### Appendix A

**Bacterial Strains Used**

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Appendix B

Media, Buffer, Solution

All media and solutions were prepared with distilled or deionised water. They were sterilized by autoclaving at 15 psi at 121°C for 20 minutes, unless otherwise stated.

B.1 Arginine Dihydrolase Test

- LB broth 100 ml
- L-arginine solution 1 ml
- Phenol red (indicator)

B.2 Alkaline Peptone Water (APW)

- Alkaline peptone water powder 12.0 g
- Distilled water up to 400 ml

B.3 Cell lysis buffer (50 mM Tris, 50 mM EDTA, pH 8.0 + 1.0% Sarcosine)

- 1.0M Tris, pH 8.0 25 ml
- 0.5M EDTA, pH 8.0 50 ml
- 10% Sarcosyl 50 ml
- Deionised water to 500 ml

B.4 Cell suspension buffer (100 mM Tris, 100 mM EDTA, pH 8.0)

- 1 M Tris, pH 8.0 10 ml
- 0.5 M EDTA, pH 8.0 20 ml
- Deionised water to 100 ml
B.5 Ethylenediaminetetraacetic acid, (0.5 M) pH 8.0

EDTA 74.44g

Deionised water to 400 ml

The pH of the solution was adjusted pH to 8.0 by adding concentrated HCl.

B.6 Luria-bertani (LB) agar

Tryptone 1.0 g
Yeast extracts 0.5 g
NaCl 0.5 g
Bacteriological agar 1.5 g

Distilled water up to 100 ml

B.7 Luria-bertani (LB) broth

Tryptone 1.0 g
Yeast extracts 0.5 g
NaCl 0.5 g

Distilled water up to 100 ml

B.8 Oxidase Test

N,N,N’,N’-Tetramethyl-p-phenylenediamine-2HCl 0.10 g

Distilled water up to 10 ml
B.9 Phosphate Buffered Saline (PBS), 10 X, pH7.4

NaCl 80 g  
KCl 2 g  
Na$_2$HPO$_4$ 14.4 g  
KH$_2$PO$_4$ 2.4 g  
Deionised water to 1000 ml  

*The pH of the stock solution was adjusted pH to 8.3 and autoclaved. It was then diluted to 1X for routine use.*

B.10 Proteinase K (10.0 mg/ml)

Proteinase K powder (Promega, Madison, USA) 100 mg  
Sterile deionised water to 10 ml

B.11 Sarcosyl (10% N-Lauryl-Sarcosine)

Sodium N-lauroyl-sarcosinate Solution 10 ml  
Deionised water to 100 ml

B.12 Sodium chloride (0.85%)

Sodium chloride 3.2g  
Distilled water up to 400ml

B.13 String-Test

Sodium deoxycholate 0.05g  
Distilled water up to 10 ml
B.14  **Thiosulphate citrate bile salt (TCBS) agar**

- Thiosulphate citrate bile salt (TCBS) powder 8.8 g
- Distilled water up to 100 ml

B.15  **Triple Sugar Iron (TSI) Agar supplemented with 3% NaCl**

- Triple Sugar Iron powder 6.5 g
- NaCl 3.0 g
- Distilled water up to 100 ml

B.16  **Tris, (1M) pH 8.0**

- Tris 48.45g
- Deionised water to 400 ml

*The pH of the solution was adjusted pH to 8.0 by adding concentrated HCl.*

B.17  **Tris-Borate EDTA Buffer (TBE), 10 X, pH8.3**

- Tris base 121.1 g
- Orthobic Acid 61.8 g
- EDTA (Ultra Pure Grade) 0.745 g
- Deionised water to 1000 ml

*The pH of the stock solution was adjusted pH to 8.3 and autoclaved. It was then diluted to 0.5X for routine use.*
B.18  Tris-EDTA (TE) buffer (10mM Tris : 1mM EDTA, pH8.0)

1 M Tris, pH 8.0          10.0 ml
0.5 M EDTA, pH 8.0        2.0 ml
Deionised water to 1000 ml

B.19  Tryptone broth with 0% NaCl

Tryptone water powder    1.0 g
Distilled water up to 100 ml

B.20  Tryptone broth with 3% NaCl

Tryptone water powder    1.0 g
NaCl              3.0 g
Distilled water up to 100 ml

B.21  Tryptone broth with 6% NaCl

Tryptone water powder    1.0 g
NaCl              6.0 g
Distilled water up to 100 ml

B.22  Tryptone broth with 10% NaCl

Tryptone water powder    1.0 g
NaCl              10.0 g
Distilled water up to 100 ml
Appendix C
Publications


### Appendix D

**Presentations**


Appendix E

Intellectual Property

1. A System for One Step Detection of Vibrio species, V. parahaemolyticus, V. cholerae and V. vulnificus (IP20092073).
Appendix F

Awards

1. **Gold Medal Award.** A Rapid and Easy PCR Assay for Differentiation of Human Pathogenic and Non-Pathogenic *Vibrio* Spp. Umexpo 2010. 1-3 April, UM.

2. **Gold Medal Award.** Molecular Approaches Towards the Differentiation of Biotypes, Serogroups and Virulence Genes in *Vibrio cholerae*. Umexpo 2010. 1-3 April, UM.


7. **Silver Medal Award.** EzPlex V - A Rapid Multiplex-PCR for Simultaneous Detection of *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and other *Vibrio* spp. 20th ITEX, 17-19 May 2009, KLCC.