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Indirect Immunofluorescence Staining of *Chlamydia trachomatis* Inclusions in Microculture Plates with Monoclonal Antibodies

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Indirect immunofluorescence (IF) staining, using a monoclonal antibody, detected two- to fourfold more inclusions than did iodine staining. Of 274 clinical specimens, 53 (19.3%) were positive by IF on passage 1 as compared with 33 (12%) by iodine staining ($P < 0.005$). IF-stained inclusions in McCoy cells in the bottom of microculture wells were readily viewed with a long-focal-length objective at a magnification of 250×.

Chlamydia trachomatis is the leading cause of sexually transmitted diseases in industrialized countries (11) and is the leading cause of blindness in underdeveloped countries (6). Chlamydial infections in children cause hyperendemic blinding trachoma, inclusion conjunctivitis, and pneumonitis; more recently, chlamydiae have been recovered from the vagina and rectum of infants (7). In adults, chlamydiae cause urethritis, epididymitis, and proctitis in men; cervicitis, salpingitis, vulvovaginitis, and pelvic inflammatory disease in women, and lymphogranuloma venereum (LGV) in both sexes. Traditional methods of chlamydial isolation involve the use of cell monolayers in shell vials and staining of chlamydial inclusions with either Giemsa stain or iodine. Since the isolation of chlamydia in shell vials with glass cover slips is cumbersome, time consuming, and costly, microculture systems recently have been introduced (1, 5, 8, 9). The microculture system has the advantage that large numbers of specimens can be processed more quickly and economically compared with the conventional shell vial method. Immunofluorescence (IF) staining of chlamydial inclusions recently has been compared with Giemsa or iodine staining (9, 10, 12).

Experiments were conducted to determine the optimal time for detecting chlamydial inclusions by IF, using monoclonal antibodies. McCoy cells propagated on the bottom of microculture wells without cover slips were infected with either LGV 434/Bu or a fresh isolate of *C. trachomatis* MU12021 (isolated from the urethra of a male with nongonococcal urethritis) and were stained by an indirect IF technique, using either anti-chlamydial monoclonal antibody or human anti-LGV serum (from a patient with LGV) at various times postinfection. Monoclonal antibodies were prepared by conventional hybridoma technology (4) by immunizing BALB/c mice with purified LGV 434/Bu elementary bodies and fusing immune spleen cells with SP 2/0 myeloma cells. One monoclonal antibody, L28 (immunoglobulin G1 [IgG1]), which reacted with *C. trachomatis* B-complex serovars (B, D, E, F, G, K, and L1–3) but not with C-complex serovars (A, C, H, I, and J) or *Chlamydia psittaci* by the micro-IF test (13), was chosen for IF staining since approximately 81% of genital tract isolates of *C. trachomatis* in North America represent B-complex serovars (3). Monolayers were washed three times with phosphate-buffered saline (PBS), fixed with methanol for 10 min at room temperature, and stained by IF. Human anti-LGV antiserum (complement fixation titer, 1:256; diluted 1:40 in PBS) or monoclonal antibody (L28 diluted 1:10 in PBS) was added for 1 h at 37°C. Plates were washed for 15 min with PBS, counterstained with 0.02% Evans blue, and rinsed in distilled water, fluorescein isothiocyanate-labeled goat anti-mouse IgG antibody (H- and L-chain specific; Cappell Laboratories, Malvern, Pa.) or fluorescein isothiocyanate-labeled goat anti-human IgG antibody (H- and L-chain specific; Behringwerke AG, Marburg, FRG) was added at a dilution of 1:30 or 1:15, respectively. Plates were then incubated for 30 min at 37°C, washed for 10 min with PBS, rinsed in distilled water, and finally rinsed with PBS-glycerol, pH 7.5. Microtiter plates were examined inverted under a Leitz fluorescence microscope, using a long-focal-length objective (LL25; Wild Leitz Canada, Toronto) at a magnification of ×250.

Chlamydial inclusions were first detected by indirect IF staining at 20 h postinfection with both monoclonal antibody L28 and anti-LGV sera. IF staining at 20 h was granular or punctate, and characteristic inclusion morphology was not evident until 36 to 48 h with both L28 antibody and anti-LGV sera. Both the morphology and the time of first appearance of IF was the same for McCoy and HeLa 229 cells infected with either L2 434/Bu or isolate MU12021. Comparison of the number of inclusions detected by iodine staining at 72 h and IF at 20 h and 48 h showed that IF staining at 48 h with antibody L28 detected two- to fourfold more inclusions than either iodine (Table 1) or IF staining at 20 h (data not shown). Since IF staining at 48 h detected more inclusions than at 20 h, it was felt that some specimens with low numbers of inclusions might be missed at 20 h; therefore, 48 h was chosen as the optimal time for comparison with iodine staining of inclusions in cell cultures inoculated with clinical specimens.

Urethral and endocervical specimens were collected with cotton-tipped swabs from patients attending a sexually transmitted disease clinic at the Hamilton General Hospital. Swabs were placed in 1.5 ml of sterile transport medium consisting of Eagle minimal essential medium, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate) (Flow Laboratories, McLean, Va.), 5.4 mM sodium bicarbonate, 10 μg of gentamicin per ml, and 1% bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.). Specimens were transported to the laboratory on wet ice and either cultured immediately or held overnight at 4°C and cultured the following day. Chlamydial isolation was performed by using a microculture technique without cover slips (1). Specimens (0.1 ml) were inoculated onto preformed

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TABLE 1. Comparison of IF staining at 48 h to iodine staining at 72 h for detection of *C. trachomatis* inclusions in microtiter plates

Dilution of inoculum of isolate MU12021	Staining technique	
	Iodine	IF
1:1	98[a]	>200[b]
1:2	24	90
1:4	8	23
1:8	4	10

[a] Number of inclusions per well (mean of three wells).

[b] Number of inclusions per well (mean of three wells), using monoclonal antibody L28 (IgG1).

monolayers of McCoy cells in 96-well plates (Costar, Cambridge, Mass.), and the plates were centrifuged at 2,400 × g and 35°C for 30 min, using microplate carriers (Dynatech Laboratories, Alexandria, Va.). Plates were incubated for 2 h at 37°C, inocula were removed by aspiration, and 0.2 ml of growth medium containing 1 μg of cycloheximide and 100 μg of vancomycin per ml was added. Plates were incubated at 37°C and stained with iodine (2) or by IF.

Of 103 clinical specimens tested by both iodine and IF staining techniques with anti-LGV serum, 12 were positive by IF, whereas 11 were positive by iodine staining (Table 2). This result suggested that IF staining, using human anti-LGV serum, was only slightly more sensitive than iodine staining. In contrast, IF staining, using monoclonal antibody L28, proved to be significantly more sensitive than iodine staining. Of 274 specimens tested, 53 (19.3%) were positive by IF staining on passage 2 as compared with 33 (12%) by iodine staining ($P < 0.005$) (Table 2); five additional specimens were positive by iodine staining on passage 2, bringing the total to 38 ($P < 0.025$). No additional positive specimens were detected by IF on passage 2. No iodine-positive, IF-negative specimens were observed, suggesting that all positive specimens belonged to the *C. trachomatis* B complex. A subset of 10 of the IF-positive, iodine-negative specimens was tested by a blocking test with human anti-LGV serum, confirming them to be true-positive specimens.

The use of monoclonal antibodies and IF staining in a microculture system without cover slips offers the advantage of rapid and economical processing of large numbers of specimens as compared with conventional methods. Since a significant proportion of positive results was detected by iodine after passage 2 (27% in a previous study [1], 13% in this study) but was detected by IF staining after passage 1, the IF staining method could eliminate the need for another passage, theoretically reducing the workload by one-half and the reporting time from 6 to 2 days.

TABLE 2. Comparison of iodine and IF staining techniques for the detection of *C. trachomatis* inclusions

No. of specimens	Staining technique		
	Iodine	IF	
		Polyclonal antibody	Monoclonal antibody
103	11[a]	12[b]	ND[c]
274	38[d]	ND	53[e]

[a] Number of specimens positive by iodine staining at 72 h.

[b] Number of specimens positive by IF staining with human anti-LGV serum stained at 48 h.

[c] ND, Not done.

[d] Five of these specimens were positive only after passage 2.

[e] Number of specimens positive by IF staining with monoclonal antibody L28 stained at 48 h.

Improved sensitivity of IF over iodine staining has been reported by other workers. Thomas et al. (12) showed that IF detected more inclusions and more positive specimens than Giemsa staining on cover slips in shell vials, and Stephens et al. (10) reported similar findings. Stamm et al. (9), using a fluoresceinated species-specific monoclonal antibody to the major outer membrane protein of *C. trachomatis*, detected eightfold higher inclusion counts and 29.4% more positive specimens than with iodine on clinical specimens grown in microculture plates. By using a *C. trachomatis* B-complex-specific monoclonal antibody and indirect IF staining, we report the detection of 37.7% more positive results in a single passage as compared with iodine staining. Both studies showed that IF staining with monoclonal antibodies in a direct or indirect test was more sensitive than iodine staining for the detection of chlamydial inclusions, and the convenience of processing specimens without cover slips is appreciable. Until such time as newer diagnostic techniques for the identification of chlamydial antigen in clinical specimens are refined and further evaluated, cell culture methods employing IF staining with monoclonal antibodies would appear to be the method of choice for detecting chlamydial inclusions in cell culture.

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LITERATURE CITED

1. **Chernesky, M., and J. Mahony.** 1982. A tissue culture procedure for the isolation of chlamydiae from large numbers of clinical specimens, p. 291–294. *In* P. A. Mardh et al. (ed.), Chlamydial infections. Elsevier Biomedical Press, Amsterdam.
2. **Gordon, F. B., I. A. Harper, A. L. Quan, J. D. Trehearne, R. S. Dwyer, and J. A. Garland.** 1969. Detection of chlamydia (*Bedsonia*) in certain infections of man. I. Laboratory procedures: comparison of yolk sac and cell culture for detection and isolation. J. Infect. Dis. **120:**451–462.
3. **Kuo, C. C., S. P. Wang, K. K. Holmes, and J. T. Grayston.** 1983. Immunotypes of *Chlamydia trachomatis* isolates in Seattle, Washington. Infect. Immun. **41:**865–868.
4. **Mahony, J., A. Bose, D. Cowdrey, T. Nusair, M. Lei, J. Harris, A. Marks, and R. Baumal.** 1981. A monoclonal antiidiotypic antibody to MOPC 315 IgA inhibits the growth of MOPC 315 myeloma cells in vitro. J. Immunol. **126:**113–121.
5. **McComb, D. E., and C. I. Puzniak.** 1974. Micro cell culture method for isolation of *Chlamydia trachomatis*. Appl. Microbiol. **28:**727–729.
6. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298:**428–435, 490–495, 540–549.
7. **Schachter, J., M. Grossman, J. Holt, R. Sweet, and S. Spector.** 1979. Infection with *Chlamydia trachomatis*: involvement of multiple anatomic sites in neonates. J. Infect. Dis. **139:**232–234.
8. **Smith, T. F.** 1977. Comparative recoveries of chlamydia from urethral specimens, using glass vials and plastic microtiter plates. Am. J. Clin. Pathol. **67:**496–498.
9. **Stamm, W. E., M. Tam, M. Koester, and L. Cles.** 1983. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. J. Clin. Microbiol. **17:**666–668.
10. **Stephens, R. S., C. C. Kuo, and M. R. Tam.** 1982. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* inclusions in cell culture. J. Clin. Microbiol. **16:**4–7.

11. **Taylor-Robinson, D., and B. J. Thomas.** 1980. The role of *Chlamydia trachomatis* in genital tract and associated diseases. J. Clin. Pathol. **33**:205–233.
12. **Thomas, B. J., R. T. Evans, G. R. Hutchinson, and D. Taylor-Robinson.** 1977. Early detection of chlamydial inclusions combining the use of cycloheximide-treated McCoy cells and immunofluorescence staining. J. Clin. Microbiol. **6**:285–292.
13. **Wang, S. P., J. T. Grayston, E. R. Alexander, and K. K. Holmes.** 1975. Simplified microimmunofluorescence test with trachoma-lymphogranuloma venereum (*Chlamydia trachomatis*) antigens for use as a screening test for antibody. J. Clin. Microbiol. **1**:250–255.