

Title: COMPARISON OF PCR BASED METHODS FOR THE DETECTION OF *Neisseria meningitidis*, *Haemophilus influenzae* AND *Streptococcus pneumoniae* IN CLINICAL SAMPLES

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Abstract:

Bacterial meningitis is a rapidly evolving illness with high mortality rates. *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* are the leading causes of meningitis and other infections. The rapid detection of bacteria is crucial for an effective treatment. Molecular approaches such as PCR are now widely used in the diagnosis and surveillance of bacterial pathogens because of its high sensitivity and specificity and high throughput capabilities. It provides an improved tool to classic phenotype-based methods such as culture, Gram stain and latex agglutination. Conventional PCR and Real-time PCR (qPCR) have been developed and optimized to detect *N. meningitidis* (*nspA*-gene), *H. influenzae* (*p6*-gene), and *S. pneumoniae* (*ply*-gene). Conventional PCR detects products at the end point of DNA amplification by visualizing amplicons using agarose gel electrophoresis while Real Time PCR can be monitored during amplification. Real time PCR was performed using Taqman: *nspA*, *p6*, and *ply* targeted primers and probes labeled at the 5' end with FAM, HEX, and Cy5 fluorescent dyes and High Resolution Melting (HRM) with Evagreen as intercalating dye. Clinical samples used in this study were collected from 2012 to 2015 and are part of the Research Collection of INCQS. Both methods were able to detect the three etiological agents. Thirty percent samples with negative results by conventional PCR, tested positive by qPCR (either Taqman or HRM), enabling the identification of the etiologic agent. The aim of this study was to determine which method would be more sensitive and cost-effective and thus could be adopted by public laboratories of developing countries for diagnostic purposes. HRM method proved to be as sensitive as Taqman, but cheaper since there is no need to use probes. HRM can be used as a novel tool for the diagnostic of invasive diseases and help to improve outcome and prognosis of patients.

Key-words: bacterial meningitis, diagnosis, Real Time PCR, HRM

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