

Comparative analysis of current diagnostic PCR assays in detecting pathogenic *Leptospira* isolates from environmental samples

ABSTRACT

Objective: To compare the efficiency of routine diagnostic PCR assays in detecting pathogenic *Leptospira* isolated from water and soils.

Methods: Seven routine assays targeting six genes (*lipL32*, *flaB*, *gyrB*, *lfb1*, *secY* and *ligB*) were evaluated and compared on the cultures of two groups of pathogenic *Leptospira* from different sources. One group included 19 described reference strains recovered from infected human or animals, and another group included 22 environmental isolates from recreational and residential sites in Malaysia. The latter have been confirmed for presence of pathogenic *Leptospira* DNA. PCR positivity or detection sensitivity of each assay was determined and compared between the two groups.

Results: Validation on reference strains showed 100.0% PCR sensitivity for all assays except *ligB*-PCR (95.0%) that failed to amplify *Leptospira interrogans* serovar Pomona. In marked contrast, there was a notable decline in sensitivity in the environmental isolates (*lipL32*-PCR, 95.5%; *flaB*-PCR, 90.9%; *gyrB*-PCR, 77.3%; *lfb1*-PCR, 59.1%; *secY*-PCRs, 40.9% G1/G2-PCR, 36.4%; *ligB*-PCR, 13.6%), implying a large genetic distance between the two groups, as well as nucleotide polymorphism among environmental isolates.

Conclusions: High proportion of false-negative PCR results suggests a need of prudent selection of primers in detecting environmental pathogenic *Leptospira*. These findings offer valuable insights on the extensive biodiversity of genus *Leptospira* and its impact on the efficacy and development of molecular detection tool.

Keyword: *Leptospira*; Pathogenic species; Environmental samples; PCR; Sensitivity