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APPENDICES

APPENDIX I

MEDIA, SOLUTION AND IDENTIFICATION PROCEDURES

1. Columbia agar with 7 % sheep blood

Columbia agar base	39 g/L
Horse serum	70 ml/L
Sheep blood	70 ml/L
Distilled water	860 ml

The medium was sterillized by autoclaving at 121°C, 15 pounds/inch^2 pressure, for 15 minutes. The sterile medium was cooled to 45°C to 50°C. Add blood and horse serum after cooling base medium. Dispense 20 ml per petri dish. Cool and store at 4°C until used.

Do not add any heat labile components (Sheep blood or antibiotic solutions) to the sterillizer.

2. Columbia agar with 7 % Sheep blood and antibiotics

Columbia agar base	39 g/L
Horse serum	70 ml/L
Sheep blood	70 ml/L
Vancomycin (1 ml of stock)	10 mg/L
Trimethoprim (0.5 ml of stock)	5 mg/L
Cefsoludin (0.5 ml of stock)	5 mg/L
Amphotericin B (0.5 ml of stock)	5 mg/L
Distilled water	860 ml

The medium was sterillized by autoclaving at 121°C, 15 pounds/inch^2 pressure, for 15 minutes. The sterile medium was cooled to 45°C to 50°C. Add blood, horse serum and antibiotic solution after cooling base medium. Dispense 20 ml per petri dish. Cool and store at 4°C until used.

Do not add any heat labile components (Sheep blood or antibiotic solutions) to the sterillizer.

3. Antibiotic solution preparation

Vancomycin, final concentration 10 mg/L

- Prepare a stock solution, dissolve 0.028 g in 5.78 ml distilled water

Cefsoludin, final concentration 5 mg/L

- Prepare a stock solution, dissolve 0.014 g in 2.82 ml distilled water.

Trimethoprim, final concentration 5 mg/L

- Prepare a stock solution, dissolve 0.018 g in 3.7 ml distilled water.

Amphotericin, final concentration 5 mg/L

- Prepare a stock solution, dissolve 0.014 g in 2.84 ml distilled water.

4. Urease test

Solution A : for 20 ml

Urea agar base (BBL)	2.9 g
Urea	4 g
Distilled water	20 ml

Dissolve in 20 ml of distilled water. Adjust to pH 6.2 Sterilize by filtration (use a 0.22 μM filter).

Solution B : for 80 ml

Bacto agar (Difco)	0.5 g
Distilled water	80 ml

Add the ingredient to 80 ml of distilled water ; heat with stirring until the agar is dissolved. Sterilize by autoclaving at 121°C, 15 ponds/inch^2 pressure, for 15 minutes. Mix solution A, 20 ml with solution B, 80 ml. Aliquot into sterile 1.5 microtube (1 ml/tube). Test the sterility of Urease medium by incubate tubes at 37°C for 24 hours. Store tubes in refrigerator at 4°C until used.

5. Brain heart infusion with 20 % glycerol

Brain heart infusion	37 g/L
Glycerol	200 ml
Distilled water	800 ml

Sterilize by autoclaving at 121°C, 15 pounds/inch^2 pressure, for 15 minutes. Aliquot into sterile screw cap tubes (1 ml/tube). Store tubes in refrigerator at 4°C until used.

6. Sterile saline solution

Sodium Chloride	8.5 g/L
Distilled water	1 L

Sterilze by autoclaving at 121°C, 15 pounds/inch^2 pressure, for minutes. Store at room temperature.

APPENDIX II

REAGENTS, MATERIALS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Agarose	(Biorad, U.S.A.)
Brain heart infusion agar	(Oxoid, U.S.A.)
EDTA	(Amresco, U.S.A.)
Ethedium bromide	(Amresco, U.S.A.)
Glacial acetic acid	(Merck, Germany)
Horse serum	(GibcoBRL, U.S.A.)
Miniral oil	(Sigma, U.S.A.)
Columbia agar base	(Oxoid, U.S.A.)
Urea agar base	(BBL, U.S.A.)
Bacto agar	(Difco, U.S.A.)
NaCl	(Merck, Germany)
$NaHCO_3$	(Merck, Germany)
$Na_2HPO_4*2H_2O$	(Sigma, U.S.A.)
Tris	(Amresco, U.S.A.)

B. MATERIALS

Anaerobic jar	(BBL, U.S.A.)
Gas pack	(Oxoid, U.S.A.)

C. INSTRUMENTS

Water bath	(Memmert, U.S.A.)
Perkin Elmer GeneAmp PCR system 9600	(Perkin Elmer, U.S.A.)
Camera Gel Doc™ MZL	(BIO-RAD, U.S.A.)
Incubator	(BIO-RAD, U.S.A.)
Microcentrifuge	(Eppendorf, U.S.A.)
Spectrophotometer	(BIO-RAD, U.S.A.)

APPENDIX III

REAGENTS AND PREPARATION

1. 5x Tris-borate buffer (TBE)

Tris base	54 g/L
Boric acid	27.5 g/L
0.5 M EDTA (pH 8.0)	20 ml

Adjust volume to 1 liter with distilled water. The solution was mixed and sterilized by autoclaving at 121°C for 15 min.

2. 0.5 M EDTA (pH 8.0)

Disodium ethylene diamine tetraacelate $2H_2O$	186.1 g/L
Distilled water	1 L

Adjust pH to 8.0 and volume to 1 liter. Store at room temperature for no longer than 1 year.

3. 10x TE buffer

Tris	12.11 g/L
0.5 M EDTA	20 ml

Adjust to pH 8.0 by adding conc. HCl, Adjust volume to 1,000 ml and sterilized by autoclaving at 121°C for 15 min.

4. 2 % Agarose gel

Agarose	0.4 g
1x TBE	20 ml

Dissolve by heating in microwave oven and occasional mix unit no granules of agarose are visible.

5. 5x Loading buffer 100 ml

Tris HCl	0.6 g
EDTA	1.68 g
SDS	0.5 g
Bromphenol Blue	0.1 g
Sucrose	40 g

Adjust volume to 100 ml with distilled water. Mix the solution, aliquot into 1.5 microtube and store at 4°C.

BIOGRAPHY

Miss Janjira Thongtem was born on September 25, 1979 in Nakornsithamarat, Thailand. She graduated with the Bachelor degree of science in Microbiology from Prince of Songkla University in 2002 and then attended to particulate in Medical Microbiology program, Graduate School, Chulalongkorn University for her Master's Degree