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Appendix I1. Preparation of media1.1 Aesculin broth (Cowan & Steel)

Aesculin	1.0 g
Ferric citrate	0.5 g
Peptone	10.0 g
Sodium chloride	5.0 g
Distilled water	1000.0 ml

Adjust pH to 7.0, dispense in test tubes.

Autoclave 115 C 10 min

1.2 Decarboxylase broth (Falkow)

Bacto peptone	5.0 g
Yeast extract	3.0 g
Dextrose	1.0 g
Bromcresol purple	0.016 g
Distilled water	1000.0 ml

Dissolve the media and divide into 4 portions.

Add 0.5% L-Lysine dihydrochloride, L-Ornithine dihydrochloride and L-Arginine monohydrochloride to each portion respectively and the remaining served as control.

Adjust pH to 6.8

Autoclave 121 C, 15lb, 15 min



### 1.3 Dextrose Starch Agar

Proteose peptone No.3	15.0 g
Bacto dextrose	2.0 g
Soluble starch	10.0 g
Sodium chloride	5.0 g
Disodium phosphate	3.0 g
Bacto gelatin	20.0 g
Bacto agar	15.0 g

Final pH  $7.3 \pm 0.1$  at 25 C. Autoclave 121 C, 15lb, 15 min

### 1.4 Gelatin agar

Gelatin	4.0 g
Distilled water	50.0 ml
Nutrient agar	1000.0 ml

Dissolve gelatin with distilled water and mix well with melted nutrient agar. Autoclave 121 C, 15lb, 15 min. Cooled and poured plates.

### 1.5 MR-VP medium

Buffer peptone	7.0 g
Dipotassium phosphate	5.0 g
Bacto dextrose	5.0 g

Dissolve and adjust pH to 6.9 at 25 C. Autoclave 121 C, 15lb, 15 min. Add 1% serum, mix and dispense in test tubes 3 ml per each.

1.6 Nitrate broth

Beef extract	3.0 g
Peptone	5.0 g
Potassium nitrate	1.0 g
Distilled water	1000.0 ml

Adjust pH to 7.0. Dispense in test tubes with inverted Durham tubes.

Autoclave 121 C, 15lb, 15 min

1.7 Nutrient broth

Beef extract	3.0 g
Peptone	5.0 g
Distilled water	1000.0 ml

Adjust pH to  $6.9 \pm 0.1$

Autoclave 121 C, 15lb, 15 min

1.8 Nutrient broth with 6% sodium chloride

Sodium chloride	6.0 g
Nutrient broth	100.0 ml

Autoclave 121 C, 15lb, 15 min.

1.9 Phenol red broth base

Bacto Beef extract	1.0 g
Proteose-peptone No.3	10.0 g
Sodium chloride	5.0 g
Bacto phenol red	0.018 g
Distilled water	1000.0 ml

Dissolved and adjust pH to 7.4 at 25 C.

Autoclave 121 C, 15lb, 15 min

Add 1% sterilized carbohydrates and dispense in test tube 3 ml per each. A Durham tube is inverted in the tube containing 1% glucose for observing gas production

#### 1.10 Urea Christensen's agar

Peptone	1.0 g
Sodium chloride	5.0 g
Monopotassium phosphate	2.0 g
Glucose (0.1%)	1.0 g
Urea (20%)	20.0 g
Phenol red	0.012 g
Agar	15.0-20.0 g
Distilled water	1000.0 ml

Weigh out accurately 29 g of the dehydrated base and dissolved in 100 ml of distilled water. Sterilize by filtration.

Dissolve 15 g of agar in 900 ml of distilled water. Autoclave 121 C, 15lb, 15 min. Cool to 50 C.

Add 100 ml of sterile urea to the agar and mix.

Dispense in sterile test tubes. Allow medium to cool in a slant position.

#### 2. Preparation of 1% agarose in PBS pH 7.0 for GDPT

NaH <sub>2</sub> PO <sub>4</sub> 0.2 M	130.0 ml
Na <sub>2</sub> HPO <sub>4</sub> 0.2 M	204.0 ml
EDTA	1.8 g
Sodium azide	0.65 g

Distilled water to	1000.0	ml
Agarose	10.0	g

### 3. Reagents

#### 3.1 Acid mercuric chloride

Mercuric chloride	12.0	g
Anhydrous sod. carbonate	10.0	g
Copper sulfate	1.73	g
Distilled water to	100.0	ml

#### 3.2 Jasmin's reagent

Phenol	1.0	ml
Serum	10.0	ml
Physiological saline to	100.0	ml

#### 3.3 Kovac's reagent

P-Dimethylaminobenzaldehyde	5.0	g
Amyl alcohol	75.0	ml
conc. HCl	25.0	ml

Keep the solution in brown bottle at 4 C.

#### 3.4 MR reagent

Methyl red	0.04	g
Absolute alcohol	40.0	ml
Distilled water to	100.0	ml

#### 3.5 Nitrate reagent

##### Reagent A

- Naphthylamine	5.0	g
Acetic acid (5N), 30%	1000.0	ml

Reagent B

Sulfanilic acid	8.0	g
(p-Aminobenzene Sulfonic acid)		
Acetic acid (5N), 30%	1000.0	ml

3.6 Oxidase reagent (Kovac's)

Tetramethyl-p-phenylenediamine dihydrochloride	1.0	g
Distilled water to	100.0	ml

Allow to stand 15 min before use. Store in a dark, glass stopper bottle.

3.7 Phosphate buffer saline 0.02 M pH 7.0Solution A :  $\text{NaH}_2\text{PO}_4$  0.2 M

$\text{NaH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$	27.6	g
Distilled water to	1000.0	ml

Solution B :  $\text{Na}_2\text{HPO}_4$  0.2 M

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	71.64	g
Distilled water to	1000.0	ml

Solution A	33.0	ml
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Solution B	67.0	ml
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Sodium chloride	5.9	g
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Distilled water to	1000.0	ml
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adjust pH to 7.0

3.8 VP solutionSolution A

- Naphthol	5.0	g
Absoluted alcohol	100.0	ml



Solution should not be darker than straw colour.

Solution B

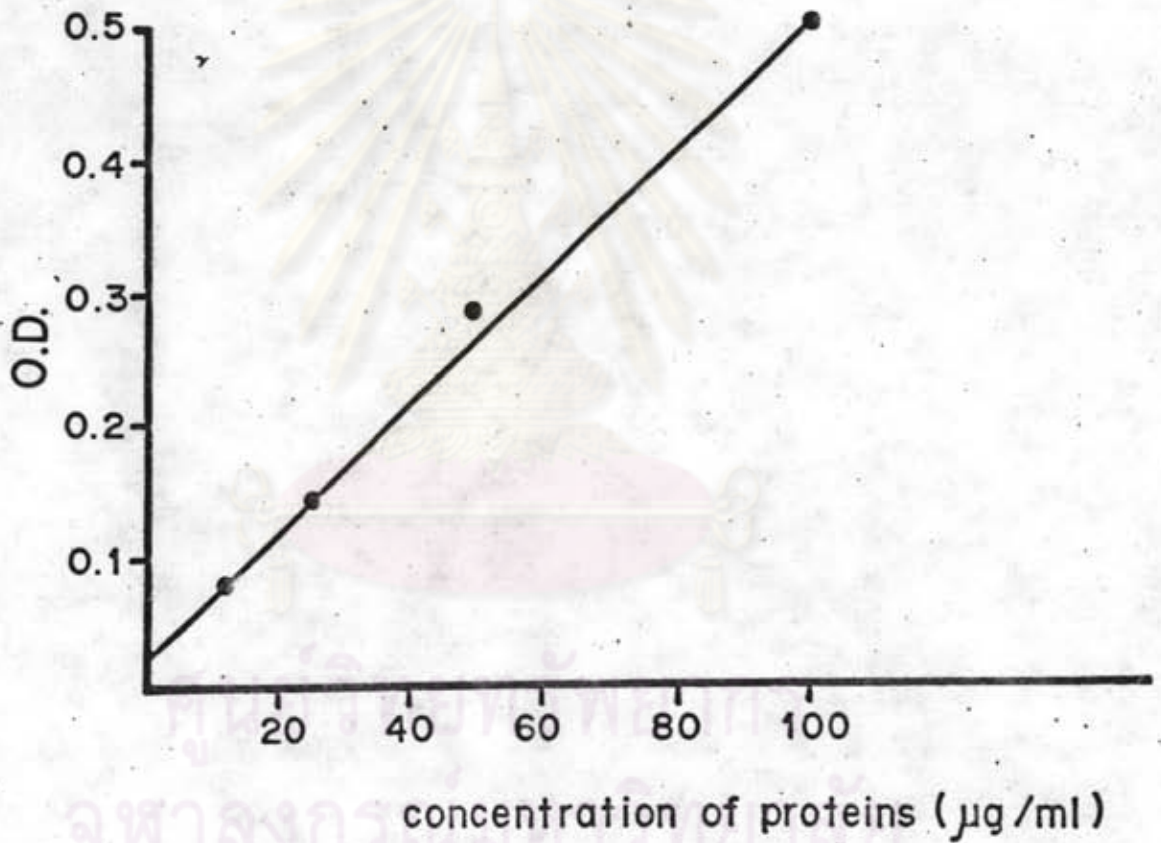
Potassium hydroxide	40.0 g
Distilled water	100.0 ml



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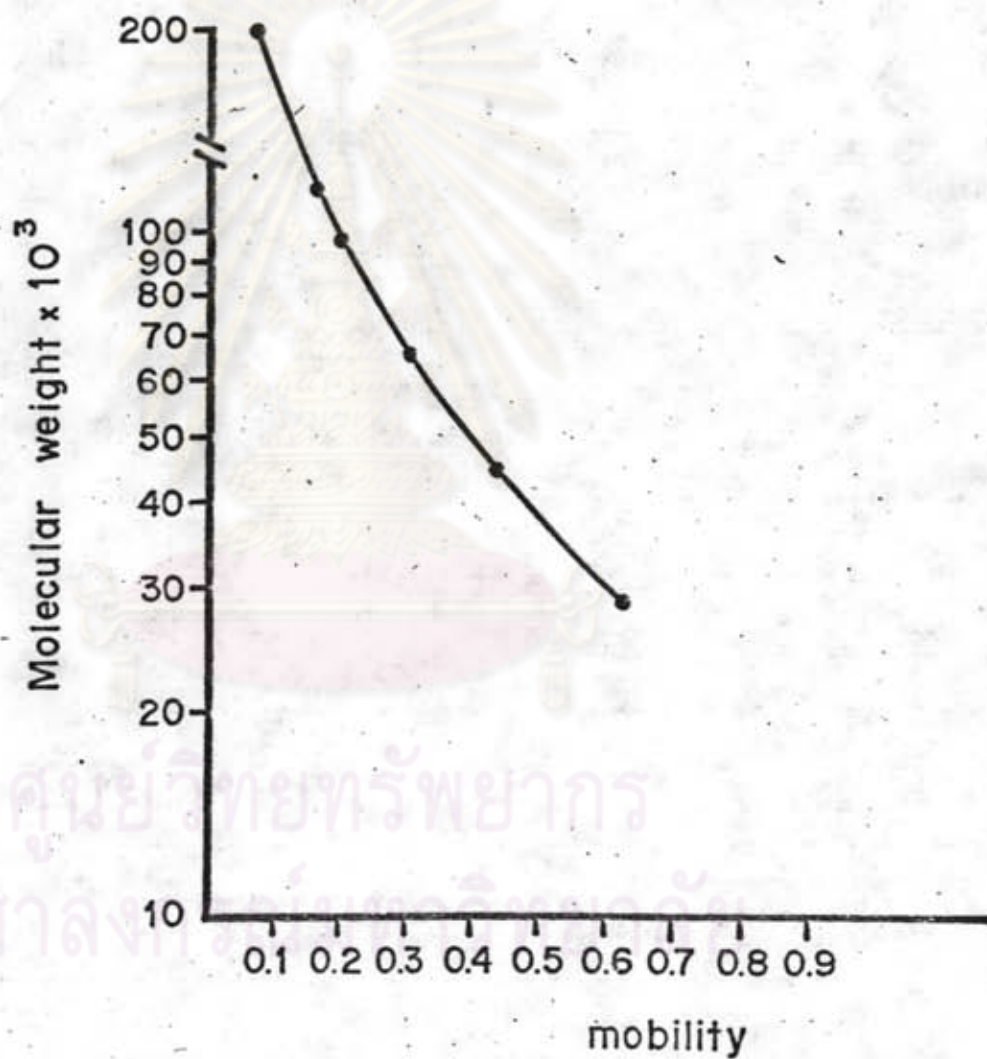
Appendix II

Standard curve for protein determination by  
method of Lowry et al (101)



Appendix III

Calibration curve for the estimation of molecular weight proteins, determined by SDS-PAGE





Miss Kanee Unchitti was born in Bangkok. She graduated with B.Sc. (Med. Tech.) from Mahidol University. She now works as a Medical Scientist at the Department of Medical Sciences, Ministry of Public Health.



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