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The detection of *Chlamydia trachomatis* by direct immunofluorescence in conjunctival smears from patients with trachoma and patients with ophthalmia neonatorum using a conjugated monoclonal antibody

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SUMMARY

Duplicate specimens were taken with cotton-wool swabs from the upper tarsal conjunctiva of 63 patients living in Gambian villages in which trachoma is endemic and from 34 infants with ophthalmia neonatorum (ON) attending an outpatient clinic in The Gambia. The detection of *Chlamydia trachomatis* by direct immunofluorescence (IF) using a conjugated monoclonal antibody to its principal outer membrane protein was compared with isolation in cycloheximide-treated McCoy cells. For trachoma, the sensitivity and specificity of the immunofluorescent technique were 62% and 100% respectively if ten elementary bodies (EBs) was taken as the minimum requirement for positivity by IF. If all cases with one or more EB were considered positive, the sensitivity was 81% and the specificity 85%. For ON the sensitivity and specificity were 100% and 95% respectively, regardless of which criterion was used. In view of its simplicity and easy applicability to field conditions it seems likely that direct IF using monoclonal antibodies may be a useful technique for the detection of *C. trachomatis* in the conjunctival epithelium of patients with trachoma.

INTRODUCTION

Demonstration of *Chlamydia trachomatis* in conjunctival epithelum is a useful adjunct to clinical examination in studying the epidemiology of trachoma. It confirms the diagnosis in doubtful cases, gives a measure of the reservoir of infection in the community and is helpful in monitoring the effect of control programmes (Dawson, Jones & Tarizzo, 1981; Jones, 1974).

Several techniques are available. Conjunctival scrapings may be examined for inclusions after staining with iodine or Giemsa, or by immunofluorescence using polyclonal antisera. All these techniques are well suited to work under field conditions, but scraping the conjunctiva with a sharp metal blade is painful, frequently causes bleeding in patients with active trachoma, and is likely to lead to resentment and lack of co-operation among populations investigated. Isolation

in irradiated or cycloheximide-treated McCoy cells is more sensitive than any of these techniques (Jones, 1974; Darougar *et al.* 1971; Schachter *et al.* 1978) and does not require conjunctival scrapings since cotton-wool swabs have been shown to yield as many positives as scrapings by this technique (Darougar & Jones, 1971). It is therefore the method of choice at present. Its disadvantage is that it requires tissue culture facilities and liquid nitrogen for storing specimens, neither of which may be available in trachoma-endemic areas, which are often remote and poor.

Elementary bodies of *C. trachomatis* (EBs) may be demonstrated by direct immunofluorescence (IF) in smears from infected epithelial surfaces using conjugated monoclonal antibodies against the principal membrane protein of the organism. This technique has been shown to be comparable to isolation in cycloheximide-treated McCoy cells for genital tract specimens. (Thomas *et al.* 1984; Ruijs *et al.* 1984; Tam *et al.* 1984), and for those taken from the eyes of experimentally infected monkeys and from infants with ophthalmia neonatorum (Taylor, Agarwala & Johnson, 1984; Taylor *et al.* 1984). We report here a comparison between the two techniques for the detection of *C. trachomatis* in eye swabs from 63 patients in a trachoma-endemic area.

PATIENTS AND METHODS

The geography and climate of The Gambia have been previously described (Sowa *et al.* 1965). This study was carried out in April 1984 in the villages of Jali, Kiang District (population about 1000) and Tuba, Wuli District (population about 600). Both are typical Gambian villages which live by subsistence farming and draw their water from wells, and in both the Mandinka tribe predominates.

Each village contains about 50 compounds; these are fenced–in areas often containing several houses and are inhabited by an extended family. In Jali the entire population was examined. In Tuba, five compounds were selected at random from a list kept by the village chief for tax purposes. Both eyes of each inhabitant were then examined using an illuminated loupe (× 4 magnification). Signs of active trachoma in the upper tarsal conjunctiva were scored according to the system of Dawson, Jones & Tarizzo (1981), scoring both follicles and papillae on a scale 0–3. On the basis of these scores the severity of active trachoma in each subject was graded as insignificant or absent, mild, moderate or severe.

In 63 cases swabs were taken from the more severely affected eye. A cotton-wood swab (MW 142, Medical Wire Company, Corsham, Wiltshire) was rubbed gently four times along the entire length of the tarsal conjunctiva. The swab was immediately immersed in sucrose phosphate (2SP) transport medium containing gentamicin 50 μg/ml and 10% foetal calf serum, and vigorously rotated before being removed. Specimens were stored on ice for up to 6 h before being transferred to liquid nitrogen. A second swab was then taken in exactly the same way, and a smear was made by rubbing the swab firmly and evenly over the whole area of one well in a ten-well multi-test slide (Flow Laboratories, UK) so that some material was visible on the slide. Smears were air-dried and kept at ambient temperature for up to 4 h before fixing in acetone. Fixed specimens were kept at 4 °C for up to 5 days and subsequently at −20 °C.

Isolation at C. trachomatis

After storage in liquid nitrogen for up to 4 weeks isolation of *C. trachomatis* was attempted in cycloheximide-treated McCoy cells as described in a previous publication. (Mabey & Whittle, 1982). Monolayers were stained with Giemsa after 48 h incubation and examined for inclusions under dark-field illumination.

Direct micro-immunofluorescence

Smears were stained for 20 min at room temperature in a humidified chamber with 20 μl of 'Microtrak' *C. trachomatis* direct fluorescent antibody reagent (Syva Company, Palo Alto, California). This contains a fluorescein-conjugated monoclonal antibody directed against the principal membrane protein of *C. trachomatis* and Evans Blue counterstain, in a protein-stabilized buffer solution. Slides were then washed for 5 min in distilled water, air dried and mounted under coverslips with 'Microtrak chlamydia direct specimen mounting fluid', and examined with a Leitz Orthoplan fluorescent microscope, each smear being scanned at $\times$ 200 magnification. Elementary bodies were identified by their characteristic size, morphology and apple-green fluorescence, which were confirmed at a magnification of $\times$ 500.

All specimens were examined by one observer (D.C.W.M.), but smears and cell monolayers were coded so that he was unaware of the clinical findings and the results obtained by the other method at the time of reading.

RESULTS

In Jali the entire population was examined, comprising 482 children aged less than 15 years. In this age group the prevalence of active trachoma was 22%. In Tuba five compounds were selected at random from the tax registrar. There were 46 children under 15 years in these compounds and the prevalence of active trachoma was 13% among them.

Duplicate swabs were taken from a total of 63 children, comprising 32 without evidence of active trachoma and 31 with sufficient subtarsal follicles and papillae to be classified as cases of active trachoma by the criteria of Dawson, Jones & Tarizzo (1981). The results of chlamydial isolation and detection by IF are shown in Table 1. If one takes as positive only those cases in which ten or more EBs were detected by IF, 6 of 16 specimens positive by culture were negative by IF. The number of inclusions identified in cell monolayers from these 6 patients were: 1 inclusion (in three cases); 2, 3 and 129 inclusions (in one case each). There were no false positives by IF.

If one takes as positive by IF every case in which one or more typical EB was seen, 13 of 16 cases positive by culture were positive by IF, but there were 7 false positives by IF.

We have also compared the two techniques for the detection of chlamydia in eye swabs from 34 infants with ophthalmia neonatorum (ON) attending the outpatients department at the M.R.C. hospital in Fajara, The Gambia; 14 were positive by both techniques, 19 were negative by both and one was positive by IF but not culture. In all positive cases more than 10 EBs were detected.

Table 1. *Results of investigation by culture (in cycloheximide-treated McCoy cells) and by direct immunofluorescence according to clinical category, taking as positive by IF only those cases in which 10 or more EBs were detected*

Intensity of active trachoma	Number of patients swabbed	Positive IF and culture	Positive IF only	Positive culture only	Negative IF and culture
Insignificant	32	1	0	1	30
Mild	10	2	0	2	6
Moderate	8	3	0	2	3
Severe	13	4	0	1	8
Total	63	10	0	6	47

DISCUSSION

By comparison with isolation in cycloheximide-treated McCoy cells, the direct IF technique described has been shown to have a specificity of 100% and a sensitivity of 62% in our patients with trachoma if one follows the manufacturer's recommendation that only cases in which ten or more EBs are detected should be considered positive. It is possible that the sensitivity of the IF method might have been higher if the swab for IF had been taken before the swab for culture.

It has been suggested that the sensitivity of the IF technique might be increased by reducing the minimum number of EBs required to constitute a positive result below the ten recommended by the manufacturer (Thomas *et al.* 1984; Taylor, Agarwala & Johnson, 1984*a*). If one counts as positive all those in the present study in whom one or more EB was seen, one increases the sensitivity to 81% but reduces the specificity to 85%. Three of the seven resulting false positives by IF were from patients with clinical evidence of active trachoma. It is therefore possible that, as has been suggested in experimental ocular infections in monkeys, the IF technique is actually more sensitive than culture in cycloheximide-treated McCoy cells (Taylor, Agarwala & Johnson, 1984).

The sensitivity of the IF technique was 100% and the specificity 95% in our patients with ON. The reason for its greater sensitivity in ON than in trachoma is clear; in ON chlamydiae are present in large numbers, whereas in trachoma they are usually not. In all but one of our patients with trachoma giving false negative results by IF, fewer than four inclusions were detected in tissue culture.

We feel that until this new technique has been more thoroughly validated in a number of different laboratories it would be wise to avoid false positives by adhering to the manufacturer's recommendation that at least ten characteristic EBs should be identified before a specimen is considered positive. This will inevitably make the technique rather less sensitive than isolation in tissue culture. Nevertheless, in view of its easy applicability to field conditions and the fact that it does not require traumatic scraping of the conjunctiva in order to demonstrate chlamydia, we feel that it will prove a useful tool in epidemiological studies of trachoma.

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