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Evaluation of Clearview and Magic Lite Tests, Polymerase Chain Reaction, and Cell Culture for Detection of *Chlamydia trachomatis* in Urogenital Specimens

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The Clearview Chlamydia test (CV; Unipath Ltd., Bedford, United Kingdom), the Magic Lite Chlamydia test (ML; CIBA Corning, Medfield, Mass.), a polymerase chain reaction (PCR), and cell culture (CC) were evaluated for detection of *Chlamydia trachomatis* in urogenital specimens. Specimens were collected from 283 men and 724 women visiting the outpatient clinic for Sexually Transmitted Diseases at the University Hospital Rotterdam, Rotterdam, The Netherlands. ML, PCR, and CC were all performed on the same sample to prevent swab-to-swab variability. CV was performed on a separate sample. Analysis of discordant results was performed by application of the following confirmatory assays: first, PCR on the CC, second, ML was repeated, and third, PCR was repeated by using a different DNA extraction protocol. If more than one test was positive, the sample was considered true positive. If only one test was positive, which was confirmed by the confirmatory assay, the sample was also considered true positive. By using these interpretations, the following results were obtained. The sensitivity and specificity of CV for samples from men were 60.4 and 86.3%, respectively. For samples from women, these values were 62.3 and 99.7%, respectively. The low specificity for samples from men was caused by unidentified substances in the swab that was used. The use of CV on samples from men is not recommended by the manufacturer. For samples from women, the specificity of CV was high, but the low sensitivity of CV limits its use for diagnostic purposes. The sensitivities of ML were low for samples from both men and women (68.8 and 50.9% respectively), while specificities were excellent for samples from both groups (100 and 99.9%, respectively). The low sensitivity of ML limits its diagnostic value. The PCR technique was highly specific for samples from both men (99.6%) and women (99.9%). The sensitivity of PCR, however, was unexpectedly low for samples from both groups (men, 87.5%; women, 79.2%), most likely because of the sample treatment method used. The sensitivity and specificity values of CC for samples from men were 95.8 and 100%, respectively. For samples from women, these values were 100 and 99.9%, respectively. In the present study, CC was the most reliable technique for the detection of *C. trachomatis*.

Chlamydia trachomatis is now the most common reportable sexually transmitted disease. Approximately 4 million cases occur annually in the United States (4). The clinical spectrum of the disease ranges from urethritis, cervicitis, and endometritis to salpingitis and perihepatitis (21). Chlamydial infections often are asymptomatic or nonspecific in their clinical course. This lack of evident symptoms contributes to the further spread of the disease. Moreover, salpingitis caused by *C. trachomatis*, although often asymptomatic, is a frequent cause of infertility in women (4). The optimal antibiotic therapy for chlamydial infections is doxycycline or erythromycin. Because of the serious sequelae, the lack of specific signs and symptoms, and the specific therapy required, it is important to have a reliable diagnostic test.

The diagnosis of chlamydial infections is mainly based on culture of the organism on HeLa 229 or McCoy cells, which takes several days and requires extensive laboratory facilities. In addition, circumstances with regard to transport and storage of the sample may influence the reliability of the cell culture result considerably (12, 13). The availability of new antigen detection techniques has overcome these disadvantages. At first, a direct fluorescent-antibody test was developed; this was soon followed by several enzyme immunoassays. Both direct fluorescent-antibody tests and enzyme immunoassays have been compared with cell culture and with each other in a number of studies. The results of those studies are summarized and discussed in three reviews (2, 7, 21).

Recently, tests based on the recognition of specific DNA or RNA sequences have been developed. A nonisotopic DNA probe for the detection of *C. trachomatis* in urogenital specimens is commercially available and has been compared with cell culture, direct fluorescent-antibody test, and polymerase chain reaction (PCR) as a reference (9, 10, 14, 27). Also, several PCRs have been described and evaluated previously (3, 5, 6, 16, 17).

In the present study, we evaluated three recently developed alternatives to cell culture. The Clearview Chlamydia test (CV; Unipath Ltd., Bedford, United Kingdom) is a rapid, solid-phase sandwich immunoassay which requires minimal laboratory facilities and hands-on time. According to the manufacturer, CV is recommended for testing only female cervical samples. The Magic Lite Chlamydia test (ML; CIBA Corning, Medfield, Mass.) is a new chemilumi-

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nometric sandwich immunoassay. This test is easy to perform and can be fully automated. The PCR used in the present study is a modification of the method we evaluated and described before (6, 10). Instead of the laborious phenol extraction method that we used previously, a simplified sample preparation procedure was applied. These modifications should make the PCR suitable for processing large numbers of specimens in routine settings.

CV, ML, and PCR were initially compared with cell culture (CC) as the "gold standard." Thereafter, analysis of discordant results was performed by using the following confirmatory assays: (i) PCR on the CC, (ii) repeat ML, and (iii) repeat PCR by using a different DNA extraction protocol. If more than one test was positive, the sample was considered true positive. If only one test was positive and this positive result was confirmed by the confirmatory assay, the sample was also considered true positive. In this way we could evaluate possible failures of CC and thus improve the value of the present evaluation.

MATERIALS AND METHODS

Patients and specimens. Specimens were collected from 283 men and 724 women visiting the outpatient clinic for Sexually Transmitted Diseases at the University Hospital Rotterdam. Male urethral and female cervical samples were collected. To eliminate swab-to-swab variability, ML, PCR, and CC were performed on the same sample. This sample was taken by using a dacron E.N.T. swab (Medical Wire and Equipment Co., Corsham, Willshire, United Kingdom), which was placed into 2 ml of 0.2 M sucrose phosphate buffer (2-SP). The portion for CC was stored at 4°C or, when not tested within 24 h after collection, at −70°C. All CCs were performed within 7 days. In a previous study, these transport conditions have been shown to give optimal sensitivity (24). The portion of the sample used for ML was stored at 4°C or, when not tested within 5 days after collection, at −20°C. All samples for ML were processed within 14 days. This procedure was approved by the manufacturer. The 2-SP portion for PCR was stored at −70°C, and all samples for PCR were processed within 7 days. In a pilot study, it was shown that the 2-SP transport medium caused both false-negative and false-positive results in the CV. Therefore, the CV was performed on a separate sample. The female samples for CV were collected by using the swab provided by the manufacturer (Unipath Ltd.). This swab was not appropriate for taking male urethral samples. To obtain samples from males, a dacron E.N.T. swab with an aluminum shaft (Medical Wire and Equipment Co.) was used. The swab was transported to the laboratory, where the samples were stored at 4°C until testing. All tests were performed within 3 days. During the first 3 months of the study, the sample for CV was taken before the 2-SP sample. The sampling order was reversed during the second part of the study. The remainder of the 2-SP sample was stored at −70°C for further reference.

CC. McCoy cells were grown as monolayers in plastic culture flasks (no. 3150; Costar, Cambridge, Mass.). Complete medium I for cell growth and maintenance consisted of Eagle minimal essential medium (Autopow; Flow Laboratories, Inc., Irvine, Scotland) containing 10% (vol/vol) fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah), 1% (vol/vol) vitamins (100× vitamins for Eagle minimal essential medium; Flow), 1% (vol/vol) glutamine (L-glutamine [200 mM] for cell culture, 29.23 mg/ml; Flow), gentamicin (20 μg/ml), amphotericin B (5 μg/ml), and $NaHCO_3$ (2.0 g/liter [pH 7.5]). For chlamydial growth, complete medium II was used; this medium contained vancomycin (25 μg/ml), glucose (4.5 g/liter), and cycloheximide (1 μg/ml) (Sigma Chemical Co., St. Louis, Mo.).

McCoy cells were suspended to a concentration of 3.5×10^5/ml, of which 200 μl was passed to a microdilution plate containing 96 wells (Falcon 3070; Becton Dickinson Labware, Oxnard, Calif.). The plates were incubated overnight at 37°C in a humidified atmosphere of 5% CO_2. Before inoculation of the specimen, the cell monolayer was inspected for confluency and was rinsed twice with 100 μl of complete medium II. A total of 45 specimens and 3 controls were cultured in duplicate in the 96-well microtiter plate. All three controls were positive controls, containing a mixture of *C. trachomatis* serotypes D, E, F, H, and K. These five serotypes are responsible for approximately 90% of the infections caused by *C. trachomatis* in Rotterdam (25). After the specimens and controls were agitated (SMI multitube vortexer; American Hospital Supply Corp., Miami, Fla.) for 30 s (speed 5), 200 μl was added to each of two wells containing McCoy cell monolayers. The inoculum was centrifuged onto the cells at 1,400 × *g* for 60 min, after which the fluid was replaced with 100 μl of complete medium II. After 48 h of incubation at 37°C in a humidified atmosphere of 5% CO_2, the monolayers were fixed with ethanol (96%) for 10 min and stained with fluorescent monoclonal Chlamydia anti-major outer membrane protein antibodies (Microtrak; Syva Co., Palo Alto, Calif.). The plates were viewed with a Leitz fluorescent microscope at a magnification of ×201.6 and were examined for inclusions. Culture results were scored as follows: 0, no inclusion per two wells; 1, 1 to 5 inclusions per well; 2, 6 to 20 inclusions per well; and 3, >20 inclusions per well. The inoculated CC plates were stored at −20°C.

CV. CV was performed according to the manufacturer's instructions for females. A different swab was used for males. Tests were performed twice a week, and one positive control was included in each run. Upon testing, the swabs were placed into a flexible reaction tube containing 0.6 ml of extraction buffer and were vortexed. The tube containing the swab was then placed into a heating block at 80°C for 10 min. After cooling for 5 min at room temperature, the swab was rotated in the buffer and was removed from the tube. Five drops (±150 μl) of the mixture was added to the absorbant pad in the sample window of a small immunochromatographic device. The part of the absorbant pad in the sample window contains dried, colored latex particles coated with monoclonal antibodies to chlamydial antigen. The absorbant pad is in contact with a membrane strip to which two lines of antibodies have been immobilized. The first immobilized line, the result window line, contains monoclonal antibodies to chlamydial antigen. The second line, the control window line, contains polyclonal antibodies to mouse immunoglobulin. If chlamydial antigen is present in the sample, it will react with the antibody bound to the latex particles, forming a complex. This complex is carried up to the immobilized monoclonal antibodies to chlamydial antigen, where the complex is captured, to form a sandwich. This gives a line in the result window. If chlamydial antigen is not present in the sample, no sandwich is built up and the result window remains clear. In either case some latex particles are carried on to the immobilized line of antibodies to mouse immunoglobulin, forming a line in the control window. If the control window remained clear, the sample was considered not evaluable.

ML. The swab and transport medium for ML were differ-

ent from those in the officially recommended system. Although the manufacturer approved the use of the system, it may have influenced the results. Furthermore, the procedure was performed exactly according to the manufacturers' instructions. In each run, one positive and one negative control were included. The negative control was determined in triplicate, and the positive control and the patient samples were determined in duplicate. Two hundred microliters of sample was added to 750 μl of reagent buffer, and the mixture was vortexed twice for 15 s. Two hundred microliters of this pretreated sample was added to the reaction tube. Then, 100 μl of reaction buffer was added and the sample was vortexed. Subsequently, 100 μl of monoclonal anti-*Chlamydia* antibody labeled with an acridinium ester was added; this was followed by the addition of 500 μl of polyclonal anti-*Chlamydia* antibody covalently bound to paramagnetic particles. The mixture was vortexed and incubated at room temperature for 90 min. After incubation, the tubes were placed on the magnetic separation base for 3 min. The supernatants were decanted and the tubes were blotted while holding the tube rack and the base of the magnetic separation unit together. One milliliter of wash buffer was added, and after vortexing, the magnetic separation procedure was repeated. Finally, 100 μl of distilled water was added and the results were read with a luminometer (MagicLite Analyzer). The results were given as relative light units (RLUs). Interpretations were made by using two cutoff factors (1.6 and 2.3), which were recommended by the manufacturer. If the mean value of the duplicate determination (test result) was <1.6 times the mean of the negative control, the sample was considered negative. If the test result was >2.3 times the mean of the negative control, the sample was considered positive. Finally, if the test result was ≥1.6 times and ≤2.3 times the mean of the negative control, the sample was retested by a blocking assay. The blocking assay used a monoclonal anti-*Chlamydia* antibody with an epitope different from those of the monoclonal and polyclonal anti-*Chlamydia* antibodies used in the routine assay. Pretreatment of the sample was identical to that described above. After the reaction buffer was added, 100 μl of blocking agent was added, and the mixture was vortexed and incubated for 30 min at room temperature. Subsequently, the routine procedure was followed starting with the addition of the acridinium ester labeled monoclonal anti-*Chlamydia* antibody. Interpretations were made by using the following calculation: $X = (A/B) \times (C/D)$, where A is the RLUs of the negative control in the assay of the blocked sample, B is the RLUs of the negative control in the assay of the unblocked sample, C is the RLUs of the unblocked sample, and D is the RLUs of the blocked sample. If X was ≥1.9, the sample was considered positive, or else the sample was considered negative. The value of 1.9 was recommended by the manufacturer.

PCR. To prepare the sample for the PCR assay, 200 μl of the patient sample was centrifuged for 10 min at 1,400 × *g* at room temperature. The pellet was resuspended in 300 μl of phosphate-buffered saline (PBS). Sixty microliters of this suspension was incubated with 10 μl of proteinase K solution (Merck) at a final concentration of 25 μg/ml and with 10 μl of 6% Triton X-100 for 1 h at 37°C. After heating to 100°C for 15 min to inactivate the proteinase K, 100 μl of sterile water distilled twice was added to the sample. Ten microliters of this solution was used for the PCR assay. The PCR assay was exactly identical to the one described previously (11). Two sets of primers recognizing sequences of the endogenous plasmid of *C. trachomatis* (20) were used. Both generated a species-specific, 517-bp amplified product with all known *C. trachomatis* serovars. One set was described previously (5): T1, GGACAAATCGTATCTCGG; T2, GA AACCA ACTCTACGCTG; probe, CGCAGCGCTAGAG GCCGGTCTATTTATGAT. The other contained the following sequences: T1, GCTAGAGCGGCATGCTACAT; T2, CGCTT GCACGAAGTACTCTG; and probe, GATTGTA CAAGGGATCCGTAAGTTAGA. Samples were considered positive if a PCR product was synthesized with at least one primer set. In each run a positive control was included. The positive control consisted of a crude phenol-chloroform extract of a McCoy cell line infected with *C. trachomatis* serovar L-2. Oligonucleotide primers and probe were synthesized on an Applied Biosystems 381A DNA synthesizer.

Analysis of discordant results by PCR. For samples with discordant results, confirmatory assays were performed (see Table 3). A confirmatory PCR was carried out by using a different DNA extraction procedure. Therefore, 200 μl of the 2-SP sample was incubated with proteinase K (final concentration, 0.15 mg/ml) in 0.5% sodium dodecyl sulfate for 30 min at 37°C. Nucleic acids were extracted with phenol, phenol-chloroform-isoamyl alcohol (25:24:1; vol/vol/vol), and chloroform-isoamyl alcohol (24:1; vol/vol). The nucleic acids were precipitated by the addition of 0.1 volume of 3 M sodium acetate and 3 volumes of ethanol and were stored at −20°C overnight. After centrifugation for 10 min at 10,000 × *g*, the pellet was washed once with 80% ethanol and was dried in a vacuum exsiccator. The pellet was resuspended in 100 μl of TE buffer (10 mM Tris, 0.1 mM EDTA). Ten microliters was used directly in the PCR assay as described above in the section on PCR.

For analysis of discordant results obtained by CC, another procedure was followed. PCR was performed on the wells of the inoculated CC plates which had been stored at −20°C. Therefore, the inoculated McCoy cells of the monolayer were suspended in 100 μl of PBS. Then, 17.5 μl of proteinase K (10 mg/ml) and 17.5 μl of Triton X-100 (6%) were added. After incubation at 37°C for 1 h, the sample was boiled for 10 min and was diluted with 135 μl of twice-distilled water. Ten microliters was used directly in the PCR assay, as described above in the section on PCR.

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).

RESULTS

Comparison with CC as the gold standard. A total of 1,007 patients were evaluated. The overall prevalence of chlamydial infections as measured by a positive CC result was 9.9%. For females (n = 724) and males (n = 283), the prevalences were 7.5 and 16.3%, respectively. Table 1 provides the results of CV, ML, and PCR compared with the results of CC for samples from males and females. Eight of the female samples for CV could not be evaluated because no line appeared in the control window. Two of these eight samples had a positive result in CC. The overall (males and females) sensitivity and specificity for CV compared with CC were 64.3 and 96.2%, respectively. These values for ML were 58.0 and 99.9%, respectively, and for PCR they were 82.0 and 99.4%, respectively. Table 2 provides the sensitivities, specificities, and positive and negative predictive values of CV, ML, and PCR in comparison with CC for samples from males and females. The correlation between the number of inclusions found in CC (CC score) and the sensitivities of CV, ML, and PCR are shown in Fig. 1. The sensitivities of

TABLE 1. CV, ML, and PCR results compared with CC results for detection of *C. trachomatis* in 283 males and 724 females

CC result	No. of samples												
	CV result					ML result				PCR result			
	Males		Females			Males		Females		Males		Females	
	+	−	+	−	NE[a]	+	−	+	−	+	−	+	−
+	28	18	35	17	2	32	14	26	28	40	6	42	12
−	32	205	2	662	6	0	237	1	669	4	233	1	669

[a] NE, not evaluable.

CV, ML, and PCR were all significantly lower when the number of inclusions in CC was low (scores of 1 and 2) ($P < 0.0001$).

Analysis of discordant results. For analysis of discordant results between the different tests, confirmatory assays on CC, ML, and PCR were performed. The sample for CV was not available for further analysis. For discordant samples, the results of initial tests with the outcomes of confirmatory assays are given in Table 3. A new standard for comparison of the tests was defined. If more than one test was positive, the sample was considered true positive. If only one test was positive and this positive result was confirmed by the confirmatory assay, the sample was also considered true positive. By this new standard, two false-negative and one-false positive CC results were detected. The false-positive CC had only one inclusion in two wells.

The final interpretations, based on the new standard, are also given in Table 3. On the basis of these interpretations, the sensitivities and specificities of CC, ML, PCR, and CV were recalculated. For samples from males, the specificity of CV was low (86.3%). All other tests had a specificity of ≥99.6%. The sensitivities for samples from males were as follows: CV, 60.4%; ML, 68.8%; PCR, 87.5%; and CC, 95.8% (Fig. 2). For samples from females, all tests were highly specific (specificities, ≥99.7%). The sensitivities for samples from females were as follows: CV, 62.3%; ML, 50.9%; PCR, 79.2%; and CC, 100%. The results for females are shown in Fig. 3.

TABLE 2. Sensitivity, specificity, predictive value of a positive test, and predictive value of a negative test of CV, ML, and PCR compared with CC as the gold standard for detection of *C. trachomatis* in 283 males and 724 females

Test and subject	Percent			
	Sensitivity	Specificity	Predictive value of positive test	Predictive value of negative test
CV				
Male	60.9	86.5	46.7	91.9
Female	67.3[a]	99.7[a]	94.6[a]	97.5[a]
ML				
Male	69.9	100.0	100.0	94.4
Female	48.1	99.9	96.3	96.0
PCR				
Male	87.0	98.6	90.1	97.5
Female	77.8	99.9	97.7	98.2

[a] Eight samples were not evaluable by CV. These samples are excluded from these estimates of the performance of CV.

DISCUSSION

In the present study, CC was compared with three alternative methods for the detection of *C. trachomatis* in urogenital specimens. Swab-to-swab variability of sampling was eliminated by performing all tests except CV on the same sample.

Of the test methods used in the present study, CV was the quickest and easiest test to perform. For samples from males, both sensitivity and specificity were low (60.4 and 86.5%, respectively). To evaluate the low specificity of CV, 25 swabs without any patient material were vortexed vigorously in the transport medium. CV was subsequently performed. Five of the 25 swabs tested positive. An unknown substance in the swab caused the false-positive results. The manufacturer does not recommend the use of CV for samples from males. Our findings confirm this policy. For samples from females, the specificity of CV was excellent (99.7%). However, its sensitivity was low (62.3%). Other studies (1, 8, 19, 22, 28) found higher sensitivities of CV ranging from 79 to 95%, while specificities were consistently lower (98.0 to 99.6%). Since CV is performed by a standardized protocol, these differences in performance are most likely caused by variations in the sensitivity of the reference method (CC) used in the studies described here. The sensitivity of our CC was probably higher than those of the CCs used in the other studies. In our setting, the patient rooms are next to the laboratory. Therefore, the problems associated with transport and storage conditions are confined. Moreover, this CC method has been used frequently as a reference method in evaluations of alternative tests for the

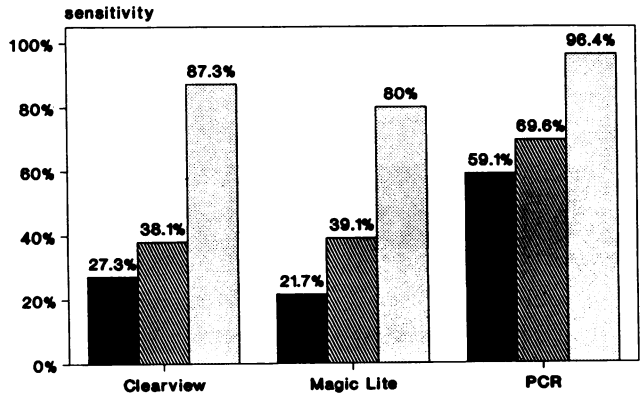

FIG. 1. Relation between CC score and sensitivity of CV, ML, and PCR. ■, CC score of 1 (1 to 5 inclusions per well), ▨, CC score of 2 (6 to 20 inclusions per well; ▩, CC score of 3 (>20 inclusions per well).

TABLE 3. Analysis of discordant results by confirmatory tests in males and females

No. of patients[a]	Test result[b]							Interpretation
	CC	CCconf	ML	MLconf	PCR	PCRconf	CV	
Male								
32	−	−	−	−	−	−	+	False-positive CV
7	+	ND[c]	+	ND	+	ND	−	False-negative CV
1	+	ND	−	−	+	ND	+	False-negative ML
2	+	ND	−	+	+	ND	+	False-negative ML
6	+	ND	−	+	+	ND	−	False-negative ML and CV
2	+	+	−	−	−	−	+	False-negative ML and PCR
1	+	+	+	+	−	+	−	False-negative PCR and CV
1	−	+	+	+	+	+	−	False-negative CC and CV
2	+	+	−	−	−	+	−	False-negative ML, PCR, and CV
1	+	+	−	−	−	−	−	False-negative ML, PCR, and CV
1	−	−	−	−	+	+	−	False-negative CC, ML, and CV
2	−	−	−	−	+	−	−	False-positive PCR
Female								
2	−	−	−	−	−	−	+	False-positive CV
2	+	ND	+	ND	+	ND	−	False-negative CV
1	+	ND	−	−	+	ND	+	False-negative ML
4	+	ND	−	+	+	ND	+	False-negative ML
4	+	ND	−	−	+	ND	−	False-negative ML and CV
5	+	ND	−	+	+	ND	−	False-negative ML and CV
2	+	ND	−	−	+	ND	NE[d]	False-negative ML and CV
1	+	+	−	−	−	−	+	False-negative ML and PCR
3	+	+	−	+	−	+	+	False-negative ML and PCR
4	+	+	−	−	−	−	−	False-negative ML, PCR, and CV
1	+	+	−	−	−	+	−	False-negative ML, PCR, and CV
2	+	+	−	+	−	+	−	False-negative ML, PCR, and CV
1	−	−	+	−	−	−	−	False-positive ML
1	−	−	−	−	+	−	−	False-positive PCR
1	+	−	−	−	−	−	−	False-positive CC

[a] Number of patients with discordant test results.
[b] CCconf, MLconf, and PCRconf are the confirmatory test results of CC, ML, and PCR, respectively.
[c] ND, not done.
[d] NE, not evaluable.

detection of *C. trachomatis* (5, 6, 10, 11, 23, 26). Therefore, a considerable amount of attention has been paid to improving the performance of this CC method over recent years. Both the transport conditions and the performance of CC in other settings may vary significantly. This problem of the gold standard has been discussed previously (10).

A comparison is more reliable if an analysis of samples with discordant results is performed. In the present study, the CV sample was not available for further analysis. Therefore, the error caused by swab-to-swab variability could not be determined. To minimize the effect of swab-to-swab variability, the sampling order was reversed. Moreover, in a previous study (10) it was shown that swab-to-swab variability did not have a major influence on the results.

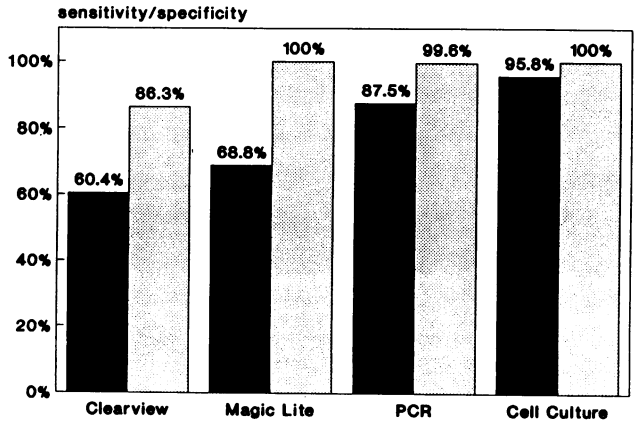

FIG. 2. Sensitivity (■) and specificity (▨) of CV, ML, PCR, and CC for samples from males after analysis of discordant results by confirmatory assays.

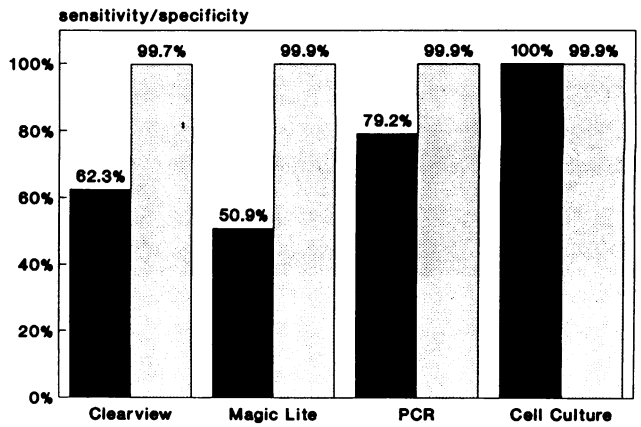

FIG. 3. Sensitivity (■) and specificity (▨) of CV, ML, PCR and CC for samples from females after analysis of discordant results by confirmatory assays.

ML was very specific. Only one false-positive result was found in samples from females, and none was found in samples from males. However, the sensitivity was low for samples from both males (68.8%) and females (50.9%). Forty-two ML samples were false negative. Upon retesting of these samples, which had been stored at −70°C, 22 (55%) samples became positive. This high number of samples with positive ML results after freezing and thawing was evaluated further. For this purpose, 25 CC-positive 2-SP samples, which were collected prospectively, were tested by ML after storage for approximately 8 weeks at both 4 and −70°C. The samples which were stored at −70°C showed significantly higher RLUs than the samples which were stored at 4°C (data not shown). These findings indicate that the performance of this highly specific technique could be improved by using a better extraction protocol. In other studies, the sensitivity of ML was higher. In a mixed population of 92 men and 102 women, Scieux et al. (18) found a sensitivity of 72.4% and a specificity of 97.7% for the ML compared with CC. Neman-Simha et al. (15) compared ML with a direct immunofluorescence technique and cell culture in 89 men and 171 women. The sensitivity was 88.5% and the specificity was 97%. These lower specificities with higher sensitivities compared with our results can also be explained by a lower sensitivity of the CC method used in the other studies. Another explanation for the low sensitivity of ML in our study could be the use of another swab and transport medium than the one supplied by the manufacturer. However, the manufacturer approved the swab and transport medium that we used in combination with the ML. The impact of the use of a different swab and transport medium cannot be determined from the results of the present study.

The sensitivity of the PCR in the present evaluation was lower than those in other studies (5, 6, 16). Furthermore, the sensitivity of the PCR assay correlated with the number of inclusions found in CC, as shown in Fig. 1. From this exercise, it can be concluded that the PCR applied in the current investigation is influenced by a limiting number of DNA targets in the patient sample. In this respect, the sample treatment protocol plays a crucial role. In the studies of Claas et al. (5, 6), a phenol extraction method was used. In addition to a purifying effect, the phenol method also has a concentration effect. Sample volumes of 1 ml or more can easily be concentrated to 100 μl, and of this concentrated DNA, a particular volume is added to the PCR mixture. Claas et al. (5, 6) actually applied 100 μl of the initial sample volume in the final PCR. In the currently applied protocol, the DNA was not purified at all and was actually diluted instead of concentrated. If the dilution factor is taken into account, only 2.2 μl of the original 200 μl was used in the PCR assay. The confirmatory PCR assay in the present study used a concentrating phenol extraction step for sample treatment. By this method, 9 of the 18 false-negative PCR results were positive. This indicates that the sample treatment method used in our study diminishes the sensitivity of the PCR. Ossewaarde et al. (16) recently reported a PCR for the detection of *C. trachomatis* that uses a simplified sample preparation procedure in which the sample is concentrated by centrifugation. They applied 24 μl of the patient sample into the PCR. A study investigating the correlation between the applied sample volume and the final PCR result is being undertaken in our institute.

CC proved to be both highly specific and sensitive. Although CC remains the gold standard, there is a definite need for alternatives to this method. Both of the immunoassays showed low sensitivities, which limits their use as diagnostic tests. The PCR was more sensitive than the immunoassays. The sensitivity of 87.5% for samples from males was higher than that for samples from females (79.3%), and specificities were excellent for samples from both groups. Nevertheless, the performance of the PCR described here is too low to replace CC for diagnostic purposes. Modifications in the sample treatment should further improve the sensitivity of the PCR.

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