Serological diagnosis with the Chlamydia Spot-IF test

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Summary. Using a set of sera for which full chlamydial micro-immunofluorescence results suggested a clear diagnosis, we have evaluated the Chlamydia Spot-IF test (bioMerieux), which allows a comparison of titres to *Chlamydia trachomatis* and *C. psittaci* antigens. A modification of the test in which the antigen slides were pre-treated with a monoclonal antibody to chlamydial lipopolysaccharide, improved its ability to differentiate infections with *C. trachomatis* from those with *C. psittaci* or *C. pneumoniae*.

Introduction

Serological confirmation of a deep-seated chlamydial infection is often based at present on appropriate clinical details and a four-fold rise in antibody titre, or a single high titre, in a test such as the complement fixation (CF) test. The CF test is genus-specific and only confirms the presumptive diagnosis of lymphogranuloma venereum (LGV) or ornithosis.¹ *Chlamydia pneumoniae* (TWAR) infection does not always produce a positive result by CF and *C. trachomatis* (CT) serovars (other than LGV) may produce a positive CF result only if deep-seated infection such as pelvic inflammatory disease (PID) is present. The whole inclusion fluorescence (WIF) test also includes a genus-specific reaction and can give positive results with TWAR infections.¹ This may cause difficulties in interpretation in that the seroprevalence of TWAR in the general population can be as high as 19.9%² and a positive WIF result may, therefore, be misinterpreted as confirming a clinically suspected CT infection (e.g., in a patient with symptoms suggestive of PID).

CT infections involving superficial sites far outnumber any other type of chlamydial infection.³ These infections are best diagnosed by culture or by antigen detection (ELISA, immunofluorescence), but with deep-seated CT infections or for screening for the late sequelae of such infections, such as infertility, these tests often give negative results and a more sensitive and specific serological test is required. Micro-immunofluorescence (MIF) fulfils this role and will reliably detect deep-seated chlamydial infections.¹ However, the technical complexities of MIF limit its availability and usage. Dependent on the choice of antigens included, the MIF test can distinguish

between the different species of chlamydia and also the different serovars of CT.⁴

The commercial availability of an immunofluorescent test based on representative CT and *C. psittaci* (CP) antigens prompted an evaluation of this test (the Chlamydia Spot-IF test; bioMerieux, Basingstoke, Hants) with a set of sera for which full MIF results and clinical details were available.

The Chlamydia Spot-IF test is presented as (PTFE)-coated microscope slides, each with 10 wells covered with CT (L2 serotype) or CP (avian) elementary bodies. Fluorescein-labelled anti-human IgG conjugate and a positive control serum are also supplied. A positive serum produces a “starry sky” appearance. The interpretation of the titres obtained is based on experimental evidence⁵ that suggests that, with chronic infection, CT and CP titres are approximately equivalent, whereas a ≥ 4 fold difference between the titres suggests infection with the species showing the higher titre.

Materials and methods

Study group

Between Feb. 1986 and Jan. 1989, a full range of chlamydial MIF tests was performed at the Institute of Ophthalmology, London on sera from 58 patients investigated at Liverpool Public Health Laboratory. The MIF results of 35 (61%) of these patients were considered diagnostically useful, i.e., showed a predominant reaction to CT, CP or TWAR. Stored sera from 24 of these 35 patients were available in sufficient quantity for further testing. On the basis of a clear diagnosis suggested by their MIF results, these 24 patients were assigned to one of three groups to be tested by the Chlamydia Spot-IF test: (1) 11 patients with CT infection (T1–11); (2) 10 patients with CP infection (P1–10); (3) three patients with TWAR infection (TW1–3).

C. trachomatis and *C. psittaci* Spot-IF test

Sera were tested in accordance with the manufacturer's instructions. The CT and CP antigen slides were removed from the refrigerator and placed at room temperature for 10–15 min before opening. Doubling dilutions of sera and control serum (Chlamyrol, bioMerieux) were made in phosphate-buffered saline (PBS) and 20- μ l volumes of each test serum dilution, the control serum and a conjugate (PBS) control were placed on a CP and CT slide. The slides were placed in a pre-warmed moist chamber for 30 min at 37°C and were then washed in PBS for 10 min.

Excess fluid was drained off the slides and 20 μ l of a 1 in 200 dilution of fluorescein-labelled anti-human IgG conjugate (Fluoline-G, bioMerieux) was applied to each well. The incubation and washing steps were then repeated, and the slides were dipped in distilled water and gently blotted to remove excess fluid. The mountant (Fluoprep) and coverslips were applied. The slides were stored in a cool dark area and were read by two independent observers (H.M., P.M.) the same day with a Zeiss standard RA fluorescent microscope at $\times 400$ magnification. Neither observer was aware of the previous MIF results. The end-point was taken as the last well in which the pattern of fluorescence was still distinct.

If the observers' results were discrepant, the slides were re-assessed until results concurred.

Pre-treatment of C. trachomatis and *C. psittaci* Spot-IF slides with anti-chlamydial lipopolysaccharide monoclonal antibody (anti-LPS MAbs)

Each slide well was treated with 20 μ l of anti-LPS (anti-LPS enzyme conjugate from the IDEIA test, Novo Biolabs Ltd). The slides were incubated for 30 min at 37°C in a pre-warmed moist chamber, washed in PBS for 10 min and then drained. Slides were used immediately in the Spot-IF test.

Results

Table I shows Chlamydia Spot-IF results for 20 patients whose clinical diagnoses matched their classification as cases of CT, CP or TWAR infection on the basis of previous MIF results. With only three exceptions (patients T8, P4, P5), antibody titres measured by the Spot-IF test were higher than those reported by MIF. In the Spot-IF test, antibody titres to CT antigen were at least four-fold higher than titres to CP antigen in all eight patients considered to have been infected with CT. Although the general tendency was for titres against CP antigen to be higher, significant four-fold or greater differences in Spot-IF

Table I. Chlamydia Spot-IF results in patients diagnosed as having CT, CP and TWAR infections

Case no.*	Sex	Age	Illness (duration, days)	Serum titres in							
				CFT	MIF test					Spot-IF test	
					TRACH			PSITT	TWAR	TRACH	PSITT
					A-C	D-K	LGV				
T1	F	...	PID	80	256	256	256	16	32	8192	2048
T2	F	19	Infertility	40	1024	4096	2048	256	256	8192	1024
T3	F	22	Vaginal discharge	320	64	256	16	16	<16	8192	2048
T4	F	27	Vaginal discharge ?Fitz-Hugh Curtis	20	256	256	256	32	128	1536	384
T5	F	28	Infertility	80	256	512	256	<16	<16	1536	128
T6	F	39	?Chlamydial infection	<20	256	256	256	<16	<16	1024	128
T7	F	31	PID	40	64	64	64	<16	<16	768	32
T8	F	24	Reactive arthritis	<20	64	64	64	<16	<16	64	<8
P1	M	60	Pneumonia (10) (46)	20 320	...	...	...	...	...	256 4096	512 8192
P2	F	48	Chest infection (24)	640	<16	<16	<16	2048	256	4096	2048
P3	M	22	PUO (22)	...	<16	<16	<16	64	16	512	512
P4	F	64	Pneumonia (23)	40	<16	<16	<16	2048	128	128	384
P5	M	48	Pneumonia (13)	40	<16	<16	<16	4096	64	64	128
P6	F	32	Pneumonia	80	16	64	64	1024	128	1024	2048
P7	M	43	Fever, chest pain	320	16	16	<16	256	256	1024	512
P8	M	55	Pneumonia (30)	80	<16	<16	<16	128	128	512	512
P9	F	82	?Psittacosis (24)	1280	<16	<16	<16	1024	1024	8192	12288
TW1	M	77	Chest infection (18)	640	<16	<16	<16	<16	256	4096	4096
TW2	M	44	Viral illness	80	<16	<16	<16	256	4096	1024	2048
TW3	M	50	Pneumonia (25)	80	<16	<16	<16	<16	64	768	768

* T1–8, *C. trachomatis*; P1–9, *C. psittaci*; TW1–3, TWAR (*C. pneumoniae*).

TRACH, *C. trachomatis* antigens; PSITT, *C. psittaci* antigens; TWAR, *C. pneumoniae* antigens.

titres to CT and CP antigens were not seen in any sera from the nine cases of CP infection or three cases of TWAR infection tested.

In an attempt to reduce the incidence of serological cross-reaction that results from the presence of genus-specific LPS in both CT and CP antigen preparations, some sera were re-tested with CT and CP Spot-IF slides which had been pre-treated ("blocked") with anti-LPS MAb (figure). For CT cases, "blocking" with anti-LPS MAb caused little or no change in titre to the CT antigen, whereas any substantial titres to the CP antigen were dramatically reduced. Differences between CT and CP titres for this group of cases of CT infections were thus accentuated in sera from five out of eight patients, including patient T3 whose serum showed no significant difference without blocking. The differentiation in the remaining three CT

cases whose CT/CP titres differed significantly in the test without blocking, did not improve in the blocking test. However, in sera from patients considered to have CP or TWAR infection, "blocking" with anti-LPS MAb caused large decreases in titres to both CT and CP antigens.

In four patients (table II) the diagnosis suggested by MIF testing was not in keeping with their clinical symptoms. For three of these patients (T9, T10, T11) Spot-IF test results (including improved resolution on blocking) were consistent with the diagnosis of CT infection supported by MIF. The fourth patient (P10) had a high CF titre consistent with active chlamydial disease; his clinical details suggested LGV. The Spot-IF test results were not consistent with CT infection and the MIF results clearly seemed to indicate a CP/TWAR infection.

Patient no.	Serum titre										
	8	16	32	64	128	256	512	1024	2048	4096	8192
T1 {				p<-----					p		t*
T2 {	p<-----							p		t<-----	t
T3 {				p<-----					t<-----	t	
T4 {				p<-----		p			t*		
T5 {			p<-----	p				t<--	t		
T6 {				p<--	p		t<-----	t			
T7 {			p*					t*			
T8 {	p*		t<--	t							
P1a {	t<-----					t					
P1b {	p<-----					p			t<-----	t	
P2 {				t<-----					p		t
P3 {			t<-----			t					
P4 {		t<-----				t					
P5 {	t<-----					t					
TW1 {	t<-----									t	
TW2 {		t<-----							t		
TW3 {	t<-----									t	

Figure. Reduction in antibody titre to *C. trachomatis* and *C. psittaci* after pre-treatment (blocking) of antigen slides with anti-LPS MAb. Titre with blocked slides <----- titre with unblocked slides; t, *C. trachomatis* antigen slide; p, *C. psittaci* antigen slide; *titre unchanged after blocking.

Table II. Chlamydia Spot-IF results in patients whose clinical illness and MIF results appeared contradictory

Case no.	Sex	Age	Illness (duration)	Serum titres in								
				CFT	MIF test				Spot-IF test			
					TRACH			PSITT	TWAR	Antigen slide pre-treatment	TRACH	PSITT
					A-C	D-K	LGV					
T9	M	48	Pyrexia Ex-Morocco (18m)	40	64	128	128	<16	<16	PBS anti-LPS	1024	128
T10	F	54	Meningitis (17d)	40	128	128	64	<16	<16	PBS anti-LPS	ND	ND
T11	F	...	Fever, sore throat (3m)	40	64	256	128	<16	<16	PBS anti-LPS	256	64
P10	M	...	Groin abscess ?LGV	640	<16	<16	<16	2048	1024	PBS anti-LPS	256	32
											256	128
											256	<8
											2048	3072
											512	512

ND, not done.

See footnote & table I.

Discussion

The optimal test at present for the serological diagnosis of deep-seated chlamydial infection is MIF, but the complexity of maintaining stock cultures of all the relevant chlamydial species and serovars limits its use to specialist centres.

The need for a species-specific test has been highlighted further by the recent interest in community-based infections with TWAR.⁶ Serological evidence of past TWAR infection may be present in up to 20% of the UK population.² This is of relevance if genus-specific serological tests are used to screen for CT or CP infection.⁷

The Spot-IF test was used to examine a panel of sera for which clinical details and serological results were available. Sera from cases of CT infection were adequately differentiated by the Spot-IF test. This was so even when clinical details suggested that samples were taken at a late stage in the infection—a stage during which the manufacturers had predicted little difference in the CT/CP titres.

In cases of CP infection, the Spot-IF titres against both antigens were equivalent, with both early and late sera. Sequential sera available from three cases of CP infection showed rising titres (≥ 4 fold) to both CT and CP antigens. Thus, the Spot-IF test seemed unable to confirm CP infections adequately. The same result was found with sera from the three cases of TWAR infection.

The presence of genus-specific LPS antigen is a useful attribute, allowing group antibody to be detected in a single CF test. However, LPS was considered to be a possible source of cross-reactivity in the Spot-IF test. Our results suggest that "blocking" of antigen slides with anti-LPS MAb may be a useful general approach to modulating such cross-reactivity in serological tests for chlamydia. Other workers have developed serological tests based on antigens treated chemically to remove the LPS.⁸ With the Spot-IF test,

whole elementary bodies are the target antigens and are supplied fixed as ready prepared slides. Therefore, blocking with a commercial anti-LPS MAb seemed a logical approach.

The anti-LPS MAb that was used reacts with LPS from both CT and CP (TWAR not having been considered at that time).⁹ We cannot exclude the possibility that steric inhibition of other epitopes that are part of, or lie in close proximity to, the LPS may occur because of the attached enzyme. The response of sera from cases of CT infection would seem to suggest that the antibody responses are predominantly against antigens specific to CT (possibly major outer-membrane protein—MOMP) with only a limited anti-LPS response. In contrast, sera from cases of CP/TWAR infection seem to have an antibody directed predominantly against genus-specific chlamydial LPS. This could relate to the strain of *C. psittaci* used in the Spot-IF test. It is likely that there is marked antigenic diversity within what is now classified as the *C. psittaci* species, and the single CP isolate used in the spot-IF test may not share many of the surface-exposed epitopes expressed by other CP strains.

This diversity of response may also relate to the timing of the serum samples. Onset dates were available for most cases of CP/TWAR infection and all the samples were obtained within 6 weeks of onset. Unfortunately, precise dates of onset were not available for the cases of CT infection, but clinical details suggested that the majority of specimens were from late in the course of the illness. Hence an initial genus-specific response could occur in all chlamydial infections, to be overtaken by a species-specific response later in the illness. Alternatively, our results would be consistent with a predominantly species-directed response in cases of CT infection and a genus-specific response in cases of CP/TWAR infection that persisted throughout the infection.

One group of workers has suggested that LPS may be lost from CT elementary bodies by the effect of

antibody binding, as a protection from immune destruction.¹⁰ Other workers have demonstrated that cells infected with CT have chlamydia-specific LPS associated with their plasma membrane.¹¹ Therefore, LPS may be the more prominent immunogen early in CT infections, whereas antibodies against MOMP develop later in the infection. However, this would be contrary to observations of a genus-specific response appearing later than the initial species-specific response during hyper-immunisation of experimental animals.¹²

In summary, with sera for which the full MIF test gave diagnostically useful information, the Spot-IF

test in its basic format confirmed deep-seated CT infections. In the "modified" form of the test the predominant reaction in CT infections was accentuated and cases of CP/TWAR infection showed a consistent (if unexpected) pattern of results. Further work is needed to assess more fully the range of titres that represent past or recent infection. The test, in its modified format, may be of help in laboratories that currently rely on a genus-specific test for the serological confirmation of deep-seated chlamydial infection.

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