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Introduction

The most common site of *Neisseria gonorrhoeae* (NG) infection is the columnar epithelium cells on the mucosal surfaces of the urethra, cervix, rectum, pharynx and conjunctiva. NG cervical infections are asymptomatic in about 50% of women which if left untreated, may result in serious complications such as pelvic inflammatory disease, ectopic pregnancy, neonatal conjunctivitis or infertility [1]. Men develop urethritis and unlike women, asymptomatic infection occurs in only 3-5% of males. Most rectal and pharyngeal gonococcal infections are also subclinical [2]. Untreated infection is an important reservoir for NG transmission.

The microbiology laboratory at St. James’s Hospital, Dublin (SJH) provides a diagnostic service for the largest Genitourinary Medicine and Infectious Diseases (GUIDE) clinic in Ireland, smaller clinics and general practitioners (GP’s) in the local catchment area. The current laboratory diagnosis of NG is based on culture, which is regarded as the gold standard and yields a positivity rate of 1% for the heterosexual population. For men who have sex with men (MSM), the positivity rate is 5%, with 17%, 21% and 35% of positives found only in the pharynx, rectum and urethra respectively.

One of the difficulties with culture, particularly from external sources is that the organism has a low viability in transport medium [3], resulting in a significant risk of false negative samples. Furthermore, in the case of pharyngeal specimens NG bacterial load maybe low and overgrown by commensal organisms [2, 4]. Isolation and identification may also be very time consuming and turnaround times can exceed 72 hours. These factors have led to the requirement for a high throughput, rapid, sensitive and specific nucleic acid amplification test (NAAT), which is independent of bacterial viability to be introduced to the diagnostic laboratory.

The Abbott CT/NG system (Abbott Molecular Inc., Il.,USA) is currently in place in the laboratory as the screening assay for the detection of CT only. As a multiplex Conformité Européene (CE) marked CT/NG assay is available, this assay therefore was chosen as the primary screening assay for the detection of NG, targeting the opacity (*opa*) gene [5]. While this assay is validated for use on urogenital specimens only, it will be evaluated in this study for use on extragenital specimens because of clinical necessity, the availability of resources and study results in current literature. However, the difficulties associated with false positive (FP) and false negative (FN) NAAT’s results due to the exchange of genetic material by *Neisseria* species (*Neisseria* spp.), is well documented [4, 6, 7]. This problem is greater in

37 specimens from pharyngeal and rectal sites than from urogenital sites where the
38 incidence of non-gonococcal *Neisseria*. spp. is significantly higher. Furthermore this
39 DNA uptake/loss is arbitrary, resulting in genetically different strains at different
40 geographic locations [8, 9, 10], hence the requirement for regional assay validation
41 before the introduction of molecular detection assays.

42 NAAT's are currently recommended for the detection of NG from urogenital
43 samples, however, supplementary testing of NG positive results is generally
44 considered best practice; provided the supplementary assay tests the original sample,
45 targets a different gene and has equal sensitivity and specificity to the screening assay
46 [11, 12, 13]. The Australian Public Health Laboratory (PHLN) have expanded their
47 guidelines to include extragenital samples with the requirement that two different
48 supplementary assays be positive before the extragenital sample is reported as truly
49 positive [12]. NAAT's are also acceptable when testing extragenital sample in the
50 US, provided the NAAT is validated to a specific standard [11], while the UK
51 recommends the use of culture-based testing [13].

52 This study evaluates the use of the Abbott CT/NG RealTime™ CT/NG assay
53 (Abbott CT/NG assay) as a screening assay and subsequent analysis of NG positive
54 template DNA using an in-house real-time PCR supplementary assay targeting the
55 *porA* pseudogene (*porA* assay). The *porA* pseudogene was selected as the target for
56 the supplementary assay as it has been shown to be highly conserved and to exist as a
57 pseudogene with no apparent function and thus far to be stable [14, 15]. The *porA*
58 pseudogene is also widespread in NG strains, absent in commensal *Neisseria*. spp [14]
59 and is sufficiently divergent from the *porA* of *N. meningitidis* to allow the design of
60 primers and probes that will differentiate the two organisms [15, 16, 17]. In addition,
61 Hjelmevoll *et al* conducted an extensive study challenging the specificity of the *porA*
62 gene sequence by testing a wide-range of organisms, (NG strains, other *Neisseria*.
63 spp. and the normal flora of genital and non-genital mucous membranes) and showed
64 the *porA* assay to be 100 % specific [18]. Further studies have confirmed the *porA* to
65 be a suitable target for NG diagnosis [19, 20, 21].

66 To evaluate the Abbott CT/NG and the *porA* assays, the NAAT results were
67 compared to culture and discrepancies were resolved using sequence analysis of the
68 *porA* gene product. It is hoped to replace the current culture-based test system with
69 the combination of the Abbott CT/NG and the *porA* assay in both urogenital and
70 extragenital specimens in our routine diagnostic service. Culture will be retained in a
71 limited manner on selected NG positive patients only.

72

73

74 **Materials and Methods**

75 **Assay evaluation**

76 *Assay sensitivity, specificity and efficiency*

77 The Abbott CT/NG and the *porA* assays were investigated as follows:

78 The limit of detection (LOD) of both assays were determined by analysing in
79 triplicate a dilution series of a 1×10^5 CFU/ml quantitated Quality Control for
80 Molecular Diagnostics (QCMD) sample diluted in a range from 10-90 copies/PCR
81 reaction. The LOD was defined as the lowest concentration to give a reproducible
82 positive result.

83

84 QCMD NG DNA-08 and 09 proficiency panels displaying a range in concentration
85 from 1×10^3 to 1×10^5 CFU/ml were analysed in accordance with QCMD instructions.

86

87 The specificity of both assays was challenged by analysing known clinical isolates.

88 *Neisseria* spp. were obtained from cultured urogenital, rectal and pharyngeal sites and

89 identified using colonial appearance, gram stain, oxidase test, sugars oxidation tests,

90 Phadebact Monoclonal GC test (Bactus AB, Huddinge, Sweden), API NH

91 (bioMerieux, Marcy l'Etoile, France) and BactiCard (Remel, Lenexa, US). These

92 clinical isolates included 36 NG, 6 *N. meningitidis*, 4 *N. lactamica*, 1 *N. flava*, 7 *N.*

93 *sicca*, 4 *N. polysaccharea* and 1 *N. cinerea*. The total non-NG strains were 26

94 isolates. *Neisseria* reference strains included were *N. lactamica* ATCC 23970

95 (American Type Culture Collection) and *N. sicca* ATCC 29193. Isolates were stored

96 at -80°C on Protect beads (Technical Service Consultants Ltd, Lancashire, UK) and

97 cultured from frozen stocks for 24hrs at 37°C in 5% CO_2 on non-selective chocolate

98 agar containing 5% defibrinated horse blood in a Columbia agar base LAB001,

99 (Lab M Ltd, Lancashire, UK). For extraction of genomic DNA, bacteria were

100 suspended in 200 μl of molecular grade nuclease free water to give a visibly turbid

101 suspension. Samples were heated at 95°C for 10min, centrifuged for 5minutes at

102 10,000 x g and the supernatant stored at -20°C .

103

104 ***Clinical Trial***

105 Specimens (n = 600) were collected from randomly selected male and female

106 patients (symptomatic and asymptomatic) attending the GUIDE clinic at SJH or the

107 Gay Men's Health Project clinic (GMHP), Dublin. First void urine (20-30 ml)

108 samples were obtained from male patients ($n = 164$) and an aliquot of each urine
109 sample was transferred into an Abbott *multi-Collect* tube. A corresponding urethral
110 swab was collected for culture-based detection. Two sets of pharyngeal swabs ($n =$
111 173) and rectal swabs ($n = 205$) were collected from MSM. Two separate
112 endocervical swabs ($n = 58$) were also collected. The first swab was directly
113 inoculated onto New York City agar* at the collection site so as not to compromise
114 the diagnostic result; the second swab was taken into individual Abbott *multi-Collect*
115 tubes. The Abbott *multi-Collect* tube samples were refrigerated and tested within
116 three days on the Abbott CT/NG system and DNA extracted on this system was used
117 as the template DNA for the *porA* assay.

118 * Containing Difco™ Dextrose Starch agar base BBL 12370 (BD, MD, USA), Agar Bacteriological (Agar No. 1) LP0011, 5%
119 defibrinated horse blood, V.C.A.T. Selective Supplement SR0104B, Yeast autolysate SR0105B (Oxoid, Basingstoke, UK)

120
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122 **Abbott CT/NG RealTime™ CT/NG assay**

123 The assay was performed according to the manufacturer's instructions with no
124 modification to the assay procedure. Briefly, the Abbott CT/NG system consists of an
125 automated sample preparation platform (*m2000sp*) that enables magnetic
126 microparticle DNA capture and extraction. An internal control (pumpkin plant DNA)
127 is also added to each sample pre-extraction and post extraction the template DNA and
128 mastermix is dispensed into an optical reaction plate. The plate is then transferred to
129 the *m2000rt* real-time PCR instrument for the homogeneous multiplexed PCR of both
130 CT and NG. The opacity (*opa*) gene (11- 13 copies/organism) [5, 22] is the chosen
131 target for amplification in NG, while two regions in the cryptic plasmid have been
132 selected to target the wild type CT (wtCT) and the Swedish variant CT (vCT). The CT
133 hydrolysis probes are both labelled with 5'FAM, as a consequence the wtCT and vCT
134 strains cannot be distinguished. The NG probe is labelled with VIC, while the
135 internal control is labelled with NED. This allows for the simultaneous differentiation
136 and detection of CT and NG. The numerical result is reported as a decision cycle
137 (DC) value, which is proportional to the starting copy number of target DNA.

138 ***porA* assay**

139 The *porA* assay was designed as a singleplex real-time PCR assay for the
140 detection of NG using *porA* pseudogene primers and probe previously described by
141 Hjelvoll *et al* [18] (Table 1). PCR amplification and detection was performed the
142 using the ABI Prism® 7500 sequence detection system (Applied Biosystems, Foster
143 City, California). The final PCR reaction volume was 25µl containing 12.5µl

144 TaqMan Universal ® Mastermix (Applied Biosystems, Foster City, California), NG
145 forward and reverse primer (900nM), NG probe (200nM) labelled with 5'FAM,
146 nuclease free water (NFW) and 10µl of extracted DNA. Real-time PCR was
147 performed using Universal Thermal Cycling Conditions. The thermal cycling protocol
148 comprised of 2 mins at 50 °C, followed by 10 mins at 95 °C (AmpliTaqGold®
149 enzyme activation), 45 cycles of 15 secs at 95 °C (denaturation) and 2 mins at 60 °C
150 (combined annealing and primer extension). A cycle threshold (Ct) value was
151 generated as the amplified sample crosses the threshold level of detection set during
152 the exponential phase of the PCR reaction. Negative and non-template controls were
153 included in each run.

154

155 **Resolution of discordant results using *porA* sequencing analysis**

156 The *porA* primers of Hjelvoll *et al* [18] were used for sequencing. Template
157 DNA extracted on the Abbott *m2000sp* was amplified using the HotStar Hi Fidelity
158 Polymerase Kit (QIAGEN GmbH, Hilden, Germany) according to manufacturers
159 instructions using the GeneAmp® PCR System 9700 thermal cycler (Applied
160 Biosystems, Foster City, California). Each reaction contained 5µl of extracted DNA.
161 A negative and non-template control was included in each run.

162 The amplified products were electrophoresed through a 2% agarose gel
163 containing Gel Red (Biotium, Hayward, US) and visualised using UV light. The
164 *porA* primer set generated a 103bp product. The PCR products were purified using
165 the QIAquick® PCR Purification Kit (QIAGEN GmbH, Hilden, Germany). Purified
166 products were sequenced using 10µM of each primer [18]. Sequencing was
167 performed using the ABI Prism®3130xl DNA analyser (Applied Biosystems, Foster
168 City, California).

169 DNASTAR® Lasergene (DNASTAR, Madison, Wisconsin) was used to carry
170 out sequence analysis. A consensus sequence was assembled and trimmed using
171 SeqMan sequence analysis software. The BLAST N sequence alignment tool was
172 used to confirm sequence homology with the *porA* pseudogene of NG in GenBank.

173 **Definition of a true positive**

174 A sample that is culture positive or positive with both the Abbott CT/NG and the
175 *porA* assays is considered to be a “true positive” (TP).

176

177 **Results**

178 **Analytical sensitivity**

179 The dilution series of a NG quantitated DNA ranging from 10-90 copies/ml
180 demonstrated an LOD of 3 organisms /PCR reaction using the Abbott CT/NG assay,
181 whereas the LOD of the *porA* assay was 40 organisms /PCR reaction.

182
183

184 **Analytical specificity**

185 In both the Abbott CT/NG and *porA* assays, all known gonococcal cultures (n = 36)
186 were detected. Amplification was not detected in any of the non-gonococcal isolates
187 from the pharyngeal or rectal swabs (n = 26) and the reference strains ATCC 23970
188 *N. lactamica* and ATCC 29193 *N. sicca*.

189

190 **QCMD NG DNA-08 and 09 panels.** A total of 20 samples were tested with the
191 expected QCMD results of 15 NG positive samples and five samples NG negative.
192 Of the Abbott CT/NG and *porA* positive samples, 14/15 were in agreement with the
193 QCMD designated results (Table 2,3). One result (09-01) with a 4.0×10^{-4} sample
194 dilution was undetected in both assays. The sample status assigned to this sample was
195 infrequently detected. Of the participating laboratories the qualitative scoring for
196 sample 09-01 using a commercial real-time PCR was 6/22 (27.3%) or 30/39 (76.9%)
197 for laboratories using an in-house real-time PCR. The five negative samples gave
198 correct results, these included two *N. lactamica* and one *N. cinera*. The *N. lactamica*
199 was shown to give false positive results using the Roche Amplicor CT/NG and/or
200 Roche COBAS Amplicor CT/NG assays in 35/37 (94.5%) of laboratories while the
201 BD ProbeTec CT/NG assay gave false positive results in 17/23 (74.0%) of
202 laboratories when tested against *N. cinera*.

203

204

205 **Clinical Trial Results**

206 A total of 600 samples were screened using both the Abbott CT/NG and the
207 *porA* assays. The sample types, culture and NAAT results are shown in Table 4.
208 There was a 100% agreement between the Abbott CT/NG assay and the *porA* assay
209 results. Of the 164 male urine samples analysed, 2/164 of the corresponding male
210 urethral swabs were positive with culture, the Abbott CT/NG and *porA* assays. The
211 positivity rate was 1.2% for male urine samples. Of the 58 endocervical swabs, 1/58
212 was positive with culture and both NAAT's, giving a positivity rate of 1.7% for
213 endocervical swabs. A total of 173 pharyngeal swabs were analysed. Five swabs were
214 positive with culture and both NAAT's. However, nine pharyngeal swabs were

215 culture negative but positive using both NAAT's. A true positive was defined as been
216 'culture positive or positive on both the Abbott CT/NG and the *porA* assays,
217 therefore, the total TP was 14/173, giving a positivity rate of 8.1% for pharyngeal
218 swabs. Of the 205 rectal swabs, nine swabs were positive with culture and both
219 NAAT's, however, three rectal swabs were culture negative but positive with both
220 NAAT's. The % positivity was 5.8 % for rectal swabs. The NAAT discrepant results
221 covered a wide range of positivity from high to low positives (DC 0.28-13.32 and Ct
222 27.2- 38.3). Analyses of the culture results on these 12 patients from different
223 specimen sites are shown in Table 5. Sequencing analysis was performed on seven
224 samples, which were all confirmed as NG.

225 Both the Abbott CT/NG and the *porA* assays with both urogenital and
226 extragenital samples and culture-based testing of urogenital samples displayed 100%
227 sensitivity, specificity. The sensitivity of culture was considerably lower for
228 pharyngeal and rectal swabs at 36 % and 75%, respectively (Table 6).

229

230

231 **Discussion**

232 The propensity of *Neisseria. spp* to alter its genome through acquisition or loss
233 of genetic material has been shown to generate false positive and false negative results
234 when tested with NAAT's [4, 6, 7]. These sequence changes are also geographically
235 specific [8, 9, 10]. This study confirms that the combined Abbott CT/NG and *porA*
236 assays were as sensitive and specific as culture when testing urogenital samples;
237 however, the combined NAAT's also demonstrated a significant increase in
238 sensitivity when testing extragenital samples and consequently a major rise in the
239 detection rate of gonococcal infection.

240

241 The LOD of the Abbott CT/NG assay was 30-copies/PCR reaction, which
242 corresponds to a detection limit of approximately three organisms/PCR reaction. This
243 compares well to the LOD demonstrated by Marshall *et al* of two organisms/PCR
244 reaction [5]. The LOD of the *porA* assay was 40 organisms/PCR reaction (one copy
245 of the *porA* gene per NG genome). Unemo *et al* demonstrated a lower detection limit
246 of 7.5 genome equivalents/PCR reaction, this increase in sensitivity could be
247 attributed to the addition of a slightly increased volume of template DNA [18].
248 Nevertheless, the LOD's of both assays permit the reliable detection of NG and even
249 though the analytical sensitivity of the *porA* assay is less than the Abbott CT/NG
250 assay, the clinical sensitivity of the assay was adequate and did not effect the

251 confirmation of NG positive results. With regard to the QCMD results, the FP's
252 generated by commercial assays further confirms the need to include supplementary
253 assays in the testing algorithm for detection of NG.

254

255 In this study, both NAAT's demonstrated a 100% sensitivity and specificity;
256 therefore both assays are satisfactory for diagnostic use even in low-prevalence
257 populations. No false positive results were observed from urogenital samples as all
258 NG positive samples were both culture positive and positive with both NAAT's. As
259 urogenital samples are validated using the Abbott CT/NG assay and as the normal
260 flora of the genital mucosa is unlikely to include commensal *Neisseria* spp.,
261 urogenital NG positive samples will be reported as positive when the initial positive
262 sample is repeat positive using the Abbott CT/NG assay. However, the situation with
263 regard to extragenital samples is not so straightforward. The normal flora of the rectal
264 and pharyngeal mucosa is more likely to be inhabited by commensal *Neisseria* spp., it
265 is therefore possible that two different commensal *Neisseria* spp. could each generate
266 a FP result with both the Abbott CT/NG and *porA* assays, resulting in a misdiagnosis
267 of NG infection. Nevertheless, this risk of FP results using NAAT's must be weighed
268 against the reporting of FN results using culture-based testing. This study showed a
269 significant decrease in the sensitivity of culture for both pharyngeal (36%) and rectal
270 (75%) when compared with the NAAT's. These results are consistent with previous
271 studies which found that NAAT's were more sensitive than culture by demonstrating
272 an increase in the number of gonococcal infections detected in both the pharynx and
273 the rectum [10, 23]. Undetected infection in these sites leaves a reservoir of untreated
274 infection and undermines the ability of public health to control NG transmission and
275 infection. This study and other studies have also revealed that NG NAAT infection at
276 multiple sites is more common in MSM than was suggested by culture diagnosis [10,
277 21]. To overcome this problem of FP from extragenital samples, the PHLN
278 guidelines recommend testing a third discriminatory gene to confirm NG positive
279 [12]. Despite this recommendation, we believe that because of the characteristics of
280 the *porA* pseudogene and the specificity of the *porA* assay in this and other studies,
281 we consider the *porA* assay to be satisfactory as a stand alone supplementary assay.
282 Extragenital specimens will therefore be reported as NG positive when positive with
283 both the Abbott CT/NG and *porA* assays. Monitoring the data of both NAAT's and
284 culture testing and occasional sequencing of the entire 16S rRNA gene maybe
285 required to ensure quality assurance [21].

286

287 The SJH NG algorithms for clinics and GP's now require that on the initial
288 visit, specimens are collected from symptomatic patients for both culture (direct
289 inoculation or Transwab MW170 MWE, Wiltshire, UK) and analyses using the
290 Abbott CT/NG assay. Gram stain is performed (clinics only) and patients receive
291 standard antimicrobial therapy. Samples from asymptomatic patients are collected for
292 Abbott CT/NG assay only and Gram maybe examined. If the NAAT's are NG
293 positive the patient is recalled and samples are taken for culture-based testing prior to
294 the administration of therapy. This algorithm has a dual role. Maintaining culture-
295 based testing in this selective, limited format will verify NAAT technology and assist
296 the lab in ensuring the quality of the service; however, a 100% compliance of the
297 algorithm is not expected. This algorithm will also permit surveillance of changes in
298 antimicrobial resistance that cannot be currently met by NAAT assays. Although not
299 investigated in this study, a further validation was performed to permit the
300 detection/reporting of *C. trachomatis* (CT) from rectal and pharyngeal samples using
301 an outer membrane protein (*ompA*) in-house real-time assay as the Abbott CT/NG
302 assay is not validated for CT testing on extragenital samples.

303

304 **Conclusion**

305 Although a limited number of samples were tested, this study provides further
306 evidence that this *porA* assay is sensitive and specific for detection of NG from
307 urogenital and extragenital samples and is satisfactory for use in a diagnostic
308 laboratory. The study also demonstrates how using an in-house *porA* assay
309 complements the commercial Abbott CT/NG assay and expands the number of
310 anatomical sites suitable for analysis. Both the Abbott CT/NG and the *porA* assays
311 will require continuous monitoring by comparing culture-based results and NAAT's,
312 also by closely observing quality assurance data and consistent review of international
313 literature. The laboratory must also maintain close communication with the GUM
314 physicians and we must all be aware of the limitation of these assays.

315

1 REFERENCES

- 2 1. Cates W, Wasserheit J N (1991) Genital Chlamydia infections; epidemiology and
3 reproductive sequelae. *Am J Obstet Gynecol* 164:1771-781.
- 4 2. Page-Shafer K, Graves A, Kent C, *et al* (2002) Increased sensitivity of DNA amplification
5 testing for the detection of pharyngeal gonorrhoea in men who have sex with men. *Clin*
6 *Infect Dis* 34:173–176.
- 7 3. Graver MA, Wade JJ (2004) Survival of *Neisseria gonorrhoeae* isolates of different
8 auxotypes in six commercial transport systems. *J Clin Microbiol* 42:4803-804.
- 9 4. Palmer H, Mallinson H, Wood RL (2003) Evaluation of the specificities of
10 five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. *J Clin*
11 *Microbiol* 41:835-837.
- 12 5. Marshall R, Chernesky M, Jang D, *et al* (2007) Characteristics of the m2000
13 automated sample preparation and multiplex real-time PCR system for
14 detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin*
15 *Microbiol* 45:747–751.
- 16 6. David M. Whiley AB, Suzanne M, *et al* (2008) Exploring best practice for nucleic acid
17 detection of *Neisseria gonorrhoeae*. *Sexual Health* 5:17-23.
- 18 7. Tapsall JW, Limnios EA, Nguyen NL, *et al* (2005) Cryptic-plasmid-free gonococci may
19 contribute to failure of *cppB* gene-based assays to confirm results of BD ProbeTec PCR for
20 identification of *Neisseria gonorrhoeae*. *J Clin Microbiol* 43:2036-2037
- 21 8. Geraats-Peters CWM, Brouwers M, Schneeberger PM, *et al* (2005) Specific and
22 sensitive detection of *Neisseria gonorrhoeae* in clinical specimens by real-time PCR. *J*
23 *Clin Microbiol* 43:5653-659.
- 24 9. Lum G, Freeman K, Nguyen NL, *et al* (2005) A cluster of gonococcal infections positive
25 on culture but with negative *cppB* gene based nucleic acid amplification assays. *Sex*
26 *Transm Infect* 81:400-402.
- 27 10. Bachmann LM, Johnson RE, Cheng H, *et al* (2009) Nucleic Acid Amplification Tests of
28 Diagnosis of *Neisseria gonorrhoeae* Oropharyngeal Infections. *J Clin Microbiol*
29 47:902-907.
- 30 11. Association of Public Health Laboratories in cooperation with the Centres for Disease
31 Control and Prevention (January 13-15, 2009, Atlanta, GA) Laboratory Diagnostic Testing
32 for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Expert Consultation Meeting
33 Summary Report. www.aphl.org/aphlprograms/.../ctgclabguidelinesmeetingreport.

- 35 12. Smith DW, Tapsall JW, Lum G, *et al* (2005) Guidelines for the use and interpretation of
36 nucleic acid detection tests for *N. gonorrhoeae* in Australia: A position paper on
37 behalf of the Public Health Laboratory Network. *CDI* 29:358-365.
- 38 13. Jonathan R, Ison C, Carder C, *et al* (2006) Sexually Transmitted Infections: UK National
39 Screening and Testing Guidelines. www.bashh.org/documents/59/59.pdf
- 40 14. Feavers IM, Maiden MC (1998) A gonococcal *porA* pseudogene: implications
41 for understanding the evolution and pathogenicity of *Neisseria*
42 *gonorrhoeae*. *Mol Microbiol* 30:647-656.
- 43 15. Unemo M, Olov N, Fredlund H, *et al* (2005) The *porA* pseudogene of *N. gonorrhoeae* –
44 low level of polymorphism and a few, mainly identical inactivating mutations. *APMIS*
45 113:410-419.
- 46 16. Whiley DM, Buda PJ, Bayliss J, *et al* (2004) A new confirmatory *Neisseria gonorrhoeae*
47 real-time PCR assay targeting the *porA* pseudogene. *Eur J Clin Microbiol Infect Dis*
48 23:705-710.
- 49 17. Whiley DM, Buda PP, Freeman K, *et al* (2005) A realtime PCR assay for the detection of
50 *Neisseria gonorrhoeae* in genital and extragenital specimens. *Diagn Microbiol Infect Dis*;
51 52:1–5.
- 52 18. Hjelmevoll SO, Olsen ME, Sollid JU, *et al* (2006) A fast real-time polymerase chain
53 reaction method for sensitive and specific detection of the *Neisseria gonorrhoeae* *porA*
54 pseudogene. *J Mol Diagn* 8:574–581.
- 55 19. McNally LP, Templeton DJ, Jin F, *et al* (2008) Low Positive Predictive Value of a Nucleic
56 Acid Amplification Test for Nongenital *Neisseria gonorrhoeae* Infection in Homosexual
57 Men. *CID* 47: 25-27.
- 58 20. Goire N, Nissen MD, LeCornec GM, *et al* (2008) A duplex *Neisseria gonorrhoeae* real-
59 time polymerase chain reaction assay targeting the gonococcal *porA* pseudogene and
60 multicopy *opa* genes. *Diag Microbiol and Inf Dis* 61:6-12.
- 61 21. Hjelmevoll SO, Olsen ME, Sollid JU, *et al* (2008) Clinical Validation of a Real-Time
62 Polymerase Chain Reaction Detection of *Neisseria gonorrhoeae* *porA* Pseudogene Versus
63 Culture Technique. *Sex Trans Dis* 35:517-520
- 64 22. Hobbs MM, van der Pol B, Totten P, *et al* (2008) From the NIH: proceedings of a
65 workshop on the importance of self-obtained vaginal specimens for detection of sexually
66 transmitted infections. *Sex Transm Dis* 35:8-13.
- 67 23. Schachter J, Moncada J, Liska S, *et al* (2008) Nucleic acid amplification tests in the
68 diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men
69 who have sex with men. *Sex Transm Dis* 35:637-642.
- 70

71 Table 1 Nucleic acid sequences of primers and probe used in the real-time PCR *porA* assay

Primer/Probe		Sequence	Reference
NGporA-F	Sense	5'CCGGAAGTGGTTTCATCTGATT 3'	(Hjelvoll <i>et al.</i> , 2006) [18]
NGporA-R	Antisense	5'GTTTCAGCGGCAGCATTCA 3'	(Hjelvoll <i>et al.</i> , 2006) [18]
NgporA- P	Probe	5'CGTGAAAGTAGCAGGCGTATAGGCGGACTT3'	(Hjelvoll <i>et al.</i> , 2006) [18]

72

73 FAM = 6-carboxy fluorescein fluorescent dye, TAMRA=Tetramethyl-6-Carboxyrhodamine dye

74

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76 Table 2 QCMD CT DNA 08A proficiency panel results

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QCMD 2008A	QCMD NG Results		Abbott CT/NG assay		<i>porA</i> assay	
	NG	Sample conc	NG	NG DC	NG	NG Ct
NG08-01	Pos	2x10 ⁻³ Diln	Pos	2.91	Pos	38
NG08-02	Weak pos	1x10 ³ CFU/ml	Pos	1.77	Pos	40
NG08-03	Pos	1x10 ⁻³ Diln	Pos	0.55	Pos	41
NG08-04	Strong pos	1x10 ⁵ CFU/ml	Pos	8.64	Pos	33
NG08-05	Neg	-	Neg	-	Neg	-
NG08-06 (<i>N.lactamica</i>)	Neg	1x10 ⁵ CFU/ml	Neg	-	Neg	-
NG08-07	Pos	1x10 ⁻² Diln	Pos	0.20	Pos	42
NG08-08	Pos	1x10 ⁻³ Diln	Pos	4.50	Pos	37
NG08-09 (<i>N.cinera</i>)	Neg	1x10 ⁵ CFU/ml	Neg	-	Neg	-
NG08-10	Strong pos	1x10 ⁻² Diln	Pos	4.02	Pos	38

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80 Table 3 QCMD CT DNA 09 proficiency panel results

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QCMD 2009	QCMD NG Results		Abbott CT/NG assay		<i>porA</i> assay	
	NG	Sample conc	NG	NG DC	NG	NG Ct
NG09-01	Pos	4.0x10 ⁻⁴ Diln	Neg	-	Neg	-
NG09-02	Pos	1.0x10 ⁻³ Diln	Pos	1.70	Pos	39
NG09-03	Pos	1.7x10 ⁻³ Diln	Pos	0.88	Pos	39
NG09-04	Pos	1.0x10 ³ CFU/ml	Pos	2.08	Pos	38
NG09-05	Neg	-	Neg	-	Neg	-
NG09-06	Strong pos	1.0x10 ⁵ CFU/ml	Pos	10.04	Pos	30
NG09-07	Pos	1.0x10 ³ Diln	Pos	0.50	Pos	39
NG09-08 (<i>N.lactamica</i>)	Neg	1.0x10 ⁵ CFU/ml	Neg	-	Neg	-
NG09-09	Pos	1.0x10 ⁻² Diln	Pos	3.15	Pos	36
NG09-10	Pos	4.0x10 ⁻⁴ Diln	Pos	0.45	Pos	40

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83 NG = *Neisseria gonorrhoeae*, DC = decision cycle, Ct = cycle threshold

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Table 4 Overview of results obtained with culture-based method and the combined Abbott CT/NG and *porA* assays from a total of 600 clinical samples: male urine samples (n=164), endocervical swabs (n=58), pharyngeal swabs (n=173) and rectal swabs (n=205)

Abbott CT/NG & <i>porA</i> assay				
		Positive	Negative	
Culture-based method	Male Urine / Urethral Swabs	Positive	2	0
		Negative	0	162
	Endocervical Swabs	Positive	1	0
		Negative	0	57
	Pharyngeal Swabs	Positive	5	0
		Negative	9	159
	Rectal Swabs	Positive	9	0
		Negative	3	193

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Table 5 Results of discrepant samples (n=12) from different specimen sites using culture-based method

	Specimens sites	Number of patients	NG culture
Pharyngeal swabs (n =9)	Urethral and rectal	1	Positive
	Urethral	2	Positive
	Rectal	4	Positive
	Urethral and rectal	2	Negative
Rectal swabs (n = 3)	Urethral	2	Positive
	Urethral and pharyngeal	1	Negative

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DC values: 0.28 – 13.32 Ct values: 38.27 – 29.36

Table 6 Performance of the culture-based method and the combined Abbott CT/NG and *porA* assays by specimen site from 600 specimens (sensitivity, 95% CI, specificity)

Test method	Male urine/Urethral swab		Endocervical swab		Pharyngeal swab		Rectal swab	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
NG culture	100% (22.4-100)	100%	100% (5.00-100)	100%	36% (14.4-62.4)	100%	75% (45.9-93.2)	100%
Abbott CT/NG and <i>porA</i> assays	100% (22.4-100)	100%	100% (5.00-100)	100%	100% (80.7-100)	100%	100% (77.9-100)	100%

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95% CI in parenthesis

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