

# Supplementary Document

## Development of a rapid, low-cost portable detection assay for enterococci in wastewater and environmental waters

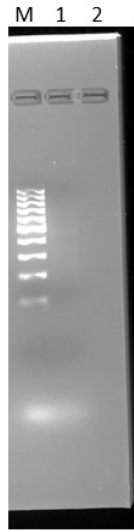
Alka Rani Batra <sup>1,2,3,\*</sup>, Darren Cottam <sup>3</sup>, Muriel Lepesteur <sup>3</sup>, Carina Dexter <sup>3</sup>, Kelly Zuccala <sup>3</sup>, Caroline Martino <sup>3</sup>, Leadin Khudur <sup>1</sup>, Vivek Daniel <sup>2</sup>, Andrew S. Ball <sup>1</sup> and Sarvesh Kumar Soni <sup>1</sup>

<sup>1</sup> ARC Training Centre for the Transformation of Australia's Biosolids Resource, School of Science, RMIT University, Bundoora West, Victoria 3083, Australia

<sup>2</sup> School of Science, RMIT University, Melbourne, Victoria 3083, Australia

<sup>3</sup> Environment Protection Authority Victoria, Centre for Applied Sciences, Ernest Jones Drive, Macleod Victoria 3085

\* *Correspondence:* alka.rani@rmit.edu.au. Tel: +61399256594

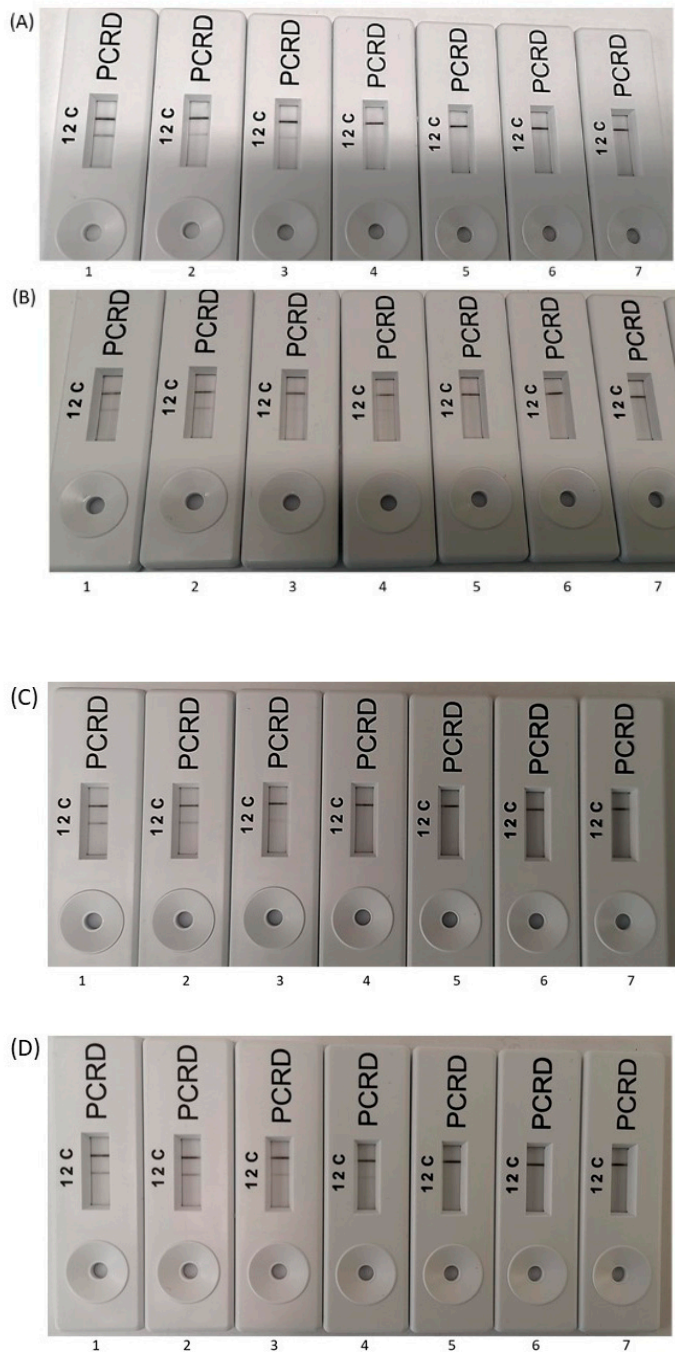


(A)



(B)

**Figure S1:** Specificity validation of Ent F1/R1 primer set. (A) RPA-AGE; Lane M: 100 bp molecular marker, Lane 1: non-template control, lane 2: empty (B) RPA-LF assay; LFA 1 – LFA 10: *E. faecalis*, *E. faecium*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E. coli* O157:H7, *Salmonella* Typhimurium, *Shigella dysenteriae*, *E. coli* K-12.



**Figure S2:** Sensitivity of RPA-LF assay for detection of *E. faecalis* using (A) Tap water; (B) Koo Wee Rup wastewater; (C) Lang Lang wastewater; (D) Saline. LFA 1 to 7:  $2.8 \times 10^6$  CFU/100 mL,  $2.8 \times 10^5$  CFU/100 mL,  $2.8 \times 10^4$  CFU/100 mL,  $2.8 \times 10^3$  CFU/100 mL,  $2.8 \times 10^2$  CFU/100 mL,  $2.8 \times 10^1$  CFU/100 mL, non-template control (NTC).



**Figure S3:** Confirmation of the presence of *E. faecalis* in Lang Lang wastewater. LFA 1 to 9: RPA- LFA of Lang Lang wastewater neat,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ; and positive template control.