A Pseudo-outbreak of *Chlamydia trachomatis* in a State Residential Facility: Implications for Diagnostic Testing

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In December 1998, an outbreak of *Chlamydia trachomatis* genital infections was reported among 18 residents of a state residential facility housing 392 mentally retarded clients. The initial patient tested positive by ligase chain reaction (LCR); 17 others tested positive by culture. Serologic test results for *C. trachomatis* antibodies in patients who had tested positive by culture were negative. Further testing showed that *C. trachomatis* DNA could not be detected in the LCR specimen or in any reportedly positive culture specimens. At the original culture laboratory, *C. trachomatis* culture was infrequently performed, and positive controls were not adequately prepared. This pseudo-outbreak highlights problems that may occur with *C. trachomatis* testing. As experience with *C. trachomatis* culture declines, laboratories performing this test should ensure quality and consider confirmatory testing. For *C. trachomatis* screening tests, the need for confirmatory testing depends on individual patient considerations (including medical-legal implications) and prevalence of infection in the tested population.

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Table 1. Tests performed by 6 laboratories that tested specimens, from clients of the Glenwood State Hospital School (GSHS; Glenwood, Iowa), for Chlamydia trachomatis infection.

<table>
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<tr>
<th>Laboratory</th>
<th>Test description</th>
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<tr>
<td>A</td>
<td>LCR test on specimen from the index patient (client 1)</td>
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<td>B</td>
<td>Culture and DNA probe tests on specimens from initial testing (392 clients in cottages I, II, and III at GSHS)</td>
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<td>C</td>
<td>LCR tests on specimens collected from 349 clients</td>
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<tr>
<td>D</td>
<td>Microimmunofluorescent assays on serum samples from 9 clients</td>
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<td>E</td>
<td>Microimmunofluorescent assays on serum samples from 44 clients</td>
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<tr>
<td>F</td>
<td>Culture tests on duplicate specimens from 9 female cottage I clients, by laboratory B</td>
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</tbody>
</table>

NOTE. Five clients were unavailable for testing. LCR, ligase chain reaction.

gation of client contacts (e.g., staff and visitors) to determine whether sexual abuse of clients had taken place.

Following the identification of the 18 apparently infected clients, the Iowa Department of Public Health on 13 December 1998 used LCR to screen the 349 clients not previously tested for C. trachomatis and for whom consent could be obtained from legal guardians. Testing of all specimens at laboratory C found that 1 endocervical specimen was weakly positive. A second subsequently obtained endocervical specimen from this untreated client was negative by LCR.

Methods

The following specimens were tested at the CDC: the original specimen from client 1, tested by LCR at laboratory A; specimens from the 10 female and 7 male clients reported to be positive for C. trachomatis by culture at laboratory B; and specimens from 6 male clients who were negative for C. trachomatis, by culture at laboratory B. Culture stock samples prepared from specimens were also tested. The specimens were tested blindly by 2 tests that employ different C. trachomatis DNA targets: a nested primer polymerase chain reaction (PCR) targeting the omp1 gene, which uses 2 different DNA extraction methods [1, 2], and the Amplicor PCR (Roche Diagnostics), which targets the cryptic plasmid [3, 4]. Control specimens included a C. trachomatis reference strain used as a control at laboratory B, a C. trachomatis laboratory strain used as a positive control at the CDC, and an original cervical swab specimen previously determined at the CDC to be culture positive.

For serologic testing, a microimmunofluorescent antibody test for C. trachomatis was done by laboratory D on serum samples from 9 clients living in cottage I and at laboratory E on serum samples from all clients living in cottages I, II, and III. At laboratory D, a patient was considered to have evidence of C. trachomatis infection if the IgG titer was ≥1:64 and the IgM titer was ≥1:20, and at laboratory E a patient was considered to have evidence of infection if the IgG titer was ≥1:16 and the IgM titer was ≥1:10.

CDC laboratory personnel visited laboratories A and B to review procedures for handling, processing, and testing of specimens and to review records. At laboratory B, blinded proficiency testing for C. trachomatis culture was conducted, and routinely used positive and negative control samples were prepared and examined by CDC and laboratory B personnel.

Results

At the CDC, 80 specimens, including a set of control specimens, were tested blindly using the DNA amplification methods described above. All 75 client specimens were negative for C. trachomatis DNA, and all 5 positive control specimens were positive.

Serologic testing by laboratory D was negative for C. trachomatis antibodies for 9 clients from cottage I who reportedly had cultures positive for C. trachomatis. Repeat serologic testing of those 9 clients, in addition to serologic testing of the 6 other clients from cottage I and all the clients in cottages II and III at laboratory E, were negative for C. trachomatis antibodies.

Laboratory A was certified under the Clinical Laboratory Improvement Amendments (CLIA) [5] for C. trachomatis LCR testing and performed 25–35 LCR tests per day. LCR proficiency testing was done 3 times yearly through the College of American Pathology (CAP); a CDC review of 1997 and 1998 proficiency test records showed that laboratory A had passed all tests. The CDC laboratory review identified no deficiencies in LCR test equipment, work flow, or procedures. Results of 2 proficiency panels, each with 6 specimens of C. trachomatis DNA in commercial LCR swab transport medium, were 100% correct.

Laboratory B was CLIA certified for viral culture, C. trachomatis culture, and C. trachomatis DNA probe. Although the laboratory performed several thousand viral cultures annually, usually only 1 or 2 C. trachomatis cultures were done each month. This laboratory performed a CAP proficiency panel for obligate intracellular (mostly viral) agents that included 1 test sample for C. trachomatis. Although this result was not scored by CAP, the laboratory results for this test were correct for all 3 panels in 1998.

A review of laboratory B found that, although the culture procedure itself was adequate, the positive control preparation used was a significant modification of established procedures. In brief, culture was done in Shell vials, using commercially obtained buffalo green monkey kidney (BGMK) cells (ViroMed) and a commercial fluorescent monoclonal antibody (Microtrak; Syva-Behring) specific for the C. trachomatis major outer-membrane protein. Positive control samples were prepared by growing C. trachomatis L2 strain (a rapid grower) on BGMK cells on a coverslip in a 1-dram vial for 4–5 days or until cytopathic effects (CPEs) were observed by visualization of the infected monolayer without fixation or staining under an inverted microscope.

When CPEs (usually in the form of large gaps in the monolayer) were observed, the culture was peeled off the coverslip, diluted, and dispensed onto multiple glass slides for fixation and staining with a C. trachomatis–specific monoclonal antibody.
This process resulted in lysis of *C. trachomatis* inclusions so that the final product used as a positive control slide contained only elementary and reticulate bodies and no typical inclusions; it is not clear what had been interpreted as positive in these specimens. Although the procedure is appropriate for viral cultures, it is not suitable for *C. trachomatis* cultures. Furthermore, *C. trachomatis* cultures, particularly with this strain, should be fixed 48 h after inoculation. However, except for preparation of the positive control, the procedure used by laboratory B for culture tests was found to be adequate and should have enabled visualization of typical inclusions when performed on patient specimens.

At the time of the site visit by CDC staff, laboratory B slides contained cell cultures that had been previously inoculated with patient specimens were not available for viewing. Staff from laboratory B did, however, correctly identify several blinded positive and negative culture slides prepared in Atlanta by CDC staff and brought for proficiency testing purposes. Laboratory B had also correctly reported that 40 of 70 culture test results for samples from clients at GSHS were negative.

During the laboratory visits, CDC staff learned that laboratory B had split the second set of specimens from the female cottage I clients they had received and sent 1 set of the specimens to laboratory F for confirmatory *C. trachomatis* culture testing. When laboratory B received negative culture results from laboratory F, they assumed that viability had been lost during storage and transport and were not concerned. The negative results were not reported to GSHS clinicians.

**Discussion**

We believe that all positive *C. trachomatis* culture and LCR test results reported for GSHS clients were spurious and that no outbreak of *C. trachomatis* genital infections occurred at GSHS during November and December 1998. Even before the intensive investigation of the laboratory, 2 curious findings suggested that there was a problem with the test results: (1) since *C. trachomatis* culture is only 56%–87% sensitive, an unusually high number of the cultures were reported to be positive (100% of the 27 cultures performed on specimens from clients in cottage I were positive), and (2) test results were clustered by test type. Except for the positive LCR test result that was not confirmed, the only positive test results from cottages I and II were from culture; all DNA probe assays were negative.

Our conclusion is supported by laboratory findings. First, none of the reportedly culture-positive clients had any detectable antibodies to *C. trachomatis* by serologic tests done in 2 different laboratories. Although the clinical utility of such serologic testing is limited, if these clients were truly infected, it is probable that some would have had positive serologic test results [6–8]. Second, nucleic acid amplification testing of the reportedly *C. trachomatis*-positive specimens at the CDC failed to detect any *C. trachomatis* DNA. Third, at the laboratory that reported the positive culture results, a method suited to viral culture but not to *C. trachomatis* culture was used to prepare control specimens. Furthermore, the context for this reported outbreak may have facilitated misinterpretation of results by laboratory workers and led them to disregard the negative results reported by laboratory F. Documented past episodes of sexual abuse at GSHS included the pregnancy of a profoundly retarded, nonambulatory client in March 1991 and the sexual assault of an ambulatory client by another client in November 1998.

This cluster of reported *C. trachomatis* infections among GSHS clients highlights several points regarding *C. trachomatis* testing. First, screening tests for *C. trachomatis* genital infection (such as the LCR test performed on the index patient) are <100% specific [9] and need to be carefully interpreted in the patient’s clinical context. In this reported outbreak, results of the first LCR test performed on client 1 should have been considered preliminary, given the previously unknown prevalence of *C. trachomatis* in this population and the potential medical-legal implications of the positive test result. The testing of additional clients may have been avoided if a confirmatory test had been done for this patient.

Second, the methodology for *C. trachomatis* culture is complex and subject to variation, even in qualified laboratories. The recommended method is to stain the infected cells with a species-specific monoclonal fluorescein-labeled antibody for *C. trachomatis* and to directly visualize the inclusions [10]. A recently published multicenter study demonstrated that differences in the sensitivity of culture varied significantly (55.6%–85.7%) across academic laboratory centers when a nucleic acid amplification test was used as the reference standard [9]. Although the appearance of typical fluorescence-stained chlamydial inclusion bodies in culture is highly characteristic of *C. trachomatis* infection, cross-contamination of specimens, misclassification due to fluorescent cell artifacts that resemble chlamydial inclusions, and failure to use a specific stain [11] can contribute to spurious results. These difficulties possibly have been compounded by a decline in both the availability of culture and the expertise in performing culture for *C. trachomatis* diagnosis, which has accompanied the increasing use of nonculture *C. trachomatis* tests since the 1980s [12], and the lack of proficiency testing programs for *C. trachomatis* culture. There is a clear need to establish a new proficiency testing program for *C. trachomatis* culture testing to improve this situation.

The implications of false-positive or spurious test results for *C. trachomatis* genital infections, whether caused by the limitations of the test itself or to errors in procedure, can be substantial. Consistent with CDC guidelines [10], we emphasize that a single positive screening test result should be considered presumptive; the need for confirmatory testing depends on the prevalence in the population screened and the possible implications of a false-positive test result. On the basis of this reported outbreak and other reports of spurious *C. trachomatis* culture test results [9, 11], we also emphasize that laboratories perform-
ing C. trachomatis culture should place increased emphasis on ensuring quality; this is especially important as use of C. trachomatis culture declines.

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