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Evaluation of the New BD Max GC Real-Time PCR Assay, Analytically and Clinically as a Supplementary Test for the BD ProbeTec GC Qx Amplified DNA Assay, for Molecular Detection of *Neisseria gonorrhoeae*

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The new BD Max GC real-time PCR assay showed high clinical and analytical sensitivity and specificity. It can be an effective and accurate supplementary test for the BD ProbeTec GC Qx amplified DNA assay, which had suboptimal specificity, and might also be used for initial detection of *Neisseria gonorrhoeae*.

Neisseria gonorrhoeae is estimated to cause 106 million gonorrhea cases every year, resulting in substantial morbidity and economic costs globally (1). The need for highly sensitive and specific laboratory diagnostics and subsequent effective therapy is crucial. Nucleic acid amplification tests (NAATs) have increasingly replaced culture diagnostics internationally, due to their higher sensitivity, speed, automation, and use of noninvasive specimens (2–7). However, due to suboptimal specificity, especially of several early generation gonococcal NAATs, in several regions (e.g., Europe and Australia), it is recommended to verify positive samples with another NAAT targeting a different genetic sequence, particularly in low-prevalence populations and in pharyngeal infections (6, 8–18). The psychological, social, and legal consequences of false-positive gonococcal test results can be substantial.

The BD Viper System with XTR technology (BD Diagnostics, Sparks, MD) is a third-generation platform that when operating in "extraction mode" provides automated DNA extraction using ferric oxide and strand displacement amplification (19). The BD ProbeTec GC Qx amplified DNA assay, targeting a pilin-inverting gene, is used on the BD Viper system to detect *N. gonorrhoeae*. However, suboptimal (particularly analytical) specificity and cross-reaction with commensal *Neisseria* species have been described for this assay as well as most other gonococcal NAATs (6, 8, 13, 20). Recently, the BD Max GC real-time PCR assay, targeting the gonococcal *opcA* gene, was developed to be run on the BD Max system, which provides automated DNA extraction and real-time PCR.

We evaluated the performance of the new BD Max GC real-time PCR assay by examining clinical specimens positive in the BD ProbeTec GC Qx amplified DNA assay and samples spiked with isolates of gonococci, nongonococcal *Neisseria* species, and other closely related bacteria.

During July to October 2014, 23,815 individuals (14,846 females and 8,969 males representing asymptomatic individuals presenting for screening and symptomatic patients) were tested with the BD ProbeTec GC Qx amplified DNA assay in a single replicate according to routine diagnostic protocol. All positive clinical specimens were subsequently stored in the primary tube (including BD transportation medium) prior to analysis (DNA extraction and real-time PCR) with the BD Max GC real-time PCR assay, which was performed within 1 to 12 h. Specimens negative in the BD Max GC real-time PCR assay were further tested with the Aptima Combo 2 assay (Hologic, Bedford, MA) and a gonococcal dual-target real-time PCR targeting the *porA* pseudogene and *opa* genes (21).

To challenge the analytical sensitivity and specificity of the BD Max GC real-time PCR assay, 460 bacterial isolates were examined. These isolates comprised gonococci ($n = 189$), nongonococcal *Neisseria* species ($n = 261$), and closely related bacteria ($n = 10$) (Table 1). Species was determined using routine phenotypic methods, including the sugar utilization test, the PhadeBact GC monoclonal test (Mkl Diagnostics AB, Stockholm, Sweden), and matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) (Microflex LT; Bruker Daltonics, Bremen, Germany), and genotypic methods (Aptima Combo 2 and Aptima GC [Hologic], a gonococcal dual-target real-time PCR [21], and 16S rRNA gene sequencing). Cultures of gonococcal and nongonococcal isolates were suspended in BD ProbeTec CT/GC Qx specimen collection tubes, and 500 μl was resuspended in a BD Max UVE sample buffer tube to concentrations of approximately 4 colonies/ml and 20 colonies/ml, respectively. All false-positive or false-negative analytical samples were retested from both the original dilution and fresh dilution using new culture from frozen stock. The retesting was also performed on different dilutions after repeated species verification, according to previously described algorithms (22). All testing using commer-

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TABLE 1 Detection of isolates of *Neisseria gonorrhoeae*, nongonococcal *Neisseria* species, and closely related species in the BD Max GC real-time PCR assay

Bacterial species	No. of isolates: By species	Positive by BD Max GC real-time PCR
Gonococcal		
N. gonorrhoeae[a]	189	189
Nongonococcal *Neisseria*		
N. meningitidis[b]	150	0
N. lactamica	20	0
N. flavescens	20	0
N. subflava	13	0
N. mucosa	12	0
N. sicca	11	0
N. cinerea	10	1
N. perflava	8	0
N. gonorrhoeae subsp. *kochii*	4	0
N. flava	2	0
N. sicca/*subflava*	2	0
N. polysaccharea	1	0
N. animalis	1	0
N. weaver	1	0
N. caviae	1	0
Other *Neisseria* species	5	0
Total	261	1
Closely related species		
Kingella denitrificans	3	0
Moraxella catarrhalis	3	0
Moraxella osloensis	1	0
Other *Moraxella* species	3	0
Total	10	0

[a] *N. gonorrhoeae* isolates reflecting temporally (isolated from 1973 to 2013), geographically (mainly a global panel), and genetically different strains.
[b] *N. meningitidis* isolates reflecting temporally, geographically, and genetically different strains.

TABLE 2 Results of supplementary testing using the BD Max GC real-time PCR assay on samples positive for *Neisseria gonorrhoeae* in the BD ProbeTec GC Qx amplified DNA assay run on the BD Viper system

No. of samples BD Viper positive	No. of samples positive/negative by BD Max GC real-time PCR confirmatory testing							
		Negative (% of BD Viper positive)[a]						
		Male			Female			
	Positive	Pharynx	Urine	Rectum	Pharynx	Vaginal	Urine	Cervix
322	252	27 (33)	19 (14)	3 (15)	8 (57)	8 (24)	4 (50)	1 (33)

[a] All 70 negative samples were negative also in the Aptima Combo 2 NAAT (Hologic); however, one of the vaginal samples was repeatedly positive in the gonococcal dual-target PCR (21).

cially available tests was performed in accordance with the manufacturer's instructions.

Of 23,815 patients tested with the BD ProbeTec GC Qx amplified DNA assay, 85 (0.6%) females and 259 (2.9%) males were positive. Of these 344 positive specimens, 322 (94%) contained sufficient material for testing with the BD Max GC real-time PCR assay. Two-hundred fifty-two (78%) and 70 (22%) specimens were positive and negative, respectively. All 70 negative specimens were negative also in the Aptima Combo 2 NAAT, and 69 of them were repeatedly negative in the gonococcal dual-target PCR. These 69 false-positive specimens were obtained from pharynx (50.0%), urine (33.0%), vagina (10%), rectum (4.3%), and cervix (1.4%) (Table 2).

In the analytical examination of the BD Max GC real-time PCR assay, all gonococcal isolates were positive, and all but one of the nongonococcal isolates (99.4%) were negative (Table 1). The cross-reacting *Neisseria cinerea* strain was also positive in retesting from the initial dilution and new culture from frozen stock, and 16S rRNA gene sequencing, MALDI-TOF, growth characteristics (colistin susceptibility and growth on tryptic soy agar and Mueller-Hinton agar [23]), and the sugar utilization test confirmed the *N. cinerea* species. The detection limit of this *N. cinerea* strain was 10 genome equivalents per reaction, which indicates clinical relevance. A sample spiked with this *N. cinerea* strain also cross-reacted in the BD ProbeTec GC Qx amplified DNA assay.

Validated, quality-assured, sensitive and specific gonococcal NAATs are crucial for accurate diagnostics and subsequent appropriate treatment of gonorrhea. However, due to suboptimal specificity of several gonococcal NAATs, in several regions (e.g., Europe and Australia), it is recommended to verify positive samples with another NAAT targeting a different genetic sequence (6, 8–18). This supplementary testing for verification of screening NAAT-positive specimens is particularly important in low-prevalence populations, if the positive predictive value (PPV) does not exceed 90%, and in pharyngeal infection. However, as shown in the present study, the number of false-positive urogenital specimens with the BD ProbeTec GC Qx amplified DNA assay can also be alarmingly high. Accordingly, supplementary testing of *N. gonorrhoeae* NAAT-positive specimens regardless of sample type can be essential, particularly in low-prevalence populations. The BD Max GC real-time PCR assay showed high clinical and analytical sensitivity and specificity, with only one cross-reacting *N. cinerea* strain, which was also false positive in the BD ProbeTec GC Qx amplified DNA assay. The performance of the BD Max GC real-time PCR was also simple and rapid (2 h and 45 min for automated DNA extraction and real-time PCR) with minimal hands-on time (20 to 30 min per 24 samples), including all appropriate controls (positive, negative, and the sample processing control).

In conclusion, the new BD Max GC real-time PCR assay showed high clinical and analytical sensitivity and specificity. However, the BD ProbeTec GC Qx amplified DNA assay had suboptimal specificity for both urogenital and extragenital clinical specimens. Accordingly, the new BD Max GC real-time PCR assay can be exceedingly valuable as a supplementary test for the BD ProbeTec GC Qx amplified DNA assay, particularly in low-prevalence populations, and might also be used for initial detection of *N. gonorrhoeae*. However, additional studies are needed to prove that the BD Max GC real-time PCR assay is sufficiently sensitive and specific for initial detection of *N. gonorrhoeae* and to investigate if the assay can be used for verification of specimens positive on NAAT platforms from other manufacturers. This study further highlights the need for supplementary testing when NAATs are used for detection of *N. gonorrhoeae*.

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REFERENCES

1. **World Health Organization.** 2012. Global incidence and prevalence of selected curable sexually transmitted infections—2008. World Health Organization, Geneva, Switzerland. http://www.who.int/reproductivehealth/publications/rtis/2008_STI_estimates.pdf.
2. **Bachmann LH, Johnson RE, Cheng H, Markowitz L, Papp JR, Palella FJ, Jr, Hook EW, III.** 2010. Nucleic acid amplification tests for diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* rectal infections. J Clin Microbiol **48:**1827–1832. http://dx.doi.org/10.1128/JCM.02398-09.
3. **Schachter J, Moncada J, Liska S, Shayevich C, Klausner JD.** 2008. Nucleic acid amplification tests in the diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men who have sex with men. Sex Transm Dis **35:**637–642. http://dx.doi.org/10.1097/OLQ.0b013e31817bdd7e.
4. **Alexander S.** 2009. The challenges of detecting gonorrhoea and chlamydia in rectal and pharyngeal sites: could we, should we, be doing more? Sex Transm Infect **85:**159-160. http://dx.doi.org/10.1136/sti.2008.035469.
5. **Hook EW, III, Handsfield HH.** 2008. Gonococcal infections in the adult, p 627–646. *In* Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, Corey L, Cohen MS, Watts DH (ed), Sexually transmitted diseases, 4th ed. McGraw-Hill, New York, NY.
6. **Unemo M, Ison C.** 2013. Gonorrhoea, p 21–55. *In* Unemo M, Ballard R, Ison C, Lewis D, Ndowa F, Peeling R (ed), Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. WHO Document Production Services, Geneva, Switzerland.
7. **Dize L, Agreda P, Quinn N, Barnes MR, Hsieh YH, Gaydos CA.** 2012. Comparison of self-obtained penile-meatal swabs to urine for the detection of *C. trachomatis*, *N. gonorrhoeae* and *T vaginalis*. Sex Transm Infect **89:**305–307. http://dx.doi.org/10.1136/sextrans-2012-050686.
8. **Tabrizi SN, Unemo M, Limnios AE, Hogan TR, Hjelmevoll SO, Garland SM, Tapsall J.** 2011. Evaluation of six commercial nucleic acid amplification tests for detection of *Neisseria gonorrhoeae* and other *Neisseria* species. J Clin Microbiol **49:**3610–3615. http://dx.doi.org/10.1128/JCM.01217-11.
9. **Bignell C, Unemo M, European Guidelines Editorial Board STI.** 2013. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS **24:**85–92. http://dx.doi.org/10.1177/0956462412472837.
10. **Golparian D, Johansson E, Unemo M.** 2012. Clinical *Neisseria gonorrhoeae* isolate with a *N. meningitidis porA* gene and no prolyliminopeptidase activity, Sweden, 2011: danger of false negative genetic and culture diagnostic results. Euro Surveill **17:**20102.
11. **Whiley DM, Limnios A, Moon NJ, Gehrig N, Goire N, Hogan T, Lam A, Jacob K, Lambert SB, Nissen MD, Sloots TP.** 2011. False-negative results using *Neisseria gonorrhoeae porA* pseudogene PCR—a clinical gonococcal isolate with an *N. meningitidis porA* sequence, Australia, March 2011. Euro Surveill **16:**19874.
12. **Ison CA, Golparian D, Saunders P, Chisholm S, Unemo M.** 2013. Evolution of *Neisseria gonorrhoeae* is a continuing challenge for molecular detection of gonorrhoea: false negative gonococcal *porA* mutants are spreading internationally. Sex Transm Infect **89:**197–201. http://dx.doi.org/10.1136/sextrans-2012-050829.
13. **Palmer HM, Mallinson H, Wood RL, Herring AJ.** 2003. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J Clin Microbiol **41:**835–837. http://dx.doi.org/10.1128/JCM.41.2.835-837.2003.
14. **Chow EPF, Fehler G, Read TRH, Tabrizi SN, Hocking JS, Denham I, Bradshaw CS, Chen MY, Fairley CK.** 2015. Gonorrhoea notifications and nucleic acid amplification testing in a very low-prevalence Australian female population. Med J Aust **202:**321–323. http://dx.doi.org/10.5694/mja14.00780.
15. **Smith DW, Tapsall JW, Lum G.** 2005. Guidelines for the use and interpretation of nucleic acid detection tests for *Neisseria gonorrhoeae* in Australia: a position paper on behalf of the Public Health Laboratory Network. Commun Dis Intell Q Rep **29:**358–365.
16. **Bignell C, Fitzgerald M, Guideline Development Group, British Association for Sexual Health and HIV UK.** 2011. UK national guideline for the management of gonorrhoea in adults, 2011. Int J STD AIDS **22:**541–547. http://dx.doi.org/10.1258/ijsa.2011.011267.
17. **Katz AR, Effler PV, Ohye RG, Brouillet B, Lee MV, Whiticar PM.** 2004 False positive gonorrhea test results with a nucleic acid amplification test: the impact of low prevalence on positive predictive value. Clin Infect Dis **38:**814–819.
18. **Fifer H, Ison CA.** 2014. Nucleic acid amplification tests for the diagnosis of *Neisseria gonorrhoeae* in low-prevalence settings: a review of the evidence. Sex Transm Infect **90:**577–579. http://dx.doi.org/10.1136/sextrans-2014-051588.
19. **Taylor SN, Van Der Pol B, Lillis R, Hook EW, III, Lebar W, Davis T, Fuller D, Mena L, Fine P, Gaydos CA, Martin DH.** 2011. Clinical evaluation of the BD ProbeTec *Chlamydia trachomatis* Qx amplified DNA assay on the BD Viper system with XTR technology. Sex Transm Dis **38:**603–609. http://dx.doi.org/10.1097/OLQ.0b013e31820a94d2.
20. **Pope CF, Hay P, Alexander S, Capaldi K, Dave J, Sadiq ST, Ison CA, Planche T.** 2010. Positive predictive value of the Becton Dickinson VIPER system and the ProbeTec GC Qx assay, in extracted mode, for detection of *Neisseria gonorrhoeae*. Sex Transm Infect **86:**465–469. http://dx.doi.org/10.1136/sti.2010.044065.
21. **Goire N, Nissen MD, LeCornec GM, Sloots TP, Whiley DM.** 2008. A duplex *Neisseria gonorrhoeae* real-time polymerase chain reaction assay targeting the gonococcal *porA* pseudogene and multicopy *opa* genes. Diagn Microbiol Infect Dis **61:**6–12. http://dx.doi.org/10.1016/j.diagmicrobio.2007.12.007.
22. **Golparian D, Tabrizi SN, Unemo M.** 2013. Analytical specificity and sensitivity of the APTIMA Combo 2 and APTIMA GC assays for detection of commensal *Neisseria* species and *Neisseria gonorrhoeae* on the Gen-Probe Panther instrument. Sex Transm Dis **40:**175–178. http://dx.doi.org/10.1097/OLQ.0b013e3182787e45.
23. **Knapp JS, Totten PA, Mulks MH, Minshew BH.** 1984. Characterization of *Neisseria cinerea*, a nonpathogenic species isolated on Martin-Lewis medium selective for pathogenic *Neisseria* spp. J Clin Microbiol **19:**63–67.