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# A rapid immunoperoxidase assay for the detection of specific IgG antibodies to *Chlamydia trachomatis*

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SUMMARY A technique, using indirect immunoperoxidase antibody (IPA), was developed for the detection of IgG antibody to *Chlamydia trachomatis*. The IPA technique employs glass slides with air-dried and acetone-fixed *C trachomatis* infected cells, which can be stored at −70°C and used for several months. Antibody titres detected by IPA were comparable to those detected by the indirect fluorescent antibody technique.

In recent years, the importance of *C trachomatis* in sexually transmitted disease has been recognised,[1,2] in addition to its long recognised role in trachoma.[3]

The diagnosis of *C trachomatis* infections is generally made after isolation in cell culture,[4] while the detection of chlamydial antibody in sera or local secretions is possible by other techniques. To date, microimmunofluorescence (MIF),[5] is the most specific but it requires the availability of many serotypes of *C trachomatis* and trained personnel. Efforts to simplify the test have involved the use of a single broadly reacting antigen, usually L2.[6] Serotype E[7] or serotype L2[8,9] have also been used in a single-antigen immunofluorescence antibody assay. Recently, ELISA[10,11] and RIA[12] assays have also been used for the detection of antibody to *C trachomatis*.

In the present study we have developed a simple immunoperoxidase technique for the detection of *C trachomatis* IgG antibody; this was compared with the immunofluorescence antibody assay (IFA).

## Material and methods

### CELLS AND MEDIA

The LLC—MK2 cell line derived from monkey kidney cells was used. These cells (which we have found to be highly susceptible to *C trachomatis*) were grown in 25 $cm^3$ tissue culture flasks with Eagle's MEM with 10% fetal calf serum (FCS) and gentamicin (10 μg/ml). When a confluent monolayer had formed, the growth medium was replaced with the same medium containing 25 μg/ml of 5-iodo-2′ deoxyuridine (IUDR). After 72 h, IUDR-containing medium was discarded, the cell monolayers were washed twice with phosphate-buffered saline (PBS) and refed with Eagle's MEM plus 2% FCS, gentamicin (10 μg/ml) and glucose (30 μmol/l).

### C TRACHOMATIS ANTIGEN PREPARATION

LLC–MK2 cell monolayers were infected with *C trachomatis* serotype L2(434–Bu strain, provided by J Schachter, San Francisco, California). The infected cultures were incubated at 37°C for 48 h. At that time, 60–80% of the cells showed the typical cytoplasmic inclusion, detectable by inverted microscope. The cells were removed from the flask with versene-trypsin solution, and washed with 0·01 *M* PBS, pH 7·4. Drops of twice-washed cell suspension, containing $10^6$ infected cells/ml mixed with $2 \times 10^5$ uninfected cells/ml, were placed on glass slides and dried overnight at room temperature. Uninfected LLC–MK2 cells, prepared in the same manner, were used as controls. The slides were fixed in cold (4°C) acetone, air dried, and stored at −70°C. The same antigen preparation was used for both the immunoperoxidase and immunofluorescence techniques.

### IMMUNOPEROXIDASE ANTIBODY ASSAY (IPA)

The procedure has been described previously.[13] Stored slides were thawed, washed in PBS and covered with test serum or control serum. After incubation at 37°C for 30 min followed by 15 min in PBS, the slides were incubated for 30 min with anti-human IgG peroxidase conjugate (Dako,

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Copenhagen, Denmark), 1/40 diluted in PBS. After washing, the enzymatic activity was detected using a modification of the method of Graham and Karnowsky.[14] The freshly prepared substrate solution was made of 4mg benzidine (Fluka AG, Buchs, Switzerland), dissolved in 0·5 ml acetone, 9·5 ml PBS, and 10 $\mu$l hydrogen peroxide from a 33% stock solution. The substrate was added for 5 min at room temperature and was followed by PBS washing. A dark blue cytoplasmic stain of *C trachomatis* inclusion was considered positive. Known positive and negative sera were included in each assay; the reproducibility of the titration was demonstrated by testing the same positive sera several times.

### IMMUNOFLUORESCENT ANTIBODY ASSAY (IFA)

This was performed as previously described,[15] but for the slides which were prepared as described above, instead of Lab-Tek (Miles Laboratories, Illinois) tissue culture chamber slides seeded with McCoy cells. Briefly, serial twofold dilutions of each sample of serum were placed on the slides and incubated for 30 min at 37°C. The slides were then washed three times in PBS and dried. Fluorescein-conjugated antibodies to human IgG (Dako, Copenhagen, Denmark) were added, and the slides were incubated for 30 min at 37°C, washed, counterstained with Evans' blue, mounted with glycerin and observed by a Zeiss UV microscope (× 400).

### HUMAN SERA

Serum samples from 90 healthy men, and five convalescent sera of men with chlamydial non gonococcal urethritis (NGU), were tested.

## Result

Fig. 1(a) shows the typical dark blue cytoplasmic staining of *C trachomatis* LLC–MK2 infected cells, by peroxidase reaction. Cytoplasmic staining was absent on uninfected LLC–MK2 cells or when *C trachomatis*-negative sera were examined (Fig. 1(b)). The use of peroxidase-conjugated antibody to human IgG at a dilution of 1/40 gave satisfactory results for specific determination of titres of antibody to *C trachomatis*. Low levels of antibody to *C trachomatis* were not detected with a conjugate dilution of 1/80. Fig. 2 shows the typical cytoplasmic fluorescence of LLC–MK2 cells infected by *C trachomatis*.

Sera of 90 healthy men and five convalescent sera of men with chlamydial NGU were examined by both IPA and IFA. Fig.3 demonstrates the comparison of IPA and IFA titres of sera from healthy adults and patients. Sixty-six sera were negative by both methods (titre <1/8) and 24 (26·6%) sera from

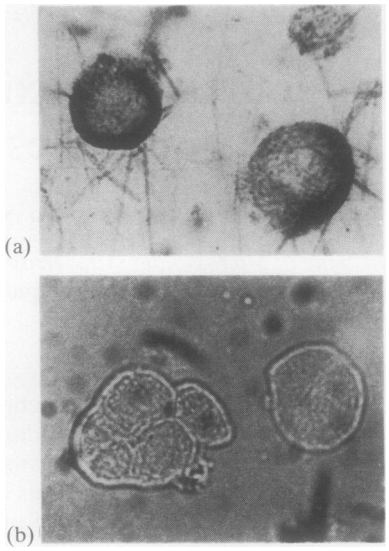


Fig. 1 *A typical dark blue cytoplasmic staining of (a) C trachomatis-positive and (b) C trachomatis-negative sera, given by peroxidase reaction.*

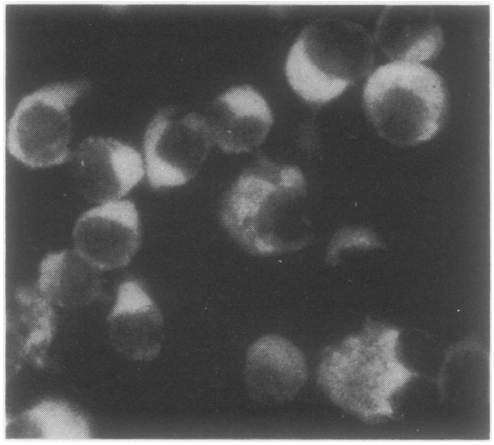

Fig. 2 *Cytoplasmic staining of C trachomatis infected cells by indirect immunofluorescence assay.*

healthy subjects had titres of 1/8–1/128 by both IPA and IFA with a mean titre of 18·6 and 21·3 by IPA and IFA respectively. Titres ≥ 1/32 were detected in convalescent sera of patients with chlamydial NGU, with a mean titre of 179·2 and 140·8 by IPA and IFA respectively.

## Discussion

With better diagnostic methods, *C trachomatis* has been found to be a widespread pathogen, involved

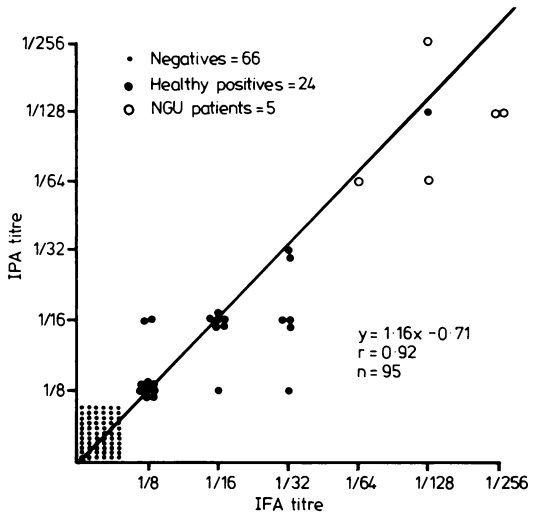


Fig. 3 *Comparison of antibody titres detected by indirect immunoperoxidase (IPA) and by direct immunofluorescence (IFA) assay.*

in many human infections. In males, *C trachomatis* is one of the most frequent causes of NGU.[16 17] In females, cervicitis[18] is the most frequent infection caused by *C trachomatis*. Chlamydial infection can also induce salpingitis.[19] There is also some serological evidence that *C trachomatis* may be linked with infertility in women[20 21] and dysplasia.[22–24] In addition, inclusion conjunctivitis by *C trachomatis* is a very common neonatal eye disease, and the commonest in the United States.[3] The association of an infant distinctive pneumonia syndrome with *C trachomatis* has also been demonstrated.[25] At present, there is no ideal method for the serological diagnosis of *C trachomatis* infections. The two most frequently employed tests are complement fixation (CF) and microimmunofluorescence. The CF test detects the group antigen that is common to all *Chlamydia* strains, but cannot differentiate type-specific infections. In addition, this test lacks sensitivity in cases of oculogenital *C trachomatis* infections. The MIF developed by Wang and Grayston[5] is more sensitive than CF and detects type-specific antibodies. This test, however, is only available in a few laboratories due to the difficulties of preparing the reagents. Richmond and Caul[7] have performed a single-antigen indirect immunofluorescence test which employs *C trachomatis* (serotype E) infected McCoy cells as the antigen. Subsequently, other authors[8 9] performed the single-antigen indirect immunofluorescence test using *C trachomatis* serotype L2, which had been estimated previously to cover 95·5%[6] of the *C trachomatis* antibodies detected in MIF.

Recently, the reliability of immunoperoxidase technique has been carefully evaluated in comparison with immunofluorescence,[13] complement fixation,[26] radioimmunoassay and ELISA[27] in our laboratory. A complete correlation, as far as positive and negative sera were concerned, was found between the different methods.

In the present study we developed an indirect peroxidase antibody technique to *C trachomatis*. The IPA described was as sensitive as IFA technique, with a good correlation (r = 0·92) between the techniques. The titre was reproducible within a twofold range. The IPA technique could detect either the low level of IgG antibody to *C trachomatis* in the normal population, as previously described by other authors using MIF[28] or IFA,[8] or higher IgG antibody level in patients with chlamydial NGU.

The method is suitable for large scale objective detection of *C trachomatis* antibodies. The slides, which can be stored at −70°C for months, are easily read by low-power light microscopy. Reagents are easily prepared and since the technique is rapid, simple and sensitive, it has the potential for widespread application.

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