

ANNEXURE

A. Composition of bacteriological media

1. Alkaline Peptone Water (APW)

Peptone	10g
Sodium chloride	5g
Distilled water (DW)	1 Litre
pH: 9.1 ± 0.1	

2. Brilliant Green Bile Broth (BGLB)

Peptone	1 g
Lactose	1 g
Bile salt	2 g
Brilliant green	0.00133g
DW	100 ml
pH: 7.4 ± 0.1	

3. Decarboxylase Broth

Basal medium

Yeast extract	0.3g
Glucose	0.1g
Bromocresol purple (BCP)	0.0016g
DW	100ml
pH : 6.5 ± 0.1	

One amino acid to 100ml of the basal medium.

1. L-Lysine Hydrochloride	0.5g
2. L-Arginine Hydrochloride	0.5g
3. L-Ornithine Hydrochloride	0.5g

4. DNase Agar with Toluidine blue

Tryptose	20g
NaCl	5g
DNA	2g
Toluidine blue	0.1g
Agar	15g
Distilled water	1litre
pH: 7.3 ± 0.2	

5. EC broth

Tryptone	2 g
Lactose	0.5 g
Bile salt (No.3)	0.15 g
K ₂ HPO ₄	0.4 g
KH ₂ PO ₄	0.15 g
NaCl	0.5 g
DW	100 ml

pH: 6.9 ± 0.1

6. Egg Yolk Agar

Nutrient Agar	100ml
50% Egg Yolk solution	5ml

pH 7± 0.2

7. Eosin Methylene Blue (EMB Agar)

Peptone	10g
Lactose	10g
K ₂ HPO ₄	2g
Eosin Y	0.4g
Methylene blue	0.065g
Agar	15g
DW	1 litre

pH : 7.1 ± 0.1

8. Fish powder agar

Peptone	10g
Beef extract	3g
Fish powder	30g
Agar	15 g
DW	1 litre

pH: 7.0 ± 0.1

Fish powder agar with different salt concentrations was prepared by adding the required quantity of NaCl to the media along with all other ingredients before autoclaving.

9. Gelatin Agar

Nutrient Agar	1000ml
KH ₂ PO ₄	0.5g
K ₂ HPO ₄	1.5g
Gelatin	4g
Glucose	0.05g

pH 7.0±
0.2

10. Gelatin Medium

Peptone	10g
Beef extract	3g
NaCl	5 g
Gelatin	120 g
DW	1 litre
pH: 7.0 ± 0.1	

11. Hugh & Leifson Glucose O/F Medium (H&L)

Peptone	1g
NaCl	0.5g
K ₂ HPO ₄	0.4g
Dextrose	1g
Agar	0.3g
DW	100ml
pH: 7.1 ± 0.1	

Add 1ml of 0.1% solution of phenol red indicator

12. Kligler Iron Agar (KIA)

Peptone	20g
Yeast extract	3g
Beef extract	3g
NaCl	5g
Lactose	10g
Glucose	1g
Ferric citrate	0.3g
Sodium thiosulphate	0.3g
Phenol red	0.05g
Agar	15g
DW	1 litre
pH 7.4 ± 0.2	

13. Methyl Red Vogues Prausker Medium (MRVP)

Peptone	0.5g
D-glucose	0.5g
K ₂ HPO ₄	0.5g
DW	100ml
pH: 6.9 ± 0.1	

14. Nitrate Broth

Peptone	1g
Potassium nitrate	0.1g
NaCl	0.5g
DW	100ml
pH: 7.1 ± 0.1	

15. Mueller Hinton Agar

Casein acid hydolysate	17.5g
Beef infusion	300g
Starch	1.5g
Agar	17g
DW	1000ml
	pH 7.3± 0.2

16. Normal Saline (NS) (Physiological Saline)

NaCl	8.5 g
DW	1 litre

17. Nutrient Agar

Peptone	10g
Beef extract	3g
NaCl	5 g
Agar	15 g
DW	1 litre
	pH: 7.0 ± 0.1

18. Nutrient Broth

Peptone	10g
Beef extract	3g
NaCl	5 g
DW	1 litre
	pH: 7.0 ± 0.1

19. ONPG (O-nitrophenyl galactopyranoside)

ONPG solution :

ONPG	6g
Na ₂ HPO ₄ buffer	1000ml

Dissolve and filter

Peptone water:

Peptone	10g
NaCl	5g
DW	1000ml

Test: Take 1 part of ONPG solution and 3 parts of peptone water in small tubes. Inoculate heavily with the test culture. Incubate at 37°C. Read after 20 min to 24h. Yellow colour indicates positive result

20. P₁ N₀ Medium

Peptone	1g
Distilled water	100ml
	pH: 7.0 ± 0.1

21. Phosphate Buffer Diluent

KH ₂ PO ₄	34g
Distilled water	500ml

Adjust pH to 7.2 ± 0.1 with 1 N NaOH and bring volume to 1 litre with distilled water. Take 1.25 ml of the above stock solution and bring volume to 1 litre with distilled water. Dispense in flasks or tubes and sterilize at 121°C for 15min.

22. Purple Broth Base (For Sugar Fermentation)

Peptone	10g
NaCl	5g
Bromocresol purple	0.02 g
	pH to 7.0 ± 0.2

Add the required sugar to 1% level.

23. Shrimp powder agar

Peptone	10g
Beef extract	3g
Shrimp powder	30g
Agar	15 g
DW	1 litre
	pH: 7.0 ± 0.1

Shrimp powder agar with different salt concentrations was prepared by adding the required quantity of NaCl to the media along with all other ingredients before autoclaving.

24. Simmon's Citrate Agar

Sodium citrate	0.2 g
NaCl	0.5g
K ₂ HPO ₄	0.1g
NH ₄ H ₂ PO ₄	0.1g
MgSO ₄	0.02g
Bromothymol blue	0.008g
Agar	1.5g
DW	100 ml
	pH: 7.0 ± 0.2

25. Skim milk agar

Casein enzymatic hydrolysate	5g
Yeast Extract	2.5g
Dextrose	1g
Skim milk powder	28g
Agar	15g
	pH 7.0 ± 0.2

26. Starch Agar

Peptone	10g
Beef extract	3g
NaCl	5 g
Soluble Starch	10g
Agar	15 g
DW	1 litre

pH: 7.0 ± 0.1

27. Sugar Fermentation Media

Peptone	10 g
Sodium chloride	5 g
DW	1 litre

pH: 7.2 ± 0.1

Add 10ml of phenol red indicator, adjust pH.
Add the required sugar to 1% level.

28. Tergitol-7 Agar (T-7)

Peptone	10g
Yeast extract	6 g
Beef extract	5 g
Lactose	20g
Tergitol-7	0.1g
Bromothymol blue	0.05g
Agar	15g
DW	1 litre

pH: 7.2 ± 0.2

Before pouring the plates, after melting and cooling, add 0.25ml of 1% solution of sterile Triphenyl Tetrazolium Chloride (TTC) per 100 ml media.

29. a) Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS)

Yeast extract	5g
Peptone	10g
Sucrose	20g
Sodium thiosulphate	10g
Sodium Citrate Dihydrate	10g
Sodium cholate	3g
Ox-gall	5g
Sodium chloride	10g
Ferric citrate	1g
Bromothymol blue (BTB)	0.04g
Thymol blue (TB)	0.04g
Agar	15g
DW	976ml

pH: 8.6 ± 0.1

b) TCBS Agar (Difco)

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Proteose Peptone No. 3	10 g
Sodium Citrate	10 g
Sodium Thiosulfate	10 g
Bacto Oxgall	8 g
Bacto Saccharose	20 g
Sodium Chloride	10 g
Ferric Citrate	1 g
Bacto Bromo Thymol Blue	0.04 g
Thymol Blue	0.04 g
Bacto Agar	15 g
Final pH 8.6 ± 0.2 at 25°C	

30. T₁N₀ and T₁N₃ Medium

Trypticase	2.0 g
DW	200 ml
pH: 7.20 ± 0.2	

Dissolve; adjust the pH; divide into two lots. To one lot add 3 g NaCl, dissolve and dispense in 5 ml quantities. This is T₁N₃ medium. The second lot is dispensed in 5 ml quantities in test tubes. This is T₁N₀ medium.

31. Tributyrin agar

Peptic digest of animal tissue	5g
Yeast Extract	3g
Agar	15g
DW	1000ml
pH 7.5 ± 0.2	

Add 10ml of Tributyrin (glycerol tributyrate) to 1litre of the medium.

32. Triple Sugar Iron Agar (TSI)

Peptone	20g
Yeast extract	3g
Beef extract	3g
Lactose	5g
Sucrose	10g
Glucose	1g
Ferric citrate	0.3g
Sodium thiosulfate	0.3g
Phenol red (0.2% soln:)	12 ml
Agar	12g
DW	988 ml
pH: 7.4 ± 0.2	

33. Trypticase Salt Broth

Trypticase	10 g
DW	1L.
pH: 7.2 ± 0.2	

Add 10, 30, 60, 80 and 100g NaCl, as the case may be, to make media with salt concentration of 3, 6, 8 and 10%. Distribute 5 ml quantities in test tubes.

34. Tryptone Broth (Indole Medium)

Tryptone	1 g
NaCl	0.5 g
DW	100 ml
pH 7.1± 0.1	

35. Tryptone Glucose Agar (TGA)

Tryptone	0.5 g
Beef Extract (Lab lemco)	0.3 g
NaCl	0.5 g
D-Glucose	0.1 g
Agar agar	1.5 g
Distilled water (DW)	100 ml
pH: 7.1± 0.1	

36. Tryptone Soy Agar (Difco™)

Pancreatic digest of casein	15g
Soybean peptone	5g
NaCl	5g
Agar	15g
DW	1000ml

37. Urea Agar

Peptone	1g
Dextrose	1g
Sodium Chloride	5g
Potassium di hydrogen phosphate	2g
Phenol red	0.012g
Agar Agar	15g
Distilled water	1000ml
pH: 6.8 ± 0.1	

After sterilizing, cool to 50°C and aseptically add 0.5 ml of a 40 % solution of Urea per tube, mix well and allowed to cool and form slants.

B. Test Reagents

1. Kovac's cytochrome oxidase reagent

N:N:N:N.Tetramethyl-p-phenylene diamine -hydrochloride	100mg
DW	10 ml

2. Kovac's Indole reagent

p-dimethyl amino benzaldehyde	5g
N-butyl alcohol (or amyl alcohol)	75ml

3. VP test reagents and test

Solution A

α -naphthol	0.25g
Alcohol	5ml

Solution B

KOH	2g
DW	5 ml

4. Reagents for nitrate reduction test

Solution A

α - Naphthylamine	0.5 g
Dilute Sulphuric Acid (1:20)	100 ml

Dissolve, filter and store in a brown bottle.

Solution B

Sulphanilic Acid	0.8 g
Dilute Sulphuric Acid (1:125)	100 ml

5. Mercuric chloride solution

HgCl ₂	15g
HCl	20ml
DW	100ml