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Vulvovaginal-Swab or First-Catch Urine Specimen To Detect *Chlamydia trachomatis* in Women in a Community Setting?[▽]

Sue Skidmore,[1] Paddy Horner,[2,3] Alan Herring,[4]† Joanne Sell,[5] Ian Paul,[5] Jane Thomas,[1] E. Owen Caul,[4]† Matthias Egger,[6,7] Anne McCarthy,[6] Emma Sanford,[6] Chris Salisbury,[8] John Macleod,[9] Jonathan A. C. Sterne,[6] and Nicola Low[6,7]* for the Chlamydia Screening Studies (ClaSS) Project Group‡

Public Health Laboratory Service, Birmingham Laboratory, Birmingham B9 5SS, United Kingdom[1]; Department of Pathology and Microbiology, University of Bristol, Bristol, United Kingdom[2]; The Milne Centre, United Bristol Healthcare NHS Trust, Bristol BS2 8HW, United Kingdom[3]; Public Health Laboratory Service Bristol Laboratory, Bristol BS2 8EL, United Kingdom[4]; Health Protection Agency, Myrtle Road, Bristol BS2 8EL, United Kingdom[5]; Department of Social Medicine, University of Bristol, Bristol BS8 2PR, United Kingdom[6]; Department of Social and Preventive Medicine, University of Bern, Bern CH-3012, Switzerland[7]; Department of Community-Based Medicine, University of Bristol, Bristol BS6 6JL, United Kingdom[8]; and Department of Primary Care, University of Birmingham, Birmingham B15 2TT, United Kingdom[9]

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Screening for chlamydia in women is widely recommended. We evaluated the performance of two nucleic acid amplification tests for detecting *Chlamydia trachomatis* in self-collected vulvovaginal-swab and first-catch urine specimens from women in a community setting and a strategy for optimizing the sensitivity of an amplified enzyme immunoassay on vulvovaginal-swab specimens. We tested 2,745 paired vulvovaginal-swab and urine specimens by PCR (Roche Cobas) or strand displacement amplification (SDA; Becton Dickinson). There were 146 women infected with chlamydia. The assays detected 97.3% (95% confidence interval [CI], 93.1 to 99.2%) of infected patients with vulvovaginal-swab specimens and 91.8% (86.1 to 95.7%) with urine specimens. We tested 2,749 vulvovaginal-swab specimens with both a nucleic acid amplification test and a polymer conjugate-enhanced enzyme immunoassay with negative-gray-zone testing. The relative sensitivities obtained after retesting specimens in the negative gray zone were 74.3% (95% CI, 62.8 to 83.8%) with PCR and 58.3% (95% CI, 46.1 to 69.8%) with SDA. In community settings, both vulvovaginal-swab and first-catch urine specimens from women are suitable substrates for nucleic acid amplification tests, but enzyme immunoassays, even after negative-gray-zone testing, should not be used in screening programs.

Screening for *Chlamydia trachomatis* in sexually active young adults is recommended as an essential component of infection control for genital chlamydial infections (10, 11), which are the most commonly reported bacterial sexually transmitted infections in the United Kingdom and United States. The increasing availability of nucleic acid amplification tests for *C. trachomatis* diagnosis has facilitated screening by use of urine and, for women, vulval or vaginal specimens. Many studies have compared the sensitivities obtained with first-catch urine and vulvovaginal-swab specimens from women, and some have found a slight advantage for swabs (2, 14, 24, 25, 33). Using the Amplicor PCR (Roche Diagnostics, Basel), the ligase chain reaction (Abbott Laboratories), and transcription-mediated amplification (APTIMA, Gene-Probe Inc., San Diego, CA) on first-catch urine specimens, Schachter et al. observed a combined sensitivity of 81%, compared to 93% for vulvovaginal-swab specimens and 91% for endocervical-swab specimens (25). Large population-based screening studies in Europe have used both urine (32) and vaginal (1) specimens obtained from women but have not directly compared the performances of these specimen types.

There is widespread agreement that nucleic acid amplification tests have superior sensitivity compared with antigen detection tests (4, 17). They are also considerably more expensive, and the costs might be prohibitive for large-scale screening programs. Chernesky recently commented that tests such as the IDEIA polymer conjugate-enhanced (PCE) enzyme immunoassay (EIA; Dako, Ely, Cambridgeshire, United Kingdom), which includes an amplification step to improve sensitivity (4, 16, 23, 29), deserved more critical evaluation (4). Studies from Japan have found that the IDEIA test performed well when used with vulvovaginal swabs (29, 30). Neither of these studies used negative-gray-zone testing, which involves retesting specimens with optical densities just below the cutoff point for a negative test with a nucleic acid amplification test. This strategy can further improve the sensitivities of enzyme immunoassays (9, 31).

The objectives of this study were to compare the performance of tests using vulvovaginal-swab specimens with that of tests using first-catch urine specimens for detecting *C. trachomatis* by using nucleic acid amplification tests with asymptom-

* Corresponding author. Mailing address: Department of Social and Preventive Medicine, University of Bern, Finkenhubelweg 11, CH-3012, Bern, Switzerland. Phone: 41 31 631 3092. Fax: 41 31 631 3520. E-mail: low@ispm.unibe.ch.
† Now retired.
‡ See Acknowledgments for list of study group members.
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atic women in a community setting and to determine whether an enhanced enzyme immunoassay with negative-gray-zone testing on vulvovaginal-swab specimens was sufficiently sensitive for use in screening programs.

MATERIALS AND METHODS

Study population. The Chlamydia Screening Studies project was a large population-based study which has been described in detail elsewhere (15, 18, 19, 20). In brief, a random sample of men and women aged 16 to 39 years from 27 family practices in and around Bristol and Birmingham, United Kingdom, was invited to participate by postal mail. Women were asked to collect 25 ml of early-morning first-catch urine in a universal container and to apply to the vulvovaginal area a double-headed cotton swab on a plastic shaft (Technical Service Consultants, Heywood, Lancashire, United Kingdom), which was transported dry. The urine specimens and vulvovaginal swabs were sent in approved packaging to the study laboratories by using first-class, prepaid envelopes. The samples were accompanied by a questionnaire which recorded time and date of specimen collection and brief demographic and sexual behavioral details.

Laboratory methods. The urine and vulvovaginal-swab specimens were tested either by a Cobas Amplicor CT Test PCR assay (Roche Diagnostics, Basel, United Kingdom) at the Bristol laboratory or by a Becton Dickinson ProbeTec ET DNA strand displacement amplification (SDA; Becton Dickinson and Company, Franklin Lakes, New Jersey) at the Birmingham laboratory. We followed the manufacturers' instructions, except that specimens with initially inhibitory results were retested with a different nucleic acid amplification test. The procedures for processing vulvovaginal swabs were the same as those recommended by the manufacturers for cervical swabs. At the Birmingham laboratory, the Roche Cobas PCR was used for retesting. At the Bristol laboratory, retesting was done using either the Becton Dickinson SDA or an in-house real-time PCR, owing to technical problems with the SDA assay, which we have described before (15).

All vulvovaginal swabs were also tested using an IDEIA PCE EIA (Dako, Ely, Cambridgeshire, United Kingdom). The cutoff value 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 below the calculated cutoff.

Specimen handling and storage. We divided first-catch urine specimens into two aliquots upon their arrival at the laboratory. At the Bristol laboratory, 1 ml of urine was removed for Cobas PCR testing, and at the Birmingham laboratory, a urine preservation pouch was added to 15 ml urine for subsequent Becton Dickinson SDA testing. The remaining urine was stored at 4°C until the results were available.

The double-headed swabs were separated upon their arrival in the laboratory: one swab was processed for nucleic acid amplification testing and the other for PCE EIA. At the Bristol laboratory, 1 ml 2-sucrose phosphate was added to one swab and eluted by vortexing, and 100 μl was removed for Cobas PCR testing. At the Birmingham laboratory, one swab was placed into a prefilled (2-ml) swab sample diluent tube, mixed by swirling it in the diluent for 5 to 10 seconds, expressed by pressing it along the inside of the tube, and then removed. This swab was then tested using the Becton Dickinson SDA. At each site, the other swab was placed in 1 ml of the enzyme immunoassay specimen diluent, eluted by vortexing, and tested according to the PCE EIA protocol. The residual of the PCE EIA eluate was kept at 4°C until the results were available. If retesting was required, we used the stored, untreated urine specimen or, for vulvovaginal swabs, the PCE EIA extract. Once all results were available and inhibitory specimens retested, the residual specimens were stored at −20°C.

Diagnostic strategy. All vulvovaginal specimens were tested using two biologically independent methods (a nucleic acid amplification test and an enzyme immunoassay). Urine specimens were tested only by the nucleic acid amplification test. We used different nucleic acid amplification tests to resolve the results for discordant specimens.

Patient infection status. True-positive and -negative results were determined according to an algorithm that required concordant results from two separate tests. A patient was considered positive if the result for a reactive nucleic acid amplification test on the vulvovaginal swab was confirmed by a reactive PCE EIA, the result for a reactive PCE EIA on the swab specimen was confirmed by a nucleic acid amplification test on either specimen type, or the result for a reactive nucleic acid amplification test on a swab or urine specimen was confirmed by another nucleic acid amplification test. A patient was considered negative if the results for the initial nucleic acid amplification test and the PCE EIA were negative.

Specimens that were initially inhibitory were retested using a different nucleic acid amplification test until a final result was obtained. PCE EIA was considered negative if the absorbance value was below the negative gray zone or if it was within the negative gray zone and negative by a nucleic acid amplification test.

Statistical analysis. For each testing strategy, we calculated the sensitivity, specificity, and predictive values with 95% confidence intervals (CI) relative to the combined standard defining true-positive and -negative results. We present the performance characteristics of nucleic acid amplification tests following retesting of inhibitory specimens and excluding the inhibitory specimens. For PCE EIA, we present the results obtained following negative-gray-zone testing and those determined as if all results in the negative gray zone had been considered negative.

RESULTS

A total of 2,754 women sent samples to a laboratory (1,479 to the Bristol laboratory, 1,275 to the Birmingham laboratory). Paired vulvovaginal-swab specimens and first-catch urine specimens were available for 2,745 different women (1,476 in the Bristol laboratory, 1,269 in the Birmingham laboratory). All nine single specimens (four swab specimens, five urine specimens) were confirmed as negative. There were 146 true-positive results (74 in the Bristol laboratory, 72 in the Birmingham laboratory). Table 1 shows how the final positive results were confirmed.

Vulvovaginal-swab specimens compared to first-catch urine specimens. After the results for inhibitory specimens were resolved, the performances of both nucleic acid amplification tests on the two specimen types were virtually identical, with closely overlapping confidence intervals: for vulvovaginal-swab specimens, the relative sensitivity for the Cobas PCR was 97.3% (95% CI, 90.6 to 99.7%), and that for the SDA was 97.2% (95% CI, 90.3 to 99.7%); for urine specimens, relative sensitivity for the Cobas PCR was 90.5% (95% CI, 81.5 to 96.1%; 67/74), and that for the SDA was 93.0% (95% CI, 84.5 to 97.7%; 67/72). We therefore combined the results to increase precision (Table 2). Using vulvovaginal-swab specimens correctly identified 142/146 true-positive specimens (relative sensitivity, 97.3%; 95% CI, 93.1 to 99.2%), compared with 134/146 identified using urine specimens (relative sensitivity, 91.8%; 95% CI, 86.1 to 95.7%). For both specimen types, relative specificity was 99.6% or higher.

Overall, 233 vulvovaginal-swab and 277 urine specimens were initially inhibitory. Almost all inhibitory swab specimens occurred with Cobas PCR: 232/1,475 (15.7%; 95% CI, 13.9 to 17.7%) compared with 1/1,269 (0.08%; 95% CI, 0.002 to 0.4%) for SDA. There were also more inhibitory urine specimens with Cobas PCR: 192/1,476 (13.0%; 95% CI, 11.3 to 14.8%) compared with 85/1,269 (6.7%; 95% CI, 5.4 to 8.2%) for SDA. If inhibitory results had not been resolved, testing using the Cobas PCR system would have failed to identify 10.8% (95% CI, 4.8 to 20.2; 8/74) of true-positive results for vulvovaginal-swab specimens and 4.1% (95% CI, 0.8 to 11.4%; 3/74) true-positive results for urine specimens. If inhibitory results with Becton Dickinson SDA had not been resolved, all true-positive vulvovaginal-swab specimens would have been picked up but 2.8% (95% CI, 0.3 to 9.7%; 2/72) true-positive urine specimens would have been missed.

PCE EIA compared with nucleic acid amplification tests on vulvovaginal-swab specimens. Of all 2,754 women who responded, paired samples were available for 2,749 different women. There were five specimens with only a PCE EIA result recorded, all of which were confirmed negative. The perfor-

TABLE 1. Tests performed to confirm positive results

Laboratory	Result for indicated test					No. of positive results	Comment(s)
	Cobas PCR		ProbeTec SDA		PCE EIA Swab		
	Swab	Urine	Swab	Urine			
Bristol	+	+			+	41	
	+	+			−	11	Real-time PCR or SDA positive
	+	+			NGZ[a]	3	NGZ confirmed by PCR
	+	−			+	5	
	+	−			−	4	Real-time PCR or SDA positive
	+	−			NGZ	0	
	−	+			+	5	
	−	+			−	4	Real-time PCR or SDA positive
	−	+			NGZ	0	
	−	−			+	1	Real-time PCR or SDA positive
	−	−			NGZ	0	
Total						74	
Birmingham			+	+	+	38	
			+	+	−	23	Roche PCR positive
			+	+	NGZ	3	
			+	−	+	1	
			+	−	−	4	Roche PCR positive
			+	−	NGZ	1	
			−	+	+	0	
			−	+	−	2	Roche PCR positive
			−	+	NGZ	0	
			−	−	+	0	
			−	−	NGZ	0	
Total						72	

[a] NGZ, negative gray zone.

TABLE 2. Results for first-catch urine and vulvovaginal-swab specimens tested using nucleic acid amplification[a]

Test result group	No. of results for nucleic acid amplification test		
	True positive	True negative	Total
First-catch urine[b]			
Positive	129	4	133
Negative	12	2,323	2,335
Inhibitory	5	272	277
Total	146	2,599	2,745
Vulvovaginal swab[c]			
Positive	134	9	143
Negative	4	2,364	2,368
Inhibitory	8	225	233
Total	146	2598[a]	2,744

[a] The total excludes one specimen with an equivocal result that could not be resolved. The specimen was confirmed negative based on the urine specimen result.

[b] Performance results after resolution of inhibitory determinations for the first-catch urine test were as follows: relative sensitivity, 91.8% (95% CI, 86.1 to 95.7%); relative specificity, 99.8% (95% CI, 99.6 to 99.9%); positive predictive value, 97.1% (95% CI, 92.7 to 99.2%); and negative predictive value, 99.5% (95% CI, 99.2 to 99.8%).

[c] Performance results after resolution of inhibitory determinations for the vulvovaginal-swab test were as follows: relative sensitivity, 97.3% (95% CI, 93.1 to 99.2%); relative specificity, 99.7% (95% CI, 99.3 to 99.8%); positive predictive value, 94.0% (95% CI, 89.0 to 97.2%); and negative predictive value, 99.8% (95% CI, 99.6 to 100%).

mances of PCE EIA differed somewhat between laboratories. Although this might have been due to chance (test for heterogeneity, $P = 0.575$), we have presented the results separately (Table 3). Of 74 true-positive specimens at the Bristol laboratory, PCE EIA correctly identified 52 (relative sensitivity, 70.2%; 95% CI, 58.5 to 80.3%). After repeat testing by Cobas PCR of 10 specimens with optical-density readings in the negative gray zone, the relative sensitivity increased to 74.3% (95% CI, 62.8 to 83.8%; 55/74) and specificity was unchanged (relative specificity, 98.8%; 95% CI, 98.1 to 99.3%).

In the Birmingham laboratory, PCE EIA identified 39/72 true-positive results (relative sensitivity, 54.2%; 95% CI, 42.0 to 66.0%) (Table 3). After the specimens in the negative gray zone were tested by SDA, the relative sensitivity of the PCE EIA test increased slightly, to 58.3% (95% CI, 46.1 to 69.8%; 42/72).

DISCUSSION

In this large population-based screening study, we found that two different nucleic acid amplification tests performed well with both self-taken vulvovaginal-swab and first-catch urine specimens. The numbers of inhibitory samples for the Roche Cobas PCR system (16% for vulvovaginal-swab specimens, 13% for urine specimens) were higher than those for the Becton Dickinson ProbeTec SDA (less than 0.1% for swab specimens, 7% for urine specimens). The sensitivity of the amplified IDEIA PCE EIA test was too low to recommend its

TABLE 3. Performance of PCE EIA tested on self-taken vulvovaginal-swab specimens

Final result	No. of results for PCE EIA			
	Positive	Negative	NGZ[a]	Total
Bristol laboratory[b]				
True positive	52	19	3	74
True negative	17	1,379	7	1,403
Total	69	1,398	10	1,477
Birmingham laboratory[c]				
True positive	39	30	3	72
True negative	7	1,188	5	1,200
Total	46	1,218	8	1,272

[a] NGZ, negative gray zone.

[b] Results including the negative-gray-zone results with nucleic acid amplification test confirmation were as follows: sensitivity, 74.3% (95% CI, 62.8 to 83.8%); specificity, 98.8% (95% CI, 98.1 to 99.3%); positive predictive value, 72.2% (95% CI, 60.4 to 82.1%); and negative predictive value, 98.1% (95% CI, 97.3 to 98.8%). Results with the negative-gray-zone results treated as negative were as follows: sensitivity, 70.2% (95% CI, 58.5 to 80.3%); specificity, 98.8% (95% CI, 98.1 to 99.3%); positive predictive value, 75.4% (95% CI, 63.5 to 84.9%); and negative predictive value, 98.4% (95% CI, 97.6 to 99%).

[c] Results including the negative-gray-zone results with nucleic acid amplification test confirmation were as follows: sensitivity, 58.3% (95% CI, 46.1 to 69.8%); specificity, 99.4% (95% CI, 98.8 to 100%); positive predictive value, 85.7% (95% CI, 72.8 to 94.1%); and negative predictive value, 97.5% (95% CI, 96.5 to 98.3%). Results with the negative-gray-zone results treated as negative were as follows: sensitivity, 54.2% (95% CI, 42.0 to 66.0); specificity, 99.0 (95% CI, 98.3 to 99.5); positive predictive value, 84.8% (95% CI, 71.1 to 93.7%); and negative predictive value, 97.3% (95% CI, 96.2 to 98.1%).

use in screening programs, and testing of specimens in the negative gray zone did not improve performance appreciably.

The strengths of this study are that it was a large population-based study looking at largely asymptomatic individuals not seeking health care, testing was carried out prospectively under routine laboratory conditions, and we compared the performances of different diagnostic tests on vulvovaginal specimens (19). The availability of two specimen types allowed us to use the status of the infected patient, rather than the infected specimen, as the reference standard. This can result in a higher positive yield but also in lower sensitivities for individual tests but provides a more realistic estimate of test performance. The advantages and disadvantages of different definitions of infection status have been discussed by Shrier et al. (27). The main disadvantage of this study is that we did not retest all specimens with negative results. Nevertheless, we accepted as true only the negative specimens that were negative from two sites and by two different tests. This avoids some of the important problems of discrepant analysis.

Many authors have advocated the use of self-taken vulvovaginal-swab specimens as suitable, acceptable, convenient, and effective in screening for *C. trachomatis* in a variety of clinical settings (5, 25, 26). Our study also showed that vulvovaginal-swab specimens collected at home and mailed to a laboratory are suitable diagnostic specimens for use with nucleic acid amplification tests. Currently, however, only the APTIMA system (Gene-Probe Inc., San Diego, CA) is approved for use with vulvovaginal specimens. At the time of the study, neither manufacturer for the tests that we evaluated had a protocol for processing vaginal swabs, so we used that for cervical swabs. Swabs can be transported dry at room temperature for the BD ProbeTec assay, but this is not recommended for the Roche PCR. Gaydos et al., however, have found that this assay performs well with both wet and dry vulvovaginal swabs when transported at 4°C (12, 13). Urine specimens were also not transported according to manufacturers' recommendations. However, we found no strong evidence for a fall in chlamydia positivity after storing female urine and vulvovaginal-swab specimens for up to 48 h (19) or after delays exceeding 96 h in testing male urine specimens (15). Taken together, these observations suggest that there is good nucleic acid stability for both vulvovaginal-swab and first-catch urine specimens stored at room temperature over a few days. As with health facility-based studies, we found slightly more positive results with vulvovaginal than with urine specimens. Despite the large numbers of specimens tested, no single study has been able to say conclusively whether or not vulvovaginal swab specimens are superior to urine specimens. A systematic review and meta-analysis of studies comparing the performances of self-collected vulvovaginal and urine specimens would be needed to resolve this issue.

The acceptability of vulvovaginal specimens to women is likely to depend on the settings and circumstances in which they are collected. In sexually transmitted disease clinics and other clinic settings in which women are likely to be expecting a physical and/or genital examination, women have expressed a preference for vaginal-swab specimens taken by clinicians (21) or by themselves (5), compared with a preference for having a pelvic examination. Self-taken vaginal swab specimens were also preferred to urine specimens in one study (5), but urine specimens were preferred to clinician-taken vaginal specimens in another (21). Our study was conducted under very different conditions. Women who were not seeking health care received specimen collection kits and instructions at home. We conducted qualitative, in-depth interviews with a random sample of these women to explore their feelings (19). Some women said that they felt more uncomfortable taking the vulvovaginal than the urine specimen, and a few said that the request for a vulvovaginal specimen contributed to their decision not to take part in the study. If specimens are being collected at home, therefore, any technical advantage of vulvovaginal specimens over urine specimens might be offset by negative attitudes toward the method of specimen collection. Gaydos et al. found that, among women responding to an offer of chlamydia testing with home-collected, mailed vulvovaginal-swab samples, women who returned the specimen said that using the swab was their preferred method, while those who did not return a specimen preferred urine specimen collections or pelvic examinations (13).

From the point of view of the laboratory, vulvovaginal swabs have also been promoted because processing them does not require a centrifugation step (24), and suboptimal processing of urine samples could lead to false-negative results (15). The empirical data that we obtained in the ClaSS project suggests that the potentially faster specimen processing time for swabs might not be important in practice. In time and motion studies conducted on 15 separate occasions at different times of day, the times taken to process urine and vulvovaginal-swab specimens were similar: 4.4 min for swab specimens and 6.6 for urine specimens tested by Cobas PCR and 3.6 min for swab specimens and 3.3 min for urine specimens tested by SDA (19).

We found that using an amplified enzyme immunoassay with negative-gray-zone testing by a nucleic acid amplification test on vulvovaginal swab specimens was not adequate for widespread screening in the general population. We tested the strategy of negative-gray-zone testing with that of the PCE EIA test to optimize the performance of an enzyme immunoassay. The best results were obtained in the Bristol laboratory, where Cobas PCR was used to retest specimens with results in the negative gray zone. The relative sensitivity of 74% was similar to the results for the same strategy used on male first-catch urine specimens in the same laboratory (15). This falls short of the 80% lower limit recommended by recent Clinical Effectiveness Group guidance and precludes the use of enzyme immunoassay results in the United Kingdom (7).

Inhibitory products in clinical specimens are well recognized as being a problem for nucleic acid amplification tests, and reported rates of inhibition can reach levels that compromise the sensitivity of the assay (3, 8, 12, 22, 28). High rates of inhibition have previously been documented with both assays and in both specimen types, but the findings are contradictory. Our results are consistent with those of a laboratory study using specimens artificially spiked with known quantities of *C. trachomatis* (6). Chernesky et al. found rates of inhibition with the Roche PCR similar to those found in our study: 10 to 13% in vulval, urine, and cervical specimens. Furthermore, with the BD ProbeTec, inhibition was more common in urine (27%) than vulval-swab (2%) specimens. The APTIMA Combo test showed low rates of inhibition in all samples (6). However, Cosentino et al. reported inhibition in about 25% of vulval-swab specimens transported dry and tested by the BD ProbeTec (8). One potential explanation is the use of dry vulvovaginal swabs transported at room temperature. Gaydos et al., however, did not encounter this problem when using a similar protocol with the Roche PCR (13). High inhibition rates might also represent specimen-associated factors or problems with certain batches of kits (28). Both of the tests that we evaluated include an internal control to detect inhibitors, but retesting is costly. One alternative is to issue no result and request a second sample, but this also requires testing and delays the definitive result for the patient. Most inhibitory specimens will be negative on retesting, and it has been suggested that the inhibitory control has a minimal impact on test performance (8). In our study, however, 1 in 10 positive vulvovaginal-swab specimens tested by the Cobas PCR would have been missed if no result had been issued and a second specimen could not be obtained.

In conclusion, both vulvovaginal-swab and first-catch urine specimens tested using nucleic acid amplification tests perform well in identifying women in the general population infected with *C. trachomatis*. A systematic review and meta-analysis of studies comparing these specimen types is required to determine definitively which female specimen has the best performance characteristics. Enzyme immunoassays, even with strategies to maximize sensitivity, are not suitable for chlamydia screening for women in community settings.

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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. **Carder, C., A. J. Robinson, C. Broughton, J. M. Stephenson, and G. L. Ridgway.** 1999. Evaluation of self-taken samples for the presence of genital *Chlamydia trachomatis* infection in women using the ligase chain reaction assay. Int. J. STD AIDS **10:**776–779.
3. **Chan, E. L., K. Brandt, K. Olienus, N. Antonishyn, and G. B. Horsman.** 2000. Performance characteristics of the Becton Dickinson ProbeTec System for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male and female urine specimens in comparison with the Roche Cobas System. Arch. Pathol. Lab. Med. **124:**1649–1652.
4. **Chernesky, M. A.** 2002. *Chlamydia trachomatis* diagnostics. Sex. Transm. Infect. **78:**232–234.
5. **Chernesky, M. A., E. W. Hook III, D. H. Martin, J. Lane, R. Johnson, J. A. Jordan, D. Fuller, D. E. Willis, P. M. Fine, W. M. Janda, and J. Schachter.** 2005. Women find it easy and prefer to collect their own vaginal swabs to diagnose *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infections. Sex. Transm. Dis. **32:**729–733.
6. **Chernesky, M. A., D. Jang, K. Luinstra, S. Chong, M. Smieja, W. Cai, B. Hayhoe, E. Portillo, C. MacRitchie, C. Main, and R. Ewert.** 2006. High analytical sensitivity and low rates of inhibition may contribute to detection of *Chlamydia trachomatis* in significantly more women by the APTIMA Combo 2 assay. J. Clin. Microbiol. **44:**400–405.
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. (Revised 2006.) http://www.bashh.org/guidelines.asp. Accessed 6 November 2006.
8. **Cosentino, L. A., D. V. Landers, and S. L. Hillier.** 2003. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by strand displacement amplification and relevance of the amplification control for use with vaginal swab specimens. J. Clin. Microbiol. **41:**3592–3596.
9. **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.
10. **Department of Health.** 2001. Better prevention, better services, better sexual health—the national strategy for sexual health and HIV. Department of Health, London, United Kingdom. [Online.] http://www.dh.gov.uk/assetRoot/04/05/89/45/04058945.pdf.
11. **Department of Health.** 2006. The National Chlamydia Screening Programme (NCSP). Department of Health, London, United Kingdom. [Online.] http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/SexualHealth/SexualHealthGeneralInformation/SexualHealthGeneralArticle/fs/en?CONTENT_ID=4084098&chk=CSLxsK.
12. **Gaydos, C. A., K. A. Crotchfelt, N. Shah, M. Tennant, T. C. Quinn, J. C. Gaydos, K. T. McKee, Jr., and A. M. Rompalo.** 2002. Evaluation of dry and wet transported intravaginal swabs in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in female soldiers by PCR. J. Clin. Microbiol. **40:**758–761.
13. **Gaydos, C. A., K. Dwyer, M. Barnes, P. A. Rizzo-Price, B. J. Wood, T. Flemming, and T. Hogan.** 2006. Internet-based screening for *Chlamydia trachomatis* to reach nonclinic populations with mailed self-administered vaginal swabs. Sex. Transm. Dis. **33:**451–457.
14. **Hjelm, E., A. Hallen, and M. Domeika.** 2001. Cervical, urine and vaginal specimens for detection of *Chlamydia trachomatis* by ligase chain reaction in women: a comparison. Acta Dermato-Venereol. **81:**285–288.
15. **Horner, P., S. Skidmore, A. Herring, J. Sell, I. Paul, O. Caul, M. Egger, A. McCarthy, E. Sanford, C. Salisbury, J. MacLeod, J. Sterne, and N. Low for the Chlamydia Screening Studies (ClaSS) Group.** 2005. Enhanced enzyme immunoassay with negative-gray-zone testing compared to a single nucleic

acid amplification technique for community-based chlamydial screening of men. J. Clin. Microbiol. **43:**2065–2069.

16. **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.
17. **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. Recomm. Rep. **51:**1–38.
18. **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.
19. **Low, N., A. McCarthy, J. Macleod, C. Salisbury, R. Campbell, T. E. Roberts, P. Horner, S. Skidmore, J. A. Sterne, E. Sanford, F. Ibrahim, A. Holloway, R. Patel, P. Barton, S. M. Robinson, N. Mills, A. Graham, A. Herring, E. O. Caul, G. Davey Smith, F. D. Hobbs, and M. Egger for the Chlamydia Screening Studies Group.** Epidemiological, social, diagnostic, and economic evaluation of population screening for genital chlamydial infection: the Chlamydia Screening Studies project. Health Technol. Assess., in press.
20. **Macleod, J., C. Salisbury, N. Low, A. McCarthy, J. A. Sterne, A. Holloway, R. Patel, E. Sanford, A. Morcom, P. Horner, S. G. Davey, S. Skidmore, A. Herring, O. Caul, F. D. Hobbs, and M. Egger.** 2005. Coverage and uptake of systematic postal screening for genital *Chlamydia trachomatis* and prevalence of infection in the United Kingdom general population: cross sectional study. BMJ **330:**940–942.
21. **Macmillan, S., H. McKenzie, and A. Templeton.** 2003. Parallel observation of four methods for screening women under 25 years of age for genital infection with *Chlamydia trachomatis*. Eur. J. Obstet. Gynecol. Reprod. Biol. **107:**68–73.
22. **Morré, S. A., I. G. M. Van Valkengoed, R. M. Moes, A. J. P. Boeke, C. J. L. M. Meijer, and A. J. C. Van den Brule.** 1999. Determination of *Chlamydia trachomatis* prevalence in an asymptomatic screening population: performances of the LCx and COBAS Amplicor tests with urine specimens. J. Clin. Microbiol. **37:**3092–3096.
23. **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.
24. **Schachter, J., M. A. Chernesky, D. E. Willis, P. M. Fine, D. H. Martin, D. Fuller, J. A. Jordan, W. Janda, and E. W. Hook III.** 2005. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. Sex. Transm. Dis. **32:**725–728.
25. **Schachter, J., W. M. McCormack, M. A. Chernesky, D. H. Martin, P. B. Van Der, P. A. Rice, E. W. Hook III, W. E. Stamm, T. C. Quinn, and J. M. Chow.** 2003. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. J. Clin. Microbiol. **41:**3784–3789.
26. **Shafer, M. A., J. Moncada, C. B. Boyer, K. Betsinger, S. D. Flinn, and J. Schachter.** 2003. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. J. Clin. Microbiol. **41:**4395–4399.
27. **Shrier, L. A., D. Dean, E. Klein, K. Harter, and P. A. Rice.** 2004. Limitations of screening tests for the detection of *Chlamydia trachomatis* in asymptomatic adolescent and young adult women. Am. J. Obstet. Gynecol. **190:**654–662.
28. **Skidmore, S., P. Horner, and H. Mallinson on behalf of the HPA Chlamydia Diagnosis Forum.** 2006. Testing specimens for *Chlamydia trachomatis*. Sex. Transm. Infect. **82:**272–275.
29. **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.
30. **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.
31. **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.
32. **van Bergen, J., H. M. Gotz, J. H. Richardus, C. J. Hoebe, J. Broer, A. J. Coenen, and the PILOT CT Study Group.** 2005. Prevalence of urogenital *Chlamydia trachomatis* increases significantly with level of urbanisation and suggests targeted screening approaches: results from the first national population based study in The Netherlands. Sex. Transm. Infect. **81:**17–23.
33. **Wiesenfeld, H. C., R. P. Heine, A. Rideout, I. Macio, F. DiBiasi, and R. L. Sweet.** 1996. The vaginal introitus: a novel site for *Chlamydia trachomatis* testing in women. Am. J. Obstet. Gynecol. **174:**1542–1546.