Identification of Chlamydia trachomatis by Direct Immunofluorescence Applied in Specimens Originating in Remote Areas

T. WILLIAMS, A. C. MANIAR, R. C. BRUNHAM, AND G. W. HAMMOND

Department of Medical Microbiology, University of Manitoba, and Cadham Provincial Laboratory, Winnipeg, Manitoba, Canada

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Culturing of Chlamydia trachomatis is costly, cumbersome, and time-consuming. Another major difficulty with culture diagnosis is the suspected failure of C. trachomatis to maintain viability with long transit times (≥48 h) to the laboratory (1). Hence, false-negative culture reports may commonly occur.

The availability of a direct immunofluorescence test with monoclonal antibodies for the detection of C. trachomatis elementary bodies (EBs) in clinical specimens (MicroTrak; Syva Co., Palo Alto, Calif.) (3-5) offered a means of assessing the role of delays in transit and processing on the results of C. trachomatis culturing.

Immunofluorescence testing was initially evaluated in comparison with optimal same-day culturing of one of duplicate specimens, and these results indicated that MicroTrak was equal in sensitivity and specificity. An evaluation of the usefulness of MicroTrak for specimens delayed in transit (i.e., under less-than-ideal circumstances) was undertaken.

Specimens for culturing of C. trachomatis were collected with plastic-shafted calcium alginate swabs and placed in sucrose-phosphate chlamydia transport medium (6).

Specimens for immunofluorescence testing were collected in accordance with the instructions of the manufacturer. After the removal of excess mucopus, an appropriate large or small dacron swab was placed in the cervix or urethra, rotated, and then rolled within the clear area of the specimen slide. After air drying, the smear was fixed with acetone and submitted to the Cadham Provincial Laboratory.

Fifty-seven duplicate specimens were obtained at a clinic on sexually transmitted disease from patients with either acute urethritis or cervicitis. One specimen was processed for immediate culturing as described below, and the other was used for direct smear examination. A total of 278 duplicate specimens were obtained from patients who had acute urethritis and cervicitis and attended city public clinics, and a further 139 duplicate specimens were from patients living approximately 350 miles north of Winnipeg. One of each paired specimen was a slide for immunofluorescence. The other was placed in transport medium for C. trachomatis culturing when delays in laboratory receipt occurred because of remoteness or because the specimen was held in city physicians’ offices overnight. On receipt at the Cadham Provincial Laboratory, specimens were kept at −70°C for up to 2 weeks before being cultured.

C. trachomatis was cultured in either of two laboratories. In the Department of Medical Microbiology Laboratory, specimens were cultured, within 24 h of collection and without prior freezing, by inoculating 24-h-old cover slip cultures of HeLa 229 cells in 1-dr (ca. 1.77-g) vials with 100 µl of transport medium as described previously (3). Cultures were incubated for approximately 60 h at 35°C, fixed with methanol, and stained with fluorescein-labeled monoclonal antibody (MicroTrak) to detect C. trachomatis inclusions. In the Cadham Provincial Laboratory, specimens were cultured by inoculating 72-h-old cover slip cultures of iododeoxyuridine-treated McCoy cells with 100 µl of transport medium (2). Cultures were incubated for 72 h at 37°C, fixed with methanol, and stained with iodine.

Fixed smears were incubated with fluorescein-labeled EB-specific monoclonal antibody (Syva Co.) for 15 min at 20°C. The presence of 10 or more apple-green EBs when smears were viewed with UV light (490-nm wavelength) in a Leitz Dialux microscope with a 1.3/40 Fluotar oil immersion objective was the criterion for a positive test. One observer read all MicroTrak slides.

Of the 57 specimens transported under optimal conditions, 23 were positive and 23 were negative for C. trachomatis. Direct examination detected 22 of 23 culture-positive specimens for a sensitivity of 96%. Direct examination was negative in 33 of 34 culture-negative specimens for a specificity of 97%.

The 278 specimens obtained from patients seen in clinics throughout Winnipeg yielded 29 cultures positive for C. trachomatis. Some of these specimens arrived at the laboratory within 24 h of procurement, but many were held overnight or longer. The sensitivity and specificity of MicroTrak under these conditions were 81 and 82%, respectively.

Of the 139 specimens originating in remote areas and delayed by weather or other transport hazards (average transit time, 3 days; maximum transit time, 16 days), only 2 were positive for C. trachomatis by culturing. The sensitivity and specificity of MicroTrak under these conditions were 100% (2 of 2) and 77% (106 of 137), respectively. Of 33
specimens positive by immunofluorescence, only 2 were positive by culturing (Table 1).

The diagnosis of microbiological pathogens from remote areas has always been a challenge. Many organisms fail to remain viable (e.g., Neisseria gonorrhoea and C. trachomatis) when transit times are long or when uncontrolled adverse ambient temperatures are encountered or both. To provide a high quality of medical care for patients with C. trachomatis infections, laboratory data are necessary to support treatment, follow-up, and contact tracing. Thus, efforts were made to provide a positive identification of C. trachomatis even under adverse conditions. Although the number of specimens cultured on the day of procurement was small (Table 1), the high correlation of MicroTrak testing with culturing under optimal conditions allowed us to consider direct immunofluorescence staining to be as sensitive and specific as optimal culturing for symptomatic patients.

A comparison of the effect of transit time on the viability of organisms could be made between city and rural specimens, as the laboratory techniques within the Cadham Provincial Laboratory were identical for these specimens. Thus, the percentages of these specimens which failed to grow in cultures but were positive by MicroTrak indicated the significant losses due to prolonged transit. A total of 94% of rural specimens and 33.3% of urban specimens that contained C. trachomatis and were identified by a positive MicroTrak test failed to yield positive results on culturing. Consequently, MicroTrak has become the test of choice for most specimens submitted for the diagnosis of C. trachomatis infections in Manitoba, with greatly improved results and turnaround times.

Specimens that contained C. trachomatis and were identified by culturing but not by MicroTrak were largely the result of the visualization of less than 10 EBs. Uyeda et al. used the presence of two EBs as the criterion for a positive test (5). We have improved the sensitivity of the test by making the visualization of 5 EBs rather than 10 EBs the criterion for a positive result. Another factor was the improvement in slide preparation, as early in the trial physicians preparing slides often failed to remove the cervical mucus before obtaining the specimen or did not air dry the slide before acetone fixing. Both factors further improve the utility of direct detection of C. trachomatis by immunofluorescence.

### LITERATURE CITED


### TABLE 1. Comparison of culturing and MicroTrak for identification of C. trachomatis

<table>
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<th>Group</th>
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* Group A, Specimens with same-day culturing; group B, urban specimens with intermediate transit times before culturing; group C, rural specimens with prolonged delay before culturing.
* Data are the percentages of specimens detected by MicroTrak but not detected by culturing were 4.3, 66.6, and 94 for groups A, B, and C, respectively.