Comparison of Immunotyping of *Chlamydia trachomatis* by Indirect Fluorescent-Antibody Staining and Radioimmunoassay

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***Chlamydia trachomatis* isolates from 58 patients were immunotyped by radioimmunoassay (RIA) and fluorescent-antibody staining (FA) by using monoclonal antibodies. Unambiguous typing was observed with 55 isolates by FA and 53 isolates by RIA, although 3 isolates were typed by RIA but not FA. FA required less antigen and was more sensitive in detecting mixed infections.**

Immunotyping of *Chlamydia trachomatis* isolates is a potentially valuable tool for studying transmission patterns. It has been used to identify newly acquired infections (3) as well as serovar-related differences in tissue tropism (1).

Procedures used previously have included microimmunofluorescence with polyclonal murine antibodies (11) and both a radioimmunoassay (RIA) (2) and an enzyme-linked immunosorbent assay (5) with monoclonal antibodies. Unfortunately, such procedures require substantial numbers of organisms and are time-consuming and technically demanding. However, simplified immunotyping of clinical isolates by indirect inclusion staining was recently reported (6, 9). Therefore, we undertook to develop and validate a similar assay in our laboratory by using a monoclonal antibody panel previously developed for typing by RIA (3, 5).

Fifty-eight stored isolates originally obtained from patients attending a sexually transmitted disease clinic were immunotyped by both fluorescent-antibody staining (FA) and RIA. They were chosen essentially at random from among isolates that were being immunotyped as part of ongoing studies of recurrent infections (2, 4). Thirteen of these isolates were from male urethral specimens, three were from female urethral specimens, and the remainder were from endocervical specimens. Cultures were originally determined to be positive for *C. trachomatis* by fluorescence staining of inclusions by using a genus-reactive monoclonal antibody (5). In order to ensure adequate antigen for the RIA, isolates were expanded for immunotyping by five to seven passes in shell vials to approximately 10^7 inclusion-forming units per ml. Reference strains from each of the 15 recognized serovars served as controls in both assays. The antibody panel that was used does not distinguish the serovariants Da from D or Ia from I (2, 7). Immunotyping by RIA has been described previously (3). Briefly, partially purified elementary bodies from a suspension of 10^5 inclusion-forming units per ml were adsorbed to strip wells overnight, and unbound antigen was blocked with phosphate-buffered saline containing Tween 20 and 2% fetal bovine serum. The wells were then incubated with monoclonal antibodies, washed, and incubated with rabbit anti-mouse immunoglobulin and were then incubated with ¹²⁵I-labeled protein A. The wells were then separated, and the bound radioactivity was determined for each sample. Sufficient antigen for typing was considered present when the

sample incubated with species-specific antibody bound $\geq 10\%$ of the radioactivity (counts per minute) bound by the control antigen incubated with species-specific antibody. Type-specific reactions were considered present when the bound radioactivity was $\geq 10\%$ of that seen with the prototype strain and appropriate controls were negative (2).

FA was performed by infecting McCoy cell monolayers in wells of a 96-well microtiter plate with isolates with titers that provided approximately 200 inclusions per well (3). After 72 h at 37°C, cultures were fixed for 10 min with methanol. Monoclonal antibodies were diluted in phosphate-buffered saline, and each monolayer was incubated with 30 μ l of 1 of 19 different antibodies for 30 min at room temperature. Wells were washed twice with phosphate-buffered saline. Then, 30 μ l of rabbit anti-mouse immunoglobulin labeled with fluorescein isothiocyanate (Wellcome Diagnostics, Dartford, England) was added to each well. Following a 30-min incubation at room temperature, wells were rinsed with phosphate-buffered saline as described above and were examined for fluorescent inclusions. The number of inclusions present in a specimen was determined by the reaction with the species-specific antibody. A reaction was considered to be type specific only if it gave bright fluorescence and could be confirmed by reaction with the B or C complex-specific or the FG subspecies-specific antibody.

For the 58 samples tested, there was complete agreement between the two methods when the serovar could be determined by both methods (Table 1). Conflicting results were not observed. However, six isolates did not bind type-specific antibody in one of the two assays. Three isolates reacted with the B-complex antibody in the FA assay, but showed no type-specific reaction by FA. Two of these typed as type E and the third typed as F type by RIA. Three isolates did not react with anything other than the C-complex antibody in the RIA, but all three were type specific for type J by FA. Two isolates were determined to be a single type by RIA but were determined to be mixed infections by FA. One isolate was type E by both methods but was also type D by FA, with a ratio of type D to E inclusions of 1:200. The other isolate was type G by both methods and also type F by FA, with a ratio of type F to G inclusions of 1:33. Typing of these two mixed-infection isolates was repeated, and similar ratios of the different inclusions were observed. None of the isolates stained ambiguously. All gave clearly positive or negative reactions by FA.

Reference strains and most clinical isolates were success-

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TABLE 1. Comparison of number of patient isolates of each immunotype determined by FA and RIA

Immunotype	No. of isolates (type) determined by:	
	FA	RIA
D	10	10
E	10	10
F	10	10
G	2	2
H	2	2
I	11	11
J	1	1
K	2	2
B complex	3	2 (E), 1 (F)
C complex	3 (J)	3
Mixed		
B-E	1	1
D-E	1	1 (E only)
D-J	1	1
F-G	1	1 (G only)

fully identified by both methods. However, there were differences. Some of these may reflect isolate-dependent differences in the surface exposure or conformation of the relevant epitopes in RIA, in which no fixative is used, as opposed to FA, in which methanol is used (8). For example, the reaction of the two type E and one type F isolates by RIA, but not by FA, suggests that, for these isolates, reactivity may be relatively more dependent on native conformation than it is for the other type E and F isolates which reacted in both assays. Similarly, the three type J isolates that did not react with the J-complex-specific antibody in RIA may have epitopes that are less exposed to this antibody when their surface proteins are in their native state than they are after fixation. Such data suggest that there may be more variability in serovar-specific epitopes (or in their surface exposure) than has been recognized to date.

The more frequent detection of mixed infections by FA reflects its greater ability to detect a small number of organisms of one serovar in the presence of a large number of organisms of a different serovar. However, the proportion of mixed infections (4 of 58) was higher than that reported previously (1-3) and needs further evaluation with larger numbers of isolates.

These observations confirm and extend those of Wang and Grayston (9). Inclusion staining requires less than half the time for expansion of isolates, and the assay can be run in about one-fifth of the time required for RIA. For these reasons, it is a convenient and cost-effective method for immunotyping large numbers of isolates, while typing by RIA may be useful when FA provides inconclusive results.

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