An assessment of the Roche Amplicor® *Chlamydia trachomatis/Neisseria gonorrhoeae* multiplex PCR assay in routine diagnostic use on a variety of specimen types

David E Leslie, Franca Azzato, Norbert Ryan, Janet Fyfe
Victorian Infectious Diseases Reference Laboratory

Abstract

The Roche Cobas Amplicor® *Chlamydia trachomatis/Neisseria gonorrhoeae* polymerase chain reaction (PCR) assay can simultaneously detect both *C. trachomatis* and *N. gonorrhoeae*, and has been cleared by United States Food and Drug Administration (FDA) for the testing of endocervical and urethral swabs and urine specimens. The Amplicor *N. gonorrhoeae* PCR target sequence is known to be present in some strains of commensal *Neisseria* species, including *N. cinerea* and *N. subflava*, necessitating the use of a second PCR assay to confirm positive results. This study analyses the performance of the assay on 7,007 unselected specimens submitted to the laboratory for the PCR diagnosis of *N. gonorrhoeae* and *C. trachomatis;* compares the PCR assay with culture for the detection of *N. gonorrhoeae*; examines the performance of the assay with specimens from different body sites; and briefly compares two confirmatory PCR assays. Confirmation rates for an initial Amplicor *N. gonorrhoeae* positive result varied widely by specimen type, ranging from 86.2 per cent for penile/urethral swabs to 5.6 per cent for oropharyngeal swabs, indicating all positive Amplicor *N. gonorrhoeae* results should be confirmed by a second method to maintain adequate specificity. Overall there was 98.1 per cent agreement between the confirmed PCR assay and culture, with confirmed PCR showing a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 81.7 per cent, 99.5 per cent, 92.7 per cent and 98.5 per cent respectively, compared with *N. gonorrhoeae* culture. When confirmed *C. trachomatis/N. gonorrhoeae* PCR assay performance was analysed against culture using only FDA-cleared specimens (553 penile/urethral swabs, urines and cervical/vaginal swabs), sensitivity, specificity, PPV and NPV and percent agreement were 96.7 per cent, 99.8 per cent, 98.9 per cent, 99.4 per cent and 99.3 per cent respectively. No significant differences were found between the two confirmatory PCR assays used during the study period. Limitations of Amplicor for the detection of *N. gonorrhoeae* and the appropriate use of combined *C. trachomatis/N. gonorrhoeae* PCR in a routine diagnostic setting are discussed. *Commun Dis Intell* 2003;27:373–379.

Keywords: Chlamydia trachomatis, Neisseria gonorrhoeae

Background

Many diagnostic laboratories now routinely use nucleic acid amplification (NAA) assays to detect a variety of pathogenic organisms. The automated Roche Cobas Amplicor® *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) PCR assay has become popular due to its ability to detect both *C. trachomatis* and *N. gonorrhoeae* simultaneously from a variety of easily collected specimen types. This allows self-collection of specimens and is less sensitive to specimen transport and storage than traditional culture methods for *N. gonorrhoeae*.[1,2] The assay has been given 510(k) listing by the United States Food and Drug Administration (FDA) for the testing of urine, endocervical and urethral swabs, but has not been fully validated or cleared for testing of specimens from other body sites.[3]

For *C. trachomatis*, there are significant advantages of NAA assays over the technically difficult and expensive culture methods and the assay can handle a much greater range of specimens than the once widely used direct immunofluorescence kits. The sensitivity of the Amplicor assay for both *C. trachomatis* and *N. gonorrhoeae* compared with

Corresponding author: Dr David Leslie, Medical Microbiologist, Victorian Infectious Diseases Reference Laboratory, Melbourne Health Network, Locked Bag 815, Carlton South, Victoria 3053. Telephone: +61 3 9342 2600. Facsimile: +61 3 9342 2666. Email david.leslie@mh.org.au

traditional methods has been demonstrated in a number of studies. Specificity for the detection of *C. trachomatis* in female genital specimens has been estimated at over 99 per cent.[4,5,6]

Recently, major problems with the specificity of the assay for detection of *N. gonorrhoeae* have been documented.[7,8,9] The Amplicor assay targets a sequence in the cytosine DNA methyltransferase gene of *N. gonorrhoeae*, however similar sequences are now known to be present in some strains of the commensal *Neisseria* species, *N. cinerea* and *N. subflava*, and possibly *N. sicca, N. lactamica* and *N. flavescens*.[7,8,9] Commensal *Neisseria* species are almost ubiquitous in the human oro-pharynx, but may also be present in the genital tracts, possibly transiently, of some healthy people.[10]

To circumvent this problem, it has been common practice to use a second polymerase chain reaction (PCR) assay targeting a different site within *N. gonorrhoeae* to confirm all initial *N. gonorrhoeae* positive results obtained by the Amplicor CT/NG assay. Two main targets have been used for the supplementary test; initially Roche released a *N. gonorrhoeae* 16S rRNA gene detection kit, however this was withdrawn for unspecified reasons in May 2001. Subsequently, various laboratories have developed other in-house methods.[7,11,12] At the Victorian Infectious Diseases Reference Laboratory (VIDRL) an in-house method was developed based on a LightCycler real-time PCR assay for detection of the cppB gene of *N. gonorrhoeae*, which is present in multiple copies in the cryptic plasmid of *N. gonorrhoeae* or as a single genomic copy in a few atypical strains.

The purpose of this study is to examine the performance of the Amplicor assay on a variety of specimen types, and to assist with the formulation of appropriate testing strategies using this assay.

All test procedures involving the detection of *N. gonorrhoeae* were analysed over a 20 month period, from 1 January 2001 to 31 August 2002. Test procedures analysed included the Roche Amplicor CT/NG assay, and culture procedures for detection of *N. gonorrhoeae* from genital, oropharyngeal, and other specimens.

Methods

Specimens

VIDRL is a tertiary referral public health laboratory and specimens were referred from all parts of the State of Victoria. In addition to referrals from other pathology laboratories, VIDRL provides primary pathology services to a number of inner Melbourne specialist clinics with high caseloads of men who have sex with men (MSM) and HIV-infected patients. This patient population is predominantly male and has very high rates of sexually transmitted and bloodborne viral infections (STI/BBVI) compared with the rates in general population. All specimens included in the study were submitted to VIDRL for diagnostic purposes; any specimens known to derive from screening or prevalence studies were excluded. First void urine specimens and swabs were either collected by medical staff or self-collected under instruction. A few unusual specimens such as intra-uterine devices, or pleural or peritoneal fluids were handled by the laboratory in a similar way to the swabs and urine specimens. Requests for *C. trachomatis* PCR only (*N. gonorrhoeae* PCR not requested) over the test period have been excluded from analysis. One thousand and thirty-eight requests for *N. gonorrhoeae* PCR with *C. trachomatis* PCR not requested were included in the study.

Cobas Amplicor® *C. trachomatis/N. gonorrhoeae* PCR assay

The details of the assay have been previously described.[13] Urine and genital swabs were processed and run on the automated Cobas instrument according to the manufacturer's instructions. Swabs from other sites were processed as for genital specimens. Swabs submitted in charcoal Amies transport medium were tested by culture and PCR, and dry swabs were tested by PCR only. An internal amplification control was included for each specimen. The criteria used for determination of a positive result were those supplied with the assay. Extracts of urine and swabs showing inhibition on initial testing were diluted, 1:2 and 1:4 respectively, for retesting. Following Amplicor testing, extracted PCR specimens were stored at –20ºC for further testing as necessary.

N. gonorrhoeae 16S rRNA confirmatory assay

Confirmatory testing of Amplicor *C. trachomatis/ N. gonorrhoeae* positive results was initially performed using a 16S rRNA assay (developed by Roche Diagnostic systems) which targets the *N. gonorrhoeae* 16S rRNA gene sequence described by Rossau, *et al*.[14] This assay used the manual Amplicor format. Use of this assay for confirmatory purposes ceased on 31 August 2001.

N. gonorrhoeae LightCycler cppB PCR assay

This assay was introduced in August 2001 following the announcement by Roche that the company would not continue to supply the *N. gonorrhoeae* 16S rRNA gene confirmatory PCR assay. The basic method for the LightCycler cppB assay for *N. gonorrhoeae* has been previously published.[15] It was optimised at VIDRL in conjunction with Gippsland Pathology Service and Roche Diagnostics Australia. Specimens already processed for the Amplicor assay were further purified using DNA extraction columns (High-Pure Purification kit, Roche Diagnostics, Australia) before the specimen was run in the cppB PCR assay. All

extracted DNA specimens were stored at –20ºC if a delay in processing was expected. This new test was run in parallel with the 16S rRNA gene assay for one month on 75 samples, of which 36 per cent were positive, but no discordant results were detected.

N. gonorrhoeae culture

Swabs submitted for culture in charcoal Amies transport medium were usually transported to the laboratory at ambient temperature on the same day of collection. Swabs were plated out on New York City Agar and Chocolate or Horse-blood agar (depending on site) and incubated at 37ºC in five per cent CO_2. The plates were read daily for up to four days. Presumptive positive *N. gonorrhoeae* isolates were identified by typical colonial and Gram-stain morphology, oxidase and superoxol reactions. Carbohydrate utilisation and other biochemical testing was determined using API NH kits (Biomerieux, Lyon France) according to the manufacturer's instructions. Any *N. gonorrhoeae* isolates identified were referred to the Victorian state *N. gonorrhoeae* reference laboratory (Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria) for confirmation and antimicrobial susceptibility testing.

Data extraction

All data relating to procedures that detect *N. gonorrhoeae* and *C. trachomatis* for the period 1 January 2001 to 31 August 2002 were downloaded from the Medipath Laboratory Information system (LRS Software Pty Ltd, Traralgon Victoria) into MS Excel spreadsheets. The data were checked for errors and non-diagnostic testing excluded from further analysis.

Statistical analysis

Categorical variables were compared using a Chi-squared analysis.

Results

N. gonorrhoeae

Overall, 7,007 specimens from 4,324 patents were tested by PCR and 4,016 specimens from 2,305 patients were tested by culture. The test and patient numbers by test type and sex are shown in Tables 1 and 2. Overall, there were more than three times as many men as women tested. When test figures were combined, culture showed a crude positive rate of 5.3 per cent compared with PCR at 3.3 per cent. Some patients had multiple episodes of infection and/or infection at multiple sites.

Specialist clinics diagnose a high proportion of the gonorrhoea cases in Victoria. Of the 214 specimens positive by culture and 229 positive by PCR, only 2 and 22 specimens respectively, were referred from sources other than inner Melbourne clinics with a known high MSM patient load.

Only five specimens from three women were found to be positive for *N. gonorrhoeae* over the period. Of the three women with gonorrhoea, one specimen was referred via a private pathology service, while the other two women, both with positive vaginal and throat swabs, were referred from a specialist STI clinic.

Table 1. Tests for the detection of *Neisseria gonorrhoeae,* 1 January 2001 to 31 August 2002

Test method	Requests				Positive tests			% +ve
	n	M	F	U*	n	M	F	
PCR	7,007	5,338	1,666	3	229	224	5	3.3
Culture	4,016	3,128	888	0	214	214	0	5.3
Total tests	11,023	8,466	2,554	3	443	438	5	4.0

* Sex unknown; n = Total number tested; +ve = positive; –ve = negative.

Table 2. Patient numbers tested for *Neisseria gonorrhoeae,* 1 January 2001 to 31 August 2002

Test method	Number of patients tested				Positive patients†			+ve %
	n	M	F	U*	n	M	F	
PCR	4,324	3,242	1,079	3	197	194	3	4.6
Culture	2,305	1,774	531	0	186	186	0	8.1
Total	5,085	3,826	1,256	3	275	272	3	5.4

* Sex unknown.

† Patients may have one or more positive results during the study period.

The initial Amplicor positive rate and subsequent *N. gonorrhoeae* confirmation rate are examined by specimen type and site in Table 3. The rate of confirmation varied significantly with specimen types ($p<10^{-7}$), with penile/urethral swabs confirmed as positive in 86.2 per cent of tests, compared with oropharyngeal swab confirmation rates of 5.6 per cent. Ano-rectal swabs confirmed positive in 20 per cent of cases. The confirmation rate of cervico-vaginal specimens (5.7%) was low, however this may be a reflection of the low rates of gonorrhoea in women (3 out of 1,256 patients tested, 0.24%) in our patient population compared with the male patients (272 out of 1,256 patients tested, 7.1%).

C. trachomatis

Results for *C. trachomatis* detection by Amplicor PCR are shown in Table 4. Of note is the rate of detection of *C. trachomatis* in ano-rectal specimens (8%), a site that was difficult to examine by *C. trachomatis* culture or direct immunofluorescence prior to the availability of PCR testing. Carriage of *C. trachomatis* at oropharyngeal sites is relatively uncommon in comparison (2.3%). Of those tests positive for *N. gonorrhoeae* by PCR (n=229), 26 (including one vaginal swab) were also simultaneously positive for *C. trachomatis*. *Chlamydia trachomatis* PCR was not requested on 15 specimens positive for *N. gonorrhoeae*.

Table 3. Amplicor CT/NG *N. gonorrhoeae* PCR detection and confirmation rate by site

Specimen type	n	All Amplicor CT/NG initial +ve	Amplicor CT/NG +ve/ confirmatory assay +ve	Amplicor CT/NG +ve/ confirmatory assay –ve	Amplicor CT/NG inhibited (No result)	Amplicor CT/NG –ve	Amplicor CT/NG initial +ve confirmed %	Specimens confirmed positive %
Urine	4,490	164	105	59	1	4,325	64.0	2.3
Penile/urethral swab	302	65	56	9	0	237	86.2	18.5
Ano-rectal swab	1,030	200	40	160	3	827	20.0	3.9
Cervical/ vaginal swab	441	35	2	33	2	404	5.7	0.5
Oropharyngeal swab	716	447	25	422	0	269	5.6	3.5
Other site*	28	2	1	1	0	26	50.0	3.6
Total	7,007	913	229	684	6	6,088	25.1	3.3

* Includes eye, iud, body fluids, and site not stated.

Table 4. Amplicor CT/NG *C. trachomatis* PCR detection rates by site and sex

Specimen type	Total* n	Amplicor +ve total	Amplicor –ve total	Positive (all) %	Male n	+ve male specimens	male specimens +ve %	Female n	+ve female specimens	Female specimens +ve %
Urine	3,521	184	3,337	5.2	2,733	145	5.3	787	39	5.0
Penile/urethral swab	286	32	254	11.2	278	32	11.5	8	0	0.0
Ano-rectal swab	1,028	82	946	8.0	1,010	81	8.0	18	1	5.6
Cervical/ vaginal swab	401	14	387	3.5	–	–	–	401	14	3.5
Oropharyngeal swab	708	16	692	2.3	602	16	2.7	106	0	0.0
Other†	25	2	23	8.0	19	2	10.5	6	0	0.0
Total	5,969	330	5,639	5.5	4,642	276	5.9	1,326	54	3.9

* CT PCR was not requested on 1,038 specimens. sex not determined for one negative urine specimen.

† Includes eye, iud, body fluids, and site not stated.

Correlation of *Neisseria gonorrhoeae* PCR and culture results

Culture was performed in parallel on 1,234 specimens tested by PCR. Results by specimen type are shown in Table 5. Of the 17 specimens culture positive but negative by PCR, 12 were initially positive by Amplicor PCR but failed to confirm on the secondary PCR. Overall, there was 98.1 per cent agreement between the confirmed PCR assay and culture, with confirmed PCR showing a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 81.7 per cent, 99.5 per cent, 92.7 per cent and 98.5 per cent respectively compared with *N. gonorrhoeae* culture.

In 146 cases, VIDRL received a urine specimen for PCR testing and a urethral swab for culture collected from the same patient on the same day. There was again excellent correlation between the two methods (99.3% agreement), with only one culture positive, Amplicor positive, but cppB PCR negative discrepant result. This gave confirmed PCR a sensitivity, specificity, PPV and NPV of 97.5 per cent, 100 per cent, 100 per cent and 99.1 per cent respectively, compared with culture.

When confirmed CT/NG PCR assay performance was analysed against culture using only FDA-listed specimens (553 penile/urethral swabs, urines and cervical/vaginal swabs), sensitivity, specificity, PPV and NPV and per cent agreement were 96.7 per cent, 99.8 per cent, 98.9 per cent, 99.4 per cent and 99.3 per cent respectively.

This compares with figures of 65.1 per cent, 99.4 per cent, 84.8, 98.1 and 97.6 per cent respectively, for non-FDA listed specimens (827 ano-rectal, oropharyngeal and other specimen types).

Confirmation assay performance

In order to assess if there were differences in performance between the two confirmatory assays used during the study period, the confirmation rates of initial Amplicor positive results were examined for the period of use of each assay. In August 2001, both methods were used in parallel, but only the results from the earlier 16S rRNA assay were used in this analysis (Table 6.) The proportion of specimens tested from sites for which the Amplicor assay has FDA approval was similar for both the 16S rRNA assay (28.6%), and the cppB assay (26.8%). Overall, no significant differences between the confirmation methods were noted (p=0.65). It should be noted that the detection limits for *N. gonorrhoeae* from clinical specimens has not been fully established for either confirmatory assay.

Table 6. *Neisseria gonorrhoeae* PCR confirmation rates by method

	Amplicor CT/NG +ve/ confirmatory assay +ve	Amplicor CT/NG +ve/ confirmatory assay –ve	Total*
16S rRNA	71	223	294
cppB	158	461	619
Total	229	684	913

Table 5. Correlation between culture and PCR results by site

Specimen type	n	Culture +ve, PCR +ve	PCR +ve, culture –ve	Culture +ve PCR –ve	Culture +ve PCR –ve initial Amplicor CT/NG result		PCR –ve Culture –ve
					–ve	+ve*	
Penile/urethral swab	216	48	1	2	1	1	165
Ano-rectal swab	491	22	1	13	3	10	455
Cervical/vaginal swab	191	0	0	0	0	0	191
Oropharyngeal swab	328	5	4	2	1	1	317
Other†	8	1	0	0	0	0	7
Total identical specimens	1,234	76	6	17	5	12	1,135
Paired urine PCR/ urethral swab culture	146	39	0	1	0	1	106

* Initial Amplicor CT/NG positive result that failed to confirm on supplementary assay.

† Includes eye, iud, body fluids, and site not stated.

Discussion

In this study culture displayed both higher sensitivity and higher crude positive rate than PCR for the detection of *N. gonorrhoeae* in contrast to previously published studies using the Amplicor assay and a confirmatory *N. gonorrhoeae* PCR.[4] However, it should be noted that confirmed PCR performed well in comparison with culture on genital specimens listed for testing in the CT/NG assay by the FDA.

There are likely to be a combination of factors influencing these findings. The apparent lower sensitivity of confirmed PCR compared with culture on paired specimens may be due to lower sensitivity of the confirmatory assays for the detection of low numbers of organisms. This is suggested by the fact that 13 of 18 identical or related specimens that were culture positive but confirmatory assay negative, were positive on the initial Amplicor PCR. The lower sensitivity of various confirmatory assays compared with Amplicor has been previously reported by a number of authors.[15,17] It has been noted that some rare *N. gonorrhoeae* strains lack multiple copies of the cppB gene, effectively reducing the sensitivity of the cppB gene PCR assay.[16]

Competitive inhibition in the multiplex PCR assay due to reagent limitation has been suggested as a reason for false-negative CT/NG PCR results, especially in specimens positive for both *C. trachomatis* and *N. gonorrhoeae*.[17] This prompted a review of the raw data printouts from the five *C. trachomatis/ N. gonorrhoeae* PCR–negative, culture-positive specimens, however, partial assay inhibition was identified as a potential problem in only one case.

The effect of specimen transport, the transport media used and the storage and handling of extracted specimens following analysis on the Cobas machine may also play a part and deserves further investigation.[11]

The low confirmation rate of cervico/vaginal specimens (5.7%) is probably a reflection of the low rates of gonorrhoea in women in our patient catchment compared with that seen in the MSM population. These findings are similar to those of Diemert, *et al* who studied the use of the Amplicor and 16S rRNA confirmatory assay in a Canadian population with a low prevalence of gonorrhoea in women.[12]

The confirmation rates for ano-rectal initial Amplicor positives was 20 per cent, with a positive rate of 3.9 per cent of specimens tested. Interestingly, the CT/NG assay revealed a rate of *C. trachomatis* carriage of 8 per cent in ano-rectal specimens; a site not traditionally associated with *C. trachomatis* infection. For this reason, we believe testing for *Neisseria gonorrhoeae* at this site by PCR should be considered in sexually active MSM populations despite the low *N. gonorrhoeae* PCR confirmation rate.

The overall higher detection rate seen with gonorrhoea culture may reflect a sampling bias by doctors working in clinics with high caseloads of symptomatic patients. In this scenario, culture is often requested in the expectation of obtaining a positive result with subsequent drug susceptibility details. A sampling bias is also suggested by the higher overall *N. gonorrhoeae* PCR detection rates noted for urethral swabs (18.5%) as compared with urine specimens submitted (2.3%) (Table 3). However, paired urine PCR and urethral swab culture results from the same, predominantly male patients showed a high degree of concordance (Table 5).

Although not subjected to a rigorous parallel comparison, the two different confirmatory assays appear to have little difference in performance when confirmation rates were compared. A recent study by Palmer, *et al*[9] identified an unusual British proline-arginine- and uracil-requiring *N. gonorrhoeae* auxotype that failed to be detected by a cppB PCR, but to the best of our knowledge, strains of this type are rare in Australia. The authors also found that no single PCR target was completely sensitive or specific for *N. gonorrhoeae* and that given the promiscuous genetic recombination that occurs within the genus,[18] any positive PCR results obtained on extra-genital specimens should be confirmed by an assay that uses a different genetic target.[9]

We believe the above data can assist in optimising the use of the available diagnostic tests for *Neisseria gonorrhoeae* and *C. trachomatis*, and that the testing strategy adopted by the laboratory should be appropriate to the patient population tested.

In a highly sexually active MSM population we believe it is appropriate to test both ano-rectal swabs and either a urine specimen or urethral swab by CT/NG PCR. We would recommend against routinely testing throat swabs by CT/NG PCR. Culture is more appropriate at this site, and will also detect other pathogens such as *Streptococcus pyogenes* that are commonly isolated in this population (data not shown). Although *C. trachomatis* may be carried at this site, it is relatively uncommon and many clinicians automatically treat for non-gonococcal STIs whenever a diagnosis of gonorrhoea is presumed or proven.

Our data on specimens from women is more limited due to the much lower rates of gonorrhoea in the study population, and the lack of data on parallel testing of urine with other types of female genital specimens. However, other authors have found either endocervical swabs, self-collected vaginal swabs or tampon specimens to be superior to urine specimens for the detection of both *C. trachomatis* and *N. gonorrhoeae* in the CT/NG assay.[1, 8,19] We found little evidence of extra-genital *C. trachomatis* infection in women in our study population, but found *N. gonorrhoeae* in throat swabs of two women who

also had a positive vaginal swab for *N. gonorrhoeae*. This implies that the CT/NG PCR need not be used routinely for testing of extra-genital sites in women, however, PCR testing of ano-rectal swabs in women with a history of exposure or symptoms would seem reasonable. As in the male population, culture is a more appropriate testing procedure for throat swabs.

It should be noted that the current Australian Medicare Benefits schedule will not fund more than one CT/NG PCR assay per patient episode. In a recent study by Donovan, *et al* in Sydney, the impact of this policy was considered as having a negative effect on STI diagnosis and control.[20] This leaves laboratories and clinicians working in practices with high rates of STIs with an ongoing testing dilemma.

Acknowledgments

The authors would like to thank Elise Walker, Roche Diagnostics Australia, and Neville Pattle, Gippsland Pathology Service for their assistance with test development, and Heath Kelly, VIDRL for assistance with statistical analysis.

References

1. Garrow SC, DW Smith, GB Harnett. The diagnosis of chlamydia, gonorrhoea, and trichomonas infections by self obtained low vaginal swabs, in remote northern Australian clinical practice. *Sex Transm Infect* 2002;78:278–281.
2. Tabrizi SN, Paterson B, Fairley CK, Bowden FJ, Garland SM. A self-administered technique for the detection of sexually transmitted diseases in remote communities. *J Infect Dis* 1997;176:289–292.
3. Johnson RE, Newhall WJ, Papp JR, Knapp JS, Black CM, Gift TL, *et al.* Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections – 2002. *MMWR Recomm Rep* 2002;51:1–38.
4. Martin DH, Cammarata C, Van Der Pol B, Jones RB, Quinn TC, Gaydos CA, *et al*. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG for *Neisseria gonorrhoeae. J Clin Microbiol* 2000;38:3544–3549.
5. Livengood CH 3rd, Wrenn JW. Evaluation of COBAS AMPLICOR (Roche): accuracy in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by coamplification of endocervical specimens. *J Clin Microbiol* 2001;39:2928–2932.
6. Gaydos CA, Crotchfelt KA, Shah N, Tennant M, Quinn TC, Gaydos JC, *et al.* Evaluation of dry and wet transported intravaginal swabs in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in female soldiers by PCR. *J Clin Microbiol* 2002;40:758–761.
7. Farrell DJ. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using cppB nested PCR and 16S rRNA PCR. *J Clin Microbiol* 1999;37:386–390.
8. Van Der Pol B, Martin DH, Schachter J, Quinn TC, Gaydos CA, Jones RB, *et al.* Enhancing the specificity of the COBAS AMPLICOR CT/NG test for *Neisseria gonorrhoeae* by retesting specimens with equivocal results. *J Clin Microbiol* 2001;39:3092–3098.
9. Palmer HM, Mallinson H, Wood RL, Herring AJ. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae. J Clin Microbiol* 2003;41:835–837.
10. Knapp JS, Hook EW 3rd. Prevalence and persistence of *Neisseria cinerea* and other *Neisseria* spp. in adults. *J Clin Microbiol* 1988;26:896–900.
11. Van Dyck E, Smet H, Van Damme L, Laga M. Evaluation of the Roche *Neisseria gonorrhoeae* 16S rRNA PCR for confirmation of AMPLICOR PCR-positive samples and comparison of its diagnostic performance according to storage conditions and preparation of endocervical specimens. *J Clin Microbiol* 2001;39:2280–2282.
12. Diemert DJ, Libman MD, Lebel P. Confirmation by 16S rRNA PCR of the COBAS AMPLICOR CT/NG test for diagnosis of *Neisseria gonorrhoeae* Infection in a low-prevalence population. *J Clin Microbiol* 2002;40:4056–4059.
13. Bassiri M, Mardh PA, Domeika M. Multiplex AMPLICOR PCR screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women attending non-sexually transmitted disease clinics. The European Chlamydia Epidemiology Group. *J Clin Microbiol* 1997;35:2556–2560.
14. Rossau R, Heyndrickx L, Van Heuverswyn H. Nucleotide sequence of a 16S ribosomal RNA gene from *Neisseria gonorrhoeae. Nucleic Acids Res* 1988;16:6227.
15. Whiley DM, LeCornec GM, Mackay IM, Siebert DJ, Sloots TP. A real-time PCR assay for the detection of *Neisseria gonorrhoeae* by LightCycler. *Diagn Microbiol Infect Dis* 2002;42:85–89.
16. Hagblom P, Korch C, Jonsson AB, Normark S. Intragenic variation by site-specific recombination in the cryptic plasmid of *Neisseria gonorrhoeae. J Bacteriol* 1986;167:231–237.
17. Hamilton MS, Otto M, Nickell A, Abel D, Ballam Y, Schremmer R. High frequency of competitive inhibition in the Roche Cobas AMPLICOR multiplex PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 2002;40:4393.
18. Kroll JS, Wilks KE, Farrant JL, Langford PR. Natural genetic exchange between *Haemophilus* and *Neisseria*: intergeneric transfer of chromosomal genes between major human pathogens. *Proc Natl Acad Sci U S A* 1998;95:12381–12385.
19. Tabrizi SN, Paterson BA, Fairley CK, Bowden FJ, Garland SM. Comparison of tampon and urine as self-administered methods of specimen collection in the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis* in women. *Int J STD AIDS* 1998;9:347–349.
20. Donovan B, Knight V, McNulty AM, Wynne-Markham V, Kidd MR. *Gonorrhoea* screening in general practice: perceived barriers and strategies to improve screening rates. *Med J Aust* 2001;175:412–414.