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Accuracy of Two Enzyme Immunoassays and Cell Culture in the Detection of *Chlamydia trachomatis* in Low and High Risk Populations in Senegal

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Two enzyme immunoassays (EIAs), Chlamydiazyme (CZ; Abbott Laboratories) and Pathfinder (PF; Kallestadt), were compared with a cell culture technique in the detection of cervical *Chlamydia trachomatis* infection in 670 women in urban settings in Senegal (377 pregnant women and 293 prostitutes). Positive CZ and positive PF specimens were tested a second time using a monoclonal antibody blocking technique. True positive specimens were defined as those positive on culture or positive on EIA with confirmation of the result after blocking. Using this definition, the prevalence of genital chlamydial infection was 14.6 % and 14.3 % in pregnant women and prostitutes respectively. An important difference between the two populations was that the pregnant women were younger than the prostitutes, which might explain the fact that the prevalence of infection among the pregnant women was as high as that among the prostitutes, although the age-adjusted prevalence was higher among prostitutes than among pregnant women. The chlamydial detection rates of cell culture, CZ and PF were 62 % (26/42), 69 % (29/42) and 86 % (36/42) respectively in prostitutes and 76 % (42/55), 40 % (22/55) and 53 % (29/55) respectively in pregnant women. Agreement between the tests was 89 %, 85 % and 88 % for culture/CZ, culture/PF and CZ/PF respectively. However, when data were adjusted for chance agreement, kappa coefficients were 0.40 for culture/CZ, 0.34 for culture/PF and 0.48 for CZ/PF. These results indicate that the accuracy of the EIAs and cell culture may vary greatly in different populations: both EIAs showed a distinctly higher detection rate than culture in prostitutes and a significantly lower detection rate in pregnant women. Confirmation of positive EIA results with a blocking assay greatly enhanced the specificity of the antigen detection tests and should be obtained when using the EIAs in a clinical setting.

Chlamydia trachomatis infection is the most common sexually transmitted disease (STD) worldwide. The standard method used in clinical and epidemiological studies for detecting *Chlamydia trachomatis* genital infection is isolation of the causative agent by cell culture. Because of the expense and limited availability of cell culture, non-culture antigen detection methods have become increasingly popular.

Many studies have determined the reliability and validity of direct fluorescence antibody techniques (DFA) and enzyme immunoassays (EIA) in comparison with cell culture (1). Most previous studies were performed in high prevalence populations, including patients attending STD clinics, and in women referred to abortion clinics or family planning facilities. The sensitivity of non-culture techniques in these populations was estimated to be 60 % to 100 % (2). However, more recently several studies performed in asymptomatic low risk populations have shown sensitivity levels for DFA and EIA of less than 60 %, and high numbers of discordant results be-

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tween different non-culture techniques used in parallel (3–5). The sensitivity determined for a non-culture test for *Chlamydia trachomatis* is always approximate since the sensitivity of the reference culture method is not 100 % (5). In addition, the sensitivity of cell culture may vary greatly in different laboratories, resulting in very different sensitivity values being reported for non-culture methods (6). Thus, in the absence of a fully valid reference method, calculations of sensitivity and specificity are only approximate. This also implies that detection rates of cell culture and non-culture methods can only be compared when both are used to test the same set of samples and that it is extremely difficult to compare data from different studies (4, 5). Even after analysis of the results from large studies, it is difficult to recommend any of the currently available tests since no ideal method exists (3, 7).

As part of a study of techniques for the rapid detection of STD prevalence conducted in urban populations in Senegal, we assessed the performance of two EIAs and cell culture in the detection of cervical *Chlamydia trachomatis* infection in pregnant women and in prostitutes.

Materials and Methods

Specimens. From 1 February to 28 March 1990, 511 consecutive pregnant women, seen in two health centers in Pikine, Senegal, and 374 female prostitutes, reporting for a routine examination at the STD clinic of the Institut d'Hygiene Sociale in Dakar, Senegal, were enrolled in a study on the rapid detection of STD. Demographic information, medical and sexual history, and symptoms were recorded. A blood specimen was taken for serological tests for syphilis. All women were examined clinically by a gynecologist. Genital specimens for microbiological investigations included a vaginal swab for wet mount and Gram stain for detection of *Trichomonas vaginalis, Candida* spp. and clue cells and examination of the bacterial flora. The pH of vaginal secretion was measured and an amine test was done using 10 % KOH. After removal of exocervical mucus and pus with a sterile gauze, a cervical swab was taken for Gram stain and culture to detect *Neisseria gonorrhoeae.* In prostitutes two endocervical swabs were taken in daily reversed order for chlamydial culture and the Chlamydiazyme EIA (CZ; Abbott Laboratories, North Chicago, IL, USA), followed by collection of a cytobrush specimen for the Pathfinder EIA (PF; Kallestadt, South Austin, Texas, USA). In pregnant women three endocervical swabs were taken for cell culture, CZ and PF, the order in which swabs were taken being reversed three times partway through the study.

Laboratory Tests. Specimens for chlamydial culture were collected in 0.7 ml 2 sucrose-phosphate transport medium (2 SP), vortexed for 30 sec and after removal of the swab immediately placed at -20 °C, and within 4 h stored at -70 °C continuously before, during and after transport to the Institute of Tropical Medicine, Antwerp, Belgium. *Chlamydia trachomatis* culture was performed in vials (with 133 mm^2 coverslips) on cycloheximide treated McCoy cells after centrifugation at 3000 x *g* for 1 h at 35 °C. Cells were stained with iodine after incubation for 72 h at 36 °C.

The CZ EIA was performed within 48 h on specimens kept at 4 °C following the test protocol included in the assay kit. The PF tube test was performed within 48 h on specimens kept at 4 °C using the appropriate equipment and following the instructions supplied by the manufacturer.

Positive CZ and positive PF specimens were tested again in a blocking assay using the blocking antibody reagents and following the instructions supplied by the respective manufacturers. A reduction of ≥ 50 % in the absorbance in the blocked assay compared to the result of the initial test was considered to confirm the presence of *Chlamydia trachomatis* antigen in the clinical specimen.

Data Analysis. Specimens with a positive result in the culture method or in at least one EIA confirmed by blocking were considered as true positive. Specimens with negative results in the culture method and both EIAs, as well as specimens with positive EIA results not confirmed by blocking were considered as true negative. The sensitivity and specificity of all tests were calculated using those criteria.

Chi-square (Yates corrected) and Fisher exact tests when appropriate were used to compare proportions. The Kruskal-Wallis test was used to compare mean values of EIA absorbance results. To compare concordance of different diagnostic tests, kappa coefficients were calculated, adjusted for chance agreement (8).

Results

The mean age of the pregnant women was 25.7 years (range 15–45) and the mean age of the prostitutes was 31.7 years (range 16–58).

Due to incomplete collection of samples EIA results were available for only 487 of 511 pregnant women and 370 of 374 prostitutes. The CZ and PF were positive in 38 (7.8 %) and 40 (8.2 %) of the pregnant women respectively, and in 41 (11.1 %) and 88 (23.8 %) of the prostitutes respectively. Specimens for *Chlamydia trachomatis* cell culture were lacking for two prostitutes. Due to contamination and/or toxic effect on tissue cells, chlamydial cultures were invalid for 114 (22.3 %) of the pregnant women and for 77 (20.6 %) of the prostitutes. For the remaining subjects cell culture was positive in 44 (11.1 %) of the pregnant women and in 26 (8.8 %) of the prostitutes. Invalid *Chlamydia trachomatis* cultures were mainly associated with older age and presence of cervical discharge: 9 of 113 (8 %)

Table 1: Results of EIA blocking assays related to the results of the other tests for detection of *Chlamydia trachomatis*.

Initially positive specimens	No. of blocked specimens/ no. of initially positive specimens		Percent of results confirmed by blocking
	Pregnant women	Prostitutes	
Chlamydiazyme			
Culture positive/PF positive	12/12	18/21	91
Culture negative/PF positive	9/10	7/8	89
Culture negative/PF negative	0/14	1/8	5
Overall	21/36	26/37	64
Pathfinder			
Culture positive/CZ positive	12/12	18/21	92
Culture positive/CZ negative	4/4	–	
Culture negative/CZ positive	10/10	7/8	95
Culture negative/CZ negative	3/11	8/42	21
Overall	29/37	33/71	58

PF: Pathfinder; CZ: Chlamydiazyme.

Table 2: Results of tests to detect *Chlamydia trachomatis* in true positive specimens.

Subjects	No. of specimens positive						No. of true positives/ no. of specimens
	Culture	Culture/CZ/PF	Culture/PF	CZ/PF	CZ	PF	
Pregnant women	26	12	4	10	–	3	55/377 (14.6 %)
Prostitutes	5	21	–	7	1	8	42/293 (14.3 %)

CZ: Chlamydiazyme; PF: Pathfinder.

pregnant women of 20 years of age or less without cervical discharge had an invalid culture result compared to 23 of 83 (28 %) with cervical discharge and older than 20 years ($p < 0.001$). Vaginal douching the morning before the examination was practised by 68 % of the prostitutes whereby 32 % had used antiseptic solutions. Invalid cell cultures were more common among users of antiseptic solutions (25 %) than among non-users (16 %), ($p = 0.09$).

Comparative analysis of results of the tests was performed in subjects with complete EIA samples and with a valid chlamydial cell culture, comprising 377 pregnant women and 293 prostitutes. Of the pregnant women 42 (11.1 %), 36 (9.5 %) and 37 (9.8 %) had a positive result in culture, CZ and PF respectively; of the prostitutes 26 (8.9 %), 37 (12.6 %) and 71 (24.2 %) had a positive result in culture, CZ and PF respectively. All specimens positive in the EIA were retested after the addition of blocking chlamydial antibody; the results of these assays are shown in Table 1.

The predictive value of an initially positive EIA was significantly higher for samples positive in the second EIA or on culture than when both other assays were negative: the CZ result was confirmed in 46 of 51 (90 %) specimens also positive in culture and/or PF versus only 1 of 22 (5 %) specimens negative in both other tests ($p < 10^{-7}$); the PF result was confirmed in 51 of 55 (93 %) specimens also positive in culture and/or CZ versus 11 of 53 (21 %) specimens with negative results in both culture and CZ ($p < 10^{-7}$). Of the initially positive CZ and PF specimens, the result in 64 % and 57.5 % respectively was confirmed by blocking.

The optical density (OD) values of initially positive EIA specimens in which the result was confirmed by blocking were significantly higher than in specimens in which the result was not confirmed by blocking: the mean OD ratio (OD/cut-off value) for CZ was 8.2 (median 6.4) for confirmed positive cases versus 2.7 (median 1.5) for non-confirmed positive cases ($p = 10^{-6}$); the mean

Table 3: Positive results of cell culture and initial antigen detection assays in women with and without *Chlamydia trachomatis* infection.

Subjects	Women with infection[a]				Women without infection[b]		
	Culture positive	CZ positive	PF positive	Total number positive	CZ positive	PF positive	Total number negative
Pregnant women	42 (76)	22 (40)	29 (53)	55	14 (4.3)	8 (2.5)	322
Prostitutes	26 (62)	29 (69)	36 (86)	42	8 (3.2)	35 (13.9)	251
Total population	68 (70)	51 (53)	65 (67)	97	22 (3.8)	43 (7.5)	573

CZ: Chlamydiazyme; PF: Pathfinder.
[a] Figures in brackets are percentage of positive results related to the total number of cases detected.
[b] Figures in brackets are percentage of false positive results.

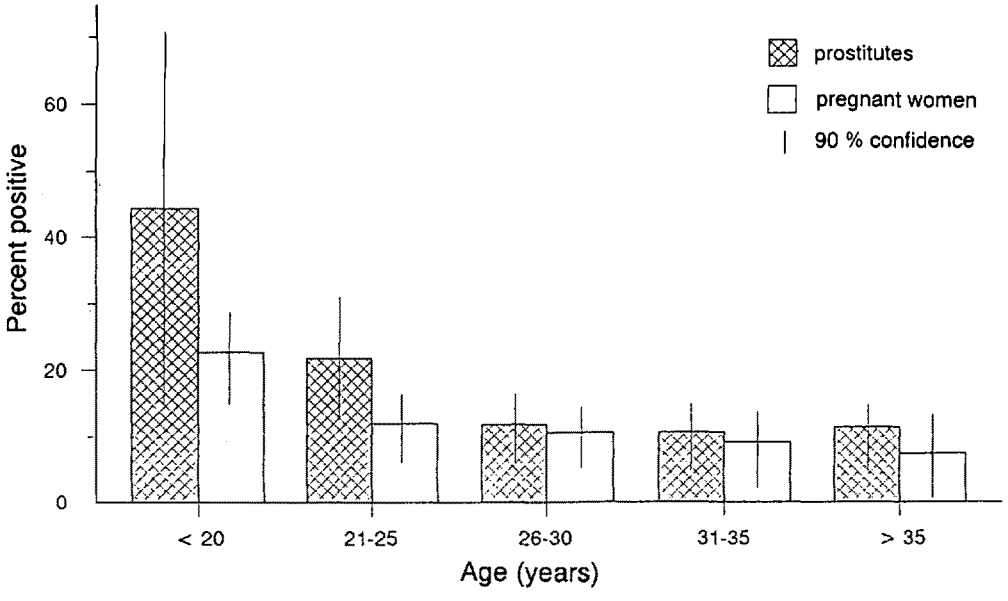

Figure 1: Association between *Chlamydia trachomatis* infection and age.

OD ratio for PF was 8.3 (median 8.1) for confirmed positive cases and 3.0 (median 1.3) for non-confirmed cases ($p < 10^{-7}$).

Specimens with a positive result on culture or at least one confirmed positive EIA result were considered as true positive. The results obtained in culture, CZ and PF for these true positive cases are shown in Table 2. The rate of detection of *Chlamydia trachomatis* by cell culture, CZ confirmed by blocking and PF confirmed by blocking were 42/55 (76 %), 22/55 (40 %) and 29/55 (53 %) respectively in pregnant women and 26/42 (62 %), 29/42 (69 %) and 36/42 (86 %) respectively in prostitutes.

The detection rates of culture and initial CZ and PF in subjects with *Chlamydia trachomatis* infection according to the definition and the prevalence of false positive EIA results in women without *Chlamydia trachomatis* infection are shown in Table 3. In pregnant women the most sensitive test for detecting cervical chlamydial infection was cell culture, whereas PF was the most sensitive assay in prostitutes. The specificity of CZ was 95.7 % in pregnant women and 96.8 % in prostitutes; the specificity of PF was 97.5 % in pregnant women and 86.1 % in prostitutes.

The strong association between *Chlamydia trachomatis* infection and young age is shown in

Table 4: Agreement between cell culture and initial EIA results in the detection of *Chlamydia trachomatis.*

	Culture/CZ	Culture/PF	CZ/PF
Positive/positive	33	37	50
Positive/negative	35	31	23
Negative/positive	40	71	58
Negative/negative	562	531	539
Level of agreement	89 %	85 %	88 %
Kappa coefficient	0.404	0.339	0.485

CZ: Chlamydiazyme; PF: Pathfinder.

Figure 1. In both pregnant women and prostitutes chlamydial infection was much more common in younger subjects (pregnant women: $p = 0.004$; prostitutes: $p = 0.017$; X^2 for trend). In addition, for each age category the prevalence of chlamydial infection was consistently higher in prostitutes than in pregnant women. The age-adjusted prevalence was 20.4 % in prostitutes and 12.5 % in pregnant women, based on direct adjustment.

Of a total of 68 positive cultures only 8 (12 %) had more than 20 chlamydial inclusions per coverslip. These were all strongly positive in both EIAs, the result being confirmed by blocking. Of the 60 cultures with less than 20 inclusions per coverslip only 25 (42 %) and 29 (48 %) were positive in CZ and PF respectively. The overall agreement between the three diagnostic tests is shown in Table 4. The rate of agreement between the different tests was at least 85 %, with a higher rate between culture and CZ. However, when adjusted for chance agreement, the calculated kappa coefficient values were less than 0.5 for all combinations due to the relatively low prevalence of chlamydial infection and to the high rate of disagreement between positive results. Thus a higher rate of agreement was obtained between the two EIAs than between each EIA and culture.

Discussion

Cell culture remains the method of choice for the diagnosis of *Chlamydia trachomatis* infection. The reported sensitivity of a single culture technique varies between 33 % and 86 % but is usually estimated to be between 70 % and 80 % (9–12). More cases are detected when blind passage and staining with fluorescent monoclonal antibodies are performed (13–16). For economic reasons we used a single-cell culture technique with iodine staining, which detected 70 % of cases with chlamydial infection. Microbial contamination, toxicity of swabs and clinical specimens, and the presence of detergents may damage or destroy culture cells (10). Such invalid cultures were much less common in subjects without cervical discharge and in the younger age group.

This study illustrates that the parallel performance of different diagnostic tests yielded a high proportion of discordant data, that for any of the combinations culture/CZ, culture/PF and CZ/PF discordant results were more common than concordant results for positive specimens, and that this discordance was not influenced by the order of specimen collection. This observation is in agreement with observations of other investigators and seems to be inherent to studies in asymptomatic women (3, 5).

Specimens with positive results in two separate non-culture assays are more likely to be truly positive than specimens positive in only one assay (3, 5). This was also shown in our study by using blocking antibody assays for confirmation of positive EIAs. The direct relationship between EIA OD values and the confirmation test outcome observed in the current study is in agreement with the findings in other studies (5, 17, 18).

As in numerous other studies, the number of chlamydial organisms in female genital specimens and the overall prevalence of *Chlamydia trachomatis* infection in symptomatic and asymptomatic women were age related, women under the age of 20 having a higher risk of infection than older women (4, 19–21.). Therefore, comparison of prevalence rates in different populations should always be based on age adjusted data.

This study suggests that none of the three diagnostic tests used was optimal for the diagnosis of cervical *Chlamydia trachomatis* infection in these populations. It is assumed that a proportion of

Table 5: Results reported in six different studies in the detection of *Chlaymdia trachomatis* in cervical specimens by EIA related to culture (original EIA results not corrected by blocking results).

Reference	Site	No. of patients	EIA	EIA positive/ culture positive (%)	95 % confidence interval
Present study	Dakar, Senegal	293 (prostitutes)	Chlamydiazyme	21/26 (80.8)	60.6–93.4
		377 (pregnant women)	Chlamydiazyme	12/42 (28.6)	15.7–44.6
		670 (all)	Chlamydiazyme	33/68 (48.5)	36.2–61.0
		293 (prostitutes)	Pathfinder	21/26 (80.8)	60.6–93.4
		377 (pregnant women)	Pathfinder	16/42 (38.1)	23.6–54.4
		670 (all)	Pathfinder	37/68 (54.4)	41.9–66.5
Amortegui et al. (27)	Pittsburgh, USA	209	Chlamydiazyme	13/18 (72.2)	46.5–90.3
Smith et al. (15)	Indianapolis, USA	1059	Chlamydiazyme	69/103 (67)	56.8–75.5
Pao et al. (35)	Taipei, Taiwan	198	Chlamydiazyme	33/48 (68.7)	53.7–81.3
van Ulsen et al. (36)	Rotterdam, Netherlands	246	Chlamydiazyme	24/36 (66.7)	49.3–81.4
Magder et al. (4)	CDC, Atlanta, USA	1417	Chlamydiazyme	93/163 (57.1)	48.8–64.6

chlamydial infections are difficult to detect by either cell culture or non-culture techniques due to low level excretion of organisms or the influence of host immunity (22, 23). Paradoxically, in some studies lower *Chlamydia trachomatis* isolation rates were found in patients with a history of STD (22, 24). These factors may partly explain why in subjects in the older age groups the prevalence of chlamydial infection was not much higher among prostitutes than in the pregnant women of this study, since prostitutes have a higher probability of having acquired immunity during previous chlamydial infections and of being exposed to STD.

In general, the number of chlamydial organisms found in symptomatic patients is greater than in asymptomatic women reporting for screening (15, 25). This means that diagnostic tests may appear fairly sensitive when evaluated in STD clinic patients, but less so in individuals with a lower prevalence of infection and fewer organisms at the infected site. In our study we found low chlamydial inclusion counts in cell culture for 88 % of culture positive specimens, although this result may also have been influenced by storage of specimens at -70 °C (26). The sensitivity of both EIAs was significantly lower in specimens with low cell culture inclusion counts than in positive specimens with more than 20 inclusions, as observed by others (4, 15, 17, 27).

Adequate female cervical specimens for detection of *Chlamydia trachomatis* by culture or non-culture assays contain metaplastic cells or columnar or cuboidal epithelial cells from the endocervix (12, 22, 28–31). It was shown that the detection rate of the CZ was almost three times higher when endocervical epithelial or metaplastic cells were present (32). The present study was performed under field conditions for rapid detection of STD and adequacy of specimens was not determined.

In this study we observed a fairly high proportion of false positive EIA results, probably due to cross-reacting antigens in the clinical specimens. Vaginal pool contamination with various bacteria was very common in the populations tested and may explain the high rate of false positive tests (6, 33, 34).

Table 5 shows the detection rates for the two EIAs related to culture obtained in various

studies. The results of our study are comparable with those of other investigations. However, our analysis of the two subpopulations shows that important differences do exist, EIA detection rates in pregnant women being below any other figures reported.

We conclude that for the diagnosis of chlamydial infection in asymptomatic women EIAs are less reliable than estimated from studies in high risk patients. On routine use of any chlamydial antigen test for primary care medicine or for screening of asymptomatic populations these limitations in sensitivity and specificity should be considered. Enthusiasm for new technical developments should be weighed up against the problems encountered with currently available tests and their interpretation (3, 4, 5, 7). However, the specificity of both EIAs tested here can be greatly enhanced by the additional performance of a blocking assay. Such confirmation tests should be performed in all EIA positive specimens to increase the accuracy of the EIA. The inclusion of such techniques may be cost-effective in screening and case finding programmes for *Chlamydia trachomatis* infection.

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