

**Annexure I**  
**Media Composition**

**1. Folic Acid Assay Medium**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Casein acid hydrolysate, vitamin free	12.00
Dextrose	40.00
Sodium citrate	20.00
L-Cystine	0.200
DL-Tryptophan	0.200
Adenine sulphate	0.020
Guanine hydrochloride	0.020
Uracil	0.020
Thiamine hydrochloride	0.020
Pyridoxine hydrochloride	0.004
Riboflavin (Vitamin B2)	0.002
Niacin	0.002
p-Amino benzoic acid (PABA)	0.0002
Biotin	0.0000008
Calcium pantothenate	0.0004
Dipotassium phosphate	1.00
Monopotassium phosphate	1.00
Magnesium sulphate	0.40
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.020
Final pH (at 25°C)	6.8±0.2

**2. Folic Acid Casei Medium**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Vitamin free casein acid hydrolysate	10.00
Dextrose	40.00
Sodium acetate	40.00
Dipotassium phosphate	1.00
Monopotassium phosphate	1.00
DL-Tryptophan	0.20
L-Asparagine	0.60
L-Cystine hydrochloride	0.50
Adenine sulphate	0.010
Guanine hydrochloride	0.010
Uracil	0.010
Xanthine	0.020
Sorbitan monooleate complex	0.100
Glutathione reduced	0.005
Magnesium sulphate	0.400
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.015
Riboflavin (Vitamin B2)	0.001
PABA	0.002
Pyridoxine hydrochloride	0.004
Thiamine hydrochloride	0.0004
Calcium pantothenate	0.0008
Nicotinic acid	0.0008
Biotin	0.00002
Final pH (at 25°C)	6.7±0.1

**3. Lactobacillus MRS Broth**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Proteose peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Polysorbate 80	1.00
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium phosphate	2.00
Final pH (at 25°C)	6.5±0.2

**4. M17 Broth**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Peptic digest of animal tissue	2.50
Casein enzymic hydrolysate	2.50
Papaic digest of soyabean meal	5.00
Yeast extract	2.50
Beef extract	5.00
Glucose	5.00
Ascorbic acid	0.50
Disodium - β - glycerophosphate	19.0
Magnesium sulphate	0.250
Final pH (at 25°C)	7.1±0.1

**5. Lactic Bacteria Differential Broth**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Casein enzymic hydrolysate	10.00
Papaic digest of soyabean meal	1.50
Casein acid hydrolysate	3.00
Yeast extract	1.00
Fructose	2.50
Monopotassium phosphate	2.50
Bromocresol green	0.055
Final pH ( at 25°C)	7.0±0.2

**6. Luria Bertani (LB) Broth**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Tryptone	10.00
Yeast extract	1.50
Sodium chloride	3.00
Final pH ( at 25°C)	7.0±0.2

**7. Nutrient Agar**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Peptic digest of animal tissue	5.00
Beef extract	1.50
Sodium chloride	5.00
Agar	15.00
Final pH ( at 25°C)	7.4±0.2

**8. Simmons Citrate Agar**

<b>Constituent</b>	<b>Concentration (g/L)</b>
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.0
Final pH ( at 25°C)	6.8 ± 0.2

**9. Eagle's Minimum Essential Medium (EMEM)**

<b>Constituent</b>	<b>Concentration (g/L)</b>
<b>Inorganic Salts</b>	
CaCl <sub>2</sub> (anhydrous)	0.20
MgSO <sub>4</sub> (anhydrous)	0.09
KCl	0.40
NaHCO <sub>3</sub>	1.50
NaCl	6.80
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.140
<b>Amino Acids</b>	
L-Alanine	0.0089
L-Arginine·HCl	0.1264
L-Asparagine·H <sub>2</sub> O	0.0150
L-Aspartic Acid	0.0133
L-Cystine·2HCl	0.0312
L-Glutamic acid	0.0147
L-Glutamine	0.2920

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Glycine	0.0075
L-Histidine·HCl·H <sub>2</sub> O	0.0419
L-Isoleucine	0.0525
L-Leucine	0.0525
L-Lysine·HCl	0.0725
L-Methionine	0.0150
L-Phenylalanine	0.0325
L-Proline	0.0115
L-Serine	0.0105
L-Threonine	0.0476
L-Tryptophan	0.0100
L-Tyrosine·2Na· <sub>2</sub> H <sub>2</sub> O	0.0519
L-Valine	0.04680
<b>Vitamins</b>	
Choline chloride	0.001
Folic acid	0.001
myo-Inositol	0.002
Nicotinamide	0.001
D-Pantothenic Acid	0.001
(hemicalcium)	0.001
Pyridoxine·HCl	0.0001
Riboflavin	0.001
Thiamine·HCl	
<b>Other</b>	
D-Glucose	1.00
Phenol red, sodium salt	0.01
Sodium pyruvate	0.011

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**10. McCoy's 5A Medium**

<b>Constituent</b>	<b>Concentration (g/L)</b>
<b>Inorganic Salts</b>	
CaCl <sub>2</sub> (anhydrous)	0.10000
MgSO <sub>4</sub> (anhydrous)	0.09770
KCl	0.40000
NaHCO <sub>3</sub>	2.20000
NaCl	6.46000
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.58000
<b>Amino Acids</b>	
L-Alanine	0.01336
L-Arginine·HCl	0.04214
L-Asparagine·H <sub>2</sub> O	0.04503
L-Aspartic Acid	0.01997
L-Cystine·HCl·H <sub>2</sub> O	0.03514
L-Glutamic acid	0.02210
L-Glutamine	0.21920
Glycine	0.00750
L-Histidine·HCl·H <sub>2</sub> O	0.02096
Hydroxy-L-Proline	0.01970
L-Isoleucine	0.03936
L-Leucine	0.03936
L-Lysine·HCl	0.03654
L-Methionine	0.01492
L-Phenylalanine	0.01652
L-Proline	0.01730
L-Serine	0.02630
L-Threonine	0.01790
L-Tryptophan	0.00310
L-Tyrosine·2Na·2H <sub>2</sub> O	0.02612
L-Valine	0.01760
<b>Vitamins</b>	

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Ascorbic acid	0.00050
p-Amino benzoic acid	0.00100
D-Biotin	0.00020
Choline chloride	0.00500
Folic acid	0.01000
myo-Inositol	0.03600
Nicotinamide	0.00050
Nicotinic acid	0.00050
D-Pantothenic acid	0.00020
Pyridoxine·HCl	0.00100
Riboflavin	0.00020
Thiamine·HCl	0.00020
Vitamin B-12	0.00200
<b>Other</b>	
D-Glucose	3.0000
Glutathione (reduced)	0.0005
Bacto-peptone	0.6000
Phenol red, Sodium salt	0.0100

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