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NOTES

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

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The AMPLICOR PCR was used to detect *Neisseria gonorrhoeae* in endocervical specimens. A 16S rRNA PCR performed on *N. gonorrhoeae*-positive samples showed sensitivities of 73.2, 64.3, and 94.4% for samples treated directly with AMPLICOR lysis buffer, samples suspended in 2-sucrose phosphate, and samples suspended in diluted phosphate-buffered saline, respectively.

For the detection of *Neisseria gonorrhoeae*, culture is still the reference diagnostic test. Nucleic acid amplification tests (NAATs) are becoming more popular for research and screening programs with centralized batch processing of clinical specimens. Because of their more recent introduction, the accuracy of NAATs for *N. gonorrhoeae* is less well documented than that of NAATs for *Chlamydia trachomatis*, although high sensitivities of *N. gonorrhoeae* NAATs with female genital specimens have been reported (1–3, 8, 10). It is proven that clinical isolates of *Neisseria subflava* and *Neisseria cinerea*, belonging to the commensal flora of the human respiratory or genital tract, may exhibit cross-reactivity in the AMPLICOR PCR (5, 11). Accurate confirmation assays include other commercially available NAATs as well as noncommercial PCRs targeting the *ccpB* gene on the 2.6-MDa cryptic plasmid or DNA encoding the 16S rRNA (a prototype of the 16S rRNA PCR was developed by Roche Diagnostics Systems, Branchburg, N.J.) (3, 5–7).

We used the AMPLICOR *C. trachomatis-N. gonorrhoeae* manual coamplification assay to diagnose chlamydial and gonococcal infection in endocervical specimens obtained from cohorts of female commercial sex workers who were participating in a multicenter study conducted in Hat Yai (HA), Thailand; Cotonou (CO), Benin; and Durban (DU), South Africa. For management of infected women, each participating center performed *C. trachomatis* enzyme immunoassay (MicroTrak; Syva, San Jose, Calif.) and *N. gonorrhoeae* culture on modified Thayer-Martin medium. An additional endocervical swab was kept dry in an empty tube, stored frozen at −20°C within 5 h, and shipped on dry ice to the Institute of Tropical Medicine, Antwerp, Belgium. After arrival, swabs were stored at −20°C until testing. We used dry swabs to avoid problems of incompatibility of commercial collection kits and transport media for multiple testing of specimens with different NAATs (12). It has been shown that samples transported on dry swabs may give higher positive rates than swabs swirled in transport media (9).

The preparation of the swabs was modified during the study.

TABLE 1. Performance of *N. gonorrhoeae* detection tests for specimens directly treated with AMPLICOR lysis buffer (method A)

Center	No. of specimens tested	No. *N. gonorrhoeae* positive[a]	Culture		AMPLICOR PCR		16S rRNA PCR	
			No. positive	Sensitivity (%)[b]	No. positive	Sensitivity (%)	No. positive	Sensitivity (%)[c]
HA	823	90	34	37.8 (27.8–48.6)	89	98.9 (94.0–100)	40	44.9 (34.5–55.9)
CO	1,166	197	128	65.0 (57–71.6)	190	96.4 (92.8–98.6)	155	81.6 (75.3–86.8)
DU	1,723	140	55	39.3 (31.1–47.9)	128	91.4 (85.5–95.5)	103	80.5 (72.5–86.9)
All	3,712	427	217	50.8 (46.0–55.7)	407	95.3 (92.7–97.1)	298	73.4 (68.8–77.5)

[a] Samples positive in culture or in AMPLICOR PCR.
[b] Numbers in parentheses are 95% confidence intervals.
[c] For AMPLICOR PCR-positive samples.

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TABLE 2. Performance of *N. gonorrhoeae* detection tests for specimens suspended in 2-SP medium (method B)

Center	No. of specimens tested	No. *N. gonorrhoeae* positive[a]	Culture		AMPLICOR PCR		16S rRNA PCR	
			No. positive	Sensitivity (%)[b]	No. positive	Sensitivity (%)	No. positive	Sensitivity (%)[c]
HA	255	16	13	81.3 (54.4–96.0)	14	87.5 (61.7–98.4)	11	78.6 (49.2–95.3)
CO	388	36	29	80.6 (64.0–91.8)	32	88.9 (73.9–96.9)	28	87.5 (71.0–96.5)
DU	523	38	28	73.7 (56.9–86.6)	38	100 (90.7–100)	15	39.5 (24.0–56.6)
All	1,166	90	70	77.8 (67.8–85.9)	84	93.3 (86.1–97.5)	54	64.3 (53.1–74.4)

[a] Samples positive in culture or in AMPLICOR PCR.
[b] Numbers in parentheses are 95% confidence intervals.
[c] For AMPLICOR PCR-positive samples.

The first 3,712 swabs were suspended directly in 500 μl of AMPLICOR lysis buffer, and further treatment and AMPLICOR PCR were performed by strictly following the instructions of the manufacturer (method A). The next 1,166 swabs were suspended in 1.0 ml of 2-sucrose phosphate (2-SP) medium. After vortexing at maximum speed for 20 s, swabs were discarded, 250 μl of each sample suspension was pipetted into a small conical tube and centrifuged at 12,000 × *g* for 10 min, and the pellet was resuspended in 250 μl of AMPLICOR lysis buffer; further treatment and PCR were similar to those for method A (method B). The last 2,195 swabs were suspended in 1.0 ml of 1:10-diluted phosphate-buffered saline (PBS) (containing 9 parts saline and 1 part PBS). Samples were vortexed at maximum speed for 20 s. After removal of the swab, 250 μl was treated in the same way as for the 2-SP suspensions (method C). The remaining samples treated with lysis buffer were stored at −20°C for later testing. The frozen DNA extracts of all AMPLICOR-positive samples were thawed and kept at room temperature for 2 h, and 50 μl was then transferred to a small conical tube for the performance of the 16S rRNA PCR according to a standard procedure provided by Roche Diagnostics Systems. Samples with weakly positive AMPLICOR or 16S rRNA results as well as samples showing inhibition were retested, and results were interpreted according to instructions of the manufacturer.

Statistical comparison of variables was done by chi-square analysis. Yates-corrected *P* values of <0.05 were considered statistically significant. Ninety-five percent confidence intervals were calculated based on the binomial distribution of the observed test results.

The data for the specimens treated by method A are shown in Table 1. The sensitivities of the AMPLICOR PCR were quite similar for the three centers. The sensitivity of culture was extremely low in HA and DU and was significantly higher in CO ($P < 0.0001$). The overall sensitivity of the 16S rRNA PCR for AMPLICOR PCR-positive samples was significantly lower for samples from HA ($P < 0.00001$) but was also lower than expected for samples from the other sites. Because of the large differences between AMPLICOR PCR and culture results, suggesting poor culture performance, major efforts were immediately made in all three centers to try to improve *N. gonorrhoeae* culture performance. The low sensitivity of the 16S rRNA PCR was striking and could not be explained by high numbers of false-positive AMPLICOR PCR results (12). It was decided to modify the specimen processing by using 2-SP medium (also recommended by Roche).

The test results for specimens treated by method B are shown in Table 2. The overall sensitivity of the AMPLICOR PCR was similar to that for specimens treated by method A. The sensitivity of the 16S rRNA PCR was lower than that for method A for samples from DU ($P < 0.00001$) but higher for samples from HA ($P = 0.05$) and CO ($P = 0.57$) The overall sensitivity of the 16S rRNA PCR was still remarkably low. The performance of culture improved greatly in all centers: HA, $P = 0.003$; CO, $P = 0.02$; and DU, $P = 0.0003$.

The data for the specimens treated by method C are shown in Table 3. The AMPLICOR PCR performance did not differ from that for specimens treated by method A or B. The sensitivity of the 16S rRNA PCR, however, was significantly higher than that for methods A and B ($P < 0.00001$). The performance of the 16S rRNA PCR was not biased by time lapses and storage periods between AMPLICOR PCR and 16S rRNA PCR testing (data not shown). The sensitivity of culture, however, decreased from 77.8 to 58.8% ($P = 0.004$), and a decrease was observed in all centers: from 81.3 to 68.4% in HA ($P = 0.46$), from 80.6 to 62.2% in CO ($P = 0.07$), and from 73.7 to 47.7% in DU (p 0.03).

The AMPLICOR *C. trachomatis-N. gonorrhoeae* PCR has been validated for use with 2-SP medium, but extended evaluation studies have not yet been published (3, 4, 6, 11). Our

TABLE 3. Performance of *N. gonorrhoeae* detection tests for specimens suspended in diluted PBS (method C)

Center	No. of specimens tested	No. *N. gonorrhoeae* positive[a]	Culture		AMPLICOR PCR		16S rRNA PCR	
			No. positive	Sensitivity (%)[b]	No. positive	Sensitivity (%)	No. positive	Sensitivity (%)[c]
HA	239	19	13	68.4 (43.4–87.4)	18	94.7 (74.0–99.9)	14	77.8 (52.4–93.6)
CO	1,075	90	56	62.2 (51.4–72.2)	84	93.3 (86.1–97.5)	81	96.4 (89.9–99.3)
DU	881	44	21	47.4 (32.5–63.3)	40	90.9 (78.3–97.5)	39	97.5 (86.8–99.9)
All	2,195	153	90	58.8 (50.6–66.7)	142	92.8 (87.5–96.4)	134	94.9 (89.2–97.5)

[a] Samples positive in culture or in AMPLICOR PCR.
[b] Numbers in parentheses are 95% confidence intervals.
[c] For AMPLICOR PCR-positive samples.

motivation to switch from 2-SP treatment to diluted PBS was based on our laboratory's extensive experience with PCR testing for other pathogens. Applying this processing to samples collected during the last study period resulted in significantly improved performance of the 16S rRNA PCR. Samples suspended in 1:10-diluted PBS showed a high correlation between AMPLICOR PCR and 16S rRNA PCR: 134 out of 142 (94.4%) AMPLICOR-positive samples were positive on 16S rRNA PCR. On the basis of these data, the 16S rRNA PCR was proven to be a very accurate confirmatory assay.

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