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# Development of A Conventional and Multiplex Polymerase Chain Reaction Assay to Detect *Burkholderia* Genus and to Differentiate the Species in Clinical Specimens

J. Suppiah, V. Mariappan, K.M. Vellasamy, J. Thimma and J. Vadivelu.

*Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.*

Recent molecular-based techniques are becoming useful tools for diagnosis of clinically important *Burkholderia* spp. There is a need to differentiate *Burkholderia pseudomallei* from *Burkholderia cepacia* due to cross-reactions especially in biochemical and serological assays. In this study, conventional Polymerase Chain Reaction assay targeting three genes was developed to detect the *Burkholderia* genus and simultaneously differentiate the two main species, *B. pseudomallei* and *B. cepacia* in various clinical specimens. Primers were designed for the amplification of *Burkholderia* genus-specific *groEL* gene, *B. pseudomallei* specific *mprA* gene and *B. cepacia* specific *zmpA* gene. The specificity of the primers was tested with a panel of gram-negative and gram-positive organisms including 40 *Burkholderia* spp. culture and blood samples. Amplification of the three genes was not observed in any other organisms except for the targeted *Burkholderia* species. In addition, all *B. pseudomallei* strains were positive for groEL and mprA amplification indicating a specificity of 100%. All *B. cepacia* strains produced corresponding amplicons for detection of *groEL* and *zmpA* except two strains. Besides that, a multiplex PCR for the detection of *B. pseudomallei* has been developed targeting the *mprA* and *groEL* genes. It was found that the developed PCR assays are specific for detection and differentiation of *B. pseudomallei* and *B. cepacia* from organisms of the same genus and other closely related organisms.