

American Biotech Labs

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Summary Of Current American Biotech Labs Product Safety Studies

ABL Product Safety Studies

1) Cyto-toxicity At Both 10 And 22 Parts Per Million (PPM).

The study found that the product at both 10 ppm and 22 ppm silver, had no negative effect on either human or Vero (African Green Monkey) cells. The product was determined to be Non-toxic to the cells.

2) LD 50 At Up To 200 Times The Normal Adult Dosage.

The study found that the product would not hurt or negatively effect the test animals at very high dosages, up to 200 times the amount normally taken by adults. The product was deemed non-toxic to the test animals even at the high levels tested.

3) Injected In Vivo Tests Of ASAP 10 PPM Silver.

The ASAP 10 ppm silver product was injected into the animals at a dose of 50 ml/kg body weight. The animals were checked at 4, 24, 48, and 72 hours. The study found that the product when injected into the test animals caused no negative reaction and thus was deemed non-toxic to the test animals at that level.

4) Injected In Vivo Tests At 32 PPM Silver.

The ASAP-AGX-32 disinfectant product was injected into the animals at a dose of 50 ml/kg body weight. The animals were checked at 4, 24, 48, and 72 hours. The study found that the product when injected into the test animals caused no negative reaction and thus was deemed non-toxic to the test animals at that level.

5) Ingested In Vivo Tests Of AGX-SilGel At 32 PPM Silver.

The AGX-SilGel 32 ppm silver product was given orally to the animals at a dose of up to 5,000 ml/kg body weight. The animals were tested at levels of 50, 500, and 5,000 ml/kg body weight. The study found that the product, when given orally to the test animals at a number of dosage levels caused no negative reactions at any of the tested levels and thus was deemed non-toxic to the test animals at all the levels tested.

6) Selective Inaction Of ASAP On Probiotics (Viridus BioPharma).

The study tested the product against a number of friendly bacteria. The type of bacteria the body uses to aid in the digestion process. It was found in the study, that the product at both 10 ppm and 22 ppm would not even inhibit the growth of probiotic bacterium. In conclusion the report states, "The results bring forth ASAP as an "antibiotic of choice" natural antibiotic, with firstly no side effects such as diarrhea and in fact will not disturb the body's natural host defense mechanism. It indeed complements therapy by sparing essential host microflora as well as concomittant oral lactobacilli therapy normally given as an adjunct."

7) Selective Antimicrobial Activity of ASAP-AGX- 32 Silver Solution Against Probiotics (Dr. Ron Leavitt).

The study found that the silver product would not harm probiotic bacterium at the levels at which it is being used inside the body. In conclusion the report states, "It can therefore be concluded that the consumption of probiotics in conjunction with ASAP silver solution would be beneficial to the health of ill and healthy people."

VIRIDIS BioPharma

**Report prepared for
American Biotech Laboratory**

**Viricidal Activity of ASAP
against Hepatitis B Virus
&
Cytotoxicity of ASAP
March 2003 – June 2003**

By:

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4 Test Procedure for Cytotoxicity

Cytotoxicity:-

Cytotoxicity test was carried out to evaluate toxic effects of ASAP in an *in vitro* system using two models Vero cells (African Green Monkey Cell line) and Hep2 (Human epithelial cell)

4.1 Aim

This Assay Method describes an *in vitro* test method for assaying cytotoxicity of ASAP solution.

4.2 Principle

Two freshly prepared cell cultures; Vero & Hep2 were inoculated with ASAP solutions and incubated under CO₂ environment. Cytopathic effects, if any are observed under inverted microscope. Cell culture with the addition of Phosphate Buffer Saline (PBS) served as reference control.

4.3 Equipment

1. Incubator having 5% CO₂ environment
2. Inverted microscope
3. Syringe
4. Microtitre wells
5. Autoclave

4.4 Materials

1. Fetal Calf serum
2. Vero cell line (African Green Monkey Cell line)
3. Hep2 (Human epithelial cell)
4. Minimum essential medium

9.61 g MEM 16 with Earle's salts

2.2 g sodium bicarbonate (NaHCO_3)17

Dissolve reagents in above two materials in 900 ml deionized water (DW).

Add 5.0 g lactalbumin hydrolysate or edamin 18 to 10 ml DW, and heat to $60^\circ \pm 2^\circ\text{C}$ until dissolved. Add to above 900ml solution with constant mixing.

4.5 Procedure

Cells are prepared from healthy, confluent Vero cells and Hep2 cells that are maintained by passing every 3 to 4 days. One day prior to test initiation, using a self-refilling repetitive syringe, cells suspended in Growth Medium are dispensed into wells. Incubate at $37^\circ \pm 2^\circ\text{C}$ in a 5% CO_2 incubator for 72 ± 12 hr.

100 μl of each substance to be tested was introduced into wells in triplicates. 100 μl of PBS served as positive control. The cell lines were reincubated at $37^\circ \pm 2^\circ\text{C}$ in a 5% CO_2 incubator for 72 ± 12 hr. Every 24 hrs wells were examined under high power of an inverted microscop to check for cytopathic effect (CPE).

Schematic Representation

Cultivate the susceptible cell lines (Vero, Hep2) in Minimum essential medium containing 10% Fetal Calf serum



Monolayer of a susceptible cell line



Inoculate 0.1 ml of the sample in the required number of wells.



Inoculate sterile 0.1 ml PBS into one of the wells as control



Incubate at 37°C, under 5% CO₂ for 72 hrs



Observe under high power for cytotoxicity (Cytopathic effect)

VIRIDIS

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Cytotoxicity Test Results

Sample Source American Biotech Lab

Sample Description ASAP 10 ppm (Lot # 02198)
ASAP 14 ppm + 1.5% H₂O₂ (No lot #)
ASAP 22 ppm (Lot # 02193)

Date of Analysis 5th May 2003

<u>Sample</u>	<u>Vero cell line</u>	<u>Hep2 cell line</u>
ASAP-10	No CPE	No CPE
ASAP-14 + 1.5% H ₂ O ₂	CPE - positive	CPE - positive
ASAP-22	No CPE	No CPE
PBS; (Control)	No CPE	No CPE

CPE - Cytopathic Effect


Quality Assurance

4.6 Conclusion:

Distinct cytopathic effect were observed in cell lines with ASAP-14. In fact, on addition of 100µl to the wells total bleaching of the vital indicator occurred. Cytopathic effects were noted as follows:

1. Rounding of cells
2. Granulation of cell cytoplasm
3. Detachment of cell monolayer from well surface.

The above can be easily seen in the attached photomicrographs (Pg. 22 & 23).

Cell lines treated with ASAP-10 & 22 were indistinguishable from the control indicating no cytotoxicity. ASAP 14 ppm displayed cytotoxicity which is likely due to H₂O₂.

(2)

**LD 50 At Up To 200 Times The
Normal Adult Dosage.**

Non-Toxicity Test Work Summary

In order to insure not only the best product, but also a safe product, American Silver LLC hired an international and independent laboratory to do a toxicology study on the ASAP Solution. The test, called an LD-50 test, was performed in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500.

In the test work, the ASAP Solution was given to a number of both male and female test rats. The amount of ASAP Solution given to the rats was 5g/kg of 22 ppm solution, or the equivalent of a 200-pound man taking 192 teaspoons or about 4 full 8 ounce bottles of the ASAP 10 ppm solution at one time (the normal adult dosage is one or two teaspoons/day).

As a result of the test work, the independent laboratory made the following conclusion, **“Under the conditions of this study, there was no mortality or significant evidence of toxicity observed in the rats. The test article (ASAP Solution) would not be considered toxic at a dose of 5g/kg by oral route in the rat.”**

STUDY TITLE:

ACUTE ORAL TOXICITY STUDY IN THE RAT

(FHSA Method)

TEST ARTICLE:

ASAP Solution

IDENTIFICATION NO.:

22 ppm Silver

TEST FACILITY:

**NAMSA
California Division**

SPONSOR:

**WILLIAM MOELLER
AMERICAN SILVER
70 WEST CANYON CREST RD.
SUITE B
ALPINE, UT 84004**

NAMSA

**Emerging Medical Device
Safety and Compliance**

Corp. Hdqrs: 2261 Tracy Road, Northwood, OH 43619-1397 / 419.886.9455 / Fax 419.886.2954
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9 Morgan, Irvine, CA 92618-2078 / 949.951.3110 / Fax 949.951.3280
Affiliates: France • Germany • Taiwan

SUMMARY

The test article, ASAP Solution, Identification No. 22 ppm Silver, was evaluated for oral toxicity in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500. A single dose of 5 g/kg of body weight was gavaged to 10 rats. The animals were then observed for up to 14 days for any signs of toxicity.

Under the conditions of this study, there was no mortality or significant evidence of toxicity observed in the rats. The test article would not be considered toxic at a dose of 5 g/kg by the oral route in the rat.

**Study and Supervisory
Personnel:**

Gina M. Johnson, B.A., LAT
Lubica Mikula, B.S.
America Salvador, B.S., LAT
Brenda Gonzales, A.S., LVT
Enrique Vazquez
David Vergil

Approved by:



Jackie Nichols, B.S.
Study Director, Toxicology

2-2-95
Date Completed

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**Injected In Vivo Tests Of ASAP 10
Parts Per Million**

Shri Vile Parle Kelavani Mandal's
SHRI C. B. PATEL RESEARCH CENTRE
FOR
CHEMISTRY AND BIOLOGICAL SCIENCES
Vile Parle (West),
Mumbai-400 056.

REPORT ON THE PROJECT

TO EVALUATE THE USP SYSTEMIC INJECTION TEST OF
ASAP SOLUTION IN MOUSE MODEL.

SPONSORED BY

VIRIDIS
VIRIDIS BioPharma Pvt. Ltd.,
6/10, Jogani Industrial Complex,
V. N. Purav Marg,
Chunabhatti, Mumbai-400 022.

Principal Investigator

Dr. A. M. Bhagwat

Co-Investigator

Mrs. Avanti S. Joshi



I. INTRODUCTION:

OBJECTIVE: To evaluate the USP systemic injection test of ASAP solution in mouse model.

STUDY GUIDELINES:

Study was conducted in full compliance with the guidelines laid down in "Requirements and Guidelines on Clinical Trials for Import and Manufacture of New Drug". under Schedule Y of The Drugs and Cosmetics Act, 1940, Government Of India.

STUDY PERSONNEL:

- 1) Dr. A. M. Bhagwat.
- 2) Mrs. Avanti S. Joshi

II. MATERIALS AND METHODS:

Test Article : ASAP solution.

TEST SYSTEM AND MANAGEMENT:

Test system : Mouse

Strain : Swiss albino mice

Source : Haffkine's Laboratory, Parel.

Age : 25 to 30 weeks.

Identification : By cage tags.



- No. of animals : 6 mice, per dose per group
- Acclimatization : At least one week in the experimental room after veterinary examination.
- Randomization : After acclimation and veterinary examination, the mice were randomly selected in mixed groups of both males and females.

Husbandary:

Environmental conditions: Temperature of the animal house was maintained in the range of 20 - 25 °C. Relative humidity close to 60 %. The mice were exposed to natural day-night cycles.

Accommodation : Groups of six in polypropylene cages with stainless-steel grill-top, facilities for food and water-bottle and bedding of clean paddy husk.

Diet : Standard pelleted rodent feed manufactured by Lipton Ltd., *ad libitum*.

Water : Water, supplied by Brihan Mumbai Municipal Corporation, filtered and kept in glass bottles, *ad libitum*.

III. STUDY DESIGN:

The study was designed to permit the assessment of USP systemic response to ASAP solution following injection into mice. In this method, the compound to be analyzed was injected at a dose of 50 ml / kg body weight.



The drug was directly injected into the peritoneal cavity in case of the experimental animals, whereas saline (0.9 %) was injected into the peritoneal cavity of the control animals.

The test material used for the analysis meets the USP requirements if none of the animals treated with the sample show a significant biological reactivity than those treated with blank. If two or more animals die or show an abnormal behavior, the test material does not meet the USP requirements.

EXPERIMENTAL :

At '0' time, the animals were given a single intraperitoneal injection, of ASAP solution 50 mg / kg. Animals were observed immediately after injection and then after 4, 24, 48 and 72 hours following injection. At the end of 72 hours, the animals were put to sleep and dissected to observe changes, if any, in gross anatomy.

IV. RESULTS AND OBSERVATIONS :

All the animals used in the experiment survived through the 72 hr observation period. No toxic effects were observed. The animals under observation showed normal feeding, drinking and grooming behavior.

At the end of 72 hours these animals were put to sleep and observed for gross anatomical changes. All mice administered the drug intra - peritoneally showed normal size, texture and colour of the organs such as liver, spleen, kidney, alimentary canal, lungs, heart, gonads etc. The average weight of the animals at the 0 hour was recorded as 33.2 g, whereas at the end of 72 hours, it averaged 33.4 g.



(4)

**Injected In Vivo Tests At 32
Parts Per Million.**

SHRI VILE PARLE KELVANI MANDAL'S
SHRI C. B. PATEL RESEARCH CENTRE FOR
CHEMISTRY AND BIOLOGICAL SCIENCES
VILE PARLE (WEST), MUMBAI - 400 056

REPORT ON THE PROJECT
TO EVALUATE THE SYSTEMIC INJECTION TEST
OF AgX (32 ppm) SOLUTION ADMINISTERED
THROUGH THE INTRA - PERITONEAL ROUTE IN
MICE

SPONSORED BY
VIRIDIS BIOPHARMA PVT. LTD.,
6 / 10, JOGANI INDUSTRIAL COMPLEX, V. N. PURAV
MARG, CHUNABHATTI, MUMBAI - 400 022.

PRINCIPAL INVESTIGATOR : DR. A. M. BHAGWAT

CO - INVESTIGATOR : MRS. AVANTI S. JOSHI



I. INTRODUCTION:

OBJECTIVE:

To evaluate the USP injection test of AgX (32 ppm) solution administered through intra - peritoneal route in mice.

STUDY GUIDELINES:

Study was conducted in full compliance with the guidelines laid down in "Requirements and Guidelines on Clinical Trials for Import and Manufacture of New Drug". under Schedule Y of The Drugs and Cosmetics Act, 1940, Government Of India.

STUDY PERSONNEL:

- 1) Dr. A. M. Bhagwat.
- 2) Mrs. Avanti S. Joshi

II. MATERIALS AND METHODS:

Test Article : AgX solution (Viridis Biopharma)

TEST SYSTEM AND MANAGEMENT:

- | | |
|-----------------|--|
| Test system | : Mice |
| Strain | : Healthy young male and female Swiss albino mice |
| Source | : Haffkine's Laboratory, Parel. |
| Age | : 25 to 30 weeks. |
| Identification | : By cage tags. |
| No. of animals | : 6 mice, per dose per group |
| Acclimatization | : At least one week in the experimental room after veterinary examination. |
| Randomization | : After acclimation and veterinary examination, the mice were randomly selected in mixed groups of both males and females. |



HUSBANDARY :

- Environmental conditions :** Temperature of the animal house was maintained in the range of 20 - 25 °C. Relative humidity close to 60 %. The mice were exposed to natural day-night cycles.
- Accommodation :** Groups of six in polypropylene cages with stainless-steel grill - top, facilities for food and water-bottle and bedding of Clean paddy husk. Standard hygiene procedures and animal welfare guidelines as prescribed by the Institutional Ethics Committee were followed
- Diet :** Standard pelleted rodent feed manufactured by Lipton Ltd., *ad libitum*.
- Water :** Water, supplied by Brihan Mumbai Municipal Corporation , filtered and kept in glass bottles, *ad libitum*.

III. STUDY DESIGN:

The study was designed to permit the assessment of USP systemic response to AgX solution following injection into mice. In this method, the compound to be analyzed was injected at a dose of 50 ml / kg. body weight.

The drug was directly injected into the peritoneal cavity in case of the experimental animals, whereas saline (0.9 %) was injected into the peritoneal cavity of the control animals.

The test material used for the analysis meets the USP requirements if none of the animals treated with the sample show a significant biological reactivity than those treated with blank. If two or more animals die or show an abnormal behavior, the test material does not meet the USP requirements.

EXPERIMENTAL:

At '0' time, the animals were given a single intraperitoneal injection, of AgX solution. Animals were observed immediately after injection and then after 4, 24, 48 and 72 hours following injection. At the end of 72 hours, the animals were put to sleep and dissected to observe changes, if any, in gross anatomy.



The organs such as liver, spleen, kidney, alimentary canal, lungs, heart, gonads were sent for histopathology. Each organ was fixed in 10 % buffered formalin, embedded in wax and cut at 5 μ thickness. The sections were stained with eosin - haematoxyline and mounted in DPX for microscopic examination.

IV. RESULTS AND OBSERVATIONS:

All the animals used in the experiment survived through the 72 hr observation period. No toxic effects were observed. The animals under observation showed normal feeding, drinking and grooming behavior.

At the end of 72 hours these animals were put to sleep and observed for gross anatomical changes. All mice administered the drug intra - peritoneally showed normal size, texture and colour of the organs such as liver, spleen, kidney, alimentary canal, lungs, heart, gonads etc. Histological observations indicate that the non - specific changes exhibited were comparable to those observed in control animals. The average weight of the animals at '0' hour was recorded as 35 g, whereas at the end of 72 hours, it averaged 35.4 g.

V. CONCLUSIONS:

From the above observations it can be concluded that the test material AgX (32 ppm) solution of Viridis Biopharma meets the USP requirements.



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**Ingested In Vivo Tests At 32 (SilGel)
Parts Per Million.**

Shri Vile Parle Kelavani Mandal's
SHRI C. B. PATEL RESEARCH CENTRE
FOR
CHEMISTRY AND BIOLOGICAL SCIENCES
Vile Parle (West),
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REPORT ON THE PROJECT

TO DETERMINE THE ACUTE TOXICITY OF SILGEL
ADMINISTERED THROUGH ORAL ROUTE IN MICE.

SPONSORED BY

VIRIDIS
VIRIDIS BioPharma Pvt. Ltd.,
6/10, Jogani Industrial Complex,
V. N. Purav Marg,
Chunabhatti, Mumbai-400 022.

Principal Investigator

Dr. A. M. Bhagwat

Co-Investigator

Mrs. Avanti S. Joshi



INTERIM REPORT – ACUTE TOXICITY OF SILGEL (B. No. – AS/RD/002)

I. INTRODUCTION:

OBJECTIVE:

To determine the acute toxicity of Silgel (B. No.- AS/RD/002) administered through oral route in mice.

STUDY GUIDELINES:

Study was conducted in full compliance with the guidelines laid down in "Requirements and Guidelines on Clinical Trials for Import and Manufacture of New Drug", under Schedule Y of The Drugs and Cosmetics Act, 1940, Government Of India.

STUDY PERSONNEL:

- 1) Dr. A. M. Bhagwat.
- 2) Mrs. Avanti S. Joshi

II. MATERIALS AND METHODS:

TEST ARTICLE:

Test Article : Silgel (B. No.- AS/RD/002), Viridis Biopharma.

TEST SYSTEM AND MANAGEMENT:

Test system : Mouse

Strain : Swiss albino mice



- Source : Haffkine's Laboratory, Parel.
- Age : 25 to 30 weeks.
- Identification : By cage tags.
- No. of animals : 6 mice, per dose per group
- Acclimatization : At least one week in the experimental room after veterinary examination.
- Randomization : After acclimation and veterinary examination ,the mice were randomly selected in mixed groups of both males and females.

Husbandary:

Environmental conditions: Temperature of the animal house was maintained in the range of 20 - 25 °C. Relative humidity close to 60 %. The mice were exposed to natural day-night cycles.

Accomodation : Groups of six in polypropylene cages with stainless-steel grill-top, facilities for food and water-bottle and bedding of clean paddy husk.

Diet : Standard pelleted rodent feed manufactured by Lipton Ltd., *ad libitum*.

Water : Water, supplied by Brihan Mumbai Municipal Corporation , filtered and kept in glass bottles, *ad libitum*.



III. STUDY DESIGN:

ACUTE TOXICITY:

The study was designed to permit the assessment of acute oral toxicity of Silgel by Acute toxic class method. (Schlede *et al*, 1992, 1995).

In this method, the compound to be analyzed was given to the animal model through the oral route (gastric intubation) at three different logarithmic concentrations, such as 50 mg / kg body weight, 500 mg / kg body weight, and 5000 mg / kg body weight.

The doses were prepared by suspending test material 0.5 % Carboxy Methyl Cellulose (CMC) to obtain the required concentration of the drug.

The animals from all the groups were observed for 24 hours, and mortality, if any, was recorded. Such a method allows allocation to the toxicity classes of very toxic, toxic, harmful, unclassified etc., the same manner as on the basis of classic LD₅₀ tests.

The advantage of this alternative method is, it uses fewer animals than the traditional LD₅₀ test and yields the same information on toxic signs in the treated animals.

At '0' time, the animals were given a single oral dose, depending on group randomization, of predetermined concentration of Silgel (Table-1). After initial 24 hours of observation, the animals will be maintained up to 14 days to observe any delayed (sub - acute) reaction to the given dose. After this period, the animals from all the groups will be put to sleep, dissected to observe changes, if any, in gross anatomy. Samples from tissues showing abnormalities will be fixed in 10 % formalin and sent for histopathological preparations.



TABLE - 1

GROUP (n = 6)	DOSE (mg / kg b.w) (Oral route)
GROUP - 1	CONTROL
GROUP - 2	50
GROUP - 3	500
GROUP - 4	5000

IV. RESULTS AND OBSERVATIONS :**RESULTS :**

Table - 2 records the results of experiments expressed as percentage mortality at given concentration of Silgel, B. No. - AS / RD / 002.

TABLE - 2

GROUP (n = 6)	DOSE (mg / kg b.w) (oral route)	PERCENTAGE MORTALITY
GROUP - 1	CONTROL	0
GROUP - 2	50	0
GROUP - 3	500	0
GROUP - 4	5000	0

From Table - 2 it can be observed that there is no mortality in all the groups treated with the drug at the end of 24 hours.



The treated mice were maintained for a period of 14 days to observe any delayed toxic expression. At the end of 14 days observation period, the animals were put to sleep and dissected open to observe gross anatomical changes, if any. It was found that there was no delayed toxic reaction and the animals under observation showed normal feeding, drinking and grooming behavior. All mice, administered the drug orally, showed normal size, texture and colour of the organs such as liver, spleen, kidney, alimentary canal, lungs, heart, gonads etc. None of the organs of animals, which received the drug orally at its highest concentration, did not present abnormal histological pattern.

CONCLUSIONS:

On the basis of the 24 hours and 14 days observation period, it may be concluded that even at an oral dose of 5000 mg / kg, Silgel produced no mortality when given to a population of male and female Swiss Albino mice. This product may therefore be assigned to unclassified category of the Acute Toxic Class Method of Schlede *et al.*, (1992, 1995).



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**Selective Inaction Of ASAP On Probiotics
(Viridus BioPharma).**

VIRIDIS BioPharma

**Report Prepared for
American Biotech Laboratory**

Selective Inaction of ASAP on Probiotics

March 2004

By:

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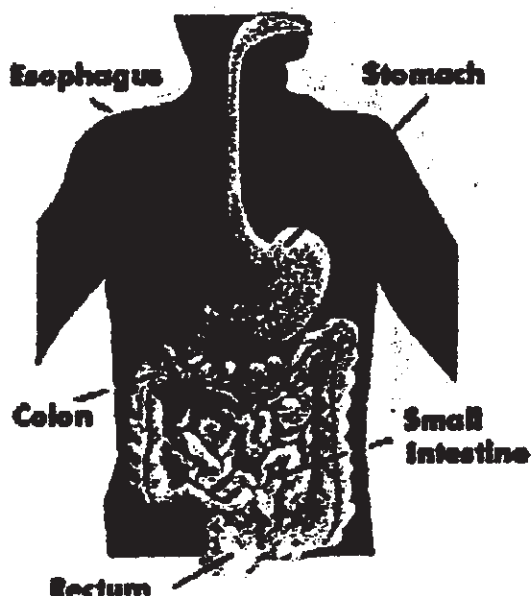
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1. Introduction & Purpose

Since the publication of "Science Digest" March 1978 issue reporting colloidal Silver in the article "Our Mightiest Germ Fighter" silver is emerging as a wonder of modern medicine. Science has traveled a long way in recognizing the fact that an antibiotic kills perhaps a half-dozen different disease organism , but silver kills some 650. Also resistant strains fail to develop against silver. Moreover, silver is virtually nontoxic to humans.

Obviously the question that would come to mind is how to deliver such colloidal silver internally. A standard dose of 10 ppm to 40 ppm aqueous colloidal silver is normally two spoons (~ 10ml) at a time. This brings up the question as to the fate of colloidal silver in the digestive systems as it travels through various zones of digestive system. It may undergo conversion in the acidic condition of the stomach. It is absorbed in the duodenum. The remaining silver as it travels further inevitably contacts intestinal flora.



All of us carry in our intestinal tracts a complex ecosystem of microbes. These bacteria are highly important to our health, providing us with protection against intestinal infection, supplying us with additional nutritional value from the food we eat, and contributing to the development of our immune system. In fact repopulation of the gut microflora, after or during antibiotic therapy is accomplished thru OTC or prescription lactobacilli formulations. The flip-side is that disturbances in this ecosystem can leave us more vulnerable to exogenous and endogenous intestinal infections.

Lacto bacilli taken as a probiotic supplementation, may also concurrently be travelling with colloidal silver through the digestive tract. The concept of ingesting live organism for the purpose of improving one's intestinal health and general well being is around as old as curds (yogurt) to capsules of Lacto bacilli.

Question 1. : Does this 'Top Gun' (colloidal silver), that destroys more than 650 microorganisms, spares the "good guys" - intestinal Lacto bacilli flora.?

Question 2. : And if indeed it spares Lacto bacilli then the question arises how does it distinguish between good and the toxic.?

Question 3. : Isn't the mechanism to spare the good bacteria applicable to any other pathogenic bacteria?

This report answers the first question while study is well into answering Questions 2 and 3.

2. Aim

To confirm the hypothesis that ASAP silver solutions are not toxic to Probiotics such as

1. Lactobacillus & Bifidobacterium supplementss.
2. Multistrain Oral Preparations

a. Lactisyn :Mfg. By Laboratories Griffon Pvt. Ltd. India

Composition :

Lactobacillus lactis

Lactobacillus acidophilus

Streptococcus lactis

Streptococcus thermophilus

b. Kyo-Dophilus :Mfg. By Wakunaga Of America Co. Ltd., USA

Marketed by : GNC, USA.

Composition :

Lactobacillus acidophilus

Bifidobacterium bifidum

Bifidobacterium longum

Table – 1 Effect of ASAP on Lactic acid Bacteria

Name of Product	Medium Used	Diameter of zone of Inhibition (mm)			Photographs
		ASAP 10ppm	ASAP 14ppm + H ₂ O ₂	ASAP 22ppm	
<i>Lactobacillus acidophilus</i>	GYEA	NI	36	NI	1
	TA	NI	30	NI	
<i>Bifidobacterium longum</i>	GYEA	NI	35	NI	2
	TA	NI	28	NI	
Lactisyn	GYEA	NI	46	NI	3
	TA	NI	36	NI	
Kyo-Dophilus	GYEA	NI	48	NI	4
	TA	NI	35	NI	

Key NI : No Inhibition

Table – 1 Effect of ASAP on Lactic acid Bacteria

Name of Product	Medium Used	Diameter of zone of Inhibition (mm)			Photographs
		ASAP 10ppm	ASAP 14ppm + H ₂ O ₂	ASAP 22ppm	
<i>Lactobacillus acidophilus</i>	GYEA	NI	36	NI	1
	TA	NI	30	NI	
<i>Bifidobacterium longum</i>	GYEA	NI	35	NI	2
	TA	NI	28	NI	
Lactisyn	GYEA	NI	46	NI	3
	TA	NI	36	NI	
Kyo-Dophilus	GYEA	NI	48	NI	4
	TA	NI	35	NI	

Key NI : No Inhibition

7. Conclusion

ASAP solutions at both 10 & 22 ppm concentrations have not demonstrated anti-probiotic activity. ASAP 14 ppm with 1.5% H₂O₂ shows appreciable killing of all the strains tested presumably due to H₂O₂.

The results bring forth ASAP as an “antibiotic of choice”, natural antibiotic, with firstly no side effects such as diarrhea and infact will not disturb the body’s natural host defense mechanism. It indeed complements therapy by sparing essential host microflora as well as concomittant oral lactobacilli therapy normally given as an adjunct.

(7)

**Selective Antimicrobial Activity of ASAP-
AGX- 32 Silver Solution Against Probiotics
(Dr Ron Leavitt).**

BYU

BRIGHAM YOUNG
UNIVERSITY

Laboratory of Dr. Ron W. Leavitt
Brigham Young University
August 2004

**Selective Antimicrobial Activity of ASAP-AGX-32
Silver Solution Against Probiotics**

Research performed by:
Jessica K. Pate

Under direction of:

Dr. Ron W. Leavitt

Table of Contents

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Appendix A Media Formulation

1. Introduction and Purpose

Since our research began years ago on the antibacterial activity of ASAP silver solutions, we have observed the bactericidal action against all microorganisms exposed to it—almost all of them being pathogens of varying biological levels. Due to our consistent observation of the ASAP silver solutions being bactericidal to varying degrees against all bacteria we have tested it with, we were skeptical about the hypothesis that silver solution could be selectively bactericidal against pathogens, but have little or no effect against probiotics.

Probiotics are non-pathogenic, non-toxic microorganisms that are able to survive the transit through the stomach and colonize the stomach and gastrointestinal tract to inhibit the colonization of any potentially pathogenic bacteria ingested. The two most recognized and used probiotics are *Lactobacillus* and *Bifidobacterium* species—found in yogurts and other dairy products. Probiotics are used frequently as prophylactic treatment in conjunction with the use of antibiotics in order to prevent the diarrhea that is so commonly caused by the use of antibiotics and are also frequently taken as a dietary supplement for the prevention of gastrointestinal bacterial infection.

Due to the lack of sufficient broad-spectrum antibiotics, and the negative side effects associated with many antibiotics, science has continued its search for an antibiotic that has no notable side effects but is an efficient bacterial killer. The ASAP Silver solution appears to be unique in its ability to kill a large number of microorganisms, including plague, tuberculosis, and anthrax, while at the same time apparently having very little antimicrobial effect against the organisms most commonly used as probiotics.

After years of research pertaining to its antimicrobial activity, scientists have been testing other ways to harness the power of silver in preventative medicine. So the question arises regarding the antimicrobial activity of ASAP silver solution against probiotics. Scientists at Viridis Biopharma have conducted limited research which shows that ASAP silver solution is ineffective against probiotic mixtures. However, their research did not use the standard MIC/MBC test methods, they did not indicate the results using pathogenic controls, nor was each microorganism tested individually with ASAP silver solution, so we felt the need to bolster the research with the above procedures in order to more effectively examine the claim that the ASAP AGX 32 Silver Solution was not antimicrobial against the probiotics.

This report demonstrates that ASAP-AGX-32 silver solution has very limited antimicrobial activity against some of the individual strains found in the probiotics, but is ineffective against others when tested extensively with American Biotech Laboratory's 32ppm silver using the standard protocols listed above.

2. Aim

To demonstrate the selective antibacterial activity of ASAP-AGX-32 silver solution against probiotics previously tested by Viridis Biopharma. Each microbe will be tested using the broth macrodilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) will be tested in plastic test tubes.

The bacteria tested in this research are:

ATCC 4356 *Lactobacillus acidophilus*

ATCC 12315 *Lactobacillus delbrueckii subsp. lactis*

ATCC 29521 *Bifidobacterium bifidum*

ATCC 15707 *Bifidobacterium longum*

ATCC 19258 *Streptococcus thermophilus*

Streptococcus lactis

ASAP-AgX-32ppm Solution

Manufactured by American Biotech Labs in Alpine, UT

3. Experiment Design

All but one of the isolates were purchased directly from ATCC and cultured in the media recommended for each specific strain by ATCC on their product information sheet.

Each of the six isolates had the broth macrodilution method of MIC/MBC performed on them in their respective media in 17mm x 12mm polystyrene capped tubes. Polystyrene were used instead of glass because silver tends to interact with glass, thereby lowering the concentration of silver available for antimicrobial activity.

ASAP-AGX-32 silver solution containing 32ppm of silver were used and serially diluted in the media specific to the organism, then each tube were inoculated with overnight culture of one organism diluted to 0.5 of the McFarland standard with sterile media.

Thereafter, the tubes were incubated for 24 – 48 hours and observed for growth. Pictures were taken of the tubes after incubation to exhibit growth. In the case of *Streptococcus thermophilus*, due to the opacity of the media, 200uL aliquots of the overnight MICs were plated onto Milk Tomato Juice Yeast Extract Agar to observe growth compared to the positive control.

Controls

It is important to have several controls performed simultaneously for comparison and analysis.

Control 1—Negative control of sterile media placed into separate tube at time of testing to show aseptic technique were demonstrated.

Control 2—Positive control of sterile media inoculated with the same culture other tubes were inoculated with at the time of testing.

Control 3—Tests were performed in triplicate to verify results

4. Method

Ten tubes were labeled from 1-10 with adhesive labels.

Protocol for Broth Macrodilution Method

Tube 1 have 1ml of antimicrobial and no media.

Tube 2 have 1ml of antimicrobial + 1ml of media, then vortexed

Tube 3 have 1ml of media + 1ml of the tube 2 mix added, then vortexed

Tube 4 have 1ml of media + 1ml of the tube 3 mix added, then vortexed

Tube 5 have 1ml of media + 1ml of the tube 4 mix added, then vortexed

Tube 6 have 1ml of media + 1ml of the tube 5 mix added, then vortexed

Tube 7 have 1ml of media + 1ml of the tube 6 mix added, then vortexed

Tube 8 have 1ml of media + 1ml of the tube 7 mix added, then vortexed. 1ml is removed and discarded.

Tube 9 positive control so it is only media plus culture, no antimicrobial.

Tube 10 negative control so it has only sterile media and is not inoculated with any culture.

After the tubes were set up according to protocol, bacteria in mid-log phase, diluted to 0.5 of McFarland standard was added in 1ml aliquots. Only tube 10 was not inoculated since it is the negative control.

Note: *Bifodobacteria* are strict anaerobes, so each inoculated tube required .04uL of Oxirase enzyme to bind all elemental oxygen and create an anoxic environment for growth. Also, the caps were double clicked to create a tight seal preventing oxygen from

altering the internal anoxic environment. They were still incubated at the same temperature.

The adjusted content of silver in each tube after dilution are as follows:

Tube 1—16ppm

Tube 2—8ppm

Tube 3—4ppm

Tube 4—2ppm

Tube 5—1ppm

Tube 6—0.5ppm

Tube 7—0.25ppm

Tube 8—0.13ppm

Tube 9—Positive control

Tube 10—Negative control

Media (see Appendix A for formulations)

Lactobacillus spp. require MRS Broth

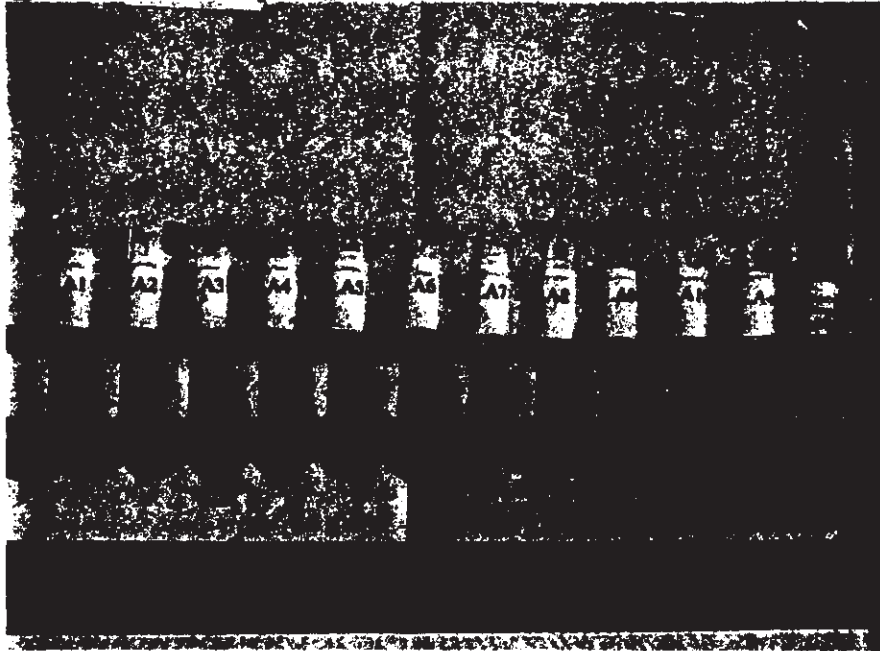
Bifidobacteria spp. require RCM

Streptococcus lactis required TSB

Streptococcus thermophilus required Tomato Skim Milk Yeast Broth

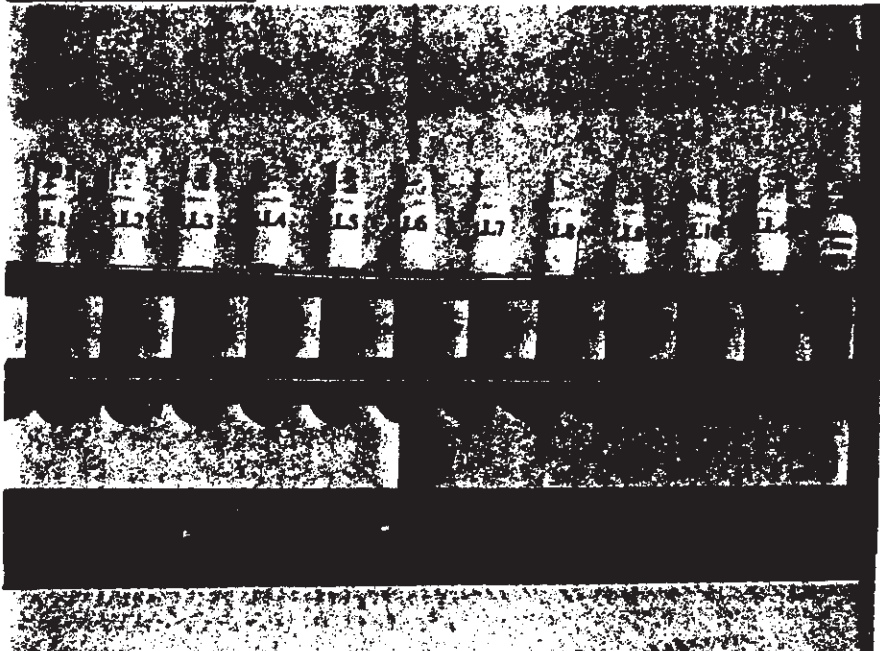
5. Results

Lactobacillus acidophilus



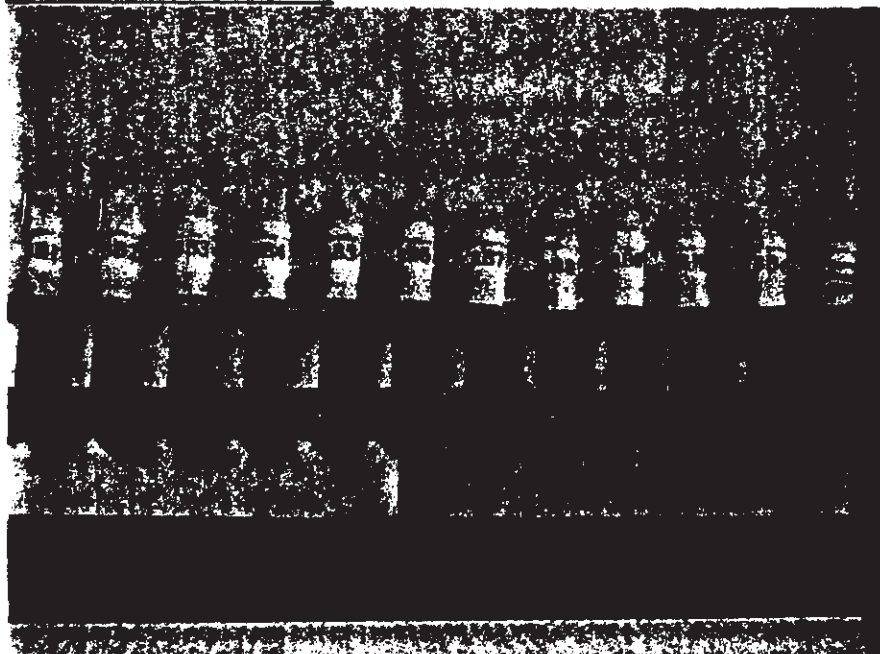
The first growth was evident in tube 4, which is 2ppm of ASAP silver solution.

Lactobacillus lactis



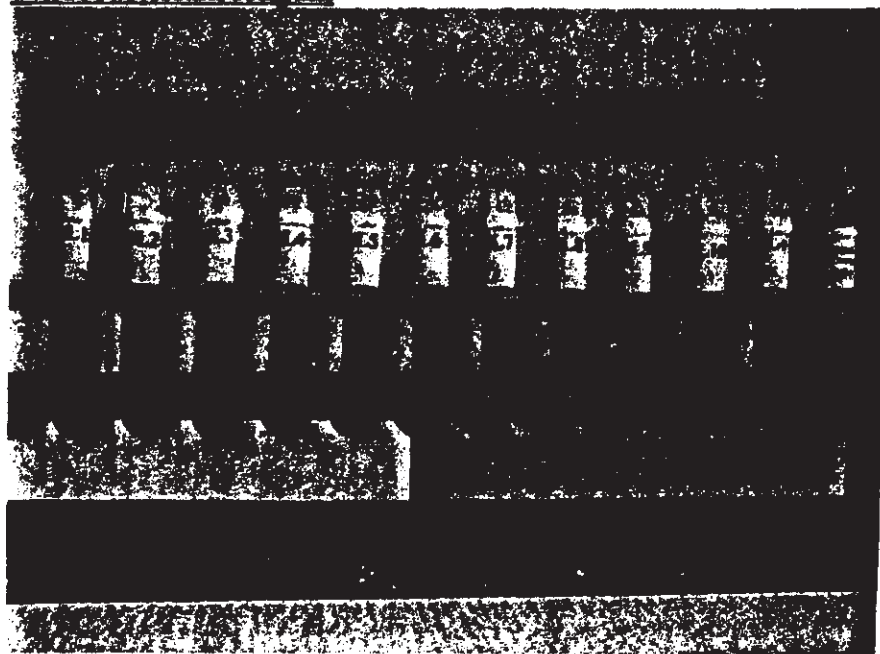
Growth was first evident in Tube 3, which is 4ppm ASAP silver solution.

Bifidobacterium bifidum



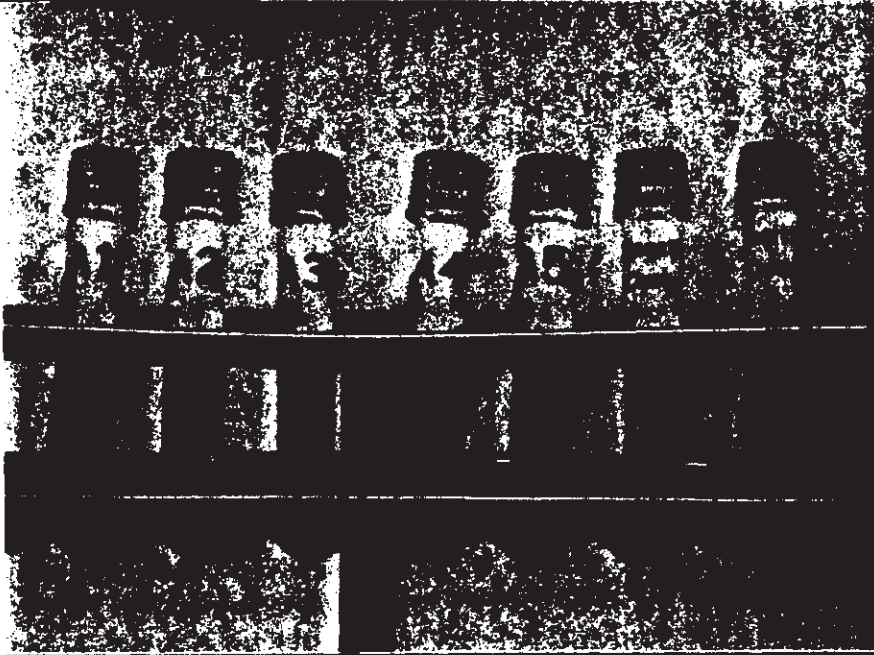
Growth was evident in Tube 1, which is 16ppm ASAP silver solution.

Bifidobacterium longum



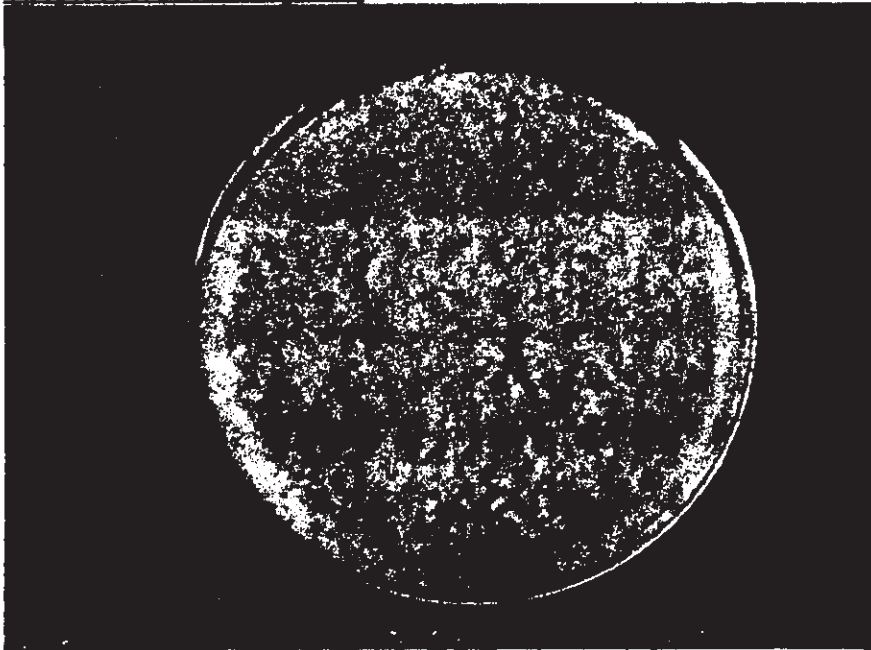
Growth was evident in Tube 1, which is 16ppm ASAP silver solution.

Streptococcus lactis



Growth began in the third tube, which is 4ppm ASAP silver solution.

Streptococcus thermophilus



Due to the nature of the media, Tomato juice Skim milk Yeast extract Agar, photographic evidence cannot accurately indicate growth. Visual inspection of plates after 24 hours of incubation and compared with positive control indicated that growth was first evident on plate 5 taken from Tube 5, which is 1ppm ASAP-AGX-32 silver solution.

Salmonella typhimurium—causative agent of food poisoning called salmonellosis. A comparison of the results of MIC testing of probiotics to a known pathogen demonstrated that the antibacterial efficacy of silver solution against probiotics is significantly less than observed in tests with the same silver solution against pathogenic bacteria.



The first evidence of growth appears in tube 5, which is 1ppm silver solution.

6. Conclusion

Results obtained from MIC testing of probiotics with ASAP-AGX-32 silver solution in comparison to pathogenic microorganisms previously tested suggest that probiotics such as *Bifidobacteria* aren't affected at all by exposure to concentrations of 16ppm ASAP silver solution, whereas pathogenic bacteria are killed at concentrations as low as 2ppm. *Lactobacilli* are killed at concentrations of silver solution between 4 and 8ppm, but not at 2ppm. Silver solution concentrations greater than ~5ppm are bactericidal to *Streptococcus lactis*, and concentrations greater than ~2ppm are bactericidal to *Streptococcus thermophilus*.

We can understand from these results that strictly anaerobic bacteria such as *Bifidobacteria* aren't harmed by the ingestion of silver solution to treat bacterial infection, and *Lactobacilli* are minimally effected in comparison to the pathogens targeted by ASAP silver solution. It can therefore be concluded that the consumption of probiotics in conjunction with ASAP silver solution would be beneficial to the health of ill and healthy people alike.

Appendix A—Media Formulation

M.R.S. Broth (de Man, Rogosa, Sharpe)

Peptone 10g/L
'Lab-Lemco' Powder 8g/L
Yeast extract 4g/L
Glucose 20g/L
'Tween' 80 1mL
Di-potassium hydrogen phosphate 2g/L
Sodium acetate trihydrate 5g/L
Tri-ammonium citrate 2g/L
Magnesium sulphate septahydrate 0.2g/L
Manganese sulphate quatrahydrate 0.05g/L
pH 6.2

Reinforced Clostridial Medium (RCM)

Yeast extract 3g/L
'Lab-Lemco' powder 10g/L
Peptone 10g/L
Soluble starch 1g/L
Glucose 5g/L
Cysteine hydrochloride 0.5g/L
Sodium chloride 5g/L
Sodium acetate 3g/L
Agar 0.5g/L
pH 6.8

Tryptic Soy Broth (TSB)

Pancreatic digest of Casein 17g/L
Papaic digest of soybean meal 3g/L
Dextrose 2.5g/L
Sodium chloride 5g/L
Dipotassium phosphate 2.5g/L
pH 7.3

Tomato Juice Skim Milk Yeast extract Broth

100mL of Tomato juice extracted from canned tomatoes through centrifugation
100g/L Skim Milk (we used Carnation)
Yeast extract 5g/L
1L of distilled water
pH 7