Rapid serological test for diagnosis of chlamydial ocular infections

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SUMMARY A rapid serodiagnostic test for the diagnosis of paratrachoma (TRIC ophthalmia neonatorum, inclusion conjunctivitis, TRIC punctate keratoconjunctivitis, and trachoma of sexually transmitted origin) has been developed. The technique is based on using a modified micro-immunofluorescence test for detecting antichlamydial IgG and IgM in the blood and IgG and IgA in tears. The blood samples are collected on cellulose sponges after a finger prick, and tears are collected by introducing small sponges into the lower conjunctival fornix of the eye. The blood and tear samples collected in this way could be sent to the diagnostic laboratory by post without special arrangements for cold storage.

In general the presence of antichlamydial IgG at a level of $\geq 1/32$ or IgM at a level of $\geq 1/8$ in blood and antichlamydial IgG or IgA at a level of $\geq 1/8$ in tears was closely associated with ocular paratrachoma. The combined results of the micro-IF test of blood and tears has yielded the highest rate of positivity (90%). In patients with acute untreated paratrachoma the sensitivity of this test was similar to that of irradiated McCoy cells. In patients with a milder infection receiving antibiotics the sensitivity of the serodiagnostic test was superior to that of the cultural test. The high sensitivity and specificity of this rapid, simple and inexpensive serodiagnostic test for the diagnosis of chlamydial ocular infections, coupled with simple and practical methods of collection and transport of blood and tear specimens, offer advantages over cultural tests for routine diagnosis and study of chlamydial ocular infections.

Infection by *Chlamydia trachomatis*, usually serotypes A-C, is responsible for hyperendemic trachoma of eye-to-eye transmission and still constitutes a major public health problem in certain developing countries. In industrially developed countries infection of the eye or genital tract by *C. trachomatis* is generally spread by sexual transmission from a genital reservoir of infection, with occasional transfer to the eye. These eye infections present as TRIC agent ophthalmia neonatorum, inclusion conjunctivitis, TRIC agent punctate keratoconjunctivitis, or as trachoma of genital transmission, and are usually caused by serotypes D-K. The incidence of this ocular paratrachoma (Jones, 1975) is likely to increase because of the apparent increase of chlamydial urethritis, cervicitis, proctitis, and salpingitis presenting as so-called 'non-specific'

urethritis, cervicitis, etc. (Catterall, 1974; Dunlop, 1974; Juhlin, 1974; Wiesner and Holmes, 1974). Accurate and rapid diagnosis of these infections and the assessment of the efficacy of treatment depend on the availability of sensitive laboratory tests.

The technique of culture in irradiated McCoy cells is the most sensitive test for the detection and isolation of *C. trachomatis* (Darougar *et al.*, 1971; Darougar *et al.*, 1977b). However, it is expensive and requires an established laboratory with adequate facilities and experienced staff.

The presence of antichlamydial antibodies in blood and tears is closely related to inflammatory changes and to the presence of *C. trachomatis* in the eye of patients with ocular chlamydial infections (Dwyer *et al.*, 1972; Treharne *et al.*, 1977b). The micro-immunofluorescence (micro-IF) test (Wang and Grayston, 1970, 1974) is sensitive for the detection of type-specific antichlamydial antibodies

(Dwyer *et al.*, 1972; Treharne *et al.*, 1977a; Wang and Grayston, 1974). Modification of this technique has resulted in the development of a simplified micro-IF test using pooled antigens instead of individual TRIC serotypes that makes it practicable to use this test for routine diagnosis and for epidemiological studies (Treharne *et al.*, 1977a).

This paper reports the results of testing sera and tears of patients with chlamydial, viral, bacterial, or allergic conjunctivitis in London using the modified micro-IF test, measuring type-specific antichlamydial antibody of various immunoglobulin classes. It also compares the sensitivity of this serological test for the diagnosis of paratrachoma with that of isolation of *C. trachomatis* in irradiated McCoy cells.

Subjects and methods

Patients included in this study were attending the External Eye Diseases Clinic, Moorfields Eye Hospital, London.

CLINICAL EXAMINATION

Eyes were examined with a Haag-Streit slit lamp. The signs observed graded from 0 to 3 following the method described elsewhere (Darougar *et al.*, 1977a; Dawson *et al.*, 1975).

CELL CULTURE

Conjunctival swabbings were collected from the whole conjunctiva (upper tarsus, upper fornix and lower lid) of both eyes using 1 swab for each eye (Darougar and Jones, 1971). The methods of culture in irradiated McCoy cells and the identification of inclusions are described previously (Darougar *et al.*, 1971).

BLOOD

Blood was taken by finger prick. A dry cellulose sponge (J. Weiss & Son Ltd) measuring $5 \times 3 \times 1$ mm was used for collection (Fig. 1a, b). When saturated, the sponge absorbed approximately 0.1 ml of blood that is, equivalent to 0.05 ml of serum. The saturated sponge was placed in a tightly-stoppered dry plastic capsule and kept at $+4^\circ\text{C}$ until transferred to the laboratory, where the sponges were stored at -70°C until tested.

TEARS

Secretions were collected from the lower conjunctival sac using 1 dry cellulose sponge (Spontex Ltd) measuring $5 \times 2 \times 1$ mm for each eye (Fig. 2a, b). When saturated, this sponge absorbed approximately 0.05 ml of fluid. The sponges collected from the right and left eye were placed in a tightly-stoppered

Fig. 1 Method of collection of blood by cellulose sponge following finger prick. (a) Dry sponge. (b) Sponge soaking with blood (full saturation of sponge is required)

dry plastic capsule and stored at $+4^\circ\text{C}$ until transferred to the laboratory, where they were stored in a refrigerator at -70°C .

MICRO-IF TEST

Using the modified micro-IF test (Treharne *et al.*, 1977a) antichlamydial IgG levels in blood were titrated in screening dilutions of 1/16, 1/32, 1/64, and 1/256. Levels of antichlamydial IgM antibodies in blood were titrated at screening dilutions of 1/8, 1/32, and 1/128, as were antichlamydial IgG and IgA levels in tears. Goat antihuman IgG, IgM, and IgA fluorescein-labelled conjugates (Hyland Division Travenol Laboratories Inc) at a dilution of 1/5 were used. The other technical procedures for the micro-IF test have been described elsewhere (Dwyer *et al.*, 1972; Treharne *et al.*, 1977a).

Results

The results of detecting type-specific antichlamydial antibodies of various immunoglobulin classes in

Fig. 2 Method of collection of tears by cellulose sponge. (a) Position of dry sponge in lower conjunctival fornix. (b) Sponge soaked with tears

blood and tears of 282 patients with various ocular infections are shown in Table 1.

Table 1 Levels of antichlamydial antibodies of various immunoglobulin classes detected by the modified micro-IF test in blood and tears of 282 patients with various ocular infections

Type of specimen	Class and titre of antibody	No. of patients	No. (%) patients	
			Active paratrachoma	Other (viral, bacterial, etc.)
Blood	IgG at 1/16	28	14 (50%)	14 (50%)
	IgG at 1/32	10	8 (80%)	2 (20%)
	IgG at 1/64 or higher	44	36 (82%)	8 (18%)
	IgM at 1/8 or higher	24	21 (88%)	3 (12%)
	No antibodies	200	4 (2%)	196 (98%)
Tears	IgG at 1/8 or higher	45	43 (96%)	2 (4%)
	IgA at 1/8 or higher	20	20 (100%)	0
	No antibodies	223	18 (8%)	205 (92%)

In blood IgG at a range of 1/16 to 1/256 was detected in 82 patients. In 28 of these the IgG level was 1/16; 14 (50%) of these were patients with clinical ocular paratrachoma (Table 1). In the remaining 54 patients the IgG level was $>1/32$. Of these, 44 (81%) were patients with clinical ocular paratrachoma (Table 1). In blood IgM at a range of 1/8 to 1/32 was found in 24 patients. Twenty-one (88%) of these were patients with ocular paratrachoma (Table 1). Antichlamydial antibodies were not detected in blood of 4 out of 52 patients with paratrachoma.

In tears IgG in a range of 1/8 to 1/128 was found in 45 patients (Table 1). Of these, 43 (96%) were patients with clinical ocular paratrachoma. IgA at a range of 1/8 to 1/32 was detected in 20 patients, all of whom were suffering from paratrachoma. Antichlamydial antibodies were not detected in tears of 18 patients with paratrachoma.

The relationship between paratrachoma and the levels of IgG and IgM in blood and the levels of IgG and IgA in tears is shown in Tables 1 and 2.

In general the presence of antichlamydial IgM in blood or antichlamydial IgG or IgA in tears was clearly associated with ocular paratrachoma (Table 1). However, the combined results of the micro-IF test of blood (IgG at a level of $\geq 1/32$ and IgM at a level of $\geq 1/8$) and/or tears (IgG and IgA at a level of $\geq 1/8$) has yielded the highest rate of positivity (90%) (Table 2). This is similar to the rate of positivity obtained from this group by isolation in irradiated McCoy cell culture (87%) (Table 2).

No chlamydia was isolated from 21 patients with clinically active paratrachoma who were under treatment with antibiotics. In these patients the micro-IF test on blood and tears were positive in 16 (76%) (Table 2).

Table 2 Relationship between levels of antichlamydial antibodies detected by the modified micro-IF test in blood and tears of 282 patients and the diagnosis of their ocular or other infections

Diagnosis	No. of patients	Positive culture for chlamydia	Blood	Tears	Blood and tears
			IgG at 1/32 or higher IgM at 1/8 or higher	IgG } IgA } at 1/8 or higher	
<i>Paratrachoma</i>					
Active untreated	39	34 (87%)	30 (77%)	33 (85%)	35 (90%)
Isolation +ve	34	34	23 (68%)	29 (85%)	31 (91%)
Treated, but actively inflamed	21	0	14 (67%)	9 (43%)	16 (76%)
Cured	8	0	0	0	0
Ophthalmia neonatorum	2	1	1	1	1
Follicular conjunctivitis of unknown etiology	3	0	0	1	1
Non-specific genital infection*	4	ND†	4	2	4
Other conjunctivitis (viral, bacterial, allergic, etc.)	205	ND	6 (3%)	1 (0.5%)	7 (3%)

*Patients attended because of adenovirus or allergic conjunctivitis. †ND — Not done

Four patients attending the clinic because of an adenovirus or allergic conjunctivitis were concomitantly suffering from a non-specific urethritis or cervicitis. The micro-IF test was positive in blood of all 4 patients and in the tears of 2.

In 205 patients suffering from non-chlamydial conjunctivitis (viral, bacterial, and others) the micro-IF test on blood as well as tears was positive in 7 (3%) (Table 2). No attempt had been made to determine the presence of concomitant chlamydial genital infections in these 7 patients.

In all patients except 2 the antichlamydial antibodies detected were against TRIC serotypes D-K. In these 2 patients the antibodies found were against TRIC serotypes A-C, responsible for hyperendemic trachoma.

Discussion

The simplified one-passage technique of culture in irradiated McCoy cells provides a rapid and sensitive test for the isolation of chlamydia. With this test chlamydial isolates were obtained from 90% of cases of TRIC ophthalmia neonatorum and inclusion conjunctivitis in London and up to 82% of cases of severe hyperendemic trachoma in Tunisia (Darougar *et al.*, 1977b). The technique of culture in irradiated McCoy cells or alternative cultural systems, namely, McCoy cells treated with idoxuridine or cycloheximide (Wentworth and Alexander, 1974; Ripa and Mardh, 1977), HeLa cells treated with DEAE-dextran (Kuo *et al.*, 1972) are also complicated methods that require an established laboratory with adequate facilities and experienced staff.

Earlier studies in this laboratory have shown that levels of antichlamydial antibody in separated serum are generally twofold higher than the levels of antibody found in whole blood collected on cellulose sponges. The technique of collection of blood on sponges, however, is far simpler and more rapid than venepuncture.

Recent studies have shown that antichlamydial IgG is present in the sera of over 80% of patients with ocular chlamydial infections (Treharne *et al.*, 1977b). In patients presenting with ocular paratrachoma the level of antichlamydial antibody found in the sample of blood collected during the acute phase of the infection is generally high, and remains at this level during the course of the disease, because of the concomitant genital chlamydial infection that usually precedes the eye infection. Therefore the diagnostic fourfold rise in the titre of antibody cannot be shown in the convalescent serum of the majority of these patients.

In this study IgG at a level of 1/16 was present in the blood of 14 patients with no obvious ocular or genital chlamydial infections (Table 1). Studies in our laboratory have shown that IgG in the blood may remain for some months after recovery from chlamydial ocular or genital infection. Hence the presence of IgG antibody at a low level may indicate a previous exposure to these infections.

In the blood antichlamydial IgG at a level of $\geq 1/32$ correlates well with clinically active paratrachoma. The presence of antichlamydial IgM in the blood and IgG or IgA in the tears is highly specific and faithfully reflects activity of ocular chlamydial infection.

The result of this work indicates that the modified micro-IF test on blood and tears together is a highly sensitive test for the diagnosis of paratrachoma. In patients with acute untreated paratrachoma the sensitivity of this test was similar to that of culture in irradiated McCoy cells. In 21 patients originally isolation-positive, who were inadequately treated with antibiotics and were still showing signs of active inflammatory changes in their eyes, the micro-IF test on blood and tears was positive in 16 (76%), whereas cell culture failed to detect chlamydia.

In addition to the high sensitivity the micro-IF test on blood and tears shows a very high degree of specificity. Of 205 patients who were suffering from a clinically or microbiologically diagnosed adenovirus, herpes virus, bacterial or allergic conjunctivitis, the micro-IF test was positive only in 7. Although these 7 patients were not investigated further, the possibility of a mild or atypical form of TRIC ocular infection or a concomitant or previous mild or asymptomatic genital chlamydial infection in them cannot be ruled out.

In the present group 4 patients with adenovirus or allergic conjunctivitis had a mild urethritis or cervicitis. The micro-IF test on blood was positive in all these cases and on tears in 2. Although this number of cases is too small to draw any conclusions, the result indicates that this test may be useful for the detection of chlamydial genital infections.

The modified micro-IF test has provided a rapid, simple, and inexpensive technique for the detection of immunoglobulin classes of antichlamydial type-specific antibody in the blood and tears. In this study 4 pooled antigens and a normal yolk-sac as control have been used. The pooled antigens are TRIC serotypes A-C (hyperendemic trachoma), TRIC serotypes D-K except J (paratrachoma), LGV 1-3 (lymphogranuloma venereum), and 4 representative isolates of *C. psittaci*. For routine purposes in selected communities the test can be further simplified by using only A-C or D-K pooled antigens and excluding the LGV and *C. psittaci* pooled antigens. This much simplified micro-IF test can be successfully used for the diagnosis of chlamydial ocular infections of sexually transmitted origin (pooled D-K antigens) or for the diagnosis of hyperendemic trachoma of eye-to-eye transmission (pooled A-C antigens).

The egg-grown antigen used in this study (Dwyer *et al.*, 1972; Treharne *et al.*, 1977a) has several advantages over cell-grown antigens (Richmond and Caul, 1975). Inoculating fertile hens' eggs with chlamydia and harvesting the infected yolk-sac is a simple procedure. One heavily infected egg provides

sufficient antigen for 2500 or more tests. A freeze-dried antigen may be stored for several months at -4°C or at room temperature without a marked loss of antigenicity. With the possibility of mass production and subsequent lyophilisation the antigen could be prepared by a national or international centre and distributed to other laboratories for routine diagnosis or study of chlamydial ocular infections.

In addition to the antigen the micro-IF test requires a fluorescent microscope and commercially available antihuman IgG, IgM, and IgA fluorescein-labelled conjugates. The staining technique and the screening of stained slides is simple and rapid, taking up to 2 hours. A technician with 2 to 3 weeks' training can test over 100 specimens a day.

Collection of blood and tear samples using dry sponges is simple, rapid, and does not require highly trained staff. We have shown that tear and blood samples collected in this way could be stored at room temperature or at -4°C for 1 week without a marked decrease in the level of antibody. Therefore specimens could be sent by post without special arrangement for cold storage.

The high sensitivity and specificity of this rapid, simple, and inexpensive serodiagnostic test, coupled with simple and practical method of collection and transport of blood and tears specimens, offer advantages over the cultural tests for routine diagnosis of ocular chlamydial infections.

Work is in hand to apply this rapid serodiagnostic test for the diagnosis of chlamydial genital infections and for hyperendemic trachoma.

The authors are grateful to colleagues at Moorfields Eye Hospital for referring their patients.

This work is financially supported by grants from the Department of Health and Social Security and an anonymous donor.

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