Supplementary Testing Is Not Required in the cobas 4800 CT/NG Test for Neisseria gonorrhoeae Weak-Positive Urogenital Samples

Collette Bromhead, Nadika Liyanarachchy, Julia Mayes, Arlo Upton, Michelle Balm
Aotea Pathology Ltd, Wellington, New Zealand; Labtests Auckland, Mt. Wellington, Auckland, New Zealand

Weak-positive Neisseria gonorrhoeae nucleic acid amplification test results are difficult to interpret. We show that the frequency of unconfirmed N. gonorrhoeae results from the cobas 4800 test rises exponentially after 38.0 cycles, where the likelihood of an unconfirmed result exceeds 29%. Supplementary testing of such samples should be avoided; instead, treatment should be based on clinical pretest probability.

The accurate diagnosis of gonorrhea demands laboratory tests that are sensitive, specific, reproducible, and robust because of variable clinical symptoms and the consequences of missed or incorrect diagnoses. The use of nucleic acid amplification tests (NAATs) for diagnosis has been a significant advance because of improved sensitivity and ease of specimen collection and transport (1).

International guidelines recommend that N. gonorrhoeae-positive NAAT results be confirmed by using supplementary assays with different targets if the positive predictive value (PPV) is <90% (2). The need for supplemental testing of urogenital samples is debated because of the high PPV of these samples (3, 4).

Labtests Auckland and Aotea Pathology are two large community laboratories in Auckland and Wellington, New Zealand, respectively. Both employ the cobas 4800 CT/NG assay, which targets the direct repeat 9 region of the N. gonorrhoeae genome, for testing in low-prevalence populations (5, 6). Confirmation of N. gonorrhoeae-positive cobas 4800 results is routinely performed with a duplex porA and opa assay (7) of samples from extragenital sites (not Communauté Européenne approved for use as an in vitro diagnostic medical device in the cobas 4800 CT/NG test) and of urogenital specimens with late threshold cycle (C_T) values. There are limitations to the use of the porA target alone, as it has been previously reported that there is a possibility of false-negative results due to the acquisition of a meningococcal porA sequence (8). Concurrent culture is now performed only in certain clinical situations.

Between September 2012 and January 2014, a total of 134 patient samples (73 urogenital, 42 pharyngeal, 19 rectal) were positive for N. gonorrhoeae in the cobas 4800 test and met the criteria for supplementary testing. Samples were referred from general practice, sexual health clinics, and other community health providers. Twenty-two of the samples were from females, 73 were from males, and no gender or date-of-birth information was available for 39 of them. The median age of the patients at the time of testing was 27 years with a range of 3 to 64 years.

Of the 134 samples in this study, 120 (90%) were positive for at least two N. gonorrhoeae targets. The remaining 14 could not be confirmed with either the porA or the opa assay and produced a significantly higher mean cobas 4800 C_T value (30.6 versus 38.4, P < 0.01). The majority of the unconfirmed samples (71%) were from extragenital sites.

Concern about the interpretation of these results arises from issues with false-positive results in low-prevalence populations, test reproducibility when there are low genome copy numbers in the specimen, and the possibility of cross-reaction with commensal Neisseria species at extragenital sites (9, 10). In order to address these issues, we undertook an investigation to determine the analytical sensitivity of the cobas 4800 CT/NG test and correlate this with the reproducibility of the results of porA and opa supplementary assays. Following this, we determined whether the C_T value in the cobas 4800 CT/NG assay was correlated with the results of supplementary assays of clinical samples.

A quantified N. gonorrhoeae DNA standard (Vircell) was reconstituted according to the manufacturer’s instructions to yield a stock concentration of 12,920 copies/μl (11). The stock DNA was then diluted in cobas collection kit buffer to achieve standards representing 1,000, 100, 50, 10, 5, 1, 0.5, 0.1, and 0.05 copies/μl. The standards were tested with the cobas 4800 instrument in accordance with the manufacturer’s instructions for swabs (12).

Standards with high N. gonorrhoeae concentrations (5 to 1,000 copies/μl) were tested in duplicate, while 1- and 0.5-copy/μl standards were tested on 10 consecutive occasions and the mean C_T value and coefficient of variation (CV) were calculated. As expected, an increasing variability of C_T values was seen at ≤1 copy/μl (mean C_T > 38.0; CV, 1.75 to 1.94%). When the standards were tested by supplementary assays, the porA target performed similarly to the cobas 4800 CT/NG test. The opa target produced earlier C_T values but without an increase in analytical sensitivity (data not shown).

The second part of the analysis determined whether cobas 4800 CT/NG test C_T values were related to the likelihood of confirmation by porA and opa supplementary assays. The frequency of negative supplementary testing associated with cobas N. gonorrhoeae C_T values was calculated from the 134 samples that were tested by all three assays (Fig. 1). The frequency of N. gonorrhoeae results
unconfirmed by porA and opa begins to rise after a C_T of 37.5 in the cobas 4800 CT/NG test and rises exponentially after 38.0 cycles. A cobas 4800 CT/NG test C_T of ≥38 represents less than one copy of the N. gonorrhoeae genome in the sample. At this level, the likelihood of a nonconfirmable result exceeds 29%.

We therefore propose that supplementary testing of cobas 4800 CT/NG test samples with C_Ts of ≥38 has limited utility. For urogenital specimens, where the PPV is high, we recommend reporting these results as indeterminate or equivocal. Treatment should be based on clinical pretest probability.

Where specimens are taken from low-risk patients for screening purposes (including testing protocols used at cervical smear or reporting purposes (including testing protocols used at cervical smear or pelvic examination), we suggest repeat testing if concern remains. Correlation not just with the clinical presentation (symptomatic or asymptomatic) but also with the laboratory-based detection of Neisseria gonorrhoeae and Neisseria meningitides.

We therefore propose that supplementary testing of cobas 4800 CT/NG test with culture for detecting Neisseria gonorrhoeae and Neisseria meningitides.

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REFERENCES