Assessment of *Chlamydia trachomatis*-Specific IgA and IgG Serum Antibodies in Genitourinary *Chlamydia trachomatis* Infection —Comparative Study between HITAZYME® and IPzyme®—

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Abstract

We assessed the C. trachomatis antibody assay kit HITAZYME® (Hitachi Chemical Co., Ltd.) using clinical specimens. This kit is based on an enzyme immunoassay (EIA) which utilized purified Chlamydia trachomatis outer membrane antigen as the solid phase antigen.

Twenty-nine untreated male urethritis patients, 816 pregnant housewives, 188 cervicitis patients, and 76 pelvic inflammatory disease patients were tested. Agreement between the HITAZYME® test and antigen detection in infected area was assessed, and a comparison was made with IPAzyme® (a commercially available indirect immunoperoxidase assay kit).

1) Summary of HITAZYME® and IPAzyme®

IgA: Agreement between the two assays was relatively good, i.e., 82.6% (916/1109). However, 5.5% (61/1109) were HITAZYME® (-), IPAzyme® (+), and 11.9% (132/1109) were HITAZYME® (+), IPAzyme® (-). Thus, in quite a few cases the results did not agree.

IgG: Agreement between the two assays was 73.7% (817/1109). Agreement was relatively low, 24.4% (271/1109) were HITAZYME® (-), IPAzyme® (+).

2) In the cases of disagreement, more specific Western blot analysis was performed to check the reactivity of the anti-C. trachomatis antibody. When IgA was used, agreement between HITAZYME® and Western blot analysis was 69.6% (16/23), and agreement between IPAzyme® and Western blot analysis was 30.4% (7/23), whereas when IgG was used, agreement between HITAZYME® and Western blot analysis was 80.0% (12/15), and agreement between IPAzyme® and Western blot analysis was 20.0% (3/15). There was significantly greater agreement with HITAZYME® than with IPAzyme®. In other words, HITAZYME® had greater specificity when reacted with C. trachomatis antigen than IPAzyme®.

3) The IgA antibody-positive rate in antigen (+) cases (male urethritis: 72.7%, pregnant housewives: 65.7%, cervicitis: 70.3%, pelvic inflammatory disease: 70.0%) was significantly (p<0.01) higher than in antigen (-) cases (male urethritis: 16.7%, pregnant housewives: 13.6%, cervicitis: 22.6%, pelvic inflammatory disease: 30.4%). Therefore, IgA antibody can serve as a suitable indicator for active infection.

4) The IgG antibody-positive rate in antigen (-) female cases was 15.5% using HITAZYME® and significantly (p<0.01) lower than with IPAzyme®. HITAZYME® had greater specificity than IPAzyme®.

In conclusion, HITAZYME® has relatively good sensitivity and specificity. Moreover, since it is an EIA assay, it allows objective evaluation of results. It permits processing of a large number of specimens because it is easy to perform. Thus, HITAZYME® is a superior antibody assay for C. trachomatis. It can be used when antigen tests are difficult to perform. It is strongly anticipated that HITAZYME® will be able to be used clinically as a screening test.

Introduction

Chlamydia trachomatis is one of the main causative microorganisms of sexually transmitted diseases (STD) and has been spreading rapidly in recent years, creating serious problems clinically.

Although C. trachomatis infection can be primarily diagnosed by detection of antigens, antigen detection is not easy in pelvic inflammatory disease, prostatitis and epididymitis. Therefore, clinical diagnosis often depends on measuring antibody titers in serum or secretions from infected areas.

Sampling of specimens from infected areas is difficult initially even in suspected cases of infection. Therefore, screening tests which measure serum antibody titers may be useful in convincing patients to undergo further clinical tests.

Conventional methods, such as the microimmunofluorescence (micro-IF) method, microplate immunofluorescence antibody technique (MFA), and indirect immunoperoxidase assay (IPAzyme) method, however, require complicated procedures, do not permit measurement of large numbers of specimens.
simultaneously, and are not objective because they are evaluated visually. These shortcomings have often been pointed out\(^8\).

In order to solve these practical problems, HITAZYME® (Hitachi Chemical Co., Ltd.), a new test kit for measuring *C. trachomatis* antibody by the enzyme immunoassay method (EIA), has been developed. HITAZYME® allows objective quantification of large numbers of samples. This method makes use of chlamydial outer membrane complex purified from the elementary body (EB) of *C. trachomatis* L\(_2\) strain as fixed antigen. Anti-*Chlamydia* antibody titers in specimens can be measured by means of their optical density\(^9\).

We investigated the *C. trachomatis* antibody-positive rate in patients with genitourinary tract infections (including pregnant housewives). Here we report our clinical assessment of this test kit.

**Materials and Methods**

1. Patients

We tested 1109 patients with untreated genitourinary tract infections (including 816 pregnant housewives undergoing routine examination) who came to the departments of urology of 3 institutions or to the departments of obstetrics and gynecology at 6 institutions between May 1990 and September 1992. There were 29 cases of male urethritis, 816 pregnant housewives, 188 cases of cervicitis, and 76 cases of pelvic inflammatory disease.

As a negative control, we tested 258 healthy adults who underwent routine annual check-ups and were negative for IgA and IgG titers by IPAzyme®, to rule out the possibility of latent infection.

2. Antibody Measurement

*C. trachomatis* antibody titers were measured in serum specimens from the patients. After collecting blood, serum was isolated, and frozen at \(-20\)°C to \(-80\)°C. *C. trachomatis* antibody titers were measured using HITAZYME®\(^2,3,7\).

1) HITAZYME®

The principle of this method is illustrated in Fig. 1. It is based on the enzyme immunoassay method (EIA), and anti-*C. trachomatis* IgA and IgG antibodies can be detected, i.e., the bottom of microtiter plate wells is coated with outer membrane antigen of *C. trachomatis* L\(_2\) strain, which reacts with anti *C. trachomatis* antibody in specimens. Anti-IgA and -IgG antibodies labeled with alkaline phosphatase react with them, and immune complexes consisting of fixed antigen-antibody-labeled antibodies are formed in each well. When substrate (p-nitrophenyl phosphate) is added, p-nitrophenol is formed. The amount of
p-nitrophenol formed is correlated with the amount of antibody. The presence or absence of anti-\textit{C. trachomatis} IgA and IgG antibodies is assessed colorimetrically by measuring optical density at 405 nm. Measurement of HITAZYME® was performed using the following protocols:

(1) Specimens were diluted 20-fold with diluting solution, and these diluted specimens were then used for IgA and IgG antibody assays.

(2) Samples (100 \(\mu\)l each of negative control, positive control, and diluted specimens) were added to the micro titerplates, and allowed to react at 37°C for 60 minutes.

(3) The microtiter plate was washed with washing solution three times, and 100 \(\mu\)l of antibody labeled with enzyme was added and allowed to react at 37°C for 60 minutes.

(4) After the microtiter plate had been washed with washing solution three times again, 100 \(\mu\)l of substrate solution (p-nitrophenyl phosphate) was added, and allowed to react at room temperature for 10 minutes.

(5) After the reaction was complete, 25 \(\mu\)l of stop solution was added, and optical density was measured at 405 nm.

(6) The cut-off value was determined by the mean absorption of the negative controls. "positive" was defined as "above the cut-off value", and "negative" as "below the cut-off value".

2) IPAzyme®

For comparison, the indirect immunoperoxidase assay IPAzyme® was also employed. The procedure was as follows. Serial 2-fold dilutions of specimens (serum and discharge) were prepared, and reacted with \textit{C. trachomatis} L2 infected cells (antigen) smeared on slides. Then, anti human IgA and IgG rabbit antiserum labeled with horseradish peroxidase was added as secondary antibody, and substrate chromogen (4-chloro-1-naphthal and hydrogen peroxidase) was added. "Positive" was defined as the presence of intracellular inclusion bodies staining dark blue on light microscopy \((\times 200)\). The antibody titer was considered positive when the IgA antibody titer was above 16-fold and when the IgG antibody titer was above 64-fold.

3. Antigen Detection

In order to check the agreement between antigen tests and antibody tests, antigen tests were performed at the same time. The procedure was as follows. Specimens were collected from the male urethra or female cervical canal in the form of smears, and antigen was detected using an EIA antigen test kit [IDEIA Chlamydia®: (Nobo Nordisk Co.)]10].

4. Western Blot Analysis

Western blot analysis was performed to determine whether the antibody detected cross-reacted with the outer membrane antigen of \textit{C. trachomatis}.

Chlamydial outer membrane antigen was isolated from the EB of \textit{C. trachomatis} L2 strain according to a method described by Caldwell et al., and antigen was separated by 10% SDS polyacrylamide gel electrophoresis in the presence of reducing agent (mercaptoethanol) according to Laemmli’s method (10 \(\mu\)g total protein was applied per lane). After gel electrophoresis, proteins were transferred to nitrocellulose membranes (Bio-Rad Co., 0.45 \(\mu\)) according to the method described by Twobin, et al. After blotting, the blot was incubated with 15 mM Tris-HCl (pH 7.4) and 0.2 M saline (Tris-saline buffer solution) containing 2% BSA (Blocking buffer) at 4°C overnight with gentle shaking (blotting step). It was then washed with Tris-saline buffer solution containing 1% Triton X-100 (washing buffer) five times, and incubated with control serum or patient serum diluted with Tris-saline buffer solution containing 0.2% BSA (binding buffer) (1000-fold dilution in the case of IgG antibody, and 500-fold dilution in the case of IgA antibody) at room temperature for 2 hours with gentle shaking. After washing with washing buffer five times, it was incubated with anti human-IgA and IgG antibodies (1 \(\mu\)g/ml) labeled with horseradish peroxidase.
Comparative Study between HITAZYME® and IPAzyme®

previously diluted with binding buffer for 2 hours. After incubation, it was washed with washing buffer five times, and incubated with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and Trissaline buffer solution containing 0.05% H₂O₂. When a color developed, the reaction solution was discarded, the blot was washed with distilled water and then air dried. The color of the samples was evaluated in comparison with a negative control and a positive control.

Results

1. Determination of the Normal Range (Cut-off Values)

The distribution of serum IgA and IgG values (determined by HITAZYME®) in 258 healthy adults is shown in Fig. 2. All patients were antibody-negative using IPAzyme®.

The mean IgA antibody titers was 0.083 with a standard deviation of 0.047, and the mean IgG antibody was 0.052 with standard deviation of 0.047, and the mean IgG antibody was 0.052 with standard deviation of 0.037.

When specimens from 258 healthy adults were assayed with HITAZYME®, the distribution of IgA and IgG antibodies was shifted to right. Hence, Geary's normal distribution test was conducted after excluding extreme values. Since a normal distribution was seen after excluding only a small percentage of the samples, the normal distribution was determined using the rest of the samples. The cut-off value (normal range) was the mean plus 2 × S.D. (standard deviation). We conducted the following investigations.

2. Comparison of HITAZYME® and IPAzyme®

1) Comparison of Antibody Values (Table 1)

All 1109 patients were assayed using HITAZYME® (enzyme immunoassay method, EIA) and IPAzyme® (indirect immunoperoxidase assay), and the data were compared.

Agreement between two assays for IgA was 82.6% (916/1109), and relatively good agreement was achieved. However, 5.5% (61/1109) were HITAZYME® (--), IPAzyme® (+), while 11.9% (132/1109) were HITAZYME® (+), IPAzyme® (--). Thus, the results for quite a few patients disagreed. In these cases, more specific Western blot analysis was performed to check the reactivity of anti-C. trachomatis antibody.

Agreement between the two assays for IgG was 73.3% (817/1109). Agreement was relatively low, 24.4% (271/1109) were HITAZYME® (–), IPAzyme® (+), comprising most of the cases of disagreement. In these cases, more specific Western blot analysis was performed to check the reactivity of anti-C. trachomatis
2) Reactivity of Specific Antibody by Western Blot Analysis in Cases of Disagreement (Fig. 3)

The reactivity of *C. trachomatis* IgA and IgG antibodies was tested by Western blot analysis in 23 specimens from patients in which there was disagreement regarding IgA antibody and 15 specimens from patients with disagreement related to IgG antibody.

Agreement between HITAZYME® and Western blot analysis with respect to IgA was 69.6% (16/23), whereas between IPAZyme® and Western blot analysis it was 30.4% (4/23). There was significantly higher agreement with HITAZYME® than with IPAZyme® ($p=0.003$).

In regard to IgG, agreement between HITAZYME® and Western blot analysis, it was 80.0% (12/15), and between IPAZyme® and Western blot analysis was 20.0% (3/15). There was significantly higher agreement with HITAZYME® than with IPAZyme® ($p\leq0.001$).

In other words, HITAZYME® had greater specificity in reactions with *C. trachomatis* antigen than IPAZyme®, as demonstrated by specificity tests for anti-*C. trachomatis* antibodies.

3. Antigen detection and Correlations with Antibody Reaction-positive Rate

The correlations between antibody (+) cases using this kit and antigen (+) cases were analyzed.

<Antibody-positive Retes in Antigen (+) Cases>

1) IgA- and IgG-positive Rates according to Disease in Male and Female Patients (Fig. 4)

In male patients, the IgA antibody-positive rate in antigen (+) cases was 72.7% (8/11) with
Comparative Study between HITAZYME® and IPAzyme®

Fig. 4 IgA- and IgG-positive rates (n=1109)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IgA</th>
<th>M</th>
<th>0</th>
<th>50</th>
<th>100%</th>
</tr>
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<tbody>
<tr>
<td>(+)</td>
<td></td>
<td>M</td>
<td>62</td>
<td>118</td>
<td>72.7%</td>
</tr>
<tr>
<td>(-)</td>
<td></td>
<td>M</td>
<td>59</td>
<td>111</td>
<td>72.7%</td>
</tr>
<tr>
<td>(+)</td>
<td></td>
<td>F</td>
<td>62</td>
<td>118</td>
<td>72.7%</td>
</tr>
<tr>
<td>(-)</td>
<td></td>
<td>F</td>
<td>59</td>
<td>111</td>
<td>72.7%</td>
</tr>
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</table>

Fig. 5 IgA-positive rates

Antigen detection: IDEIA

<table>
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<th>n=18</th>
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</thead>
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<tr>
<td>(+)</td>
<td>72.7%</td>
<td>66.7%</td>
</tr>
<tr>
<td>(-)</td>
<td>18.3%</td>
<td>16.7%</td>
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</table>

<table>
<thead>
<tr>
<th>Pregnant housewives</th>
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<th>n=749</th>
</tr>
</thead>
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<tr>
<td>(+)</td>
<td>65.7%</td>
<td>63.9%</td>
</tr>
<tr>
<td>(-)</td>
<td>34.3%</td>
<td>36.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cervicitis</th>
<th>n=64</th>
<th>n=124</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>70.3%</td>
<td>67.4%</td>
</tr>
<tr>
<td>(-)</td>
<td>29.7%</td>
<td>32.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PID</th>
<th>n=20</th>
<th>n=56</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>70.0%</td>
<td>64.3%</td>
</tr>
<tr>
<td>(-)</td>
<td>30.0%</td>
<td>35.7%</td>
</tr>
</tbody>
</table>

HITAZYME®, and 16.7% (3/18) in antigen (−) cases. Thus, the IgA antibody-positive rate in antigen (+) cases was significantly higher than in antigen (−) cases (p<0.01).

IPAzyme® assays, on the other hand, yielded an IgA antibody-positive rate in antigen (+) cases of 18.2% (2/11) and in antigen (−) cases, of 16.7% (3/18). No statistical significance was detected.

The IgG antibody-positive rate in antigen (+) cases was 63.6% (7/11) with HITAZYME®, and 72.7% (8/11) with IPAzyme®, while in antigen (−) cases it was 33.3% (6/18) using HITAZYME® and 33.3% (6/18) using IPAzyme®. No statistical significance was detected in any of these instances because of the small number of cases. We need to increase the number of cases.

In female patients, the IgA antibody-positive rate in antigen (+) cases was 68.2% (103/151) with HITAZYME® and 58.3% (88/151) with IPAzyme®, and while in antigen (−) cases the rates were 15.8% (147/929) and 12.5% (116/929), respectively. Therefore, the IgA antibody-positive rate in antigen (+) cases was significantly higher than in antigen (−) cases (p<0.01).

The IgG antibody-positive rate in antigen (+) cases was 75.5% (114/151) with HITAZYME® and 88.1% (133/151) with IPAzyme®, while among antigen (−) cases is was 15.5% (144/929) with HITAZYME®, and 40.5% (376/929) with IPAzyme®. The two assays were comparable in antigen (+) cases. The IgG antibody-positive rate in antigen (−) cases was significantly higher using IPAzyme® (p<0.01). This may have been
due to cross-reactivity of *C. pneumoniae* and *C. psittaci* as mentioned earlier.

2) IgA Antibody-positive Rates According to Disease (Fig. 5)

No significant difference was detected between antigen (+) cases and antigen (−) cases when male urethritis patients were assayed by IPAzyme®. The IgA antibody-positive rate was significantly (p<0.01) higher in antigen (+) cases than in antigen (−) cases in all other diseases. Although these data may indicate that IgA antibody can serve as an indicator for active infection by *C. trachomatis*, approximately 10% of antigen (−) cases were positive for IgA antibody, while approximately 30% of antigen (+) cases were negative for IgA antibody. Thus, the diagnosis of individual cases on the basis of IgA antibody data alone is unreliable.

3) IgG Antibody-positive Rates According to Disease (Fig. 6)

In male urethritis alone, no significant difference in percentage of IgG (+) cases was detected among antigen (+) patients and antigen (−) patients. The IgG antibody-positive rate was significantly (p<0.01) higher in antigen (+) cases than antigen (−) cases in all other diseases.

However, even among male urethritis patients in which antigen detection is relatively easy, approximately 30% of antigen (−) patients were positive for IgG antibody, suggesting that IgG antibody was elevated due to previous infections or considerable number of cases were false-positive due to cross-reactions.

4. *C. trachomatis* Antigen-positive Rates According to Group Classified by Antibody Findings

In the previous sections, antibody-positive rates were investigated on the basis of antigen-positive findings, whereas in this section, the antigen-positive rates were investigated on the basis of antibody-positive findings.

1) Male Urethritis (Fig. 7)

Considerable variability can be seen probably because only 29 patients were tested. In the IgA (+), IgG (+) group, 63.6% of the patients were positive for antigen with HITAZYME®, and 40.0% of the patients were positive for antigen with IPAzyme®. Eight out of 11 antigen (+) patients were positive for IgA antibody with HITAZYME®, but only 2 out of 11 antigen (+) patients were positive for IgA antibody with IPAzyme®. Therefore, HITAZYME® reveals the activity of infection better than IPAzyme®.

2) Pregnant housewives (Fig. 8)

In the IgA (+) IgG (+) group, the antigen-positive rate was 38.6% with HITAZYME® and 35.9% with IPAzyme®, and significantly higher than the 1.4% with HITAZYME® and 1.9% with IPAzyme® in the IgA
Fig. 7 C. trachomatis antigen-positive rates according to groups classified by antibody findings

<table>
<thead>
<tr>
<th>Male urethritis (29 cases)</th>
<th>Distribution of antibody findings in 11 antigen-positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen detection : IDEIA</strong></td>
<td><strong>HITAZYME</strong></td>
</tr>
<tr>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
<td><strong>No. of cases</strong></td>
</tr>
<tr>
<td>25.0%</td>
<td>3</td>
</tr>
<tr>
<td>50.0%</td>
<td>2</td>
</tr>
<tr>
<td>63.6%</td>
<td>7</td>
</tr>
<tr>
<td>0%</td>
<td>3</td>
</tr>
<tr>
<td><strong>37.9%</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>

Fig. 8 C. trachomatis antigen-positive rates according to groups classified by antibody findings

<table>
<thead>
<tr>
<th>Pregnant housewives (816 cases)</th>
<th>Distribution of antibody findings in 67 antigen-positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen detection : IDEIA</strong></td>
<td><strong>HITAZYME</strong></td>
</tr>
<tr>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
<td><strong>No. of cases</strong></td>
</tr>
<tr>
<td>1.4%</td>
<td>9</td>
</tr>
<tr>
<td>18.2%</td>
<td>12</td>
</tr>
<tr>
<td>38.6%</td>
<td>32</td>
</tr>
<tr>
<td>36.8%</td>
<td>14</td>
</tr>
<tr>
<td><strong>8.2%</strong></td>
<td><strong>67</strong></td>
</tr>
</tbody>
</table>

(−) IgG (−) group. Moreover, when either IgA or IgG was positive, 86.6% of antigen (+) cases can be detected. Therefore, antibody tests are useful as screening tests.

3) Cervicitis (Fig. 9)

In the IgA (+), IgG (+) group, the antigen-positive rate was 60.3% with HITAZYME® and 61.2% with IPAzyme®, both the which were high values. In any event, whether either IgA or IgG is positive, most of antigen (+) cases can be detected.
Fig. 9 *C. trachomatis* antigen-positive rates according to groups classified by antibody findings

### Cervicitis (188 cases)

Antigen detection: IDEIA

<table>
<thead>
<tr>
<th>HITAZYME</th>
<th>IPAzyme</th>
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<tbody>
<tr>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
</tr>
<tr>
<td>100 (%) 80 60 40 20 No. of cases</td>
<td>20 40 60 80 (%) 100 No. of cases</td>
</tr>
<tr>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>11.4%</td>
<td>0 79</td>
</tr>
<tr>
<td>16.7%</td>
<td>1 6</td>
</tr>
<tr>
<td>60.3%</td>
<td>47 78</td>
</tr>
<tr>
<td>28.0%</td>
<td>7 25</td>
</tr>
<tr>
<td>34.0%</td>
<td>54 188</td>
</tr>
</tbody>
</table>

Fig. 10 *C. trachomatis* antigen-positive rates according to groups classified by antibody findings

### PID (76 cases)

Antigen detection: IDEIA

<table>
<thead>
<tr>
<th>HITAZYME</th>
<th>IPAzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
<td><strong>C. trachomatis antigen-positive Rates</strong></td>
</tr>
<tr>
<td>100 (%) 80 60 40 20 No. of cases</td>
<td>20 40 60 80 (%) 100 No. of cases</td>
</tr>
<tr>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>10.3%</td>
<td>3 29</td>
</tr>
<tr>
<td>12.5%</td>
<td>1 8</td>
</tr>
<tr>
<td>56.0%</td>
<td>14 25</td>
</tr>
<tr>
<td>14.3%</td>
<td>2 14</td>
</tr>
<tr>
<td>26.3%</td>
<td>20 76</td>
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4) Pelvic Inflammatory Disease (Fig. 10)

In the IgA (+), IgG (+) group, the antigen-positive rate was 56.0% with HITAZYME® and 50.0% with IPAzyme®. Since it is difficult to detect antigen in pelvic inflammatory disease cases, the actual number of cases of *C. trachomatis* infection may be even higher. Since antibody test data are so useful in making a diagnosis, in IgA (+), IgG (+) group, the possibility of having *C. trachomatis* infections may be higher even if antigen-negative.
Discussion

*C. trachomatis* infections have been causing serious problems in Japan, since the disease has been spreading very rapidly. Consequently, developments in the diagnosis and prevention of the disease have been greatly anticipated. *C. trachomatis* infection often produces relatively mild symptoms, and many female patients in particular have asymptomatic infection.

Therefore, it is nearly impossible to convince patients to collect genitourinary tract specimens themselves when asymptomatic, even among high risk people. Under these circumstances, the only possible way is to perform antibody tests. Our data did not prove that antibody (+)=antigen (+). Nevertheless, antibody tests are meaningful as screening tests because of their high correlation with antigen tests.

Additionally, even when samples can be obtained from the genitourinary tract of symptomatic patients, it is often difficult to detect antigen in cases of pelvic inflammatory disease, prostatitis and epididymitis. Therefore, antibody tests on secretions or serum may often corroborate the diagnosis.

Since Sarov et al. demonstrated that IgA antibody increases with local antibody in the initial phase of *Chlamydia* infection, many investigators have gradually demonstrated the clinical significance of diagnosis by IgA antibody assays of secretions or serum. In short, antibody reactions involving IgAs play a major role in local immunological responses because genitourinary infection by *C. trachomatis* is a mucosal infection. IgA antibody can serve as an indicator of active infection as an acute antibody. On the other hand, IgG antibody reflects a past history of infection and does not necessarily mean the presence of active infection. Hence, IgG is regarded as a chronic antibody.

In the past, the main assay methods for *C. trachomatis* antibody were the microimmunofluorescence (micro-IF) method, microplate immunofluorescence antibody technique (MFA), and indirect immunoperoxidase (IPAzyme) method.

The micro-IF method is an indirect immunofluorescence antibody method developed by Wang et al. in 1970. It utilizes *C. trachomatis* elementary body as the antigen, and is an excellent method in terms of sensitivity and specificity. It can determine immunotypes and requires a great deal of work to prepare the antigens because of their variability. It also requires special skills and facility in evaluating the data. Hence, it is not suitable as a clinical test.

The MFA method is an indirect immunofluorescence antibody method developed by Matsumoto et al. Since it utilizes inclusion bodies in cells infected with *C. trachomatis* as the antigen, it is not necessary to purify the antigen. It can be evaluated under a low magnification microscope. The problems are cross-reactivity with antibody against *C. psittaci* and *C. pneumoniae*, because of the use of *C. trachomatis* infected cells, and lack of objectivity because the results depend on visual evaluation.

IPAzyme is a commercially available test kit which is based on an indirect immunoperoxidase method. Although it has been the most widely used method in Japan, since it also utilizes *C. trachomatis* L2 strain infected cells as antigen, it has the same problems as the MFA method, i.e., cross-reactivity with antibodies against *C. psittaci* and *C. pneumoniae*, and lack of objectivity in evaluation. Therefore, development of simpler, more sensitive and specific assays for *C. trachomatis* antibodies had been highly anticipated.

Since this new test kit, HITAZYME®, utilizes Chlamydial outer membrane complex purified from *C. trachomatis* L2 elementary body as the solid phase antigen, it seldom cross-reacts with antibodies against *C. psittaci* or *C. pneumoniae*. Since it is based on an EIA method, the results can be evaluated semi-quantitatively by measuring optical density. Thus, HITAZYME® is a more objective and quantitative assay capable of processing many samples.

We therefore assessed this test kit for *C. trachomatis* antibodies.
After testing 258 specimens from healthy individuals with HITAZYME®, the distribution of both IgA antibody and IgG antibody was shifted slightly to the right. When a normal distribution is sought, the cut-off value should be determined after identifying a single peak and symmetrical sample distribution. A normal distribution is usually hypothesized, but when raw data are used, the distribution patterns tend to be shifted either to the right or to the left, and symmetrical distribution often cannot be achieved. In such cases, Geary’s symmetry tests can be performed after excluding extreme values. If a symmetrical distribution can be seen after excluding less than a small percentage of the samples, a normal distribution can be obtained from the rest of raw data. On the other hand, if a symmetrical distribution is not apparent after excluding less than a small percentage of the samples, the raw data can be transformed (log, exponent, root), and extreme values can be excluded until a symmetrical distribution is achieved. Then, a normal distribution can be obtained from the rest of the transformed values of samples. Hence, Geary’s symmetry tests were performed repeatedly after excluding extreme values, and a symmetrical distribution was achieved after excluding less than a small percentages of the samples [IgA; 245 samples (excluded: 5.0%), IgG: 236 samples (excluded: 8.5%)]. Thus, a normal distribution was obtained from the rest of the samples, i.e., the cut-off value was the mean value plus \(2 \times \text{S.D.}\) (standard deviation) (IgA: 0.176, IgG: 0.126).

[Comparison with the Results using IPAzyme®]

Although agreement of IgA data was relatively good, i.e., 82.6% (916/1109), 5.5% (61/1109) were HITAZYME® (-), IPAzyme® (+), while 11.9% (132/1109) were HITAZYME® (+), IPAzyme® (-). Thus, there was disagreement in quite a few cases. In these cases, more specific Western blot analysis was performed to check the reactivity of anti-\emph{C. trachomatis} antibody, and agreement of Western blot analysis was tested. Agreement between HITAZYME® and Western blot analysis was 69.6% (16/23), and agreement between IPAzyme® and Western blot analysis was 30.4% (7/23). HITAZYME® yielded significantly higher agreement than IPAzyme® (p=0.03).

In the case of IgG, agreement between the two assays was 73.7% (817/1109). Agreement was relatively low; 24.4% (271/1109) were HITAZYME® (-), IPAzyme® (+), which was significantly higher than HITAZYME® (+), IPAzyme® (-) cases (1.9%, 21/1109). In these cases, more specific Western blot analysis was performed to check the reactivity of anti-\emph{C. trachomatis} antibody in a similar manner. Agreement between HITAZYME® and Western blot analysis was 80.0% (12/15), and agreement between IPAzyme® and Western blot analysis was 20.0% (3/15). HITAZYME® had significantly higher agreement than IPAzyme® (p<0.001).

HITAZYME® had higher specificity in reacting with \emph{C. trachomatis} antigen than IPAzyme®, because HITAZYME® utilizes the purified outer membrane complex of \emph{C. trachomatis} L2 strain as the antigen, whereas IPAzyme® utilizes \emph{C. trachomatis} L2 infected cells as the antigen.

However, there were some cases in which HITAZYME® and Western blot analysis did not agree. One of the reasons for disagreement may be cross-reactivity with \emph{C. pneumoniae} or \emph{C. psittaci} which are in same genus as \emph{C. trachomatis}.

Matsumoto et al. compared the reactivity of human serum containing antibodies against \emph{C. pneumoniae}, \emph{C. trachomatis}, and \emph{C. psittaci} with purified outer membrane complex from \emph{C. trachomatis}. Their data showed that cross-reactivity with \emph{C. pneumoniae} was small, if not negligible. This low level of cross reactivity is not likely to influence detection of \emph{C. trachomatis} antibody. On the other hand, cross-reactivity with \emph{C. psittaci} was found to be relatively high. However, since \emph{C. psittaci} infection is much rarer than \emph{C. trachomatis} or \emph{C. pneumoniae} infection, it may not influence detection of \emph{C. trachomatis} antibody clinically.
On the other hand, since IPAzyme® utilizes L₂ strain-infected cells as antigen, lipopolysaccharide (LPS, a genus-specific antigen) and reticulate body (RB) in inclusion bodies which has genus-specific antigenicity increase cross-reactivity with C. pneumoniae, C. psittaci, and this may explain the disagreement mentioned above.

[Antibody-positive Rates in Antigen (+) Cases]

The IgA antibody-positive rate in antigen (+) cases was significantly (p<0.01) higher than in antigen (-) cases except for male urethritis (IPAzyme®). Therefore, IgA antibody reflects the activity of infection as an acute antibody.

There are some cases, however, in which IgA antibody is positive and antigen is negative, and in which IgA antibody is negative and antigen is positive.

This type of disagreement can be explained as follows. In antigen (+), IgA antibody (-) cases, the infection is in its initial phase, and IgA antibody has not increased yet. In antigen (-), IgA antibody (+), cases, the infection has taken place in superior regions, and consequently no antigen is detected in the urethra or cervical canal, the amount of antigen in the infected areas was small, or specimens were collected improperly.

In contrast, IgG antibody was approximately 30 to 60% positive in antigen (-) cases, and was correlated with a post history of C. trachomatis infection. IgG antibody is not suitable as an indicator for active infection.

[C. trachomatis Antigen-positive Rates According to Groups Classified by Antibody Findings]

Generally, it is more difficult to detect antigen in female patients than male patients because specimens cannot be collected properly due to mucous discharge in the cervical canal, the fact that the antigen detection rate varies despending on the menstrual cycle, and that C. trachomatis travels superiorly, and so it may not be present in cervical canal in pelvic inflammatory diseases. Therefore, antibody tests are particularly useful in female cases. Antigen tests may not be sufficient for the diagnosis of C. trachomatis infection, and serum antibody tests should be performed at the same time. A diagnosis should be made on the basis of all these clinical data.

Nevertheless, even among female patients at high risk, it is not possible to perform sampling in every patient. In female patients, as shown in Figures 8,9 and 10, when either IgA antibody or IgG antibody is positive, and particularly in those who are IgA (+), IgG (+), approximately 40 to 60% of patients were antigen-positive. When both IgA antibody and IgG antibody were negative, only 1 to 10% of cases were antigen-positive. Therefore, antibody tests should be performed first. If antibody tests prove positive, antigen tests should be performed. This diagnostic protocol may be useful clinically as a screening method for C. trachomatis infection.

References


尿路性 Chlamydia trachomatis 感染症における
特異的血清 IgA, IgG 抗体についての検討
—HITAZYME®と IPAzyme®の比較—

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要 旨
精製 Chlamydia trachomatis（以下 C. trachomatis）が外膜抗原を固定抗原として用いた酵素免疫抗体法（EIA）による C. trachomatis 抗体測定法——HITAZYME®（日立化成工業株式会社）一の有用性を臨床検体を用いて検討した。

1) HITAZYME®とIPAzyme®の検出成績では、IgAで陽性・陰性一致率は82.6％（916/1,109）と比較的良く一致していた。しかし、HITAZYME®陰性・IPAzyme®陽性症例が5.5％（61/1,109）、また、逆にHITAZYME®陽性・IPAzyme®陰性症例が11.9％（132/1,109）と一致症例も少なく認められた。

IgGの陽性・陰性一致率は73.7％（817/1,109）とかなり低く、HITAZYME®陰性・IPAzyme®陽性症例が24.4％（271/1,109）も認められた。

2) これらのHITAZYME®とIPAzyme®の一致率について、より特異性の高いWestern blot法で C. trachomatis 特異抗体の確認試験を行った。Western blot法との一致率は、IgAでHITAZYME®が69.6％（16/23）、IPAzyme®が30.4％（7/23）、IgGでHITAZYME®が80.0％（12/15）、IPAzyme®が20.0％（3/15）となり、HITAZYME®の方が有意に高い一致率であった。すなわち、HITAZYME®の方がIPAzyme®より,
C. trachomatis 抗原特異性が高いと証明された。

3）抗原陽性症例の HITAZYME®による IgA 抗体陽性率（男子尿道炎：72.7％，妊娠65.7％，女子子宮頸管炎：70.3％，女子骨盤内感染症：70.0％）は、抗原陰性例の IgA 抗体陽性率（男子尿道炎：16.7％，妊娠：13.6％，女子子宮頸管炎：22.6％，女子骨盤内感染症：30.4％）よりも有意（p＜0.01）に高値であり，IgA 抗体は活動性感染の指標となり得ると考えられた。

4）女子抗原陰性症例の HITAZYME®による IgG 抗体陽性率（15.5％）は，IPAzyme®による IgG 抗体陽性率（40.5％）よりも有意（p＜0.01）に低かった。

以上より，HITAZYME®は感度，特異性も比較的良好，しかも EIA 法であるため，客観的判定が可能で，しかも操作が簡便なため多量の検体を処理できる優れた C. trachomatis 抗体測定法と考えられる。抗原検査の困難な症例や，スクリーニング検査として臨床応用されるものと期待される。