TECHNICAL NOTE

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

G. P. LEONARDI*‡, A. M. STAVROULAKIS§, J. CRUZ* and K. SZABO*

*Department of Pathology, Nassau County Medical Center, East Meadow, New York, ‡Sunrise Medical Laboratory, Hauppauge, New York and §Department of Biological Sciences, Kingsborough Community College, Brooklyn, New York, USA

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 chlamydia 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.
been impractical to obtain a repeat specimen for the initial EIA) and mouse-derived LPS antibodies.

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.

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

<table>
<thead>
<tr>
<th>S/CO</th>
<th>Number of tests</th>
<th>Verification assay results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-1.9</td>
<td>42</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>2.0-2.9</td>
<td>18</td>
<td>27 (64)</td>
</tr>
<tr>
<td>3.0-3.9</td>
<td>14</td>
<td>14 (100)</td>
</tr>
<tr>
<td>4.0+</td>
<td>21</td>
<td>21 (100)</td>
</tr>
</tbody>
</table>
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.

The authors gratefully acknowledge the efforts of Ms Maggie George in editing this manuscript.

**References**