

Evaluation of an enzyme-linked immunoassay and confirmatory test for the detection of *Chlamydia trachomatis* in male urine samples

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Abstract

First pass urine (FPU) samples were compared with urethral swab culture from 304 males attending a genitourinary medicine clinic using an enzyme immuno assay (EIA). All of the EIA positive samples were retested by incorporating a novel blocking reagent into the EIA protocol; 101 were positive by culture of which 83 were also positive by FPU EIA, an additional four were detected in FPU only and not by culture; 86 of these 87 were also confirmed positive by the blocking reagent. Discrepant results were evaluated by Syva MicroTrak. The sensitivity and specificity of FPU EIA as compared with urethral swab culture was 82.2% (83/101) and 98% (199/203) respectively with positive and negative predictive values of 95.4% (83/87) and 91.7% (199/217). Male urethral swab culture is more sensitive than FPU EIA; however, when culture is not available then FPU offers a reliable non-invasive alternative to swab EIA which may be of enormous benefit in community screening of asymptomatic as well as symptomatic patients.

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Introduction

The reference standard for the detection of *Chlamydia trachomatis* from urogenital samples is by tissue culture. Although this is by no means 100% sensitive it is still the "benchmark" by which other methods are assessed. However, when utilising first pass urine (FPU) samples as an antigen source, tissue culture is of little value and enzyme immunoassay (EIA) and direct immunofluorescence (DIF) are the methods of choice.¹⁻³ A difficulty arises when investigating FPU as a suitable *alternative* to urethral swabs as to which samples and methods are employed to compare results. Since our original study where urethral swab culture was compared with FPU amplified EIA,⁴ the relative merits of these techniques have been evaluated by many investigators using various 'benchmarks'.⁵⁻⁷ In this study we have compared the urethral swab culture status with FPU conventional EIA (AntigEnz Chlamydia, Shield Diagnostics Ltd., Dundee, U.K.) and confirmed the EIA positives with a novel blocking reagent (AntigEnz Chlamydia Confirmatory Test, Shield Diagnostics Ltd.)

and DIF. In a multicentre study using urogenital swabs, the AntigEnz Chlamydia EIA test has been shown to have a sensitivity of 87% in men and 86% in women, with specificities of 94% and 97% respectively when compared with swab culture.⁸

Methods

Specimen collection

Urethral swabs and FPU samples were collected from 304 males with symptoms of dysuria and/or urethral discharge, attending the Department of Genitourinary Medicine, General Hospital, Birmingham over a 10 week period. In the first group (A) of 151 patients the urethral swabs were collected prior to the urine samples and in the second group (B) of 153 patients, this procedure was reversed. The urethral swab was placed in 2 ml of chlamydia culture transport medium and both the FPU and urethral swab were stored at +4°C for no longer than 24 hours before transportation to the laboratory.

Laboratory procedures

The urethral swabs were vortex mixed and inoculated onto two coverslip cultures of McCoy cells (ICN Flow, High Wycombe, UK) treated with cycloheximide and incubated at 35°C for 48-72 hours. After fixing one of the coverslip cultures in methanol for 10 minutes it was stained by a group specific chlamydia monoclonal antisera (Imagen Dako Ltd., High Wycombe, UK) and examined by epifluorescence microscopy for typical intracytoplasmic inclusions. The remaining culture was passaged into fresh McCoy cells and incubated for a further 48 hours.

Any urines that were cloudy in appearance were warmed at 37°C to dissolve cold precipitins prior to all being cultured for bacterial contaminants by inoculating onto blood agar using a standard 10 µl loop and incubating aerobically at 37°C for 24 hours.

If excess urine had been passed, the volume was adjusted to 15 mls, and centrifuged at 2,500 g for 20 minutes, the supernatant discarded and deposit resuspended in 1 ml of AntigEnz Chlamydia transport medium. After vortex mixing 200 µl was removed from each sample and stored in microvials at -70°C. In the event of discrepant results these stored samples, together with remaining EIA and culture specimens, were examined for *C trachomatis* elementary bodies (EBS) using species specific monoclonal antisera (Syva MicroTrak, Palo Alto USA). AntigEnz

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Table 1 Summary of results

Urethral culture	+	+	-	-
Urine EIA	+	-	+	-
	83	18	4	199 = 304

Chlamydia was performed according to the manufacturer's protocol and all results were recorded photometrically.

Results

Bacterial cultures

Although a wide variation in results were recorded from the blood agar culture the majority were of "no growth" or "scanty skin flora". There were no specimens that were EIA positive and chlamydia culture negative that could not be confirmed as true positives, and none of those samples with bacterial growth caused any significant rise in the recorded optical density (OD).

Chlamydia cultures

There were seven swab samples that caused very slight toxicity in the first monolayers of both negative and positive cultures, but as none of these were thought toxic enough to warrant exclusion from the trial all of the collected samples were included.

In the first group of 151 patients, where the swab was taken first, the overall detection rate by both methods was 28.4% (43/151) and by tissue culture alone 27.1% (41/151). In the second group of 153, where the urine was collected first the detection rates were 40.5% (62/153) and 39.2% (60/153) by tissue culture. There were two cases where the chlamydia culture was only positive after subculture, both were in group B and both were also positive by urine EIA. As shown by ourselves and other workers,^{2,4} the order of collection made no significant difference to the urine EIA sensitivity compared with positive urethral cultures, being 82.9% (34/41) in the first group and 81.7% (49/60) in the second. A summary of the results is given in table 1.

Confirmation of positive results using blocking antibody

In addition to the clinical evaluation of AntigEnz Chlamydia for urine testing a concurrent study utilising a novel blocking antibody was carried out on all of the positive EIAs. Briefly, a polyclonal rabbit anti-chlamydia serum and equivalent concentration of normal rabbit serum was added to two wells after the addition of the test sample. Those samples that had given an initial high OD reading (> 2.0 absorbance units) were diluted 1:11 with freshly boiled transport

medium prior to retesting. A 50% or greater reduction in the OD reading in the blocked well, as compared to the control well, confirmed the presence of chlamydia antigen. Of the 89 specimens that were re-tested using the blocking reagent, 86 were confirmed as positive by blocking and in one there was insufficient sample remaining. In the other two specimens that did not block, one was culture positive but with no visible EBs in the stored aliquot and the other was culture negative but had EBs in the stored aliquot and remaining swab sample. All three had OD readings within the "grey zone" (the threshold plus 0.05 absorbance units), but with reproducibility being a signal of positivity, coupled with a finite amount of specimen remaining, they were classified as EIA/blocking test negative according to the manufacturer's protocol, even though they were proved positive by the other means.

Discordant results

Besides analysing the discordant results of EIA positive and culture negative samples it was considered that all the culture positive and EIA negative should also be examined. There were four of the former (table 2) and 18 of the latter (table 3).

Of the 22 samples that initially gave negative EIA results with positive cultures, four became EIA positive when repeated and all proved to be true positives by either or both the blocking test and stored aliquot.

Comparison of urine EIA with urethral swab culture

As there was no significant difference between the first and second groups of patients the overall detection rate by both methods was 34.5% (105/304) with 28.6% (87/304) by urine EIA and 33.2% (101/304) by urethral culture. The sensitivity of the urine EIA as compared with the urethral swab culture was 82.2% (83/101) with a specificity of 98% (199/203). The positive and negative predictive values were 95.4% (83/87) and 91.7% (199/217) respectively with overall agreement of 92.8% (282/304) (see table 4). After resolution of the discrepant results the sensitivity of the urine EIA rose slightly to 82.8% (87/105). These results compare favourably with other EIA test evaluations on male FPU samples.^{4,5,6,7}

Discussion

In this study the sensitivity of tissue culture was 96.2% (101/105) as compared with the

Table 2 Four EIA positive/culture negative samples

Lab No.	Urine EIA	EIA Blocked	Urethral Culture	EBs Detected		
				Stored aliquot	EIA	Swab
2810	+	No	Neg	Yes	N/a	Yes
2963	+	Yes	Neg	Yes	N/a	Yes
4041	+	Yes	Neg	No	Yes	Yes
4568	+	Yes	Neg	Yes	N/a	No

Table 3 Eighteen EIA negative/culture positive samples

Samples	Urethral Culture	Urine EIA	EBs Detected	
			Stored aliquot	Swab
8*	+	-	-	+
10	+	-	+	+

*In two specimens the repeat EIA OD readings fell in the "grey zone" (the threshold plus 0.05 absorbance units) but as there was insufficient remaining sample to block in one and the other was negative on blocking they were classified as negative.

Table 4 Urethral swab culture v urine EIA

	Urethral swab culture	
	Positive	Negative
Urine EIA Positive	83	4
Urine EIA Negative	18	199

overall detection rate by both methods. If this new figure is used for comparison of the EIA sensitivity then the adjusted results are 79.8% (82.9%) in the first group and 78.6% (81.7%) in the second (original results in brackets). This lower sensitivity represents a more accurate figure and should be considered when evaluating differing methods of detection. EIA is probably only 85–95% as sensitive as culture which, in turn is thought to detect only 80–90% of actual infections.⁹

Immunofluorescence on the other hand, is a very sensitive technique, whether used in a direct manner or as a confirmatory test. Thomas *et al*¹⁰ has recently shown that DIF on 1 ml of FPU deposit was as sensitive as EIA and may be the most sensitive method for utilising FPU samples, a conclusion we also came to when investigating sterile pyuria, but for differing reasons.¹¹ However, the factors governing the choice of method for the detection of *C trachomatis* is not only sensitivity but also cost and level of technical competence required to satisfy quality assurance. DIF may be the most sensitive method for FPU but it also requires the highest degree of skill, is time consuming and is often a task undertaken by senior staff with increased revenue consequences.

In this present study we also evaluated a simple confirmatory kit with two prediluted, blocking and control reagents that were added to the usual assay scheme when confirming previously detected positives.

All of the initial 89 EIA positives detected in FPU were confirmed either by culture or DIF and 86 were confirmed by the blocking test. It was noted that in those samples that gave an original high OD reading and were diluted prior to retesting the strength of the new OD signal was not always reduced in proportion to the dilution and in a few cases actually increased. Although this phenomenon has been seen in other centres (personal communication, EO Caul) and is related to the initial volume of urine/antigen chosen, it had no detrimental effect on the outcome of the blocking tests.

The confirmation of true positives by an EIA blocking mechanism is to be encouraged in all laboratories and especially in those that cannot confirm by DIF on the remaining sample. This is particularly important in low prevalence populations where the positive predictive value can be very low. For this reason it would be useful to evaluate AntigEnz Chlamydia blocking reagent in a low prevalence group to see if these results can be maintained.

In our 1987 study using an amplified EIA system (IDEIA Dako Diagnostics Ltd)⁴ we concluded that urethral swab culture was superior to FPU EIA and this present study

utilising conventional EIA technology, confirms this view. However, since then, the use of tissue culture in the United Kingdom for the detection of *C trachomatis* has declined. The results of questionnaires by the National External Quality Assessment Scheme^{12,13} show that the number of responding laboratories offering culture has fallen from 36 in 1988 to 24 in 1990. Moreover, only two of the 24 offered culture alone and the other 22 also performed EIA or DIF. In the same period of time, the number using EIA has risen from 105 to 135 and DIF from 141 to 154.

The accepted use of EIA when screening large numbers of samples as a suitable, albeit less sensitive method than tissue culture, is a reflection of kit manufacturers' continued refinement and quality assurance of the product. Given this alternative it is not surprising that many laboratories have forsaken culture in favour of EIA.

It therefore follows that if chlamydia culture is not generally available for swab samples from male urethra then FPU should be considered for the advantages, not only for the patient, but also for the clinician and laboratory. From the patients' point of view there is no trauma or pain involved in collecting the specimen, which may then be supervised by nursing staff after the initial medical consultation. This relieves the clinician of swab collection duties and could create a greater throughput of patients. For the laboratory, FPU is a more uniform specimen that can be divided, examined by more than one method and removes the inherent "sampling error" often associated with inadequate swabbing. This is an important point when diagnosing genital chlamydial infection from males in the community; especially general practice, where there are often inappropriate swabs or transport medium available. Furthermore, utilising a non-invasive means of diagnosing *C trachomatis* infection would be of enormous benefit in mass-screening of asymptomatic men to provide hitherto unavailable epidemiological information as well as aiding the diagnosis of asymptomatic cases and thus the control of genital chlamydial infection.

The Chlamydia AntigEnz test gave good positive and negative predictive values (95.4% and 91.7% respectively) on a highly selected group of males attending a GUM clinic; however, caution should be taken when extrapolating these findings to a lower prevalence population. Taking all these factors into consideration and if EIA is the only method available for the detection of *C trachomatis* from male urogenital specimens then there is little to be gained from taking urethral swabs when FPU samples will give the same, or slightly reduced, sensitivity.

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