

Title: EVALUATION OF A REAL-TIME PCR BASED DIAGNOSTICS ASSAY FOR THE DETECTION AND IDENTIFICATION OF *Haemophilus influenzae* FROM CLINICAL ISOLATES

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Abstract:

Haemophilus influenzae is a commensal microorganism which usually can be found in humans respiratory tract in a commensal lifestyle, but in some cases can be responsible for causing pneumonia and others opportunistic infections. For this reason it is extremely important to detect the microorganism correctly particularly if it is suspected of causing infection. Conventional microbial culture diagnostics techniques are time-consuming and labour-intensive. The faster way to detect and identify microorganisms of interest is to utilize nucleic acid based diagnostics assays. Nucleic acid based diagnostics assays are used to detect this microorganism and the *fuculokinase* gene (*fucK*) has been described as a marker for detecting and identifying *H. influenzae*. There is a published diagnostic assay based on this methodology, however, some studies have indicated that occasional isolates often lack of this gene in the *H. influenzae* genome. In an attempt to test this published assay, the aim of this study was to test some clinical isolates to determine the number of *fucK* negative *H. influenzae* strains from the collection of clinical isolates at the Nucleic Acid Diagnostics Research Laboratory (NADRL). The clinical isolates were tested in a Real-Time Polymerase Chain Reaction (qPCR), the template *H. influenzae* DNA was quantified and diluted to 10^{-4/5} uL rxn for each isolate. Thirty six clinical isolates, all which were previously tested and confirmed to contain the microorganism, were tested in triplicate using the *fucK* diagnostic assay. The results generated show that the most of them could be detected by the published methodology, however three clinical isolates were negative for this gene target. PCR products from clinical isolates which were not detected by the real-time were run on a 1.5% agarose gel electrophoresis. These three isolates tested did not have the *fuculokinase* gene, and consequently they could not be identified as *H. influenzae* using this published diagnostic assay.

Keywords: *Haemophilus influenzae*; clinical isolates; *fuculokinase* gene; nucleic acid diagnostic.

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