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# Certificate of Analysis

**Project:** "Comparative Bacteriology Analysis: Particulate vs. Ionic Silver"
**EMSL Reference Number: 240500586**

**Experimental Design Summary:**

The antibacterial activities of two different silver products were compared using two strains of *Staphylococcus aureus.* One silver product is composed of 95% silver ions ($Ag^+$) and the other is a suspension of particulate silver. The experiment was designed to test the inhibitory effects of gastric hydrochloric acid on the silver suspensions, based upon the hypothesis that it is the ionic silver that is antibacterial. The test protocol is dependent upon ionic silver being inhibited by hydrochloric acid (HCl) because of the reaction between $Ag^+$ and HCl to form AgCl, which would be inactive against the bacteria. It is also dependent upon the fact that particulate silver will not react with HCl.

**Experimental Protocol:**

Two strains of *Staphylococcus aureus*, one wild type/ normal strain (S1) and one antibiotic resistant Methycillin-Resistant *Staphylococcus aureus* (S2), were used as challenge organisms. Each organism was grown on a standard, all-purpose bacterial growth medium containing yeast extract, tryptone, NaCl, and agar. An overnight culture of each organism was suspended in sterile water and serially diluted to provide 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000 dilutions in sterile water.

Each silver product was diluted in sterile water to form 20 part-per-million (ppm) suspensions. The silver suspensions were mixed with three different concentrations of HCl at 9:1 ratios to form final concentrations of:

18 ppm Ag/ 3 ppm HCl  18 ppm Ag/ 4 ppm HCl  18 ppm Ag/ 7 ppm HCl

The silver solutions were then added to the bacterial preparations at 1:10 ratio to provide final Ag/HCl dilutions in the reaction tubes of:

1.8 ppm Ag/ 0.3 ppm HCl  1.8 ppm Ag/ 0.4 ppm HCl  1.8 ppm Ag/ 0.7 ppm HCl

Each silver preparation was added to a complete series of bacterial dilutions.

Negative controls consisted of bacterial suspension at each dilution with sterile water added instead of silver solution. Positive controls consisted of bacterial suspensions at each dilution treated with silver suspension without adding HCl (sterile water substituted for volume of HCl).

After the silver suspensions were added to the bacterial dilutions, the mixture was agitated and allowed to react for two time points, 4 and 8 minutes. The bacterial dilutions were then spotted on an agar plate in 10 microliter (µL) aliquots to test for growth. Agar plates were incubated at 35-37 degrees C overnight and observed qualitatively for growth.

Growth was graded grossly as +, ½, +/- and – for each bacterial dilution. This process seems to mimic the Minimum Inhibitory Concentration (MIC) technique used by EMSL Analytical Inc. to evaluate antibacterial activity of antibiotics or products, except that instead of varying the concentration of the antibacterial product, the bacterial concentration is the variable. In methods recognized in the industry, the bacterial concentration remains constant and the product dilutions are the variable. A classical MIC experiment determines which concentration of product inhibits a standard concentration of the organism, and does so quantitatively, not grossly as in this experiment.

**Findings and Conclusions:**

Results from this experiment indicate that HCl affects the antibacterial performance of the particulate silver suspension and HCl does not affect that of the ionic silver suspension. The negative control is used as a baseline measurement of untreated bacterial growth. Conclusions were drawn that the silver ions were responsible for antibacterial activity because concentrations of HCl used were responsible for binding all of the available Ag+ ions present in the particulate suspensions but not in the ionic suspension, because of the greater availability of ionic $Ag^+$ in the ionic suspension.

According to the investigators, it is the $Ag^+$ ions that are responsible for the antibacterial activity of the products, since there is a significant difference in the bacterial growth using particulate silver vs. ionic. However, when compared to in vivo conditions, this conclusion is not valid. Actual gastric levels of HCl are in the range of 3600 ppm[1], a $10^4$ increase over the parameters of this experiment. This experiment does not simulate actual in vivo usage conditions with respect to HCl concentrations. The discussion of the experiment refers to an earlier experiment that was used to determine the range of HCl concentrations used, but does not provide details of this rationale or discuss whether these concentrations do approximate gastric HCl. The discussion also mentions that this range of HCl is not the limiting factor and that the availability of $Ag^+$ ions is the limiting factor. That would only hold at this low concentration of HCl. But, the level of HCl might be the limiting factor in actual usage of the product, i.e., gastric HCl.

This experiment tests only the effects of HCl as an inactivating agent for these two products at one concentration of silver, 1.8 ppm. If actual usage levels of Ag were significantly higher, there may not be this complete quenching effect of HCl on the particulate silver. Since the concentration of Ag remained constant, this possibility cannot be evaluated from this experiment. An experiment to evaluate this variable would involve testing progressive dilutions of the Ag, keeping HCl constant. This should be repeated at different HCl

concentrations that actually reflect gastric HCl, providing various Ag/HCl combinations. This is the protocol involved in classical testing methods.

The fact that there were only two time points seems inconsequential in the conclusions drawn by the investigators because there is no significant difference in products at these time points. However, the narrow and unrealistically short time points do not reflect actual usage of either product. If there were a time-sensitive chemical reaction that would take place between particulate Ag and HCl over extended time involving $Ag^+$ ions, and their binding with HCl, or if, over time, particulate Ag provided antibacterial activity by another mode, these time points would be meaningless. From this data, conclusions regarding this possibility cannot be drawn. The product efficacy testing performed by EMSL involved standard methods for evaluation of antibacterial effectiveness that reflect the true nature of bacterial inhibition in vivo. This reaction is time-dependent on the bacteria and its interaction over time with the product, not the product's interaction with interfering agents before incubation. The only reaction involved in this experiment that could occur quickly is the combination of $Ag^+$ and HCl. But a maximum time point of 8 minutes is invalid when evaluating product efficacy.

In reviewing the product efficacy testing performed by EMSL using *Staphylococcus aureus* and MRSA, the concentrations of particulate silver products effective in inhibiting the growth of both strains was in the range of 1.0% to 10.0% of a 20 ppm suspension of silver (0.2-2.0 ppm). In this experiment, the final working concentration of 1.8 ppm is very close to the minimal inhibitory concentration for *S. aureus* as tested. It may be that the Ag concentration used was so close to the minimal antibacterial activity concentration for the particulate product, that even a small effect of HCl on the concentration of available $Ag^+$ was enough to reduce efficacy. That the ionic suspension is active at a lower concentration in the presence of insufficient HCl to quench its available ions is meaningless if taken out of context with the realistic parameters of usage. The greater inhibition of the organisms at this 1.8 ppm concentration of ionic silver than at the same concentration of particulate is probably because this concentration is so close to the inhibitory concentration for the particulate product. This is a parameter seemingly designed to mislead.

The objective of this experiment was to prove that it is the ionic $Ag^+$ that provides antibacterial activity. The experiment was not designed according to nationally standardized methods used to test product efficacy. The test parameters were designed outside of actual usage parameters and according to non-standardized methods. EMSL's experiments were designed according to widely accepted methods. Conclusions drawn from this experiment that ionic silver is less inhibited than colloidal by HCl at low concentrations for 8-minute exposure are meaningless with regard to product efficacy. To scientifically evaluate the effect of HCl on the two products, the experiment should be conducted according to verifiable methods generally accepted by the industry and should reflect actual conditions of usage.

[1] http://web.jjay.cuny.edu/~acarpi/NSC/7-ph.htm
http://www.newton.dep.anl.gov/askasci/zoo00/zoo00114.htm
http://www.phmeters.com/basic_ph_tutorial.htm

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