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APPENDIX

Media and reagents used for isolation and identification of Campylobacter species

1. Brucella blood agar (Gibco, Madison, WI, U.S.A.)

Brucella agar (Gibco, Madison, U.S.A.)	43 g
Distilled water	1000 ml

Heat to boiling with agitation to completely dissolve the medium. Sterilize by autoclaving for 15 minutes at 121°C. Cool the agar to 48°C, pipette 50 ml of sterile sheep blood to the media. Mix well, and pour approximately 20 ml amount into a sterile Petri dish. The medium is used for isolating of Campylobacter species and used to examine the growth at 25°C and 42°C of Campylobacter species.

2. Brucella broth (Gibco, Madison, WI, U.S.A.)

Brucella broth (Gibco, Madison, WI, U.S.A.	29 g
Distilled water	1000 ml

Dispense into appropriate containers and sterilze by autoclaving for 15 minutes at 121°C. The medium is used for growing Campylobacter in order to detect the reaction of reduction of triphenyl-tetrazolium chloride

3, 0.04% 2,3,5-triphenyl tetrazolium chloride (TTC) (Nachamkin et al. 1984)

2,3,5-triphenyl-tetrazolium chloride	4	g
(TTC; Sigma, St. Louis, MO, U.S.A.		
Distilled water	100	ml

4. Doyle's medium

Brucella broth (Gibco, Madison, WI, U.S.A.) 930 ml (preparation by dissolving 29 g of anhydrous Brucella broth in 970 ml of distilled water and sterilize by autoclaving for 15 min at 121°C and adding the followings:-)

Lysed horse blood 7%	70	ml
0.3% sodium succinate	10	ml
(Sodium succinate 30 g/100 ml)		
0.01% cysteine hydrochloride	1	ml
(Cysteine hydrochloride 10 g/100 ml)		
Vancomycin (15 ug/ml)	1	ml
Trimethoprim (5 ug/ml)	1	ml
Polymyxin B (20 IU/ml)	1	ml
Cycloheximide (50 ug/ml)	1	ml

Mix well, and distribute aseptically in approximately 5 ml amounts in sterilized 16 X 125 mm Pyrex screw-capped tubes. The medium is enrichment for growth of Campylobacter spp.

5. Heart infusion agar

Dehydrate Heart infusion broth (Gibco, Madison, WI, U.S.A.)	25	g
Agar, powdered	25	g
Distilled water	1000	ml

Suspend the ingredients in the water, and dissolve by boiling. Distribute in approximately 3 ml amounts in 13X100 mm screw-capped tubes. Autoclave at 121°C for 15 min and slant before solidifying. The medium is used for growing Campylobacter spp. in order to test catalase production.

6. 3% hydrogen peroxide (3% H_2O_2)

Hydrogen peroxide 30% (E. Merck, Darmistadt, Germany)	10	ml
Distilled water	10	ml

The reagent is stored at 4°C. It is used for performing catalase test.

7. Nitrate broth medium

Heart infusion broth (Difco, Detroit, Michigan, U.S.A.)	25	g
Potassium nitrate	2	g
Distilled water	1000	ml

Before adding the potassium nitrate, boil the other ingredient in water and adjust pH to 7.3 to 7.4 Dispense

4 ml per tube and sterilized at 15 pounds pressure for 15 min. The medium is used for nitrate reduction test.

8. Nitrate reagents

Solution A :	Sulfanilic acid	8 g
	5 N acetic acid	1000 ml
Solution B :	Dimethyl- -naphthylamine	6 ml
	5 N acetic acid	1000 ml

The reagents are used for nitrate reduction test.

9. 1% tetramethyl-para-phenylene diamine dihydrochloride

NNN'N'-tetramethyl-p-phenylenediammine hydrochloride (BDH, Poole, England)	1 g
Distilled water	1000 ml

This reagent is used for oxidase test. Pepared freshly before testing.

10. Nalidixic acid disc (NA disc)

NA disc with a concentration of 30 ug of nalidixic acid (BBL, Cockeysville, MD. U.S.A.) are stored in a cool dry place at 4°C until use. The disc is used for susceptibility test in identification step.

11. Cephalothin disc (CF disc)

CF disc with a concentration of 30 ug of cephalothin (BBL, Cockeysville, MD, U.S.A.) are stored in a cool dry place at 4°C until use. The disc is used for susceptibility test in identification step.

12. 1% Basic fuchsin

Basic fuchsin (EM Diagnostic system, New Jersey, U.S.A.)	1 g
Distilled water	100 ml

Basic fuchsin is dissolved in distilled water and filtered by using Whatman filter paper no. 1. The solution is used to stain the smear of Campylobacter organism.

13. Triple sugar iron (TSI) agar

Dehydrated triple sugar iron agar (TSI, BBL, Cockeysville, M.D., U.S.A.)	59.4 g
Distilled water	1000 ml

Suspend the powder in distilled water. Mix thoroughly. Heat with frequent agitation and boil for 1 min to completely dissolve the powder. Tube (3 ml per tube) and sterilize by autoclaving at not over 118°C for 15 min. Cool in a slanted position such that deep butts are formed. The medium is freshly prepared and used

within 3 days to detect hydrogen sulfide production.

14. 1% sodium hippurate

Sodium hippurate (Sigma, St. Louis, MO, U.S.A.)	1 g
distilled water	100 ml

The solution is dispensed 0.4 ml amounts into small test tubes and frozen at -20°C until use.

15. 3.5% ninhydrin solution

Ninhydrin (Sigma, St. Louis, MO, U.S.A.)	0.35 g
Acetone	5 ml
Butanol	5 ml

Ninhydrin was dissolved in 1:1 mixture of acetone and butanol and kept at room temperature until use.

16. FBP semisolid medium

- Medium A

Brucella broth (Difco, Detroit, Michigan, U.S.A.)	2.9 g
$Na_2 HPO_4$ (anhydrous)	0.118 g
KH_2PO_4 (anhydrous)	0.023 g
Agar (Gibco, Madison, WI, U.S.A.)	0.2 g
Distilled water	97 ml

Midium A was autoclaved for 15 min at 15 lbs and

allowed to cool to 45°C

The following solutions were freshly prepared and sterilized by filtration :

- Solution B Ferrous sulphate .$7H_2O$ 10%
- Solution C Sodium metabisulphite 10%
- Solution D Sodium pyruvate 10%

One ml of solution B was added to 1 ml of solution C, mixed well and this mixture was added to 1 ml of solution D. The whole mixture was then added to medium A. Final pH was adjusted to about 7.3. The medium was dispensed 3 ml into 16X150 mm-screw-capped tubes. Fresh media was prepared every two weeks. The medium was used for rapid test of hydrogen sulfide production

17. Methyl green (MG)-Deoxyribonucleic acid test agar (DTA),

DTA (BBL, Cockeysville, MD., U.S.A.)	42	g
Distilled water	1000	ml

pH 7.3±0.2

The medium was prepared and added 1.35 ml of 0.5% aqeuous MG solution (see 18) to 100 ml agar media. The mixture was autoclaved and 25 ml was poured into Petri-dishes. The medium was used for DNA hydrolysis test.

18. 0.5% Methyl green (MG) solution (Lior 1984)

Mg (Sigma, St. Louis, MO, U.S.A.)	0.5 g
Distilled water	100 ml

MG powder was dissolved in water and washed 3-5 times with chloroform until washings were colorless, then keep at 4°C

19. 1.5% NaCl (Benjamin et al. 1983)

Yeast extract nutrient agar	100 ml
NaCl	1.5 g

The media was dissolved and autoclaved. Poured the media into Petri dishes, 25 ml per plate. This media was used for NaCl tolerance test.

20. 3.5% NaCl

Brucella albimi broth (Gibco, Madison, WI, U.S.A.)	100 ml
Agar	0.16 g
NaCl	3.5 g

The media was dissolved and dispensed 3 ml into 13X100 mm screw-capped tubes and autoclaved.

21. 1% glycine medium

Agar	0.16 g
Glycine	1 g
Brucella broth	100 ml

The medium was dissolved and dispensed 3 ml into 13X100 mm screw-capped tubes and autoclaved.

22. 15% glycerol-Brain heart infusion broth

Dehydrated Brain heart infusion broth (BBL, Cockeysville, MD, U.S.A.)	3.7 g
Distilled water	85 ml

The BHI broth was dissolved by heating, and added with glycerol 15 ml. Then the media was autoclaved at 121°C for 15 min. Dispensed 0.5 ml of media into sterile small glass vial. The medium was used as a stock medium for Campylobacter spp.

23. Urea agar (Urease test medium) (Christensen 1946).

Peptone	1 g
Glucose	1 g
Sodium chloride	5 g
Monopotassium phosphate	2 g
Phenol red	0.12 g
Agar	20 g
Distilled water	1000 ml

Final pH 6.8-6.9

Prepare the agar base, and sterilize in the autoclave at 121°C for 15 min in flasks containing 100-200 ml amounts. Store until needed. Prepare a 29% solution of urea. Sterilize by filtering through a

sterile bacterilogic filter. Add the sterile urea solution in a final concentration of 10% to a flask of agar base that has been melted and cooled to a temperature of 50°C. Mix well, and distribute aseptically in sterile small tubes in amount of 2-3 ml. Allow the medium to solidify in a slanting position in such a way as to obtain an agar butt of 1/2 inch and an agar slant of 1 inch.

24. MacConkey agar slant

Suspend 25 g of dehydrated MacConkey agar in 500 ml of distilled water and heat to boiling to dissolve the medium completely. Distribute in tubes in amount 3 ml. Sterilized in the autoclave at 121°C for 15 min. Cool the medium to solidify in a slanting position. The medium was used to determine the ability of Campylobacter spp to grow on this medium.

25. Wang's media (Wang et al. 1980)

Brucella broth (prepared by dissolving dehydrate Brucella broth 2.9 g in 90 ml of of distilled water	90 ml
Agar	0.5 g
Sheep blood	10 ml

To completely dissolve agar in Brucella broth by

heating and autoclave at 121°C for 15 min. Cool the medium to 48°C, pipette 10 ml of sheep blood to the medium. Mix well, and dispense the medium 0.5 ml amount into steriled microtubes.

This medium was used for transportation of Campylobacter spp.

BIOGRAPHY

Miss Keskaew Pienthaweechai was born on October 1, 1953, in Bangkok, Thailand. She graduated the degree of Bachelor of Science in Medical Technology from Faculty of Medical Technology, Mahidol University in 1975. She had been a medical technologist of Division of Microbiology, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University from March 1, 1975 to July 30, 1983. Up to now, she has been an instructor of Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Khonkaen University. During her occupational experience, she has had publications :

1. Jayanetra, P., M. Vorachit, K. Pienthaweechai, B. Pongpanich and S. Niemsiri, "Aetiologic agents of septic sore throat in Thai children," Southeast Asian J. Trop. Pub. Hlth., 9, 549-557, 1978.

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