

# 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*

Daniel Golparian,<sup>a</sup> Stina Boräng,<sup>b</sup> Martin Sundqvist,<sup>a</sup> Magnus Unemo<sup>a</sup>

WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden<sup>a</sup>; Department of Clinical Microbiology, Karolinska University Hospital, Huddinge, Sweden<sup>b</sup>

**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 gonorrhoea 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  $\mu$ l 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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Address correspondence to Magnus Unemo, magnus.unemo@regionorebrolan.se.

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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
<b>Gonococcal</b>		
<i>N. gonorrhoeae</i> <sup>a</sup>	189	189
<b>Nongonococcal <i>Neisseria</i></b>		
<i>N. meningitidis</i> <sup>b</sup>	150	0
<i>N. lactamica</i>	20	0
<i>N. flavescens</i>	20	0
<i>N. subflava</i>	13	0
<i>N. mucosa</i>	12	0
<i>N. sicca</i>	11	0
<i>N. cinerea</i>	10	1
<i>N. perflava</i>	8	0
<i>N. gonorrhoeae</i> subsp. <i>kochii</i>	4	0
<i>N. flava</i>	2	0
<i>N. sicca/subflava</i>	2	0
<i>N. polysaccharea</i>	1	0
<i>N. animalis</i>	1	0
<i>N. weaver</i>	1	0
<i>N. caviae</i>	1	0
Other <i>Neisseria</i> species	5	0
Total	261	1
<b>Closely related species</b>		
<i>Kingella denitrificans</i>	3	0
<i>Moraxella catarrhalis</i>	3	0
<i>Moraxella osloensis</i>	1	0
Other <i>Moraxella</i> species	3	0
Total	10	0

<sup>a</sup> *N. gonorrhoeae* isolates reflecting temporally (isolated from 1973 to 2013), geographically (mainly a global panel), and genetically different strains.

<sup>b</sup> *N. meningitidis* isolates reflecting temporally, geographically, and genetically different strains.

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

**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								
	Positive	Negative (% of BD Viper positive) <sup>a</sup>						Urine	Cervix
		Pharynx	Urine	Rectum	Pharynx	Vaginal			
322	252	27 (33)	19 (14)	3 (15)	8 (57)	8 (24)	4 (50)	1 (33)	

<sup>a</sup> 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).

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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