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Enhanced Enzyme Immunoassay with Negative-Gray-Zone Testing Compared to a Single Nucleic Acid Amplification Technique for Community-Based Chlamydial Screening of Men

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We evaluated a low-cost diagnostic strategy for detecting *Chlamydia trachomatis* in a low-prevalence population. We used an amplified enzyme immunoassay (EIA) with a reduced-cutoff "negative gray zone" to identify reactive specimens for confirmation by a nucleic acid amplification test. As part of the Chlamydia Screening Studies project, men provided a first-pass urine specimen, which they returned by post for testing. We tested 1,003 specimens by IDEIA PCE EIA (Dako) and Cobas PCR (Roche). There were 32 (3.2%) true positive specimens according to a combined standard using an algorithm requiring concordant results from at least two independent tests. All of these were positive by Cobas PCR and 24 were confirmed to be positive by PCE EIA, including 2 that gave results in the negative gray zone. There were 971 true negative specimens, 2 of which were positive by Cobas PCR and 19 of which were initially inhibitory for PCR. The relative sensitivity, specificity, positive predictive value, and negative predictive value of PCE EIA with PCR confirmation were 75.0% (95% confidence interval [CI], 56.6 to 88.5%), 100% (95% CI, 99.7 to 100%), 100% (95% CI, 88.3 to 100%), and 99.2% (95% CI, 98.4 to 99.6%), respectively. The corresponding values for Cobas PCR were 100% (95% CI, 89.1 to 100%), 99.8% (95% CI, 99.3 to 100%), 94.1% (95% CI, 76.9 to 98.2%), and 100% (95% CI, 99.6 to 100%), respectively, with 1.9% (19/1003) of the samples being initially indeterminate. When the prevalence of *C. trachomatis* is low, the use of an amplified EIA on urine specimens, with confirmation of results in the negative gray zone by use of a nucleic acid amplification technique, is not suitable for screening asymptomatic men. In addition, positive nucleic acid amplification test results should be confirmed and an inhibition control should be used.

Chlamydia trachomatis is the most common bacterial sexually transmitted infection in the developed world (4, 22). It is associated with significant morbidity, particularly for women, who are at risk of developing pelvic inflammatory disease and consequent tubal factor infertility (15). Population-based surveys in Europe have found prevalence rates of asymptomatic chlamydial infections in men under 25 years of 2.2 to 5.7%, similar to those for women of the same age (1, 10, 17, 34). The importance of including men in screening programs to control chlamydial infection is therefore being increasingly acknowledged (13). In addition, it has been argued that screening that excludes men could have negative social and psychological consequences for women and could further reduce men's responsibility for sexual and reproductive health (9).

There is intense debate about the most useful test for use in population screening programs, since the prevalence of chlamydia is likely to be lower than that in sexually transmitted disease clinics and since infected people are more likely to be asymptomatic with potentially lower chlamydia loads (11). It is generally agreed that urine is the most acceptable specimen type for men and that nucleic acid amplification tests (NAATs) are more sensitive than enzyme immunoassays (EIAs) for detecting *C. trachomatis* (7, 15). However, the high costs of NAATs have hindered their widespread use in countries such as the United Kingdom, although they have been shown in modeling studies to be cost-effective in the medium to long term (15, 35). Enhanced antigen detection tests such as IDEIA PCE EIA (Dako, Ely, Cambridgeshire, United Kingdom), which includes an amplification step to improve sensitivity (6, 21), have been suggested as alternative tests in the United Kingdom when NAATs are unavailable (7). Another way to improve sensitivity at a low cost is to use an EIA to screen all samples and to retest specimens with optical densities just below the cutoff point for a negative test (negative gray zone) by using a NAAT (7, 15). The only published studies to have directly compared the PCE EIA with a NAAT for male urine

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samples have shown conflicting results for men with urethritis (26, 29).

The objective of this study was to compare the performance characteristics of an amplified EIA with those of a NAAT to detect *C. trachomatis* in male urine specimens in a pragmatic study in a low-prevalence community setting. We also wanted to determine whether retesting by NAAT of specimens with optical density readings in the negative gray zone increased the sensitivity of this approach.

MATERIALS AND METHODS

Study population. The ClaSS project involved a population-based survey of the prevalence of genital chlamydial infections. The sampling strategy has been described in detail elsewhere (17). Briefly, a random sample of men and women aged 16 to 39 years from selected family practices in Bristol and Birmingham, England, were invited to participate by post. Men were asked to collect a first-pass urine specimen in a 25-ml universal container at home and to complete a brief questionnaire recording demographic and sexual behavioral details and the time and date of specimen collection. They sent specimens, triple wrapped in protective packaging, back to the study laboratories in first-class, prepaid envelopes. Only men from the Bristol site were involved in this part of the study.

Laboratory methods. The urine specimens were tested by both PCE EIA and the Cobas Amplicor CT test (Roche Diagnostics, Basel, Switzerland). Tests were performed according to the manufacturer's instructions, except that specimens with initially inhibitory results by Cobas PCR were retested with a second NAAT rather than with Cobas PCR. Detailed protocols for the handling, processing, and evaluation of the diagnostic tests can be found at http://www.chlamydia.ac.uk/evaldiag.htm (laboratory protocol, sections 6 and 10). Briefly, upon arrival at the laboratory, each specimen was divided into three aliquots as follows: 15 ml for PCE EIA, 1 ml for Cobas PCR, and the rest for storage at 4°C until the results of the tests were known. If a sample was inhibitory in Cobas PCR, it was retested by either Becton Dickinson ProbeTec ET DNA strand displacement amplification (SDA; Becton Dickinson Company, Franklin Lakes, N.J.) or real-time PCR (see below).

Negative-gray-zone testing by PCE EIA. The cutoff value for the PCE EIA assay was calculated by adding 0.05 absorbance units to the mean value of the negative control values. A sample was regarded as being in the negative gray zone if it fell within 0.025 absorbance units (50%) below the calculated cutoff (7, 8).

Confirmatory testing. A second NAAT was used to retest specimens that were inhibitory in Cobas PCR or for which the results of PCE EIA and Cobas PCR were discrepant (Table 1). The Becton Dickinson SDA assay or an in-house NAAT was used. The in-house confirmation NAAT was a real-time PCR test utilizing a LightCycler instrument (Idaho Instruments, Idaho). The use of this test as a confirmatory assay has been previously described (36). Both real-time assays used for this test have a detection limit of approximately 10 genome copies (36).

In brief, the method was as follows. Nucleic acids were prepared from urine pellets from 1 ml of first-pass urine by one of two silica binding techniques (2). Early in the study, a QIAamp viral RNA extraction kit (QIAGEN Ltd., Crawley, Sussex, United Kingdom) was used (23). The columns were eluted with 100 μl distilled H_2O. For later analyses of discrepant results, binding to MagPrep magnetic silica particles (Merck BDH, Poole, Dorset, United Kingdom) was used. Specimens were dissolved in guanidinium isothiocyanate-containing L6 buffer (2), and 10 μl of beads was added. After incubation with shaking at room temperature for 15 min, the beads were washed three times after being immobilized with a magnet, using 20 mM Tris-Cl, pH 7.4, 1 mM EDTA, 100 mM NaCl mixed with an equal volume of 100% ethanol. The beads were then dried at room temperature and eluted in 50 μl 10 mM Tris, pH 8.0, for 10 min at 80°C. Column or bead eluates (4 μl) were assayed in 10-μl reactions in a LightCycler instrument. The confirmatory PCR was performed by using the following primers directed at the major outer membrane protein gene: MOMPfp2, 5′-CCAGAAAAAGATAGCGAGCACAAA-3′; and MOMPrp1, 5′-AGCAGAACTCAAAGCGGCAAAT-3′.

A plasmid-based PCR adapted from the work of Loeffelholz et al. (16) used the same primers as the Roche Cobas PCR assay. This assay was only used to resolve samples that were inhibitory in Roche Cobas PCR. For both assays, 50 cycles of PCR were performed on the LightCycler in the presence of the SYBR green I intercalating dye. Only those specimens that gave a clear peak in a melting point analysis of the PCR product, with a melting point within 0.2°C of that of the positive control, were considered positive.

TABLE 1. Algorithm for determining results of tests for *C. trachomatis*[a]

PCE EIA result[b]	Cobas PCR result	Comment[c]	Final report
+	+		True positive
NGZ	+		True positive
−	+	If confirmatory NAAT on 2nd aliquot is positive	True positive
+	−	If confirmatory NAAT on 2nd aliquot is positive	True positive
NGZ	−	If confirmatory NAAT on 2nd aliquot is positive	True positive
−	+	If confirmatory NAAT on 2nd aliquot is negative	True negative
+	−[d]	If confirmatory NAAT on 2nd aliquot is negative	True negative
NGZ	−[d]	If confirmatory NAAT on 2nd aliquot is negative	True negative
−	−[d]		True negative

[a] Adapted from section 10 of the Chlamydia Screening Studies Laboratory Protocol (www.chlamydia.ac.uk/evaldiag.htm) with permission.
[b] NGZ, negative gray zone.
[c] Original urine was tested by confirmatory NAAT if the volume allowed it (1 ml was required for real-time PCR). Otherwise, the residual urine was used.
[d] Internal control used to detect inhibitory specimens. Inhibitory specimens were retested by using a second NAAT (see Materials and Methods).

Diagnostic strategies. All specimens were tested by both tests, which used biologically independent methods. True positive and negative results were determined according to a testing algorithm that required concordant results from two tests, with a second NAAT used to resolve the results of discordant specimens (Table 1), and these were the final results that were issued to patients.

PCE EIA testing strategy. All specimens that were reactive by PCE EIA (absorbance value above the cutoff or within the negative gray zone) required repeat testing and confirmation by a NAAT for the result to be considered positive. The PCE EIA result was considered negative if the absorbance value was below the negative gray zone or within the negative gray zone and negative by a NAAT.

Cobas PCR testing strategy. A reactive Cobas PCR result was considered positive if it was confirmed by either a reactive PCE EIA or another NAAT result. The result of the Cobas PCR was considered negative if the PCE EIA was negative, without the need for a confirmatory real-time PCR test. Cobas PCR specimens that were initially inhibitory were retested by a second NAAT. Cobas PCR-inhibitory specimens were considered positive if the confirmatory NAAT was positive and were considered negative if the confirmatory NAAT was negative.

We calculated the sensitivity, specificity, and predictive value, with confidence intervals (CI), for each testing strategy relative to the combined standard defining true positive and negative results.

RESULTS

One thousand thirteen men returned first-pass urine specimens by post. Of these specimens, results for both PCE EIA and Cobas PCR were available for 1,003. Thirty-two specimens (3.2%) were classified as true chlamydia-positive specimens and 971 were classified as true negative specimens.

Twenty-seven samples were initially PCE EIA reactive, and 22 were confirmed as positive by NAAT. Six samples were in the negative gray zone, and two were confirmed as positive by Cobas PCR. PCE EIA with negative-gray-zone testing and confirmation by Cobas PCR therefore identified 24/32 true positive specimens (relative sensitivity, 75.0%; 95% CI, 56.6 to 88.5%) (Table 2). Without negative-gray-zone testing, the relative sensitivity of PCE EIA was 68.8% (95% CI, 50.0 to

TABLE 2. Performance of PCE EIA for detection of *C. trachomatis*, with negative-gray-zone testing and confirmation by NAAT, in first-pass urine specimens from men[c]

EIA result	No. of true positive specimens	No. of true negative specimens	Total
Confirmed positive[a]	22	0	22
Gray zone, confirmed positive[b]	2	0	2
Negative	8	971	979
Total	32	971	1,003

[a] There were 27 initially reactive samples by PCE EIA. Five specimens were negative by Cobas PCR. Of these, four were confirmed to be negative by a second NAAT and one was negative upon repeat EIA testing.

[b] There were six PCE EIA results in the negative gray zone. The four true negative specimens were confirmed to be negative by a second NAAT on two specimens, and two were negative by EIA upon repeat testing.

[c] The relative sensitivity, calculated by including gray-zone-positive specimens in the positive EIA results, was 75.0% (95% CI, 56.6 to 88.5%). The specificity, calculated by including unconfirmed positive EIA results and unconfirmed gray-zone-positive specimens in negative EIA results, was 100% (95% CI, 99.7 to 100%). The positive predictive value was 100% (95% CI, 88.3 to 100%), and the negative predictive value was 99.2% (95% CI, 98.4 to 99.6%).

83.9%). There were 962 negative specimens by PCE EIA, and a further five initially reactive specimens and 4 specimens in the negative gray zone were confirmed as negative by subsequent NAATs. The specificity of the PCE EIA with confirmatory NAAT testing was therefore 100% (95% CI, 99.7 to 100%). Without NAAT testing of initially reactive specimens, the specificity of the PCE EIA was 99.5% (966/971; 95% CI, 98.8 to 99.8%).

Cobas PCR identified 34 positive specimens, including all 32 true positive specimens (relative sensitivity, 100%; 95% CI, 89.1 to 100%). The other two (5.9%) positive specimens by Cobas PCR were negative by PCE EIA and real-time PCR, giving a specificity of 99.8% (95% CI, 99.3 to 100%) (Table 3). Nineteen (1.9%) samples were initially inhibitory in Cobas PCR. All were negative by PCE EIA and were confirmed to be negative when retested by either SDA or real-time PCR. The estimate of specificity was unchanged if inhibitory specimens were excluded from the calculations (Table 4) or if we assumed that no inhibition control had been used and inhibitory specimens were classified as negative (data not shown).

Fifty-seven percent of specimens were tested within 48 h of sample collection. Increasing the time from collection to testing did not affect the positivity rate (the odds ratio for specimens collected more than 96 h before testing compared to those collected less than 48 h before testing was 1.0; 95% CI, 0.4 to 2.3).

TABLE 3. Results of the Cobas Amplicor CT test for detection of *C. trachomatis* in first-pass urine specimens from men[a]

Cobas PCR result	No. of true positive specimens	No. of true negative specimens	Total
Positive	32	2	34
Negative	0	950	950
Inhibitory	0	19	19
Total	32	971	1,003

[a] The values presented in this table were used to analyze the performance of the test, reflected in the values presented in Table 4.

DISCUSSION

For the low-prevalence, largely asymptomatic population used for this study, the relative sensitivity of the PCE EIA on male urine samples, with negative-gray-zone testing and PCR confirmation, was 75.0% (95% CI, 56.6 to 88.5%). Negative-gray-zone testing with NAAT confirmation increased the relative sensitivity of the PCE EIA alone by 6%. The Cobas PCR test identified all specimens with true positive results (relative sensitivity, 100%; 95% CI, 89.1 to 100%). However, approximately 1 in 15 positive results with the Cobas PCR was a false-positive result, and 19 (1.9%) of 1,003 first-pass urine specimens were inhibitory for PCR. The specificities of both tests were >99.5%.

Methodological issues. The main strength of this study is that it involved a random sample of >1,000 men from the general population, the majority of whom were asymptomatic. It therefore simulates the conditions of screening of a low-prevalence population. The study was designed to evaluate low-cost diagnostic strategies for low-prevalence populations. Mailed urine specimens were used for this study. Urine specimens perform as well as urethral swabs from men for the detection of *C. trachomatis* by use of a NAAT (25, 31–33). Although postal delays have been suggested to reduce the chance of detecting *C. trachomatis* (3), with this study we found no evidence of a deterioration in test performance for delays exceeding 96 h.

Our results need to be considered in the context of discussions about the evaluation of tests for which no perfect gold standard exists (12, 20). We calculated sensitivities and specificities relative to a standard incorporating the results of both EIA and PCR tests and a second NAAT, which was used to test discordant specimens. We intentionally used repeat testing with NAATs in our diagnostic strategy for the PCE EIA to reclassify false-negative results and indeterminate results in the negative gray zone to maximize performance. This resulted in an increase in relative sensitivity of 6% and in specificity of 0.5%. As recommended, we have also reported the results before discrepancy analysis (19). Although all negative specimens by Cobas PCR were also negative by the PCE EIA, our estimate of the relative sensitivity of the Cobas PCR test might be biased since a single NAAT on a single specimen cannot identify all infections (6, 14, 15, 32), and further testing of all negative specimens with independent NAATs might have identified additional infections. The evidence suggests that approximately 10% of positive specimens may be missed (14, 25, 32, 33) by the strategy used in this study. If further testing identified 10% more positive specimens, then the estimated sensitivity of Cobas PCR would be 32/35 (91.4%; 95% CI, 76.9 to 98.2%).

In the original study design, both Becton Dickinson SDA and real-time PCR could be used as independent NAATs to confirm positive results and to resolve discrepant results (http://www.chlamydia.ac.uk/evaldiag.htm; laboratory protocol, pages 9 and 33). This study was conducted in a busy routine laboratory, and the Becton Dickinson SDA, which was already in routine use, was the test of choice. During the study,

TABLE 4. Performance of the Cobas Amplicor CT test for detecting *C. trachomatis*[a]

Inhibitory specimens	No. of specimens	Relative sensitivity[d]	Specificity[d]	Positivepredictive value[d]	Negative predictive value[d]
Included[b]	1,003	100 (89.1–100)	99.8 (99.3–100)	94.1 (76.9–98.2)	100 (99.6–100)
Excluded[c]	984	100 (91.1–100)	99.8 (99.2–100)	94.1 (76.9–98.2)	100 (99.6–100)

[a] The test results appear in Table 3.
[b] Calculated values include results for specimens for which an inhibitory control was used; when necessary, the true result was resolved by a second NAAT.
[c] Calculated values exclude results for 19 inhibitory specimens.
[d] Values are percentages. Values in parentheses are 95% confidence intervals.

however, the Becton Dickinson SDA assay developed technical problems, resulting in positive results which were not repeatable upon retesting. In addition, occasionally the kit's negative controls were positive. This problem was investigated by Becton Dickinson and was observed by other sites in the United Kingdom, where it subsequently resolved itself.

Comparison between PCE EIA and NAAT results for specimens from men. The PCE EIA is a dually amplified EIA since it incorporates a polymer conjugate to increase the sensitivity compared to that of an earlier version of the same assay (IDEIA) (27). In clinical settings, this test has been found to have relative sensitivities of 91.8% (95% CI, 80.4 to 97.7%) with endocervical swabs (5) and 91.4% (95% CI, 81.1 to 97.1%) for men with urethritis compared to a NAAT (26). A second study of men with urethritis, however, found a relative sensitivity of 53% (no confidence intervals were provided) (29). Our study of a low-prevalence population estimated an intermediate level of relative sensitivity of 75.0%.

Asymptomatic men are suggested to have chlamydial loads that are approximately half those of men with clinical urethritis (11). The detection limit of the PCE EIA for urethritis-associated serovars is about half that of the previous assay (21). It has been argued that even with the gain in sensitivity, it is still about 2 log above the detection limit of NAATs (28). This might explain the lower sensitivity of the assay in our study than that obtained by Tanaka et al. (26), but it does not explain the even lower sensitivity found by Templeton et al. (29). However, a direct comparison of the detection limits of NAATs and EIA in vitro is difficult, as lipopolysaccharide, the target antigen for EIAs, is present in both structural (elementary body associated) and nonstructural (not associated with elementary bodies) forms. Thus, in vitro studies using purified elementary bodies will contain less target antigen per elementary body than would occur in vivo: this might underestimate the analytical sensitivities of EIAs.

If PCE EIA absorbance values are associated with the chlamydial load, then in a low-prevalence, largely asymptomatic population the absorbance readings would be expected to be towards the lower end of the distribution, and many values might fall close to the cutoff value for a negative test. Previous studies with conventional EIAs have found that retesting specimens with results in the negative gray zone by using a NAAT increased the sensitivity 5 to 30% (8, 30). In our study, only six specimens gave results in the negative gray zone, and further NAAT testing of these specimens increased the relative sensitivity 6%, which is at the lower end of this range.

Implications for screening. The United Kingdom National Clinical Effectiveness Guideline for *C. trachomatis* recommends that EIAs should not be used in situations in which their sensitivity is <80% (7). In our study, the sensitivity of a strategy to optimize PCE EIA performance by using negative-gray-zone testing with confirmation by a NAAT was below this level, although the upper confidence interval included this value. In a population with a prevalence of 3.2%, however, a study sample of >9,000 men would have been required to exclude a sensitivity of 80% with 95% confidence. Our findings suggest that even the best-performing EIA is not suitable for chlamydia screening of men in the community.

Zenilman et al. have highlighted the potentially serious implications for individuals and their partners of using a single NAAT for chlamydia testing (37). Mallinson et al. also observed reproducibility problems with the ligase chain reaction (now withdrawn) and Cobas PCR: they were only able to confirm 237 (84%) of 282 initially positive (by Roche PCR) urine samples (18). The Centers for Disease Control and Prevention advise that when the positive predictive value of a test is <90%, repeat testing should be undertaken, preferably with a different NAAT (15). Because specimens with low levels of target DNA are known to give nonrepeatable results for NAATs (24), they also advise that therapy should be offered to all patients with reactive NAAT results which were not confirmed upon repeat testing (15) but that individuals should then also be informed of the possibility of errors and offered repeat testing (37). In our study, the Cobas PCR identified two false-positive results. One was retested by SDA and the other was retested twice by Cobas PCR (before real-time PCR was introduced), and all repeat tests were negative. Our findings of false-positive PCR and unreproducible SDA results support the advice that chlamydia-positive NAAT results should be confirmed.

The presence of amplification inhibitors in clinical specimens, which can result in false-negative results, is also a recognized potential disadvantage of NAATs (15). Only 19 samples (1.9%) in our study with the Cobas PCR assay were inhibitory, and they were all subsequently found to be negative for chlamydia. Nevertheless, it is likely that with the large number of specimens obtained in clinical practice, some chlamydia-positive specimens would be missed if an inhibition control were not used.

The cost-effectiveness of PCE EIA and Cobas PCR will be formally compared by the ClaSS project (17) to see if savings due to cheaper diagnostic tests are outweighed by the costs of undiagnosed infections in terms of reproductive tract morbidity and ongoing transmission of *C. trachomatis*.

Conclusions. We found evidence that the relative sensitivity of an amplified EIA with additional negative-gray-zone testing and NAAT confirmation was lower than that of PCR for screening urine samples from asymptomatic men in a low-

prevalence community setting for *C. trachomatis*. A testing strategy that used a nucleic acid amplification test incorporating an inhibition control and confirmation of initially positive results provided a reliable method for screening in this setting.

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REFERENCES

1. **Andersen, B., F. Olesen, J. K. Moller, and L. Ostergaard.** 2002. Population-based strategies for outreach screening of urogenital *Chlamydia trachomatis* infections: a randomized, controlled trial. J. Infect. Dis. **185:**252–258.
2. **Boom, R., C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
3. **Caul, E. O., P. J. Horner, J. Leece, T. Crowley, L. Paul, and G. Davey Smith.** 1997. Population-based screening programmes for *Chlamydia trachomatis.* Lancet **349:**1070–1071.
4. **Centers for Disease Control and Prevention.** 1997. *Chlamydia trachomatis* genital infections—United States, 1995. Morb. Mortal. Wkly. Rep. **46:**193–198.
5. **Chernesky, M., D. Jang, D. Copes, J. Patel, A. Petrich, K. Biers, A. Sproston, and J. Kapala.** 2001. Comparison of a polymer conjugate-enhanced enzyme immunoassay to ligase chain reaction for diagnosis of *Chlamydia trachomatis* in endocervical swabs. J. Clin. Microbiol. **39:**2306–2307.
6. **Chernesky, M. A.** 2002. *Chlamydia trachomatis* diagnostics. Sex. Transm. Infect. **78:**232–234.
7. **Clinical Effectiveness Group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases).** 1999. National guideline for the management of *Chlamydia trachomatis* genital tract infection. Sex. Transm. Infect. **75**(Suppl. 1)**:**S4–S8.
8. **Dean, D., D. Ferrero, and M. McCarthy.** 1998. Comparison of performance and cost-effectiveness of direct fluorescent-antibody, ligase chain reaction, and PCR assays for verification of chlamydial enzyme immunoassay results for populations with a low to moderate prevalence of *Chlamydia trachomatis* infection. J. Clin. Microbiol. **36:**94–99.
9. **Duncan, B., and G. Hart.** 1999. Sexuality and health: the hidden costs of screening for *Chlamydia trachomatis.* BMJ **318:**931–933.
10. **Fenton, K. A., C. Korovessis, A. M. Johnson, A. McCadden, S. McManus, K. Wellings, C. H. Mercer, C. Carder, A. J. Copas, K. Nanchahal, W. Macdowall, G. Ridgway, J. Field, and B. Erens.** 2001. Sexual behaviour in Britain: reported sexually transmitted infections and prevalent genital *Chlamydia trachomatis* infection. Lancet **358:**1851–1854. (Erratum, **359:**174, 2002.)
11. **Geisler, W. M., R. J. Suchland, W. L. Whittington, and W. E. Stamm.** 2001. Quantitative culture of *Chlamydia trachomatis*: relationship of inclusion-forming units produced in culture to clinical manifestations and acute inflammation in urogenital disease. J. Infect. Dis. **184:**1350–1354.
12. **Hadgu, A.** 1997. Bias in the evaluation of DNA-amplification tests for detecting *Chlamydia trachomatis.* Stat. Med. **16:**1391–1399.
13. **Hart, G. J., B. Duncan, and K. A. Fenton.** 2002. Chlamydia screening and sexual health. Sex. Transm. Infect. **78:**396–397.
14. **Johnson, R. E., T. A. Green, J. Schachter, R. B. Jones, E. W. Hook III, C. M. Black, D. H. Martin, M. E. St. Louis, and W. E. Stamm.** 2000. Evaluation of nucleic acid amplification tests as reference tests for *Chlamydia trachomatis* infections in asymptomatic men. J. Clin. Microbiol. **38:**4382–4386.
15. **Johnson, R. E., W. J. Newhall, J. R. Papp, J. S. Knapp, C. M. Black, T. L. Gift, R. Steece, L. E. Markowitz, O. J. Devine, C. M. Walsh, S. Wang, D. C. Gunter, K. L. Irwin, S. DeLisle, and S. M. Berman.** 2002. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections—2002. Morb. Mortal. Wkly. Rep. **51:**1–38.
16. **Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon.** 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. J. Clin. Microbiol. **30:**2847–2851.
17. **Low, N., A. McCarthy, J. MacLeod, C. Salisbury, P. J. Horner, T. E. Roberts, R. Campbell, A. Herring, S. Skidmore, E. Sanford, J. A. C. Sterne, G. Davey Smith, A. Graham, M. Huengsberg, J. Ross, and M. Egger for the Chlamydia Screening Studies Group.** 2004. The chlamydia screening studies: rationale and design. Sex. Transm. Infect. **80:**342–348.
18. **Mallinson, H., J. Hopwood, and K. Mutton.** 2002. Resolution of the recent performance problem of Abbott LCx *Chlamydia trachomatis* assay. Issues of repeat testing for confirmation of chlamydial infection. Sex. Transm. Infect. **78:**225–226.
19. **McAdam, A. J.** 2000. Discrepant analysis: how can we test a test? J. Clin. Microbiol. **38:**2027–2029.
20. **Miller, W. C.** 1998. Bias in discrepant analysis: when two wrongs don't make a right. J. Clin. Epidemiol. **51:**219–231.
21. **Okadome, A., T. Notomi, S. Nomura, and A. Nagayama.** 1999. Reactivity of a dual amplified chlamydia immunoassay with different serovars of *Chlamydia trachomatis.* Int. J. STD AIDS **10:**460–463.
22. **PHLS, DHSS&PS, and Scottish ISD(D)5 Collaborative Group.** 2004. Sexually transmitted infections in the UK: new episodes seen in genitourinary medicine clinics, 1995–2000. Public Health Laboratory Service, London, United Kingdom.
23. **Shattock, R. M., C. Patrizio, P. Simmonds, and S. Sutherland.** 1998. Detection of *Chlamydia trachomatis* in genital swabs: comparison of commercial and in house amplification methods with culture. Sex. Transm. Infect. **74:**289–293.
24. **Smieja, M., J. B. Mahony, C. H. Goldsmith, S. Chong, A. Petrich, and M. Chernesky.** 2001. Replicate PCR testing and probit analysis for detection and quantitation of *Chlamydia pneumoniae* in clinical specimens. J. Clin. Microbiol. **39:**1796–1801.
25. **Sugunendran, H., H. D. Birley, H. Mallinson, M. Abbott, and C. Y. Tong.** 2001. Comparison of urine, first and second endourethral swabs for PCR based detection of genital *Chlamydia trachomatis* infection in male patients. Sex. Transm. Infect. **77:**423–426.
26. **Tanaka, M., H. Nakayama, K. Sagiyama, M. Haraoka, H. Yoshida, T. Hagiwara, K. Akazawa, and S. Naito.** 2000. Evaluation of a new amplified enzyme immunoassay (EIA) for the detection of *Chlamydia trachomatis* in male urine, female endocervical swab, and patient obtained vaginal swab specimens. J. Clin. Pathol. **53:**350–354.
27. **Tanaka, M., H. Nakayama, H. Yoshida, K. Takahashi, T. Nagafuji, T. Hagiwara, and J. Kumazawa.** 1998. Detection of *Chlamydia trachomatis* in vaginal specimens from female commercial sex workers using a new improved enzyme immunoassay. Sex. Transm. Infect. **74:**435–438.
28. **Taylor-Robinson, D., and B. J. Thomas.** 2000. Sensitivity of chlamydial diagnostic tests. Int. J. STD AIDS **11:**204–205.
29. **Templeton, K., J. Roberts, D. Jeffries, G. Forster, and C. Aitken.** 2001. The detection of *Chlamydia trachomatis* by DNA amplification methods in urine samples from men with urethritis. Int. J. STD AIDS **12:**793–796.
30. **Tong, C. Y., C. Donnelly, and N. Hood.** 1997. Lowering the cut off value of an automated chlamydia enzyme immunoassay and confirmation by PCR and direct immunofluorescent antibody test. J. Clin. Pathol. **50:**681–685.
31. **Toye, B., R. W. Peeling, P. Jessamine, P. Claman, and I. Gemmill.** 1996. Diagnosis of *Chlamydia trachomatis* infections in asymptomatic men and women by PCR assay. J. Clin. Microbiol. **34:**1396–1400.
32. **Van Der Pol, B., D. V. Ferrero, L. Buck-Barrington, E. Hook III, C. Lenderman, T. Quinn, C. A. Gaydos, J. Lovchik, J. Schachter, J. Moncada, G. Hall, M. J. Tuohy, and R. B. Jones.** 2001. Multicenter evaluation of the BDProbeTec ET system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. J. Clin. Microbiol. **39:**1008–1016.
33. **Van Der Pol, B., T. C. Quinn, C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Jungkind, D. H. Martin, B. Turner, C. Peyton, and R. B. Jones.** 2000. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis.* J. Clin. Microbiol. **38:**1105–1112.
34. **van Valkengoed, I. G., A. J. Boeke, A. J. van den Brule, S. A. Morre, J. H. Dekker, C. J. Meijer, and J. T. van Eijk.** 1999. Systematic home screening for *Chlamydia trachomatis* infections of asymptomatic men and women in family practice by means of mail-in urine samples. Ned. Tijdsch. Geneeskd. **143:**672–676.
35. **Watson, E. J., A. Templeton, I. Russell, J. Paavonen, P. A. Mardh, A. Stary, and B. S. Pederson.** 2002. The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. J. Med. Microbiol. **51:**1021–1031.
36. **Winter, A. J., G. Gilleran, K. Eastick, and J. D. Ross.** 2000. Comparison of a ligase chain reaction-based assay and cell culture for detection of pharyngeal carriage of *Chlamydia trachomatis.* J. Clin. Microbiol. **38:**3502–3504.
37. **Zenilman, J. M., W. C. Miller, C. Gaydos, S. M. Rogers, and C. F. Turner.** 2003. LCR testing for gonorrhoea and chlamydia in population surveys and other screenings of low prevalence populations: coping with decreased positive predictive value. Sex. Transm. Infect. **79:**94–97.