Rapid diagnosis of \textit{Chlamydia trachomatis} infection in patients attending an ophthalmic casualty department

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SUMMARY Direct immunofluorescence (DIF) techniques were compared with conventional cell culture for the diagnosis of ocular infections with \textit{Chlamydia trachomatis}. The DIF test was found to have a sensitivity of 100\% and a specificity of 97.5\%. Of 178 patients studied, 19 patients (11\%) were positive by DIF and 15 (8.4\%) by conventional cell culture technique. Four patients who had previous treatment with chloramphenicol eye drops were negative by cell culture but positive by the DIF test. The DIF test is considered to be a rapid, accurate test with a number of advantages over culture techniques for the detection of \textit{C. trachomatis}. The importance of appropriate referral of positive patients to prevent more serious sequelae due to \textit{C. trachomatis} infection is discussed.

Much interest has recently been shown in the diagnosis of acute conjunctivitis, with emphasis on the importance of viral and chlamydial infections and the necessity to diagnose them accurately.\(^{1,3}\)

One important cause of follicular conjunctivitis seen in an ophthalmic casualty department is infection by \textit{Chlamydia trachomatis}. Other causes are associated with adenovirus and herpes simplex infections, and serious hospital outbreaks of adenovirus type 8 have occurred in the UK.\(^{4,5}\)

\textit{C. trachomatis} infections of the eye have been linked with genital tract infections.\(^6\) As this organism is not eradicated by chloramphenicol, the usual broad spectrum antibiotic used in ‘blind’ therapy of bacterial eye infections, accurate differentiation between chlamydial and other bacterial infections is essential.\(^{3,5}\)

It is now well recognised that genital infections due to \textit{C. trachomatis} are increasing in the western world,\(^7\) and it can be reasonably expected that a concomitant increase in \textit{C. trachomatis} infections of the eye will occur. Genital infection due to \textit{C. trachomatis} in women is often asymptomatic,\(^8\) and the first indication of such infection may be conjunctivitis in the newborn. Infected infants will also carry \textit{C. trachomatis} in the pharynx, and, if the eye infection is recognised and treatment withheld, pharyngeal infection may lead to severe pneumonia in the first few months of life.\(^9\)

Until recently the only reliable method of identifying \textit{C. trachomatis} infections was by isolation of the organisms in cell culture.\(^10\) With the development of monoclonal antibody specific to \textit{C. trachomatis} techniques have been devised for the identification of antigen directly in clinical specimens by means of fluorescein labelled antibody.\(^10-12\)

This study compares isolation of ocuogenital strains of \textit{C. trachomatis} in cell culture with their direct identification in conjunctival smears.

Subjects and methods

One hundred and sixty-three patients (age range 13–55 years) presenting to the Casualty Department of the Bristol Eye Hospital with signs of acute follicular conjunctivitis between November 1984 and May 1985 were included in this study. Any patient who had clinical signs of bacterial conjunctivitis was excluded. In addition 12 neonates (10 days to 4 weeks) and three babies (10 weeks, 4 months, and one year) were also included.

A clinical history was taken and any previously used medication recorded. Patients were examined with a slit-lamp and any abnormality in the tarsal and bulbar conjunctivae, fornix, and lids noted. The cornea was examined and stained for signs of epithelial and subepithelial abnormalities or pannus formation.

Sterile cotton-wool swabs were applied vigorously to the lower lid conjunctivae of affected eyes to

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obtain a good yield of epithelial cells. Smears were prepared by rolling half the swab on to the centre of a 6 mm well of a Teflon-coated glass slide. The swab was then placed into sorbitol transport medium for isolation of chlamydia in cell culture. All specimens were placed at 4°C for up to four hours before being transported twice daily to the laboratory, where they were either immediately inoculated into cell cultures or snap frozen at −70°C.

Patients with suspected \textit{C. trachomatis} infections were initially treated with tetracycline eye drops. All patients were asked to attend again approximately seven days later for re-examination and assessment of laboratory results. Where there was laboratory evidence of \textit{C. trachomatis} infection, the diagnosis and implications were discussed with the patients before a two-week course of oral tetracycline was begun. Such patients were referred to the Department of Genito-Urinary Medicine at the Bristol Royal Infirmary for further investigation and contact tracing. Neonatal \textit{C. trachomatis} infections were treated with both topical tetracycline eye drops and oral erythromycin and the parents referred for investigation and treatment.

\section*{Laboratory Methods}

\textbf{Chlamydia trachomatis isolation.} Our standard methods of isolation in McCoy cell monolayers were used as previously described.\textsuperscript{4} Briefly, specimens were inoculated on to coverslip monolayers of McCoy cells pretreated with cytochalasin B. After centrifugation at 3000 g for one hour at 34°C cultures were incubated for a further hour at 35-5°C before the inoculum and medium were removed and replaced with maintenance medium and reincubated for 68–72 hours. Cultures were washed in phosphate buffered saline (PBS), fixed in methanol, and stained with Giemsa. Stained coverslip monolayers were mounted and examined systematically by dark ground microscopy for typical intracytoplasmic inclusion bodies of \textit{C. trachomatis}.

\textbf{Direct immunofluorescence (DIF) of conjunctival smears.} Smears were fixed in acetone for 5 minutes before being stained with a genus specific fluorescein isothiocyanate labelled chlamydia monoclonal anti-

<table>
<thead>
<tr>
<th>Culture</th>
<th>DIF +</th>
<th>DIF −</th>
<th>Total</th>
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<tbody>
<tr>
<td>Culture +</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Culture −</td>
<td>4</td>
<td>159</td>
<td>163</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>159</td>
<td>178</td>
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Sensitivity 100-0%. Specificity 97-5%.

Results

\textbf{C. trachomatis isolation in cell culture.} Of 178 patients with follicular conjunctivitis \textit{C. trachomatis} was isolated from 15 (8-4\%) in McCoy cell culture. These represented four males (18, 19, 22, and 23 years), three females (17, 18, and 20 years) and eight newborn babies.

\textbf{Immunofluorescence of conjunctival smears.} On examination of the 178 conjunctival smears \textit{C. trachomatis} antigen was detected in 19 by the direct monoclonal fluorescein labelled (DIF) reagent. Fifteen of these positive smears were confirmed by isolation of \textit{C. trachomatis} in cell culture (see above). The four remaining smears (from 2 females and 2 neonates) were taken from patients who had received recent chloramphenicol treatment. Overall the examination of smears by the DIF technique had a sensitivity of 100\% and a specificity of 97-5\% (Table 1). Characteristically positive smears contained large numbers (>100) of brightly fluorescing, apple green, spherical elementary bodies. Occasionally cells contained intracellular antigen which resembled \textit{C. trachomatis} inclusions in cell monolayers. These smears also contained copious amounts of a smaller particulate antigen similar to that observed previously in urogenital specimens and were present only in smears containing >1000 elementary bodies.\textsuperscript{10}

\textbf{Discussion.} Previous studies have shown that \textit{C. trachomatis} infections of the urogenital tract can be diagnosed
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rapidly by examination of clinical specimens by *C. trachomatis* genus specific\(^5\) or species specific\(^1\)-\(^12\) monoclonal antibodies in a direct immunofluorescent (DIF) system. We have extended the application of this technique to the diagnosis of *C. trachomatis* in patients presenting with follicular conjunctivitis and have shown that the diagnosis can be made from conjunctival smears in under one hour from receipt of the sample in the laboratory.

At the time of this study using the genus reactive monoclonal antibody, 10 or more elementary bodies in a smear were recommended by the manufacturer as being diagnostic of a *C. trachomatis* infection. We consider that, provided sufficient experience has been gained, diagnosis of *C. trachomatis* infections can be made when five or six cell associated elementary bodies are present in a smear.\(^8\) Although we used this criterion, low levels of elementary bodies were never detected in this study. Positive smears containing more than 1000 elementary bodies often contained large numbers of brightly fluorescing particulate bodies, which probably represent the glycolipid, genus reactive, heat stable antigen.\(^8\) At present further studies are in progress to clarify this observation.

A particular advantage that direct immunofluorescence has over conventional isolation of the organism in cell culture is the ability to detect chlamydia antigens in patients who have had inappropriate antibiotic treatment such as chloramphenicol. It is recognised that chloramphenicol is unreliable in resolving *C. trachomatis* in vivo but does interfere with the replication of *C. trachomatis* in vitro.\(^6\) Perhaps it is the number of viable elementary bodies in the eye which is the limiting factor in determining whether *C. trachomatis* may be isolated in cell culture following treatment with chloramphenicol. In our study four of 19 patients with *C. trachomatis* infections had had previous chloramphenicol treatment and the organism was not isolated in cell culture. These infections would not have been diagnosed without the introduction of the DIF test. Following laboratory diagnosis of *C. trachomatis* infection in these four patients appropriate antimicrobial therapy was instigated, with rapid resolution of the clinical symptoms. In contrast *C. trachomatis* was isolated in cell culture from one patient following chloramphenicol treatment. A further important aspect of the laboratory diagnosis of *C. trachomatis* is in those instances where herpes simplex virus infections may be diagnosed clinically without laboratory verification. Under such circumstances patients have been treated empirically with 5-iodo-2-deoxyuridine (IUDR), which is recognised as enhancing the replication of *C. trachomatis* in vitro. In vivo the severity of *C. trachomatis* infection has been shown to be increased after treatment with IUDR.\(^13\)

In a previous study in Bristol *C. trachomatis* was isolated from 13 (3%) out of 449 patients studied.\(^1\) In that study neonatal *C. trachomatis* infections were not considered. Apart from our neonatal study group *C. trachomatis* was identified in 9 (5.5%) out of a total of 163 adult patients tested.

The laboratory confirmed diagnosis of *C. trachomatis* in our adult population occurred in the age range 17–23 years, which is in agreement with previous observations\(^8\) and confirms that these infections occur in young, sexually active, often single adults. The importance of this diagnosis indicates the need for referral to a urogenital clinic for further investigations and appropriate treatment of their partners to avoid more serious sequelae in females such as acute salpingitis\(^14\)-\(^15\) or infertility.\(^16\)-\(^17\)

Similarly the identification of neonatal *C. trachomatis* conjunctivitis may be the first indication of an asymptomatic cervical infection in the mother, which also requires further investigation as outlined above. Furthermore prompt diagnosis is essential in newborn babies to enable the appropriate antimicrobial therapy (oral erythromycin and topical tetracycline) to be instigated to prevent those rare cases of lower respiratory tract involvement\(^16\)-\(^17\) which have been reported in the UK. Neonatal infections are acquired at birth and conjunctivitis may appear five to 14 days later.\(^20\) This incubation period is a useful indicator and allows differentiation from other bacterial infections. In rare cases chlamydia conjunctivitis appears earlier than five days if the membranes rupture shortly before term.\(^20\)

Before the development of the DIF test for detecting antigen directly in clinical samples, serology was also used as a presumptive indicator of a current *C. trachomatis* infection of the eye.\(^2\) We consider that direct antigen detection in smears prepared from infected conjunctiva has advantages over serological techniques and has an associated higher degree of specificity. Furthermore this technique gives a more rapid result and does not suffer from the drawbacks of storage, transport, and contamination which are associated with a culture based service.\(^10\)

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References


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