

Detection of *Chlamydia trachomatis* in Male and Female Urine Specimens by Using the Amplified *Chlamydia trachomatis* Test

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The amplified *Chlamydia trachomatis* test (AMP-CT; Gen-Probe), a new diagnostic test for the detection of *Chlamydia trachomatis*, was evaluated with urine specimens from 1,000 patients visiting the outpatient department for sexually transmitted diseases at the University Hospital Rotterdam, Rotterdam, The Netherlands, by comparing the results to those of cell culture. From February 1996 to July 1996, urine samples for the AMP-CT test and urethral swabs for cell culture were collected from 544 men, while cervical swabs from 456 women were also taken for cell culture. Positive test results were obtained for 130 (13%) of the patients. AMP-CT test and cell culture results were discordant for 70 (7%) specimens. Analysis of the samples with discordant results was performed by an in-house PCR. After resolution of the discordant results, the sensitivity, specificity, and positive and negative predictive values of the AMP-CT test were 84.3, 98.8, 89.6, and 98%, respectively, for samples from females and 100, 99.2, 93.1, and 100%, respectively, for samples from males, while for cell culture these values were 72.5, 99.2, 92.5, and 98%, respectively, for samples from females and 57.4, 99.0, 86.1, and 95.4%, respectively, for samples from males. We conclude that the AMP-CT test is a fast and reliable test for the detection of *C. trachomatis* in urine specimens from females and, in particular, males.

Infection with *Chlamydia trachomatis* is one of the most important sexually transmitted diseases and has potentially serious sequelae (9). *C. trachomatis* can cause infections of the cervix, urethra, and upper genital tract in women, infections of the urethra and epididymis in men, and conjunctivitis and pneumonia in newborns (23). The most sensitive method for the diagnosis of a genital *C. trachomatis* infection was, until recently, based on culture of the microorganism on HeLa 229 or McCoy cells, which 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 (17). A second major disadvantage is that it takes several days before the test result is known. These disadvantages of the cell culture method have led to the search for alternative techniques for the detection of *C. trachomatis*. Apart from the development of antigen detection techniques, such as direct fluorescent-antibody tests and enzyme immunoassays (3, 11, 21, 22), nucleic acid detection techniques have been developed (7, 16, 18). These nucleic acid amplification tests have generally been more sensitive than traditional tests for the detection of *C. trachomatis*. The disadvantage of all these tests still remained that the majority of assays had to be performed with samples obtained from cervical or urethral smears. A sensitive, reliable, and inexpensive assay for the detection of *C. trachomatis* in specimens that can be easily collected was urgently needed. Two such assays have been marketed recently: the urine LCx assay from Abbott Laboratories based on the ligase chain reaction (LCR) (10) and the AMPLICOR assay from Roche based on PCR (13). These assays are based on the detection and amplification of *C. tra-*

chomatis DNA in urine and have proved to be reliable and reproducible (4–6, 10, 13). A disadvantage of both of these assays, however, is the need for thermocycling and, because both assays are based on DNA amplification, the need for stringent measures to prevent contamination. In this study, we evaluated a new test, the Amplified *Chlamydia trachomatis* (AMP-CT) test from Gen-Probe. This new test is based on amplification of rRNA, which has several potential advantages. Since the breakdown of one cell will result in the release of up to 10,000 copies of rRNA, giving a head start in the amplification process, the amplification time is short (2 h or less). Detection uses the hybridization protection assay with acridinium ester-labeled DNA probes which are specific for the target organism (1, 2). The results of the AMP-CT test were compared with the results of cell culture. An in-house PCR technique was used for analysis of samples with discordant results (12).

MATERIALS AND METHODS

Patient population and specimen handling. Specimens were collected from 544 men and 456 women visiting the outpatient department for sexually transmitted diseases at the University Hospital of Rotterdam, Rotterdam, The Netherlands, from February 1996 to July 1996. Samples were collected in a block design, with 250 samples in each block, in the following order for females: block one, cervical swab (CS), urethral swab (US), and first-void urine (FVU); block two, FVU, CS, and US; block three, CS, US, and FVU; block four, FVU, CS, and US. For males the order of collection was US and FVU over all blocks. The chlamydial culture samples were collected with ENT Dacron swabs (Medical Wire, Corsham, United Kingdom) and were placed in 1 ml of 0.2 M sucrose phosphate buffer and stored at 4°C or, when not tested within 24 h after collection, at –70°C. Four Eppendorf tubes were filled with 1.5 ml of FVU for the AMP-CT test and one tube was filled with 3 ml of FVU for the in-house PCR by using aerosol-resistant tips (Molecular Bio-Products Inc., San Diego, Calif.). This latter tube was stored at –20°C until processing. Of the four 1.5-ml tubes, three were stored at –70°C for later reference and one was stored at 4°C until processing, within 7 days.

Cell culture. Chlamydial culture was performed with cycloheximide-treated McCoy cells in microtiter plates and has been described previously (14, 15, 24). Briefly, two wells per plate were each inoculated with 0.2 ml of a patient sample. After centrifugation at 1,400 × g, for 60 min, the supernatant was replaced with

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0.1 ml of complete growth medium (Eagle minimal essential medium with Glutamax [Flow Laboratories Inc., Paisley, Scotland]) containing 10% fetal calf serum (Flow), 1% vitamins (Flow), 20 mg of gentamicin per liter, 5 mg of amphotericin B per liter, 25 mg of vancomycin per liter, 4.5 g of glucose per liter, and 1 mg of cycloheximide (pH 7.5; Sigma Chemical Co., St. Louis, Mo.) per liter. The plates were incubated at 37°C in 5% CO₂ for 48 h. Thereafter, the monolayers were fixed with ethanol (96%) for 10 min and stained with a fluorescent monoclonal antibody (Microtrak; Syva Co., Palo Alto, Calif.) specific for *C. trachomatis* and examined for inclusions. Culture results were scored as follows: 0, no inclusions per two wells; 1, 1 to 5 inclusions per two wells; 2, 6 to 20 inclusions per two wells; and 3, >20 inclusions per two wells.

AMP-CT test. The whole AMP-CT test procedure was carried out according to the instructions of the manufacturer. The urine samples were incubated for 10 min at 37°C. After centrifuging the specimens at 8,000 × g for 5 min, the supernatant was decanted. The pellet was resuspended in 200 µl of specimen diluent buffer, and 25 µl of amplification reagent was added to the propylene reaction tubes. After adding 200 µl of oil reagent to the reaction tube, 50 µl of processed specimen was pipetted under the oil reagent. The tubes were incubated for 10 min at 95°C in a heating block (Diagnostics Grifols S.A., Barcelona, Spain). After cooling down in a heating block at 42°C for 5 min, 25 µl of enzyme reagent was added. After incubation for 1 h at 42°C in the same heating block, 20 µl of termination reagent was added and the mixture was incubated for 10 min at 42°C in the heating block. After the addition of 100 µl of probe reagent, the tubes were briefly vortexed and incubated for 15 min at 60°C in a water bath. After adding 300 µl of selection reagent, the tubes were incubated for 10 min at 60°C in the water bath and were cooled down for 10 min at room temperature. The hybridization protection assay with acridinium-ester-labeled DNA probes (2) which are specific for the target organism was used for detection. Positive and negative controls were included in every run.

PCR. (i) Urine specimens. The urine specimens were thawed at room temperature and vortexed, and 1.5 ml was transferred to a clean tube and incubated for 15 min at 37°C. The specimens were then centrifuged at 14,000 × g for 30 min at 37°C. The supernatant was removed and the pellet was treated with 80 µl of lysis buffer (50 mM Tris-HCl [pH 7.5], 1% Triton X-100, 1 mM EDTA, 250 µg of proteinase K per ml). After incubation at 37°C for 1 h, the DNA in the lysates was extracted and purified with 4 M guanidine isothiocyanate and Celite by the method of Boom et al. (8). At the final step the DNA was eluted in 100 µl of 10 mM Tris-HCl (pH 8.0).

(ii) Cervical and urethral swabs. A 400-µl sample of specimen was centrifuged at 14,000 × g for 30 min, and the pellet was treated with 40 µl of lysis buffer (50 mM Tris-HCl [pH 7.5], 1% Triton X-100, 1 mM EDTA, 250 µg of proteinase K per ml). After incubation at 37°C for 1 h, the lysates were boiled for 10 min and centrifuged briefly. From each lysate, 8 µl was added to 92 µl of the PCR mixture.

Primers and PCR protocol. PCR was performed in a 100-µl volume under standard conditions with 10 µl of DNA sample. The following primer set, generating a 517-bp fragment, was used: T1, GGACAAATCGTATCTCGG; T2, GAAACCAACTCTACGCTG. PCR was performed in 100 µl of PCR solution containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 200 µM (each) deoxynucleoside triphosphate, 50 pmol of each primer, 0.1 mg of gelatin per ml, 0.2 U of *Taq* DNA polymerase (Sphaero-Q, Leiden, The Netherlands), and 10 µl of sample. The reaction mixture was overlaid with 100 µl of mineral oil (Sigma) to prevent evaporation and was preincubated for 5 min at 94°C for DNA denaturation. Thirty-five cycles of amplification were performed with a PCR processor (Biomed GmbH, Theres, Germany). Each cycle consisted of a denaturation step at 94°C for 1 min, a primer annealing step at 55°C for 1 min, and a chain elongation step at 74°C for 2 min. After 35 cycles, a temperature delay step of 5 min at 74°C completed the elongation. A 40-µl volume of the amplified PCR product was analyzed by agarose (1.5%; wt/vol) gel electrophoresis.

Southern blot analysis of PCR products. DNA was transferred from agarose to Hybond plus nylon filters (Amersham International, plc, Amersham, United Kingdom) by electrophoretic transfer. The PCR products were analyzed with a *C. trachomatis*-specific probe (CGCAGCGCTAGAGGCCGGTCTATTATGAT).

Analysis of results. Analysis of samples with discordant results was performed as follows. A result was considered to be true positive when cell culture results were comparable to the AMP-CT test results. The results for all samples with discrepant results were confirmed by using the in-house PCR. The in-house PCR was performed with FVU, US, and CS, if applicable. A cell-culture-negative and AMP-CT test-positive result was considered to be true positive if the PCR with FVU was positive. If the PCR result was negative, the AMP-CT test result was considered to be false positive. A cell culture-positive and AMP-CT test-negative result was considered to be false negative if the result with FVU PCR was positive. If the PCR result with FVU was negative and the US and/or CS specimen was PCR positive, the AMP-CT test was considered to be false negative due to sample-to-sample variation. DNA was isolated from cells in the inoculated cell culture monolayers, which was followed by PCR if the FVU and US (and CS, if applicable) remained negative by PCR. The cell culture result was considered true positive if the inoculated monolayer was PCR positive.

The SAS statistical program, version 6.08 (20), was used for analysis of the results. The Fisher test was used for statistical evaluation. Statistical significance was accepted at $P \leq 0.05$ (two-tailed).

TABLE 1. AMP-CT test and cell culture results for detection of *C. trachomatis* in males and females

Gender and cell culture result	No. of subjects with the following AMP-CT test result:	
	Positive	Negative
Males ($n = 544$)		
Positive	31	6
Negative	33	474
Females ($n = 456$) ^a		
Positive urethra and positive cervix	12	3
Positive urethra and negative cervix	1	1
Negative urethra and positive cervix	16	7
Negative urethra and negative cervix	20	396
Positive urethra or positive cervix	29	11
Overall ($n = 1,000$)		
Positive ^b	60	17
Negative	53	870

^a One female had no cervix.

^b Positive culture result for either cervical or urethral sample.

RESULTS

The results of cell culture compared to those of the AMP-CT test are presented in Table 1. The prevalence of chlamydial infection for the entire group ($n = 1,000$), as measured by a positive culture result, was 7.7%. For males ($n = 544$) and females ($n = 456$) the prevalences were 6.8 and 8.8%, respectively.

In a comparison of the AMP-CT test results with those of cell culture, the sensitivity, specificity, and positive and negative predictive values were 77.9, 94.2, 46.9, and 98.1%, respectively.

AMP-CT test and cell culture results were discordant for 70 (7%) of the samples. Table 2 presents the results of the analysis of samples with discordant results. In total, results for eight samples were discordant due to sample-to-sample variation. More than half of the samples with discordant results (37 of 70), were considered to be false negative by culture. Of the eight samples that were false negative by the AMP-CT test, all were from females.

After resolution of discordant results by PCR, the sensitivity, specificity, and predictive values were determined for both the AMP-CT test and cell culture (Table 3). The sensitivity of the AMP-CT test appears to be much higher than that of cell culture (92.4 versus 64.8%). The specificities of both techniques were quite similar. To determine whether the sensitivities of the assays were related to the order of sampling, the results were analyzed for samples within each block. There were no significant differences in sensitivity between the blocks for either culture or the AMP-CT test ($P > 0.2$) (data not shown). To determine whether the sensitivity of the AMP-CT test was related to the load of *C. trachomatis* in the samples, the sensitivity was compared for samples for which different numbers of inclusions were found in cell culture. There was no significant difference in the sensitivity of the AMP-CT test between samples with scores of 1 and those with scores of 2 or 3 ($P > 0.2$).

TABLE 2. Number of patients whose samples had shown discrepant cell culture and AMP-CT test results and interpretation after resolution by PCR analysis

Discordance		PCR result		No. of samples		Explanation
AMP-CT test result	Culture result	Urine	CS/US	Male	Female	
+	-	+	-	6	1	Sample-to-sample variability
		+	+	22	9	False-negative culture
		-	+	1	5	False-negative culture
		-	-	4	5	False-positive AMP-CT test
-	+	-	+	1	0	Sample-to-sample variability
		+	+	0	5	False-negative AMP-CT test
		+	-	0	1	False-negative AMP-CT test
		-	-	5	5	False-positive culture ($n = 8$) or false-negative AMP-CT test ($n = 2$) ^a

^a These two samples (both from females) were PCR positive in the culture wells.

DISCUSSION

In this study, we evaluated the value of a new technique, the AMP-CT test, for detecting *C. trachomatis* in urine samples. One of the remarkable findings in this study was the number of false-negative culture results. More than half of the samples with discordant results had false-negative culture results. Other amplification techniques, such as the commercially available AMPLICOR PCR and the LCx, have provided similar results (4, 5, 10, 13). There are several explanations for this. Theoretically, one inclusion would be enough to show a positive result in a PCR-based test, while culture has been shown to need multiple elementary bodies to be positive. Another argument which is often used is that culture techniques need viable elementary bodies to become positive, while amplification techniques only show the presence of DNA (or RNA). Patients who have been successfully treated with anti-chlamydial agents can remain positive for some time. Since the AMP-CT test is based on RNA amplification, in contrast to the AMPLICOR and the LCx, it could be expected that this issue is of less importance for this technique. RNA is much less stable than DNA in the environment and is degraded quickly by naturally occurring RNases (19). On the other hand, the AMP-CT test is based on amplification of rRNA, making use of the thousands of copies of the sequence to be detected, while only 10 copies per inclusion are available for the DNA amplification techniques. Which of the two forgoing mechanisms is going to prevail remains to be investigated. Alternatively, it has been shown that *C. trachomatis* can persist in mononuclear phagocytes, which can be responsible for the prolonged positive amplification reaction. It is then the longevity of the phagocytes rather than the persistence of DNA or RNA in situ which determines the duration of positivity.

Therefore, although the amplification techniques are probably more sensitive overall than cell culture, the sensitivity is still not 100%. After discrepancy analysis, eight samples yielded false-negative AMP-CT test results, and these were all from females. One explanation may be that inhibitory substances were present in the sample. Inhibitory substances have been shown to influence PCR-based results considerably (25). This finding warrants further study.

In this study, we chose to analyze the results after analysis of samples with discordant results but not including those samples with discordant results due to sample-to-sample variation (a total of eight samples). This could be debated, since it could be argued that the purpose of testing is to find whether a person is infected, irrespective of the method used. In that case the figures in this study would have provided slightly different results. Seven of the eight sample-to-sample variations oc-

curred with samples from the AMP-CT test-positive and culture-negative group, mostly with samples from males (Table 2). The sensitivity of the AMP-CT test would therefore increase. Among the samples from the AMP-CT test-negative and culture-positive group, results for only one of the samples varied among the tests. The sensitivity for samples from males would be slightly lower if this sample is taken into consideration as a false-negative AMP-CT test result and would decrease from 100 to 98.4%.

The overall prevalence found by cell culture was somewhat lower than that found in earlier studies in our hospital. In a study performed in 1991, we found prevalences of 8.7 and 13.5% for females and males, respectively, compared to prevalences of 8.8 and 6.8%, respectively, in this study. It appears that the lower prevalence is due to a shift in the prevalence in males only. Although this figure increases after resolution of the results for samples with discordant results between the tests, we have no explanation for this. It seems, however, that the prevalence of *Chlamydia* in the male population is decreasing. This may be explained by the difference in clinical symptoms between males and females. Most male *C. trachomatis* infections are symptomatic, while 75% of the female *C. trachomatis* infections remain asymptomatic (9). Successful prevention strategies and the availability of *C. trachomatis* diagnostic tools may also contribute to this decline.

Both PCR with the original sample and PCR with material obtained from the culture wells were negative for eight subjects, whose samples were initially culture positive and AMP-CT negative, yielding a specificity of less than 100% for

TABLE 3. Sensitivity, specificity, and positive and negative predictive values for the AMP-CT test and cell culture after resolution of discordant results

Group and test	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Females				
AMP-CT	84.3	98.8	89.6	98.0
Cell culture	72.5	99.2	92.5	96.6
Males				
AMP-CT	100	99.2	93.1	100
Cell culture	57.4	99.0	86.1	95.4
Overall				
AMP-CT	92.4	99.0	91.5	99.1
Cell culture	64.8	99.1	89.5	96.0

cell culture. There are several explanations for this. One possibility would be that only one *C. trachomatis* elementary body was present in the whole sample. However, in that case the PCR with material from the culture wells should have been positive for at least some of the samples and is further mainly of theoretical interest. A second possibility would be that there is aspecific binding of the *C. trachomatis*-specific monoclonal antibodies or other fluorescent artifacts. We are evaluating the sensitivity and specificity of several other assays, including the PACE-2 assay (Gen-Probe), for the detection of *C. trachomatis*, and although more assays are used and thus the overall sensitivity increases, preliminary results indicate there are still some false-positive culture results, probably on the basis of the last of the two foregoing explanations (data not shown).

We conclude that the AMP-CT test is a fast and reliable test for the detection of *C. trachomatis* in urine specimens from females and, in particular, males.

REFERENCES

- Abe, C., M. Hirano, W. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and S. Goto. 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. *J. Clin. Microbiol.* **31**:3270-3274.
- Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson. 1989. Assay formats involving acridinium-ester-labeled probes. *Clin. Chem.* **35**:1588-1594.
- Barnes, R. C. 1989. Laboratory diagnosis of human chlamydial infections. *Clin. Microbiol. Rev.* **2**:119-136.
- Bassiri, M., H. Y. Hu, M. A. Domeika, J. Burczak, L. O. Svensson, H. H. Lee, and P. A. Mardh. 1995. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. *J. Clin. Microbiol.* **33**:898-900.
- Bauwens, J. E., A. M. Clark, M. J. Loeffelholz, S. A. Herman, and W. E. Stamm. 1993. Diagnosis of *Chlamydia trachomatis* urethritis in men by polymerase chain reaction assay of first-catch urine. *J. Clin. Microbiol.* **31**:3013-3016.
- Bianchi, A., C. Scieux, N. Brunat, D. Vexiau, M. Kermanach, P. Pezin, M. Janier, P. Morel, and P. H. Lagrange. 1994. An evaluation of the polymerase chain reaction amplicor *Chlamydia trachomatis* in male urine and female urogenital specimens. *Sex. Transm. Dis.* **21**:196-200.
- Bobo, L., F. Coutlee, R. H. Yolken, T. Quinn, and R. P. Viscidi. 1990. Diagnosis of *Chlamydia trachomatis* cervical infection by detection of amplified DNA with an enzyme immunoassay. *J. Clin. Microbiol.* **28**:1968-1973.
- 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.
- Centers for Disease Control and Prevention. 1993. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. *Morbidity Mortal. Weekly Rep.* **42**(RR-12):1-39.
- Chernesky, M. A., H. Lee, J. Schachter, J. D. Burczak, W. E. Stamm, W. M. McCormack, and T. C. Quinn. 1994. Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. *J. Infect. Dis.* **170**:1308-1311.
- Ehret, J. M., and F. N. Judson. 1989. Genital Chlamydia infections. *Clin. Lab. Med.* **9**:481-500.
- Goessens, W. H., J. A. Kluytmans, N. den Toom, T. H. van Rijsoort-Vos, B. G. Niesters, E. Stolz, H. A. Verbrugh, and W. G. Quint. 1995. Influence of volume of sample processed on detection of *Chlamydia trachomatis* in urogenital samples by PCR. *J. Clin. Microbiol.* **33**:251-253.
- 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.
- Kluytmans, J. A., W. H. Goessens, J. W. Mouton, J. H. Van Rijsoort Vos, H. G. Niesters, W. G. Quint, L. Habbema, E. Stolz, and J. H. Wagenvoort. 1993. Evaluation of Clearview and Magic Lite tests, polymerase chain reaction, and cell culture for detection of *Chlamydia trachomatis* in urogenital specimens. *J. Clin. Microbiol.* **31**:3204-3210.
- Kluytmans, J. A., H. G. Niesters, J. W. Mouton, W. G. Quint, J. A. 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.
- Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon. 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J. Clin. Microbiol.* **30**:2847-2851.
- 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.
- Ossewaarde, J. M., M. Rieffe, M. Rozenberg Arska, P. M. Ossenkoppele, 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.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed, p. 7.1-7.87. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- SAS Institute Inc. 1990. *SAS user's guide*. SAS Institute Inc., Cary, N.C.
- Scholes, D., A. Stergachis, F. E. Heidrich, H. Andrilla, K. K. Holmes, and W. E. Stamm. 1996. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N. Engl. J. Med.* **334**:1362-1366.
- Stamm, W. E. 1988. Diagnosis of *Chlamydia trachomatis* genitourinary infections. *Ann. Intern. Med.* **108**:710-717.
- Stamm, W. E. 1990. Diagnostic tests should be used for sexually transmitted chlamydia. *West. J. Med.* **153**:559-560. (Editorial.)
- Thewissen, E. A., I. Freundt, J. H. Van Rijsoort Vos, E. Stolz, M. F. Michel, and J. H. Wagenvoort. 1989. Comparison of HeLa 229 and McCoy cell cultures for detection of *Chlamydia trachomatis* in clinical specimens. *J. Clin. Microbiol.* **27**:1399-1400.
- Verkooyen, R. P., A. Luijendijk, W. M. Huisman, W. H. F. Goessens, J. A. J. W. Kluytmans, J. H. van Rijsoort-Vos, and H. A. Verbrugh. 1996. Detection of PCR inhibitors in cervical specimens using the AMPLICOR *Chlamydia trachomatis* assay. *J. Clin. Microbiol.* **34**:3072-3074.