Comparative Bacteriology Analysis:
Particulate vs. Ionic Silver

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Purpose

Claims have been made by the manufacturer of Mesosilver that suggest "ionic" silver potency is compromised by Hydrochloric Acid (HCl) in the stomach, and that only the particulate [species], elemental silver (i.e. the primary content in Mesosilver) will survive and therefore be effective in inhibiting microorganisms. Whether or not HCl in the stomach is an issue is well beyond the scope of this paper. Herein we are going to deal with only one issue: the antimicrobial effect of 'particulate' (elemental) vs. (free) 'ionic' silver.

The hypothesis of Natural-Immunogenics, Corp. is that, contrary to the claims above, it is the silver ion [species] that is primarily responsible for silver's antimicrobial efficacy. Natural-Immunogenics, Corp.'s products (Argentyn 23 and Sovereign Silver) are composed in excess of 95% silver ions.

The purpose of this study, then, is to determine the antibacterial efficacy of both species, ionic vs particulate. This was to be achieved by comparing the two products, Argentyn 23 and Mesosilver, after the free ion content in both products was reduced or eliminated equally to the extent that only the particulate content in Mesosilver remained.
This of course would reduce the ionic content of Argentyn 23 by the same amount.

This was accomplished by first exposing both products to the same amount of HCl. Identical bacterial concentrations and dilutions of two strains of Staphylococcus aureus (S-1 and S-2) were then used to test each product. This testing was accomplished by exposing healthy strains of the bacteria (in dilution series) to the two products after adding 10µl of HCl solution (in various concentrations).

Materials and Methods
Summary:

The antibacterial activity of both Argentyn 23 and Mesosilver were compared by treating healthy cultures of the bacterial strains, separately, with each of the two products (to which was added 10µl of HCl solution, in various concentrations, to make 1 ml samples of each).

Source, cultivation, and preparation of bacterial samples:

1. The YT media was autoclaved and poured into sterile plates and allowed to dry.

2. Two strains of Staph received from the New York Hospital of Queens.
    - B14192 - wild type/ normal strain (S-1)
    - B14310 - antibiotic resistant MRSA (S-2)

3. The bacteria was grown on YT media for 16 to 24 hours before being harvested.

4. A 3 mm confluent inoculum of S-1 was resuspended in 1,250µl of sterile, 17 to 18 MegOhm (M?) purified lab water.

    - Then a standard 10:1 dilution series was performed on each strain, using sterile purified lab water
    - 1/10, 1/100, 1/1,000, 1/10,000, and 1/100,000 dilutions of each stock bacterial suspension were prepared.

5. The bacterial strain, S-2, was prepared identically, as was strain S-1.

Preparation of Test Media (YT):

1. Each 95mm sterile polystyrene culture plate (Petri dish) was filled with 5mm of YT media (which consists of 0.5% NaCl, 0.6% yeast extract, 0.8% tryptone and 2% agar).

Preparation of the silver products:

1. Argentyn 23 was diluted to 20 PPM (so as to have an equal concentration of silver) using sterile, 18 MegOhm (M?) purified lab water.

    - Mesosilver was determined (separately, by atomic absorption) to have a concentration of 20 PPM.

2. 9 parts of Argentyn 23 (final concentration 18 PPM) and one part of HCl (33, or 44, or 77 PPM, finally diluted to 3, 4, and 7 PPM, respectively) were added to eachother and mixed.

3. Mesosilver and HCl were added to eachother and mixed, as above.

Treatment of Bacterial Strains with Silver Products

1. 10 µl. of the silver product/ HCl mixture (9µl. each of Silver product and 1 µl. of HCl) were added to 90µl. of each of the sets of dilutions (1/100 through 100,000) of the two bacterial strain, above.

2. The mixture was agitated and allowed to react until spotted onto the YT plate.

    - One 10µl sample of each mixture of bacterial dilution/Silver/HCl of Strain S-1 was spotted onto the YT plates.

    - One 10µl sample of each mixture of bacterial dilution with Silver/HCl of Strain S-2 was spotted onto YT plates.

    - The exposure times were limited to 4 or 8 minutes, respectively, for each set of treatments.

3. This protocol was performed identically for Argentyn 23 and Mesosilver.

4. Negative control plate (positive growth) - No silver/HCl being added to S-1 or S-2 cultures. Sterile, purified water replaced the volume (10µl) of Silver/HCl.

5. Positive control plate - Treatment of bacterial strains with respective silver products without HCl.

6. Plates then placed in a 35°C to 37°C incubator overnight (16 to 24 hours).

Results

Qualitative results can easily be seen on each plate.

The negative control for S-1 grew out 4.5 spots, represented by (++++ ½), as did the negative control for S-2. These samples did not contain the silver or silver/ HCl mix. A (-) represents no Staph growth and (1/2) represents some Staph growth. The efficacy of the silver/HCl in the various ratios can be compared by the number of (+) vs. (-) spots observed.

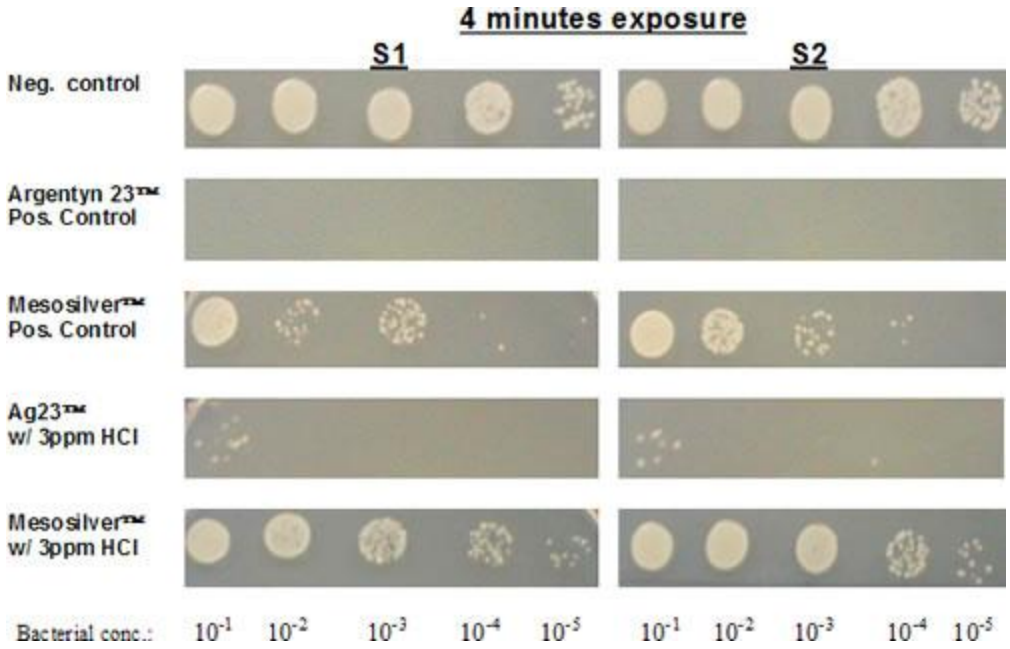
The positive control (silver and NO HCl) for S-1 and S-2. This should have shown the greatest degree of kill/inhibition (since there was no HCl to degrade/inactivate the silver.

A + designation is given for complete or near-complete spot-growth. A ½ is given for mottled/speckled appearing growth. A ½ is given for only a few spots of growth. A - is given for NO growth. The right-most + or - character represents a 1/100,000 dilution of the stock bacterial suspension; the left-most + or - represents a 1/10 dilution. The more - characters from the right, the more potent the antibacterial activity. The negative control (representing NO silver/HCl treatment should show 4 + and one ½ characters, demonstrating the viability of the untreated bacterial employed.

Inhibition results

	S-1 4min	S-2 4min	S-1 8min	S-2 8min
Negativecontrol	++++ ½	++++ ½	++++ ½	++++ ½
Argentyn23, 0 HCl	- - - - -	- - - - -	- - - - -	- - - - -
Ag23 w/ 3ppm HCl	+/- - - - -	+/- - - - -	- - - - -	½ - - - -
Ag23 w/ 4ppm HCl	+ - - - -	+ - - - -	+ - - - -	+ - - - -
Ag23 w/ 7ppm HCl	+ - - - -	+ - - - -	+ - - - -	+ - - - -
Mesosilver, 0 HCl	+½ ½ +/- -	++½ +/- -	+ ½ ½ - -	+ ½ ½ - -
Meso w/ 3ppm HCl	+++½ +/-	+++½ +/-	++ ½ ½ -	++++ ½
Meso w/ 4ppm HCl	++++ ½	++++ ½	+++ ½ -	++++ ½
Meso w/ 7ppm HCl	++++ ½	++++ ½	++++ ½	++++ ½

The photographs of the results for the 4 minute exposure of the Silver/HCl (at 3 PPM) are shown below. The observations of the 4 PPM and 7 PPM and for both 4 and 8 minute exposures of Silver/HCl mixtures were virtually identical to those for the illustrated 3 PPM HCl (shown below).



Discussion

The concentrations of HCl added to each of the two products were such that the concentrations of HCl in the final volumes would be 3ppm, 4ppm, and 7ppm. The range of concentration was determined in an earlier experiment. These concen-trations were selected because, if the HCl were to react with the silver, the conversion of Ag+ to AgCl would not make HCl the limiting reagent. The amount of remaining active silver would be the true limiting factor.

The following conclusions can be drawn from the data:

1. It is evident from the results that the antimicrobial efficacy of both products decreases as the concentration of HCl increases.

2. When comparing the plates where no HCl was added, it can be seen that Argentyn 23™ has far greater antibacterial efficacy than Mesosilver™. Mesosilver's™ antibacterial efficacy is almost negated by adding just 4ppm of HCl, whereas Argentyn23™ 's antibacterial efficacy is only slightly reduced but still strong, even with the addition of 7 PPM HCl.

3. Adding HCl to the silver products, causes the chloride ion to bind the silver ion forming Silver chloride (AgCl). Since Argentyn23™ is primarily an ionic product there is still a sufficient number of Ag+ ions left to kill the bacteria.

4. Mesosilver™ on the other hand is primarily particulate in nature, and has significantly little (compared to Argentyn 23™) silver ion content. Once the silver ions in Mesosilver™ react with the chloride ions, they become almost completely inactivated. We see an almost complete loss of antimicrobial activity when Mesosilver™ is exposed to even modest quantities of HCl.

5. This experiment serves as proof that **it is the active, available silver ions** – not the particulate silver – in which the antibacterial property of silver resides.

An Afterward

Clearing the Confusion Spread by Mesosilver

Date: May 25, 2005

Natural-Immunogenics Corp. addresses the obfuscations published by Mesosilver relative to its study: "**Particulate vs. Ionic Silver – The End of the Debate**".

In its website, Mesosilver gives quite a good definition of "techno-babble"; unfortunately, the definition is the only thing about which it was correct. Its entire posting about the recent Natural-Immunogenics Corp. study conforms perfectly to its definition of "techno-babble."

We apologize for the length of this response. However, misinformation should not be left unchallenged.

The Purpose and Design of the Natural-Immunogenics Corp. Study:

Let us get the purpose of the study straight. We quote from the Natural-Immunogenics Corp. study:

"The purpose of this study, then, is to determine the antibacterial efficacy of both species, ionic vs. particulate. This was to be achieved by comparing the two products, Argentyn 23 and Mesosilver, after the free ion content in both products was reduced or eliminated equally to the extent that only the particulate content in Mesosilver remained. This, of course, would reduce the ionic content of Argentyn 23 by the same amount."

It is clear from the above that our purpose was NOT to compare products, nor to mimic physiological or in vivo conditions. The manufacturer of Mesosilver keeps ignoring this. Obviously he doesn't like the results, or he would not have tried to cloud the issues and discredit the Natural-Immunogenics Corp. study.

We chose Mesosilver™ for this experiment because it is typical of what is known in the marketplace as 'particulate' [colloidal] silver, as the site itself proclaims. Argentyn 23™, on the other hand, is typical of what is known, perhaps simplistically, as 'ionic' [colloidal] silver (analyzed at the U. of Miami as being approximately 97% ionic).

So now, let us continue to clear away the mists of confusion generated by Mesosilver and its hired lab, EMSL Analytical, Inc., point-by-point. Perhaps we can help them to understand scientific method at the same time. We will accomplish this by addressing the most egregious of their errors, quoting from the website, communications with the Silver List, and the "intellectual analysis" that was commissioned.

- **"The experiment was not designed according to nationally standardized methods used to test product efficacy."**

We have already addressed the study's purpose. We reiterate: it was not to test products, but to test species [of silver]. Can't Mesosilver or EMSL Laboratory see this?
Now, about the study design…. For a study to be accurate and valid, it does not have to be based upon a standard test procedure. Science 101, even grade school science, teaches that, for a scientific study to be valid, it must be:

1. Accurate,

2. Precise,

3. Controlled,

4. Reproducible, and, of course,

5. Even handed. Which means that, as in this case, comparative reactants must be treated identically, and equalized/normalized for concentration:

The more concentrated material (Argentyn 23 - 23 PPM), was diluted to the concentration of the less concentrated material (Mesosilver - 20 PPM). Both were tested at 20 PPM.

The microbial concentrations of the two S. aureus strains were adjusted to the same concentration (by spectrophotometrically-measured % transmittance), and then confirmed by actual plate count of dilutions of the stock suspensions. Each of the silver specimens was challenged by the same stock suspension of each S. aureus strain.

In order to understand how silly the idea is that no test method is valid unless it is nationally or internationally recognized, consider the following: Before the advent of modern analytical equipment, the pigeon vomiting reflex was the standardized method of evaluating batches of digitalis for potency! Thankfully for the pigeons and for heart patients, better new analytical methods have been developed! The FDA approves non-standard test methods all the time - after they are proven/validated as accurate, precise and reproducible, etc. A newly developed test method for assaying something new cannot yet be a national standard, but it can certainly be valid.

The test method described in the Natural-Immunogenics.com-published article has been thoroughly validated.

Are the test methods and comparative studies which Mesosilver does in house and those of its hired laboratory validated to the standards of 21 CFR part 58 (Good Laboratory Practices) and/or 21 CFR part 211 (cGMP regs.) ? Come to think of it, Mesosilver doesn't publish comparative studies, does he? Is it because they know something they don't want anyone else to know?

- **By Mesosilver: "The test parameters were designed outside of actual usage parameters"**

- **By EMSL: "The narrow and unrealistically short time points do not reflect actual usage of either product"**

Absolutely correct! Both Mesosilver and EMSL seem to be very concerned about this. However, their objections ignore fundamental principles that are well known in the scientific disciplines of enzyme kinetics and the pharmacokinetics of biologically active substances: that efficacy is determined by overall potency (a complex combination of concentration, molecular structure, surface area, etc.) and by related exposure/ response time (our italics). All silver species (products) will kill bacteria given a sufficiently long exposure. Only the most potent will kill in

shorter exposure times. It is for this reason that the parameter of exposure time selected becomes the primary criteria for differentiating the bactericidal effect of the silver species. This parameter is totally independent of concentrations and proportions of experimental reactants.

Consider the logic: You have two products, both of the same concentration, both exposed to the same number of microorganisms and for the same duration of time. But one product either kills more microorganisms, or kills the same number in a shorter exposure time…. would you not, logically, conclude that the product killing MORE microorganisms FASTER was more potent? Obviously!

The EMSL analyst ignores this reality and wants readers to give Mesosilver more exposure time…Why? Because it obviously needs more exposure time (to kill the same number of microbes Argentyn 23 kills faster). But, again, the purpose of the study was not to compare products, only species.

- **"Used parameters that were "seemingly designed to mislead".**

Well, the EMSL reviewer did not dispute the experimental controls used in the study. However, the reviewer did ignore the test reality that both species [the particulate of Mesosilver and the ionic of Argentyn 23] were exposed to the same identical conditions, to the same numbers of microorganisms and for the same duration of time. Yet the EMSL reviewer does not mention the fact that the ionic species outperformed the particulate species. Fact is: Argentyn 23 killed all S. aureus microorganisms with only a 4 minute exposure (and, of course, again, with the 8 minute exposure) while the particulate species product, Mesosilver™, was only marginally bactericidal.

- **"This experiment tests only the effects of HCl as an inactivating agent for these two products at one concentration of silver."**

Again the **true** purpose of the study is obscured. It had nothing to do with testing the effects of HCl on silver; that effect is already a well-known and accepted fact. HCl was used only as a tool, and only at a concentration, to precipitate out the ionic content in Mesosilver, and (at the same time) the equally proportionate ionic content in Argentyn 23. Thus the experiment design ensured that only Mesosilver's ™ 'particulate' silver remained.

Obviously, the EMSL reviewer was grasping at straws to explain why the performance of Argentyn 23 was not significantly diminished under identical conditions.

- **One EMSL straw: "The only reaction involved in this experiment that could occur quickly is the combination of Ag + and HCl. But a maximum time point of eight minutes is invalid when evaluating product efficacy."**

If this were true, then why did Argentyn 23 bring about complete microbial kill and Mesosilver did NOT? Obviously, 4 minutes is sufficient for Argentyn 23 to kill the massive numbers of S. aureus organisms used as a challenge in this study.

- **A second EMSL straw: "In this experiment the final working concentration of 1.8 ppm is very close to the minimum inhibitory concentration for S. aureus as tested. It may be that the Ag concentration used was so close to the minimum and antibacterial activity concentration for the particulate product..."**

Is 1.8 ppm close to the minimum inhibitory concentration (MIC) for S. aureus? Perhaps for the less potent 'particulate' product, MesoSilver, this is true. It is clear that, from the test results of the Natural-Immunogenics Corp. study, the MIC for S. aureus using Argentyn 23 would be much less ! (By the way, the 1.8 PPM silver alluded to is the final, experimental concentration used for both Argentyn 23 and Mesosilver reaction mixtures, including microbes and HCl).

- **A third straw: "If actual usage levels of silver were significantly higher there may not be this complete quenching effect of HCL on the particulate silver."**

Apparently, the EMSL reviewer thought that more Mesosilver™ in the reaction mixture would overwhelm the amount of HCl used, permitting sufficient, unconverted, ionic silver to then act as the reactive bacteria-killer. ONCE MORE, the purpose of the study was to determine the effect of eliminating the ionic silver from the particulate silver product so that we could determine once and for all whether it is 'ionic' or 'particulate' silver that is primarily responsible for silver's antimicrobial activity. The unassailable answer is that it is the ionic, not the particulate component of silver that is responsible for silver's antimicrobial activity!

- **The last EMSL straw: "This experiment does not simulate actual in vivo usage conditions with respect to HCl concentrations."**

Repeating now, so many times, HCl was used as a tool, not to simulate in vivo conditions.

The barrier of straw that Mesosilver and EMSL try to erect to obscure/hide the findings and realities of the Natural-Immunogenics Corp.'s study is blown away by the study's valid science and its logic.

- **But look at this from EMSL: "Results from this experiment indicate that HCl affects the antibacterial performance of the particulate silver suspension and does not affect that of the ionic silver suspension."**

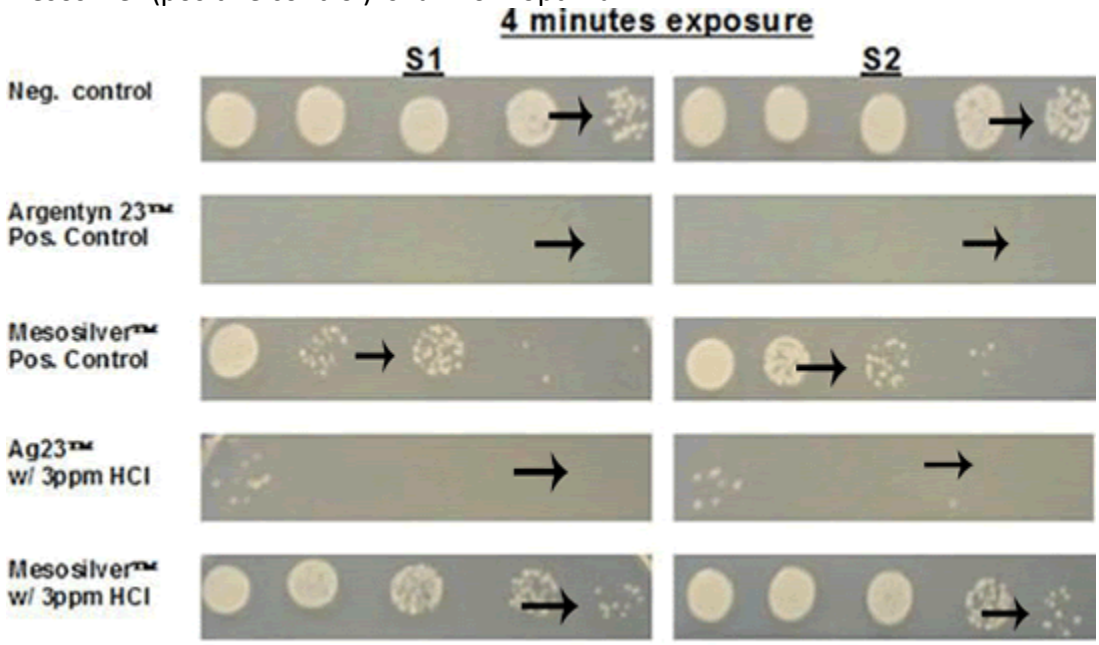
Not quite right, EMSL**!** Although the study proved the superiority of 'ionic' over 'particulate' silver relative to antimicrobial efficacy, it also suggested that greater quantities of HCl would, in fact, progressively diminish the 'ionic' product too. But Mesosilver's antibacterial performance was so devastated by the low concentration of HCl because its antibacterial performance is only due to the minor ionic silver content in it. THAT is the real issue that has been resolved: that

antimicrobial potency is NOT, as claimed by Mesosilver, due to particulate silver, but, rather to *ionic* silver!

It is unfortunate that Mesosilver performed so poorly under scientifically controlled conditions. It is doubly unfortunate for this industry, and for the public at large, that Mesosilver has not been adequately taken to task before for the nonsense it contributes to the public understanding of colloidal silver.

Unavoidable Conclusions:

As can be seen below (reproduced from the Natural-Immunogenics Corp. study):
Removal of the ionic silver content from Mesosilver, by the addition of only 3 ppm HCl, diminished its antibacterial potency to virtually nothing. Even untreated, the performance of Mesosilver (positive control) is far from optimal.



Bacterial conc: 10*5* 104 103 102 101 105 104 103 102 101
Dilution factor: 10-1 10-2 10-3 10-4 10-5 10-1 10-2 10-3 10-4 10-5

In the photos above, [taken from the published experimental paper], each spot, from left to right, is a ten-fold dilution of the preceding spot of the two cultures used. On the left (S1) is Wildtype Staph. aureus; and on the right (S2) MRSA (Methicillin-resistant S. aureus). Each spot cleared from the right represents 10 times more bacteria killed; a 2 spot difference is equal to 100 times more killed, etc.). The more blank (non-growth) spots from the right, the greater the bacterial kill.

The principal conclusions from the study, as illustrated by the photos, above are:

- Before being treated with HCl (as was also shown in the experiment), Argentyn 23 achieves total kill in four minutes, and is at least 100 times more potent than untreated Mesosilver.

- Mesosilver loses almost all its antibacterial potency when the ionic silver component is removed.

- Comparative antibacterial potency loss by Argentyn 23, due to the same HCl treatment, is negligible.

- After being treated with HCl, under the same experimental conditions, Argentyn 23 is now up to 10,000 times(a4 spot difference: 10X10X10X10) more potent than Mesosilver.

- Therefore, it is obvious from the Natural-Immunogenics Corp. study:

The almost total loss of antibacterial activity (when the ionic component has been removed from particulate Mesosilver), proves that 'ionic', NOT 'particulate', silver is the basis of silver's antibacterial activity. The lack of ionic content after HCl treatment was the limiting factor in Mesosilver's poor performance.

This should be instructive to Mesosilver's audience, since Mesosilver has been espousing the opposite -- without proof -- for years. During which time, the manufacturer of Mesosilver has discredited other products and represented it as the only true colloid, and one that outperforms all the others. Therefore…..

We would like make a modest proposal…

Let us unquestionably resolve the 'differences of opinion' in the only meaningful way possible. Thus we, Natural-Immunogenics Corp., challenge Mesosilver (and any other silver product manufacturer for that matter) to*:*

- Submit their product to a scientifically-designed study, to be performed by a FDA-licensed preclinical testing laboratory which has, to date, done no business with any of the parties (the cost would be shared by the participating companies);

- The design of the study would be agreed upon by the participating companies and the FDA-licensed preclinical testing laboratory's Study Director and would focus upon maximizing the differentiation of antimicrobic performance… period!

- Such differentiation would be accomplished by test method modification of normalized product concentration (in PPM), microbial challenge level, and/or by exposure time.

- Results may be freely published by either party with the express approval of the other.

Who will join Natural-Immunogenics Corp. in challenging Mesosilver to agree to this comparative study?

We are ready to seek and agree upon a laboratory. Is Mesosilver?

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http://www.hydrosolinfo.com/articles/silver-2001-09-17.php
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