Use of fully automated real-time PCR platform (BD MAX) to perform a confirmatory in house real-time multiplex PCR for detection of *N. gonorrhoeae*.

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### Aims and Introduction

Nucleic acid amplification tests (NAAT) are now commonly used by clinical laboratories to diagnose gonococcal infection and are more sensitive than culture for *Neisseria gonorrhoeae*. NAATs provide timely and accurate results but may occasionally cross react with other Neisseria species. Guidance by the (HPA & CDC) suggests if a test for *N. gonorrhoea* has a positive predictive value (PPV) of less than 90%, then a confirmatory test should be performed on a second assay, using a different target (1,2). Although the specificity of these tests is high they may have unacceptably low PPV when testing non-genital samples (rectal or throat), in a low prevalence setting.

We routinely use the VIPER GC\(^+\) assay to diagnose *N. gonorrhoea* and confirm positive results with a second reference laboratory NAAT assay for extragenital samples as we have previously demonstrated that the PPV from these sites is poor (3).

The BD MAX (BD, New Jersey, USA) is a new flexible platform for performing automated extraction and real time polymerase chain reaction, using a microfluidic cartridge (PCR) on a range of patient specimens. This platform allows in house PCRs to be performed using with user defined primers and probes.

### Methods

A multiplex real time confirmatory PCR *N. gonorrhoeae* porA and opaA PCR assays into an in house multiplex NAAT for use, as a confirmatory PCR, on the BD MAX platform (BD, USA).

### Results

Between December 2010 and August 2011 two 200 µl aliquots were saved for all rectal (33 samples) and throat swabs (98 samples) tested routinely for GC/CT using the VIPER GC Q laboratories to diagnose gonococcal infection and are more sensitive than GC/CT using the VIPER GC\(^+\) assay. To perform routine confirmation one aliquot was immediately sent to the Sexually Transmitted Bacteria Reference Tube position to be anonymised stored at -70 °C until batch testing using the in house porA/opa multiplex on the BDMax.

A titration was performed to optimise the concentration of primer and probe. Final reaction primer concentration 0.5 µM and probe concentration 0.125 µM.

**PCR Cycling parameters**

5 sec 95.0°C
12 sec 60.0°C

Rectal and throat swabs in VIPER diluent were extracted using the Swab in Transport media Extraction kit.

Sensitivity of the two NAATs was determined by comparing results with bacterial culture (VCAT media, Oxoid, Basingstoke UK) and API NH in three ways

1) GC culture positive at that site
2) GC culture positive at any site in that subject
3) A combined result where a positive was defined as a samples in which 2 out of 3 of the tests were positive

Performance data was determined using Stata 12.0 (College Stn. Tx, USA)

### Conclusions

It was a simple process to convert an existing real time PCR assay into use on the BDMax platform for confirmation of *N. gonorrhoeae*. Limited hands on time.

No statistically significant difference between sensitivity of the in house multiplex porA/opa BDMax assay PCR and the STBRL porA and opa assays when culture positive samples were compared.

It was not possible to determine specificity as neither NAAT assay nor bacterial culture were validated.

HPA and BASHH guidelines for the diagnosis of gonococcal infection state that positive throat and rectal specimens should be confirmed using a NAAT with a different target. We have developed and validated a GC real-time multiplex PCR using the BD Max platform. This assay will now be used to perform a *N. gonorrhoeae* confirmatory PCR.

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**References**


