Conventional tissue culture compared with rapid immunofluorescence for identifying *Chlamydia trachomatis* in specimens from patients attending a genitourinary clinic

E L TEARE,* C SEXTON,* F LIM,† T McMANUS,† A H C UTTLEY,* AND J HODGSON*

From the *Public Health Laboratory, Dulwich Hospital, and the †Department of Genitourinary Medicine, King's College Hospital, London

SUMMARY Specimens collected from 182 patients with histories suggesting chlamydial disease were examined by conventional culture and direct immunofluorescence techniques. *Chlamydia trachomatis* was identified by both methods in 57% of all patients. There was no significant difference between the two methods in detecting *C trachomatis*.

Where a tissue culture service is not already established, cost analysis in individual departments may justify the use of the immunofluorescence method.

Introduction

Chlamydia trachomatis is widely recognised as the most common sexually transmitted agent in the United Kingdom,¹ with an incidence nearly three times that of *Neisseria gonorrhoeae*.² *C trachomatis* is associated with urethritis, salpingitis, epididymitis, perihepatitis, conjunctivitis, and pneumonia.³⁻⁵ In addition, there is accumulating evidence of a major role for *C trachomatis* in the pathogenesis of infertility, which may be associated with asymptomatic infection.^{6,7} Asymptomatic women in particular are an important reservoir of infection. Their sexual partners are likely to contract non-gonococcal urethritis and their infants conjunctivitis or pneumonia, or both. Infection with *C trachomatis*, whether clinical or subclinical, may persist for many months.⁸ The importance of this organism therefore led us to reconsider our own methods of identification, and we were stimulated by the report of Thomas *et al*⁹ and the potential applications of their findings in our clinical unit. These workers compared a conventional tissue culture technique with the use of a fluorescein labelled monoclonal antibody to detect *C trachomatis* elementary bodies in smears. They concluded that the

immunofluorescence technique was highly specific and slightly more sensitive than tissue culture. In addition, they found it quicker and simpler.

The aims of the present study were: firstly, to assess the incidence of chlamydial infection at the Alexandra Clinic, Kings College Hospital, London (the genitourinary department for the Camberwell Health Authority, which serves a resident population of around 227 000.) Secondly, we aimed to compare the tissue culture and immunofluorescence methods in the context of a busy clinic.

Patients and methods

STUDY POPULATION

The population studied consisted of consecutive patients attending the Alexandra Clinic who either had signs and symptoms of chlamydial infection or were known contacts of men with non-gonococcal urethritis (NGU). Presenting features in the women included cervicitis, mucopurulent discharge (with no Gram negative diplococci seen in Gram stained endocervical specimens), and lower abdominal pain with clinical pelvic inflammatory disease. All the men in the study were diagnosed as suffering from NGU on clinical grounds and had >5 pus cells per high power field (magnification $\times 1000$) in Gram stained urethral smears.

Address for reprints: Dr E L Teare, Public Health Laboratory, Dulwich Hospital, East Dulwich Grove, London SE22 8QF

Accepted for publication 11 March 1985

COLLECTION OF SPECIMENS

Men

We took urethral swabs for microscopy and culture for *N gonorrhoeae* with 1 µl inoculating loops (NUNC Products, Kamstrup, Denmark). Specimens for gonococcal culture were directly plated on to Thayer-Martin medium (Southern Group Laboratory, London, England). A sterile pernasal swab (Medical Wire & Equipment Co, Corsham, Wiltshire, England) was used for collecting specimens to identify *C trachomatis*. Swabs were inserted a few centimetres into the urethra and rotated several times. The swab was then rolled over a circular area (4 mm in diameter) of a teflon coated slide, which was checked for even coverage of material left on the slide. Finally, the swab was placed in a bijoux bottle containing 3 ml of chlamydia transport medium. The transport medium consisted of Eagle's minimum essential medium supplemented with 0.13% sodium bicarbonate, 10% fetal calf serum, 0.5% glucose, and additional amino acids, vitamins, and glutamine. It contained gentamicin (10 mg/l) and amphotericin B (2.5 mg/l).

Women

The cervical area was first cleared of excess mucus and exudate with a large cotton wool swab. We then used 10 µl inoculating loops (NUNC) to obtain urethral and cervical specimens for microscopy, wet preparations for *Trichomonas vaginalis*, and endocervical specimens for *N gonorrhoeae* culture, directly inoculated on to Thayer-Martin media. Finally, a cotton wool swab was inserted into the endocervical canal, rotated for five to 10 seconds, and withdrawn without touching the vaginal surface. This swab was rolled on to a teflon coated slide and placed in chlamydia transport medium as described for the men. Thayer-Martin plates were incubated in a candle jar with 2% carbon dioxide at 37°C until transported to the laboratory.

Slides were air dried and flooded with acetone, which was allowed to evaporate. When completely dried, the slides were placed in a slide box. All specimens were kept at 4°C for a maximum of 18 hours before transport to the laboratory. On receipt in the laboratory swabs were stored at 4°C if they were to be inoculated on the day of receipt or stored in liquid nitrogen (at -192°C) if inoculation was to occur subsequently. Slides were held at -70°C if not examined on the day of receipt.

CHLAMYDIAL ISOLATION

Isolation was attempted in McCoy cells grown on 13 mm coverslips in culture tubes, each coverslip being seeded with 5×10^4 cells. The McCoy cells were seeded in growth medium (Eagle's minimum essential medium containing 10% fetal calf serum, 0.11% bicarbonate, 10 mg/l gentamicin, and additional

amino acids, vitamins, and glutamine) containing 25 mg/l idoxuridine and were incubated for two days before use.¹⁰ For isolation, the growth medium was removed, replaced with 1 ml of the specimen, and centrifuged at $3000 \times g$ for one hour at 35°C. After further incubation for two hours at 35°C, the supernatant was removed and replaced with fresh growth medium containing idoxuridine. Tubes were incubated for a further two to three days before the coverslips were fixed with methanol and stained with iodine. *C trachomatis* was considered to be present if characteristic intracytoplasmic inclusions were seen. All specimens were examined by the same observer (CS).

EXAMINATION OF SLIDES

Fixed slides were allowed to reach room temperature, and 15 µl of direct monoclonal antibody (MicroTrak, Syva, Palo Alto, California, United States of America) was spread on the smear. The slide was incubated at room temperature in a moist chamber for 15 minutes. Reagent was removed by immersing the slide in distilled water for 10 seconds, excess water being absorbed by blotting round the smear with filter paper. Finally, the slide was allowed to dry at room temperature. The smear was mounted under a coverslip with MicroTrak chlamydia direct specimen mounting fluid and examined with a polyvar immunofluorescence microscope (Reichert-Jung). We used a B1 filter block epi-illumination system with $\times 10$ magnification eye pieces, a $\times 40$ magnification aperture, and with $\times 1.25$ intermediate magnification. All slides were examined by one observer (CS).

STATISTICAL COMPARISON

We used the following definitions: true positives (results positive by both methods), true negatives (results negative by both methods), false positives (results positive by test method and negative by standard method), and false negatives (results negative by test method and positive by standard method).

We defined sensitivity as:

$\frac{\text{true positives}}{\text{true positives plus false negatives}}$

and specificity as:

$\frac{\text{true negatives}}{\text{true negatives plus false positives}}$

We calculated the probability value using the χ^2 test.

Results

A total of 216 patients (93 men, 123 women) entered the study. Thirty four (19 men and 15 women) were excluded from the analysis; 24 because of insufficient material on the slides to perform the immunofluorescence tests, seven because of contaminated tissue culture cells, and three because specimens failed to reach the laboratory. The table summarises the presenting clinical features and

may not return. With an "on site" immunofluorescence service, a result could be available within 40 minutes. If there is sufficient clinical awareness for patients to be treated on clinical grounds, then a tissue culture method may suffice if that is deemed necessary. The avoidance of delay in diagnosis and effective treatment of patients and sexual contacts is, however, of paramount importance. A rapid diagnostic method will undoubtedly be more efficient in achieving this.

TABLE Summary of presenting clinical features and microbiological results of culture and immunofluorescence

Clinical diagnosis	No of patients positive by:				No (%) positive by:	
	Both methods	Culture only	Immunofluorescence only	Neither method	Both methods	One or both methods
Cervicitis and vaginal discharge (n = 17)	7	0	1	9	7 (41)	8 (47)
Pelvic inflammatory disease (n = 45)	28	0	4	13	28 (62)	32 (71)
Contact of men with NGU (n = 46)	20	0	1	25	20 (43)	21 (46)
Men with NGU (n = 74)	48	1	7	18	48 (65)	56 (76)
Total (n = 182)	103	1	13	65	103 (57)	117 (64)

NGU = non-gonococcal urethritis.

microbiological results obtained in the remaining 182 patients. The difference between the two tests in detecting *C trachomatis* was not significant ($p > 0.1$). If tissue culture was taken to be the reference method, the sensitivity of the immunofluorescence test was 99% and the specificity 83%.

Discussion

C trachomatis was identified in a total of 64% of patients in this study by culture or immunofluorescence, or both. The immunofluorescence method had high sensitivity but only moderate specificity compared with the standard culture method, a finding that other workers have shown.¹¹ This may be accounted for by detecting non-viable organisms by the immunofluorescence technique, and the apparent lack of specificity may really be due to the enhanced sensitivity of this method. Positive by both methods were: 41% of women with cervicitis and discharge, 62% of women with clinical pelvic inflammatory disease, 43% of sexual contacts of men with NGU, and 65% of men with NGU. It therefore became apparent during the course of this study that the incidence of chlamydial infection in our district was much greater than had hitherto been thought, and clinical awareness of the condition increased dramatically.

Many advantages may be gained from the use of a rapid diagnostic technique, not least because patients attending sexually transmitted disease (STD) clinics

The incidence of chlamydial infection in our study population justifies providing an identification service and, though local incidences may vary, we suspect that this is generally true. In our hands, there was no significant difference between the two methods used for identifying *C trachomatis*, but it should be pointed out that we already had a well established tissue culture service at the start of the study. Such a service requires considerable expenditure and technical expertise, and cost analysis in an individual unit may well justify the capital outlay for a fluorescence microscope. In such an analysis, consideration must be given to factors such as possible delays between collecting specimens and inoculating tissue cultures in the laboratory. Even if transport medium is used there may be loss of viability of *C trachomatis* and hence false negative results. None of the nursing or technical staff associated with this study found either the collection and transport of specimens or practical procedures difficult to learn effectively. The most common difficulty encountered was the finding of insufficient material on the slides for the immunofluorescence procedure. This was greatly reduced by careful instruction and explanation to those taking the specimens and occurred rarely towards the end of the study.

In this study we highlighted a need for clinical awareness of chlamydial infection and for a microbiological service to genitourinary clinics. There was no significant difference between tissue culture and immunofluorescence methods in detecting *C trachomatis*, and the choice of method must rest with

an analysis of cost, expertise, and practicalities in individual departments.

References

1. Dunlop EMC. Chlamydial genital infection and its complications. *Br J Hosp Med* 1983;29:6-11.
2. Chief Medical Officer of the DHSS. On the state of the public health for the year 1983. London: HMSO, 1984:56-60.
3. Terho P. *Chlamydia trachomatis* in non-specific urethritis. *British Journal of Venereal Diseases* 1978;54:251-6.
4. Holmes KK. The *Chlamydia* epidemic. *JAMA* 1981;245:1718-23.
5. Schachter J, Holt J, Goodner E, Grossman M, Sweet R, Mills J. Prospective study of chlamydial infection in neonates. *Lancet* 1979;ii:377-9.
6. Jones RB, Andrey BR, Hui SL, Cleary RE. Correlation between serum antichlamydial antibodies and tubal factor as a cause of infertility. *Fertil Steril* 1982;38:553-8.
7. Cevenini R, Possati G, LaPlaca M. *Chlamydia trachomatis* infection in infertile women. In: Mardh P-A, Holmes KK, Oriel JD, Piot P, Schachter J, eds. *Chlamydial infections*. New York, Oxford: Elsevier Biomedical Press, 1982:189-92.
8. McCormack WM, Alpert S, McCombe DE, Nichols RL, Semine DZ, Zinner SH. Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*. *N Eng J Med* 1979;300:123-5.
9. Thomas BJ, Evans RT, Hawkins DA, Taylor-Robinson D. Sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of a fluorescein labelled monoclonal antibody: comparison with conventional chlamydial isolation *J Clin Pathol* 1984;37:812-6.
10. Reeve P, Owen J, Oriel JD. Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital tract. *J Clin Pathol* 1975;28:910-4.
11. Taylor HR, Agarwala N, Johnson SL. Detection of experimental *Chlamydia trachomatis* eye infection in conjunctival smears and in tissue culture by use of fluorescein-conjugated monoclonal antibody. *J Clin Microbiol* 1984;20:392-5.