Premarket evaluation of Monofluor reagent for detecting *Chlamydia trachomatis* in adolescent outpatients

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SUMMARY A new direct fluorescent antibody reagent, Monofluor, was evaluated for detecting *Chlamydia trachomatis* in fresh specimens. Monofluor was compared with MicroTrak and with cultivation in McCoy cells. Both direct systems were slightly less sensitive than culture, but no significant differences in specificity or sensitivity were noted between culture, Monofluor, or MicroTrak results.

Chlamydia trachomatis is one of the most prevalent sexually transmitted organisms and is responsible for at least 20% of cases of pelvic inflammatory disease in some patient populations.¹ Endometritis and salpingitis due to *C trachomatis* are often referred to as the silent infections. In many instances, the patient does not know that she has an infection until she is evaluated for infertility. The need for efficient and reliable detection methods is therefore imperative if treatment is to be instituted in patients who are at risk for asymptomatic infection with *C trachomatis*. Currently available techniques for detecting *C trachomatis* include cultivation of the organism in tissue cell cultures, antigen detection by enzyme linked immunoassay, and fluorescent antibody staining. The most commonly used fluorescent antibody technique has been MicroTrak (Syva Company, Palo Alto, California, USA).

We conducted a comparative evaluation of Monofluor (Synbiotics, San Diego, California, USA), MicroTrak, and tissue culture. The high incidence of positivity for *C trachomatis* in the asymptomatic population studied emphasises the need for rapid and reliable diagnostic alternatives.

Patients, materials, and methods

STUDY POPULATION

We screened 116 sexually active adolescent girls aged 13 to 19 who attended a Teen Health Clinic at

Jefferson Davis Hospital in Houston, Texas, from 13 May to 6 June 1986. Eight patients were white, 21 hispanic, and 87 black. Most were indigent and lived in the inner city. Ninety three were attending the clinic for family planning counselling and yearly pelvic examination or for postpartum examinations.

COLLECTING AND PROCESSING SPECIMENS

Two endocervical specimens were collected from each patient on sterile calcium alginate swabs after the tip of the cervix had been cleansed of vaginal discharge and debris with a large cotton tipped applicator. The first swab was used to make smears of the sample within the perimeter of two circular areas (wells) located at opposite ends of a glass slide, one end of which was frosted. The slide was allowed to air dry and then fixed in absolute methanol for 10 minutes. It was removed from the methanol, allowed to air dry, fixed, and frozen at -70°C . Slides were stained in batches of 20 within one week after preparation.

A second swab was placed in chlamydial transport medium and kept refrigerated (at $2-8^{\circ}\text{C}$) until delivered to the laboratory within 18 hours. On receipt in the laboratory, each transport tube was vortexed for 30 seconds to remove cellular material from the swab. Excess moisture was removed from the swab by pressing it against the side of the tube as it was withdrawn. The swab was then discarded. The tubes were centrifuged at $800 \times g$ for five minutes to remove debris. The overlay medium was removed from commercially prepared McCoy cell monolayers grown on cover slips contained in 1 fluid dram (3.7 ml) vials. A 0.5 ml volume of the inoculum was added, the vials were centrifuged for one hour at $3000 \times g$ at 30°C , and

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then incubated at 35°C for one hour. Fresh isolation medium was added, and the vials were incubated for 48–72 hours before staining. The chlamydial transport medium, McCoy cell monolayers, and isolation medium were obtained from Bartels Immunodiagnosics (Bellevue, Washington, USA).

STAINING AND INTERPRETATION

The well nearest the frosted end of each slide was stained with the Monofluor reagent, and the other well was stained with the MicroTrak reagent. A 40 µl drop of the Monofluor reagent or a 30 µl drop of the Syva reagent was added to the appropriate well and spread over the entire surface of the specimen. The slides were incubated for 15 minutes in a humidified chamber at room temperature. They were then removed from the chamber and the edges were gently tapped on absorbent paper to remove excess reagent. The slides were rinsed by gentle swirling in deionised water for 10 seconds. The edges of the slides were gently tapped on absorbent paper and excess water was carefully removed from the area around each specimen. One drop of mounting fluid was added to the centre of each well, and a coverslip was added.

All the specimens were examined with a Nikon Labophot EPI fluorescent microscope with an IF 535–550 interference excitation filter, DM575 dichroic mirror, and 580 W barrier filter. The well containing the specimen stained with Monofluor was read by one technologist, and the well containing the specimen stained with MicroTrak was read by another. Neither technologist knew the results obtained by the other reagent or the culture results, though both rechecked discrepancies. Specimens were scanned at 400 × magnification for the presence of counterstained epithelial cells. Only slides containing adequate cellular material (at least 20 parabasal, basal, or columnar epithelial cells) were included in the analysis. The slides were then scanned for apple green elementary bodies (or more rarely reticulate bodies). Elementary bodies were round with smooth edges and were about 0.2 µm in diameter. The presence of elementary bodies was confirmed at 1000 × magnification. Each well was given a score (0, <10, <100, or >100) according to the number of elementary bodies observed. Specimens were considered to be positive if there were at least two elementary bodies present. All positive specimens, however, contained at least five elementary bodies. Background fluorescence was given a score on a scale of 0 (no background fluorescence) to ++ (moderate background fluorescence).

The McCoy cell monolayers were stained with the Ortho culture confirmation reagent (Ortho Diagnostics, Raritan, New Jersey, USA) and by the Synbiotics culture confirmation reagent. Both culture staining systems required the removal of growth media, fixa-

Phillips, Faro, Smith, Martens, Riddle, Goodrich

tion with methanol for 10 minutes, removal of methanol, adding one drop of fluorescein labelled antibody to *C trachomatis* to the monolayer, incubating for 30 minutes at 37°C in a humidified chamber, rinsing, and mounting the coverslip cell-side-down on a clean glass slide. The stained monolayers were scanned at 250 × to 400 × magnification for typical apple green inclusion bodies, which measured 8 to 15 µm in diameter. It was also possible to detect the smaller elementary bodies, if the inclusions had ruptured, by the use of 1000 × magnification. Blind subcultures were not performed.

The method used for comparing results was that described by Galen.²

Results

Of the 116 culture specimens, 29 yielded *C trachomatis*. All 116 slides contained adequate cellular material for comparison, but two stained with the MicroTrak reagent and four stained with Monofluor were uninterpretable due to excessive background fluorescence. Three specimens yielded a toxic and uninterpretable culture result. Thus a total of 107 specimens were compared by the three systems.

Table 1 compares detection by the three systems. Of the 107 specimens examined, two were negative by the Monofluor reagent that were positive by culture and MicroTrak. All specimens positive by Monofluor were also positive by MicroTrak. Six specimens positive by culture were negative by both direct systems.

Five specimens were negative by culture and positive by both MicroTrak and Monofluor. Four of these contained fewer than 10 elementary bodies and the fifth contained 10–100 elementary bodies. Table 2 shows that, if these five specimens were considered to be true positives (false negative by culture), the sensitivity of culture would be 84%, that of Monofluor 75%, and that of MicroTrak 81%. The specificity and predictive value of a positive result by all three systems would then be 100%. The predictive values of a

Table 1 Comparison of Monofluor and MicroTrak with culture results for detecting *Chlamydia trachomatis* in 107 endocervical specimens

Culture	Monofluor		MicroTrak	
	Positive	Negative	Positive	Negative
Positive	19	8	21	6
Negative	5	75	5	75
Sensitivity	70% (19/27)		78% (21/26)	
Specificity	94% (75/80)		94% (21/27)	
Positive predictive value	79% (19/24)		81% (21/26)	
Negative predictive value	90% (75/83)		93% (75/81)	

Table 2 Results obtained by culture, MicroTrak, and Monofluor compared with confirmed positive results*

	Culture		Monofluor		MicroTrak	
	Positive	Negative	Positive	Negative	Positive	Negative
Confirmed results:						
Positive	27	5	24	8	26	6
Negative	0	75	0	75	0	75
Sensitivity†		84% (27/32)		75% (24/32)		81% (26/32)
Specificity		100% (75/75)		100% (75/75)		100% (75/75)
Positive predictive value		100% (27/27)		100% (24/24)		100% (26/26)
Negative predictive value†		94% (75/80)		90% (75/83)		93% (75/81)

*Five specimens positive by MicroTrak and Monofluor were counted as true positives (culture misses).

†Differences not significant by χ^2 test.Table 3 Monofluor compared with MicroTrak for detecting *Chlamydia trachomatis* in 107 endocervical specimens

MicroTrak	Monofluor	
	Positive	Negative
Positive	24	2
Negative	0	81
Sensitivity		92% (24/26)
Specificity		100% (81/81)
Predictive value of positive result		100% (24/24)
Predictive value of negative result		98% (81/83)

negative result would be 94% by culture, 90% by Monofluor, and 93% by MicroTrak.

Table 3 shows that, when compared with MicroTrak, Monofluor had a sensitivity of 92%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 98%.

Three specimens stained with MicroTrak and 15 stained with Monofluor showed a + level of background fluorescence. One specimen stained with Monofluor showed a ++ level of background fluorescence.

There were no discrepant results between monolayers stained with the Ortho fluorescent antibody reagent and those stained with Monofluor.

Discussion

The cost, labour, and potential lack of sensitivity due to loss of viability in delayed transport of specimens has made procedures other than cultivation for *C trachomatis* desirable. Some investigators who compared MicroTrak with culture results have found an acceptable level of performance with that reagent.³⁻⁵ We undertook the present study to compare the methodology and ease of interpretation of a new reagent, Monofluor, with that of MicroTrak and to compare both fluorescent stains with our standard cell culture technique.

Monofluor, MicroTrak, and the Ortho culture confirmation reagent all contain monoclonal

antibodies directed against the major outer membrane protein (MOMP) of *C trachomatis*. The Ortho reagent also contains monoclonal antibodies against the lipopolysaccharide (LPS) portion of the cell wall. The presence of two antibodies in the Ortho reagent offered no apparent increase in sensitivity, as all the specimens positive by the Ortho reagent were also positive by the Monofluor reagent. The discrepancies between the direct tests and culture results may have been due to sampling error or inappropriate storage of the transport tube after specimen collection. The latter would result in loss of viability and hence a negative culture, whereas the direct tests would have given positive results due to staining of antigen. Blind subculture of initially negative specimens was not performed, as we have not found that to increase positive cultures. Thus we have found that the large inoculum size, the high speed of centrifugation, and use of a fluorescent antibody reagent (rather than iodine) for staining provides optimum detection of *C trachomatis*.

The results obtained with both fluorescent reagents were comparable to those obtained by other investigators who compared results of MicroTrak with culture results from asymptomatic female patients.^{4,6-9} The culture procedures used by different investigators have varied somewhat, making it difficult to make clear cut comparisons with other studies. Some investigators used McCoy cell monolayers contained in 96 well microtitre plates.⁶⁻⁸ Stary *et al* used the MicroTrak culture confirmation stain for staining McCoy cells,⁶ whereas Quinn *et al* and Chernesky *et al* used iodine for staining McCoy cells.^{7,8} Other investigators used McCoy cell monolayers contained in one dram vials for culture.^{4,9} Uyeda *et al* used the MicroTrak culture confirmation stain,⁴ whereas Lipkin *et al* used iodine for staining McCoy cells.⁹ Fluorescent staining of McCoy cells has been shown to be more sensitive than iodine staining in detecting positive cultures.^{7,10,11} Furthermore, cultivation in vials has been shown to be more sensitive than in microtitre plates for recovering *C trachomatis*.^{9,12}

Perhaps the most accurate results of comparisons are obtained by comparing new reagents or test systems with the reference method under optimum conditions, which is cultivation in vials and staining with fluorescent antibody reagents. In that respect, the present study most closely resembles that of Uyeda *et al.*⁴ with the difference that the Ortho and Monofluor, rather than MicroTrak, fluorescent stains were used to confirm culture. The differences in sensitivity between the study of Uyeda *et al.* (96%) and the present study (78%) may possibly be explained by differences in specimen collection techniques for culture or direct examination, differences in time between collection of specimens and inoculation of McCoy cells, differences in performance characteristics of the Ortho culture confirmation test and the MicroTrak culture confirmation test, or a combination of these and other factors.

We found that the Monofluor reagent was only slightly less sensitive than the MicroTrak reagent compared with culture results. The other variables considered (specificity and predictive value of positive or negative results) were nearly equal. Both manufacturers recommend that 10 elementary bodies should be seen after staining the specimen directly for a result to be considered positive. We found, however, that the size, shape, and colour of elementary bodies stained with either reagent were easily recognised even when present in small numbers. To use 10 as the cutoff point would unnecessarily decrease the sensitivity of both tests. Although the technical procedure of Monofluor was as simple as MicroTrak, Monofluor stained specimens were slightly more difficult to interpret because of background fluorescence. This difficulty was overcome with practice, but may prove quite frustrating and fatiguing to technologists not accustomed to reading fluorescence.

The prevalence of infection with *C. trachomatis* imposes the necessity of diagnostic techniques for recognition of that organism even in settings where cultivation is not available. Both fluorescent reagents studied are designed to be interpreted within 40 minutes after collection of the specimen. Slides are usually sent to a laboratory and stained in batches, however, so the results are often not available until after the patient has left. Furthermore, direct antigen detection is reliable in patient populations where the incidence of *C. trachomatis* is high.⁷⁹ Such settings include sexually transmitted disease (STD) clinics or other large scale screening facilities that may process over 20 specimens a day. As pointed out by other investigators,¹³ accurate interpretation of fluorescent

stained slides requires well trained microscopists. The efficiency of even the best trained microscopists will diminish with large numbers of slides to read.¹⁴ Consequently, we consider the best setting for direct fluorescent staining to be a high prevalence population of low volume. In those settings Monofluor offers an alternative to the MicroTrak reagent.

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