Appendices

APPENDIX 1

MEDIA

1.1. Nutrient Agar

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000

Final pH (at 25°C) 7.4±0.2

Directions

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desiredand sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Mix well before pouring.

1.2. Peptone Water

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Sodium chloride	5.000

Final pH (at 25°C) 7.2±0.2

Directions:

Suspend 15.0 grams in 1000 ml distilled water. Add the test carbohydrate in desired quantity and dissolve completely.Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

1.3. Simmons Citrate Agar

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000

Final pH (at 25°C) 6.8±0.2

Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

1.4. Starch Agar

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Yeast extract	1.500
Beef extract	1.500
Starch, soluble	2.000
Agar	15.000

Final pH (at 25°C) 7.4±0.2

Directions

Suspend 30 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

1.5. Urea Agar Base (Christensen)

Ingredients	Gms / Litre
Peptic digest of animal tissue	1.000
Dextrose	1.000
Sodium chloride	5.000
Disodium phosphate	1.200
Monopotassium phosphate	0.800
Phenol red	0.012
Agar	15.000

Final pH (at 25°C) 6.8±0.2

Directions

Suspend 24.01 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize byautoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% UreaSolution (FD048) and mix well. Dispense into sterile tubes and allow setting in the slanting position. Do not over heator reheat the medium as urea decomposes very easily.

1.6. Nutrient Broth

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000

Beef extract	1.500
Yeast extract	1.500

Final pH (at 25℃) 7.4±0.2

Directions

Suspend 13 grams in 1000 ml distilled water. Heat, if necessary, to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

APPENDICES TWO

REAGENT

2.1. Lugol's iodine solution

To make 1 litre:

Potassium iodide	20 g
Iodine	10 g
Distilled water	1000ml

Direction

Weigh the potassium iodide, and transfer to abrown bottle premarked to hold 1 litre. Add about a quarter of the volume of water, and mix until the potassium iodide is *completely* dissolved. Weigh the iodine, and add to the potassiumiodide solution. Mix until the iodine is dissolved.

2.2. Oxidase reagent

Prepare freshbefore use.

To make 10 ml:

Tetramethyl-*p*-phenylenediamine dihydrochloride*	0.1 g
Distilled water	10 ml

Direction

Dissolve the chemical in the water

2.3. Crystal violet Gram stain

To make 1 litre:

Crystal violet	20 g
Ammonium oxalate	9 g

Ethanol or methanol, absolute 95 ml

Distilled water 1000ml

Direction

Weigh the crystal violet on a piece of clean paper (pre weighed). Transfer to a brown bottle premarked to hold 1 litre. Add the absolute ethanol or methanol (technical grade is suitable) and mix until the dye is completely dissolved.

Weigh the ammonium oxalate and dissolve in about 200 ml of distilled water. Add to the stain. Make up to the 1 litre mark with distilled water, and mix well.

2.4. Kovac's Reagent for indole

P-dimethylaminobenzaldehyde 5g

Amyl alcohol 75ml

ConcHcl 25ml

Direction

Disolve the aldhyde in alcohol by gently warming in water path (50-55c), cool and add acid with care. Protect from the light,store at 4°C.

2.5. Nitrate Broth

Beef extract 3.0 g

Prptone 5.0g

Potassium nitrate 1.0g

Direction

This medium used concentration 0.9 g in 100ml of distilled water.

Sterlize by autoclaveing 121˚C for 15mintes.

2.6.Sulphanilic acetic acid Reagent

To make 20ml

Sulphanilic acid	0.16g
Acetic acid	20ml

Direction

Prepare by mixing 5.7ml of glacial acetic acid with 14.3 ml of distilled water.

Weight the sulphanilic acid transfer to clean bottle, then add acetic acid mix and dissolve the chemical.

Lable the bottle, store at room temperature.

2.7. Alpha Naphthylamine Reagent

To make 20 ml

Alpha Naphthylamine	0.1g
Acetic acid, 5mol/l	20ml

Direction

Prepare by mixing 5.7 ml of glacial acetic acid with 14.3ml of Distelled water

Dissolve and mix, label the bottle, store at 2-8°C.