Rapid identification of melioidosis agent by an insulated isothermal PCR on a field–deployable device

ABSTRACT

Background. Burkholderia pseudomallei causes melioidosis, a serious illness that can be fatal if untreated or misdiagnosed. Culture from clinical specimens remains the gold standard but has low diagnostic sensitivity. Method. In this study, we developed a rapid, sensitive and specific insulated isothermal Polymerase Chain Reaction (iiPCR) targeting bimA gene (Burkholderia Intracellular Motility A; BPSS1492) for the identification of B. pseudomallei. A pair of novel primers: BimA(F) and BimA(R) together with a probe were designed and 121 clinical B. pseudomallei strains obtained from numerous clinical sources and 10 ATCC nontargeted strains were tested with iiPCR and qPCR in parallel. Results. All 121 B. pseudomallei isolates were positive for qPCR while 118 isolates were positive for iiPCR, demonstrating satisfactory agreement (97.71%; 95% CI [93.45– 99.53%]; k = 0.87). Sensitivity of the bimA iiPCR/POCKIT assay was 97.52% with the lower detection limit of 14 ng/µL of B. pseudomallei DNA. The developed iiPCR assay did not cross-react with 10 types of non-targeted strains, indicating good specificity. Conclusion. This bimA iiPCR/POCKIT assay will undoubtedly complement other methodologies used in the clinical laboratory for the rapid identification of this pathogen.