Rapid diagnosis of chlamydial infection of the cervix

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SUMMARY A rapid serodiagnostic test for the presumptive diagnosis of chlamydial infection of the cervix has been developed. The method used is based on the modified micro-immunofluorescence test using pooled chlamydial antigens and the detection of different immunoglobulin classes of chlamydial antibody in sera and cervical secretions. The presence of IgG chlamydial antibody at a level of 1/64, or IgM antibody at a level of 1/8 or greater, or both in sera and IgG or IgA antibody at a level of 1/8 or more or both in cervical secretions was closely associated with the isolation of *Chlamydia trachomatis* and non-specific genital infection. In general, serodiagnosis was three to nine times more sensitive than cultural methods, and the detection of IgG chlamydial antibody in cervical secretions alone provided the most sensitive of the serological tests. This sensitive, low-cost, rapid, and simple serodiagnostic test for the presumptive diagnosis of chlamydial infection of the cervix, coupled with transportation of specimens by post, offers advantages over conventional isolation techniques for the routine diagnosis and management of chlamydial genital infections.

Introduction

Chlamydia trachomatis is now generally recognised as a pathogen in the male genital tract and as the primary cause of non-specific urethritis (NSU) in up to 68% of such patients (Dunlop *et al*, 1965; Dunlop *et al*, 1972; Oriel *et al*, 1972; Richmond *et al*, 1972; Alani *et al*, 1977).

In the female genital tract *C. trachomatis* was first isolated from the cervix of the mother of a baby suffering from TRIC-agent ophthalmia neonatorum (Jones *et al*, 1959). Since that time more sensitive cell-culture isolation techniques have become available, and the *C. trachomatis* isolation rate from the cervix of unselected women presenting at clinics for sexually transmitted diseases (STD) has been reported as varying between 12% and 31% (Hilton *et al*, 1974; Oriel *et al*, 1974; Burns *et al*, 1975; Nayyar *et al*, 1976).

Recent studies have shown that the modified micro-immunofluorescence (micro-IF) test for the detection of different immunoglobulin classes of type-specific antichlamydial antibody can provide information on the prevalence of these infections

(Treharne *et al*, 1977a). Studies on ocular chlamydial infections have shown that the detection of different immunoglobulin classes of type-specific chlamydial antibody in eye secretions (tear fluids) as well as in sera can be used successfully for diagnosis (Treharne *et al*, 1977b; Darougar *et al*, 1978).

The present study was carried out to assess the sensitivity of the modified micro-IF test for the detection of antichlamydial antibody in both serum and local cervical secretions for the diagnosis of chlamydial cervical infections and to compare these with the results of cultural tests for the isolation of *Chlamydia*.

Materials and methods

SELECTION OF PATIENTS

All women presenting at the clinic between 10 February and 26 May 1977 with a presumed infection of the cervix were included in the study. Specimens were collected from the cervix for the identification of *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Candida* species, and *C. trachomatis*. In addition, all women who were sexual contacts of men with genital herpes, or with any genital lesion suggesting genital herpes, had culture specimens collected from the cervix for herpesvirus isolation.

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Received for publication 19 June 1978

For the purposes of this study these women have been divided into five separate groups.

Group 1

This group consisted of: female sexual contacts of men with proved or suspected NSU; women with excess urethral or cervical leucocytes, irrespective of epithelial inflammatory changes, in whom no pathogens were found (non-specific genital infection [NSGI]); and women with evidence of salpingitis in whom no pathogens were found (non-specific salpingitis).

Group 2

This group consisted of women with evidence of infection by *N. gonorrhoeae*.

Group 3

This group consisted of women with candidal infection as determined by microscopy or culture or both.

Group 4

This group consisted of: women with culture-positive herpesvirus infection; women with trichomoniasis or genital warts; and women with vaginitis in whom no pathogens were found.

Group 5

This group consisted of women with no apparent venereal disease (NVD) on historical, clinical, or microbiological grounds.

COLLECTION OF SPECIMENS AND LABORATORY TESTS

Collection of specimens and investigations for *N. gonorrhoeae*, *T. vaginalis*, and *Candida* species have been described elsewhere (Burns *et al*, 1975).

Swabs for herpesvirus were collected from the cervical os and inoculated on to human embryonic lung cells (Thin *et al*, 1975).

ISOLATION OF *C. TRACHOMATIS*

The irradiated McCoy cell culture technique used was that described by Darougar *et al* (1971).

COLLECTION OF BLOOD SAMPLES

Blood was collected by venepuncture from all women. The serum was separated and stored at -20°C until tested.

COLLECTION OF CERVICAL SECRETIONS

Cervical secretions were also collected from all patients by means of a $10 \times 5 \times 1$ mm cellulose sponge (John Weiss, London), which was held at the tip with a pair of forceps; these were then inserted into the cervical os so that the whole sponge was within the lower canal. The sponge remained in contact with the cervical secretions for at least 10 seconds; it was then removed, placed in a tightly stoppered plastic capsule, and stored at -180°C . Before antibody titration cervical secretion (CS) sponges were eluted with 0.7 ml of 0.1 mol/l phosphate-buffered saline (pH 7.2) which represented a starting dilution of 1/8.

SEROLOGY

The modified micro-IF test (Treharne *et al*, 1977a) was used to detect antichlamydial IgG and IgM antibodies in sera and IgG and IgA in cervical secretions. Statistical comparisons were made using Student's *t* test.

Results

In this study all investigations for *Chlamydia* were performed on a total of 272 women. *C. trachomatis* was isolated from the cervix of 35 (13%) out of 272 women. The distribution of culture-positive results in different patient groups is shown in Table 1.

Table 1 Results of cultural and serodiagnostic tests for the presence of *C. trachomatis*

Patient groups	Total no. of patients	Positive results to									
		Any test		Chlamydial isolation		Micro-IF test* (serum)		Micro-IF test† (CS)		Micro-IF test (Serum or CS or both)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Group 1	60	42	70	15	25	34	57	28	47	41	68
Group 2	21	15	71	10	48	9	41	8	38	10	48
Group 3	90	20	22	2	2	18	20	9	10	19	21
Group 4	41	17	40	3	7	15	37	11	27	17	41
Group 5	60	18	30	5	8	14	23	10	17	17	28
Total	272	112	41	35	13	90	33	66	24	104	38

*IgG 1/64 or IgM 1/8 or both

†IgG 1/8 or IgA 1/8 or both

[illegible]

1/8 or more, or cervical secretion IgG or IgA levels of 1/8 or more there was a much greater probability of positive chlamydial isolation than in patients with antibody levels at lower titres (Table 3). Furthermore, the frequency with which a diagnosis of NSGI occurred was greater in patients with higher levels of antichlamydial antibody. Conversely, the greatest proportion of patients with low antichlamydial antibody levels were in the group with diagnoses other than NSGI.

Finally Table 4 shows a close relationship between cervical secretion titres and serum geometric mean titres (GMT). Clearly those patients with the highest titres of CS antibody (1/128) are those from whom *C. trachomatis* is most likely to be isolated (83%). Furthermore, 83% of patients with this level of antichlamydial antibody in the cervical secretions were diagnosed as belonging to the group with NSGI.

Discussion

In the present study the overall isolation rate of *C. trachomatis* was 13%, which compares well with the 12% rate in a previous study based on women from the same hospital (Burns *et al*, 1975). In this group of women 118 (43%) out of 272 had antichlamydial IgG antibody at a titre of 1/16 or

greater. Richmond and Caul (1977) demonstrated antichlamydial antibody in 150 (61%) out of 245 women attending an STD clinic. In their study, however, a level of 1/8 or greater was taken as the criterion of positivity against a single *C. trachomatis* serotype. We have previously shown (Treharne *et al*, 1977a) that the GMT of antibody in 117 women with cervical isolation-positive results was 1/66, and Wang *et al* (1977) demonstrated a GMT of 1/101 in 49 women with cervical isolation-positive results. Thus we considered an antichlamydial IgG titre of 1/64 was a more realistic criterion of serological positivity.

In ocular chlamydial infections in the United Kingdom, the presence in blood of IgG at a titre of 1/64 or IgM at a level of 1/8 and IgG or IgA in eye secretions at a level of 1/8 or both closely correlated with the clinical diagnosis and presence of *C. trachomatis*. Accepting these criteria of serological positivity in the present study, 90 (33%) out of 272 patients had positive antichlamydial antibody in sera (that is, IgG $\geq$ 1/64 or IgM $\geq$ 1/8 or both) and 68 (25%) out of 272 patients had positive antibody in cervical secretions (IgG or IgA $\geq$ 1/8 or both), thus giving a total of 104 (38%) out of 272 patients when the two criteria are combined.

The presence, albeit a transient one, of IgM antichlamydial antibody has been shown to be

Table 3 Correlation of levels of antichlamydial immunoglobulins in sera and cervical secretions with isolation of *C. trachomatis* and clinical diagnosis

<i>Immunoglobulins</i>	<i>Reciprocal antichlamydial antibody titre</i>	<i>No. of patients</i>	<i>Isolation- positive (%)</i>	<i>With non-specific diagnoses (%)</i>	<i>With other diagnoses (%)</i>	
Serum	IgG	<16	220	9	16	84
		≥64	52	27	46	54
	IgM	<8	201	8	18	82
		≥8	71	24	36	64
Cervical secretion	IgG	<8	206	5	14	86
		≥8	66	39	49	51
	IgA	<8	224	7	16	84
		≥8	48	42	52	48

Table 4 Relationship of antichlamydial antibody levels in cervical secretions with antibody levels in serum and isolation of *C. trachomatis*

No. of patients	Cervical secretion IgG titre	Geometric mean titre*				Patients with NSGI or as contacts of NSU (%)
		Serum*		Isolation-positive patients (%)		
		IgG	IgM			
6	128	161	10	83	83	
16	32	84	5	38	44	
44	8	29	4	34	45	
206	0	<2	<2	4	16	

*Reciprocal titre

associated with early response to infection with *C. trachomatis* (Wang and Grayston, 1971). In this study IgM serum antibody at a titre of 1/8 or greater was present in 49% of isolation-positive women but in only 23% of isolation-negative women ($P < 0.001$). Richmond and Caul (1977), using their single antigen test on similar groups of patients, demonstrated IgM serum antibody in only 29% of isolation-positive women and 7% of isolation-negative women. The overall figure in our study showed that we could demonstrate IgM antichlamydial serum antibody in 71 (26%) out of 272 women and IgG antibody (at 1/64 or more) in 52 (19%) out of 272 women. When these two criteria of serodiagnosis are considered together a considerably greater proportion of women seem to have chlamydial genital infections than is suggested by isolation studies.

The finding of antichlamydial antibodies in cervical secretions is not surprising since these antibodies have been found in local secretions, such as tear fluids in patients with hyperendemic trachoma (Treharne *et al*, 1978) and paratrachoma infection (Darougar *et al*, 1978). In 26 (74%) out of 35 isolation-positive patients antichlamydial IgG antibody was detected at a titre of 1/8 or more. A strong correlation between the presence of *C. trachomatis* and the titre of CS IgG antibody was demonstrated, which is consistent with the evidence from ocular infections with this agent, where it was shown that over 75% of children with positive chlamydial isolation in a hyperendemic trachoma area in southern Tunisia had detectable type-specific antibody in their conjunctival secretions compared with only 20% in the isolation-negative group (Treharne *et al*, 1978).

Of 112 patients with cultural or serological evidence of chlamydial infection, 104 (93%) showed evidence of genital infection with *C. trachomatis* when the detection of antichlamydial antibody in either sera or cervical secretions is considered together. This compares with the figure of 35 (31%) out of 112 women from whom *C. trachomatis* was isolated. If we had used solely serodiagnostic tests as our criteria for *C. trachomatis* infection we would have excluded only eight isolation-positive women out of 112 who showed evidence of infection by any method.

Furthermore, our studies suggest that in such a group of unselected women those who show the most evidence of chlamydial genital infections are those who are diagnosed as having either NSGI or are sexual contacts of men with NSU (70%) and those who are infected with *N. gonorrhoeae* (71%). In agreement with others (Hilton *et al*, 1974; Burns *et al*, 1975) we found a higher isolation rate of

C. trachomatis in women with concomitant gonococcal infections (48%) than in any other group. There were fewer women in this latter group, however, in whom we could detect antichlamydial antibody compared with those who had NSGI or were contacts of men with NSU. A possible explanation is that in women with acute gonorrhoea there is a relatively short incubation period and, although they may also harbour *C. trachomatis*, antichlamydial antibody has not yet been formed. This hypothesis was confirmed in this study by the fact that the women with a history of gonorrhoea for four weeks or longer more often had antichlamydial antibody than the women with gonorrhoea who presented at one to two weeks.

In agreement with most others we consider *C. trachomatis* to be a significant genital pathogen (Lancet, 1974) and that laboratory facilities should be available for the screening of such infections in women attending STD clinics. Until recently, the only available diagnostic test has been the isolation of *C. trachomatis* in susceptible tissue culture cells. This has not generally found favour with microbiologists as it is a time-consuming and extremely labour-intensive test. The collection of specimens for serodiagnosis on the other hand (particularly of cervical secretions or of venous blood with cellulose sponges) requires no great expertise (Darougar *et al* 1978) and, provided specimens are posted directly to the laboratory, requires no special refrigerated transportation. Results can often be obtained within two or three hours, and when the specialised treatment needed in the various sexually transmitted diseases is considered such a test seems to have a valuable place in diagnosis.

The authors are grateful to Professor Barrie R. Jones for his encouragement and advice and to Mr R. J. Dines for his excellent technical assistance. We are indebted to the Department of Health and Social Security and to an anonymous donor for financial support.

References

- Alani, M. D., Darougar, S., Burns, D. C. MacD., Thin, R. N., and Dunn, H. (1977). Isolation of *Chlamydia trachomatis* from the male urethra. *British Journal of Venereal Diseases*, 53, 88-92.
- Burns, D. C. MacD., Darougar, S., Thin, R. N., Lothian, L., and Nicol, C. S. (1975). Isolation of *Chlamydia* from women attending a clinic for sexually transmitted diseases. *British Journal of Venereal Diseases*, 51, 314-318.
- Darougar, S., Kinnison, J. R., and Jones, B. R. (1971). Simplified irradiated McCoy cell culture for isolation of chlamydiae. In *Trachoma and Related Disorders*, pp. 63-70. Edited by R. L. Nichols. Excerpta Medica: Amsterdam.
- Darougar, S., Treharne, J. D., Minassian, D., El-Sheikh, H., Dines, R. J., and Jones, B. R. (1978). Rapid serological test for diagnosis of chlamydial ocular infections. *British Journal of Ophthalmology*, 62, 503-508.

- Dunlop, E. M. C., Al-Hussaini, M. K., Garland, J. A., Treharne, J. D., Harper, I. A., and Jones, B. R. (1965). Infection of urethra by TRIC agent in men presenting because of 'non-specific' urethritis. *Lancet*, 1, 1125-1128.
- Dunlop, E. M. C., Vaughan-Jackson, J. D., Darougar, S., and Jones, B. R. (1972). Chlamydial infection: incidence in 'non-specific' urethritis. *British Journal of Venereal Diseases*, 48, 425-428.
- Hilton, A. L., Richmond, S. J., Milne, J. D., Hindley, F., and Clarke, S. K. R. (1974). *Chlamydia* A in the female genital tract. *British Journal of Venereal Diseases*, 50, 1-10.
- Jones, B. R., Collier, L. H., and Smith, C. H. (1959). Isolation of virus from inclusion blennorrhoea. *Lancet*, 1, 902-905.
- Nayyar, K. C., O'Neill, J. J., Hambling, M. H., and Waugh, M. A. (1976). Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *British Journal of Venereal Diseases*, 52, 396-398.
- Oriel, J. D., Reeve, P., Powis, P., Miller, A., and Nicol, C. S. (1972). Isolation of *Chlamydia* from patients with non-specific genital infection. *British Journal of Venereal Diseases*, 48, 429-436.
- Lancet* (1974). Editorial: *Chlamydia* and genital infection. *Lancet*, 2, 264-266.
- Oriel, J. D., Powis, P. A., Reeve, P., Miller, A., and Nicol, C. S. (1974). Chlamydial infections of the cervix. *British Journal of Venereal Diseases*, 50, 11-16.
- Richmond, S. J., Hilton, A. L., and Clarke, S. K. R. (1972). Role of *Chlamydia* Subgroup A in non-gonococcal and post-gonococcal urethritis. *British Journal of Venereal Diseases*, 48, 437-444.
- Richmond, S. J., and Caul, E. O. (1977). Single-antigen indirect immunofluorescence test for screening venereal disease clinic populations for chlamydial antibodies. In *Non-gonococcal Urethritis and Related Infections*, pp. 259-265, edited by K. Holmes and D. Hobson. American Society for Microbiology: Washington, D.C.
- Thin, R. N. T., Atia, W. A., Parker, J. D. J., Nicol, C. S., and Canti, G. (1975). Value of Papanicolaou-stained smears in the diagnosis of trichomoniasis, candidiasis, and cervical herpes simplex virus infection in women. *British Journal of Venereal Diseases*, 51, 116-118.
- Treharne, J. D., Darougar, S., and Jones, B. R. (1977a). Modification of the micro-immunofluorescence test to provide a routine sero-diagnostic test for chlamydial infection. *Journal of Clinical Pathology*, 30, 510-517.
- Treharne, J. D., Dines, R. J., and Darougar, S. (1977b). Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In *Non-gonococcal Urethritis and Related Infections*, pp. 249-258, edited by K. Holmes and D. Hobson. American Society for Microbiology: Washington, D.C.
- Treharne, J. D., Dwyer, R. St. C., Darougar, S., Jones, B. R., and Daghsous, T. (1978). Antichlamydial antibody in tears and sera, and serotypes of *Chlamydia trachomatis* isolated from school-children in Southern Tunisia. *British Journal of Ophthalmology*, 62, 509-515.
- Wang, S-P., and Grayston, J. T. (1971). Local and systemic antibody response to trachoma infection in monkeys. In *Trachoma and Related Disorders*, pp. 217-232. Edited by R. L. Nicols. Excerpta Medica: Amsterdam.
- Wang, S-P., Grayston, J. T., Kuo, C-C., Alexander, E. R. and Holmes, K. K. (1977). Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test. In *Non-gonococcal Urethritis and Related Infections*, pp. 237-248. Edited by K. Holmes and D. Hobson. American Society for Microbiology: Washington, D.C.