Comparison of the cobas 4800 CTNG test with culture for detecting Neisseria gonorrhoeae in genital and non-genital specimens in a low prevalence population in New Zealand.

Collette Bromhead *PhD, Amanda Miller BBMedSci, Mark Jones MD, FRCPath, FRCPA, David Whiley PhD

1. Aotea Pathology Ltd, Wellington, New Zealand
2. Queensland Paediatric Infectious Diseases Laboratory, Queensland Children’s Medical Research Institute, The University of Queensland & SASVRC, Royal Children’s Hospital, Brisbane, Australia
3. *89 Courtenay Place, Wellington 6011, New Zealand

Phone: +64 4 381 5900
Fax: +64 4 381 5948
Email: cbromhead@apath.co.nz
Abstract
To assess the clinical utility of replacing microbial culture for *Neisseria gonorrhoeae* with a NAAT, we compared *Neisseria gonorrhoeae* (NG) culture with the cobas 4800 CTNG test in 18,247 urogenital and 666 non genital samples. In urogenital specimens the sensitivity, specificity, positive and negative predictive values of the cobas NG PCR were 98.7%, 100%, 95.6% and 100% respectively, and for non-genital specimens 100%, 99.8%, 92.9% and 100% respectively.

In our testing population, 37% (10,185) of patients tested over the study period were screened for *Chlamydia trachomatis* by PCR, but were not screened for gonorrhoea by culture. Of these, 43 were NG positive by PCR and therefore went undiagnosed.

The cobas 4800 CTNG test diagnosed 33% (n=30) more urogenital and 25% (n=3) more rectal gonorrhoea infections than culture, and based on the above performance indicators, does not require supplementary testing for urogenital or rectal specimens. The ability to test non-invasive specimens (such as urine and self-taken vulvovaginal swabs) for NG will enable more patients to be screened for infection, thus bringing significant positive public health benefits.

Introduction
Nucleic acid amplification tests (NAATs) have been recommended for the diagnosis of *Chlamydia trachomatis* (CT) in New Zealand since 2008 (1). However, problems with the specificity of particular NAATs for *Neisseria gonorrhoeae* (NG) (2,3) meant that microbial culture remained the method of choice in New Zealand’s low prevalence population (67 per 100,000)(4). Well established clinical advantages of NAATs for diagnosing Sexually Transmitted Infections (STIs), including higher sensitivity, detection of non-viable organisms and testing of non-invasive specimens, are driving a change to molecular testing.
Replacement of culture with automated NAATs brings significant workflow benefits, a reduction in reporting time and the ability to test a single specimen for both CT and NG. Also, the positive public health impacts of increased diagnoses are important for tackling increasingly difficult to treat gonorrhoea infections.

Universal screening using NAATs in non-genital sites, particularly in men who have sex with men (MSM), is encouraged, to minimise the morbidity associated with gonorrhoea and potentially to enhance control of HIV transmission (5). However there may be issues with the specificity of NAATs in these anatomic sites due to the potential presence of commensal Neisseria species (6,7).

Since 2009, CT testing in our laboratory has been performed on the cobas 4800 using the CTNG (PCR) test (Roche Diagnostics, NZ), which simultaneously detects CT and NG. However, the NG test was not initially reported and culture was maintained for detection of gonorrhoea. Recent studies report improved clinical and analytical specificity of the cobas 4800 NG assay over previous assays (8,9) indicating that NG confirmatory testing may not be required on some specimens (10). This study compared the cobas 4800 NG test to culture, for genital and non-genital specimens, to investigate whether its performance would be acceptable without supplementary confirmatory testing in our low prevalence population.

Methods

Study Design

We retrospectively compared culture and cobas 4800 NG results obtained for patients from whom 2 specimens were collected; a cervical or urethral swab taken by a physician for microbial culture, and a physician taken cervical, urethral, rectal, or throat swab or a urine or self-taken vulvovaginal swab for CT PCR between 1/1/11 and 13/9/11. NG PCR results
were obtained from the raw data (Extensible Markup Language (XML) files), generated by
the cobas 4800 for all CT specimens tested, with the assistance of Roche Diagnostics, NZ.

Testing methods

Amies media swabs direct culture plates were inoculated onto NYC or Thayer Martin agar
(Fort Richard) and incubated in 5% CO₂ at 37°C for 48 hours. Suspect isolates were
identified by gram stain and oxidase testing, and confirmed using BactiCard (Remel, USA),
and/or MicroTrak Direct fluorescence antibody test (Syva, USA). Specimens submitted for
routine CT NAAT analysis were tested on the cobas 4800 as per the manufacturers’ protocol
(11) with the exception that swab specimens were vortexed for 1 minute and the swabs
removed prior to testing. Physician collected non-genital swab specimens were processed in
the same manner as genital swabs. The recommended procedure for collection of rectal
swab is via a proctoscope and for pharyngeal specimens a swab should be taken from the
back of the throat/tonsillar area.

Testing for NG was simultaneously performed by the cobas 4800; however, the results for
NG were not reported clinically during the study period. Any specimen with a non-valid
result (i.e. failed due to pipetting error or clot in the specimen; or invalid due to
amplification failure) was treated with Sputasol (1.4% DTT, Oxoid, United Kingdom) and re-
tested as previously described (12).

Urine specimens were not cultured for NG in our laboratory, but were tested for CT by PCR,
therefore where urine was sent for CT, an accompanying cervical or urethral culture swab
result was compared (‘urogenital’ specimens) (n=18,247).

Assay performance was further investigated using 97 local isolates grown from directly
inoculated plates (from Wellington Sexual Health Clinics) or swabs in Amies transport
media, from the rectum (n=24), throat (n=32), urethra (n=26), penis (n=2) and cervix (n=13) inoculated into cobas PCR media.

**Analytical specificity panel**

Analytical specificity was evaluated using 6 gonococcal and 34 commensal or pathogenic bacterial and fungal isolates grown from reconstituted freeze-dried organisms from the Institute of Environmental Science and research Limited (ESR) culture collection. Gonococcal isolates included antibiotic sensitive and resistant strains: (ATCC 43069 (Media quality control strain), ATCC 49226 (Antibiotic sensitivity reference strain), AGSP QC 00/3 (Ciprofloxacin MIC 2.0mg/L), AGSP QC 36 (Penicillin MIC 2.0mg/L), AS 84/417 (“NZ plasmid”), AS 91/376 (Tetracycline MIC >16mg/L, penicillinase producing). *N. meningitidis* isolates included a representative strain from each of the groups A, B and C. Also included were *Acinetobacter colcoaecticus*, *Candida albicans*, *C. glabrata*, *Citrobacter freundii*, *Enterococcus faecalis*, *E. faecium*, *Escherichia coli*, *Fusobacterium necrophorum*, *Gardnerella vaginalis*, *Haemophilus influenzae* (n=3), *H. parainfluenzae*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Micrococcus luteus*, *Neisseria cinerea*, *N. flava*, *N. lactamica*, *N. meningitidis* (n=3), *N. sicca*, *N. subflava*, *P. mirabilis*, *Serratia marcescens*, *Streptococcus agalactiae*, *S. pneumoniae*, *S. viridans*, *Staphylococcus aureus* (n=2), *S. epidermis*, *S. pyogenes*, and *S. saprophyticus*. Organisms were grown on tryptic soy agar with sheep blood, supplemented chocolate agar or sabouraud dextrose agar with antibiotics (Fort Richard, Auckland, NZ) under optimal conditions (36°C 5% CO2, 36°C O2 or ANO2 for 24-48 hours).

A pure isolate of each organism was inoculated into sterile saline to 0.5 MacFarland standard turbidity (approximately 1x10⁸ CFU/mL of bacteria). 50μL of the resulting
inoculation was added to a 4.2mL tube of cobas PCR media. Panels included 2-5 organisms per tube of cobas PCR media.

Confirmatory Testing

Where results were discordant between culture and PCR, available specimens were sent for testing at the Queensland Paediatric Infectious Diseases (QPID) laboratory, Brisbane. DNA was extracted on the MagNA Pure LC (Roche) using the Total Nucleic Acid Isolation Kit (Roche), then sent for confirmatory testing by a NG duplex PCR test targeting the porA pseudogene, and opa genes (8,13).

Discrepant Analysis

This study was a retrospective audit; therefore some specimens with discrepant results were not available for testing. A board certified microbiologist determined the expected result of these specimens by reviewing available clinical information under the following criteria: Specimens were ruled positive if there was previous or subsequent testing history of a NG positive result (by culture and/or PCR), clinical details from the referrer indicated probable NG infection, the NG PCR specimen tested positive by supplementary testing for porA/opa gene targets, a companion specimen tested NG positive (by culture and/or PCR) or if the patient was a sexual contact of a patient diagnosed with NG infection. Specimens were ruled negative if there was no clinical history/possibility of infection, if culture was performed on an inappropriate anatomical site (e.g. external genitalia). As culture was considered the gold-standard for this evaluation, where no information was available discrepant PCR results were ruled false.

Results
Between 1/1/11 and 13/9/11, 27,585 specimens were tested on the cobas 4800 for CT, of which 187 (0.68%) were positive for NG during our retrospective analysis. During the same period, 144/18,789 (0.77%) (unpaired) cultures grew NG.

CT samples with valid results were separated into “orphan CT specimens” (where there was no request for NG culture n= 10,185) and “study specimens” which were accompanied by a request for NG culture (n=17,400), and direct culture plates (n=97). As multiple sites were tested in some patients, there were 18,913 specimens with comparable test results, including 16,692 genital swabs, 1,555 urines, 531 rectal, 19 throat and 84 eye swabs. Non-genital sites are not validated for testing on the cobas 4800 by the manufacturer. However, a significant proportion of specimens from Sexual Health clinics are from these sites therefore it was important to analyse assay performance in these specimen types. A total of 79/187 (42.2%) patients infected with NG were found to be co-infected with CT at the time of testing. In contrast, only 79/2,165 (3.6%) of patients infected with CT were positive for NG.

Samples with failed or invalid cobas 4800 results were excluded from the analysis of assay performance.

**Assay Performance**

The performance of the cobas 4800 NG assay was assessed by comparing valid NG PCR results with microbial culture for 17,400 specimens tested on the same day.

This data showed 122 concordant NG positive results and a further 37/17,400 (0.2%) discrepant results. Of the discrepant cobas specimens, 35 were PCR positive/culture negative (5 urines, 24 cervical, 1 urethral, 1 penile, 1 vaginal and 3 rectal swabs) and 2 were PCR negative/culture positive (both urine).
Before the resolution of discrepant results, the sensitivity, specificity, positive and negative predictive values for the performance of the cobas 4800 CT/NG test on urogenital specimens were 98.5% (95% confidence intervals; 94.6% – 99.8%), 99.8% (99.7% – 99.9%), 80% (73.0% – 85.9%) and 100% (99.9% – 100%) respectively. In non-genital specimens the sensitivity, specificity and positive and negative predictive values were 100% (71.5% - 100%), 99.5% (98.7% - 99.9%), 78.6% (49.2% - 95.3%) and 100% (99.4% - 100%) respectively.

Further testing and analysis

There were 10 discrepant samples (NG positive by PCR but negative by culture) available for confirmatory PCR testing at QPID. The cobas NG results were considered correct if they tested positive for the porA and/or opa targets. In total, 9 samples were positive by these criteria, while 1 was not confirmed. All remaining discrepant results were evaluated by the clinical microbiologist with regard to history, clinical details and companion specimen results.

Of the 37 discrepant samples, 27 positive PCR results were confirmed, while 2 results were ruled false negative and 8 were ruled false positive. In total there were 164 concordant NG positive results, and 18,739 samples with concordant negative results (Table 1). The assay performance was recalculated and showed that in urogenital specimens the sensitivity, specificity and positive and negative predictive values were 98.7% (95% confidence intervals; 95.4% – 99.8%), 100% (99.9% – 100%), 95.6% (91.1% – 98.2%) and 100% (99.9% – 100%) respectively. For non-genital specimens the sensitivity, specificity and positive and negative predictive values were 100% (75.3% – 100%), 99.8% (99.1% - 100%), 92.9% (66.1% – 99.8%) and 100% (99.4% - 100%) respectively (Table 2). A separate analysis of performance in the 564 female and 950 male urine specimens showed that the cobas 4800 NG test has a sensitivity of only 86.7% for female urines.
compared to cervical or urethral swab culture, while it has 100% sensitivity for male urines compared to urethral swab culture (Table 3).

**Missed opportunities for screening and diagnosis**

A total of 10,185 (37%) of CT specimens in the study were not accompanied by a culture swab for gonorrhoea, and were excluded from the analysis of assay performance. However, of these specimens 43 were NG positive by PCR (8 vaginal swabs, 6 cervical swabs, 1 penile swab, 1 urethral swab and 27 urines), with 36/43 of these being “non-invasive” specimen types. Only 1,389 (7.4%) of gonorrhoea culture specimens were not accompanied by a swab for CT PCR.

During the period of the study the number of extra infections that were identified by PCR was 65 (35%). These patients were managed by the Clinical Microbiologist.

**Specificity**

The analytical specificity of the cobas 4800 CTNG assay was firstly determined for local gonococcal isolates including both antibiotic resistant and sensitive strains, including a NG prolyl iminopeptidase (PIP) negative clinical isolate (14). All were identified correctly as positive for NG. A further 34 reference laboratory organisms (including non-gonococcal Neisseria’s and commensal and pathogenic bacterial and fungal isolates) were tested and correctly identified as negative by this assay.

**Discussion**

For routine supplementary confirmatory testing not to be necessary, the performance indicators of specificity and PPV should reach >95% and >90% respectively, which can be difficult in a low prevalence population (15,16). As prevalence decreases, PPV decreases and therefore the likelihood of a false positive result increases and the effect of test specificity is magnified. Our testing population exhibits a low frequency of cultured NG
infections (0.77%, data not shown) which is comparable with reported national rates of infection in New Zealand (67 NG cases/100,000 population) (4). Previous reports have shown that 35-41% of patients may be co-infected with CT and NG (17) and we found that 42.2% of those infected with NG had a concurrent CT infection. These high rates of co-infection confirm the importance of testing both STI’s and the ability to test both organisms from a single specimen is a benefit of multiplex NAAT tests.

The performance of the cobas 4800 NG assay was assessed by comparison with culture in 18,905 paired specimens obtained from 17,400 visits, and we initially found 37 (0.2%) discrepant results between PCR and culture. Ten of these samples were available for supplementary confirmatory testing, of which 9 were positive for both *opa* and *porA*, and 1 did not give consistent results (presumably due to low organism load). After supplementary testing and clinical review of the remaining discrepancies, 10 samples were deemed to have false NG PCR results (8 false positives, 2 false negatives).

The performance indicators calculated from this data reached >95% sensitivity and specificity, and >90% PPV and NPV. Notably, our PPV reached 95.6% in urogenital specimens and 92.9% in non-genital specimens. It should however be noted that a limitation and potential bias of this study was that the PCR and culture were, for the most part, not performed on the same sample type (ie. PCR was performed on urine and bacterial culture on genital swabs). Nevertheless our findings are supported by two recent studies which showed the cobas NG test had lower levels of false positivity than predecessor assays (9), and does not require a confirmation test for NG in urine specimens (10).

Despite recent calls to move to NAAT routinely for pharyngeal and rectal testing, it is still recommended that positive results from these sites be confirmed by supplemental assays (5). The presence of potentially cross-reacting commensal Neisseria species in non-genital
sites may compromise the specificity of NAATs, and the cobas 4800 CTNG test is not commercially validated for these specimen types. While our results showed that there was no cross-reactivity with either local or reference laboratory strains of commensal Neisseria species, it should be noted that we only tested limited numbers of throat samples (n=51) compared to rectal samples (n=531), and had no positive eye swabs in the validation. Therefore, supplementary testing of cobas 4800 NG positive results from throat and eye samples is still warranted (13).

While different sample types were used for much of the PCR and culture-based testing, the results suggest that if PCR were the gold-standard test against which the sensitivity of culture was assessed, the sensitivity of culture for urogenital and non-genital specimens would be low at 80% and 79% respectively. The poor sensitivity of culture may not only be contributing to a higher prevalence of gonorrhoea but also to higher rates of HIV transmission (5). The limited sensitivity of culture compared with NAAT, particularly from pharyngeal and rectal samples, is well substantiated (6,7,18).

Self taken swabs and urine are now widely used for CT testing but in our laboratory, culture for NG was only carried out on cervical, urethral, throat and rectal swabs. The comparative difficulty of obtaining invasive specimens may have led to a decrease in testing for NG. This is evidenced by the one third of patients who provided “non-invasive” specimens for CT PCR testing but no culture specimen during the study period. Indeed 43 of these patients were found to be positive for gonorrhoea by NG PCR with the majority of these results (36/43) coming from “non-invasive” specimen types.

The cobas 4800 NG PCR assay has several limitations. As in previous studies on CT/NG NAAT assays, our data shows that a urine specimen alone is not sufficiently sensitive (86.7%) to screen for NG in females (19,20). We have previously shown that interference from
mucopurulent discharge in NG positive patients causes failed cobas 4800 results (12), and this has also been reported by Hopkins et al (2012) (10). Most failed results can be resolved by treatment with Sputasol (1.4% DTT, Oxoid) (12). Methods to overcome this technical problem are essential to the reliable use of the cobas 4800 CT/NG assay when secondary assays are not available. Extensively drug resistant (XDR) NG strains displaying high level resistance to third generation cephalosporins (Ceftriaxone and Cefixime) may pose a problem for transferring NG diagnosis to NAAT methods and empirically treating patients. Therefore, enhanced surveillance of antibiotic susceptibility is recommended (21,22). However, maintaining a viable organism for this testing is not possible in the high salt cobas PCR collection media. Until molecular methods for detection of gene sequences that confer resistance are validated for routine laboratory use, testing by both culture and PCR of NG is required to continue surveillance of antibiotic susceptibility (23). The conundrum of a diagnostic laboratory arises when it is not economically viable to provide both tests. In this situation, questions remain over whether diagnostic sensitivity is subordinate to antimicrobial resistance surveillance.

In summary, this audit comprehensively evaluated the performance of the cobas 4800 CTNG test for Neisseria gonorrhoeae compared to microbial culture. The cobas CTNG test diagnosed 33% more urogenital and 25% more rectal NG infections than culture, and, based on performance indicators, neither urogenital nor rectal specimens require supplementary confirmatory testing in our low-prevalence population.

Acknowledgements.

We thank Associate Professor Stephen Lambert, Queensland Children’s Medical Research Institute, The University of Queensland for statistical assistance.
References


5. **Fairley CK, Chen MY, Bradshaw MS and 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? Sexual Health. **8**: 9-11.


Table 1: Summary of NG PCR results compared to culture following discrepant analysis and resolution. Numbers of males and female are provided in parentheses (M/F)

<table>
<thead>
<tr>
<th>Location</th>
<th>Culture +</th>
<th>Culture -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital</td>
<td>PCR + 151(79F/72M)</td>
<td>PCR - 7(F)</td>
</tr>
<tr>
<td>Rectal</td>
<td>PCR + 12(1F/11M)</td>
<td>PCR - 1(M)</td>
</tr>
<tr>
<td>Throat</td>
<td>PCR + 1(M)</td>
<td>PCR - 0</td>
</tr>
<tr>
<td>Eye</td>
<td>PCR + 0</td>
<td>PCR - 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Culture +</th>
<th>Culture -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital</td>
<td>18,087(16,517F/1570M)</td>
<td>7(F)</td>
</tr>
<tr>
<td>Rectal</td>
<td>518(24F/494M)</td>
<td>1(M)</td>
</tr>
<tr>
<td>Throat</td>
<td>50(9F/41M)</td>
<td>0</td>
</tr>
<tr>
<td>Eye</td>
<td>84(11F/73M)</td>
<td>0</td>
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</table>
Table 2. Performance of the cobas 4800 NG assay by specimen type (post-discrepant resolution). 95% confidence intervals are provided in parentheses.

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urogenital (all)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18,247</td>
<td>98.7% (95.4 – 99.8)</td>
<td>97.5% (91.4 – 99.7)</td>
<td>100% (95.0 – 100)</td>
<td>100% (99.9 – 100)</td>
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<tr>
<td>Male</td>
<td>16,605</td>
<td>97.5% (91.4 – 99.7)</td>
<td>91.9% (83.9 – 96.7)</td>
<td>100% (95.0 – 100)</td>
<td>100% (99.9 – 100)</td>
</tr>
<tr>
<td>Non-genital (all)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>666</td>
<td>100% (75.3 – 100)</td>
<td>99.8% (99.1 – 100)</td>
<td>99.8% (99.1 – 100)</td>
<td>92.9% (66.1 – 99.8)</td>
</tr>
<tr>
<td>Male</td>
<td>86</td>
<td>100% (73.5 – 100)</td>
<td>92.3% (64.0 – 99.8)</td>
<td>100% (99.4 – 100)</td>
<td>100% (95.7 – 100)</td>
</tr>
<tr>
<td>Total</td>
<td>18,913</td>
<td>98.8% (95.7 – 99.9)</td>
<td>99.8% (99.9 – 100)</td>
<td>95.3% (91.0 – 98.0)</td>
<td>100% (99.9 – 100)</td>
</tr>
</tbody>
</table>
Table 3: Performance of the cobas 4800 NG assay in male and female urine specimens

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female urine</td>
<td>570</td>
<td>86.7% (59.5 – 98.3)</td>
<td>100% (99.3 – 100)</td>
<td>100% (75.3 – 100)</td>
<td>99.6% (98.7 – 100)</td>
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<tr>
<td>Male urine</td>
<td>985</td>
<td>100% (91.0 – 100)</td>
<td>100% (99.6 – 100)</td>
<td>100% (91.0 – 100)</td>
<td>100% (99.6 – 100)</td>
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</tbody>
</table>