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
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.

**ACKNOWLEDGMENT**

We thank Kevin Barratt at Canterbury Health labs for Abbott m2000 real-time PCR testing.

**REFERENCES**


**TABLE 1 Summary of NAAT and culture results***

<table>
<thead>
<tr>
<th>Week collected</th>
<th>Anatomical site/type of sample</th>
<th>Result with each <em>N. gonorrhoeae</em> diagnostic method</th>
<th>In-house porA and opa PCR assays</th>
<th>Bacterial culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (17/09/12)</td>
<td>Urine</td>
<td>Negative</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>1 (17/09/12)</td>
<td>Rectal swab</td>
<td>Negative</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>1 (17/09/12)</td>
<td>Throat swab</td>
<td>Positive (35.7, 35.3)*</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7 (7/11/12)</td>
<td>Throat swab</td>
<td>Positive (39.4)</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>8 (23/11/12)</td>
<td>Throat swab (× 2)</td>
<td>NP</td>
<td>NP</td>
<td>Negative</td>
</tr>
<tr>
<td>8 (23/11/12)</td>
<td><em>Neisseria macacae</em> isolate**</td>
<td>Positive (28.2)</td>
<td>NP</td>
<td>NA</td>
</tr>
</tbody>
</table>

*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 (P515PPI and p13B [9]). GenBank nucleotide blast analysis of a 787-bp 16S rRNA sequence provided a 100% match with two *Neisseria flavia* 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 AJ39293.1). 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 pharyngeal samples in which commensal *Neisseria* strains are prevalent.


Fairley CK, Chen MY, Bradshaw CS, Tabrizi SN. 2011. Is it time to move to nucleic acid amplification tests screening for pharyngeal and rectal *gonorrhoea* in men who have sex with men to improve gonorrhoea control? Sex. Health 8:9–11.