PP042

Development and Evaluation of a Recombinase Polymerase Amplification Lateral-Flow Assay (RPA-LFA) for Species Level Identification of *Burkholderia pseudomallei*

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Introduction

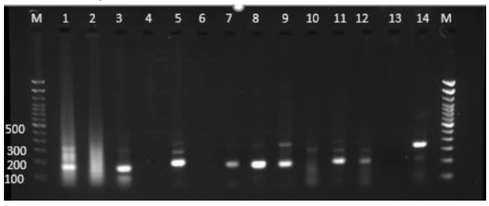
- Melioidosis is a common, severe infection among high-risk hospitalised adults in India, with nearly one in eleven patients presenting with syndromes of sepsis/community-acquired pneumonia/multi-focal abscess during the monsoon and immediate post-monsoon season having culture-confirmed melioidosis.[1]
- High rates of morbidity and mortality (20-50%) highlight the substantial clinical impact of delayed or missed diagnosis.
- Culture, the cornerstone of microbiological diagnosis, is time-consuming and has an estimated sensitivity of 60%.[2]
- Accurate species-level identification of *B. pseudomallei* remains challenging due to its phenotypic similarity to other *Burkholderia* species, such as *B. cepacia complex*, and to other non-fermenting Gram-negative bacilli.
- Existing commercial biochemical identification platforms are inadequate, leading to frequent misidentification.

Aims and objectives

The present study aimed to develop and optimise a recombinase polymerase amplification combined with a lateral flow assay (RPA-LFA) in identifying genomic DNA of *B.pseudomallei* and to assess the detection limit of RPA-LF in comparison to real-time PCR in the detection of *B. pseudomallei.*

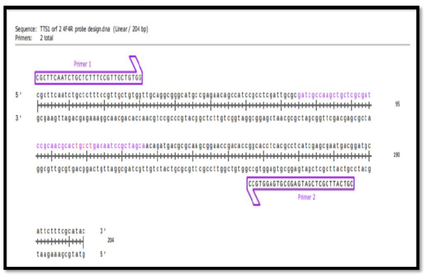
Material & Methods

- Genomic DNA extraction of *B. pseudomallei*.
- Real-time PCR assay targeting the Type III secretion system 1 (TTS1) gene for confirmation of *B. pseudomallei,*
- Determination of the lower limit of detection (LOD) for the TTS1 real-time PCR using *B. pseudomallei* genomic DNA.
- Screening of RPA Primers and Standardisation of Basic RPA, Reaction time, and temperature.



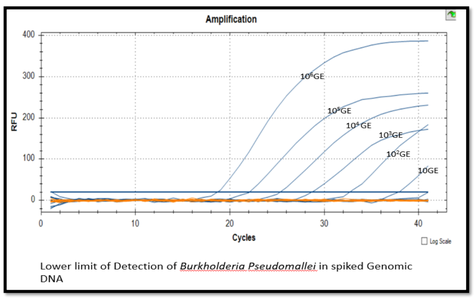
M- DNA ladder. Lane 1,2 are 1F/1R. Lane 3,4 are 2F/2R. Lane 5,6 are 3F/3R. Lane 7,8,9 are 4F/4R. Lane 10,11,12 are 5F/5R. Lane 13 is 4F/5R.Lane 14 is 5F/4R.

- Design of RPA Primer probes (for LFA endpoint reactions), targeting the orf2 gene of *B. pseudomallei* type III secretion system (T3SS) cluster genes.

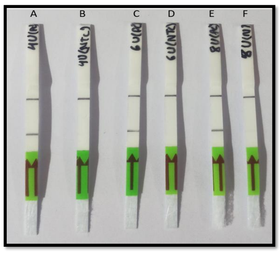


- Standardisation of RPA-LF, Reaction time, and temperature
- Determination of LOD for RPA-LF using *B. pseudomallei* genomic DNA.

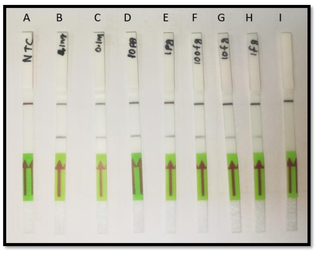
Results



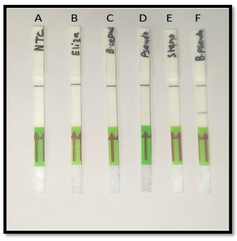
Linear Dynamic Range analysis of Serially Diluted genomic DNA of *B. pseudomallei*



Standardization of RPA-LF assay (at 40˚C for 20 minutes) with varying units of Endonuclease IV enzyme. A-4U, C-6U, E-8U, B, D, and F are template controls of the respective units.



The limit of detection of the LF-RPA (at 40˚C for 20 minutes), using genomic DNA of *B. pseudomallei.* Serial diluted gDNA of *B. pseudomallei* (from 1 ng to 0.1 fg/reaction) was tested by LF-RPA at 40˚C for 20 minutes; NTC – no template control.



LF-RPA reactions (at 40˚C for 20 minutes) were conducted with gDNA of non- *B. pseudomallei* strains. Lane A- No template control, B -*Elizabethkingia meningoseptica,* C- *B. cepacia, D - Pseudomonas aeruginosa*, E- *Stenotrophomonas maltophilia*, *F- B. pseudomallei.* This experiment was repeated three times with the same result.

Discussion

- The RPA-LFA method offered a high sensitivity (LOD of 0.1 fg of pure gDNA of *B. pseudomallei)*, compared to the analytical LOD of *B. pseudomallei* real-time PCR. [Approx 10 genome equivalents (GE) per reaction, corresponding to roughly 10(fg) of purified bacterial DNA], with minimum equipment dependence.
- Prior studies by Peng et al. and Li et al, using TwishAmp nfo kit for LF-RPA, have reported analytical sensitivities in the ranges of 20 fg and 30 f, of genomic DNA, respectively.[3,4] We used the Endonuclease IV enzyme due to the unavailability of the TwishAmp nfo kit, with improved LOD
- In addition, there was no cross-reactivity with other non- *B. pseudomallei* GNB, suggesting that LF-RPA has good specificity.
- A constraint of the LF-RPA for routine field application is the DNA extraction step.
- Further studies, in various clinical samples, are required to evaluate its practicability over other currently available DNA diagnostic systems for melioidosis diagnosis.

Funding

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References

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