3

Enterobaccteriaceae

Types of *Enterobaccteriaceae*

Enterobaccteriaceae is a family of bacteria containing gram negative rod shaped bacteria. This family includes *Escherichia coli, salmonella, proteus, shigella* and *citrobacter*. Some bacteria in the *enterobaccteriaceae* family such as *E. coli* colonize the normal intestinal flora in humans and animals. Although some types of these bacteria are inhabitants of the human body, they are capable of causing severe disease.
Escherichia Coli is probably the most well known *enterobaccteriaceae*. Although people associate them with dangerous gastrointestinal illnesses many types are completely benign and live symbiotically in mammals. *Salmonella* is also a well publicized member of the family causing many counts of food poisoning.

Common infections and current treatments

E. Coli

There are 4 types of *E. coli* that cause gastroenteritis, inflammation of the stomach and intestinal lining, in humans. There is enterotoxigenic *E. coli*, enteropathogenic, enterohemolytic *E. coli,* also known as *E. coli* O157:H7, and enteroinvasive *E. coli*. All of these types cause different gastrointestinal illnesses.

Enterotoxigenic *E. coli*:

This type of enterovirulent *E. coli* causes what is known as traveler's sickness. It is generally found in food that has been prepared with contaminated water in a country where sanitation practices are not high. Water contaminated with human sewage could be used to prepare food and cause the spread of the bacteria. Contaminated food handlers could also be responsible for infecting others. This is not a common infection in the United States but it is common among travelers in foreign countries. The *E. coli* causes illness by attaching to the intestinal lining. Once bound it releases a toxin that is absorbed by the intestinal cells. The toxin causes the cells to release large amounts of electrolytes along with water into the intestines.

Enteropathogenic *E. coli*

This strain causes symptoms similar to dysentery. The bacteria cause physical changes to the intestines and a toxin attaches to the tissue and destroys it. Countries with poor sanitation habits have the highest outbreaks. It is usually associated with raw beef and chicken, or anything that has been exposed to fecal contamination.

Enterohemolytic *E. coli*

Infections caused by these bacteria are of great importance and will be discussed in the following section.

Enteroinvasive *E. coli*

These organisms invaded the cells in the lining of the intestines and cause a mild form of dysentery. Infection caused by this type of *E. coli* is rare but only a few bacteria are needed to cause infection, as few as 10. The disease is typically caused by contaminated food that is ingested. It is difficult to determine the source of the bacteria because it is hard culture the potentially few bacteria from food that are cause illness.

Urinary Tract Infections

Uropathogenic *E. Coli* are responsible for causing bladder infections. Women are more likely to develop bladder infections than men due to the shortened urethra. The bacteria get into the bladder and invade the cells. There they form protective layers over them called pods and colonize. The colonies then shed creating an infection. When the bacteria are shed there and some remaining in the pods that cause recurrent infections. Most people are put on antibiotics such as amoxicillin, a mix of trimethoprim and sulfamethoxazole, or ciprofloxacin. These antibiotics are not as effective any more because of high levels of resistance. In one study performed by Hernandez-Porras el al. (2004) 83% of the *E. coli* found to cause UTIs were resistant to ampicillin and 76% were found resistant to the trimethoprim-sulfamethoxazole mix. Regardless of resistance these antibiotics cure the shedding bacteria but do not eliminate the pods where bacteria will continue to grow and shed causing recurrent infections.

Salmonella

Salmonella is generally found in chicken and pigs. Infection is usually caused by food and contaminated water. Illness can occur when contaminated raw meat is not thoroughly cooked or the area where the meat was prepared was not cleaned. There are other sources of *salmonella* such as eggs, milk, coconut, cream filled desserts, peanut butter or any food that has come in contact with or made with contaminated water. The main forms of Salmonella that cause disease are *S. typhi*, *S. paratyphi*, and *S. enteritidis*.

Food Poisoning

Salmonella is most associated with food poisoning in the US. *S. enteritidis* is the most common form of food poisoning generally a result of raw or undercooked eggs. Undercooked poultry and swine are also common sources of the bacteria. Generally food poisoning caused by salmonella is not treated with antibiotics because this course of action does not decrease the length of time that symptoms are present. For severe cases chloramphenicol and ampicillin are generally used. *S. enteritidis* causes less severe food poisoning symptoms.

Typhoid

S. typhi and *S. paratyphi* are rare in the US, but cause serious illnesses such as typhoid and typhoid like fever. Typhoid is a dangerous and sometimes life-threatening illness. Only humans carry the infecting bacteria *S. typhi*. Most infections occur in developing countries where water sources are contaminated with sewage. For infection to occur the bacteria have to be eaten or drank in contaminated food or water. Once in the intestines the bacteria bind and invade the intestinal cells. They then pass to the lymphatic system and are propelled throughout the body by the blood. White blood cells, the body's defense system, engulf the bacteria to try and kill them off but *S. Typhi* does not die. Instead the bacteria multiply in the white blood cells and then infect the cells of the spleen, liver, or kidneys. This infection can be fatal. It can be treated with antibiotics such as ciprofloxacin or pefloxacin but there is increasing resistance to these and other drugs used to treat the infection. Once the symptoms of infection disappear a person can continue to carry the bacteria and transmit the disease to others.

Salmonella also causes other infections such as infection of the liver, pneumonia, and meningitis.

Silver colloid lab Results

E. Coli Lab Tests

Abstract:

Lab tests were performed on *E. coli* K12 to determine if silver colloid is a successful antibacterial. Lab tests were done with 5 ml of silver colloid and 100 μl of an approximate 10^6-10^7 CFU/ml culture unless otherwise stated. Three tests were performed. Test 1 determined the minimum time needed to kill the bacteria with a 50 PPM silver colloid + peppermint oil solution, test 2 determined the minimum concentration of silver required to eliminate bacteria in a silver colloid + peppermint oil solution, and test 3 determined if KC-20 and peppermint oil increased the antibacterial properties of the colloid. It was found that the minimum time needed for a silver colloid plus peppermint oil solution was about 40 minutes. The minimum concentration needed was 25 PPM at 40 minutes. Test 3 determined that KC-20 and peppermint oil were effective in amplifying the antibacterial capabilities by 100 fold. A 4^{th} test was performed on a test subject with a urinary tract infection. This test was designed to determine how many bacteria were killed in vivo in one day of treatment. The test shows that an infection of 10^4 CFU/ml can be decreased to a 10^2 CFU/ml contamination in one day with Nature's Rite's silver colloid and oil product. These test were designed to evaluate the antibacterial capabilities of silver colloid on *E. Coli* and proved that the various colloid combinations were indeed quite lethal to the bacteria.

Test 1: Establishing the minimum time needed to kill 100% of *E. Coli*

The purpose of this test was to determine how long the bacteria needed to be exposed to the silver colloid to be killed.

Methods:

One hundred μl of bacteria was added to tubes containing 5 mls of silver colloid-peppermint oil solution. The solutions were incubated at 37° C for 10, 15, 20, 30, 40, 60 minutes. One hundred μl of the solution was then plated on LB-agar plates and grown overnight at 37° C. Dilutions were made at the lower times to account for a low kill. Dilutions were made after the incubation time was completed by added 100 μl of the silver colloid bacteria solution to 900 μl of DI water. This was done a series of times depending on the dilution desired.

Control Plates:

The original culture was diluted by serial dilution. One hundred μl of bacteria was added to 900 μl of DI water, and 100 μl of the new DI water-bacteria solution was added to another 900 μl of DI water. This was repeated a total of 5 times to obtain a –5. One hundred μl of the -4 and -5 dilutions were then plated on LB-agar plates and grown over night at 37° C.

Results:

Control plates:

plate	description	CFU/mL	average
-4 dilution	520	5.2×10^7	**5.85×10^7**
-5 dilution	65	6.5×10^7	

One hundred μl of bacteria was added to 5 ml of 50 PPM silver colloid. Approximately 5.85×10^6 cells were placed into each 5 mL tube. One hundred μl of the solution was plated. If no bacteria were killed there would be approximately 1.17×10^5 cells would be plated.

Colony counts on Plates:

time	no dilution	-1	-2	total CFU
10 min	uncountable	uncountable	325	3.25×10^4
15 min	uncountable	260	*	2600
20 min	600	*	*	600
30 min	36	*	*	36
40 min	1	*	*	1
60 min	0	*	*	0

* Solutions not plated

Log Kill Ratio:

The log kill ratio was determined by dividing 1.17×10^5 by the remaining colonies. The resulting number was multiplied by Log_{10}.

time	colonies $\frac{\text{count}}{\text{total colonies}}$	log kill ratio
10	$\frac{3.25 \times 10^4}{1.17 \times 10^5}$	0.56
15	$\frac{2600}{1.17 \times 10^5}$	1.65
20	$\frac{600}{1.17 \times 10^5}$	2.29
30	$\frac{36}{1.17 \times 10^5}$	3.5
40	$\frac{1}{1.17 \times 10^5}$	5.07
60	$\frac{0}{1.17 \times 10^5}$	5.1

From this experiment it was determined that approximately a 40 minute incubation is needed to kill 1×10^5 CFU.

Test 2: Determining the minimum PPM silver

This test is designed to evaluate the minimum PPM of silver colloid needed to kill *E. coli*. The incubation times were 40 and 60 minutes because test 1 showed that a 1×10^5 culture could be killed in 40 minutes with 50 PPM of silver colloid.

Methods:

Each solution was 5 ml. The 25 PPM solution of the silver colloid-peppermint oil solution was made by a 1:1dilution of the 50 PPM and DI water. The 12.5 solution was made in the same manner but with the 25 PPM solution rather than the 50. The 6.25 solution was also made from a 1:1 dilution of the 12.5 PPM solution and DI water. These solutions were heated to 37° C before bacteria was added. One hundred µl of an unknown concentration of bacteria was added to the solutions and incubated for 40 or 60 minutes. After incubation 100 µl of the solution with bacteria was plated and grown over night at 37° C.

Control Plates:

One hundred µl of the bacteria culture was added to 5 ml of DI water. This was incubated at 37° C for 40 and 60 minutes to determine if *E. coli* survive in DI water. After incuabtion 100 µl of bacteria was added to 900 µl of DI

water, and 100 µl of the new DI water-bacteria solution was added to another 900 µl of DI water. This was repeated a total of 5 times to obtain a –4and –5dilution. One hundred µl of these dilutions were then plated on LB-agar plates and grown over night at 37° C.

Results:

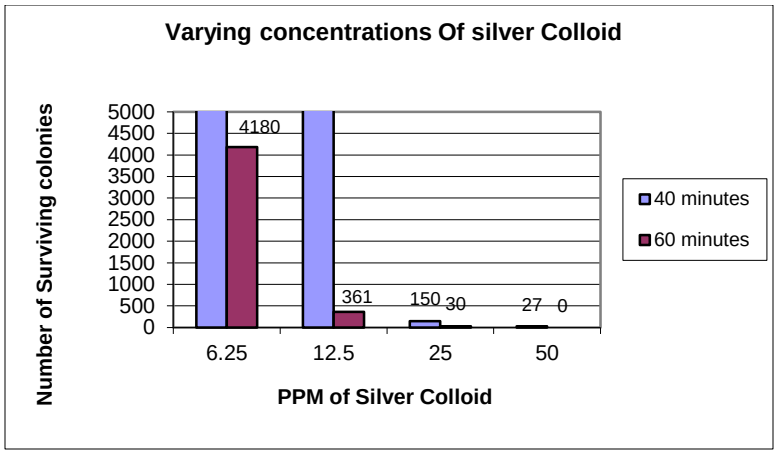
Control Plates:

Time	-4 Dilution	-5 Dilution
40 min	37	4
60 min	63	5
Average	50	4.5

One hundred µl of the original culture was added to 5 ml of DI water and incubated at 37° C for 40 or 60 minutes. One hundred µl of the DI water was then diluted in a serial dilution. One hundred µl of the dilution was then plated and grown overnight at 37° C. There is an average of 50 CFU/plate at the –4 dilution. In 100 µl there would be approximately 5.0×10^5 CFU. The original culture contained approximately 2.5×10^8 CFU/ml. On each experimental plate approximately 5.0×10^5 CFU were plated.

Experimental Plate Counts:

PPM	40 min	60 min
0	5.0×10^5	5.0×10^5
6.25	2.04×10^4	4180
12.5	7200	361
25	150	30
50	27	0



This experiment shows that 50 PPM and 25 PPM silver colloid are effective antibacterials at 40 and 60 minutes. A 60 minute exposure time kills better than 40 minutes at all concentrations of silver.

Test 3: Silver Colloid Plus KC-20

This test is designed to determine if KC-20 increases the effectiveness of silver colloid as an antibacterial.

Methods:

One hundred μl of an unknown concentration of bacteria was added to 5 ml of silver colloid, silver colloid with 1% KC- 20, Silver colloid + peppermint oil, and silver colloid + peppermint oil with KC-20. All solutions contained approximately 50 PPM of silver. All solutions were incubated at 37° C before the addition of bacteria.

The bacteria in the silver colloid solutions incubated at 37° C for 30 minutes. After the given amount of time 100 μl of the bacteria-silver colloid solution was plated on LB agar plates and incubated overnight at 37° C. The following day the colonies were counted.

Control Plates:

The original culture was diluted by serial dilution. One hundred μl of bacteria was added to 900 μl of DI water, and 100 μl of the new DI water-bacteria solution was added to another 900 μl of DI water. This was repeated a total of 6 times to obtain a –5 and –6 dilution. One hundred μl of the –5 and -6 dilutions were then plated on LB-agar plates and grown over night at 37° C.

Results:

Control plates:

Dilutions of –5 and –6 were plated and grown over night at 37° C. The colonies were counted and the quantity of bacteria in the original culture was determined.

Dilution	colonies	CFU in 100 µl of original culture	average	CFU/ml in original culture
-5	123	1.23×10^7		
-6	16	1.6×10^7	1.45×10^7	1.45×10^8

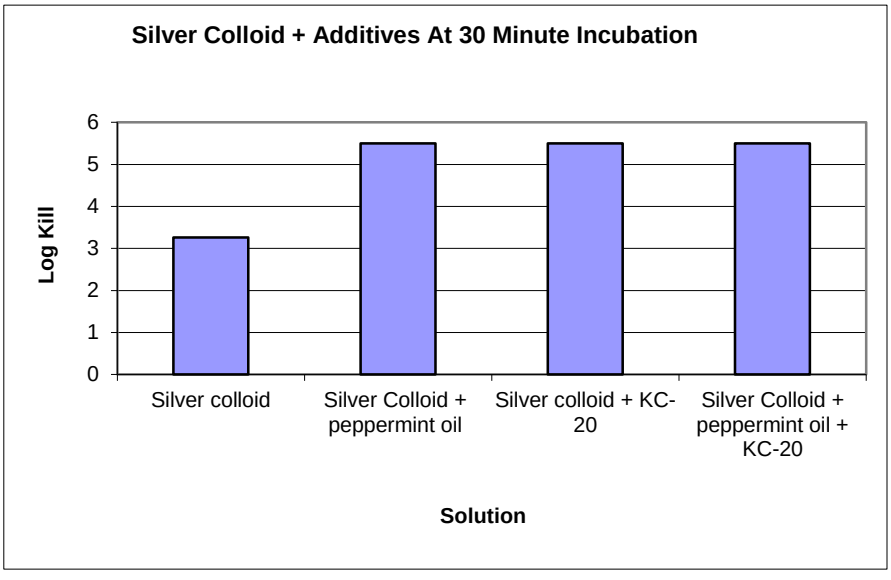
Experimental plates:

One hundred µl of the bacteria colloid solution was plated after a 30-minute incubation at 37° C. In 100 µl there should have been approximately 2.9×10^5 CFU.

30 minute

solution	Colonies Remaining / Total Colonies	Log Kill Ratio
Silver colloid	$\frac{158}{2.9 \times 10^5}$	3.26
Silver Colloid + peppermint oil	$\frac{0}{2.9 \times 10^5}$	5.5
Silver colloid + KC-20	$\frac{0}{2.9 \times 10^5}$	5.5
Silver Colloid + peppermint oil + KC-20	$\frac{0}{2.9 \times 10^5}$	5.5

The results show that the peppermint oil and KC-20 increase the antibacterial capabilities of silver colloid.



Since each of the colloids with performance enhancing additives showed a complete kill, it cannot be determined which additive is more effective but all additives are about 100 times as effective at killing *E. coli* than silver colloid alone. Additional testing with greater inoculum counts or lower exposure times would allow a more quantitative comparison of the effectiveness of the performance enhancing additives.

Test Summary

The conclusions that can be made about these 3 tests are that about a 40 minute incubation time is needed to eliminate a 10^5 culture of *E. coli*, the lowest concentration of silver colloid that retains its antibacterial capabilities in 40 minutes is 25 PPM, and the peppermint and KC-20 increase silver colloids kill.

Test 4: Treating a UTI

Methods:

Day 1

Urine was obtained from a female with a suspected bladder infection. Jars were not sterile but were wiped out with ethanol. One hundred μl of the urine sample was plated on an LB-agar plate and grown for 24 hours. Two hundred μl was grown in 1ml of sterile LB broth for testing. Urine was stored at 4° C after use.

Day 2

Another urine sample was obtained at 9:15 am the next day and 10 μl was plated. 4 oz of Naure's Rite colloid was taken before this sample. The plate was grown overnight at 37° C. A third urine sample was obtained at 3:00 pm and 10 μl, 50 μl, and 100 μl of the urine were plated and incubated overnight at 37° C.

Day 3

A fourth urine sample was aquired at 10:00 am on the 3rd day and 100 μl was plated and grown overnight at 37° C.

Day4

A fifth urine sample was obtained at 9:00 am and 100μl was plated and grown overnight at 37° C.

Results:

Day 1

Colonies were counted on the plate

1300 colonies in 100μL = 1.3×10^4 CFU/ml

Day 2

From the 9:15 am sample there were 500 CFU/ml

From the 3:00 pm sample there were ~ 100 CFU/ml

Day 3

Colonies were ~ 150 CFU/ mL

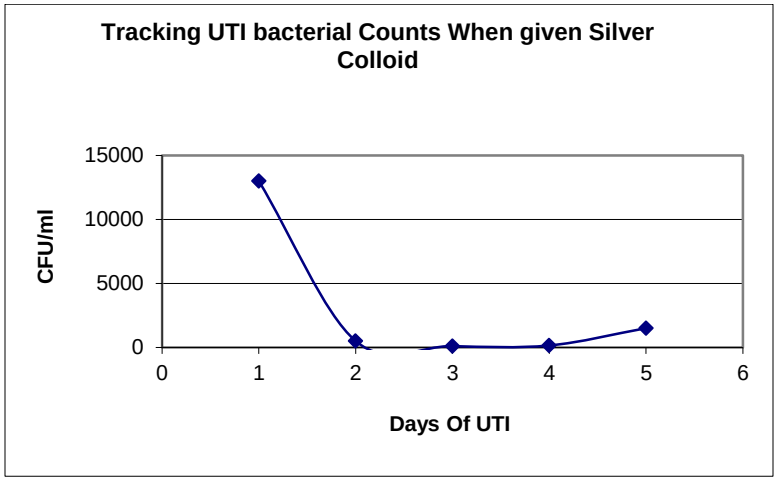
Day4

Counts 143 colonies on plate 1 and 165 colonies on plate 2

CFU/ml= 1500 (10^3)

Overall Results:

date	time sample taken	CFU/mL	magnitude
10/13/04	9:15 AM	13000 CFU/mL	10^4
10/14/04	9:15 AM	500 CFU/mL	10^2
10/14/04	3:00 PM	100 CFU/mL	10^2
10/15/04	10:00 AM	150 CFU/mL	10^2
10/18/04	9:00 AM	1500 CFU/mL	10^3



These results show the level of bacteria was greatly reduced in the first 24 hours. The bacteria levels did increase after a few days but this could be considered contamination rather than infection.

UTI Test Summary

The UTI experiment shows that an infection can be cleared in one day with the silver colloid-peppermint oil solution in a human subject. The bacterial counts were decreased by a factor of 2 log, or 100 times in 24 hours.

Salmonella Lab Test

This test was conducted at Warren Analytical Labs in Greeley, Colorado. The test was performed in peptone water solution, which is known to inactivate the silver particles and cause the silver to precipitate out of solution within 30 minutes. Although the silver particles were inactivated over time, the solutions were still able to kill the bacteria. The kill counts may be lower than ones not performed in peptone water solution due to the reduced exposure time.

Methods:

Ten milliliters of Nature's Rite colloid was added to test tubes. One hundred fifty mg of dehydrated peptone water media was added to each tube and vortexed for 2 minutes. One hundred µl of an approximate 10^7 CFU/100µl was added to each tube and incubated for 30 minutes at 37° C. After incubation 500 and 100 µl was plated on sheeps blood agar plates and grown overnight at 37° C.

Control Plates:

A serial dilution was done to accurately count the number of bacteria added to each tube. One hundred µl of bacteria was added to 900 µl of DI water, and 100 µl of the new DI water-bacteria solution was added to another 900 µl of DI water. This was repeated a total of 7 times to obtain -7 dilution. One hundred µl of these dilutions were then plated on LB-agar plates and grown over night at 37° C.

Results:

30 minute incubation

solution	0.1 ml plated	0.5 ml plated	Survival/ml	Log Kill/ml
control for Nature's Rite Colloid	35	86	1.7×10^6	0.15
Nature's Rite Colloid un-dilute	0	0	0	6.15

In this experiment the Nature's Rite colloid was very successful at killing *salmonella*. With a very short exposure in a saline rich environment, the Nature's Rite colloid was able to attenuate the population by 6 orders of magnitude or 1 million to one. The limited exposure time in this experiment made it more akin to the exposure environment in a stomach.

Enterohemolytic *E. coli*

The stereotypic *E. coli* O157:H7 is a member of the enterohemolytic group. It produces harmful toxins that destroy the lining of the intestines. When infected with *E. coli* O157:H7 a person will have severe cramping and hemorrhaging in the intestines and kidneys. These types of infections are rare but serious. The center for disease control and prevention estimates that 73,000 people in the US are infected and 60 people die each year. The illness is usually attributed to raw or undercooked beef although outbreaks have occurred from contaminated vegetables, and un-pasteurized juice or milk. In severe cases Hemolytic uremic syndrome (HUS) can occur. HUS is a condition where there is hemorrhaging in the kidneys potentially causing a loss of kidney function. In this case a person is hospitalized and generally has to endure transfusions and kidney dialysis. This is more likely to be life threatening in children or elderly people. Antibiotics are not generally used for enterohemolytic *E. coli* because they can induce HUS in patients by killing the *E. coli* too quickly and causing a great release of toxins.

Toxins

Enterohemolytic E. coli produce multiple toxins called shiga toxins. E. coli generally produce shiga toxin 1 and 2. The toxin is a protein that consists of two subuints labeled A and B. The B subuint is responsible for binding to the targeted cell and allows the toxin to enter. The A subunit then interacts with the ribosome, the component of the cell that builds proteins, and inactivates it. This inactivation causes the cell to no longer be able to produce proteins and die. The cells that are targeted are the vascular endothelium. The rapid death of these cells causes a breakdown in the lining of blood vessels and hemorrhaging. The vessels that are destroyed are smaller blood vessels found in the kidney, digestive tract, or lungs. It is thought that the hemorrhaging causes platelet activation and clogs the vessel. Exactly how the toxins cause HUS is not known.

Tests on E. coli O157:H7

Purpose:

An MBC was performed on *E. coli* O157:H7 to determine the minimum exposure time of a 50-PPM silver colloid-peppermint oil solution with 0.2 % KC-20 additive needed to kill 100% of the bacteria. Approximately a 1×10^6 colony forming units (CFU)/ml concentration of bacteria will be added to 5 ml of silver colloid-peppermint solution with 0.2% KC-20 additive and incubated for 20, 30, and 40 minutes.

Methods:

This experiment was performed twice. Everything was done in triplicate for an accurate measurement. One hundred micro liters of a 5×10^6 CFU/ml culture was added to 5 ml of 50 PPM silver colloid with 100 PPM

peppermint oil and 0.2% KC-20 and incubated at 37° C for 20, 30, and 40 minutes. After incubation 100 µl was plated on LB agar plates and grown over night at 37° C. The original CFU/ml was determined by optical density reading and by plating a –4 and –5 dilution of the original culture.

Results:

If 5×10^6 CFU/ml were the concentration of the original culture, 5×10^5 CFU were added to the 5-ml of silver colloid solution. In the 100 µl plated there should have been 1×10^4 CFU plated if all the bacteria survived.

Control

Dilution	colonies	CFU/ml
-4	42	4.2×10^6
-5	5	5.0×10^6

Trial 1

	Plate 1	**Plate 2**	**Plate 3**
20 minutes	0	0	0
30 minutes	0	0	0
40 minutes	0	0	0

Control

Dilution	colonies	CFU/ml
-4	52	5.2×10^6
-5	6	6.0×10^6

Trial 2

	Plate 1	**Plate 2**	**Plate 3**
20 minutes	0	0	0
30 minutes	0	0	0
40 minutes	0	0	0

Kill Ratio

Since approximately a 5×10^5 CFU concentration was used in both trials the log kill ratio is the same. In 20 minutes a log kill ratio of 6.2 was achieved.

Conclusion:

Silver colloid with peppermint oil and KC-20 is extremely effective at killing E. coli O157:H7. All of the *E. coli* was killed with a 20-minute exposure to the silver colloid solution.

Future Tests:

Certain types of antibiotics cause the E. coli cell wall to burst and release all their cytotoxins. Penicillin is one of these antibiotics. Penicillin prevents the *E. coli* from forming a cell wall. As the bacteria grows in preparation to divide a new cell wall is never made. Instead the *E. coli* doubles in size as it is replicating and the cell wall eventually ruptures due to the stress. This can happen within 30 minutes of exposure to Penicillin. Preliminary microscopic comparison of *E. coli* killed by penicillin and by silver colloid suggest that the colloid leaves the cell wall intact. This could reduce the shock-impact of the endotoxin and prove to be superior method for dealing with these infections.