JOURNAL OF CLINICAL MICROBIOLOGY, May 2005, p. 2231–2235 Vol. 43, No. 5
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.5.2231–2235.2005

Evaluation of Conventional and Real-Time PCR Assays Using Two Targets for Confirmation of Results of the COBAS AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* Test for Detection of *Neisseria gonorrhoeae* in Clinical Samples

C. H. E. Boel,[1]* C. M. C. van Herk,[1] P. J. M. Berretty,[2] G. H. W. Onland,[1] and A. J. C. van den Brule[1]

PAMM Laboratories, Laboratory for Medical Microbiology, Veldhoven,[1] and Catharina-Hospital, Department of Dermatology, Eindhoven,[2] The Netherlands

Received 24 July 2004/Returned for modification 4 November 2004/Accepted 17 January 2005

Two conventional PCR-enzyme immunoassays (PCR-EIAs) and two real-time PCR assays (LightCycler system; Roche Diagnostics) were evaluated as confirmation assays with *cppB* and 16S rRNA genes as targets. Of 765 male and female genitourinary and nasopharyngeal specimens positive for *Neisseria gonorrhoeae* in the COBAS AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* PCR test (Roche Diagnostics), 229 (30%) were confirmed positive; 13 of these (5.7%) were lacking the *cppB* gene. Of the 534 samples (70%) that could not be confirmed, 81 (15%) showed a positive crossing point. However, melting curve analysis revealed an aberrant melting temperature in the LightCycler 16S rRNA assay; therefore, these samples were considered non-*N. gonorrhoeae Neisseria* species. Both of the 16S rRNA assays performed well, with positive predictive values of 99.1% and 100% for the PCR-EIAs and the real-time assays, respectively, and a negative predictive value of 99.8% for both. The *cppB* assays were compromised by the absence of the *cppB* gene in 5.7% of the *N. gonorrhoeae*-positive samples, resulting in negative predictive values of 96.8% and 97.6% for the PCR-EIAs and the real-time assays, respectively. Therefore, the 16S rRNA gene is preferable to the *cppB* gene as a target for confirmation assays. The melting curve analysis of the real-time assays provides useful additional information.

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the two most common causes of sexually transmitted infections (STI). Infection by these microorganisms can lead to clinical syndromes ranging from mild urethritis to pelvic inflammatory disease. However, both organisms can also cause asymptomatic infections in women and men; these infections can proceed in women to an ascending infection of the fallopian tubes (3, 10, 14). The outcome of the ascending infection can be reduced fertility, infertility, or even extrauterine pregnancies. Therefore, early diagnosis is necessary to prevent these complications and control the spread of infection.

Several studies have shown that nucleic acid amplification tests are superior to conventional tests for the detection of *C. trachomatis* and *N. gonorrhoeae* (4, 5, 8). Because these organisms are regularly found together, the COBAS AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CA CT/NG) multiplex PCR test (Roche Diagnostics, Mannheim, Germany) provides a useful platform for STI evaluation. An internal control greatly reduces false-negative results. The CA CT component has proven to be a useful diagnostic tool for the detection of *C. trachomatis* in cervical scraping and urine specimens (13, 22, 23). The CA NG component, however, lacks specificity, as some non-*N. gonorrhoeae Neisseria* species (NgNS) (8, 16) and even lactobacilli (23) have been reported to give false-positive results. Therefore, confirmation by a second, more specific PCR assay is required, especially in low-prevalence communities (8, 15, 22). This second PCR assay should be convenient and fast to ensure that the delay introduced by required confirmation is kept to a minimum. Moreover, extra attention should be directed toward the prevention of contamination, as the prevalence of positive samples is much higher in collections of CA NG-positive samples sent to a reference laboratory for confirmation. Real-time technology is promising in this respect.

In this study, we compared two in-house PCR-enzyme immunoassays (PCR-EIAs) and two in-house real-time PCR assays (LightCycler [LC] system; Roche Diagnostics, Mannheim, Germany) with 16S rRNA and *cppB* genes as targets for 765 CA NG-positive samples.

MATERIALS AND METHODS

Cultured strains. For specificity analysis, 17 *N. gonorrhoeae* strains and 15 NgNS strains (18) were grown on nonselective gonococcal plates for 48 h at 37°C in an incubator containing 5% CO_2 and were suspended to a 1.0 McFarland standard in 20 mM Tris-HCl (pH 8.3). Aliquots of 200 μl were subsequently used for DNA extraction according to Boom et al. (1). Aliquots of 5 μl were subsequently used in the LC assays, and 10-μl aliquots were used in the PCR-EIAs.

Clinical specimens. A total of 526 CA NG-positive specimens were sent at ambient temperature from 12 laboratories located throughout The Netherlands to our laboratory for confirmation testing. In addition, 239 CA NG-positive specimens from our laboratory were included. Of the 765 specimens, 561 (73%) were from women; 261 were from cervical origin, and 102 were from nasopharyngeal origin. For 138 specimens, no origin was provided by the referring laboratory. Of the 204 specimens (27%) from men, the origin was not provided for 109; the urethra was the origin for 46 specimens, and urine was the origin for 27 specimens.

Clinical specimens were split equally, and from both parts 200 μl was used for DNA extraction according to Boom et al. (1). DNA was analyzed with the CA

* Corresponding author. Mailing address: Laboratory for Medical Microbiology, PAMM Laboratories, P.O. Box 2, NL-5500 AA Veldhoven, The Netherlands. Phone: 31-40-8888177. Fax: 31-40-8888182. E-mail: e.boel@pamm.nl.

TABLE 1. Primers and probes used in this study

Designation	Sequence (5′ to 3′)	Target gene	Reference or source
PCR-EIA			
cppB			
HO1	GCT ACG CAT ACC CGC GTT GC	*cppB*	11
HO2	CGA AGA CCT TCG AGC AGA CA	*cppB*	11
Roy1 probe	AAT ACT GCC TTG CTG ATG CGT AAC TGC CGG	*cppB*	18
16S rRNA			
SL67	TAT CGG AAC GTA CCG GGT AGC	16S rRNA	8
SL59	GTA TTA CCG CGG CTG CTG GCA	16S rRNA	8
SL80E probe	GGC CGC CGA TAT TGG CAA CA	16S rRNA	8
LC			
cppB			
HO1	GCT ACG CAT ACC CGC GTT GC	*cppB*	11
HO2	CGA AGA CCT TCG AGC AGA CA	*cppB*	11
NG-LC1 probe	CGT TCT TGA CGC TCC ATA TCG CTA TGA A	*cppB*	24
NG-LC2 probe	AGC CCT GCT ATG ACT ATC AAC CCT GCC	*cppB*	24
16S rRNA			
NG16SF	TAT CGG AAC GTA CCG GGT AG	16S rRNA	This study
NG16SR	GCT TAT TCT TCA GGT ACC GTC AT	16S rRNA	This study
NG16SFL probe	CGG GTT GTA AAG GAC TTT TGT CAG GGA A	16S rRNA	This study
NG16SLC probe	AAG GCT GTT GCC AAT ATC GGC GG	16S rRNA	This study

CT/NG for inhibition (internal control) and for degradation (the NG component of the CA CT/NG). One DNA sample was used for the two 16S rRNA assays, and the other was used for the two *cppB* assays.

PCR-EIAs. For 16S rRNA, PCR primers SL67 and SL59 and probe SL80E, directed at the same region as in the confirmation assay previously available from Roche, were used (8, 17) (Table 1). A 10-μl aliquot was processed in a 100-μl reaction volume containing PCR Gold buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl_2, 0.2% gelatin), 200 μM each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dUTP), 1.5 U AmpliTaq Gold polymerase, and 0.25 μg of each primer. Amplification parameters for the 16S rRNA PCR were as follows: an initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min; and finally 5 min at 72°C. PCR products were analyzed by EIAs with digoxigenin-labeled probe SL80E as described previously (19). Briefly, streptavidin-coated wells were incubated with 0.2 ml diluted PCR product, washed, incubated with 0.1 M NaOH, washed again, and incubated with 5′-digoxigenin-labeled probe. After incubation with conjugate (antidigoxigenin peroxidase) and substrate (2,2-azino-di-[3-ethylbenzthiazoline sulfonate]), the optical density (OD) was read at 405 nm.

The *cppB* assays were performed as described previously (18). Briefly, a 10-μl aliquot of purified DNA was amplified in a 100-μl reaction volume with primers HO1 and HO2 (11) (Table 1) by using AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) in a PCT200 processor (MJ Research, Waltham, MA). PCR products were analyzed by EIAs with digoxigenin-labeled probe Roy1 (18) (Table 1) as described previously (19).

LC assays. For 16S rRNA, LC assay primers NG16SF and NG16SR and fluorescence resonance emission transfer probes NG16SFL and NG16SLC were developed (Table 1). Primers and probes were synthesized by Tib-Molbiol, Berlin, Germany. A 5-μl aliquot of DNA was added to 15 μl of PCR mixture in each reaction capillary. The optimized PCR mixture consisted of 2 μl LC hybridization probe master mix (Roche Diagnostics, Mannheim, Germany), 4 mM $MgCl_2$, 0.25 μM each primer, and 0.25 μM each probe.

Reaction capillaries were capped with plastic plugs, placed into the carousel, centrifuged, and placed into the LC machine (Roche Diagnostics, Mannheim, Germany). The cycling protocols were identical for the *cppB* and the 16S rRNA amplification reactions. Amplification and detection of target DNA were performed with the following parameters: an initial denaturation step of 30 s at 95°C, followed by 45 cycles of denaturation at 95°C for 5 s, annealing at 55°C for 10 s, and extension at 72°C for 20 s. The fluorescence response data were obtained during the annealing period in the "single" mode with the channel setting F2/F1.

The *cppB* LC assays were performed as described by Whiley et al. (24), but melting curves were generated with the following parameters: 95°C for 20 s, 35°C for 10 s, and then an increase in temperature to 85°C at a rate of 0.2°C/s. Analysis of PCR amplification curves and melting curves was carried out with LC software. The *cppB* hybridization probes were designed to dissociate from the specific target at a temperature of 68°C, and the 16S rRNA probes were designed to dissociate at a melting temperature (T_m) of 66°C. A T_m below 60°C for the 16S rRNA probes was considered negative for *N. gonorrhoeae* but positive for NgNS.

Definition of confirmation-positive and confirmation-negative samples. When the four assays were not in agreement with each other, the set of four assays was repeated with both aliquots. Samples were considered positive for the presence of *N. gonorrhoeae* DNA when three or more assays were positive. Because *cppB*-lacking *N. gonorrhoeae* has been described (2, 7, 15), samples for which both 16S rRNA assays were positive and both *cppB* assays were negative were also considered positive. Samples were considered to not contain *N. gonorrhoeae* DNA when the above criteria were not fulfilled. In addition, samples with a 16S rRNA T_m below 60°C were considered to contain NgNS but not *N. gonorrhoeae* DNA (see the description of LC assays for details). Throughout this study, samples considered positive or negative according to these criteria are referred to as confirmation positive or confirmation negative, respectively.

RESULTS

Specificity analysis. Purified DNA preparations from 15 well-defined NgNS and 17 *N. gonorrhoeae* strains were tested in all four assays (Table 2). All *N. gonorrhoeae* strains were detected, and none of the NgNS strains gave a positive result; however, seven of these NgNS strains scored negative in the LC 16S rRNA assay after melting curve analysis. Therefore, it can be concluded that LC 16S rRNA amplification primers and probes are not completely *N. gonorrhoeae* specific. However, subsequent melting curve analysis clearly distinguished NgNS from *N. gonorrhoeae*, with the *N. gonorrhoeae* T_m always being above 64°C and the NgNS T_m always being below 57°C. These results were confirmed in a study in which 450 well-characterized *N. gonorrhoeae* strains interspersed with negative controls and 20 NgNS strains were tested (2).

Confirmation assays. Of the 765 CA NG-positive specimens tested, 715 (93%) were concordant in all four confirmation

TABLE 2. Specificities of PCR-EIAs and LC assays

Organism (no. of strains)	Result[a] of:			
	PCR-EIA		LC assay	
	cppB	16S rRNA	*cppB*	16S rRNA
Neisseria gonorrhoeae (17)	+	+	+	+
Neisseria subflava subsp. *flava* (2)	−	−	−	−/S
Neisseria flavescens (2)	−	−	−	−/S
Neisseria subflava (2)	−	−	−	−/S
Neisseria sicca (2)	−	−	−	−/S
Neisseria mucosa (1)	−	−	−	−
Neisseria elongata (1)	−	−	−	−
Neisseria lactamica (1)	−	−	−	S
Neisseria denitrificans (1)	−	−	−	−
Neisseria cinerea (1)	−	−	−	S
Neisseria perflava (2)	−	−	−	−/S

[a] +, positive; −, negative; −/S, one strain negative and one strain positive with a T_m below 60°C and therefore considered NgNS; S, NgNS.

assays (Table 3). Considering all available data, 763 samples could be labeled confirmation positive or negative, while the remaining 2 samples gave inconsistent results: 1 was found positive in both LC assays with a correct 16S rRNA T_m, and 1 was found positive in both *cppB* assays only.

A total of 229 samples (30%) out of 763 were determined to be confirmation positive; 13 of these (5.7%) were considered to lack the *cppB* gene because they were found positive in both 16S rRNA assays only.

Of 534 confirmation-negative samples, 81 (15%) had positive crossing points but aberrant T_m values in the LC 16S rRNA assay and were therefore regarded as NgNS. Most of these samples ($n = 45$) were of nasopharyngeal origin, which is a well-known habitat for many nonpathogenic *Neisseria* species.

The performance of the confirmation assays is depicted in Table 4. Both 16S rRNA assays performed well, with sensitivity and specificity scores well above 99%. It can be clearly seen that the LC 16S rRNA assay is highly dependent on the melting curve analysis, as specificity drops to 85% when only the amplification characteristics are considered. The performance of both *cppB* assays is compromised by the 5.7% of strains lacking the *cppB* gene, resulting in a drop in the negative predictive value to 97%.

TABLE 3. Results of PCR-EIAs and LC assays

No. of specimens	Result[a] of:				Conclusion[a]	Comment
	PCR-EIA		LC assay			
	cppB	16S rRNA	*cppB*	16S rRNA		
214	+	+	+	+	P	
13	−	+	−	+	P	Lacking *cppB*
1	+	+	+	−	P	
1	+	−	+	+	P	
1	+	+	+	S	N	NgNS
1	+	−	+	S	N	NgNS
1	+	+	−	S	N	NgNS
15	+	−	−	S	N	NgNS
63	−	−	−	S	N	NgNS
1	+	−	+	−	?	
1	−	−	+	+	?	
15	+	−	−	−	N	
438	−	−	−	−	N	

[a] +, positive; −, negative; S, T_m below 60°C and therefore considered NgNS; P, confirmation positive; N, confirmation negative; ?, no conclusion could be drawn from the combination of results, and the samples were therefore excluded from the analysis.

TABLE 4. Sensitivity, specificity, positive predictive value, and negative predictive value of PCR-EIAs and LC assays

Parameter	Value (%) for:				
	PCR-EIA		LC assay		
	cppB	16S rRNA	*cppB*	16S rRNA + T_m	16S[a]
Sensitivity	92.9	99.6	94.3	99.6	99.6
Specificity	94.0	99.6	99.6	100	84.8
Positive predictive value	87.1	99.1	99.1	100	73.8
Negative predictive value	96.8	99.8	97.6	99.8	99.8

[a] Results of amplification without consideration of the melting curve analysis.

NgNS. Remarkably, 30 (5.6%) of the 534 CA NG-positive samples considered confirmation negative were found positive in the *cppB* PCR-EIA only, and 19 of these were derived from 17 female occupational sex workers all employed in the same building. All were throat swab samples, and 12 were considered to contain NgNS. This group of sex workers was routinely screened for STI every 6 weeks. To further investigate the nature of these false-positive results, we tried to culture *Neisseria* species responsible for these findings. At one of the STI screening rounds, throat and cervical swab samples were obtained from 35 women for culturing and nucleic acid amplification by the CA NG assay. None of these samples was found positive in either the CA NG assay or the *cppB* PCR-EIA (Table 5). However, we were able to isolate five NgNS strains from four throat swab samples; some of these were CA NG positive, and one was *cppB* PCR-EIA positive. As shown in Table 5, additional characterization (performed by the National Institute for Public Health and the Environment, Bilthoven, The Netherlands) revealed the following NgNS culture identifications: *Neisseria meningitidis*, *Neisseria perflava*, and *Neisseria cinerea*.

DISCUSSION

Confirmation assays for the CA NG assay should be both specific and sensitive. The lack of an amplifiable *cppB* gene in a subset of *N. gonorrhoeae* strains (7, 15) makes this an unsuitable target for *N. gonorrhoeae* detection. Indeed, in the present study, 5.7% of the samples containing *N. gonorrhoeae* were found negative in both *cppB* assays. In a recent study by Bruisten et al. (2), the *cppB* gene was not found in 5.8% of 450 well-characterized *N. gonorrhoeae* strains isolated from patients attending an STI clinic in Amsterdam. Remarkably, the *cppB*-lacking *N. gonorrhoeae* strains found in the present study were not evenly distributed throughout The Netherlands. Eleven of these 13 strains were sent by a laboratory in Rotterdam serving a population comparable to that served by the STI clinic in Amsterdam (2). In this Rotterdam STI population, 11 (9.1%) of 121 *N. gonorrhoeae* strains showed no amplifiable *cppB* gene. This finding is in great contrast to what we found

TABLE 5. COBAS AMPLICOR NG PCR and *cppB* PCR-EIA performed directly on specimens and on *Neisseria*-like colonies cultured from specimens from 35 female occupational sex workers

No. of specimens (source)	Result[a] of:				Organism isolated
	Direct testing		Testing of *Neisseria*-like colonies		
	CA NG	*cppB* PCR-EIA	CA NG	*cppB* PCR-EIA	
25 (cervix)	−	−	−		
10 (cervix)	+	−	−		
14 (throat)	−	−	−		
17 (throat)	+	−	−		
1 (throat)	−	−	+	−	*N. meningitidis* group A
3[b] (throat)	+	−	+	−	*N. perflava*
1[b] (throat)	+	−	−	+	*N. cinerea*

[a] −, negative; +, positive.
[b] From one sample, *N. perflava* and *N. cinerea* were isolated.

for CA NG-positive samples sent to us by the remaining laboratories for confirmation: only 2 (1.9%) of 108 *N. gonorrhoeae* strains were found to lack the *cppB* gene. A possible explanation for this difference in the epidemiology of *cppB*-lacking *N. gonorrhoeae* strains might be that these strains are circulating in certain networks of people practicing unsafe sex in particular urban societies, such as Amsterdam and Rotterdam. The investigation of a cluster of lymphogranuloma venereum proctitis in men having sex with men in Rotterdam revealed such a network with connections to neighboring countries in Western Europe (9). It would be worthwhile to further investigate this difference in the proportions of *N. gonorrhoeae* lacking the *cppB* gene in the two largest cities of The Netherlands compared to the rest of the country by culturing and DNA fingerprinting of *N. gonorrhoeae* strains and combining these data with background information for patients, such as sexual behavior, contacts, and site of infection.

In addition to the compromised negative predictive value of the *cppB* assays due to the absence of the *cppB* gene in some *N. gonorrhoeae* strains in certain populations, our results seem to confirm that *cppB*-like genes are not confined to *N. gonorrhoeae* (12, 20). A total of 33 confirmation-negative samples (5.9%) were found positive in the *cppB* PCR-EIA; 18 of these were considered to be NgNS because of a T_m below 60°C in the LC 16S rRNA assay. The choice of the *cppB* primers is critical, as shown by the fact that the LC assay was negative in most of these cases.

Because of the large number of CA NG and *cppB* PCR-EIA false-positive throat swabs from female sex workers employed in the same building, we attempted to culture the organisms responsible for these results. We succeeded in recovering five *Neisseria* species from four throat swabs (Table 5). One of these, determined to be *N. cinerea*, was found positive in the *cppB* PCR-EIA. This assay amplifies a 390-bp fragment of the *cppB* gene as described by Ho et al. (11), but instead of MspI digestion of the amplicon, probe hybridization is used as a specificity control (18). The amplification product of the *N. cinerea* isolate was of the expected length but was not digested by MspI (data not shown). This *N. cinerea* isolate was also found positive in the LC *cppB* assay, but the T_m was 63°C instead of 68°C, which would be typical for *N. gonorrhoeae*. These data show that *cppB*-like sequences closely related to those appearing in *N. gonorrhoeae* can compromise confirmation assays. A similar *N. cinerea* strain showed a 390-bp amplification product digested by MspI (15). The combination of *cppB*-like sequences in NgNS and the absence of the *cppB* target in a substantial subset of isolates in certain populations make the *cppB* target unsuitable for the detection or confirmation of *N. gonorrhoeae*.

Both the 16S rRNA PCR-EIA and the LC assay performed well (Table 4). However, without melting curve analysis, the LC assay was not reliable, because many NgNS isolates were also shown to be amplified. Most NgNS isolates were found in samples of nasopharyngeal origin, a finding which is explained by the fact that the nasopharynx is a habitat for many nonpathogenic *Neisseria* species. The reliability of distinguishing *N. gonorrhoeae* from NgNS by use of the T_m was confirmed in a recent study in which 450 confirmed *N. gonorrhoeae* strains, interspersed with negative samples and other bacteria, including 20 NgNS strains, were tested (2).

The distinction by T_m of *Neisseria* species provides an easy means to develop an internal control for this LC assay. The addition of an NgNS strain or its DNA in a low quantity will provide an amplification signal easily distinguishable from those of *N. gonorrhoeae* strains. Monitoring of this internal control will provide information on the efficiency of the amplification and/or DNA extraction and thereby prevent possible negative results due to inhibitors in the sample or loss of DNA during extraction.

A considerable number of samples found positive by either our laboratory ($n = 99$) or referring laboratories ($n = 168$) were found negative in the CA NG assay on repeated testing and were also found negative in all confirmation assays. It is possible that either *N. gonorrhoeae* bacteria or their DNA in some of these samples had been degraded during transport to our laboratory. However, the percentages of CA NG-negative samples for which degradation could play a role were not different in our laboratory and the referring laboratories (41% and 32%, respectively). Another explanation might be that false-positive results in the CA NG assay are not reproducibly positive. Therefore, an algorithm has been proposed in which only samples that have an A_{660} of ≥2.0 in two of three tests are accepted as confirmation positive (21). However, Diemert et al. (6) showed that this algorithm does not apply to low-prevalence populations, like that in Montreal, Canada, or The Netherlands. They reported positive predictive values of 65.1%

for CA NG-positive samples with ODs of ≥3.5 and of 10.1% for positive samples with ODs consistently below 3.5. These values are close to the positive predictive values of 69.4% and 13.8% for CA NG-positive samples with ODs of ≥3.5 and <3.5, respectively, after Boom extraction, as in this study (data not shown). These data confirm the need for additional confirmation testing of CANS-positive samples in populations with a low *N. gonorrhoeae* prevalence. In many cases, background information for the patient and the possible impact of a false-positive diagnostic test for STI on a patient and the patient's family and social network are not forwarded to the laboratory. Therefore, all CA NG-positive samples should be confirmed, even in high-prevalence populations. The lack of specificity is a major drawback of the CA NG assay, while the multiplex CA CT/NG format, on the other hand, is convenient. However, whether the advantages of the multiplex format outweigh the need for confirmation testing depends on local circumstances.

In conclusion, we showed in the present study that the *cppB* gene cannot be recommended as a target for the confirmation of CA NG-positive samples. In certain populations, a substantial portion of strains lack this target. Moreover, evidence for *cppB*-like genes has been found for NgNS. Both 16S rRNA assays performed well, and T_m proved very useful as an additional specificity control for LC assays. Because LC assays are less laborious, faster, and less contamination prone than PCR-EIAs, the LC 16S rRNA assay is at present our confirmation test of choice.

ACKNOWLEDGMENTS

We thank A. Erceg for providing information and swabs for culturing and DNA amplification from the female occupational sex workers and H. T. Tjhie for critically reading the manuscript.

REFERENCES

1. **Boom, R., C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
2. **Bruisten, S. M., G. T. Noordhoek, A. J. C. van den Brule, B. Duim, C. H. E. Boel, K. El-Faouzi, R. du Maine, S. Mulder, D. Luijt, and J. Schirm.** 2004. Multicenter validation of the *cppB* gene as a PCR target for detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**4332–4334.
3. **Centers for Disease Control and Prevention.** 2002. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. Morb. Mortal. Wkly. Rep. **51:**1–39.
4. **Ching, S., H. Lee, E. W. Hook III, M. R. Jacobs, and J. Zenilman.** 1995. Ligase chain reaction for detection of *Neisseria gonorrhoeae* in urogenital swabs. J. Clin. Microbiol. **33:**3111–3114.
5. **Crotchfelt, K. A., L. E. Welsh, D. DeBonville, M. Rosenstraus, and T. C. Quinn.** 1997. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. J. Clin. Microbiol. **35:**1536.
6. **Diemert, D. J., M. D. Libman, and P. Lebel.** 2002. 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. **40:**4056–4059.
7. **Dillon, J. R., and M. Pauzé.** 1981. Relationship between plasmid content and auxotype in *Neisseria gonorrhoeae* isolates. Infect. Immun. **33:**625–628.
8. **Farrell, D. J.** 1999. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using *cppB* nested PCR and 16S rRNA PCR. J. Clin. Microbiol. **37:**386–390.
9. **Gotz, H. M., J. M. Ossewaarde, R. F. Nieuwenhuis, W. I. van der Meiden, J. Dees, B. Thio, O. de Zwart, and M. J. van de Laar.** 2004. A cluster of lymphogranuloma venereum among homosexual men in Rotterdam with implications for other countries in Western Europe. Ned. Tijdschr. Geneeskd. **148:**441–442.
10. **Hammerschlag, M. R.** 2003. Implications of inappropriate sexually transmitted disease testing go beyond pure diagnostics. ASM News **69:**74–79.
11. **Ho, B. S., W. G. Feng, B. K. Wong, and S. I. Egglestone.** 1992. Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. J. Clin. Pathol. **45:**439.
12. **Ison, C. A., C. M. Bellinger, and J. Walker.** 1986. Homology of cryptic plasmid of *Neisseria gonorrhoeae* with plasmids from *Neisseria meningitidis* and *Neisseria lactamica*. J. Clin. Pathol. **39:**1119–1123.
13. **Morre, S. A., I. G. M. Van Valkengoed, R. M. Moes, A. J. Boeke, C. J. L. M. Meijer, and A. J. C. Van den Brule.** 1999. Determination of *Chlamydia trachomatis* prevalence in an asymptomatic screening population: performances of the LCx and COBAS Amplicor tests with urine specimens. J. Clin. Microbiol. **37:**3092–3096.
14. **Palladino, S., J. W. Pearman, I. D. Kay, D. W. Smith, G. B. Harnet, M. Woods, L. Marshal, and J. McCloskey.** 1999. Diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* genitourinary infections in males by the Amplicor PCR assay of urine. Diagn. Microbiol. Infect. Dis. **33:**141–146.
15. **Palmer, H. M., H. Mallinson, R. L. Wood, and A. J. Herring.** 2003. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **41:**835–837.
16. **Roche Diagnostics.** 1996. AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) test package insert. Roche Diagnostics, Mannheim, Germany.
17. **Rossau, R., L. Heyndrickx, and H. Van Heuverswyn.** 1988. Nucleotide sequence of a 16S ribosomal RNA gene from *Neisseria gonorrhoeae*. Nucleic Acids Res. **16:**6227.
18. **Roymans, R., G. Onland, A. Jansz, W. Quint, and E. Boel.** 1999. Evaluation of an in-house polymerase chain reaction for detection of Neisseria gonorrhoeae in urogenital samples. J. Clin. Pathol. **52:**411–414.
19. **Roymans, R., G. Onland, and B. H. Postma.** 1996. One-day detection of PCR amplified Chlamydia trachomatis DNA in clinical samples: ELISA versus Southern blot hybridisation. J. Clin. Pathol. **49:**581–583.
20. **Totten, P. A., K. K. Holmes, H. H. Handsfield, J. S. Knapp, P. L. Perine, and S. Falkow.** 1983. DNA hybridization technique for the detection of *Neisseria gonorrhoeae* in men with urethritis. J. Infect. Dis. **148:**462–471.
21. **Van Der Pol, B., D. H. Martin, J. Schachter, T. C. Quinn, C. A. Gaydos, R. B. Jones, K. Crotchfelt, J. Moncada, D. Jungkind, B. Turner, C. Peyton, J. F. Kelly, J. B. Weiss, and M. Rosenstraus.** 2001. Enhancing the specificity of the COBAS AMPLICOR CT/NG test for *Neisseria gonorrhoeae* by retesting specimens with equivocal results. J. Clin. Microbiol. **39:**3092–3098.
22. **Van der Pol, B.** 2002. COBAS Amplicor: an automated PCR system for detection of *C. trachomatis* and *N. gonorrhoeae*. Expert Rev. Mol. Diagn. **2:**379–389.
23. **van Doornum, G. J. J., L. M. Schouls, A. Pijl, I. Cairo, M. Buimer, and S. Bruisten.** 2001. Comparison between the LCx probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. J. Clin. Microbiol. **39:**829–835.
24. **Whiley, D. M., G. M. LeCornec, I. M. Mackay, D. J. Siebert, and T. P. Sloots.** 2002. A real-time PCR assay for the detection of Neisseria gonorrhoeae by LightCycler. Diagn. Microbiol. Infect. Dis. **42:**85–89.