Sensitive Detection and Quantification of *Burkholderia pseudomallei* in Soil Samples Using Culture-Dependent and-Independent Approaches

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Abstract. *Burkholderia pseudomallei*, the causative agent of melioidosis, is a soil-dwelling Gram-negative bacterium. Although the bacterium has been known for a century, its worldwide distribution as well as its natural lifestyle still remains uncertain. Detection of *B. pseudomallei* using culture-dependent and-independent methods has been previously reported. However, these methods have demonstrated either lack sensitivity or inaccurate quantification; therefore, environmental samples containing low numbers of *B. pseudomallei* will likely remain undetected or unquantifiable. This study aimed to develop different methods for sensitive detection and quantification of *B. pseudomallei* in soil samples. With an improved culture method based on soil dispersion in a polyethylene glycol and sodium deoxycholate solution, we found a significantly higher recovery (p < 0.001) of *B. pseudomallei* colony forming units (CFU) compared to the conventional culture method. With a culture-independent method, we established DNA isolation and purification protocols for soil samples allowing direct detection and quantification of *B. pseudomallei* cells by real-time PCR assay targeting the type three secretion systems 1 (TTSS 1) gene. PCR inhibition test was constructed to monitor for possible PCR inhibition and to exclude false-negative PCR results and inaccurate quantifications. Out of 40 studied environmental soil samples collected in Northeast Thailand 33 (82.5%) were qPCR-positive whereas only 26 (65.0%) were culture-positive. The sensitivity of the method was 97%. Confirmation by nested PCR approach revealed three (7.5%) soil samples positive by real-time PCR assay but negative by culture method. Quantification of *B. pseudomallei* DNA by real-time PCR assay was significantly higher than the CFU values obtained by culture method (p < 0.001). The presented assays provide highly specific and sensitive tools for environmental surveillance of *B. pseudomallei* in order to fully understand the epidemiology of melioidosis.

Keywords: *Burkholderia pseudomallei*, meliodosis, polyethylene glycol, sodium deoxycholate, culture-dependent method, culture-independent method