

Methods for Testing Antimicrobial Effectiveness

Protocol for performing this test is found in the National Committee for Clinical Laboratory Standards (NCCLS) publication M7-T2.

TEST #1

October 4, 2001- **MSP Research and Treatment Foundation (Silver 400) sample received at Laboratory.**

Broth Dilution method, decreasing concentrations of the Silver 400 to be tested, are placed in tubes of a broth medium that will support growth of **Pseudomonas aeruginosa, K-12 E. coli, & Staphylococcus aureus, B. subtilis.** ANTHRAX is a subtype of B. subtilis.

Test procedure: 1ml of Silver 400 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Muller-Hintont broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml liquid at this point.

1/10 Dilution with Distilled Water (1ml of Silver 400 sample is diluted with 9 ml distilled water then vortexed).

Test procedure: 1ml of Silver 400 at 1/10 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Muller-Hintont broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid.

1/100 Dilution with distilled water (1ml of Silver 400 1/10 dilution with distilled water sample, is diluted with 9ml distilled water then vortexed).

Test procedure: 1ml of Silver 400 at 1/100 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Muller-Hintont broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid.

1/1000 Dilution with distilled water (1ml of Silver 400 1/100 dilution with distilled water sample, is diluted with 9ml of distilled water then vortexed).

Test procedure: 1ml of Silver 400 at 1/1000 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Muller-Hintont broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid.

1 ml Muller-Hintont broth added to all tubes.

0.1 ml of test organism suspension (1×10^6 CFU/ml) is added to tubes containing 1 ml broth and 1 ml of concentrations of Silver 400.

All tubes contain 2.1 ml of liquid with 2.5×10^4 CFU/ml immediately
0.001 ml from control tube is subcultured to agar, after overnight
incubation = 250 colonies.

OVERNIGHT TEST TUBE INCUBATION AT 35C

+ = Turbid (growth) - = Nonturbid (no growth)

Tube	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	+	+	+	+	+
1/1000 dilution 1	2	3	4	5	6	7	8	9	10	
	+	+	+	+	+	+	+	+	+	+

0.01 ml from control tube subcultured to agar = 250 CFU

October 5, 2001-Negative growth in all tubes, except 5-10 of the 1/100 which contained 000.5%-000.1% of the Silver 400 solutions. Minimum inhibitory concentration (MIC) = .5 ml of the 1.100 solution + .5 ml Nutrient broth +(1 ml Nutrient broth added to all tubes) + 0.1 ml of test organism suspension = 000.227%.

MINIMUM INHIBITORY CONCENTRATION = 000.227% OF ORIGINAL FORMULATION.

The MIC measures the ability of the antimicrobial agent to inhibit multiplication of the organisms. Thus, organisms in the inoculum are merely inhibited by the antimicrobial agent and will be able to recommence growing if the antimicrobial influence is removed. (AGENTS THAT ARE USUALLY BACTERIOSTATIC: CHLORAMPHENICOL, ERYTHROMYCIN, NALIDIXIC ACID, SULFONAMIDES, and TETRACYCLINES).

October 5, 2001- Visual turbidity is noted, and 0.1 ml from nonturbid tubes is subcultured to Nutrient agar, Mannitol – Salt agar & Mac Conkey agar.

October 6, 2001-CFU on subcultures made from nonturbid tubes are determined.

Nutrient agar

OVERNIGHT PLATE INCUBATION AT 35C

+ = Turbid (growth) - = Nonturbid (no growth)

Plate	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	+
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	+	+	+	+	+	+	+	+	+	+
1/1000 dilution 1	2	3	4	5	6	7	8	9	10	
	+	+	+	+	+	+	+	+	+	+

Mannitol-Salt agar

OVERNIGHT PLATE INCUBATION AT 35C

+ = Turbid (growth) - = Nonturbid (no growth)

Plate	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/1000 dilution 1	2	3	4	5	6	7	8	9	10	
	-	-	-	-	-	-	-	+	+	+

Mac Conkey Agar

OVERNIGHT PLATE INCUBATION AT 35C

+ = Turbid (growth) - = Nonturbid (no growth)

Plate	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	+	+	+
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	+	+	+	+	+	+	+	+	+	+
1/1000 dilution	1	2	3	4	5	6	7	8	9	10
	+	+	+	+	+	+	+	+	+	+

MINIMUM BACTERICIDAL CONCENTRATION BINARY = 00.454%

MINIMUM BACTERICIDAL CONCENTRATION GRAM + = 000.018%

MINIMUM BACTERICIDAL CONCENTRATION GRAM - = 001.81%

The MIC measures the ability of the antimicrobial agent to inhibit multiplication of the organisms. Thus, organisms in the inoculum are merely inhibited by the antimicrobial agent and will be able to recommence growing if the antimicrobial influence is removed. The MBC measures the ability of the antimicrobial agent to KILL the organisms.

Methods for Testing Antimicrobial Effectiveness

Protocol for performing this test is found in the National Committee for Clinical Laboratory Standards (NCCLS) publication M7-T2.

TEST #2 LABORATORY METHODS IN BASIC MYCOLOGY/IN VITRO
ANTIFUNGAL ACTIVITIES OF MSP RESEARCH AND TREATMENT FOUNDATION
SILVER 400

October 4, 2001- Silver 400 sample received at Laboratory

Broth dilution method, decreasing concentrations of the Silver 400 to be tested, are placed in tubes of a broth medium that will support growth of Pathogenic yeast *Candida albicans*, Filamentous fungi *Aspergillus* sp., Dermatophytic fungi *Microsporum* sp. Control organism *Saccharomyces cerevisiae*.

Test procedure: 1ml of Silver 400 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Sabouraud Dextrose broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid at this point

1/10 Dilution with distilled water (1ml of Silver 400 sample is diluted with 9 ml distilled water then vortexed).

Test procedure: 1ml of Silver 400 at 1/10 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Sabouraud Dextrose broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid

1/100 Dilution with distilled water (1ml of Silver 400 1/10 dilution with distilled water sample, is diluted with 9 ml distilled water then vortexed).

Test procedure: 1 ml of Silver 400 at 1/100 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 ml into #10.

9/10 ml of Sabouraud Dextrose broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid

1/1000 Dilution with distilled water (1ml of Silver 400 1/100 dilution with distilled water sample, is diluted with 9 ml distilled water then vortexed).

Test procedure: 1 ml of Silver 400 at 1/1000 was placed into #1 test tube, 9/10 ml into #2, 8/10 ml into #3, 7/10 ml into #4, 6/10 ml into #5, 5/10 ml into #6, 4/10 ml into #7, 3/10 ml into #8, 2/10 ml into #9, 1/10 into #10.

9/10 ml of Sabouraud Dextrose broth was placed into #10, 8/10 ml into #9, 7/10 ml into #8, 6/10 ml into #7, 5/10 ml into #6, 4/10 ml into #5, 3/10 ml into #4, 2/10 ml into #3, 1/10 ml into #2.

All tubes contain 1 ml of liquid.

1 ml Sabouraud Dextrose broth added to all tubes.

0.1 ml of test organism suspension (1x10⁶ CFU/ml) is added to tubes containing 1 ml broth and 1 ml of concentrations of Silver 400.

All tubes contain 2.1 ml liquid with 2.5 x 10⁴ CFU/ml immediately 0.001 ml from control tube is subcultured to agar, after overnight incubation = 250 colonies.

OVERNIGHT TEST TUBE INCUBATION AT 35C

+ = Turbid (growth) - = Nonturbid (no growth)

Tube	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/1000 dilution	1	2	3	4	5	6	7	8	9	01
	-	-	-	-	-	-	-	+	+	+

0.01 ml from control tube subcultured to agar = 250 CFU.

October 5, 2001- Negative growth in all tubes, except 8-10 of the 1/1000 which contained .04%-.01% of the Silver 400. Minimum inhibitory concentration (MIC) = .4 ml of the 1/1000 solution + .6 ml Sabouraud Dextrose Broth +(1 ml Sabouraud Dextrose broth added to all tubes) + 0.1 ml of test organism suspension = 000.018%.

MINIMUM INHIBITORY CONCENTRATION = 000.018 % OF ORIGINAL FORMULATION.

The MIC measures the ability of the antimicrobial agent to inhibit multiplication of the organisms. Thus, organisms, in the inoculum are merely inhibited by the antimicrobial agent and will be able to recommence growing if the antimicrobial influence is removed. **(AGENTS THAT ARE USUALLY BACTERIOSTATIC: CHLORAMPHENICOL, ERYTHROMYCIN, NALIDIXIC ACID, SULFONAMIDES AND TETRACYCLINES).**

October 5, 2001- Visual turbidity is noted, and 0.1 ml from nonturbid tubes is subcultured to Sabouraud Dextrose agar.

October 6, 2001- CFU on subcultures made from nonturbid tubes are determined.

Sabouraud Dextrose agar
 OVERNIGHT PLATE INCUBATION AT 35C
 + = Turbid (growth) - = Nonturbid (no growth)

Tube	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/100 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	-	-	-
1/1000 dilution	1	2	3	4	5	6	7	8	9	10
	-	-	-	-	-	-	-	+	+	+

Minimum Mycoidal concentration = .4 ml of the 1.1000 Solution + .6 ml Sabouraud Dextrose Broth + (1ml Sabouraud Dextrose broth added to all tubes + 0.1 ml of test organism suspension = 000.018%

MINIMUM FUNGICIDAL CONCENTRATION = 000.018%

The MIC measures the ability of the antimicrobial agent to inhibit multiplication of the organisms. Thus, organisms in the inoculum are merely inhibited by the antimicrobial agent and will be able to recommence growing if the antimicrobial influence is removed. The MFC measures the ability of the antimicrobial agent to KILL the organisms.

I have tested over 100+ different silver solution form colloidal silver to stabilized silver to soluble silver, however Silver 400 is undoubtedly the finest product I have ever tested.

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