

Evaluation of Three *Chlamydia trachomatis* Immunoassays with an Unbiased, Noninvasive Clinical Sample

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First-catch early morning urine samples from patients attending a genitourinary medicine clinic were tested by three different enzyme immunoassays. The results suggest significant differences in the sensitivities and specificities of the different assays. The direct visualization of elementary bodies in urine deposits by direct immunofluorescence was used as the "gold standard," using a monoclonal antibody with a different epitope specificity from those of antibodies used in the enzyme-linked immunosorbent assays. We report for the first time that urine specimens represent an unbiased sample, free of the inherent sampling errors associated with other genital specimens. We feel that urine is a valid specimen for use in any evaluation study of new assays directed towards the detection of *Chlamydia trachomatis*.

Cell culture techniques are traditionally used as the "gold standard" for the detection of *Chlamydia trachomatis* in clinical specimens. In recent years, tests using new biotechnology have been compared with cell cultures to evaluate the sensitivities and specificities of these recently introduced techniques (1, 2, 6-8, 10-12). Many of these studies have highlighted the problems associated with the taking of replicate swabs from individual patients, a procedure which is prone to sampling errors (4, 5, 7, 9) and therefore variations in the apparent sensitivities of assays under evaluation.

We have recently established that first-voided early morning urine samples are suitable specimens for demonstrating *C. trachomatis* and represent a very acceptable noninvasive technique for male populations (3). The advantage of urine samples is that they allow unbiased antigen presentation to any of the new assays available and overcome the problems of sampling errors that have plagued all earlier comparative studies. We present our results of three different enzyme-linked immunoassays (ELISAs) in which positive and negative specimens were confirmed by the presence or absence of elementary bodies in urine deposits by direct immunofluorescence. A particularly important aspect of this study was the choice of monoclonal antibody used for confirmation and identification of elementary bodies in the urine deposits. We felt that this monoclonal antibody should have an epitope specificity to the major outer membrane protein of *C. trachomatis* rather than to the lipopolysaccharide epitope of antibodies used in ELISAs.

Study group. Patients requesting appointments at the Genito-Urinary Medicine Clinic in Bristol were allocated standard laboratory urine containers and were asked to hold their urine overnight before collecting approximately 50 ml of first-voided early morning urine. Appropriate urethral or endocervical swabs were taken from each patient at the clinic.

Urine samples, for comparative studies, from 91 unselected patients (29 females and 62 males) were used on the basis of an unequivocal positive (21 patients) or negative (70 patients) urethral or endocervical swab when tested by amplified ELISA (IDEIA). We have previously demonstrated by direct immunofluorescence (3) that elementary bodies are invariably present in freshly voided early morning

urine samples from patients with positive urethral or endocervical swabs, and this was used as our "gold standard" for the reason outlined in the discussion.

MATERIALS AND METHODS

Urine samples. On arrival at the laboratory, well-mixed urine samples were divided into 4 equal volumes (10 ml). Each aliquot was centrifuged at $3,000 \times g$ for 30 min. The deposit from three aliquots was suspended in either 1 ml of ELISA transport medium or buffer supplied with assays from each of the manufacturers. The deposit from the remaining aliquot was washed in phosphate-buffered saline, centrifuged as described above, and suspended in phosphate-buffered saline (0.2 to 0.5 ml) to achieve a turbid suspension. We found that the final concentration of cells was important to achieve a single layer of cells for final examination by direct immunofluorescence. Smears were then prepared by adding 25 μ l of suspended deposit to a 6-mm well of a Teflon-coated slide. These smears were dried at 37°C before fixation in acetone for 5 min at room temperature.

Urethral and endocervical swabs. Detection of *C. trachomatis* antigen was carried out, using our standard ELISA technique (IDEIA; Novo Bio) (2).

Immunofluorescence techniques. All smears were stained by a direct immunofluorescence technique (Syva Microtrak). The whole area of the wells was systematically scanned at a magnification of $\times 1,000$, using incident blue light microscopy.

ELISA. Three commercially available ELISAs were used to detect *C. trachomatis* antigen in urine deposits. They were Chlamydiazyme (Abbott Laboratories), which uses a polyclonal antibody as an antigen capture, and IDEIA and the Pharmacia chlamydia ELISA, both using a genus-specific monoclonal antibody for antigen capture. All assays were carried out according to the recommendations of the manufacturers.

RESULTS

Assay 1 (IDEIA). All 21 patients with positive urethral or endocervical swabs were also positive when early morning urine samples were tested. Of these 21 positive urine samples, 19 were confirmed by the presence of typical elemen-

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TABLE 1. Comparison of three ELISAs with direct immunofluorescence on first-voided early morning urine deposits^a

Occurrence of <i>C. trachomatis</i> in urethral or cervical swab ^b	No. of patients with urine samples tested by:							
	Assay 1		Assay 2		Assay 3		Direct immunofluorescence	
	+	-	+	-	+	-	+	-
+	21	0	16	5	8	13	19	2 ^c
-	0	70	10	60	0	70	0	70

^a +, *C. trachomatis* present; -, *C. trachomatis* absent.

^b When tested by amplified ELISA (IDEIA).

^c Two female patients with *C. trachomatis*-positive endocervical swabs.

tary bodies in smears prepared from the urine deposit after staining with Syva Microtrak (Table 1).

Assay 2 (Chlamydiazyme). Chlamydiazyme was less sensitive, as only 16 of the 21 positive patients had detectable antigen in their early morning urine samples. In addition, this assay had a poorer specificity, as 10 false positives were detected among the 70 negative patients. Of these 10, 4 false-positive urine samples had optical densities between 1.0 and 2.0. Direct immunofluorescence failed to confirm any of these 10 false positives (Table 1).

Assay 3 (Pharmacia chlamydia ELISA). The Pharmacia chlamydia ELISA did not detect any false-positive urine samples. However, sensitivity was markedly less than that of either of the other assays (Table 1): only 8 of the 21 positive samples were detected.

Direct immunofluorescence. Of the 91 patients screened for *C. trachomatis*, 19 male patients had elementary bodies (>5) detected in their urine deposits (Table 1). Elementary bodies were not detected in the remaining 72 smears.

DISCUSSION

The introduction of recent biotechnology for the detection of *C. trachomatis*, as an alternative to cell culture methods, has resulted in many workers carrying out comparative studies. Many of these studies have rightly discussed the problems associated with the sampling errors which arise from the collection of multiple urethral or endocervical swabs. An additional but less-well-documented problem is the inadequacy of urethral swab sampling, which may result in 10 to 20% of poorly collected swabs. As a result, it is still unclear which assays have acceptable sensitivities and specificities.

We previously reported on the suitability of first-voided early morning urine samples as sources of *C. trachomatis* antigen (3). As a result, we undertook the present comparative study to evaluate three commercially available ELISAs, using freshly voided early morning urine as an unbiased sample, free from the inherent sampling errors associated with urethral or endocervical swabbing; it is the first report of its kind.

In this study, 21 patients had positive urethral (19 males) or endocervical (2 females) swabs. Swabs from the remaining 70 patients were negative when tested by amplified ELISA (IDEIA). We chose to confirm the presence or absence of *C. trachomatis* antigen in urine samples from all patients by using a monoclonal antibody in a direct immunofluorescence assay (Syva Microtrak) which had a different epitope specificity from that of the monoclonal antibody used in the ELISAs under evaluation (i.e., lipopolysaccharide specificity). Direct immunofluorescence examination of

smears from urine deposits, rather than culture confirmation, was chosen as the "gold standard," since chlamydiae lose infectivity in urine samples.

During the course of our early studies, it became apparent that the method of preparation of smears from urine deposits was important in the final visualization of elementary bodies. Washing the deposit in phosphate-buffered saline before smears were prepared prior to staining and microscopical examination was critical. The density of the suspended deposit was also important, and smears which contained too many cells had to be repeated to achieve a single layer of cells for visualization of elementary bodies. All urine samples in this study contained numerous squamous epithelial cells to which the *C. trachomatis* elementary bodies adhere, allowing for their deposition on centrifugation. It is possible that acellular urine from a positive patient is difficult to confirm by direct visualization of the organism. We have not yet experienced this problem. Given these criteria, we could confidently identify one or two elementary bodies, although this low cutoff was not necessary in this study.

Our comparative studies of the three commercially available ELISAs demonstrated significant differences in their sensitivities and specificities. It was apparent that assay 2 detected an unacceptable number of false positives in our negative group of patients, which is in agreement with a previous report (8). At the time of our study, a neutralization assay was unavailable for confirmation of these positive samples. This assay also failed to detect approximately 25% of the true positives (5 of 21). In contrast, assay 3 did not detect any false positives (high specificity) but failed to detect approximately 62% of the true positives (poor sensitivity). In this respect, it was apparent that this assay detected antigen in all urine samples that contained >10 elementary bodies. It should be stated that the manufacturer of neither assay 2 nor assay 3 has claimed that the assay detects *C. trachomatis* in urine samples. Further studies by the manufacturers may improve and optimize these assays for the detection of antigen in urine. The IDEIA incorporates an amplification step and also uses a genus-specific lipopolysaccharide monoclonal antibody (7) which may have a better avidity for *C. trachomatis* antigen in clinical samples. Both these explanations would account for the superior sensitivity and specificity of this assay. This is further emphasized by the lack of correlation between the number of elementary bodies present when Syva Microtrak (species-specific monoclonal antibody) is used in smears prepared from urine deposits and the optical density readings obtained in the IDEIA (data not presented). This suggests that free lipopolysaccharide is present in clinical samples and is available for enhancing the sensitivities of assays which use a lipopolysaccharide monoclonal antibody epitope (1). We have noted a similar lack of correlation in our previous studies when optical density readings are compared with the number of inclusion-forming units in cell cultures (6).

In the study described here, two female patients with positive endocervical swabs in an ELISA were not confirmed by direct immunofluorescence of elementary bodies in urine deposits. This is not surprising, as we have previously shown that urine samples are not reliable for the detection of *C. trachomatis* infection in all infected women (3). The reasons are clear if urine samples collected from female patients with cervical (rather than urethral) infection are assayed.

In conclusion, these studies have demonstrated significant differences in the specificities and sensitivities of different commercially available assays. We believe that the validity

of these results is due entirely to the use of a single urine sample which is free of the sampling errors known to occur with other genital specimens when multiple swabs are collected. Finally, the collection of first-voided early morning urine samples represents a very acceptable noninvasive technique for the evaluation of new assays. In addition, our current prospective studies suggest that first-voided early morning urine samples are better than urethral swabs for screening male populations and will prove important as a further step in the control of genital *C. trachomatis* infections.

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