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APPENDIX 1

PREPARATION OF MEDIA

Brain Heart Infusion Dehydrate

Calf Brains, Infusion form	200	g
Beef Heart, Infusion form	250	g
Proteose Peptone, Difco	2	g
Sodium chloride	5	g
Disodium Phosphate	25	g

To rehydrate, dissolve 37 grammes in 1 litre distilled or deionized water. Dispense as desired for 15 minutes at 15 pounds pressure 121°C.

Trypticase Caseine Soy Broth

Pancreatic Digest of Caseine	17.0	g
Papaic Digest of Soybean Meal	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Dextrose	2.5	g

Final pH 7.3 ± 0.2

Dissolve 30 grammes of powder in 1 litre of Purified Water, Mix thoroughly and then warm gently until solution is complete.

Dispense and autoclave at 121°C, 15 pounds for 15 minutes.

Blood Agar Plate for Streaking

Beef extract agar	1000	ml
Defibrinated blood	200	ml
(Calf, sheep or human)		

The defibrinated blood may be substitute by citrated blood, Cowan and Steel has been described the strain of staphylococci may be inhibit by citrate.

Beef Extract Broth (Cowan and Steel)

Beef extract	10	g
Peptone	10	g
NaCl	5	g
Distilled water	1000	ml

Final pH 7.2 - 7.4

Dissolve the ingredients by hot water, filtered and sterilized at 115°C for 20 minutes.

Nutrient Agar

Beef extract	3	g
Agar	15	g
Peptone (glystate pancreatic digest of gelatin)	5	g
Distilled water to	1000	cc
Adjust pH to	6.8	

Enrichment media for Vibrio cholera

(Balley & Scott 158)

Peptone	10	g
NaCl	5	g
H ₂ O qs	1000	cc
Adjust pH to	9.2	
Sterile 121°C 5 minutes		

Enrichment media for Vibrio parahaemolyticus

Peptone	5	g
NaCl	10	g
Glucose	5	g
Methyl violet	2	mg
H ₂ O qs	1000	cc
pH adjusted to	9.0	
Sterile 121°C 15 minutes		

Muller-Hinton Agar (MH)

Dehydrated infusion from	300	g beef
Acid digest of casein	17.5	g
Corn starch	1.5	g
Agar	17	g
Distilled water to	1000	cc
Adjust pH to	7.4	

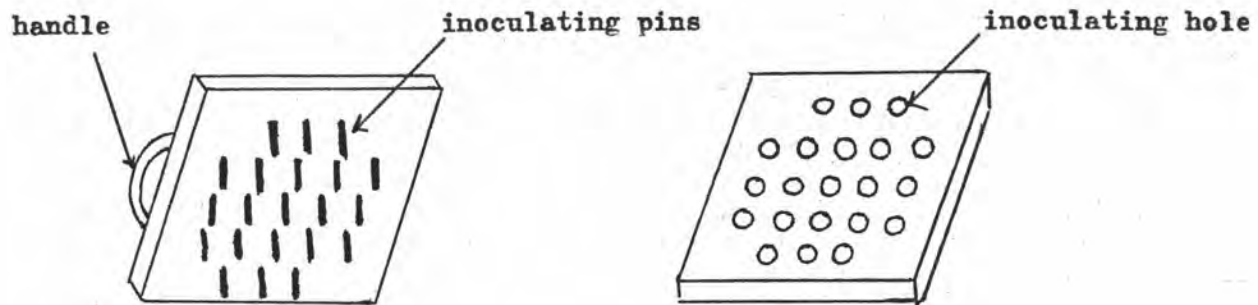
APPENDIX 2

STEER REPLICATOR (51)

In case of testing many bacterial strains for a certain antibiotic, a steer replicator can be applied for the Agar Dilution Test.

The steer replicator is composed of

1. Replicator - a square metal with handle at one side and 32-36 inoculating pins with 3 mm in diameter at another side.
2. Seed tray - a plastic tray with 32-36 inoculating holes.



REPLICATER

SEED TRAY

Application of the Steer replicator

Fill the bacterial suspension into the inoculating holes, one strain per hole. Press the inoculating needle into the filled inoculating holes. The inoculum on the surface of each needle is estimated about 0.001 cc. Touch the needle on to the surface of the antibiotic containing agar. Repeat the same process with other plates ranking from a lower concentration to the higher one. Incubate all plates at 35°C 18 hours. The sensitive strain will show no growth on the surface of antibiotic plate. The resistant strain will show a growing colony on the agar surface at the area where the pin attached.

Advantage of the Steer replicator 1) Less time consuming, because 32-36 strains of bacteria can be tested at one plate. 2) Economic. 3) Result can be interpreted as MIC also.

APPENDIX 3

The Distribution of t^* (Two-Tailed Tests)

Degrees of Freedom	Probability of a Larger Value, Sign Ignored								
	0.500	0.400	0.200	0.100	0.050	0.025	0.010	0.005	0.001
1	1.000	1.376	3.078	6.314	12.706	25.452	63.657		
2	0.816	1.061	1.886	2.920	4.303	6.205	9.925	14.089	31.598
3	.765	0.978	1.638	2.353	3.182	4.176	5.841	7.453	12.941
4	.741	.941	1.533	2.132	2.776	3.495	4.604	5.598	8.610
5	.727	.920	1.476	2.015	2.571	3.163	4.032	4.773	6.859
6	.718	.906	1.440	1.943	2.447	2.969	3.707	4.317	5.959
7	.711	.896	1.415	1.895	2.365	2.841	3.499	4.029	5.405
8	.706	.889	1.397	1.860	2.306	2.752	3.355	3.832	5.041
9	.703	.883	1.383	1.833	2.262	2.685	3.250	3.690	4.781
10	.700	.879	1.372	1.812	2.228	2.634	3.169	3.581	4.587
11	.697	.876	1.363	1.796	2.201	2.593	3.106	3.497	4.437
12	.695	.873	1.356	1.782	2.179	2.560	3.055	3.428	4.318
13	.694	.870	1.350	1.771	2.160	2.533	3.012	3.372	4.221
14	.692	.868	1.345	1.761	2.145	2.510	2.977	3.326	4.140
15	.691	.866	1.341	1.753	2.131	2.490	2.947	3.286	4.073
16	.690	.865	1.337	1.746	2.120	2.473	2.921	3.252	4.015
17	.689	.863	1.333	1.740	2.110	2.458	2.908	3.222	3.965
18	.689	.862	1.330	1.734	2.101	2.445	2.878	3.197	3.922
19	.688	.861	1.325	1.725	2.086	2.423	2.845	3.153	3.850
20	.687	.860	1.325	1.725	2.086	2.423	2.845	3.153	3.850
21	.686	.859	1.323	1.721	2.080	2.414	2.831	3.135	3.819
22	.686	.858	1.321	1.717	2.074	2.406	2.819	3.119	3.792
23	.685	.858	1.319	1.714	2.069	2.398	2.807	3.104	3.767
24	.685	.857	1.318	1.711	2.064	2.391	2.797	3.090	3.745
25	.684	.856	1.316	1.708	2.060	2.385	2.787	3.078	3.725
26	.684	.856	1.315	1.706	2.056	2.379	2.779	3.067	3.707
27	.684	.855	1.314	1.703	2.052	2.373	2.771	3.056	3.690
28	.683	.855	1.313	1.701	2.049	2.368	2.763	3.047	3.674
29	.683	.854	1.311	1.699	2.045	2.364	2.756	3.038	3.659
30	.683	.854	1.310	1.697	2.042	2.360	2.750	3.030	3.646
35	.682	.852	1.306	1.690	2.030	2.342	2.724	2.996	3.591
40	.681	.851	1.303	1.784	2.021	2.329	2.704	2.971	3.551
45	.680	.850	1.301	1.680	2.014	2.319	2.690	2.952	3.520
50	.680	.849	1.299	1.676	2.008	2.310	2.678	2.937	3.496
55	.679	.849	1.297	1.673	2.004	2.304	2.669	2.925	3.476
60	.679	.848	1.296	1.671	2.000	2.299	2.660	2.915	3.460
70	.678	.847	1.294	1.667	1.994	2.290	2.648	2.899	3.435
80	.678	.847	1.293	1.665	1.989	2.284	2.638	2.887	3.416
90	.678	.846	1.291	1.662	1.985	2.279	2.631	2.878	3.402
100	.677	.846	1.290	1.661	1.982	2.276	2.625	2.871	3.390
200	.677	.845	1.289	1.658	1.980	2.270	2.617	2.860	3.373
∞	.677	.8416	1.2816	1.6448	1.9600	2.2414	2.5758	2.8070	3.2905

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APPENDIX 4

Usual Minimum Inhibitory Concentration (ug/ml) (41)

	E. coli	Salmonella	Shigella
Streptomycin	2.8	2-16	2-8
Tetracycline	1	1-2	1-8
Chloramphenicol	0.8-8	0.5-10	2.5-6.0
Colistine	0.01-25	0.01-0.8	0.01-0.8
Neomycin	8	2	8
Co-trimoxazole			
Vanamycin	2-4	1-2	2-4
Penicillin	20-32	4-16	16
Polymycin	0.02-11	0.02-0.4	0.01-0.75
Gentamycin	8	1-8	4-8
Sulphadiazine	8	16	4

119 ✓



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