Journal of Infectious Diseases and Research

JIDR, 6(S4): 06 www.scitcentral.com ISSN: 2688-6537

Abstract: Open Access

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

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Published November 28, 2023

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 inhouse Opa primers, Opa 1 performed the best as opposed to Opa 2 and Opa 3. With the Opa 1 assay, 100% of the culturepositive 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 crossreactivity 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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Citation: Naicker D & Abbai NS. (2023) Evaluation of an *Opa* Gene-Based Real-Time PCR Assay for Detection of *Neisseria gonorrhoeae* in South African Populations. J Infect Dis Res, 6(S4): 06.

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