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Evaluation of an In-House PCR Assay for the Detection of *Neisseria Gonorrhoeae*

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ABSTRACT

Introduction: Sexually transmitted infections (STIs) are a major public health concern worldwide, affecting quality of life and causing serious morbidity and mortality. *Neisseria gonorrhoeae* is challenging to diagnose by culture due to its fastidious nature. A rapid, highly sensitive PCR assay is urgently needed for routine *N. gonorrhoeae* detection to prevent widespread infection and reproductive complications. The aim of this study was to evaluate the sensitivity of an in-house PCR assay specifically for the detection of *N. gonorrhoeae*.

Methods: For the design of the in-house PCR, the *16S rRNA* of a *N. gonorrhoeae* isolate was accessed from GenBank (AJ247239.2). Using a primer design tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), the primers were selected and used in a qPCR SYBR green assay. The in-house *16S rRNA* assay was compared to the commercially available TaqMan assay which uses primers and probes specific for *N. gonorrhoeae*. The 16SrRNA primers were tested for cross reactivity towards six Neisseria strains. The DNA from the following Neisseria isolates: *N. elongate, N. cinera, N. weaver* and *N. sicca* were tested.

Results/Findings: The analysis showed successful *16S rRNA* amplification in all clinical *N. gonorrhoeae* isolates. Positive amplification matched in-house and TaqMan results, displaying strong correlation. Cross-reactivity analysis confirmed assay specificity for *N. gonorrhoeae* detection, excluding non-gonococcal Neisseria strains.

Conclusion: This study showed that the in-house PCR had successfully detected *N. gonorrhoeae*. The in-house assay showed a good correlation with a commercially available assay. This assay needs to be validated using different clinical samples to show its promise as a diagnostic test.

Keywords: *Neisseria gonorrhoeae*, Cross-reactivity, TaqMan assay, In-house PCR assay

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