TECHNICAL PAPER 79

Concordance of chlamydia trachomatis infections within sexual partnerships


Reference Citation

**Concordance of *Chlamydia trachomatis* infections within sexual partnerships**

S M Rogers,1 W C Miller,2 C F Turner,1,3 J Ellen,4 J Zenilman,5 R Rothman,6 M A Villarroel,1 A Al-Tayyib,2 P Leone,2 C Gaydos,5 L Ganapathi,7 M Hobbs,2 D Kanouse8

**ABSTRACT**

**Objectives:** The enhanced sensitivity of nucleic acid amplification tests (NAAT) provides an opportunity for estimating the prevalence of untreated *Chlamydia trachomatis* infections. The transmissibility and public health significance of some NAAT-identified infections are, however, not known.

**Methods:** Adults attending an urban emergency department provided specimens for *C. trachomatis* screening using NAAT. Participants testing positive were offered follow-up including re-testing for *C. trachomatis* using NAAT and traditional methods, eg culture and direct fluorescent antibody, and were treated. Overall, 90 % of NAAT-positive participants had one or more sexual partners enrolled.

**Results:** Evidence of transmission, as defined by infection concordance between partners, was observed among 75% of partners of index cases testing positive by both NAAT and traditional assay but only 45% of partners of index cases testing positive by NAAT only (prevalence ratio 1.7, 95% CI 1.1 to 2.5). Among index participants returning for follow-up, 17% had no evidence of *C. trachomatis* infection by NAAT or traditional assay (median follow-up three weeks).

**Conclusions:** A substantial proportion of positive NAAT results for chlamydial infection may be of lower transmissibility and may not persist after a short follow-up. The long-term health effects of some positive NAAT are uncertain.

Unrecognised *Chlamydia trachomatis* infection is common among US young adults.1–3 Estimates of the prevalence of *C. trachomatis* have been facilitated by the development of non-invasive nucleic acid amplification tests (NAAT). NAAT provide substantial improvements in test sensitivity while retaining the high specificity of traditional methods.4 The use of NAAT, compared with culture, increases the yield of infections detected by 20–40%.5 Although the enhanced sensitivity of NAAT is well recognised, the significance and transmissibility of the additional infections detected by NAAT are unknown. It is possible that NAAT is detecting clinically inconsequential infections involving low levels of viable organisms, or perhaps amplifiable residual DNA from a recently controlled infection.

We conducted a cross-sectional study with recruitment of sexual partners to examine the potential transmissibility of chlamydial infections identified by NAAT but not by traditional assay. We tested the hypothesis that chlamydial infections that are detectable only by NAAT are less transmissible, as evidenced by infection concordance within partnerships, than infections that are also detectable by traditional methods. As a secondary aim, we examined the persistence of NAAT-positive infections among participants after a short follow-up.

**METHODS**

Between November 2002 and February 2005, trained interviewers approached adult patients attending the Johns Hopkins Hospital Emergency Department to assess eligibility for *C. trachomatis* screening, eg age between 18 and 35 years, English speaking, and sexually active in the past 90 days. Eligible adults who consented to screening also completed a brief audio computer-assisted self interview about recent sexual and health behaviours.6 Participants screened in the emergency department after March 2003 received a US$10 food coupon. We obtained contact information from all index participants undergoing *C. trachomatis* screening in the emergency department to facilitate the follow-up of positive test results by trained research disease intervention specialists. Disease intervention specialists offered follow-up examination to detect clinical evidence consistent with chlamydial infection, additional *C. trachomatis* testing (NAAT and traditional assay), and treatment at the Johns Hopkins Hospital General Clinical Research Center (GCRC). Disease intervention specialists also contacted up to five named sexual partners within the past 60 days and offered evaluation and treatment procedures identical to those provided to index participants. Index participants and partners presenting to the GCRC for follow-up completed a detailed behavioural audio computer-assisted self interview and received US$50–200 in compensation for their time and travel costs. Partners not attending the GCRC were offered the option of a home visit to complete the questionnaire and to collect a specimen for NAAT.

The Research Triangle Institute, University of North Carolina, and Western (for the Johns Hopkins University School of Medicine) Institutional Review Boards approved all study procedures. Study participants with positive test results for chlamydial infection were reported to the Baltimore City Health Department.

**Specimen collection and laboratory testing**

**NAAT for *C. trachomatis***

US Food and Drug Administration-approved NAAT were performed according to the manufacturers’ instructions at the University of North Carolina.
Initially, urine specimens were tested using a ligase chain reaction (LCR) assay (Abbott Laboratories, North Chicago, Illinois, USA). After the LCR was withdrawn from the market in 2003, male urine specimens and female vaginal swabs were tested using the COBAS Amplicor PCR assay (Roche Diagnostic Systems, Indianapolis, Indiana, USA). Positive NAAT results were confirmed by repeating the assay. Infection with Chlamydia trachomatis as detected by NAAT (N+) was defined as a repeatedly positive test on the same specimen.

At follow-up, NAAT were performed on urine (men throughout the study and women initially), urethral swabs (men), endocervical swabs (women), and self-administered vaginal swabs. Each index thus received three NAAT: one upon initial recruitment in the emergency department and two repeat tests on follow-up. Sexual partners received two NAAT during their GCRC visit.

Traditional assay for C trachomatis
Traditional testing was performed at GCRC follow-up using culture and direct fluorescent antibody (DFA) from urethral and endocervical specimens. Specimens were stored at –80°C until testing by the International STD Research Laboratory, Johns Hopkins University. Culture was performed in McCoy cells. Culture-negative specimens were tested using DFA of the sediment from the centrifuged culture transport media; a slide was considered DFA positive if three or more elementary bodies were present. Culture-positive specimens and DFA-positive/culture-negative specimens were considered positive for C trachomatis by traditional assay (T+). Specimens that tested culture and DFA negative were considered traditional assay negative (T–).

Statistical analyses and outcomes
Chi-square and t-tests were used to compare characteristics of NAAT-positive index participants who did and did not present for follow-up.

We assessed the concordance of infection between sexual partners as a surrogate for the transmissibility of NAAT-identified infections, restricted to partnerships in which both NAAT and traditional test results were available. We defined concordance as either a positive NAAT (N+) or traditional assay (T+) result for C trachomatis among partners of C trachomatis-positive index participants. All index participants were considered NAAT positive on the basis of their emergency department test result.

Our primary hypothesis was that chlamydial infections detectable by NAAT but not by traditional assay (N+T–) in the index participants would be less transmissible to sexual partners than infections that were also detectable by traditional assay (N+T+). Non-concordance was considered as evidence of lower transmissibility. We tested this hypothesis by examining prevalence ratios with 95% CI that compared the proportion of partnerships with concordant C trachomatis infections (N+ and/or T+) between index participants who tested N+T– and those who tested N+T+.

We also examined characteristics of index participants and their partners that may be associated with infection concordance. For all partnership analyses, we used generalised estimating equations with a log link and binomial error distribution to estimate prevalence ratios for concordance. Generalised estimating equations account for within-group correlation, such as that that exists between multiple partners of the same index participant.

As a secondary aim, we examined the persistence of NAAT-identified chlamydial infections at follow-up among individuals with positive NAAT in the emergency department. Factors potentially influencing persistence were examined in binomial regression analyses. In addition, we examined the association between the persistence of infection within partnerships. All statistical analyses were conducted using Stata version 8 (Stata Corp., College Station, Texas, USA).

RESULTS
Study recruitment: C trachomatis screening and follow-up
Over a 27-month period, 21 trained interviewers identified 6952 eligible adults attending the emergency department (fig 1); 6094 (87.7%) consented to screening. The prevalence of chlamydial infection was 7.0% in emergency department participants. The prevalence of C trachomatis in male urine specimens (7%) was comparable as determined by LCR and PCR (8.4% versus 6.6%, p>0.10). Among female urine specimens tested by LCR (November 2002 to August 2003), 6.8% were C trachomatis positive; 7.3% of female swab specimens tested positive by PCR (p=0.10).

Of the 419 index participants who tested NAAT positive, 81 (19.3%) received antibiotic treatment during their emergency department visit. Of the remaining 338, 166 (49%) participated in follow-up. The mean number of disease intervention specialist contacts was two (range one to 10) and the average number of days between emergency department testing and follow-up was 21.5 (range eight to 46 days). Participants at follow-up were slightly younger (mean age 22.5 versus 23.5 years; p=0.04) and more were women (62% versus 50.6%, p=0.03) compared with those who did not participate (table 1).

Most index participants (87%) named one or more recent sexual partners at follow-up; 15% refused to provide partner information. Of 175 partners identified, 152 (86.9%) were contacted successfully by disease intervention specialists and 102 (58.3%) attended follow-up. Nearly half (48%) of the partnerships presented for follow-up together.

Partner concordance of C trachomatis infections
Our concordance analyses are limited to the 83 heterosexual couples (72 index participants with one partner, four indexes with two partners, and one index with three partners) for whom both NAAT and traditional assay results were available. We excluded 17 partnerships because of missing results, inadequate specimen collection, transcription error, or multiple enrollment of a positive index subject. Two exclusively male partnerships were omitted as we did not collect anal or throat specimens.

Evidence of infection transmission, as defined by concordance within partnerships, was more common among index cases testing positive by both NAAT and traditional assay (N+T+) than among index cases testing positive by NAAT alone (N+T–; table 2). Evidence of transmission was observed in 39 of 52 index cases (75%) of N+T+ index cases, but only 14 of 31 partners (45%) of N+T– index cases (prevalence ratio 1.7, 95% CI 1.1 to 2.5).

The relationship between index test result (N+T– or N+T+) and partner concordance did not vary by gender. Among the male partners of N+T+ female indexes, 82% were concordant for C trachomatis, compared with 46% of male partners of N+T– women. Similarly, 70% of female partners of N+T+ index men were concordant, in comparison with 45% of female partners of men testing positive by NAAT only. There was no difference by index’s or partner’s age, the number of new
CT screening of index subjects

14,188 ED patients approached

6952 (49%) eligible for participation

858 ineligible

6094 (87.7%) received NAAT screening

86 specimens unprocessed

419 (7.0%) CT +

81 received antibiotic therapy in ED

Follow-up and evaluation of CT + subjects

338 CT + index subjects contacted for follow-up

172 did not participate

166 CT + index subjects participated in follow-up

Partner evaluation

175 partners identified

152 partners contacted by disease intervention specialists

50 partners did not participate

102 partners of CT + index subjects participated in follow-up

19 partnerships excluded

83 partnerships valid NAAT and traditional assay results

Figure 1  Subject participation in screening and follow-up, November 2002 to February 2005. *Patients attending the emergency department (ED) were eligible for C. trachomatis (CT) screening if they were between 18 and 35 years of age, English-speaking, sexually active in the past three months, and a non-Hopkins employee or student. For the first five months of data collection, respondents reporting antibiotic use within the past three months were excluded. Patients were also ineligible if they were critically ill or unable to participate as a result of a physical condition or cognitive impairment, or they had been previously enrolled in the study and tested positive for C. trachomatis. A total of 700 individuals did not consent, 82 completed the audio computer-assisted self interview only, 57 individuals were released from the emergency department before completing the study, and 19 patients enrolled twice. **Subject consented and provided urine or self-administered swab specimen for sexually transmitted infection testing using the nucleic acid amplification test (NAAT). As determined by repeatedly positive NAAT on the same specimen. Initially male and female urine specimens were tested using ligase chain reaction (LCR) assay. After August 2003, male urine and female self-administered vaginal swabs were tested using the COBAS Amplicor assay. This change was necessitated by the specimen requirements of the Roche Amplicor assay, which was used after Abbott Laboratories discontinued the LCR. *C. trachomatis-positive index subjects who received antibiotic therapy during their emergency department visit were not re-contacted for follow-up. **Forty subjects provided insufficient locating information, 44 received healthcare elsewhere, 40 received treatment only at the Johns Hopkins Hospital General Clinical Research Center (GRCRC), 16 did not show for their scheduled GRCRC appointment, and 22 either refused treatment, were incarcerated, a non-resident of Baltimore, or in substance abuse rehabilitation. †After enrollment IDs were switched on two index partners in the past three months, a history of chlamydial infection, a history of any sexually transmitted infection, antibiotic use before the follow-up visit, or time to index follow-up. Adjusting for the timing of the partner visit, however, reduced the prevalence ratio to 1.5 (95% CI 1.03 to 2.2). Partners presenting at the same time as the index participant were more likely to test concordant than partners presenting after the index visit.

Limiting traditional test results to culture only decreased our overall estimate of concordance from 64% to 54% (prevalence ratio 1.4, 95% CI 0.93 to 2.0). Differences in the type of NAAT (LCR versus PCR) or specimen type (female urine versus vaginal swab) could also influence our transmission estimates. Although all women screened in the emergency department provided vaginal swabs for C. trachomatis testing using PCR, initially women also provided urine specimens for LCR testing. When the LCR was withdrawn in 2005, male urine samples and female swabs were tested using PCR. Restricting our concordance analysis to include only female indexes with positive swab results (n = 91 couples, prevalence ratio 1.7, 95% CI 1.1 to 2.5) or male and female index subjects with positive PCR results alone (n = 75 couples, prevalence ratio 1.7, 95% CI 1.2 to 2.5) had no effect on our transmission estimates.

Non-persistence of NAAT-positive results

Index participants were screened initially in the emergency department and re-evaluated at follow-up, thus it is possible to examine the short-term persistence of NAAT-identified C. trachomatis. Among participants who had not received antibiotic therapy during the emergency department visit and who had both NAAT and traditional assay results available for follow-up (n = 163; three participants had missing traditional assay results), 27 (17%) individuals had no evidence of C. trachomatis infection by NAAT or culture/DFA. Nine (5%) individuals were NAAT negative, but positive by culture/DFA. The remaining 127 (78%) participants were NAAT positive (table 3).

In bivariable analyses, women were significantly more likely than men to test negative for C. trachomatis after a short follow-up (25% versus 6%, risk ratio 3.6, 95% CI 1.3 to 9.9) as were individuals who had used antibiotics in the three months before their emergency department visit (52% versus 12%, risk ratio 2.6, 95% CI 1.2 to 5.5). In multivariable analyses, only gender remained significantly associated with the persistence of NAAT-positive results.

Non-persistence and partner concordance

We also examined the association between the persistence of NAAT-positive results and partnership concordance. Within the 83 partnerships, only one partner of nine (11%) index participants without evidence of C. trachomatis infection at follow-up tested positive. In contrast, 52 partners of 74 (70%) index participants with evidence of C. trachomatis infection at
follow-up tested positive (prevalence ratio 6.3; 95% CI 0.98 to 40.8).

**DISCUSSION**

NAAT provides enhanced sensitivity to detect chlamydial infection. Understanding the clinical and public health implications of the additional infections identified by NAAT is critical for the appropriate use of these tests. Using concordance as an estimate of transmission, we observed that individuals who were positive by NAAT, but not by traditional assay, were significantly less likely than those who were positive by both NAAT and traditional assay to have a concordantly infected sexual partner. Nonetheless, partner concordance among individuals with infections detected only by NAAT was moderate (45%). In addition, a substantial proportion of individuals (17–22%) who screened positive by NAAT for *C. trachomatis* had no evidence of chlamydial infection after a short follow-up period (median three weeks).

A likely explanation for the diminished partner concordance among infections detectable only by NAAT is reduced organism burden. The enhanced sensitivity of NAAT increases the likelihood of detecting infections with relatively few organisms. Alternatively, NAAT may detect the “passive presence” of the organism after exposure, without a true, established infection. Consequently, individuals with infections detectable only by NAAT may be inherently less likely to transmit *C. trachomatis* to their partners.

We observed a surprisingly high incidence of infection clearance, especially among women. This observation, coupled with our findings regarding reduced concordance among partners of index participants whose follow-up NAAT was negative, suggests that at least some NAAT infections may be cleared relatively rapidly, perhaps through antibiotic exposure or natural immune response, and not transmitted. We observed that many individuals without detectable infection at follow-up had been treated with antibiotics in the three months preceding their initial screening, suggesting that NAAT may

### Table 1 Characteristics of index subjects positive for *C. trachomatis* who did and did not participate in follow-up

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>C. trachomatis</em>-positive index participated in follow-up (N = 166)* N (%)</th>
<th><em>C. trachomatis</em>-positive index did not participate in follow-up (N = 172)* N (%)</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, in years, mean (SD)</td>
<td>22.5 (4.2)</td>
<td>23.5 (4.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Women</td>
<td>103 (62%)</td>
<td>87 (51%)</td>
<td>0.03</td>
</tr>
<tr>
<td>African American</td>
<td>137 (91%)</td>
<td>132 (88%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Never married</td>
<td>132 (88%)</td>
<td>135 (89%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Completed less than high school</td>
<td>60 (40%)</td>
<td>61 (40%)</td>
<td>0.98</td>
</tr>
<tr>
<td>Health behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous <em>C. trachomatis</em> or gonorrhoea infection</td>
<td>57 (38%)</td>
<td>61 (40%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Dysuria and/or discharge past 3 months</td>
<td>48 (32%)</td>
<td>38 (25%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Antibiotic use past 3 months</td>
<td>25 (17%)</td>
<td>29 (19%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Condom use, past 5 sexual acts, mean (SD)</td>
<td>2.1 (2.0)</td>
<td>2.1 (2.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>Illicit drug use past 30 days</td>
<td>79 (53%)</td>
<td>86 (57%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Sexual behaviours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+ Partners past 3 months</td>
<td>49 (33%)</td>
<td>62 (41%)</td>
<td>0.15</td>
</tr>
<tr>
<td>New partner past 3 months</td>
<td>55 (37%)</td>
<td>58 (40%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Age of most recent partner, mean (SD)</td>
<td>24.2 (5.7)</td>
<td>25.4 (7.7)</td>
<td>0.12</td>
</tr>
<tr>
<td>Only heterosexual partners past 2 years</td>
<td>131 (90%)</td>
<td>130 (90%)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*11% of index patients did not complete emergency department interview.

†Estimate for difference in measured characteristic between *C. trachomatis*-positive index subjects who did and did not participate in follow-up, based on chi-square and t-tests for categorical and continuous outcomes, respectively.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>C. trachomatis</em>-positive index did not participate in follow-up (N = 172)* N (%)</th>
<th>p†</th>
</tr>
</thead>
</table>

### Table 2 Estimate of transmission, as defined by partner concordance, *C. trachomatis*

<table>
<thead>
<tr>
<th>Partner</th>
<th>N+T+ (N = 52)</th>
<th>N−T− (N = 31)</th>
<th>Total (N = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N+T+</td>
<td>18 (35%)</td>
<td>5 (16%)</td>
<td>23 (28%)</td>
</tr>
<tr>
<td>N−T−</td>
<td>7 (13%)</td>
<td>5 (16%)</td>
<td>12 (14%)</td>
</tr>
<tr>
<td>N−T+</td>
<td>14 (27%)</td>
<td>4 (13%)</td>
<td>18 (22%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (75%)</td>
<td>14 (45%)</td>
<td>53 (64%)</td>
</tr>
<tr>
<td>Discordant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N−T−</td>
<td>13 (25%)</td>
<td>17 (55%)</td>
<td>30 (36%)</td>
</tr>
</tbody>
</table>

N+, Nucleic acid amplification test (NAAT) positive; T+, traditional assay positive; T−, traditional assay negative.

Concordant defined as NAAT and/or traditional assay positive for *C. trachomatis*.

Disconcordant defined as NAAT and traditional assay negative for *C. trachomatis*.

Tabulations include all partnerships with valid NAAT and traditional assay results: 72 index subjects with one partner, four indexes with two partners, and one index with three partners. Fourteen index subjects with N− results at follow-up (five N−T+ and nine N−T−) are considered N+ (based on their emergency department test result).

Prevalence ratio and 95% CI represent the outcome of partner status of concordant versus discordant by index status and were estimated from a generalised estimating equation logistic model that accounts for a lack of independence among index patients with multiple partners.
have detected residual DNA, rather than viable organisms, at the initial screening evaluation. A proportion of the results could be false positives, although we re-tested all initial positive results to reduce this possibility. Sampling variability associated with the repeated testing of low-level infections is another possibility, but appears less likely because of the multiple specimens taken at follow-up. The likelihood of each of these possibilities is worthy of further clinical investigation.

The widespread application of NAAT has “resulted in considerable revision of our views of the clinical epidemiology of C trachomatis”. Only recently, however, have we begun to question the significance of some additional infections identified by NAAT. In this study, we examined partner concordance as a marker of the clinical significance of NAAT-identified infections. Further study of the association between asymptomatic NAAT-identified chlamydial infections and other clinical consequences, eg the incidence of pelvic inflammatory disease, or inflammation, is needed.

Few studies have examined infection transmission within partnerships. We chose a non-sexually transmitted infection clinic population as we were interested in understanding the transmissibility of largely asymptomatic, unrecognised C trachomatis infections. Consequently, we screened over 6000 individuals. In this cross-sectional sample, the timing of the prevalent infection in the index and the direction of transmission between index and partner could not be established. Generalisation of our findings may be constrained by our sample selection and by incomplete recruitment, although index participants had a similar risk profile to individuals who did not participate.

The advent of NAAT screening has expanded opportunities for prevention of the serious consequences of untreated C trachomatis infection. Although we strongly believe that individuals with a positive NAAT should be informed and treated, our study demonstrates that some NAAT-detected infections may not represent clinically active disease or transmissible infections. Failure to diagnose a chlamydial infection can negatively affect health, although incorrectly identifying individuals as infected can damage relationships. Patients, especially those with low-risk profiles or those screened from low C trachomatis prevalence populations, should be counselled about their test result accordingly. Guidelines for testing, patient counselling and management should be formulated so that users of this remarkable and powerful tool—doctors, patients, and researchers—are aware of the advantages of NAAT and also its limitations.

### Acknowledgements
The authors would like to thank Don Orr, Martina Morris, and Heather Miller for serving as scientific advisors to this project. They also thank Sarah Mobius for her managerial contribution to the study and Sheping Li at RTI for programming and data management; Ambreen Khaliq and Chadd Krauss for their oversight of interviews at the Johns Hopkins Adult Emergency Department; Joan Bess, Kenya S. Steward and Nancy Willard of the Johns Hopkins Adolescent Health Research Group for outreach support; and Mary Ann Knott-Grasso, MS, CPNP, for providing patient care at the Johns Hopkins General Clinical Research Center. The authors also wish to thank the laboratory personnel from the Department of Medicine, University of North Carolina at Chapel Hill, including Marcia Stedman, John Schmitz, and Dana Lapple; and Jeff Younger, Billie Jo Wood, and Hope L Johnson from the Johns Hopkins School of Medicine, Department of Infectious Diseases.

### Funding
Primary support for this research was provided by National Institutes of Health (NIH) grant R01-HD039633 to SMR. RR was supported in part by a grant from NCRR NIH 3MD01RR00052-39(S1).

### Competing interests
None.

### Author contributions
SMR, WCM, CFT, PL, JE, RR and DK contributed to the conception and design of the study. SMR, WCM, CFT, JE, RR and CFT contributed to writing the manuscript. SMR, WCM, CFT, PL, JE, RR and DK contributed to the analysis and interpretation of the data. SMR, WCM, CFT, PL, JE, RR and DK contributed to the analysis and interpretation of the data. All authors contributed to writing the manuscript.

### Role of the funding source
The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

### REFERENCES

BMJ Clinical Evidence—Call for contributors

**BMJ Clinical Evidence** is a continuously updated evidence-based journal available worldwide on the internet which publishes commissioned systematic reviews. **BMJ Clinical Evidence** needs to recruit new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine, with the ability to write in a concise and structured way and relevant clinical expertise.

### Areas for which we are currently seeking contributors:
- Secondary prevention of ischaemic cardiac events
- Acute myocardial infarction
- MRSA (treatment)
- Bacterial conjunctivitis

However, we are always looking for contributors, so do not let this list discourage you.

#### Being a contributor involves:
- Selecting from a validated, screened search (performed by in-house Information Specialists) valid studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we will publish.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with **BMJ Clinical Evidence** editors to ensure that the final text meets quality and style standards.
- Updating the text every 12 months using any new, sound evidence that becomes available. **BMJ Clinical Evidence** in-house team will conduct the searches for contributors; your task is to filter out high quality studies and incorporate them into the existing text.
- To expand the review to include a new question about once every 12 months.

In return, contributors will see their work published in a highly-rewarded peer-reviewed international medical journal. They also receive a small honorarium for their efforts.

If you would like to become a contributor for **BMJ Clinical Evidence** or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to CECommissioning@bmjgroup.com.

### Call for peer reviewers

**BMJ Clinical Evidence** also needs to recruit new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity and accessibility of specific reviews within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge).

Reviews are usually 1500–3000 words in length and we would ask you to review between 2–5 systematic reviews per year. The peer review process takes place throughout the year, and our turnaround time for each review is 10–14 days. In return peer reviewers receive free access to **BMJ Clinical Evidence** for 3 months for each review.

If you are interested in becoming a peer reviewer for **BMJ Clinical Evidence**, please complete the peer review questionnaire at www.clinicalevidence.com/ceweb/contribute/peerreviewer.jsp
Concordance of *Chlamydia trachomatis* Infections Within Sexual Partnerships

Susan M. Rogers, PhD
William C. Miller, MD, PhD, MPH
Charles F. Turner, PhD
Jonathan Ellen, MD
Jonathan Zenilman, MD
Richard Rothman, MD, PhD
Maria Villarroel, MA
Alia Al-Tayyib, MSPH
Peter Leone, MD
Charlotte Gaydos, DrPH
Laxminarayana Ganapathi, PhD
Marcia Hobbs, PhD
David Kanouse, PhD

Author Affiliations: Program in Health and Behavior Measurement, Research Triangle Institute, Washington, DC (Drs. Rogers, Turner, Ms Villarroel); Division of Infectious Diseases, Department of Medicine and Epidemiology, University of North Carolina, Chapel Hill (Drs Miller, Leone, Hobbs, Ms Al-Tayyib); City University of New York, Queens College and Graduate Center (Dr Turner); Division of Adolescent Medicine, School of Medicine, Johns Hopkins University (Dr Ellen); Division of Infectious Diseases, School of Medicine, Johns Hopkins University (Drs Zenilman and Gaydos); Department of Emergency Medicine, Johns Hopkins Medical Institution (Dr Rothman); Research Computing Division, Research Triangle Institute, Research Triangle Park, NC (Dr Ganapathi); RAND (Dr Kanouse).
Corresponding Author: Susan M. Rogers, Program in Health and Behavior Measurement, Research Triangle Institute, 701 13th St NW, Suite 750, Washington, DC 20005 (smr@rti.org)

Funding/support: Primary support for this research was provided by NIH grant R01-HD039633 to Dr Rogers. Dr. Rothman was supported in part by a grant from NCRR NIH 3M01RR00052-39-5(S1).
Summary

Background Nucleic acid amplification tests (NAATs) offer new opportunities for estimating the prevalence of untreated Chlamydia trachomatis infections (Ct), providing substantial improvements in test sensitivity while maintaining the high specificity of traditional testing methods. However, the transmissibility and public health significance of some NAAT-identified infections are not known. We conducted a cross-sectional and short-duration prospective cohort study with follow-up of participants with NAAT-identified chlamydial infection and their sexual partners to examine the potential transmissibility and short-term persistence of NAAT-identified chlamydial infections.

Methods 6,094 adults aged 18 to 35 years attending an urban Emergency Department (ED) from November 2002 through February 2005 provided specimens for Ct screening using NAATs. Unrecognized Ct infections were identified in seven percent of ED participants using NAAT. Participants testing positive were offered follow-up including re-testing for Ct using NAAT and traditional methods, e.g. culture and direct fluorescent antibody, and treated. Partners were offered identical evaluation and treatment services. Overall, 90 Ct-positive participants had one or more sexual partners enrolled.

Results Evidence of transmission, as defined by concordance of infection between sexual partners, was observed among 75% of partners of index cases testing positive by both NAAT and traditional assay (N+T+) but only 45% of partners of index cases testing positive by NAAT only (N+T-) (prevalence ratio 1.7, 95% CI 1.1, 2.5). Among index participants returning for follow-up who had not received antibiotic therapy during the ED visit, 17% had no evidence of Ct infection by NAAT or traditional assay (median follow-up = 3 weeks).

Interpretation A substantial proportion of positive NAAT results for chlamydial infection may be of lower transmissibility and may not persist after a short follow-up. The public health significance and long-term health effects of some positive NAATs are uncertain.
Introduction

Unrecognized and untreated *C. trachomatis* infection is common among young adults in the United States. Nationwide, 4.7% of women and 3.7% of men aged 18 to 26 years were estimated to have an untreated chlamydial (Ct) infection in 2001-2002.\(^1\) Among adults aged 18 to 35 years in Baltimore, MD, untreated chlamydial infections were detected in 6.4% of African American females; the majority of infections were asymptomatic.\(^2\) Given the significant potential morbidity associated with chlamydial infection, including pelvic inflammatory disease and its consequences, controlling and preventing undiagnosed *C. trachomatis* is a major public health concern.\(^3\)

Estimates of the prevalence of *C. trachomatis* at the population level have been facilitated by the development of non-invasive nucleic acid amplification tests (NAATs) that can use urine specimens or self-collected vaginal swabs. NAAT provides substantial improvements in test sensitivity while retaining the high specificity of traditional methods such as culture. The enhanced sensitivity of NAAT is due to an extremely low limit of detection, with the potential to detect DNA (or RNA) of approximately 10 organisms per milliliter of sample.\(^4\) In contrast to culture, NAAT does not require viable organisms. Given the high sensitivity and low limit of detection, the use of NAAT, as compared to culture, increases the yield of infections detected by 20 to 40 percent.\(^5\)

Although the enhanced sensitivity of NAAT is well-recognized, the significance and transmissibility of the additional infections detected by NAAT are unknown. If the additional infections detected by NAAT are as transmissible as infections detected using traditional methods, such as culture or DFA of culture transport media, the findings from
population studies call for new public health strategies to reduce infection rates. On the other hand, it is possible that NAAT is detecting clinically inconsequential infections involving extremely low levels of viable organisms, or perhaps amplifiable residual DNA from a recently treated or controlled infection. In a previous population-based study, we observed that many persons with NAAT-identified infection had few behavioral risk factors and most were asymptomatic. This raises the possibility that certain NAAT infections may be of long duration and, presumably, low organism burden. Given the extensive use of NAAT for the detection and diagnosis of chlamydial infection, a better understanding of the clinical and public health significance of the additional infections detected by NAAT is needed.

We conducted a cross-sectional study of adults attending an urban Emergency Department and offered NAAT screening for chlamydial infection. We conducted follow-up of participants with untreated chlamydial infection and recruitment of their sexual partners to explore the potential transmissibility of NAAT-identified infections. We tested the hypothesis that chlamydial infections that are detectable only by NAAT are less transmissible, as evidenced by infection concordance within partnerships, than infections that also are detectable by traditional methods. In addition, a short-duration prospective cohort study examined persistence of chlamydial infections among the NAAT-positive participants who did and did not re-test Ct-positive (by NAAT and/or traditional assay) when returning for follow-up treatment.

Methods

Study Population

Between November 2002 and February 2005, trained interviewers approached adult patients attending the Johns Hopkins Hospital Emergency Department (JHH-ED) in
Baltimore, MD, USA, to assess eligibility for the study. We selected an ED population, rather than a sexually transmitted infection (STI) clinic population, because our focus was on largely asymptomatic and untreated NAAT-identified infections. Patients were eligible for Ct screening if they were between 18 and 35 years of age, English-speaking, and sexually active in the past 90 days. Employees and students of Johns Hopkins, and patients who were critically ill (level-1 acute trauma patients), intoxicated, or presenting for acute psychiatric or STI-related care were excluded. Initially, patients were not eligible if they had used antibiotics within the past 30 days, but in April 2003 this exclusion criterion was discontinued to enhance enrollment. Index participants enrolled after March 2003 received a $10 food coupon for their participation.

We obtained contact information from all participants undergoing *C. trachomatis* testing to facilitate notification and follow-up of positive test results by trained research Disease Intervention Specialists (DIS). The DIS informed participants who did not receive antibiotics during their ED visit of their positive result and offered follow-up examination, additional Ct testing (NAAT and traditional assay), and treatment at the JHH General Clinical Research Center (GCRC). Positive participants also were informed that they could seek care from their private physician or the local health department.

After providing written informed consent, Ct-positive participants presenting to the GCRC were asked to provide names of up to 5 sexual partners in the last 90 days. DIS contacted named partners and offered evaluation and treatment procedures identical to those provided to index participants. Index participants and partners were offered $50-200 in compensation for their time and travel costs. Participants who presented to the GCRC but did not wish to complete the study were provided a free medical examination and treatment.
The Research Triangle Institute, University of North Carolina, and Western (for The Johns Hopkins University School of Medicine) Institutional Review Boards approved all study procedures and modifications to the original protocol. Study participants with positive test results for chlamydial infection were reported to the Baltimore City Health Department.

Participant Interviews and Examinations

Participants in the ED completed a brief, approximately 8 minute, audio computer-assisted self interview (ACASI) about recent sexual and health behaviors. At follow-up, Ct-positive index participants presenting to the GCRC underwent physical examination to detect clinical evidence consistent with chlamydial infection, including visible discharge, genital ulcers, and lower abdominal and testicular tenderness. Detailed self-reports of current and recent sex partners, partner-specific sexual behaviors and STI history, use of antibiotics, STI symptoms, drug and alcohol use were collected using a touch-screen ACASI. The follow-up interview took an average of 23 minutes to complete.

Sexual partners located by DIS staff were offered physical examinations, testing, and treatment identical to index participants and asked to complete the ACASI. Partners who did not attend the GCRC (and had not sought care elsewhere) were offered the option of a home visit by a study DIS to complete the questionnaire and to collect a urine or vaginal swab specimen for Ct testing.

Specimen Collection and Laboratory Testing
NAAT for Ct: FDA-approved NAAT was performed according to the manufacturers’ instructions at the University of North Carolina at Chapel Hill. In the first nine months of the study during ED screening, male participants provided a urine specimen and females provided urine and self-administered vaginal swab specimens for Ct NAAT; however, after August 2003, women provided only swabs. Initially, male and female urine specimens were tested using a ligase chain reaction (LCR) assay (Abbott Laboratories, North Chicago, Ill). After the LCR was withdrawn from the market in 2003 and the laboratory’s supply of LCR kits was depleted, male urine specimens and female self-collected vaginal swabs were tested using the COBAS Amplicor polymerase chain reaction (PCR) assay (Roche Diagnostic Systems, Indianapolis, IN). Comparisons of the performance characteristics of the NAATs suggest that the assays are similar for the detection of chlamydial infection in urine and vaginal swab specimens. Positive NAAT results were confirmed by repeating the assay. Infection for *C. trachomatis* as detected by NAAT (N+) was defined as a repeatedly positive test.

At follow-up, index participants with chlamydial infection and their sexual partners provided specimens for multiple NAATs. Repeat NAAT was performed on urine (men throughout the study and women during the initial study period), urethral swabs (men), endocervical swabs (women), and self-administered vaginal swabs. Each index participant thus received three NAATs: one upon initial recruitment in the ED and two repeat tests on follow-up. Sexual partners received two NAATs during their GCRC clinic visit.

Traditional assay for Ct: Traditional testing for *C. trachomatis* was performed at follow-up using culture and direct fluorescent antibody (DFA) test from urethral and endocervical specimens in 2-sucrose phosphate culture transport media obtained from
male and female participants, respectively. Specimens were stored at -80C until testing by the International STD Research Laboratory, Johns Hopkins University. Culture was performed in McCoy cells. Culture-negative specimens were tested using DFA of the sediment from the centrifuged culture transport media; a slide was considered DFA-positive if three or more elementary bodies were present. Culture-positive specimens and DFA-positive/culture-negative specimens were considered positive for Ct by traditional assay (T+). Specimens that were both culture and DFA negative were considered traditional assay negative (T-).

Ct Genotyping

To determine the *C. trachomatis* serovar for organisms detected by NAAT, we amplified variable portions of the *ompA* gene encoding the major outer-membrane protein (MOMP) from a subset of NAAT-positive specimens using previously described primers and sequenced the resulting PCR products on an ABI 3730 analyzer. We compared sequences from clinical samples with *ompA* sequences of 17 *C. trachomatis* serovars in the Gen-Bank database and assigned the serovar of the best match to each specimen.

Statistical Analyses and Outcomes

We used chi-square and t-tests to compare demographic and behavioral characteristics of index participants with Ct-positive NAAT test results who did and did not present for follow-up.

We assessed concordance of infection between sexual partners as a surrogate for transmissibility of NAAT-identified chlamydial infections. We restricted this analysis to
partnerships in which both NAAT and traditional test results were available. We defined concordance as either a positive NAAT (N+) or traditional assay (T+) result for \textit{C. trachomatis} among partners of Ct-positive index participants. All index participants were considered NAAT-positive based on their ED test result.

Our primary hypothesis was that chlamydial infections detectable by NAAT but not by traditional assay (N+T-) in the index participants would be less transmissible to sexual partners than infections that also were detectable by traditional assay (N+T+). Non-concordance was considered as evidence of lower transmissibility. We tested this hypothesis by examining prevalence ratios with 95% confidence intervals (CIs) that compared the proportion of partnerships with concordant Ct infections (N+ and/or T+) between index participants who tested N+T- and those that tested N+T+.

We also examined characteristics of index participants and their partners that may be associated with infection concordance. These variables included age, number of recent sexual partners, new partners within the past 90 days, history of chlamydial infection, recent antibiotic use, reporting of symptoms, time to follow-up (days between index screening in the ED and follow-up, days from index follow-up to partner follow-up, and days between index screening and partner follow-up), and specimen type (female urine vs vaginal swab, LCR vs PCR).

For all partnership analyses, we used generalized estimating equations (GEE) with a log link and binomial error distribution to estimate prevalence ratios for concordance. GEE account for within-group correlation, such as that which exists between multiple partners of the same index participant. Additional analyses of partner concordance using only
single partnerships, e.g., the index and the first enrolled partner, yielded similar results and are not presented.

As a secondary aim, we examined the persistence of NAAT-identified chlamydial infections at follow-up among persons with positive NAAT in the ED. We defined persistent infection as a positive NAAT in one or both specimens (urine or self-administered vaginal swab and clinician administered endocervical/urethral swab) or a positive traditional test (culture or DFA) at follow-up. Factors potentially influencing persistence of infection, including the number of days from initial screening to follow-up, type of NAAT, respondent's gender, age, and previous diagnosis of chlamydial infection (ever and within the past year) were examined in bivariable and multivariable binomial regression analyses. In addition, we examined the association between persistence and concordance of infection within partnerships. All statistical analyses were conducted using Stata version 8 (Stata Corp., College Station, TX).

Role of the funding source

The US National Institutes of Health did not participate in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript. The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Results

Study Recruitment: Ct Screening and Follow-up

Over a 27-month period, 14,188 adult patients attending the JHH-ED were screened for eligibility by 21 trained interviewers. Screening identified 6,952 English-speaking
sexually active 18-35 year old adults eligible for participation (Figure 1). Of these, 6,094 participants (87.7%) consented to chlamydial screening.

Overall, the prevalence of chlamydial infection was 7.0% in the ED participants. The prevalence of Ct in male urine specimens (7%) was comparable as determined by LCR and PCR (8.4% vs 6.6%, \( p > 0.10 \)). Among female urine specimens tested by LCR between November 2002 through August 2003, 6.8% were Ct positive; 7.3% of female swab specimens collected during September 2003 through February 2005 tested positive by PCR (\( p > 0.10 \)).

Of the 419 index participants who tested NAAT positive for chlamydial infection during ED screening, 81 (19.3%) received antibiotic treatment during their ED visit and were not eligible for follow-up. Of the remaining 338 eligible index participants, 166 (49%) returned and enrolled in follow-up. Among those enrolled, the mean number of DIS contacts was 2 (range, 1 to 10) and the average number of days between ED testing and follow-up was 21.5 (range, 8 to 46 days). Participants who did and did not enroll in follow-up were similar based on education, race/ethnicity, marital status, previous STI, and recent sexual behaviors (Table 1). In comparison to participants who did not enroll, participants enrolled in follow-up were slightly younger (mean age, 22.5 vs 23.5 years; \( p = 0.04 \)) and more were female (62% vs 50.6%, \( p = 0.03 \)).

Most index participants enrolled at follow-up (87%) named one or more recent sexual partners; 22 (13%) refused to provide partner information. Of the 175 partners identified, 152 (86.9%) were contacted successfully by the DIS and 102 (58.3%) were enrolled. One hundred partners were examined and treated; two partners refused evaluation and were interviewed at home. Overall, 90 Ct-positive index participants had one or more
sexual partners enrolled; 79 index participants had one partner, 10 indexes had two partners, and 1 index had three partners enrolled. Nearly one-half (48%) of the partnerships enrolled in the study presented for follow-up together.

Partner Concordance of Ct Infections

Our analyses of Ct partnerships are limited to the 83 heterosexual couples (72 index participants with one partner, four indexes with two partners, and one index with three partners) for whom both NAAT and traditional assay results were available. We excluded 17 partnerships because of missing NAAT or traditional assay results for the index subject or their partner(s), inadequate specimen collection, transcription error, or multiple enrollment of a positive index subject. In addition, two exclusively male partnerships were omitted from these analyses as we did not collect anal or throat specimens.

Evidence of transmission of chlamydial infection, as defined by concordance within partnerships, was more common among index cases testing positive by both NAAT and traditional assay (N+T+) than among index cases testing positive by NAAT only (N+T-) (Table 2). Evidence of transmission was observed in 39 of 52 partners (75%) of N+T+ index cases, but only 14 of 31 partners (45%) of N+T- index cases (prevalence ratio 1.7, 95% CI: 1.1, 2.5).

The relationship between test result of the index (N+T- or N+T+) and partner concordance did not vary by gender. Among male partners of N+T+ female indexes, 82% were concordant for Ct, compared to 46% of male partners of N+T- females. Similarly, 70% of female partners of N+T+ index males were concordant, in comparison to 43% of female partners of males testing positive by NAAT only. There was no
difference by the index’s or partner’s age, number of new partners in the past 3 months, history of chlamydial infection, history of any STI, or antibiotic use prior to the follow-up visit. Adjusting for time from screening of the index to follow-up visit also had no substantial effect. However, adjusting for the timing of the partner visit reduced the prevalence ratio to 1.5 (95% CI: 1.03, 2.2). Partners presenting at the same time as the index participant were the most likely to test concordant. Partners presenting after the index participants were less likely to demonstrate concordance (1 – 7 days: prevalence ratio 0.62; 95% CI: 0.41, 0.93; > 7 days: prevalence ratio 0.65; 95% CI: 0.39, 1.07; referent = 0 days).

Limiting traditional test results to culture only decreased our overall estimate of concordance from 64% to 54% (prevalence ratio = 1.4, 95% CI 0.93, 2.0). It is possible that differences in NAAT results by type of NAAT (LCR vs PCR) or specimen type for females (urine vs vaginal swab) could influence our transmission estimates. Although all women screened in the ED provided vaginal swabs for Ct testing using PCR, initially women provided urine specimens, in addition to swabs, for testing by LCR. When the LCR was withdrawn in 2003, male urines and female swabs were tested using PCR. Restricting our concordance analysis to include: 1) only female indexes with positive swab results (n=81 couples, prevalence ratio 1.7, 95% CI 1.1, 2.5) or 2) male and female index subjects with positive PCR results alone (n=75 couples, prevalence ratio 1.7, 95% CI 1.2, 2.5) had no effect on our transmission estimates.

Non-persistence of NAAT Positive Results

Index participants were screened initially in the ED and re-evaluated at follow-up; thus it is possible to examine the short-term persistence of NAAT-identified Ct. Among participants who had not received antibiotic therapy during the ED visit and who had
both NAAT and traditional assay results available for follow-up (n=163; 3 participants had missing traditional assay results), 27 (17%) persons had no evidence of Ct infection by NAAT or culture/DFA. Nine (5%) persons were NAAT-negative, but positive by culture/DFA; the remaining 127 (78%) participants were NAAT-positive (Table 3).

In bivariable analyses, women were significantly more likely than men to test negative for Ct after a short follow-up (23% versus 6%, risk ratio 3.6, 95% CI: 1.3, 9.9). Persons who had used antibiotics in the 3 months prior to their visit in the ED were also more likely to test negative at follow-up (32% versus 12%, risk ratio 2.6, 95% CI: 1.2, 5.5). Index participant’s age, previous chlamydial infection, type of NAAT, and time between ED testing and the follow-up visit were not associated with infection status at follow-up. In multivariable analyses, only gender remained significantly associated with persistence of NAAT-positive results.

Non-persistence and Partner Concordance

We also examined the association between persistence of NAAT-positive results and concordance of infection within partnerships. Within the 83 partnerships, only one partner of 9 (11%) index participants without evidence of Ct infection at follow-up tested positive. In contrast, 52 partners of 74 (70%) index participants with evidence of Ct infection at follow-up tested positive for Ct (prevalence ratio 6.3; 95% CI: 0.98, 40.8).

C. trachomatis typing

To determine whether the nucleic acids amplified by NAAT in concordant partnerships represented infection by the same chlamydial strain, we compared C. trachomatis serovars established from the DNA sequences of variable portions of the ompA gene, encoding the major outer-membrane protein amplified in specimens from NAAT-positive
concordant couples. In 19 of 21 couples with identifiable serovars, chlamydial
genotypes matched exactly. Serovar D was the most commonly identified (30%),
followed by Ia (25%), F (20%), and E (10%). Serovars J (5%), Ja (5%), and K (5%)
each were identified in one couple.

Discussion

NAAT technology provides enhanced sensitivity to detect chlamydial infection and
increased opportunities for chlamydial screening compared to traditional testing
methods. Understanding the clinical and public health implications of the additional
infections identified by NAAT is critical for appropriate use of these tests. Using
concordance as an estimate of transmission, we observed that persons who were
positive by NAAT, but not by traditional assay, were significantly less likely than persons
who were positive by both NAAT and traditional assay to have a concordantly infected
sexual partner. Nonetheless, partner concordance among persons with infections
detected only by NAAT was moderate (45%). In addition, a substantial proportion of
persons (17%-22%) who screened positive by NAAT for Ct in the ED setting had no
evidence of chlamydial infection by NAAT and/or traditional assay after a short follow-up
period (median = 3 weeks).

These results suggest that while many C. trachomatis infections detected by NAAT
persist and are transmissible within sexual partnerships, the significance of some
infections detected by these tests is unclear. This uncertainty derives from the same
factors that are responsible for the advantages of the assay – a low limit of detection and
the ability to detect DNA without viable organisms.
A likely explanation for the diminished partner concordance among infections detectable only by NAAT is reduced organism burden. The enhanced sensitivity of NAAT increases the likelihood of detecting infections with relatively few organisms. Alternatively, NAAT may detect ‘passive presence’ of the organism after exposure, without a true, established infection. Consequently, persons with infections detectable only by NAAT may be inherently less likely to transmit Ct to their partners.

The probability of transmission is influenced by factors other than organism burden, such as frequency of intercourse and previous exposure. Unfortunately, because of ambiguities in partner specification for participants with multiple partners, we did not have precise quantitative information on the frequency of intercourse within partnerships. In our study, previous self-reported chlamydial infection did not influence the relationship between test result of the index and partner concordance.

We observed a surprisingly high incidence of infection clearance, especially among women. Our observed clearance rate (7.8 cases per 1000 person-days) is consistent with an estimated average duration of infection of 128 days, considerably shorter than the commonly cited 365 days. This observation, coupled with our findings regarding reduced concordance among partners of index participants whose follow-up NAAT was negative, suggests that at least some NAAT-identified infections may be cleared relatively rapidly and not transmitted. Some of these may be infections that are close to being resolved, whether through antibiotic exposure or natural immune response.

We observed that many persons without detectable infection at follow-up had been treated with antibiotics in the 3 months preceding their initial screening, suggesting that NAAT may have detected residual DNA, rather than viable organisms, at the initial screening evaluation. A proportion of the screening results could be false positives,
although we re-tested initial positive results to reduce this possibility. Sampling variability associated with repeated testing of individuals with low-level infections \(^{19}\) is another possibility, but appears less likely because of the multiple specimens taken at follow-up. The likelihood of each of these possibilities is not known but is worthy of further clinical investigation.

Since their widespread introduction more than a decade ago, the use of NAAT has “resulted in considerable revision of our views of the clinical epidemiology of \(C. trachomatis\)”. \(^{20}\) Not only has NAAT suggested an increased prevalence of infection in nearly every population tested, but it has also allowed expansion of screening programs to non-clinical settings, thereby enhancing screening services available to men and to asymptomatic individuals. \(^{21}\) Only recently, however, have we begun to question the clinical and public health significance of some additional infections identified by NAAT. \(^{11,22}\) In this study, we examined partner concordance as a marker of the clinical significance of NAAT-identified infections. Further study of the association between asymptomatic NAAT-identified chlamydial infections and other clinical consequences, e.g., incidence of PID, inflammation, is needed.

Very few studies have examined infection transmission within partnerships \(^{23}\). Not only are such studies logistically and technically challenging, but they are expensive to conduct. Our study design was intended to enhance our understanding of the likelihood of transmission of NAAT-diagnosed infection in a sexual partnership – and to do so with minimal risk to our study subjects. Several alternate study designs were considered by our research team, but ultimately rejected. For ethical reasons, we chose not to test index subjects for chlamydia using traditional assay. Only subjects who tested positive for chlamydia by NAAT and their recent sexual partners had urethral or cervical samples
obtained for culture during the clinical examination. We chose an Emergency Department population rather than a STI clinic setting as we were interested in understanding the clinical consequences of largely asymptomatic and unrecognized Ct infections detectable by NAAT but not by traditional methods. As a result, it was necessary to screen over 6,000 individuals. We used concordance as an estimate of transmission. Because we began with a cross-sectional screening evaluation, the timing of the prevalent infection in the index case was unknown. Consequently, the direction of transmission between index and partner could not be established. Generalization of our findings may be constrained by our sample selection – patients recruited from an urban Emergency Department – and by the incomplete recruitment of index participants and their partners. In general, index participants had a similar risk profile to persons who were not recruited into the study. We were unable to compare characteristics of partners who did and did not enroll; however, it seems unlikely that characteristics on which selection might occur would bias enrollment with respect to traditional test status, the basis for our primary hypothesis.

The advent of NAAT screening for chlamydial infection has vastly expanded opportunities for prevention of the serious consequences of untreated infection. However, questions remain regarding the clinical and public health consequences of some infections detected by NAAT. The interpretation of a positive NAAT or any other screening method as an ipso facto indicator of disease can have important personal implications. While failure to diagnose a chlamydial infection can negatively affect health, incorrectly identifying individuals as infected can damage relationships. Because many Ct infections identified by NAAT screening are asymptomatic and easily treated, there has been a tendency to presume that a positive result indicates the presence of disease, with the responsibility to inform and provide treatment. While we strongly
believe that persons with a positive NAAT should be informed and treated, our study
demonstrates that some NAAT-detected infections may not represent clinically active
disease or transmissible infections. Patients, especially those with low risk profiles or
those screened from a low Ct-prevalence population,\textsuperscript{21} should be counseled about their
test result accordingly. Guidelines for testing, patient counseling and management
should be formulated so that users of this remarkable and powerful tool -- physicians,
patients, and researchers -- are aware of not only NAAT's advantages, but also its
limitations.

Contributors: S Rogers, W Miller, C Turner, P Leone, J Ellen, R Rothman, and D Kanouse contributed to the conception and design of the study. S Rogers, W Miller, C Turner, J Ellen, J Zenilman, R Rothman, C Gaydos, M Hobbs contributed to the acquisition of data. S Rogers, W Miller, M Villarroel, A Al-Tayyib, and D Kanouse contributed to questionnaire design. S Rogers, M Villarroel, and L Ganapathi contributed to data management. S Rogers, W Miller, M Villarroel and C Turner contributed to the analysis and interpretation of data. All authors contributed to writing the manuscript.

Conflicts of Interest: We declare that we have no conflicts of interest.

Acknowledgment: We thank Don Orr, Martina Morris, and Heather Miller for serving as scientific advisors to this project. We also thank Sarah Mobius for her managerial contribution to the study and Sheping Li at RTI for programming and data management; Ambreen Khalil and Chadd Krauss for their oversight of interviews at the Johns Hopkins Adult Emergency Department; Joan Bess, Kenya S. Stewart and Nancy Willard of the Johns Hopkins Adolescent Health Research Group for outreach support; and Mary Ann Knott-Grasso, MS, CPNP for providing patient care at the Johns Hopkins General
Clinical Research Center. We also thank the laboratory personnel from the Department of Medicine, University of North Carolina at Chapel Hill, including Marcia Stedman, John Schmitz, and Dana Lapple; and Jeff Younger, Billie Jo Wood, and Hope L. Johnson from the Johns Hopkins School of Medicine, Department of Infectious Diseases.


11 Lysen M, Osterlund A, Rubin CJ, et al. Characterization of ompA genotypes by sequence analysis of DNA from all detected cases of Chlamydia trachomatis infections


13 Gen-Bank Accession numbers AF118868, AY535166, DQ064279, DQ064289, DQ064291, DQ064292, DQ064293, DQ064299, M14738, M17342, M17343, M36533, U78528, X52080, X55700, X62919, X62921.


Figure 1. Subject participation in screening and follow-up, November 2002-February 2005

### CT Screening of Index Subjects

<table>
<thead>
<tr>
<th>Event</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,188 ED patients approached</td>
<td></td>
</tr>
<tr>
<td>6,952 (49%) eligible for participation</td>
<td>858 ineligible</td>
</tr>
<tr>
<td>6,094 (87.7%) received NAAT screening</td>
<td>86 specimens unprocessed</td>
</tr>
<tr>
<td>419 (7.0%) CT+</td>
<td>81 received antibiotic therapy in ED</td>
</tr>
</tbody>
</table>

### Follow-up and Evaluation of CT+ Subjects

<table>
<thead>
<tr>
<th>Event</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>338 CT+ index subjects contacted for follow-up</td>
<td>172 did not participate</td>
</tr>
<tr>
<td>166 CT+ index subjects participated in follow-up</td>
<td></td>
</tr>
</tbody>
</table>

### Partner Evaluation

<table>
<thead>
<tr>
<th>Event</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>175 partners identified</td>
<td></td>
</tr>
<tr>
<td>152 partners contacted by Disease Intervention Specialists</td>
<td>50 partners did not participate</td>
</tr>
<tr>
<td>102 partners of CT+ index subjects participated in follow-up</td>
<td>19 partnerships excluded</td>
</tr>
<tr>
<td>83 partnerships valid NAAT and traditional assay results</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**

- **a** Patients attending the Emergency Department were eligible for Ct screening if they were between 18 and 35 years of age, English-speaking, sexually active in the past 3 months, and a non-Hopkins employee or student. For the first 5 months of data collection, respondents reporting antibiotic use within the past 3 months were excluded. Patients also were ineligible if: they were critically ill or unable to participate due to a physical condition or cognitive impairment, or they had been previously enrolled in the study and tested positive for CT.
- **b** 700 subjects did not consent, 82 completed the ACASI only, 57 subjects were released from the ED prior to completing the study, and 19 patients enrolled twice.
- **c** Subject consented and provided urine or self-administered swab specimen for STI testing using NAAT.
As determined by repeatedly positive NAAT on the same specimen. Initially male and female urine specimens were tested using LCR assay (Abbott Laboratories). After August 2003, male urine and female self-administered vaginal swabs were tested using the COBAS Amplicor assay (Roche Diagnostics). This change was necessitated by the specimen requirements of the Roche Amplicor assay which was used after Abbott Laboratories discontinued the LCR.

CT+ index subjects who received antibiotic therapy during their ED visit were not re-contacted for follow-up.

40 subjects provided insufficient locating information, 44 received healthcare elsewhere, 40 received treatment only at the GCRC, 16 did not show for their scheduled GCRC appointment, and 22 either refused treatment, were incarcerated, a non-resident of Baltimore, or in substance abuse rehabilitation.

Following enrollment, IDs were switched on 2 index subjects’ specimens, 2 indexes were actually partners of 2 previously enrolled Ct-positive index subjects, and 1 subject did not have complete NAAT and traditional assay results.

17 partners were treated elsewhere, 20 were out of jurisdiction or not located, 8 refused treatment, and 5 received treatment and an examination only at the GCRC.

Two partners were enrolled at home and provided specimens for NAAT only. An additional 14 partnerships were missing complete NAAT and traditional assay results, 1 partner was enrolled 6 months after the index, and specimen IDs were mislabeled during collection for 2 partnerships.
Table 1. Characteristics of index subjects positive for *C. trachomatis* who did and did not participate in follow-up

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CT+ Index participated in Follow-up (N=166) $^a$</th>
<th>CT+ Index did not participate in Follow-up (N=172) $^a$</th>
<th>$p$ $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$ (%)</td>
<td>$N$ (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, in years (mean ± SD)</td>
<td>22.5±4.2</td>
<td>23.5±4.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Female</td>
<td>103 (62%)</td>
<td>87 (51%)</td>
<td>0.03</td>
</tr>
<tr>
<td>African American</td>
<td>137 (91%)</td>
<td>132 (88%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Never married</td>
<td>132 (88%)</td>
<td>135 (89%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Completed less than high school</td>
<td>60 (40%)</td>
<td>61 (40%)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Health behaviors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior CT or GC infection</td>
<td>57 (38%)</td>
<td>61 (40%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Dysuria and/or discharge past 3 mos</td>
<td>48 (32%)</td>
<td>38 (25%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Antibiotic use past 3 mos</td>
<td>25 (17%)</td>
<td>29 (19%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Condom use, past 5 sexual acts (mean ± SD)</td>
<td>2.1±2.0</td>
<td>2.1±2.0</td>
<td>0.50</td>
</tr>
<tr>
<td>Illicit drug use past 30 days</td>
<td>79 (53%)</td>
<td>86 (57%)</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Sexual behaviors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+ partners past 3 mos</td>
<td>49 (33%)</td>
<td>62 (41%)</td>
<td>0.15</td>
</tr>
<tr>
<td>New partner past 3 mos</td>
<td>55 (37%)</td>
<td>58 (40%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Age of most recent partner (mean ± SD)</td>
<td>24.2±5.7</td>
<td>25.4±7.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Only heterosexual partners past 2 years</td>
<td>131 (90%)</td>
<td>130 (90%)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

**Notes:**

$^a$ 11% of index patients did not complete ED interview.

$^b$ Estimate for difference in measured characteristic between CT+ index subjects who did and did not participate in follow-up, based on chi-square and t-tests for categorical and continuous outcomes, respectively.
Table 2. Estimate of transmission, as defined by partner concordance, *Chlamydia trachomatis*

<table>
<thead>
<tr>
<th>Partner</th>
<th>Index N+T+ (N=52)</th>
<th>Index N+T- (N=31)</th>
<th>Total (N=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N+T+</td>
<td>18 (35%)</td>
<td>5 (16%)</td>
<td>23 (28%)</td>
</tr>
<tr>
<td>N+T-</td>
<td>7 (13%)</td>
<td>5 (16%)</td>
<td>12 (14%)</td>
</tr>
<tr>
<td>N-T+</td>
<td>14 (27%)</td>
<td>4 (13%)</td>
<td>18 (22%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (75%)</td>
<td>14 (45%)</td>
<td>53 (64%)</td>
</tr>
<tr>
<td>Discordant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-T-</td>
<td>13 (25%)</td>
<td>17 (55%)</td>
<td>30 (36%)</td>
</tr>
</tbody>
</table>

**Prevalence ratio: 1.70 (95% CI, 1.1-2.5)**

**Notes:**
N+, NAAT positive; T+, traditional assay positive; T-, traditional assay negative
Concordant defined as NAAT and/or traditional assay positive for *C. trachomatis*.
Disconcordant defined as NAAT and traditional assay negative for *C. trachomatis*.

Tabulations include all partnerships with valid NAAT and traditional assay results: 72 index subjects with 1 partner, 4 indexes with 2 partners, and 1 index with 3 partners. Fourteen index subjects with N- results at followup (5 N-T+ and 9 N-T-) are considered N+ (based on their ED test result).

Prevalence ratio and 95% CI represent the outcome of partner status of concordant versus discordant by index status and were estimated from GEE logistic model that accounts for lack of independence among index patients with multiple partners.
Table 3. Results of testing for *C. trachomatis* at follow-up among NAAT positive index participants

<table>
<thead>
<tr>
<th>Follow-up test result</th>
<th>NAAT positive index participants</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total N-T-</td>
<td>Male 6%</td>
<td>Female 23%</td>
</tr>
<tr>
<td></td>
<td>Total N-T+</td>
<td>Male 5%</td>
<td>Female 6%</td>
</tr>
<tr>
<td></td>
<td>Total N+T+</td>
<td>Male 57%</td>
<td>Female 47%</td>
</tr>
<tr>
<td></td>
<td>Total N+T-</td>
<td>Male 32%</td>
<td>Female 24%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>63</td>
<td>100</td>
</tr>
</tbody>
</table>

*Fisher's exact p=0.03*

Notes:
N+, NAAT positive; T+ traditional assay positive; N- NAAT negative; T- traditional assay negative