>2x10 ⁷	8.8x10 ⁵	>2x10 ⁷	Pure Colloid
2	1.7x10 ⁵	9x10 ⁴	8.9x10 ⁴	Source Natural Throat Spray
3	>2x10 ⁷	>2x10 ⁷	>2x10 ⁷	Chloraseptic
4	>2x10 ⁷	9.7x10 ⁵	2.9x10 ⁵	Peaceful Mountain Throat Rescue

Source Natural (1.7x10⁵-8.9x10⁴)/1.7x10⁵ = 48% reduction

Chloraseptic (2x10⁷-2x10⁷)/2x10⁷ = 0% reduction

PMTR (2x10⁷-2.9x10⁵)/2x10⁷ =>99% reduction

Discussion:

As can be seen in subject 1, the **pure colloid** offered a reduction in the bacterial load after 10 minutes. The population then returned to a beyond countable level. The **Source Natural Throat Spray** offered a 48% reduction in the natural fauna. The **Peaceful Mountain Throat Rescue** reduced the natural fauna by >99% and maintained this reduction to the 20 minute point. The **Chloraseptic** offered no detectable antimicrobial activity.

Indentification

Subject	Initial	10 minutes	20 minute
1	Streptococcus constellatus Streptococcus mitis Streptococcus intermedius	Streptococcus intermedius Streptococcus oralis	Neisseria sp. Streptococcus oralis Streptococcus intermedius
2	Neisseria sp. Streptococcus mitis	Neisseria sp. Streptococcus mitis	Neisseria sp. Streptococcus intermedius
3	Staphylococcus capitis Streptococcus intermedius	Staphylococcus aureus	Staphylococcus aureus Streptococcus intermedius Streptococcus constellatus
4	Staphylococcus epidermidis	Streptococcus mitis Streptococcus uberts	Streptococcus agalactiae

Summary Analysis:

The in vitro testing clearly showed the capability of the Peaceful Mountain Throat Rescue to kill a very heavy Strep. challenge. In test after test, the plates for the Throat Rescue showed no surviving bacteria.

The comparisons to Penicillin and Erythromycin showed the superiority of the Throat Rescue in its ability to kill Strep. on contact. Clearly the oral antibiotics would be preferred for dealing with a systemic infection. In a chronic systemic infection the heavy antibiotic dosing (1g/day) would offer longer term contact with the Strep. and would allow a more satisfactory response from the antibiotics. Since the Throat Rescue would be applied roughly 12 times/day, only to the site of the infection, the total dose would be <100mcg/day. This allows a very limited dose to offer dramatic results.

The in vivo test simply serves to show a reasonable parallel performance of the product in the target environment (human throat). To this end, it showed quite clearly that the Throat Rescue product kills a broad spectrum of bacteria in a manner that would be expected based upon the laboratory results.

Another note of interest is that since the salts of the beef peptone buffer will cause the silver to precipitate within 30 minutes, all of the killing that is done by the Throat Rescue product had to be accomplished in a very short period of time. It is this speed that is what makes the Throat Rescue so effective. Before it can be completely flushed from the throat environment, it has already killed hundreds of millions of bacteria.