Measurement of IgG antibodies to Chlamydia trachomatis by commercial enzyme immunoassays and immunofluorescence in sera from pregnant women and patients with infertility, pelvic inflammatory disease, ectopic pregnancy, and laboratory diagnosed Chlamydia psittaci/Chlamydia pneumoniae infection

C S Jones, P A C Maple, N J Andrews, I D Paul, E O Caul

Background: Screening for Chlamydia trachomatis specific antibodies is valuable in diagnosing asymptomatic pelvic inflammatory disease (PID) and tubal damage following repeated episodes of PID. The assays in current use are unsuitable for screening large numbers of samples so there is a need to develop more suitable assays.

Aims: To compare the performance of several commercial C trachomatis enzyme immunoassays (EIAs) [SeroCT, C trachomatis IgG EIAs] using major outer membrane protein (MOMP), an inactivated organism EIA (Genzyme Virotech EIA), and a genus specific EIA (Platelia Chlamydia IgG) with the whole cell inclusion immunofluorescence (WIF) assay. In addition, to adapt, using time resolved fluorescence technology, the assay showing the highest correlation with WIF.

Methods: Ninety sera from patients presenting with ectopic pregnancies, 187 sera from those with a variety of types of infertility, 33 sera from cases of PID where a fourfold rise in WIF titre occurred, and 90 sera from antenatal clinic attenders were tested. A panel of 36 sera from laboratory diagnosed cases of Chlamydia psittaci/Chlamydia pneumoniae infection was also tested.

Results: The Genzyme Virotech EIA showed the highest rank correlation coefficient (0.82) with WIF, particularly at high WIF titres. The MOMP specific assays varied in their correlation with WIF, with rank correlation coefficients ranging from 0.70 (Medac p-EIA) to 0.80 (Vircell EIA). The Genzyme Virotech assay showed poor specificity (5.6%; 95% confidence interval (CI), 0.68% to 18.7%)—it was reactive with 34 of the panel of 36 C psittaci/ C pneumoniae positive sera. The MOMP based EIAs showed high specificity, particularly the Medac p-ELISA (97.2%; 95% CI, 85.5% to 99.9%)—only one serum was reactive. In view of the good correlation between WIF and the Genzyme Virotech EIA, a time resolved fluorescence immunosassay (TRFIA) was developed using the Genzyme Virotech antigen. Using an appropriate cut off the TRFIA assay showed excellent correlation with WIF.

Conclusions: The TRFIA assay may be useful as a screening assay, possibly in conjunction with one of the highly specific EIAs studied (for example, Medac p-EIA) to confirm the antibody specificity of sera selected by the screening assay.

Chlamydia trachomatis infection is the most common sexually transmitted bacterial disease in England, Wales, and Northern Ireland, with 64 000 diagnoses made in the year 2000.1 Most C trachomatis lower genital tract infections are asymptomatic and the most common clinical presentation in women is mucopurulent cervicitis, and in men urethritis. For lower genital tract infection, the detection of specific antibodies in a single serum specimen is held to be of little value because such antibodies are frequently found in sera from women who do not have active infection.2 Despite the difficulty of differentiating between previous and current lower genital tract infection, there is a considerable amount of evidence that the presence of C trachomatis specific antibody is significantly associated with upper genital tract infection, particularly when the antibody is at a high titre.3 4 Screening for C trachomatis specific antibodies is valuable in diagnosing asymptomatic pelvic inflammatory disease (PID) and tubal damage following repeated episodes of PID, particularly because it has been shown that C trachomatis is rarely isolated from the upper genital tract and clinical diagnosis requires invasive procedures not routinely available in general practice.5

There are two accepted reference assays for measuring C trachomatis specific antibodies, the microimmunofluorescence assay (MIF) of Wang and colleagues6 and the whole cell inclusion immunofluorescence assay (WIF) of Richmond and Caul.7 The WIF assay is a single antigen immunofluorescence test in which cytochalasin B treated McCoy cells infected with an LGV type 2 strain of C trachomatis are placed in wells on slides coated with polytetrafluoroethylene. In this system, the whole chlamydial inclusion acts as the antigen, in contrast to the MIF test in which elementary bodies act as the antigen. The WIF test detects both genus specific (lipopolysaccharide; LPS) antibody and species specific major outer membrane protein (MOMP) antibody and, like MIF, it is a subjective, labour intensive assay not suited to screening large numbers of sera. Our laboratory uses the WIF assay because we have found it to be more reliable for the diagnosis of upper genital tract infection than MIF, and also because inclusions are easier to visualise than cell free elementary bodies.7

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; LPS, lipopolysaccharide; MIF, microimmunofluorescence assay; MOMP, major outer membrane protein; PID, pelvic inflammatory disease; TRFIA, time resolved fluorescence immunoassay; WIF, whole cell inclusion immunofluorescence.
Time resolved fluorescence immunoassay is suitable for the measurement of low and high amounts of antibody, even with single dilutions of specimen, because of its large linear dynamic range, and has previously been assessed for population screening of anti-\textit{C. pneumoniae} IgG, where its objectivity, reproducibility, and amenability to automation were distinct advantages compared with MIF.

Our study had two objectives: (1) to compare the performance of several commercial \textit{C. trachomatis} antibody assays in relation to WIF, with a view to assessing their specificity and sensitivity for \textit{C. trachomatis}; and (2) to adapt, using time resolved fluorescence technology, the commercial assay showing highest correlation with WIF.

**MATERIALS AND METHODS**

**Sera tested**

A set of 310 sera was assembled, comprising 90 sera from ectopic pregnancies, 187 sera from patients with infertility, and 33 sera from cases of PID shown by at least a fourfold increase in titre. In addition, a set of 36 sera from WIF diagnosed cases of \textit{C. psittaci} or \textit{C. pneumoniae}, as shown by rising complement fixation test/WIF titres, and a control set of 90 antenatal sera were also assembled to produce a test panel of 436 sera.
Table 1  Specificity of the commercial enzyme immunoassays (EIAs) for detecting Chlamydia trachomatis antibody when tested against a panel of Chlamydia psittaci/Chlamydia pneumoniae antibody positive sera as diagnosed by whole cell immunofluorescence assay

<table>
<thead>
<tr>
<th>Commercial EIA</th>
<th>Number negative out of 36</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medac Diagnostica</td>
<td>35</td>
<td>97.2% (85.5% to 99.9%)</td>
</tr>
<tr>
<td>Labsystems</td>
<td>34</td>
<td>94.4% (81.3% to 99.3%)</td>
</tr>
<tr>
<td>PBS Orgenics (Tracho PEP)</td>
<td>34</td>
<td>94.4% (81.3% to 99.3%)</td>
</tr>
<tr>
<td>Savoy Diagnostics (SeroCT)</td>
<td>33</td>
<td>91.7% (77.5% to 98.3%)</td>
</tr>
<tr>
<td>Vircell SL</td>
<td>21</td>
<td>58.3% (40.8% to 74.5%)</td>
</tr>
<tr>
<td>Genzyme Virotech</td>
<td>2</td>
<td>5.6% (0.68% to 18.7%)</td>
</tr>
<tr>
<td>Sanofi Pasteur (Platelia)</td>
<td>0</td>
<td>0% (0% to 9.7%)</td>
</tr>
</tbody>
</table>

CI, confidence interval.

RESULTS

Figure 1 shows the correlation between commercial EIA results (log₂ scale) and WIF results. From the graphs, a clustering effect of EIA values could be seen with WIF low and high titre sera to varying degrees, depending on the EIA kit used. A substantial number of sera had low titre WIF and high titre EIA results (false positives) and high titre WIF with low titre EIA results (false negatives). Overall, the Genzyme Virotech assay, which had the highest rank correlation coefficient of 0.82, correlated most closely with WIF, particularly with WIF high titre sera. The lowest rank correlation coefficient (0.70) was obtained with the Medac p-EIA.

Using a panel of WIF diagnosed C. psittaci and C. pneumoniae sera, the C. trachomatis antibody specificity of the commercial assays was evaluated (table 1). The Medac p-EIA had the highest specificity when compared with WIF (97.2%; 95% CI, 85.5% to 99.9%), with only one serum testing C. trachomatis antibody positive. Both Labsystems and PBS Orgenics assays had specificities of 94.4% (95% CI, 81.3% to 99.3%), followed by the Savoy Sero CT assay with a specificity of 91.7% (95% CI, 77.5% to 98.3%). Despite having the highest rank correlation coefficients with WIF, the Genzyme Virotech and Vircell assays showed the lowest specificities—5.6% and 58.3%, respectively—of the species specific assays tested. The Sanofi Platelia EIA had a specificity of 0%, which reflects the fact that it is a genus specific assay.
between the genus specific LPS antigen of thereby compromising specificity. For example, crossreaction may detect antibodies unrelated to a wide variety of antibodies and therefore confer enhanced because they use whole organisms/native antigens they detect whole cell inclusion immunofluorescence assays is that vars. One advantage of the microimmunofluorescence and produced at different stages of the life cycle and by different sero-

infection must differentiate between infection by other species.

Any serological test that is used to detect serovars of the lipopolysaccharides of three species of chlamydia, yet retain the capacity to detect antibodies produced at different stages of the life cycle and by different serovars. One advantage of the microimmunofluorescence and whole cell inclusion immunofluorescence assays is that because they use whole organisms/native antigens they detect a wide variety of antibodies and therefore confer enhanced sensitivity. A potential limitation of these assays is that they may detect antibodies unrelated to Chlamydomatiss infection, thereby compromising specificity. For example, crossreaction between the genus specific LPS antigen of Chlamydia spp and the lipopolysaccharides of Porphyromonas gingivalis, Escherichia coli O19, and Salmonella newington has been reported for both immunofluorescence and EIAs utilising chlamydial LPS.

“...The specificity of the Medac p-EIA may be attributed to its use of a highly immunogenic species specific epitope, which shares no sequence homology with Chlamydia pneumoniae”

MOMP has been used extensively in EIA based tests for Chlamydomatis infection because the organism has a unique biphasic life cycle, alternating between infectious elementary bodies and a replicating, reticulate body. There are three species of chlamidia that cause infection in humans, namely: Chlamydomatis, Ch pneumoniae, and C psittaci and several serovars of Chlamydomatis (D–K) that infect the urogenital tract. Any serological test that is used to detect Chlamydomatis infection must differentiate between infection by other species of chlamydia, yet retain the capacity to detect antibodies produced at different stages of the life cycle and by different serovars. One advantage of the microimmunofluorescence and whole cell inclusion immunofluorescence assays is that because they use whole organisms/native antigens they detect a wide variety of antibodies and therefore confer enhanced sensitivity. A potential limitation of these assays is that they may detect antibodies unrelated to Chlamydomatiss infection, thereby compromising specificity. For example, crossreaction between the genus specific LPS antigen of Chlamydia spp and the lipopolysaccharides of Porphyromonas gingivalis, Escherichia coli O19, and Salmonella newington has been reported for both immunofluorescence and EIAs utilising chlamydial LPS.

DISCUSSION

A wide range of antibodies has been reported to be produced following Chlamydomatis infection because the organism has a unique biphasic life cycle, alternating between infectious elementary bodies and a replicating, reticulate body. There are three species of chlamydia that cause infection in humans, namely: Chlamydomatis, Ch pneumoniae, and C psittaci and several serovars of Chlamydomatis (D–K) that infect the urogenital tract. Any serological test that is used to detect Chlamydomatis infection must differentiate between infection by other species of chlamydia, yet retain the capacity to detect antibodies produced at different stages of the life cycle and by different serovars. One advantage of the microimmunofluorescence and whole cell inclusion immunofluorescence assays is that because they use whole organisms/native antigens they detect a wide variety of antibodies and therefore confer enhanced sensitivity. A potential limitation of these assays is that they may detect antibodies unrelated to Chlamydomatiss infection, thereby compromising specificity. For example, crossreaction between the genus specific LPS antigen of Chlamydia spp and the lipopolysaccharides of Porphyromonas gingivalis, Escherichia coli O19, and Salmonella newington has been reported for both immunofluorescence and EIAs utilising chlamydial LPS.

“The specificity of the Medac p-EIA may be attributed to its use of a highly immunogenic species specific epitope, which shares no sequence homology with Chlamydia pneumoniae”

MOMP has been used extensively in EIA based tests for Chlamydomatis because this antigen is considered to be species and serovar specific, and the Savoyon SeroCT, PBS Orgenics C trachco, Lystems Chlamydomatis, and Medac C trachomatis p-EIA assays use peptides mimicking regions of this protein. From fig 1 it can be seen that the species specific peptide assays performed variably in relation to WIF, with rank correlations ranging from 0.70 to 0.76. The VirCELL assay, which uses extracted and purified MOMP had a higher rank correlation with WIF (0.80) than the synthetic MOMP peptide assays, which may reflect the fact that the antigen used is native, and therefore possesses more epitopes. The assay that had the best correlation with WIF, particularly at high WIF titres, was the Genzyme Virotech C trachomatis EIA, which uses C trachomatis LGV type II strain, cultured in mouse L cells, and inactivated using γirradiation. It is possible that the reduced correlation of the synthetic peptide assays results from conformational differences in the epitopes presented by the peptides certain with native antigens.

The Medac p-EIA showed the highest specificity for C trachomatis specific antibody (97.2% with WIF when tested against sera from WIF diagnosed C psittaci and C pneumoniae infection. The specificity of the Medac p-EIA may be attributed to its use of a highly immunogenic species specific epitope, which shares no sequence homology with C pneumoniae. The Labsystems C trachomatis EIA was the next most specific assay, and this test is based on four synthetic peptides derived from the variable domain IV of the MOMP of C trachomatis serotypes C, G, E, and L2. The Genzyme Virotech EIA, although showing the best correlation with WIF, particularly at high titres, had a specificity of only 5.6% with the C psittaci/C pneumoniae antibody positive sera. This would be expected because the antigen used has not had chlamydial LPS, which is a group specific antigen, extracted. The antigen used for WIF is also not LPS extracted and LPS antibodies will be detected in both these assays, but not in the MOMP synthetic peptide assays. There is evidence to suggest that, in many instances, anti-LPS antibody rapidly declines following Chlamydomatis infection, and in cases of chronic infection associated with tubal factor infertility and ectopic pregnancy little LPS antibody is found. In our laboratory, comparison of complement fixing antibodies (that is, anti-LPS antibody) with WIF titres is used to differentiate recent and chronic active infections in patients with high WIF titres (≥ 512). The absence of complement fixing antibody or its presence at low titres in sera with WIF titres ≥ 512 is suggestive of tubal damage with no evidence of active infection; however, if complement fixing antibody is found at titres ≥ 12, PID is suspected.

The Genzyme Virotech EIA was selected as our screening assay; however, we needed to refine the assay so that the cut off used correlated with that used for WIF (≥ 512) in our laboratory. The assay was modified using time resolved fluorescence technology because this technique, as a result of its large linear dynamic range, has particular application to screening single serum dilutions. This approach appeared to be highly successful in differentiating between WIF positive sera (titres ≥ 512) and WIF negative sera (titres < 512). Using the modified, time resolved Genzyme Virotech assay, high chlamydial antibody titre sera can be selected from our screening programmes for further testing for C trachomatis specific MOMP antibody. Our studies of specificity have shown that the Medac p-EIA is the most appropriate assay to use; however, there are occasions when sera have high screening antibody titres but are not p-EIA positive. Such incidents may be the result of genuine C psittaci/C pneumoniae infection, crossreactivity with LPS from Gram negative bacteria, or a failure of C trachomatis antibodies in the serum to recognise the MOMP epitopes in the Medac p-EIA.

Our ultimate goal is to develop a non-invasive screening assay for chlamydial infection which, with appropriate refinements, will be specific for C trachomatis mediated upper genital tract disease. The amount of IgG in whole saliva is about one thousandth of that in plasma, and using the TRFIA cut off...
Screening for Chlamydia trachomatis specific antibodies is valuable in diagnosing asymptomatic pelvic inflammatory disease (PID) and tubal damage following repeated episodes of PID.

Because the currently used assays are unsuitable for screening activities, several commercial Chlamydia trachomatis specific enzyme immunoassays (EIAs) against the most commonly used assay (WIF) have been modified, using time resolved fluorescence technology, for use as a screening assay.

Some of the EIAs studied (for example, Medac p-EIA) were highly specific for Chlamydia trachomatis antibody and can be used to confirm the antibody specificity of sera selected by the screening assay.

Further studies have been initiated to assess the usefulness of this screening approach.

“Two ultimate goals is to develop a non-invasive screening assay for chlamydial infection which, with appropriate refinements, will be specific for Chlamydia trachomatis mediated upper genital tract disease.”

To conclude, we have evaluated several commercial Chlamydia trachomatis specific EIAs against WIF and the correlation has ranged between 0.70 and 0.80. The best correlation (0.82) was seen with an EIA using a cultured, inactivated organism and this assay has been modified, using time resolved fluorescence technology, for use as a screening assay. Some of the EIAs studied (for example, Medac p-EIA) were highly specific for Chlamydia trachomatis antibody and can be used to confirm the antibody specificity of sera selected by the screening assay. Further studies have been initiated to assess the usefulness of this screening approach.

Take home messages

- Screening for Chlamydia trachomatis specific antibodies is valuable in diagnosing asymptomatic pelvic inflammatory disease (PID) and tubal damage following repeated episodes of PID.
- Because the currently used assays are unsuitable for screening activities, several commercial Chlamydia trachomatis specific enzyme immunoassays (EIAs) against the most commonly used assay (WIF) have been modified, using time resolved fluorescence technology, for use as a screening assay.
- Some of the EIAs studied (for example, Medac p-EIA) were highly specific for Chlamydia trachomatis antibody and can be used to confirm the antibody specificity of sera selected by the screening assay.
- Further studies have been initiated to assess the usefulness of this screening approach.

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REFERENCES


