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Neisseria gonorrhoeae False-Positive Result Obtained from a Pharyngeal Swab by Using the Roche cobas 4800 CT/NG Assay in New Zealand in 2012

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The Roche cobas 4800 CT/NG assay is a commonly used commercial system for screening for *Neisseria gonorrhoeae* infection, and previous studies have shown the method to be highly sensitive and specific for urogenital samples. We present the first confirmed clinical *N. gonorrhoeae* false-positive result using the cobas 4800 NG assay, obtained from testing a pharyngeal swab sample and caused by cross-reaction with a commensal *Neisseria* strain.

Nucleic acid amplification tests (NAATs) are widely used for the detection of gonorrhea. However, the specificity of these methods can be undermined by ongoing genetic exchange between species within the *Neisseria* genus, leading to commensal *Neisseria* strains acquiring *Neisseria gonorrhoeae* (NG) NAAT target sequences. For these reasons, supplementary "confirmatory" testing for *N. gonorrhoeae* NAATs has been widely adopted (1, 2). The Roche cobas 4800 CT/NG assay is a later-generation NAAT method, and the NG component of the assay utilizes a dual-target approach, using two assays to detect sequences within the direct-repeat (DR-9) region (3). Performance data to date show excellent sensitivity and specificity for urogenital specimens (3–7), and it has been suggested that the assay does not require a second test to confirm urogenital positive results (6). Also, to our knowledge, there have been no definitive reports of the assay cross-reacting with commensal *Neisseria* strains (3, 8); while initial testing in a study by Tabrizi et al. (8) showed that the cobas 4800 NG assay cross-reacted with two commensal *Neisseria* strains, both were negative upon retesting using fresh cultures (8). Herein, we report the first clinical demonstration of a Roche cobas 4800 NG false-positive result obtained from a pharyngeal swab sample and caused by a reproducible cross-reaction with a commensal *Neisseria* strain.

On 17 September 2012, as part of the heightened awareness program sponsored by the New Zealand AIDS Foundation, self-referred throat and rectal swabs and a first-void urine were received from a 38-year-old male presenting to a sexual health clinic in Auckland, New Zealand. The samples were tested by PCR for *N. gonorrhoeae* and *Chlamydia trachomatis* (CT) on the Roche cobas 4800 CT/NG assay at Labtests, Auckland, New Zealand (Table 1). The three specimens were all negative for *C. trachomatis* DNA; the urine and rectal swabs were also negative for *N. gonorrhoeae* DNA. The throat swab was positive for *N. gonorrhoeae* DNA with a cycle threshold value of 35.7, which was confirmed upon retesting at a second laboratory (Aotea Pathology, Wellington; cobas 4800 NG assay positive with a cycle threshold value of 35.3), and the patient was treated with 500 mg intramuscular ceftriaxone. As part of an ongoing study investigating the specificity of the cobas 4800 NG assay in our population, the specimen was subsequently referred for supplementary testing by the Abbott m2000 real-time PCR (Canterbury Health Laboratories) and in-house PCR methods targeting the gonococcal *porA* and *opa* genes (Aotea Pathology, Wellington); the specimen was negative by all assays.

Approximately 7 weeks later, the patient presented to his general practitioner with a sore throat and had a throat swab taken for routine bacteriology and *C. trachomatis*/*N. gonorrhoeae* PCR. No beta-hemolytic streptococci, *Arcanobacterium haemolyticum*, or *N. gonorrhoeae* was isolated by routine culture. Again, the cobas 4800 assay detected *N. gonorrhoeae* DNA (cycle threshold value, 39.4) and did not detect *C. trachomatis* DNA. In response to this result, the patient received another dose of ceftriaxone. After concerns over the validity of the result were raised by laboratory staff with the patient's general practitioner (GP), the patient was then contacted by his GP and he agreed to provide a further two throat swabs for inoculation on sheep blood agar, chocolate agar, and New York City agar. While no *N. gonorrhoeae* grew, three species of commensal *Neisseria* grew and were identified as *Neisseria flavescens*, *Neisseria macacae*, and *Neisseria perflava* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany). Colonies of each strain were tested in the cobas 4800 assay, and a positive result was obtained for the *N. macacae* isolate (cycle threshold value, 28.2) while the other two isolates were negative. Four individual suspensions of the *N. macacae* isolate (prepared by inoculating 2 colonies each into 1.0 ml sterile water) were subsequently retested in the cobas 4800 NG assay at both the Labtests, Auckland, and Aotea Pathology laboratories; positive results were obtained for all suspensions in both laboratories (cycle threshold values ranged from 30.9 to 34.5 cycles; mean, 32.4 cycles).

The *N. macacae* isolate was further investigated by testing the four above-mentioned suspensions by an in-house *N. gonorrhoeae* *porA* and *opa* PCR; negative results were obtained for all suspensions. To further investigate species identification, a partial 16S

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TABLE 1 Summary of NAAT and culture results[a]

Week collected (day/mo/yr)	Anatomical site/type of sample	Result with each *N. gonorrhoeae* diagnostic method			
		cobas 4800 NG (threshold cycle value)	Abbott m2000 real-time PCR	In-house *porA* and *opa* PCR assays	Bacterial culture
1 (17/09/12)	Urine	Negative	NP	NP	NP
	Rectal swab	Negative	NP	NP	NP
	Throat swab	Positive (35.7, 35.3)*	Negative	Negative	NP
7 (7/11/12)	Throat swab	Positive (39.4)	NP	Negative	NP
8 (23/11/12)	Throat swab (× 2)	NP	NP	NP	Negative
	N. macacae isolate**	Positive (28.2)	NP	Negative	NA

[a] NA, not applicable; NP, not performed; *, tested at two separate laboratories; **, isolated from a throat swab taken in week 8.

rRNA sequence of the isolate was subject to PCR and DNA sequencing using previously described primers (P515PPl and p13B [9]). GenBank nucleotide blast analysis of a 787-bp 16S rRNA sequence provided a 100% match with two *Neisseria flava* sequences (GenBank accession numbers GU561419.1 and AJ239301.1), two unidentified *Neisseria* species (GenBank accession numbers FJ976424.1 and EU663609.1), and one *Neisseria sicca* sequence (GenBank accession number AJ239293.1). The closest *N. gonorrhoeae* match was 98% (768/787 nucleotides; GenBank accession number CP002440.1). The isolate was also sent to the Environmental and Science Research (ESR) reference laboratory (Porirua, New Zealand) and was identified as *Neisseria subflava* biovar *perflava* by standard phenotypic testing.

The above-described data provide clear evidence that the *N. gonorrhoeae*-positive results provided by the cobas 4800 assay for the throat swabs from this patient were false-positive results and that the problem arose through cross-reaction with a *Neisseria* species strain present in the throat of this particular patient. Since March 2012, we have had only eight other patients from whom pharyngeal swabs have provided positive PCR results in the cobas 4800 NG assay, and all eight have been confirmed as *N. gonorrhoeae* positive by the Abbott m2000 real-time PCR. Based on these limited data, the confirmation rate of the method for pharyngeal swabs is 88.9%. This is lower than the overall positive predictive value (PPV) of 97.1% (95% confidence interval, 95% to 98.5%) previously observed in our population based on testing of over 40,000 mainly urogenital specimens, of which 361 were positive for *N. gonorrhoeae* (10). While the identifications of the isolate provided by the MALDI-TOF, 16S rRNA, and phenotypic testing were not in complete agreement, the results clearly indicate that this was a commensal *Neisseria* strain and not *N. gonorrhoeae* and that it had likely acquired the DR-9 sequence targeted by both *N. gonorrhoeae* reactions of the cobas 4800 NG assay. We did not, however, seek to derive the DR-9 sequence from this isolate, as the precise region targeted by the cobas 4800 assay is not in the public domain.

It should be noted that most *N. gonorrhoeae* NAATs are neither validated nor marketed for use on extragenital sites, including pharyngeal swabs, but that NAAT testing of these sites is driven by the relatively poor sensitivity of culture for nongenital specimens (11). Overall, this case provides yet another example of specificity problems faced with molecular detection of *N. gonorrhoeae* and shows that false-positive results can be obtained from later-generation *N. gonorrhoeae* NAATs, including the cobas 4800 assay. These data further highlight the ongoing need for supplementary testing for *N. gonorrhoeae* NAATs so as to avoid unnecessary treatment and patient anxiety, particularly when they are applied to pharyngeal samples in which commensal *Neisseria* strains are prevalent.

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