Detection of Serum Antibodies to *Chlamydia trachomatis* in Patients with Chlamydial and Nonchlamydial Pelvic Inflammatory Disease by the IPAzyme Chlamydia and Enzyme Immunoassay

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A novel serological test, IPAzyme Chlamydia (Savyon Diagnostics Ltd., Beer Sheva, Israel), was compared with an enzyme immunoassay (EIA) for the ability to detect serum immunoglobulin G and A antibodies in the diagnosis of acute chlamydial pelvic inflammatory disease. In comparison with cell culture, which is the "gold standard," IPAzyme Chlamydia and EIA exhibited sensitivities of 63 and 68% and specificities of 76 and 87%, respectively. Thus, IPAzyme Chlamydia offers no advantages over the EIA, and neither serological test can be recommended for the diagnosis of acute *Chlamydia trachomatis* infection. So far, conventional cell culture remains the most reliable diagnostic test for chlamydial pelvic inflammatory disease.

Postinflammatory sequelae following acute pelvic inflammatory disease (PID), such as tubal scarring and peritubal adhesions, are considered the most important preventable causes of infertility and ectopic pregnancy (16). Seroepidemiologic studies have demonstrated a strong link between *Chlamydia trachomatis* infection and tubal factor infertility or ectopic pregnancy. The vast majority of women with tubal damage have no history of frank PID, suggesting that most chlamydial PID is subclinical (3, 4).

Cell culture is still considered the "gold standard" for the diagnosis of *C. trachomatis* infection, although it is time-consuming, labor-intensive, and costly (1). The specificity of the technique is 100%, while the sensitivity varies from 70 to 90% (12). Recently, direct fluorescent-antibody tests and enzyme immunoassays (EIAs) for the rapid diagnosis of *C. trachomatis* infection have been introduced, but their sensitivities and specificities vary considerably (5, 13, 15). Polymerase chain reaction (PCR) is reported to be the most sensitive method for detecting *C. trachomatis* infection (8). However, it is thought that further improvements to simplify the PCR method must be made before it can be used routinely for the diagnosis of *C. trachomatis* infection.

Several tests can be used to detect antibodies to *C. trachomatis* in human serum samples, including complement fixation, various immunofluorescence tests, EIAs, and radioimmunoassays. While the antigens used in these tests vary considerably, the tests all measure antibodies against different antigenic determinants of *Chlamydia* species. Most women with acute PID have serum antibodies to *C. trachomatis*, often in high titers. Since serum antibodies persist for several years after the acute infection (10, 14), it is difficult to use serum antibody testing in the diagnosis of acute chlamydial PID. However, some studies suggest that specific short-lived immunoglobulin A (IgA) antibodies may be a potential marker of active chlamydial infection (7).

The IPAzyme Chlamydia test (Savyon Diagnostics Ltd., Beer Sheva, Israel) is a novel indirect immunoperoxidase assay using *C. trachomatis* serotype L2-infected cells as the antigen for the detection of serum IgG and IgA antibodies. This test is intended for the diagnosis of chlamydial infections, including acute PID. In this study, we compared the test performance of the IPAzyme Chlamydia with an EIA in the detection of serum IgG and IgA antibodies in patients with proven PID. Conventional cell culture was used for the isolation of *C. trachomatis* in samples obtained from the lower and upper genital tract.

The study population consisted of 45 women with suspected acute PID who presented to the Department of Obstetrics and Gynecology, University Hospital, Tampere, Finland. All patients were hospitalized and underwent laparoscopy and endometrial biopsy. *C. trachomatis* was isolated from specimens collected from the cervix, endometrium, fallopian tubes, and cul-de-sac. Altogether, 35 patients had either laparoscopic evidence of salpingitis or histopathological evidence of endometritis. These patients thus had proven PID. The laparoscopic findings in 10 women who did not have PID were as follows: ovarian cysts in 6 women, pelvic adhesions in 2 women, ectopic pregnancy in 1 woman, and periaudipendicular abscess in 1 woman. In addition, 11 women undergoing laparoscopic sterilization (tubal ligation) were enrolled as controls.

An unlubricated speculum was used to expose the cervix. Cervical specimens were obtained with cotton-tipped swabs for culture. Endometrial, cul-de-sac, and tubal specimens were obtained as described previously (6, 9). *C. trachomatis* was isolated on cycloheximide-treated McCoy cells (11). The inclusion bodies were visualized with iodine.

The serum samples were drawn upon admission of the patients and stored at −20°C until tested. For each serum sample, a dilution of 1:16 for the IPAzyme IgA assay and dilutions of 1:64 and 1:128 for the IgG assay were prepared. The IPAzyme Chlamydia assay was performed according to the manufacturer's instructions. After the slides were rinsed, they were dried with compressed air. A microscope magni-
fication of $\times 250$ was used to observe the results. The presence of a blue precipitate in the chlamydia-infected cells was considered a positive reaction.

The EIA was performed with highly purified elementary body-derived protein antigen (Chlamydia trachomatis IgG EIA; Labsystems Ltd., Helsinki, Finland). The IgG assay was performed as specified by the manufacturer. To detect serum IgA antibodies, the procedure was modified by substituting the provided anti-human IgG conjugate with a rabbit anti-human IgA conjugate (Dakopatts A/S, Glostrup, Denmark). The bound rabbit antibodies were detected by horseradish peroxidase-conjugated anti-rabbit antibodies produced in swine sera (Dakopatts). The samples were analyzed with a Titertek Multiscan spectrophotometer (Efflab, Helsinki, Finland) set at 405 nm. In the IgG assay, the cutoff level for seropositivity was set according to the manufacturer’s instructions at 60 relative EIA units (EIUs), derived from negative and positive standard serum samples containing 0 and 100 EIUs of antibodies, respectively. This level roughly corresponded to the mean plus 2 standard deviations for the values from the patients and controls who had no evidence of chlamydial infection or PID. In the modified IgA assay, the cutoff level for seropositivity was determined as 20 EIU, corresponding to the mean plus 3 standard deviations of the values for the patients and controls who had no evidence of chlamydial infection or PID.

C. trachomatis was isolated from the cervix in 17 (49%) of the 35 patients with PID. Of these patients, C. trachomatis was isolated from the endometrium in six patients and from both the endometrium and fallopian tubes in five patients. C. trachomatis was also isolated from the cervix in 1 of the 10 patients without PID and 1 of the 11 women undergoing tubal ligation.

The results of the two serological tests were compared with the results of cell culture (Table 1). The IPAzyme Chlamydia test correctly identified 12 of 19 patients with chlamydial infections, giving a sensitivity of 63%. The test showed 9 false-positive findings among the 37 chlamydia-negative patients and controls, giving a specificity of 76%. The positive predictive value for the IPAzyme Chlamydia was 57%, and the negative predictive value was 80%.

If only IgA positivity of the IPAzyme Chlamydia was considered, the number of false-positive results decreased from 9 to 3. However, the IgA assay detected only 9 of the 19 chlamydial infections, giving a sensitivity of 47%.

The EIA for IgG antibodies correctly detected 9 of the 19 chlamydial infections, and the EIA for IgA antibodies detected 11 of the 19 infections. The IgG assay gave five false-positive results, and the IgA assay gave three false-positive results. By combining the IgG and IgA assay results, the combined EIA had an overall sensitivity of 68%, a specificity of 87%, a positive predictive value of 72%, and a negative predictive value of 84%.

Our results are in agreement with the generally accepted view that serological tests are of limited value in the diagnosis of acute chlamydial infection. Although the IPAzyme Chlamydia is recommended for patients with suspected acute PID, our results showed an unacceptably low specificity, suggesting that a considerable number of false-positive results will occur both in high-risk patient populations and especially in low-risk patient populations. The IgA assay performed with higher specificity but detected only half of the proven chlamydial infections, which means that many patients would thus go undetected and untreated.

Some of the discordant findings obtained by serological tests and cell culture can be explained by the <100% sensitivity of the cell culture method. However, in our study, culture specimens were taken from several anatomic sites from all patients, making this explanation unlikely. Another plausible explanation for the discordant findings is the potential cross-reactivity between antibodies to C. trachomatis and other chlamydial species, and other bacteria (2).

There is a definite need for rapid diagnostic tests for screening C. trachomatis infections. However, when one considers the social and psychological consequences of a false-positive test result, the accuracy of the test is of fundamental importance. Thus, no serological test alone can be recommended for the diagnosis of C. trachomatis infection. So far, chlamydial cell culture remains superior to any less sensitive and less specific tests.

### TABLE 1. Correlation of serum antibody determination by IPAzyme test and EIA with chlamydial cell culture in 45 patients with suspected acute PID and 11 controls undergoing tubal ligation

<table>
<thead>
<tr>
<th>Patients with suspected PID</th>
<th>IPAzyme</th>
<th>EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PID present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis positive (17)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>C. trachomatis negative (18)</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>PID not present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis positive (1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C. trachomatis negative (9)</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Women undergoing tubal ligation (controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis positive (1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C. trachomatis negative (10)</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

* A positive reaction with a serum dilution of 1:64 and/or 1:128 in the IgG assay or a positive reaction with a serum dilution of 1:16 in the IgA assay.