

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 4th 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 the 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 count 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 1:1 dilution 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 incubation 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:

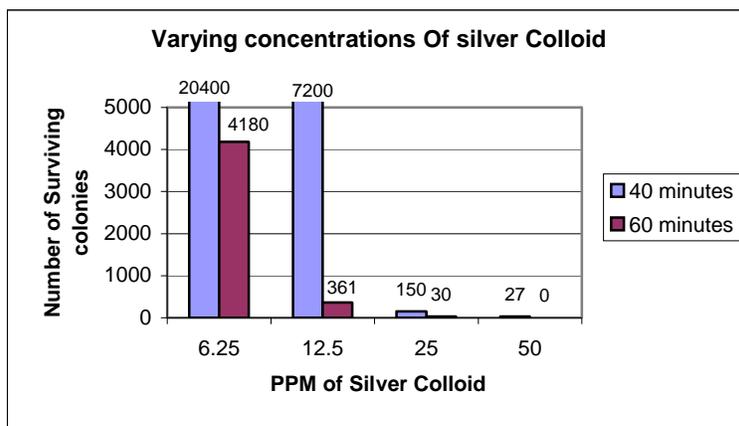
Control Plates:

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

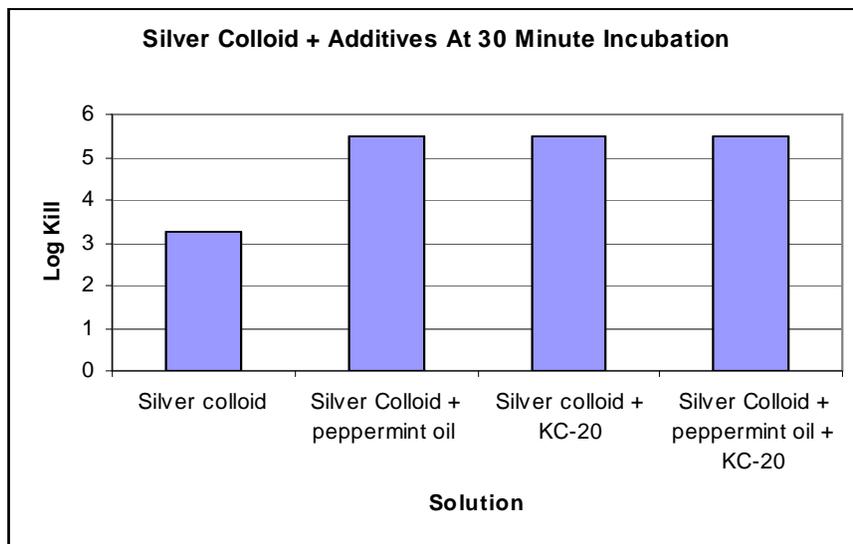
Experimental plates:

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.