Test 4: In vitro and in vivo testing of Super Neti Juice on Streptococcus

Purpose:

The testing reported herein was intended to evaluate the effectiveness of the Nature's Rite, Super Neti Juice product against Streptococcus A. Hemolytic bacteria. Quantifying the minimum bactericidal 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.

Methods:

Samples of the Super Neti Juice, 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° 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 of the Nature's Rite Super Neti Juice product to a simple silver colloid, a competitive silver colloidal product 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**	**Log_{10}Kill/ml**
30 mcg/ml Penicillin VK	$2.9x10^5$	**1.1**
10 mcg/ml Erythromycin	$2.6x10^6$	**0.1**
Super Neti Juice	<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 had their 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	$>2x10^7$	$9.7x10^5$	$2.9x10^5$	Super Neti Juice

Source Natural	$(1.7x10^5-8.9x10^4)/1.7x10^5$	= 48% reduction
Chloraseptic	$(2x10^7-2x10^7)/2x10^7$	= 0% reduction
Nature's Rite	$(2x10^7-2.9x10^5)/2x10^7$	=>99% reduction

Indentification

Subject	*Initial*	*10 minutes*	*20 minute*
1	Streptococcus constellatus Streptocuccus mitis Streptocuccus intermedius	Streptococcus intermedius Stretococcus oralis	Neisseria sp. Streptococcus oralis Streptococcus intermedius

2	Neisseria sp. Sterptococcus mitis	Neisseria sp. Streptococcus mitis	Neisseria sp. Streptococcus intermedius
3	Staphylococcus capitis Streptococcus intermedius	Staphylococcus aureus	Staphyloccus aureus Streptococcus intermedius Streptococcus constellatus
4	Staphylococcus epidermidis	Streptococcus mitis Streptococcus uberts	Streptococcus agalactiae

Conclusion:

The in vitro testing clearly showed the capability of the Super Neti Juice to kill a very heavy Strep. challenge. In test after test, the plates for the Super Neti Juice showed no surviving bacteria.

The comparisons to Penicillin and Erythromycin showed the superiority of the Super Neti Juice 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 Super Neti Juice could 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 shows that Super Neti Juice was the most effective throat spray to eliminate streptococcus. 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 Nature's Rite Super Neti Juice reduced the natural fauna by >99% and maintained this reduction to the 20 minute point. The Chloraseptic offered no detectable anti-microbial activity.

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 Super Neti Juice 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 Super Neti Juice product had to be accomplished in a very short period of time. It is this speed that is what makes the product so effective. Before it can be completely flushed from the treatment environment, it has already killed hundreds of millions of bacteria.