Failure of Multiple Passages To Increase Chlamydial Recovery

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Because of a previous publication indicating that increasing the number of blind passages to five resulted in a marked increase in *Chlamydia trachomatis* isolation rates, we evaluated a multiple-blind-passage system in vials. Cervical specimens from 119 pregnant women and 82 specimens from other sources were tested for chlamydial infection. The recovery rate for *C. trachomatis* was not markedly increased after the first blind passage. Thus, we conclude that multiple blind passages are not necessary to maximize the *C. trachomatis* isolation rate in a vial system.

Chlamydia trachomatis is the most common sexually transmitted pathogen in the United States (8). Recovery of the organism in cell culture is generally considered the diagnostic method of choice (6). The sensitivity of chlamydial isolation is recognized as less than 100%, even under optimal conditions (5). The importance of a blind passage in increasing isolation rates has been stressed (7). Blind passage has been shown to have a greater effect on increasing recovery rates in the screening of asymptomatic individuals than it has on the diagnostic evaluation of symptomatic patients.

A variation in the isolation method involves the use of microtiter plates rather than the traditional 1-dram (3.7-ml) vials (11). Microtiter plates offer the advantages of easier processing of large numbers of specimens and reduced costs of reagents. However, direct comparison has shown that microtiter plates are less sensitive than 1-dram vials, with the difference between the two being greater when screening studies involved asymptomatic individuals (7).

A recent study, in which microtiter plates were used, has indicated that increasing the number of blind passages from the commonly used single blind passage to five passages resulted in a marked increase in recovery of *C. trachomatis* (2). Many of the specimens which were negative in passage 1 or 2 became positive in passages 3, 4, or 5. For us this observation had important ramifications. Thus, we decided to repeat the multiple-passage experiment with the vial systems that are standard in our two laboratories. In the San Francisco (SF) laboratory, the effects of multiple passage had been evaluated previously. The initial evaluation occurred in the early 1970s, when irradiated L-929 cells were used with the cell culture system and specimens came from symptomatic patients being evaluated for sexually transmitted diseases. No new isolates were obtained beyond passage 3, and the yield in passage 3 was inadequate to justify its routine use.

The second evaluation, in the late 1970s with cycloheximide-treated McCoy cells, produced the same result, and a parallel evaluation, done with microtiter plates, showed a marked superiority in sensitivity of the vial system over the microtiter plates (7). A 0.5% cross-contamination rate to uninoculated wells in the microtiter plates was also found.

The third evaluation in the SF laboratory was performed in a 6-month period in 1983 when women attending family planning clinics were screened for chlamydial infection (9). The reappraisal of the multiple-passage technique was done because the population was asymptomatic and it was thought that the sensitivity of culture might be improved by a second blind passage. *C. trachomatis* was isolated in a vial system from 90 (7.3%) of 1,229 specimens. Of the 90 isolates, 62 were detected in the original inoculation, 25 were detected after a single blind passage, and 3 were detected after two blind passages. It was concluded that the yield from blind passage 2 did not justify the cost or effort involved in doing the passage. Therefore, we had never performed more than two blind passages (either on routine clinical specimens or in the course of our research), but because of the report by Jones et al. (2), the SF and New Orleans (NO) laboratories each did a small series of *Chlamydia* cultures by using five passages.

MATERIALS AND METHODS

Patient populations. Pregnant women were the sources of most of the specimens. A total of 69 specimens in NO and 50 specimens in SF were endocervical swabs from pregnant women. In addition, the SF laboratory processed 82 diagnostic specimens from a variety of clinics. These included specimens from the male urethra and the respiratory tracts of infants and autopsy specimens from infants who had died of sudden infant death syndrome.

Laboratory procedures. Laboratory procedures were based on the use of cycloheximide-treated McCoy cells. The standard procedures in use in the two laboratories have been published elsewhere (3, 7). These procedures differed in some minor details. Staining for detection of the inclusions was with either a commercially available (Ortho Pharmaceutical Corp., Raritan, N.J.) fluorescein-conjugated monoclonal antibody (at the NO laboratory) or iodine stain (at the SF laboratory). Specimens were processed within 24 h of collection. Specimens were collected in 0.2 M sucrose-phosphate-glutamate (at the NO laboratory) or in tissue culture medium containing antibiotics and 10% fetal calf serum (at the SF laboratory). Inoculation of cells was performed by centrifugation for 1 h at $1,200 \times g$ at 30°C (in NO) or at ambient temperatures and $2,800 \times g$ (in SF). The monolayers were evaluated microscopically after 3 days of incubation at 35°C. Sealed vials were used in NO, whereas in

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TABLE 1. Multiple passage in the isolation of *C. trachomatis*

Time of sampling	No. of pregnant women testing positive at:		No. of positive diagnostic specimens at SF (n = 82)
	NO (n = 69)	SF (n = 50)	
Primary inoculation	16	2	3
Passage 1	2	1	1
Passage 2	0	1	1 ^a
Passage 3	0	0	0
Passage 4	0 ^b	0	0

^a Cytopathic effect and iodine-stained material noted at passage 1.^b Only 36 of the 51 specimens underwent passage 4.

benchtop mixer (in SF) or by scraping with a pipette tip (in NO) and inoculating fresh cells 3 to 4 days postinoculation.

RESULTS

The results of our study are presented in Table 1. In the NO laboratory, 18 (26%) of the 69 specimens were isolate positive and all were identified on primary inoculation or in passage 1. Of the 51 specimens that were negative in passage 1, none were found to yield inclusions with subsequent passages. Three additional passages were performed with 36 of the 51 specimens; the other 15 specimens received only two more passages because the cells became contaminated in the final run.

In the SF laboratory, 9 (1.8%) of the 132 specimens yielded *C. trachomatis*. Seven of the isolates were positive on primary inoculation or on passage 1. Two were first identified definitively in passage 2, but one of these two had a cytopathic effect and iodine-stained material that could not be definitively identified as chlamydial inclusions in passage 1. According to standard laboratory procedure in SF, such questionable specimens are passaged once to resolve the identification of the iodine-stained material. Thus, by routinely performing passage 2, only one isolate was obtained in addition to that which would have been detected by primary inoculation and one additional passage. None of the negative specimens in passage 2 were found to have inclusions in passage 3 or 4.

DISCUSSION

Of the 201 specimens that were tested in this evaluation, only 1 of 27 infections was detected because we performed more than a single blind passage. None of the specimens became positive in passage 3 or 4. Thus, in this study, there was no benefit from performing multiple blind passages to enhance chlamydial recovery. Jones and colleagues observed a 77% increase (19.5 to 34.7%) in recovery from the male urethra and a 48% increment (37.8 to 56%) in isolation rate from the female genital tract by increasing the number of passages to four (2). As they noted, these results could have been a function of the microtiter plate tissue culture system used. A comparison of vials versus microtiter plates has shown previously that microtiter plates are only 60% as sensitive as vials in screening for asymptomatic cervical chlamydial infection (7). Therefore, it appears that what Jones and his colleagues have shown is that multiple passages may be required in the microtiter plate system for it to approach the sensitivity of the vial system. If, however, microtiter plates are that much less sensitive than the vials for isolating *C. trachomatis*, the benefits of the former in terms of ease of handling and decreased cost are negated.

It is also possible that the results of Jones et al. reflect the inadvertent presence in their system of an inhibitor that can delay chlamydial replication. The five-passage protocol resulted in a recovery rate of about 35% from males considered at risk for chlamydial infection. This rate is well within the expected range and similar to previous results reported by that group in studies using only one passage (4). The 56% recovery from females is, however, unexpectedly high.

The current study was performed because the investigators were concerned that the report by Jones et al. would have a chilling effect on laboratories, clinicians, and clinical investigators seeking to isolate *C. trachomatis* from patients and research subjects. If a standard single-passage system was only 50% sensitive compared with the multiple-passage system, as the Jones results suggested, it probably would deter clinicians from relying on laboratories that use a single-blind-passage method and would cast considerable doubt on the results of research on the epidemiology and pathogenesis of *C. trachomatis* infections. In addition, laboratorians would likely balk at the necessity of performing five passages. The delay in reporting positive results (>2 weeks for four passages) would make the results clinically irrelevant in most settings.

A further concern was that the currently used nonculture methods for diagnosing chlamydial infection (fluorescein-conjugated monoclonal antibody or enzyme immunoassay for detection of chlamydial antigens in clinical specimens) are generally considered to be 60 to 90% sensitive when compared with the standard culture system (1, 7, 10). If these methods were really only 50% sensitive, then by inference, the accepted sensitivity of the nonculture methods would have to be reduced correspondingly. Such low sensitivities would make these tests worthless. Our results do not support such conclusions. While we agree with Jones and colleagues that chlamydial culture can be improved and that more sensitive diagnostic methods are needed, the vial culture system, with one blind passage, appears to provide the maximum sensitivity achievable given present-day technology.

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