Rapid diagnosis of chlamydial infection of the cervix

J. D. TREHARNE,* S. DAROUGAR,* P. D. SIMMONS,† AND R. N. THIN†
From the *Virus Laboratory, WHO Collaborating Centre for Reference and Research on Trachoma and other Chlamydial Infections, Department of Clinical Ophthalmology, Institute of Ophthalmology; and the †Department of Genital Medicine, St Bartholomew’s Hospital, London

SUMMARY A rapid serodiagnostic test for the presumptive diagnosis of chlamydial infection of the cervix has been developed. The method used is based on the modified micro-immunofluorescence test using pooled chlamydial antigens and the detection of different immunoglobulin classes of chlamydial antibody in sera and cervical secretions. The presence of IgG chlamydial antibody at a level of 1/64, or IgM antibody at a level of 1/8 or greater, or both in sera and IgG or IgA antibody at a level of 1/8 or more or both in cervical secretions was closely associated with the isolation of Chlamydia trachomatis and non-specific genital infection. In general, serodiagnosis was three to nine times more sensitive than cultural methods, and the detection of IgG chlamydial antibody in cervical secretions alone provided the most sensitive of the serological tests. This sensitive, low-cost, rapid, and simple serodiagnostic test for the presumptive diagnosis of chlamydial infection of the cervix, coupled with transportation of specimens by post, offers advantages over conventional isolation techniques for the routine diagnosis and management of chlamydial genital infections.

Introduction

Chlamydia trachomatis is now generally recognised as a pathogen in the male genital tract and as the primary cause of non-specific urethritis (NSU) in up to 68% of such patients (Dunlop et al, 1965; Dunlop et al, 1972; Oriel et al, 1972; Richmond et al, 1972; Alani et al, 1977).

In the female genital tract C. trachomatis was first isolated from the cervix of the mother of a baby suffering from TRIC-agent ophthalmia neonatorum (Jones et al, 1959). Since that time more sensitive cell-culture isolation techniques have become available, and the C. trachomatis isolation rate from the cervix of unselected women presenting at clinics for sexually transmitted diseases (STD) has been reported as varying between 12% and 31% (Hilton et al, 1974; Oriel et al, 1974; Burns et al, 1975; Nayyar et al, 1976).

Recent studies have shown that the modified micro-immunofluorescence (micro-IF) test for the detection of different immunoglobulin classes of type-specific antichlamydial antibody can provide information on the prevalence of these infections (Treharne et al, 1977a). Studies on ocular chlamydial infections have shown that the detection of different immunoglobulin classes of type-specific chlamydial antibody in eye secretions (tear fluids) as well as in sera can be used successfully for diagnosis (Treharne et al, 1977b; Darougar et al, 1978).

The present study was carried out to assess the sensitivity of the modified micro-IF test for the detection of antichlamydial antibody in both serum and local cervical secretions for the diagnosis of chlamydial cervical infections and to compare these with the results of cultural tests for the isolation of Chlamydia.

Materials and methods

SELECTION OF PATIENTS
All women presenting at the clinic between 10 February and 26 May 1977 with a presumed infection of the cervix were included in the study. Specimens were collected from the cervix for the identification of Neisseria gonorrhoeae, Trichomonas vaginalis, Candida species, and C. trachomatis. In addition, all women who were sexual contacts of men with genital herpes, or with any genital lesion suggesting genital herpes, had culture specimens collected from the cervix for herpesvirus isolation.
For the purposes of this study these women have been divided into five separate groups.

**Group 1**
This group consisted of: female sexual contacts of men with proved or suspected NSU; women with excess urethral or cervical leucocytes, irrespective of epithelial inflammatory changes, in whom no pathogens were found (non-specific genital infection [NSGI]); and women with evidence of salpingitis in whom no pathogens were found (non-specific salpingitis).

**Group 2**
This group consisted of women with evidence of infection by *N. gonorrhoeae*.

**Group 3**
This group consisted of women with candidal infection as determined by microscopy or culture or both.

**Group 4**
This group consisted of: women with culture-positive herpesvirus infection; women with trichomoniasis or genital warts; and women with vaginitis in whom no pathogens were found.

**Group 5**
This group consisted of women with no apparent venereal disease (NVD) on historical, clinical, or microbiological grounds.

**COLLECTION OF SPECIMENS AND LABORATORY TESTS**
Collection of specimens and investigations for *N. gonorrhoeae, T. vaginalis,* and *Candida* species have been described elsewhere (Burns et al, 1975).

Swabs for herpesvirus were collected from the cervical os and inoculated on to human embryonic lung cells (Thin et al, 1975).

**ISOLATION OF C. TRACHOMATIS**
The irradiated McCoy cell culture technique used was that described by Darougar et al (1971).

**COLLECTION OF BLOOD SAMPLES**
Blood was collected by venepuncture from all women. The serum was separated and stored at -20°C until tested.

**COLLECTION OF CERVICAL SECRETIONS**
Cervical secretions were also collected from all patients by means of a 10 × 5 × 1 mm cellulose sponge (John Weiss, London), which was held at the tip with a pair of forceps; these were then inserted into the cervical os so that the whole sponge was within the lower canal. The sponge remained in contact with the cervical secretions for at least 10 seconds; it was then removed, placed in a tightly stoppered plastic capsule, and stored at -180°C. Before antibody titration cervical secretion (CS) sponges were eluted with 0.7 ml of 0.1 mol/l phosphate-buffered saline (pH 7.2) which represented a starting dilution of 1/8.

**SEROLOGY**
The modified micro-IF test (Treharne et al, 1977a) was used to detect antichlamydial IgG and IgM antibodies in sera and IgG and IgA in cervical secretions. Statistical comparisons were made using Student's *t* test.

**Results**
In this study all investigations for *Chlamydia* were performed on a total of 272 women. *C. trachomatis* was isolated from the cervix of 35 (13%) out of 272 women. The distribution of culture-positive results in different patient groups is shown in Table 1.

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Total no. of patients</th>
<th>Any test</th>
<th>Chlamydial isolation</th>
<th>Micro-IF test* (serum)</th>
<th>Micro-IF test† (CS)</th>
<th>Micro-IF test (Serum or CS or both)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Group 1</td>
<td>60</td>
<td>42</td>
<td>70</td>
<td>15</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>Group 2</td>
<td>21</td>
<td>15</td>
<td>71</td>
<td>10</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Group 3</td>
<td>90</td>
<td>20</td>
<td>22</td>
<td>2</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Group 4</td>
<td>41</td>
<td>17</td>
<td>40</td>
<td>3</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Group 5</td>
<td>60</td>
<td>18</td>
<td>30</td>
<td>5</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>112</td>
<td>41</td>
<td>35</td>
<td>13</td>
<td>90</td>
</tr>
</tbody>
</table>

*IG G 1/64 or IgM 1/8 or both
†IgG 1/8 or IgA 1/8 or both
Antichlamydial IgG antibody at a level of 1/16 or more was present in the sera of 118 (43%) women. Antichlamydial IgG antibody in serum at a level of 1/64 or more or IgM antibody at a level of 1/8 or more or both was present in 90 (33%) patients. Antichlamydial IgG or IgA antibody at a titre of 1/8 or greater was present in the cervical secretions (CS) in 66 (24%) women. The total number of patients with positive results to any of the chlamydial tests was 112 (41%). Of these, 35 (31%) had positive results to isolation of C. trachomatis and 104 (93%) positive results by serodiagnosis (that is, serum IgG >1/64 or serum IgM >1/8 or both or CS IgG or IgA >1/8 or both). The percentage of women with antichlamydial antibody in either sera or cervical secretions in the different groups of patients is shown in Table 1, evidence of infection by C. trachomatis being more common in group 1 (70%) and group 2 (71%) than in any other group of patients.

The association of antichlamydial antibody with the isolation of C. trachomatis is shown in more detail in Table 2. The highest incidence (74%) of antibody associated with isolation was found in the IgG-positive cervical secretions. Antichlamydial antibody was present in either the sera (IgG >1/64 or IgM >1/8) or the cervical secretions (IgG or IgA >1/8) in 27 (77%) out of 35 women with positive C. trachomatis-isolation and in 77 (32%) out of 237 of those with isolation-negative results. Thus in 104 (38%) out of 272 women antichlamydial antibody was detected. Even though in the isolation-positive group there was a degree of significance in the finding of IgG in the cervical secretions compared with the detection of other immunoglobulin classes of antichlamydial antibody in serum there was no significant difference in the number of patients with positive results to combined serodiagnostic tests when compared with the number of patients with IgG antibody in cervical secretions alone (p>0.07).

In all cases the finding of any immunoglobulin class of antibody in either serum or cervical secretions in those patients with positive C. trachomatis isolation was highly significant (p>0.001 in all comparisons) when compared with the findings in those patients who were isolation-negative (Table 2).

The relative sensitivity of chlamydial isolation compared with detection of antichlamydial antibody in sera or cervical secretions is shown in the Figure and Table 1. With the exception of group 2 (patients infected by N. gonorrhoeae) chlamydial serodiagnosis was three to nine times more sensitive than chlamydial isolation.

In patients with serum IgG antichlamydial antibody levels of 1/64 or more, serum IgM levels of

---

**Figure Relative sensitivity of isolation and serodiagnosis for the detection of Chlamydia trachomatis in women attending an STD clinic**

**Table 2 Relationship of antichlamydial immunoglobulins with the presence of C. trachomatis in cell culture**

<table>
<thead>
<tr>
<th>Culture results</th>
<th>No. of patients</th>
<th>Serum IgG (1/64)</th>
<th>Serum IgM (1/8)</th>
<th>CS IgG (1/8)</th>
<th>CS IgA (1/8)</th>
<th>Serum or CS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>C. trachomatis isolation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>35 (13%)</td>
<td>14</td>
<td>40</td>
<td>17</td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td>Negative</td>
<td>237</td>
<td>38</td>
<td>16</td>
<td>54</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1/8 or more, or cervical secretion IgG or IgA levels of 1/8 or more there was a much greater probability of positive chlamydial isolation than in patients with antibody levels at lower titres (Table 3). Furthermore, the frequency with which a diagnosis of NSGI occurred was greater in patients with higher levels of antichlamydial antibody. Conversely, the greatest proportion of patients with low antichlamydial antibody levels were in the group with diagnoses other than NSGI.

Finally Table 4 shows a close relationship between cervical secretion titres and serum geometric mean titre (GMT). Clearly those patients with the highest titres of CS antibody (1/128) are those from whom C. trachomatis is most likely to be isolated (83%). Furthermore, 83% of patients with this level of antichlamydial antibody in the cervical secretions were diagnosed as belonging to the group with NSGI.

**Discussion**

In the present study the overall isolation rate of C. trachomatis was 13%, which compares well with the 12% rate in a previous study based on women from the same hospital (Burns et al, 1975). In this group of women 118 (43%) out of 272 had antichlamydial IgG antibody at a titre of 1/16 or greater. Richmond and Caul (1977) demonstrated antichlamydial antibody in 150 (61%) out of 245 women attending an STD clinic. In their study, however, a level of 1/8 or greater was taken as the criterion of positivity against a single C. trachomatis serotype. We have previously shown (Treharne et al, 1977a) that the GMT of antibody in 117 women with cervical isolation-positive results was 1/66, and Wang et al (1977) demonstrated a GMT of 1/101 in 49 women with cervical isolation-positive results. Thus we considered an antichlamydial IgG titre of 1/64 was a more realistic criterion of serological positivity.

In ocular chlamydial infections in the United Kingdom, the presence in blood of IgG at a titre of 1/64 or IgM at a level of 1/8 and IgG or IgA in eye secretions at a level of 1/8 or both closely correlated with the clinical diagnosis and presence of C. trachomatis. Accepting these criteria of serological positivity in the present study, 90 (33%) out of 272 patients had positive antichlamydial antibody in sera (that is, IgG >1/64 or IgM >1/8 or both) and 68 (25%) out of 272 patients had positive antibody in cervical secretions (IgG or IgA >1/8 or both), thus giving a total of 104 (38%) out of 272 patients when the two criteria are combined.

The presence, albeit a transient one, of IgM antichlamydial antibody has been shown to be

---

**Table 3** Correlation of levels of antichlamydial immunoglobulins in sera and cervical secretions with isolation of C. trachomatis and clinical diagnosis

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>Reciprocal antichlamydial antibody titre</th>
<th>No. of patients</th>
<th>Isolation-positive (%)</th>
<th>With non-specific diagnoses (%)</th>
<th>With other diagnoses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>&lt;16</td>
<td>220</td>
<td>9</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>IgM</td>
<td>≥1/64</td>
<td>52</td>
<td>27</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>≥1/8</td>
<td>201</td>
<td>8</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>&gt;8</td>
<td>71</td>
<td>24</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>Cervical secretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>≥1/8</td>
<td>200</td>
<td>5</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>IgA</td>
<td>≥1/8</td>
<td>224</td>
<td>7</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>&lt;1/8</td>
<td>48</td>
<td>42</td>
<td>52</td>
<td>48</td>
</tr>
</tbody>
</table>

**Table 4** Relationship of antichlamydial antibody levels in cervical secretions with antibody levels in serum and isolation of C. trachomatis

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Cervical secretion IgG titre</th>
<th>Geometric mean titre*</th>
<th>Serum*</th>
<th>Isolation-positive patients (%)</th>
<th>Patients with NSGI or as contacts of NSU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>128</td>
<td>161</td>
<td>10</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>16</td>
<td>32</td>
<td>84</td>
<td>5</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>44</td>
<td>8</td>
<td>29</td>
<td>4</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>206</td>
<td>0</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

*Reciprocal titre
Rapid diagnosis of chlamydial infection of the cervix

associated with early response to infection with C. trachomatis (Wang and Grayston, 1971). In this study IgM serum antibody at a titre of 1/8 or greater was present in 49% of isolation-positive women but in only 23% of isolation-negative women (p<0.001). Richmond and Caul (1977), using their single antigen test on similar groups of patients, demonstrated IgM serum antibody in only 29% of isolation-positive women and 7% of isolation-negative women. The overall figure in our study showed that we could demonstrate IgM antichlamydial serum antibody in 71 (26%) out of 272 women and IgG antibody (at 1/64 or more) in 52 (19%) out of 272 women. When these two criteria of serodiagnosis are considered together a considerably greater proportion of women seem to have chlamydial genital infections than is suggested by isolation studies.

The finding of antichlamydial antibodies in cervical secretions is not surprising since these antibodies have been found in local secretions, such as tear fluids in patients with hyperendemic trachoma (Treharne et al., 1978) and paratuberculosis (Darougar et al., 1978). In 26 (74%) out of 35 isolation-positive patients antichlamydial IgG antibody was detected at a titre of 1/8 or more. A strong correlation between the presence of C. trachomatis and the titre of CS IgG antibody was demonstrated, which is consistent with the evidence from ocular infections with this agent, where it was shown that over 75% of children with positive chlamydial isolation in a hyperendemic trachoma area in southern Tunisia had detectable type-specific antibody in their conjunctival secretions compared with only 20% in the isolation-negative group (Treharne et al., 1978).

Of 112 patients with cultural or serological evidence of chlamydial infection, 104 (93%) showed evidence of genital infection with C. trachomatis when the detection of antichlamydial antibody in either sera or cervical secretions is considered together. This compares with the figure of 35 (31%) out of 112 women from whom C. trachomatis was isolated. If we had used solely serodiagnostic tests as our criteria for C. trachomatis infection we would have excluded only eight isolation-positive women out of 112 who showed evidence of infection by any method.

Furthermore, our studies suggest that in such a group of unselected women those who show the most evidence of chlamydial genital infections are those who are diagnosed as having either NSGI or are sexual contacts of men with NSU (70%) and those who are infected with N. gonorrhoeae (71%). In agreement with others (Hilton et al., 1974; Burns et al., 1975) we found a higher isolation rate of C. trachomatis in women with concomitant gonococcal infections (48%) than in any other group. There were fewer women in this latter group, however, in whom we could detect antichlamydial antibody compared with those who had NSGI or were contacts of men with NSU. A possible explanation is that in women with acute gonorrhoea there is a relatively short incubation period and, although they may also harbour C. trachomatis, antichlamydial antibody has not yet been formed. This hypothesis was confirmed in this study by the fact that the women with a history of gonorrhoea for four weeks or longer more often had antichlamydial antibody than the women with gonorrhoea who presented at one to two weeks.

In agreement with most others we consider C. trachomatis to be a significant genital pathogen (Lancet, 1974) and that laboratory facilities should be available for the screening of such infections in women attending STD clinics. Until recently, the only available diagnostic test has been the isolation of C. trachomatis in susceptible tissue culture cells. This has not generally found favour with microbiologists as it is a time-consuming and extremely labour-intensive test. The collection of specimens for serodiagnosis on the other hand (particularly of cervical secretions or of venous blood with cellulose sponges) requires no great expertise (Darougar et al. 1978) and, provided specimens are posted directly to the laboratory, requires no special refrigerated transportation. Results can often be obtained within two or three hours, and when the specialised treatment needed in the various sexually transmitted diseases is considered such a test seems to have a valuable place in diagnosis.

The authors are grateful to Professor Barrie R. Jones for his encouragement and advice and to Mr R. J. Dines for his excellent technical assistance. We are indebted to the Department of Health and Social Security and to an anonymous donor for financial support.

References


