

## CHATER V

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## APPENDIX I

### 1. The Isotonic Balance Salt Solution (for the collection of intestinal lavage)

Sodium Chloride	6.5	g.
Sodium Bicarbonate	2.5	g.
Potassium Chloride	0.75	g.
Distilled Water to	1000	ml.
Resulting ionic concentration were :		
Sodium	141.0	mEq/L
Potassium	10.0	mEq/L
Chloride	121.0	mEq/L
Bicarbonate	30.0	mEq/L

(from Levy A.G., Gastroenterol, 70(2), 157, 1976)

### 2. The Stool Extract Buffer Solution

Sodium Chloride	8.0	g.
Potassium Chloride	0.2	g.
di-potassium hydrogen phosphate	1.15	g.
Potassium di-hydrogen phosphate	0.2	g.
Tween - 20	0.5	ml.
Bovine Serum Albumin	1.0	g.
Polymyxin B	20.0	mg.
Distilled Water to	1000	ml.

(from Evans D.G., Clin. Microbiol., 12(6), 738, 1980)



## APPENDIX II

### The reagents for ELISA method

#### 1. Coating Buffer (Carbonate-bicarbonate buffer, pH 9.6)

Sodium-carbonate	1.59	g.
Sodium-bicarbonate	2.93	g.
Sodium Azide	0.2	g.
Distilled Water to	1000	ml.

The pH was adjusted to 9.6 and stored at 4° c for not more than 2 weeks.

#### 2. Washing Buffer (PBS-Tween pH 7.4)

Sodium Chloride	8.0	g.
Potassium di-hydrogen phosphate	0.2	g.
di-sodium hydrogen phosphate	2.9	g.
Potassium Chloride	0.2	g.
Tween -20	0.5	ml.
Distilled Water to	1000	ml.

The pH was adjusted to 7.4 and stored at 4° c.

#### 3. Enzyme Diluent (Triethanolamine buffer)

Sodium Chloride	8.0	g.
0.25 M triethanolamine pH 7.6	50.0	ml.

20 mg/ml BSA	10.0 ml.
10 % Sodium Azide	10.0 ml.
100xMg/Zn	10.0 ml.
(100 mM Magnesium Chloride/0.25 mM Zinc Chloride)	
Distilled Water to	1000 ml.
Stored at 4° c.	

4. Sample Diluent (PBS - Tween pH 7.4)

5. Substrate Diluent for alkaline phosphatase  
(Diethanolamine buffer pH 9.8 )

Diethanolamine	97.0 ml.
Sodium Azide	0.2 g.
Magnesium Chloride	100.0 ml.
Distilled Water	800.0 ml.
1.0 M HCl is added until the pH reaches 9.8 and then distilled water is added to final volume of 1 liter and stored at 4° c in the dark.	

6. Alkaline Phosphatase substrate

p-nitrophenyl phosphate (sigma 104 ) is used for convenience. Tablets(5 mg) of the substrate are stored below 0° c in the dark. Immediately before use, one 5 mg tablet is dissolved in each 5 ml of diethanolamine buffer

already warmed to room temperature. Any substrate remaining one day after preparation was rejected.

7. Stopping Solution for Alkaline Phosphatase (3 M NaCl)

Sodium Chloride	1.2	g.
Distilled Water to	10.0	ml.

8. Urease Substrate ( Urea-BCP pH 4.8 )

Urea-BCP consists of 8 mg of bromocresol purple powder (Gurr) was dissolved in 1.48 ml of 0.01 M NaOH and the volume made to 100 ml with deionized water. After addition of 100 mg urea, EDTA was added to a final concentration of 0.2 mM to chelate any heavy metal ions which might inhibit the urease activity and to provide slight buffering. The pH of the substrate was adjusted to 4.8 using 0.1 M NaOH or 0.1 M HCl and the solution was stored at 4° c until used.

9. Stopping Reagent for Urease (1 % merthiolate solution)

Merthiolate	0.1	g.
Distilled Water to	10.0	ml.



## BIOGRAPHY

Miss Kamolwan Sukprasert was born on April 23, 1962 in Bangkok, Thailand. She graduated with a Bachelor of Science (Medical Technology) from the Faculty of Medical Technology at Cheingmai University in 1984.