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Improved Performance of PACE 2 with Modified Collection System in Combination with Probe Competition Assay for Detection of *Chlamydia trachomatis* in Urethral Specimens from Males

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The Gen-Probe PACE 2 assay (GP) in combination with a modified collection system was compared with cell culture (CC) for the detection of *Chlamydia trachomatis* in urethral specimens from males. Analysis of discordant results was performed by PCR. The modifications, i.e., application of a more rigid swab type and a 50% reduction in the amount of transport medium, were made to improve the sensitivity of the assay. By using the modified GP on 302 urethral specimens from males, a sensitivity of 89.5% and a specificity of 100% were determined. In addition, performance of a probe competition assay on all GP samples with a result >0.6 and <1.0 times the cutoff factor (gray zone) detected three more true-positive samples. The sensitivity of GP in combination with the probe competition assay increased to 94.9%, with a specificity of 100%. This was identical to the performance of CC. The modified GP offers a very sensitive and specific alternative to CC.

Chlamydia trachomatis is now the most common cause of sexually transmitted diseases (2). Among other serious complications, it frequently leads to infertility in women (4). Cell culture (CC) is considered the reference method for detection of *C. trachomatis* in clinical specimens. However, CC requires at least 48 h of incubation and extensive laboratory facilities. Furthermore, the reliability of CC may be influenced substantially by transport and storage conditions (9). Alternative methods aimed at overcoming these disadvantages have been developed. Direct immunofluorescent-antibody tests and enzyme immunoassays were the first alternatives. In several studies, variable sensitivities and specificities were found (1, 5, 12). The reliabilities of these methods are considered to be inferior to that of CC. More recently, molecular biological methods have been developed. Both a DNA probe and a PCR assay are now commercially available (3, 7, 13). In a former study (8), we evaluated the GenProbe PACE 2 assay (GP), a nonisotopic DNA probe assay, for the detection of *C. trachomatis* in urogenital specimens. For samples from women, the performance was comparable to that of CC (sensitivity, 95.2%; specificity, 98.2%). However, for samples from men, the sensitivity was lower (77.2%), but the specificity was high (99.6%). The low level of sensitivity for samples from males prompted the manufacturer to modify the swab and transport system by reducing the volume of the transport medium and using a more rigid swab. To evaluate whether these modifications had improved the sensitivity of the assay, we repeated the study with samples from males using the modified GP. In addition, all GP-positive samples and all samples showing discrepancies between CC and GP results and all samples in a gray zone were tested by a probe competition assay (PCA) to improve the performance of the assay.

Patients. Urethral samples were taken from 302 men visiting the outpatient clinic for sexually transmitted diseases at the University Hospital in Rotterdam, The Netherlands.

Sample collection. Samples for both GP and CC were collected with dacron swabs and were placed into GP transport medium or 0.2 M sucrose phosphate buffer, respectively. The sampling order was, first, the sample for GP and, thereafter, the sample for CC during the first half of the study (149 samples). Then, the sampling order was reversed (153 samples). The sample for GP was stored at 4°C. The sample for CC was stored at 4°C or, when not tested within 24 h, at −70°C. All specimens were processed within 7 days. The remainders of both samples were stored at −70°C for further evaluation by PCA and PCR.

CC. CC was performed on cycloheximide-treated McCoy cells in microtiter plates exactly as described previously (8). Briefly, two wells per plate were each inoculated with 0.2 ml of sample. After centrifugation, the supernatants were replaced with 0.1 ml of growth medium. The plates were incubated at 37°C for 48 h, after which the monolayers were fixed, stained with a fluorescent monoclonal antibody, and examined for inclusions. CC results were scored as follows: 0, no inclusions per two wells; 1, 1 to 5 inclusions per well; 2, 6 to 20 inclusions per well; and 3, >20 inclusions per well.

GP. GP was performed according to the manufacturer's instructions as described in our previous study (8). The modifications in comparison with the previous version were (i) the use of a more rigid swab and (ii) a 50% reduction in the amount of transport medium that was used. The gray zone was defined as >0.6 and <1.0 times the cutoff factor.

PCA. PCA is a rapid DNA probe test which can be used in combination with GP. It uses competitive nucleic acid hybridization to differentiate a true-positive signal from a false-positive signal. All samples which tested positive in the

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TABLE 1. GP results in comparison with CC results for detection of *C. trachomatis*[a]

GP result	No. of samples with the following CC result:		
	Positive	Negative	Total
Positive	33	1	34
Negative	6	262	268
Total	39	263	302

[a] Sensitivity of GP, 33 of 39 samples (84.6%); specificity of GP, 262 of 263 samples (99.6%); predictive value of a positive GP result, 33 of 34 samples (97.1%); predictive value of a negative GP result, 262 of 268 samples (97.8%).

GP, all samples showing discrepancies between CC and GP, and all samples in the gray zone were retested by both GP and PCA in the same run. For PCA, 100 μl of patient sample was added to the PCA tube. This tube contained lyophilized probe reagent that was identical to the GP probe reagent except that it lacked the chemiluminescent label. Then, 100 μl of standard chemiluminescent-labeled probe was added to the PCA tube. Because the labeled and unlabeled probes are complementary to the rRNA of the target organism, they compete with one another to form a stable RNA-DNA hybrid with the target. Replacement of the labeled probe by the unlabeled probe results in a reduction of the signal. If this reduction is 70% or greater in comparison with the GP result from the same run, the sample is considered true positive. A lower level of reduction of the signal indicates that the positive GP result was caused by nonspecific hybridization and should be considered negative.

PCR. PCR was used for the analysis of discordant GP and CC results. The remainders of both the samples for GP and CC, which were stored at −70°C, were therefore examined without knowledge of the initial test results. The PCR technique used in the present study was identical to that used in the former study (8).

Statistics. Statistical evaluation of the collected data was performed by using Fisher's exact test. Statistical significance was accepted at $P \leq 0.05$ (two-tailed).

Urethral samples from 302 men were evaluated by CC and GP. Thirty-nine samples were positive by CC, giving a prevalence of 13%. The results of GP in comparison with those of CC are given in Table 1. With CC as the reference method, the sensitivity of GP was 84.6% and the specificity was 99.6%. The results of PCA for all samples positively GP were positive. Ten samples tested by GP and CC were either discordant or had a GP result in the gray zone. Of these 10 samples, the sample tested by GP was retested by PCA and the samples for both GP and CC were retested by PCR. The results of this analysis are given in Table 2. Results for patients 1 through 3 showed a discordance, which was confirmed by PCR. Because the outcomes of both CC and GP were confirmed by PCR for these samples, the discordances were considered to be a result of swab-to-swab variability and were omitted from further analysis. This left 299 evaluable patients. The following interpretations were made. If the CC result was not confirmed by PCR on the sample for CC, this was considered a false-positive CC result. Accordingly, if the GP result was not confirmed by PCR of the sample for GP, this was considered a false-positive GP result. The performances of both GP and CC were recalculated. Table 3 shows the sensitivities, specificities, and positive and negative predictive values of CC and GP and of CC and GP in combination with PCA for the samples in the gray zone.

For evaluation of the performances of noncultural methods of detecting *C. trachomatis*, CC has long been considered the "gold standard." However, several studies have clearly demonstrated that the sensitivity of CC is insufficient for this purpose (8, 11, 13). Most of the recent studies therefore use either a combination of tests (3, 13) or confirmatory tests on the initial sample (8, 11) as a reference method. In the current study, we used a confirmatory PCR on the initial samples to analyze discordant results. By this method, the one false-positive GP result in the comparison with CC was shown to be caused by a failure of CC (Table 2, sample 10). This increased the specificity of GP and decreased the sensitivity of CC. Two of the six false-negative GP results were caused by swab-to-swab variability (Table 2, samples 1 and 2). The other four false-negative GP results were indeed false negative (Table 2, samples 5 to 8). The recalculated sensitivity of GP was 89.5% (34 of 38 samples), in comparison with 97.4% (37 of 38 samples) for CC. The specificities of both GP and CC were 100% (Table 3). In comparison with the results from the former study (8), before the modifications, the sensitivity of GP increased from 77.2 to 89.5%. This difference was not statistically significant.

TABLE 2. Patient samples showing discrepancy between CC and GP results and with a GP result in the gray zone and results of PCA and interpretation after analysis by PCR

Sample no.	GP result			CC result			PCA category	Interpretation
	RLU[a]	Category	PCR	Score[b]	Category	PCR		
1	218	−	−	1	+	+	−	Swab-to-swab variability
2	286	±[c]	−	1	+	+	−	Swab-to-swab variability
3	379	±	+	0	−	−	+	Swab-to-swab variability
4	286	±	−	0	−	−	−	True-negative GP result
5	191	−	+	1	+	+	−	False-negative GP result
6	186	−	+	2	+	+	+	False-negative GP result
7	256	±	+	1	+	+	+	False-negative GP result[d]
8	367	±	+	1	+	+	+	False-negative GP result[d]
9	346	±	+	0	−	+	+	False-negative CC result
10	23,834	+	+	0	−	+	+	False-negative CC result

[a] RLU, GP result, in relative light units.
[b] Score, score of CC for interpretation; see text.
[c] ±, GP result in the gray zone (>0.6 and <1.0 times the cutoff factor).
[d] False-negative GP result but positive by PCA on the GP samples in the gray zone.

TABLE 3. Sensitivities, specificities, and predictive values of CC and GP and of CC and GP in combination with PCA on samples in the gray zone

Test	Percent			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
CC versus GP				
CC	97.4	100	100	99.6
GP	89.5	100	100	98.5
CC versus GP and PCA				
CC	94.9	100	100	99.2
GP	94.9	100	100	99.2

Since no false-positive GP results obtained by GP were found in the present study, we could not evaluate the value of PCA for improving the specificity of GP. However, application of PCA on the samples for GP in an arbitrarily chosen gray zone (>0.6 and <1.0 times the cutoff factor) improved the sensitivity of the assay. Six samples in the gray zone were identified. One sample (Table 2, sample 2) was negative by PCA but positive by CC. Another sample (Table 2, sample 3) was positive by PCA but negative by CC. Analysis by PCR showed that both of these discrepancies were caused by swab-to-swab variability. One sample (Table 2, sample 4) was negative by PCA. PCR of the samples for GP and CC was negative. This was a true-negative GP result. Two samples in the gray zone (Table 2, samples 7 and 8) were positive by PCA and CC. These were initially negative by GP. So, PCA was able to detect two more true-positive samples, and thus increased the sensitivity of the GP. The last sample in the gray zone (Table 2, sample 9) was negative by CC. PCR analysis was positive for the samples that tested positive by GP and negative by CC. This was an additional false-negative CC result and the third additional true-positive PCA result. Table 3 shows the sensitivity, specificity, and positive and negative predictive values of both CC and GP after application of PCA on the samples in the gray zone. Both CC and GP detected 37 of 39 true-positive samples (sensitivity, 94.9%). The specificities of both assays were 100%. The sensitivity of GP with PCA of samples in the gray zone was statistically significantly greater than the sensitivity obtained in the former study (8).

Blocking assays were evaluated in combination with enzyme immunoassays. Blocking assays are primarily used to increase the specificities of these assays (6, 10), but the sensitivities can also be improved by applying blocking assays to samples that test in the gray zone (6). In this evaluation, the specificity of GP was already optimal without the application of PCA. In our previous study (8), the specificity of GP for cervical samples was 98.2%. PCA could improve the specificity for samples from females; this application should be evaluated. Although the number of samples in the gray zone was small, the application of PCA on these samples improved the sensitivity from 89.5 to 94.9%. The application of PCA in combination with GP looks promising and deserves further evaluation to optimize the diagnosis of *C. trachomatis* in urogenital specimens. PCA is not yet available for diagnostic purposes.

REFERENCES

1. **Barnes, R. C.** 1989. Laboratory diagnosis of human chlamydial infections. Clin. Microbiol. Rev. **2**:119–136.
2. **Bell, T. A., and J. T. Grayston.** 1986. CDC guidelines for prevention and control of *Chlamydia trachomatis* infections. Ann. Intern. Med. **104**:524–526.
3. **Blanding, J., L. Hirsch, N. Stranton, T. Wright, S. Aarnaes, L. M. de la Maza, and E. M. Peterson.** 1993. Comparison of the Clearview Chlamydia, the PACE 2 assay, and culture for detection of *Chlamydia trachomatis* from cervical specimens in a low prevalence population. **31**:1622–1625.
4. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections. Policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **35**:535–574.
5. **Ehret, J. M., and F. N. Judson.** 1989. Genital chlamydia infections. Clin. Lab. Med. **9**:481–500.
6. **Hallander, H., P. Jonsson, and B. Gästrin.** 1992. Value of confirmation of Chlamydiazyme enzyme immunoassay results in the detection of *Chlamydia trachomatis*. Eur. J. Clin. Microbiol. Infect. Dis. **11**:550–552.
7. **Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn.** 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. J. Clin. Microbiol. **31**:1209–1212.
8. **Kluytmans, J. A. J. W., H. G. M. Niesters, J. W. Mouton, W. G. V. Quint, J. A. J. IJpelaar, J. H. van Rijsoort-Vos, L. Habbema, E. Stolz, M. F. Michel, and J. H. T. Wagenvoort.** 1991. Performance of a nonisotopic DNA probe for detection of *Chlamydia trachomatis* in urogenital specimens. J. Clin. Microbiol. **29**:2685–2689.
9. **Mahony, J. B., and M. A. Chernesky.** 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J. Clin. Microbiol. **22**:865–867.
10. **Moncada, J., J. Schachter, G. Bolan, J. Engelman, L. Howard, I. Mushahwar, G. Ridgway, G. Mumtaz, W. Stamm, and A. Clark.** 1990. Confirmatory assay increases specificity of the Chlamydiazyme test for *Chlamydia trachomatis* infection of the cervix. J. Clin. Microbiol. **28**:1770–1773.
11. **Ossewaarde, J. M., M. Rieffe, M. Rozenberg-Arska, P. M. Ossekoppele, R. P. Nawrocki, and A. M. van Loon.** 1992. Development and clinical evaluation of a polymerase chain reaction test for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **30**:2122–2128.
12. **Stamm, W. E.** 1988. Diagnosis of *Chlamydia trachomatis* genito-urinary infections. Ann. Intern. Med. **108**:710–717.
13. **Warren, R., B. Dwyer, M. Plackett, K. Pettit, N. Rizvi, and A. Baker.** 1993. Comparative evaluation of detection assays for *Chlamydia trachomatis*. J. Clin. Microbiol. **31**:1663–1666.