

Enzyme immunoassay for the detection of *Chlamydia trachomatis* antigen in urethral and endocervical swabs

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SUMMARY An enzyme immunoassay technique based on the direct detection of *Chlamydia trachomatis* antigen in urethral or cervical swabs was used for the rapid diagnosis of chlamydial genital infection. Urethral and cervical samples from 140 patients were tested in parallel by enzyme immunoassay and cell culture using iodine staining. The direct test had a sensitivity of 92.5% and specificity of 97.2% when compared with the cell culture system. The enzyme immunoassay technique provides a rapid and simple method for diagnosing chlamydial genital infection and may be performed on a large number of samples in laboratories which do not have tissue culture facilities or a trained microscopist.

Chlamydia trachomatis is the commonest cause of preventable blindness in the world.¹ It is also an important genital pathogen of men and women and may cause ophthalmia neonatorum and respiratory infection in neonates. Lower genital tract infections in both sexes may be followed by serious complications such as epididymitis, pelvic inflammatory disease (with the attendant risk of infertility), and perihepatitis. Even mild and asymptomatic infections with *C trachomatis* may have serious consequences and early diagnosis and treatment is clearly important.¹

The traditional method for detecting *C trachomatis* entails the use of tissue cultures grown on coverslips in vials or microtitre plates followed by staining with iodine, Giemsa stain, or fluorescent antibody dyes. These techniques are expensive, laborious, and time consuming, requiring strict quality control of both the cell line and the media. Recently, a direct (culture independent) immunofluorescence test for the diagnosis of *C trachomatis* has been described.^{2,3} The test uses a monoclonal antibody and has both high sensitivity and specificity. This direct technique is based on the detection of extracellular *C trachomatis* elementary bodies. Although simple and rapid, the test is technically demanding and the skill of the microscopist plays a major part in its sensitivity and specificity.²

We describe here an enzyme immunoassay technique (Abbott Laboratories) for the rapid detection of *C trachomatis* antigen in urethral and cervical specimens. The immunoassay technique has been compared with an established cell culture technique in routine use in this laboratory.

Material and methods

PATIENTS

All the patients attended the Department of Genito-urinary Medicine, University College Hospital, London. Endourethral swabs from 61 men with non-gonococcal urethritis and cervical swabs from 79 women who were contacts of men with non-gonococcal urethritis were studied. Patients who had received antimicrobial chemotherapy during the previous four weeks were excluded. Endourethral specimens were obtained by inserting a cotton wool tipped wire swab 4-5 cm into the urethra; cervical swabs were taken with a cotton wool tipped wooden swab rotated in the cervical canal.

SPECIMENS

Two swabs were collected from each patient. One was inserted into the Abbott's enzyme immunoassay transport medium and the other was taken into a chlamydial transport medium.¹ Swabs were placed first or second into the transport media in a known but unpredictable sequence according to a random number table; this avoided bias of the identification system associated with the quality of specimens.

CHLAMYDIA CULTURE

Monolayer preparations of McCoy cells were prepared in Eagle's minimum essential medium with 10% fetal calf serum on glass coverslips in plastic tubes. Specimens were collected into chlamydial transport medium consisting of the minimum essential medium growth medium plus 1% 3M glucose, gentamicin (10 µg/ml), vancomycin (10 µg/ml), and amphotericin B (2.5 µg/ml).¹

To culture the organism the supernatant growth medium was removed from the coverslip preparation and the cells were treated with cycloheximide at a final concentration of 1 µg/ml.⁴ After treatment, 0.5 ml of infected chlamydial transport medium was added to each of two coverslip preparations.

The inoculated cells were then centrifuged at 3000 g for 1 h at 33°C and this was followed by incubation at 36°C for up to 72 h. After incubation one of the pair of tubes was removed and the cells were fixed with neat methanol for 10 min, stained with Lugol's iodine for 5 min, blotted, dried, and mounted. Slides were examined by bright field microscopy for the presence of the distinctive brown inclusion bodies. The number of inclusion forming units seen per low power field using a ×16 objective was estimated. Cells from the remaining tube were passaged into fresh cells. The cells were then centrifuged, and the supernatant medium replaced with 1 ml of fresh chlamydial transport medium containing 1 µg/ml of cycloheximide. Tubes were incubated and examined in the same manner as the original tube.

ENZYME ASSAY PROCEDURE

Samples were collected from the patient into the transport medium supplied by Abbott Laboratories. Specimens were examined within 72 h of collection after storage at +4°C.

Before testing, 1 ml of specimen dilution buffer (supplied with the kit) was added to each specimen tube, which was then left at room temperature for 10 min. Samples were then vortex mixed three times for 15 s each time on a Fisons Whirlimixer at maximum setting. All samples and controls were treated in this manner. After mixing, the liquid in the swab was expressed by pressing and rotating it against the side of the tube; the swab was then discarded. Immediately after this procedure the samples were assayed.

Assay

Two hundred microlitres of sample or control specimen was pipetted into one well of a plastic reaction tray, to which was added a commercially prepared polystyrene bead labelled with polyclonal antibody to *C trachomatis*. One positive and three

negative control specimens (supplied with the kit) were included with every run. After sealing, the tray was incubated at 37°C for 45 min. The beads were then washed three times in distilled water using an Abbott Pentawasher. Two hundred microlitres of rabbit serum containing antibody to *C trachomatis* was added to each bead, and the plate was resealed and reincubated at 37°C for a further 45 min. The beads were washed again three times in distilled water, and then 200 µl of horseradish peroxidase conjugated goat serum containing antibodies to rabbit IgG was added to each bead. Plates were resealed and reincubated at 37°C for a further 45 min. After three further washes in distilled water the beads were transferred to clean, clear plastic tubes (supplied with the kit) and 300 µl of substrate (o-phenylenediamine 2 HCl prepared in citrate phosphate buffer with 0.02% hydrogen peroxide) was added to each tube containing a bead and to two further clean tubes to act as substrate blanks. All tubes were left in the dark at room temperature for 30 min, after which the reaction was stopped by the addition of 1 ml of 1N sulphuric acid to each tube.

Tests were read on a Quantum spectrophotometer at 492 nm. Negative controls were required to have an absorbance of less than 0.150 and positive controls an absorbance of greater than 0.800 to validate the test. Samples from patients giving an absorbance of greater than 0.100 plus the negative control value were considered reactive. Samples were retested if the reading fell within 20% of the gross cut off range (determined as 0.100 plus the average negative reading).

Cell culture and enzyme immunoassay were carried out by different personnel in order to avoid bias in interpretation.

Statistical correlates were determined using the χ^2 test with Yates' correction.

Results

C trachomatis was isolated from 27.1% of samples (38/140), which corresponds to an isolation rate of 15.2% (12/79) for women and 42.6% (26/61) for men. The number and percentage of positive specimens using the enzyme immunoassay system were 15.2% (12/79) for women and 41% (25/61) for men. The Table shows results obtained by both culture and the enzyme immunoassay system. There were five discordant results. Two of these were positive by enzyme immunoassay and negative by culture (one man and one woman). Conversely, there were three culture positive samples which were negative by enzyme immunoassay (one woman and two men). Two of the optical density values for enzyme immunoassay negative specimens showing

Positive and negative results obtained by enzyme immunoassay and cell culture of specimens from 61 men and 79 women

	Positive		Negative	
	Men	Women	Men	Women
Results of enzyme immunoassay:				
Positive	24	11*	1	1
Negative	2	1	34	66

*One woman positive on passage only.

discordance (culture positive, enzyme immunoassay negative) were well below the retest range, and one which fell within the retest range initially had a low optical density value when retested. One of the enzyme immunoassay positive specimens showing discordance (culture negative, enzyme immunoassay positive) gave an optical density value within the retest range when the test was repeated.

Discussion

The high sensitivity and specificity values found by enzyme immunoassay compared with the culture system were impressive. There were only two possible false positive results obtained by enzyme immunoassay which could not be confirmed by culture (1.4% of total number). These may have been because of the presence of non-viable antigenic material in the specimen or may have resulted from the presence of some interfering agents in the enzyme immunoassay test system. On the other hand, there were three definite false negative results found by enzyme immunoassay (2.1% of total number). In these three cases the initial inoculum gave <1 inclusion forming units per low power field ($\times 16$ objective) and the second swab had been used for culture. The order in which the first or second swab was used for either system did not make a significant difference to the results obtained for specimens from men or women ($p > 0.5$ for men and $p > 0.1$ for women).

It is reasonable to compare the results obtained from the initial inoculation in the culture system with the enzyme immunoassay result because in most diagnostic laboratories only one tube is usually

set up for culture as duplication of the culture system and subsequent passage is expensive and time consuming. Passage yielded only one further positive culture. When compared with the enzyme immunoassay system the results from the initial inoculum showed an overall sensitivity of 92.5% and specificity of 97.2%. The enzyme immunoassay system was slightly more sensitive for urethral specimens from men (92.6% for men and 92.3% for women) but had a higher specificity for cervical specimens (98.5% for women, 97.3% for men). This difference was not significant ($p > 0.5$).

The sensitivity and specificity of the enzyme immunoassay method found in this study are comparable to the results obtained by Jones *et al.*⁵ who obtained a sensitivity of 81% and a specificity of 98% during phase II of their study using a modified enzyme immunoassay kit similar to the kit evaluated by us.

The results obtained with the Abbott's enzyme immunoassay system for the detection of *C trachomatis* antigen are encouraging. Although there is some scope for improvement of the sensitivity of the enzyme immunoassay test, it proved to be a simple and rapid diagnostic technique, taking only three and a half hours to perform, which could easily be used to process a large number of samples.

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