Appendix

Preparation of culture media and biochemical test

1.1. Culture media:

Preparation of Media

Sterilization of all media was accomplished at 15Ib pressure (121^{o}C) for 15 minutes unless otherwise specified.

1.1.1. Buffered charcoal yeast extract (BCYE) agar

Composition:

Ingredients	Grams / Liter
Norit SG charcoal	2.0 g
Yeast extract	10.0 g
ACES buffer	10.0 g
Ferric pyrophosphate (soluble)	0.25 g
L-cysteine HCl-H20	0.40 g
Agar, bacto (Hardy)	17.0 g
Potassium alpha-ketoglutarate	1.0 g
Distilled water	1,000 ml

DGVB medium

Ingredients	Grams / Liter
Glycine	0.3%
Polymyxin B	50 units/ml
Vancomycin	1 mg/l
Dyes	10 mg/l

Directions:

Suspend 40 grams in 1000 ml distilled water. The medium dissolved by heat to boiling completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add sterile rehydrated contents of one vial of Legionella Supplement. Mix well and pour into sterile Petri plates with constant agitation to ensure that charcoal particles were evenly distributed.

1.1.2. Blood agar:

It was prepared by dissolving 28g blood agar base powder in one liter of distilled water, and Sterilized by autoclaving at 121°C for 15 minutes. Transfer to a 50°C water bath, add aseptically the sterile blood and mix gently but well. Avoid forming air bubbles Important. Dispensed under aseptically in 25 ml amounts in sterile petri dishes. The poured media were left to solidify at room temperature.

1.1.3. Mueller - Hinton agar:

It was prepared by dissolving 38g Muller and Hinton powder in one liter of distilled water and Sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C and poured into sterile petri dishes. The poured media were left to solidify at room temperature.

1.2. Biochemical test

1.2.1. Nitrocefin disks:

The rapid detection of B-lactamase enzymes in solution colonies *of Neisseria gonorrheae, Legionella pneumophila* and *Staphylococcus spp.* and anaerobic bacteria.

Composition:

(I package contain 50 discs in a light resistant plastic vial.) 6mm diameter filter paper impregnated with Nitrocefin.

1.2.2. Nutrient Gelatin

Nutrient Gelatin was recommended for detection of gelatin liquefaction by proteolytic microorganisms.

Composition:

Ingredients	Grams / Liter
Peptic digest of animal tissue	5.000
Beef extract	3.000
Gelatin	120.000

Final pH (at 25°C) 6.8±0.2

Formula adjusted, standardized to suit performance parameters

Directions:

Suspend 128 grams in 1000 ml of warm (50°C) distilled water. The medium dissolved by heat to boiled completely. Dispense into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to cool in an upright position.

1.2.3. Sodium Hippurate Hydrolysis Broth

Hippurate Hydrolysis Broth was recommended for detection of hippurate hydrolyzing bacteria.

Composition:

Ingredients	Grams / Liter
Heart infusion powder	10.000
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Sodium hippurate	10.000

Final pH (at 25°C) 7.4±0.2

Ferric Chloride Test Reagent

Ferric chloride solution, 12%

Ferric Chloride .....12.0 g

Concentrated Hydrochloric Acid .....5.4 mL

Deionized Water .....94.6 mL

**Formula adjusted, standardized to suit performance parameters

Directions:

Suspend 35 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amounts in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Add approximately 75 ml of distilled water to a 100 ml volumetric flask. With a transfer pipette, add 5.4 ml of HCl to the flask, running down the acid along the sides of the flask. Add 12 gram of ferric chloride. Dissolve by warming the flask gently, swirling the contents to mix well. Bring the volume up to 100 ml with distilled water.