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Confirmatory assays for detection of *Neisseria gonorrhoeae* using porA pseudogene real-time PCR base method

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Background: Since the advent of molecular techniques, diagnosis of *Neisseria gonorrhoeae* has been ruin by false positive results when compared with culture, which is currently the gold standard. False positive results are often due to the cross-reaction of nucleic acid amplification test (NAAT) with closely related non-pathogenic Neisseria species. Regardless of the availability of commercial NAATs for *N. gonorrhoeae*, issues surrounding the specificity of these platforms persist.

Objectives: This research aims to institute, heighten and compare the sensitivity and specificity of previously available *N. gonorrhoeae* real-time assays which target the porA pseudogene.

Methods: In the course of investigation, 156 gonococci specimens and 30 non-gonococci culture specimens were used. Optimization of the PorA pseudogene real-time PCR was carried out by varying the concentration of magnesium chloride as follows: 5 mM ranges between 19.08 (4.31) and 23.27 (17.57), 4 mM ranges from 17.18 (1.15) and 22.01 (16.43) and for 3 mM the range is from 21.71 (2.20) and 27.33 (15.27) with the standard deviation in bracket and as well as the forward and reverse primers which has varying concentration as 50 mM, 300 mM and 900 mM for both.

Results: The results obtained show the high specificity of the assays for all 156 gonococci culture specimens gave positive results, whilst the 30 non gonococci specimens gave negative results. This shows that PorA pseudogene real-time PCR is a suitable assay for the confirmation of putative *N. gonorrhoeae* cultures and can assist in identification, particularly in cases where traditional biochemical and immunology tests have failed. The potential of the PorA pseudogene real-time PCR to detect the presence of *N. gonorrhoeae* specific DNA directly from clinical samples was then evaluated. An initial experiment was performed which involved the addition of a primer and probe set which acted as an internal control, it was determined that the internal control did not compromise the sensitivity of the PorA pseudogene real-time PCR assay and could be used reliably to screen for assay inhibition. The PorA pseudogene real-time PCR was then used to examine some clinical specimens which had been examined previously at three laboratories, each of which different commercial *N. gonorrhoeae* NAAT platforms was used. The results from this investigation show a high specificity evidence of PorA pseudogene real-time PCR when compared to previous results obtained from the other laboratories.

Conclusion: The study has succeeded in establishing to very large extent that the PorA pseudogene real-time PCR is a very valuable assay for the detection and confirmation of *N. gonorrhoeae* specific DNA from both putative cultures and directly from clinical samples.

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