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Evaluation of an *Opa* Gene-Based Real-Time PCR Assay for Detection of *Neisseria gonorrhoeae* in South African Populations

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ABSTRACT

Molecular-based assays have shown promise for detecting *Neisseria gonorrhoeae* (*N. gonorrhoeae*) with a higher sensitivity and specificity when compared to culture. Rapid detection is essential for effective treatment and controlling transmission. The objective of the study was to develop and evaluate the performance of an in-house *Opa*-based real-time PCR assay for the detection of *N. gonorrhoeae*. Three primer sets targeting the *Opa* gene of *N. gonorrhoeae* were designed and evaluated against published *Opa* gene primers [reference assay] (Verma et al., 2012). The in-house and published primers were tested against laboratory and clinical isolates of *N. gonorrhoeae* as well as non-gonococcal Neisseria control isolates. For the in-house *Opa* primers, *Opa* 1 performed the best as opposed to *Opa* 2 and *Opa* 3. With the *Opa* 1 assay, 100% of the culture-positive samples produced positive amplification and were classified as true positives when compared to the reference assay which classified 90.9% of the culture isolates as true positives. For the endocervical samples, 82.8% of samples were classified as true positives compared to 27.6% for the reference assay. For the vaginal samples, the *Opa* 1 assay classified 95.0% of the samples as true positives when compared to 25.0% by the reference assay. All 11 (100%) of the urine samples were classified as true positives for *N. gonorrhoeae* when compared to 36.4% with the reference assay. There was no cross-reactivity with non-gonococcal isolates with the *Opa* 1 assay, however, for the reference assay cross-reactivity was detected. The *Opa* 1 assay also had a higher limit of detection when compared to the other assays. In conclusion, the study demonstrates that the *Opa* 1 assay was the superior assay when compared to *Opa* 2, *Opa* 3, and the reference *Opa* assay and can be further evaluated for its use as a diagnostic assay.

Keywords: *Neisseria gonorrhoeae*, *Opa* gene, Nucleic acid amplification tests, Sexually transmitted infections

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