



**LARRY WAYNE HARRIS, RM (A.A.M.)
REGISTERED MICROBIOLOGIST (N.R.M.) 3053**

FUNGAL / FUNGUS REPORT
Laboratory Methods in Basic Mycology

Methods for Testing Anti-Microbial Effectiveness

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

Test #1

March 19, 2001, Received Sample Bottle A, Ultra-Safe Super 4x cc at Laboratory.

One part of Ultra -Safe Super 4x cc was mixed and vortexed with three parts distilled water to make test sample. Broth Dilution method, decreasing concentrations of the Ultra -Safe Super 4x cc to be tested are placed in tubes of a broth medium that will support growth of *Saccharomyces Cerebisiae* for effectiveness against Mycological agents.

Test procedure: 1 ml. of Ultra -Safe Super 4x cc 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 distilled water 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 (1 ml. of Ultra-Safe Super 4x cc Sample is Diluted with 9 ml. Distilled Water then Vortexed).

Test procedure: 1 ml. of Ultra - Safe Super 4x cc at 1/10 was placed i nto #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 distilled water 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

1 ml. Nutrient broth added to all tubes.

0.1 ml. of test organism suspension (1×10^6 CFU/ml.) is added to tubes containing 1 ml. of broth and 1 ml. of concentrations of Ultra-Safe Super 4x cc.

ALL TUBES CONTAIN 2.1 ML. 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
	-	-	-	-	-	-	-	-	-	+
Cell per field	8	8	9	9	11	15	17	20	23	33
1/10 dilution	1	2	3	4	5	6	7	8	9	10
	+	+	+	+	+	+	+	+	+	+
Cells per field	33	35	40	48	51	59	80	85	90	100+

0.001 ML. FROM CONTROL TUBE SUBCULTURED TO AGAR = 250 CFU

MARCH 20, 2001 – Negative growth in all tubes, except 10 of the 1/10, which contained .1 ml of the Ultra-Safe Super 4x cc. Minimum inhibitory concentration (MIC) = 1 ml. of the Ultra-Safe Super 4x cc solution.

MINIMUM INHIBITORY CONCENTRATION (MIC) = 20% OF ORIGINAL FORMULATION

The (MIC) measures the ability of the anti-microbial agent to inhibit multiplication of the organisms. Thus, organisms in the inoculums are merely inhibited by the anti-microbial agent and will be able to recommence growing if the anti-microbial agents influence is removed.

Agents that are usually bacteriostatic are:

(Chloramphenicol, Erythromycin, Nalidixic, Acid, Sulfonamides, and Tetracycline's).

The (MBC) measures the ability of the anti-microbial agent used to KILL the organisms!!

Larry Wayne Harris, RM (A.A.M.)



Registered Microbiologist (N.R.M.) 3053

