TECHNICAL NOTE

Evaluation of verification assays in EIA specimens presumptively positive for *Chlamydia trachomatis*

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Verification of specimens positive for *Chlamydia trachomatis* by enzyme immunoassay (EIA) has been recommended when testing low prevalence populations. This study compared direct fluorescent antibody (DFA) and blocking antibody (BLA) verification assays in specimens presumptively positive for *C. trachomatis* by the Syva Microtrak II EIA. Of 1785 specimens originally tested by EIA, 96 were presumptively positive for *C. trachomatis*. Verification assays were concordant in 86 specimens (69 positive, 17 negative); nine of the remaining samples gave positive results in a second EIA and one was unresolved. Both verification assays gave some false-negative results. When initial EIA absorbance values were correlated with verified results, all EIA false positive results had absorbances in the low range (less than a three-fold increase over assay cut-off values). Verification of EIA results by both DFA and BLA was effective in detecting false positive results, but confining verification to low-value positive specimens could be considered for cost-effective *C. trachomatis* testing.

Introduction

Recognised as the most prevalent agent of sexually transmitted disease, *Chlamydia trachomatis* is associated with significant health problems and health-care costs. The development of practical, population-specific strategies for the detection and management of infection with this agent is critical [1].

Although imperfect, cell culture has long been considered the 'gold standard' for *C. trachomatis* diagnosis. Difficulties associated with culture, including stringent requirements for specimen collection and transport, relatively high labour and procedural costs, the need for technical expertise in interpretation and delays in reporting results, make it impractical as a screening method. Consequently, rapid diagnostic methods such as direct fluorescent antibody (DFA) tests and enzyme immunoassay (EIA) have become 'a cornerstone of chlamydia prevention strategies' [1].

EIA kits based on antibodies that detect the genus-specific lipopolysaccharide (LPS) component of chla-

mydia can cross-react with other micro-organisms including *Acinetobacter* spp., *Klebsiella* spp., *Streptococcus* spp. and *Gardnerella* spp. [2]. Assay performance can also be compromised by improper specimen collection [3, 4]. Because of these potential specificity problems, the Centers for Disease Control and Prevention (Atlanta, GA, USA) has recommended verification of EIA-positive results in situations where reporting a false-positive result could have adverse social, medical or psychological consequences [1]. Verification is also recommended in low prevalence populations (<5%) because the predictive value of a positive result declines with prevalence.

In the present study, a DFA and a blocking antibody assay (BLA) were compared for the verification of presumptively positive EIA results in a low prevalence population. Both assays are practical choices for verification because they are relatively inexpensive and can be performed on the residue of the EIA specimen.

The MicroTrak DFA (Syva Co., San Jose, CA, USA) contains monoclonal antibodies directed against the major outer-membrane protein (MOMP) of *C. trachomatis*. Thus, verification is accomplished by detecting a chlamydial antigen other than that detected by EIA

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(LPS). The MicroTrak II Chlamydia EIA Blocking Reagent contains both rabbit- (also a component of the initial EIA) and mouse-derived LPS antibodies. The mouse and rabbit antibodies compete for chlamydial LPS binding sites, but only the latter is subsequently detected by anti-rabbit immunoglobulin conjugate, which contains the colorimetric enzyme. A reduction in the initial absorbance value of >50% is accepted as verification of a presumptively positive result.

A second LPS-detecting EIA (Prima EIA, Dade Diagnostics, Issaquah, WA, USA) was used to evaluate specimens that produced discrepant results in DFA and BLA verification. This assay was chosen rather than cell culture because it permitted re-testing of the original EIA specimen, whereas it would have been impractical to obtain a repeat specimen for culture.

Selective confirmation may help to limit costs while maintaining overall screening accuracy in low prevalence populations. In an effort to predict which EIA-positive specimens are most likely to be false positive and, therefore, to warrant verification, the results of verification tests were compared with absorbance values in the initial EIA.

Materials and methods

Patient population and specimen collection

A total of 1785 specimens (cervical, urethral, urine, conjunctival) were collected over a 4 month period from symptomatic and asymptomatic male and female patients attending various clinics and units (obstetrics, gynaecology, sexually transmitted disease, family planning, eye and adolescent clinics; medical emergency room) at the Nassau County Medical Center. Syva MicroTrak EIA collection kits were used for all specimens except male urine specimens, which were collected and processed as described previously [5]. All specimens were maintained at 2–8°C and tested within 7 days. Physicians were not informed of the study to prevent specimen collection bias which may have influenced EIA results.

Screening and verification

All specimens were tested by the MicroTrak II Chlamydia EIA (Syva Co.) according to the manufacturer's protocol. Presumptively positive specimens were re-tested by a blocking assay performed according to the protocol in the MicroTrak II Chlamydia EIA Blocking Reagent package insert. For DFA verification, 0.2 ml of the EIA specimen was prepared and examined as described previously [5]. Specimens were considered positive for *C. trachomatis* if 10 or more elementary bodies were observed. This cut-off value

was chosen because it was used by the manufacturer in the collection of sensitivity and specificity data and its use is recommended when first using DFA. DFA slides were examined without knowledge of BLA results.

Resolution of discrepant results

A further 0.2 ml of the original specimen was re-tested with a second polyclonal LPS-based EIA kit (Prima System EIA *C. trachomatis*; Baxter Diagnostics, Issaquah, WA, USA) according to the manufacturer's instructions.

Results and discussion

Of the 1785 specimens tested, 96 were presumptively positive for *C. trachomatis* by MicroTrak II EIA. The distribution of positive specimens by gender (85 female and 11 male) reflected the ratio of male and female specimens submitted for EIA testing.

Verification assays were concordant in 86 of the 96 specimens (Fig. 1). Of the 10 specimens with discrepant results, nine gave positive results by Prima EIA. Since these specimens were positive in three out of four assays, they were considered true positives. The remaining specimen (urethral) was considered unresolved, since it was positive by two assays and negative by two assays, and it was excluded from data analysis.

At the completion of all testing, 78 (82.1%) of 95 presumptively positive specimens were found to be true positive (4.4% prevalence of *C. trachomatis* infection). Positive specimens were obtained from cervical (68), urethral (five) and eye (one) body sites and from male urine (four). All 17 specimens presumptively positive by EIA (17.9%), but subsequently negative on confirmatory testing were obtained from the cervix. This may be due to the presence of other bacterial species in the female genital tract that cross-react with chlamydial LPS. The small number of male specimens in the present study precludes statistical analysis of any possible gender difference in false-positive EIA results.

One advantage of DFA over BLA verification is that specimen quality, and, therefore, the need for training in specimen collection, can be assessed. Drawbacks to DFA testing include the equipment and technical expertise required, which may preclude its use in large volume laboratories. In contrast, BLA antibody verification can be easily incorporated into the normal EIA testing routine.

The distribution of verified positive and false-positive specimens *versus* absorbance in the initial EIA, expressed as the signal to cut-off ratio (S/CO), was evaluated (Table 1). The absorbance values of true

Fig. 1. Planning sequence and results of screening and verification assays for *C. trachomatis* on 1785 clinical specimens.

positive specimens were distributed over a wide range of S/CO values. In contrast, the S/CO ratio for 15 of the 17 false-positive specimens and for the unresolved urethral specimen was <2 . A strong correlation between EIA false-positive results and low absorbance values has been demonstrated previously with the Chlamydiazyme EIA [4] and the MicroTrak EIA [6]. These findings may permit laboratories to confine

verification testing to a range of weak-positive EIA specimens.

The effectiveness of both DFA and BLA assays in verifying presumptively positive EIA results in a low prevalence population is clearly demonstrated by the present study. Unfortunately, both assays had some false negative results (three for DFA, six for BLA),

Table 1. Distribution of signal to cut-off values (S/CO) by Syva MicroTrak II EIA for presumptively positive specimens and their corresponding verification assay results

S/CO	Number of tests	Verification assay results	
		Positive (%)	Negative (%)
1.0–1.9	42	27 (64)	15 (36)
2.0–2.9	18	16 (89)	2 (11)
3.0–3.9	14	14 (100)	0 (0)
4.0 +	21	21 (100)	0 (0)

but this difference between the DFA and BLA assays was not significant. Infection with *C. psittaci* or *C. pneumoniae*, which lack MOMP antigen, could account for the DFA false negativity. However, this explanation is unlikely as these specimens were collected from genital sites, which are not normally infected by these chlamydial species. Further studies should be undertaken to determine the common characteristics (e.g. absorbance, specimen type), if any, of true positive specimens that give negative results by verification assays.

In summary, verification assays enhance the specificity of the MicroTrak II EIA, thus permitting positive results to be confidently accepted in low prevalence populations.

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