APPENDIX I

Formulation and preparation of stains, reagents, culture media and routine solutions

Gram staining procedure

1) Crystal violet
   - Solution 1: Crystal violet (2g)  
     Ethanol 95% (20ml)
   - Solution 2: Ammonium oxalate (0.8g)  
     Distilled water (80ml)
   - Mix solution 1 and 2, and allow to stand for at least 24 hours, and then filter before use

2) Lugol’s Iodine
   - Potassium iodide (2g)
   - Iodine (1g)
   - Distilled water (300ml)

3) Diluted carbol fuschin
   - Stock solution: Safranine O (2.5g)  
     Ethanol 95% (100ml)
   - Working solution: Stock solution (10ml)  
     Distilled water (90ml)

Catalase reagent:
   - Hydrogen peroxide (3%)
   - Store in refrigerator

Blood Agar
   - Tryptone / peptic digest of animal tissue (10g)
   - Brain heart infusion (500g)
   - Sodium chloride (5g)
   - Agar (15g)
   - Distilled water (1000 ml)
   - Sterilize by autoclaving
   * Cool media to 45-50°C, then add preheated (45°C) sterile blood

Mueller Hinton Agar:
   - Beef infusion (300g)
   - Casein (17.5g)
   - Starch (1.5g)
   - Agar (17g)
   - Distilled water (1000ml)
   - Sterilize by autoclaving

* Blood may be added as described for blood agar
Brain Heart Infusion (BHI) broth:
- Calf Brain infusion (12.5g)
- Beef heart infusion (5g)
- Proteose peptone (10g)
- Glucose (2g)
- Sodium chloride (5g)
- Disodium phosphate (2.5g)
- Distilled water (1000ml)
- Sterilize by autoclaving

Mc Farland 0.5 Turbidity Standard:
- 0.048 M BaCl₂ (0.5ml)
- 0.35 M NH₄SO₄ (99.5ml)

Saline
- Sodium chloride (0.9g)
- Distilled water (100ml)
- Sterilize by autoclaving

Tris-acetate EDTA (TAE) buffer (50X):
- Tris base (242g)
- Glacial acetic acid (57.1ml)
- EDTA (18.61g)
- Distilled water to 1000ml
- Sterilize by autoclaving

Tris-borate EDTA (TBE) buffer (10X):
- Tris base (121.2g)
- Boric acid (61.8g)
- EDTA (0.745g)
- Distilled water (1000ml)
- Sterilize by autoclaving

Tris-EDTA (TE) buffer:
- 1M Tris-HCL, pH 8.0 (1ml ; final concentration = 10mM)
- 0.5 M EDTA (200 µl ; final concentration = 1mM)
- Distilled water to 100ml
- Sterilize by autoclaving

0.5 M EDTA, pH 7.0, 7.5, or 8.0
- Disodium EDTA.2H₂O (18.61g)
- Distilled water 60ml
- The pH is adjusted as desired with 1 M NaOH
- Distilled water to 100 ml
- Sterilize by autoclaving

* EDTA will not dissolve completely until the pH is adjusted with NaOH pH 7.0
1 M NaOH
- NaOH (4g)
- Distilled water (100 ml)
- Sterilize by autoclaving

10mM Tris-HCl, pH 7.5
- Tris base (0.12g)
- Distilled water (80 ml)
- The pH is adjusted as desired with concentrated HCL
- Distilled water to 100 ml
- Sterilize by autoclaving

Phenol-chloroform mixture
1) Phenol :
- water saturated redistilled phenol (100 ml)
- 0.5 M Tris-HCL pH 8.0 containing 1mM EDTA (100 ml)
- Mix well and allow the layers to separate
- Remove upper aqueous phase and repeat step 2 and 3 once more
- 10 mM Tris-HCL pH 8.0 containing 1 mM EDTA (100 ml)
- Mix well and layers to separate
- Remove upper aqueous phase and repeat step 5 and 6 once more
- 10 mM Tris-HCL pH 8.0 (100 ml)
- Store at 4°C in the dark

2) 24:1 (v/v) chloroform-isoamyl alcohol
- Chloroform (96 ml)
- Isoamyl alcohol (4 ml)
- Store in a capped bottle

3) Phenol-chloroform mixture
- Lower phase of the phenol solution (5 ml)
- 24:1 (v/v) chloroform–isoamyl alcohol (5 ml)
- TE, pH 8.0 (10 ml)
- Store at 4°C in the dark. Use the lower organic layer.

Phosphate-buffered saline (PBS)
- NaCl (10 g)
- KCL (0.25 g)
- Na2HPO4 (1.43 g)
- KH2PO4 (0.25 g)
- Adjust pH to 7.3
- Distilled water (1000 ml)
- Sterilize by autoclaving

Sodium dodecyl sulphate (SDS)
- Sodium dodecyl sulphate (10 g)
- Distilled water (100 ml)
Proteinase K buffer:
- 0.5 M EDTA, pH 8.0 ( autoclaved) (100 ml; final concentration = 0.5 M)
- 25 % Sarkosyl (4 ml; final concentration = 1 %)

Restriction buffer:
- 10 X RE buffer (provided by manufacturer) (10 µl)
- Bovine serum albumin 100 mg/ml (provided by manufacturer) (1 µl)
- Distilled water (89 µl)

Component preparation for cDNA synthesis, Fragmentation, Labelling, Hybridization, Washing and Staining for the Microarray technique.

75 ng/µL Random Primers
For 1000 µL:
- 25 µL of 3 µg/µL Random Primers
- 975 µL of Nuclease-free H2O
- Store at -20°C in a non-frost-free freezer.

2 mg/mL NeutrAvidin
- Resuspend 10 mg NeutrAvidin in 5 mL PBS solution. Store at 4°C.

12X MES Stock Buffer
- (1.22M MES, 0.89M [Na+])
For 1,000 mL:
- 64.61g of MES hydrate
- 193.3g of MES Sodium Salt
- 800 mL of Molecular Biology Grade water
- Mix and adjust volume to 1,000 mL.
- The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter.

2X Hybridization Buffer (50 mL)
- (Final 1X concentration is 100mM MES, 1M [Na+], 20 mM EDTA, 0.01% Tween-20)
For 50 mL:
- 8.3 mL of 12X MES Stock Buffer
- 17.7 mL of 5M NaCl
- 4.0 mL of 0.5M EDTA
- mL of 10% Tween-20
- 19.9 mL of water
- Store at 2°C to 8°C, and shield from light.

Wash Buffer A: Non-Stringent Wash Buffer
- (6X SSPE, 0.01% Tween-20)
For 1,000 mL:
- 300 mL of 20X SSPE
- mL of 10% Tween-20
- 699 mL of water
- Filter through a 0.2 µm filter.
- Store at room temperature.
APPENDIX

APPENDIX II

PUBLICATIONS (INTERNATIONAL)


   Selected as Science Direct Top 25 Hottest Articles within the journal counted by article downloads from Science Direct (APR-JUN 2006)


SCIENTIFICAL MEETING POSTERS (INTERNATIONAL)

Simultaneous species identification and detection of methicillin-resistance in staphylococci using multiplex PCR and Real time PCR (ISAAR 2003, Korea, Award Winner).

Differentiation of *S. aureus* and coagulase negative staphylococci and simultaneous detection of methicillin resistance using triplex real-time PCR assay. (Presented at the 9th Western Pacific Congress on Chemotherapy and Infectious Disease, at Bangkok, Thailand (1 – 5th December, 2004)

PATENTS (2005)

Differentiation of *S. aureus* and coagulase negative staphylococci and the method for simultaneous detection of methicillin resistance using triplex real-time PCR assay (Patent No - PI20053073)


AWARDS
