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Development of a multiplex PCR assay for the diagnosis of Whooping cough (Pertussis disease)

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Whooping cough or pertussis disease is caused by two *Bordetella* species; *B. pertussis* and *B. parapertussis.* Although the disease has been under control after vaccines were introduced, it has re-emerged in some countries within the last two decades causing around 300,000 deaths globally per annum. Culture, PCR and serological methods are used to detect pertussis infection. Although culture is considered to be the gold standard for its diagnostic specificity, PCR is widely used due to its high specificity and sensitivity. In Sri Lanka, only the culture method is currently used for pertussis detection. However, due to high intake of antibiotics by patients with symptoms of pertussis infection, the culture method often gives false negative results. Therefore, in this study, a multiplex PCR method was developed to detect clinically relevant *B. pertussis* and *B. parapertussis*, using the repetitive insertion sequences IS481 and IS1002 of the two species. Both insertion elements IS481 and IS1002 are present in the genome of *B. pertussis* while, only IS1002 is present in *B. parapertussis*. For each insertion sequence, two pairs of primers were designed with one set of primers nested within the other primer pair. Part of the sequence of each insertion element was PCR amplified using genomic DNA isolated from killed *B. pertussis* vaccine (DTwP) and cloned in to pGEM®-T easy vector system. The nested primer pair from each element was used for multiplex PCR. The sensitivity of the multiplex PCR assay was determined by using serial dilutions of linearized cloned plasmid from each insertion element. The PCR assays amplified 142 bp and 266 bp fragments from the insertion element IS481 and IS1002 respectively. The individual PCR assays were capable of detecting 200 copies of IS481 and 1 copy of IS1002 . A multiplex reaction that amplified both fragments was also developed by optimizing the $MgCl_2$ concentration and the use of a PCR enhancer. The assay was capable of detecting 200 copies of IS481 and 10 copies of IS1002 insertion elements. In *B. pertussis*, the IS481 and IS1002 insertion elements are present in 200 and 10 copies per genome respectively while in *B. parapertussis*, IS1002 insertion element is present in 10 copies per genome. Therefore, the multiplex PCR assay has the potential to detect a single organism of either *B. pertussis* or *B. parapertussis.* Currently, work is underway to test clinical samples using the optimized assay.

Keywords: Whooping cough, pertussis disease, multiplex PCR, PCR detection kit

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