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Comparison of Transcription-Mediated Amplification and PCR Assay Results for Various Genital Specimen Types for Detection of *Mycoplasma genitalium*

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***Mycoplasma genitalium* is now recognized as a possible cause of several idiopathic sexually transmitted disease (STD) syndromes. However, due to the difficulty of culture of this fastidious bacterium, nucleic acid amplification tests (NAATs) are necessary for its detection in patient specimens. In the current study we compared a newly developed research-only transcription-mediated amplification (TMA) assay (Gen-Probe Incorporated) to our in-house DNA-based PCR assay for detection of *M. genitalium*. The relative performance characteristics of these two NAATs were assessed with genital specimens from 284 women and 352 men reporting to an STD clinic in Seattle, WA. Among the women, *M. genitalium* was detected by the TMA and PCR assays in 36 (13%) and 39 (14%) vaginal swab specimens, respectively ($\kappa = 0.923$); 26 (9%) and 23 (8%) cervical swab specimens, respectively ($\kappa = 0.843$); and 25 (9%) and 28 (10%) urine specimens, respectively ($\kappa = 0.687$). Among the *M. genitalium*-positive women, the relative sensitivities of detection for the TMA and PCR assays were 84% and 91%, respectively, for vaginal swab specimens; 60% and 53%, respectively, for cervical swab specimens; and 58% and 65%, respectively, for urine specimens. By using an infected patient (a woman positive at any site by TMA assay and at any site by PCR) as a proxy for a "gold standard," the specificities of detection were >99.5% for both the TMA and the PCR assays. Among the men, *M. genitalium* was detected in 24 urine specimens (6.8%) by the TMA assay, 26 (7.4%) urine specimens by PCR assay, and 32 urine specimens (9%) by either test ($\kappa = 0.791$). We conclude that the *M. genitalium* TMA and PCR assays are highly specific and that vaginal swab specimens are the most sensitive specimen type for the detection of *M. genitalium* in women.**

Mycoplasma genitalium was first isolated from urethral exudates from two men with urethritis in 1981 (38), yet the isolation and culture of this fastidious organism are extremely difficult and time-consuming. Although culture techniques for *M. genitalium* have improved in the last two decades (20), efficient isolation and cultivation of this organism remain elusive (2, 20), and thus, identification of infected individuals has relied on the use of PCR tests (16, 36). The application of *M. genitalium*-specific PCR tests has allowed studies that assess reproductive tract disease in men and women, including urethritis, cervicitis, endometritis, and pelvic inflammatory disease (7, 14, 16, 19, 25, 27, 36, 37), suggesting a disease spectrum similar to those caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Detection of DNA from this organism by PCR in fallopian tube tissue from a woman with salpingitis (8) and the association of *M. genitalium* with tubal factor infertility by serologic tests (6) suggest an even broader range of disease associations and sequelae for this emerging pathogen. These results are particularly disconcerting, considering that *M. genitalium* has been detected in 1% of young adults in the U.S. general population, a prevalence intermediate between those of *N. gonorrhoeae* (0.4%) and *C. trachomatis* (4.2%) (24). Additional studies are needed to assess the disease associations, optimal treatment regimens, risk factors, and risk markers for infection and the effect on human immunodeficiency virus acquisition and transmission. High-throughput nucleic acid amplification tests (NAATs) would facilitate such studies, as would the determination of the optimal genital specimen type for detection.

More than 10 PCR assays have been used to detect *M. genitalium* in patient specimens (3, 9, 10, 16, 21, 22, 27, 29, 35, 36, 41, 42). However, few studies have assessed the relative sensitivities and specificities of different specimen types for the detection of *M. genitalium*. Because these assays differ in their target DNA sequences, specimen preparation techniques, amplicon detection methods, and the use of an internal control to measure inhibition, they may differ in their sensitivities and specificities for the detection of *M. genitalium*. As an alternative to PCR, Gen-Probe Incorporated (San Diego, CA) developed a research-use-only, transcription-mediated amplification (TMA)-based test for the detection of *M. genitalium*. The TMA assay targets rRNA, a molecule present in multiple copies per cell, thereby potentially increasing the sensitivity of detection relative to the sensitivities of PCR assays that target single-copy genes. The TMA assay also incorporates target capture technology, which serves to isolate the target RNA and remove interfering substances. This NAAT is semiautomated and thus would increase the specimen throughput compared to that of PCR for laboratory testing of this organism.

The optimal genital specimen type for the detection of *M.*

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genitalium in men and women has not been thoroughly assessed. Although cervical and urethral swab specimens have traditionally been used to detect other genital pathogens, such as *C. trachomatis* and *N. gonorrhoeae*, patient-obtained urine and vaginal swab specimens are often as sensitive as cervical swab specimens, are the specimen types preferred by patients, and can be obtained without a clinical examination, facilitating screening programs (13, 15). To our knowledge, no studies have compared cervical swab, vaginal swab, and urine specimens for the detection of *M. genitalium*.

In the current study we compared the research-use-only Gen-Probe TMA assay to our in-house PCR test (10) for *M. genitalium* detection and assessed the relative sensitivities and specificities of vaginal swab, cervical swab, and urine specimens for the detection of this organism by both assays. The ability to test each specimen type by two different NAATs allowed us to more accurately define the relative sensitivity and specificity of each assay with each specimen type. Our results should inform the selection of the genital specimen type and NAAT for use in future studies for the assessment of this emerging pathogen and its disease associations, the risk factors for its acquisition, optimal treatment regimens, and the sequelae of infection with the organism.

MATERIALS AND METHODS

Study population and specimen handling. Women (n = 321) and men (n = 352) were recruited among 18- to 27-year-olds presenting to the Public Health Seattle-King County Sexually Transmitted Disease Clinic with symptoms suggestive of or a sexual partner with a sexually transmitted infection between November 2001 and May 2004. Study participants provided written informed consent, completed a computer-assisted survey instrument interview, and underwent a routine clinical examination in an ongoing study to evaluate the disease associations, risk markers and risk factors, and prevalence of *M. genitalium* infection. Subsequent to their clinical examination, the men provided a first-void urine specimen to screen for *M. genitalium*, *C. trachomatis*, and *N. gonorrhoeae*. Prior to their clinical examination, the women provided a self-obtained vaginal swab specimen, which was placed in a dry tube, and a first-void urine specimen. During the female clinical examination, a cervical specimen was collected on a Dacron-tipped swab for routine screening for *N. gonorrhoeae* and *C. trachomatis*. A second cervical swab specimen was collected for *M. genitalium* testing and placed in 1 ml of 2SP (0.2 M sucrose, 0.02 M potassium phosphate buffer, 0.001% phenol red [pH 7.5]).

Following the clinical examination, all three specimen types were refrigerated until further processing occurred, typically within 12 h, although in rare cases the specimens were held for up to 1 week before they were tested. Upon receipt at the laboratory, the vaginal swabs were hydrated in 1 ml of 2SP for 1 h at room temperature and vortexed. After the swab was removed from the medium, the retained fluid was gently suctioned from the swab head with an aerosol barrier pipette tip and combined with the 2SP in the collection tube. The cervical swab, first-void urine, and hydrated vaginal swab specimens were frozen and maintained at −80°C until analysis. The laboratory personnel performing the assays were blinded to the results of the TMA and PCR assays for all specimens.

Specimen amplification and detection of *Mycoplasma genitalium* by PCR. The vaginal swab and urine specimens were prepared for PCR by using a MasterPure DNA purification kit, according to the manufacturer's protocol (Epicenter, Madison, WI). A 150-μl aliquot of each specimen was subjected to cellular lysis, the proteins were precipitated and removed, and DNA was isolated and eluted in 30 μl of Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA, pH 8.5). Each PCR mixture included a 12.5-μl aliquot of the eluted DNA, which corresponds to 62.5 μl of the original specimen. Cervical swab specimens were processed by using the COBAS AMPLICOR CT/NG specimen preparation kit, according to the manufacturer's instructions (swab procedure; Roche Diagnostics Corporation, Indianapolis, IN), as reported previously (10). By this method, an aliquot of 100 μl of the specimen was added to 100 μl of lysis buffer, briefly incubated, and mixed with 200 μl of diluent. From this mixture, 50 μl, which corresponded to 12.5 μl of the original specimen, was added to each PCR mixture.

All processed specimens were amplified by the MgPa-IMW PCR assay with biotinylated primers modMgPa1 and modMgPa3, which target the *mgpB* gene of the MgPa adhesin operon (10). Detection of the PCR products was performed by a microwell-plate-based colorimetric assay (10). An internal control (IC) (10) was used to identify specimens containing inhibitors that might result in falsely negative results. Each PCR was performed in conjunction with three controls: (i) a specimen processing control (processed *M. genitalium* strain G-37 from culture), (ii) a positive control (*M. genitalium* G-37 DNA), and (iii) a negative control (PCR reagents alone). Following the PCR assay, amplified specimens were assayed for the presence of *M. genitalium* and IC DNA by using a color detection-based microwell assay, according to our standard procedure (10). Each microwell assay included the PCR controls as well as a development control (titer of previously positive *M. genitalium* and IC amplicons adjusted to achieve an optical density at 405 nm [OD_{405}] of approximately 1.0) to ensure the efficiency of the procedure. If specimens contained PCR inhibitors, the corresponding specimen preparations were diluted 1:5 and reamplified.

TMA assay. The TMA assay used to detect *M. genitalium* was a modification of the APTIMA assay kit protocols established for *C. trachomatis* and *N. gonorrhoeae* (Gen-Probe Incorporated). Briefly, 200 μl of unprocessed specimen (urine or vaginal/cervical swab specimens in 2SP) was added to a tube containing either 200 μl of Urine Transport Medium or Swab Transport Medium (Gen-Probe), as appropriate for the specimen type. The contents were mixed by gentle shaking with 100 μl of target capture reagent, which consisted of magnetic particles coated with oligonucleotides complementary to a region of *M. genitalium* 16S rRNA. This mixture was subjected to 62°C for 25 min, during which time the target rRNA was captured, and was then cooled to room temperature for 30 min; placed on a Gen-Probe Target Capture system magnetic base; and washed to remove unbound nucleic acids, cell debris, and buffers. A 100-μl aliquot of the amplification mixture containing *M. genitalium*-specific primers and silicone oil (200 μl) was added to the magnetic beads, and the mixture was heated at 62°C for 10 min, cooled to 42°C for 5 min, and incubated for 45 min with reverse transcriptase and T7 RNA polymerase. One hundred microliters of an acridinium ester-labeled DNA probe was added to the RNA amplicon mixture, and the mixture was incubated at 62°C for 20 min and then cooled at room temperature for 5 min. Selection reagent (200 ml) was added to the probe-amplicon hybrids, and the mixture was incubated at 62°C for 7.5 min. A chemiluminescent signal was read in a luminometer. The signal corresponded to hybridized target and is expressed as relative light units (RLUs) after the injection of detection reagents. Following this technique, 200 μl (or 6.6%) of the requested 30-ml urine specimen and 100 μl (or 10%) of the 1-ml vaginal and cervical specimens were used in the TMA assay.

Interpretation of results. Specimens were considered *M. genitalium* positive (i) by the TMA assay if the RLU values were ≥50,000 and (ii) by PCR if the OD_{405} was ≥0.25. Specimens were *M. genitalium* negative (i) by the TMA assay if the RLU values were ≤5,000 and (ii) by PCR if the result for the IC was valid (OD_{405} ≥ 0.25) and the *M. genitalium* OD_{405} was ≤0.10. Specimens were classified as *M. genitalium* intermediate (i) by the TMA assay if the RLU values were between 5,001 and 49,999 and (ii) by PCR if the OD_{405} was between 0.11 and 0.24 and the result for the inhibition control was valid. Specimens containing PCR inhibitors were diluted 1:5 and reamplified. Intermediate specimens were processed from the original specimen in duplicate and retested by the relevant NAAT. Initially intermediate specimens scoring positive by one or both of these repeat assays were classified as positive.

Statistical analysis. The kappa statistic (κ) was used to evaluate the agreement between the TMA and the PCR assays for each specimen type, such that the closer to "1" that the κ value was, the more perfect the agreement between the tests was, beyond that which would be expected from chance alone (23). McNemar's test was used to determine the significance of the differences in the detection of *M. genitalium* in the different specimen types by either assay. We also calculated the relative sensitivities and specificities of the tests and specimen types compared to the results obtained with an infected-patient standard (IPS), which we used as a proxy for a gold standard for these patients. By these criteria, a woman was considered *M. genitalium* positive if any specimen type from the woman was positive for *M. genitalium* by the TMA assay and any specimen type was positive by PCR. For example, for an IPS-positive woman, the TMA assay positive could be for the vaginal specimen and the PCR could be positive for the vaginal specimen or a specimen from any other site.

RESULTS

Of the 321 women enrolled in the study, sufficient volumes for analysis of vaginal swab, cervical swab, and first-void urine specimens were collected from 284 women, all of whom were

TABLE 1. Agreement of TMA and PCR test results among vaginal swab, cervical swab, and urine specimens from 284 women and among urine specimens from 352 men

Specimen type and TMA assay result	No. of specimens with the following PCR result:		κ value
	Positive	Negative	
Vaginal (n = 284)			0.923
Positive	35	1[a]	
Negative	4	244	
Cervical (n = 284)			0.843
Positive	21	5	
Negative	2[a]	256	
Female urine (n = 284)			0.687
Positive	19	6	
Negative	9[a]	250	
Male urine (n = 352)			0.698
Positive	18	6	
Negative	8	320	

[a] One of these specimens was not from an infected patient standard-positive woman.

TABLE 2. Agreement of results among vaginal swab, cervical swab, and urine specimens by TMA and PCR tests for 284 women for whom all three specimen types were tested

Specimen combination result	Result for the following specimen[a]:			No. (%) of specimens positive by:	
	Vaginal swab	Cervical swab	Urine	TMA	PCR
Results for all three specimen types concordant	+	+	+	16 (5.6)	18 (6.3)
	−	−	−	243 (85.6)	242 (85.2)
Result for at least one specimen type discordant	+	−	−	9 (3.2)[b]	9 (3.2)
	+	+	−	5 (1.8)	3 (1.1)
	+	−	+	6 (2.1)	9 (3.2)
	−	+	−	2 (0.7)	2 (0.7)[b]
	−	+	+	3 (1.1)	0 (0)
	−	−	+	0 (0)	1 (0.4)[b]
Total no. detected by TMA	36	26	25	41	NA[c]
Total no. detected by PCR	39	23	28	NA	42

[a] +, positive result for the indicated specimen type by TMA or PCR; −, negative result for the indicated specimen type by TMA or PCR.
[b] One of these specimens was from an IPS-negative woman.
[c] NA, not applicable.

included in the current analysis and for whom all three specimen types were tested for *M. genitalium* by TMA and PCR assays. Among the men (n = 352), only urine specimens were collected and tested for *M. genitalium* by TMA and PCR assays. The overall *M. genitalium* prevalences, determined by either a positive TMA result or a positive PCR test result at any site by any test, were 15% (43/284) for women and 9% (32/352) for men in the sexually transmitted disease clinic population studied.

Performance of TMA and PCR assays with each genital specimen type. The TMA and the PCR assay results were concordant for 279 (98.2%) of the vaginal swab specimens, 277 (97.6%) of the cervical swab specimens, and 269 (94.7%) of the urine specimens from the 284 women tested and for 338 (96%) of the 352 male urine specimens (κ = 0.687 to 0.923) (Table 1). The highest correlation was among the vaginal swab specimens (κ = 0.923), followed by the cervical swab specimens (κ = 0.843) and then by the male (κ = 0.698) and the female (κ = 0.687) urine specimens. Among the 27 female specimens with discordant TMA and PCR test results for a single specimen type, only 3 specimens were from IPS-negative women (Table 1).

Concordance of results and relative sensitivities of the different genital specimen types for detection of *M. genitalium*. The results for all three specimen types (vaginal swab, cervical swab, and urine specimens) were concordant for 259 (91%) and 260 (92%) of the 284 women by the TMA and PCR assays, respectively (Table 2). Among the 25 women with discordant TMA results for the three specimen types, 20 (80%) were positive for *M. genitalium* in the vaginal specimen: as the sole positive specimen in 9 (36%) women and in combination with either the cervical swab or the urine specimen in 5 (20%) and 6 (24%) women, respectively. Similarly, of the 24 women with discordant PCR results between specimen types, 21 (88%) were positive for *M. genitalium* in the vaginal specimen: as the sole positive specimen in 9 (38%) women and in combination with either the cervical swab or urine specimen in 3 (13%) and 9 (38%) women, respectively.

Of the 41 infections detected by the TMA assay, 36 (88%) were detected in the vaginal swab specimens, 26 (64%) were detected in the cervical swab specimens, and 25 (61%) were detected in the urine specimens, indicating that the vaginal swab specimens were more sensitive than the cervical swab specimens (P = 0.04) and the urine specimens (P = 0.02) for the detection of *M. genitalium*-infected women by this NAAT. Similarly, of the 42 *M. genitalium* infections detected by PCR, 39 (93%) were detected in the vaginal swab specimen, 26 (55%) were detected in the cervical swab specimen, and 28 (67%) were detected in the urine specimen ($P < 0.01$ for the difference in the rates of detection between the vaginal swab specimen and either cervical swab or urine specimens). There was no significant difference in the rates of detection of *M. genitalium* between urine and cervical swab specimens among vaginally infected women (P = 1.0 for TMA and P = 0.3 for PCR).

Of the 43 IPS-positive women, *M. genitalium* was detected in vaginal specimens from 36 (84%) and 39 (91%) women by the TMA and the PCR assays, respectively. The use of both vaginal and cervical swab specimens to screen for *M. genitalium* would have detected 41 (95%) of the 43 infected women by either the TMA or the PCR assay. Similarly, the use of both vaginal swab and urine specimens to screen the women would have detected 39 (91%) and 40 (93%) of the 43 infected women by the TMA and the PCR assays, respectively.

Relative specificity of *M. genitalium* detection by TMA and PCR assays. In the absence of an established reference NAAT for the detection of *M. genitalium*, we used the IPS (see above) to evaluate the relative specificities of the NAATs for each specimen type. Only three specimens from women who were not IPS positive were positive by TMA or PCR: one by the TMA assay for the vaginal specimen, one by the PCR assay for the cervical swab specimen, and one by the PCR assay for the urine specimen (these are indicated in Table 1). For the purposes of this analysis, the NAATs from these women were

TABLE 3. Relative sensitivities and specificities of TMA and PCR tests by specimen type or combination of specimen types

Specimen tested	Relative sensitivity[a] (% [no.] positive)		Relative specificity[b] (% [no.] positive)	
	TMA (n = 43)	PCR (n = 43)	TMA (n = 244)	PCR (n = 244)
Single specimen				
Vaginal swab	84 (36)	91 (39)	99.6 (243)	100 (244)
Cervical swab	60 (26)	53 (23)	100 (244)	99.6 (243)
Urine	58 (25)	65 (28)	100 (244)	99.6 (243)
Two specimens				
Vaginal swab and urine	91 (39)	93 (40)	99.6 (243)	99.6 (243)
Vaginal and cervical swabs	95 (41)	95 (41)	99.6 (243)	99.6 (243)
Cervical swab and urine	74 (32)	77 (33)	99.6 (243)	99.6 (243)
All three specimens	95 (41)	98 (42)	99.6 (243)	99.2 (242)

[a] The relative sensitivity was calculated as the number of specimens positive for *M. genitalium* for each specimen type or combination of specimen types relative to the total number of women positive at any site by the TMA assay or at any site by PCR.

[b] The relative specificity was calculated as the number of specimens negative for *M. genitalium* for each specimen type or combination of specimen types relative to the total number of women positive at any site by the TMA assay and at any site by PCR.

considered "false positives." Despite the few discrepant results, the relative specificities of the TMA and the PCR assays were 99.6% and 100%, respectively, for the vaginal swab specimens; 100% and 99.6%, respectively, for the cervical swab specimens; and 100% and 99.6%, respectively, for the urine specimens (Table 3). Among the total number of specimens from women tested, the relative specificities were 99.9% (851/852) for the TMA assay and 99.8% (850/852) for the PCR.

Repeatability of assay and resolution of intermediate results. The TMA and the PCR tests with initial positive or intermediate results were retested by the same NAAT in duplicate by using two separate preparations of the original patient specimen. Retesting of these specimens served to assess the repeatabilities of the NAATs, to identify possible false-positive results caused by cross-contamination of the PCR assay tubes during processing or amplification, and to resolve intermediate test results as either positive or negative. Among the specimens initially positive by the PCR or the TMA assays, the majority retested positive: 72 of 73 the vaginal swab specimens, 48 of 48 the cervical swab specimens, and 45 of 51 female urine specimens. All but two samples that retested positive were from IPS-positive women, consistent with their interpretation as true positives. In addition, the seven positive specimens that did not have positive results in retesting were from IPS-positive women. We concluded that repeat testing of specimens to confirm positive results served only to decrease the sensitivity and did not increase the specificity.

Specimens with intermediate values by PCR most often (92% [66/72]) resolved as negative or intermediate and were thus classified as negative. All but 2 of the 57 female specimens with intermediate results that did not retest positive were from IPS-negative women, confirming their interpretation as negative. Four of the five female specimens that resolved as positive were from IPS-positive women. In contrast to the PCR results, only two TMA test results were intermediate: one, from an IPS-positive woman, did not resolve as positive; the other, from a male urine specimen, that was also positive by PCR resolved as positive.

DISCUSSION

In the current study, we evaluated a newly developed research-use-only TMA assay for the detection of *M. genitalium* in four genital specimen types (female vaginal swab, cervical swab, and urine specimens and male urine specimens) and compared its performance with that of the PCR assay routinely used for the detection of this organism in our laboratory. We found the results of the TMA and the PCR tests to be highly concordant with the four specimen types analyzed. The results for the vaginal swab specimens were the most concordant (κ = 0.923), followed by those for the cervical swab specimens (κ = 0.843), male urine specimens (κ = 0.698), and female urine specimens (κ = 0.687). In addition, we determined that vaginal swab specimens were relatively more sensitive than cervical swab or urine specimens for the detection of *M. genitalium* in women. Finally, by using an infected-patient standard, we determined that both the PCR and the TMA NAATs had a high relative specificity (>99%) for the detection of this emerging pathogen.

To our knowledge, our study is the most extensive comparison of *M. genitalium* NAATs with different specimen types conducted to date. Although numerous PCR assays have been developed for the detection of *M. genitalium*, few have been validated relative to an independent PCR assay and none have been compared to a different type of NAAT. In 1995, Deguchi et al. (9) found a 100% concordance between the results of two PCR assays that targeted different portions of the MgPa gene using urethral swab specimens from 108 men with a 17% prevalence of this organism. In 2003, Jensen et al. (19) found that of 41 urogenital specimens positive by a PCR assay targeting the 16S rRNA gene, the results for 40 (97.5%) were confirmed by an independent MgPa PCR assay. Using urethral swab specimens and the same MgPa PCR assay described above (19) as a comparator, Svenstrup et al. (35) found a concordance of 89% (κ = 0.91, according to our calculations) compared to the results of a newly developed quantitative PCR assay that targets the *M. genitalium* glyceraldehyde-3-phosphate dehydrogenase (*gap*) gene. Similarly, a concordance of 98% (κ = 0.71, according to our calculations) was found among conventional and quantitative PCR assays that used the same primer sets to detect *M. genitalium* in 301 endocervical swab and/or first-void urine specimens (22). The comparisons described above may be biased toward concordance, because several of the analyses cited above grouped different sample types in the same analyses (22); included only men with nongonococcal urethritis (9), who presumably have higher organism loads than controls (35); compared the quantitative and conventional PCR assays by use of the same primers (21); or used the same purified specimen in the two comparator PCR assays (3). In contrast, our comparison of the *M. genitalium* TMA and PCR assays is particularly robust because these two NAATs target different sequences in different genetic molecules (16S rRNA versus *mgpB* DNA) and use very different methods for specimen preparation (magnetic bead capture versus the Epicenter MasterPure DNA purification kit), am-

plification (TMA versus PCR), and detection (chemiluminescent hybridization versus microwell absorbance).

The use of vaginal swab, cervical swab, and urine specimens from each woman allowed us to establish an infected-patient standard to more accurately evaluate the relative sensitivity and specificity of detection by each NAAT with each specimen type. In an elegant study comparing *C. trachomatis* detection assays, Martin et al. (26) assayed cervical swab and urine specimens from 1,410 women by each of three NAATs (PCR, ligase chain reaction, and TMA) and showed that the apparent sensitivity and specificity of each of these assays was drastically affected by the infected-patient gold standard used. In the study of Martin et al. (26), the best sensitivity and specificity estimates were obtained by using a definition of at least one specimen positive by each of two comparator assays, which was the same definition that we used to define sensitivity and specificity. In our study, the most sensitive specimen type for the detection of *M. genitalium* was a self-obtained vaginal swab (84% by TMA, 91% by PCR), followed by a clinician-obtained cervical swab (60% by TMA, 53% by PCR) and patient-collected urine (58% by TMA, 65% by PCR). Because only three positive specimens (two by PCR, one by TMA) were from patients who were not positive for *M. genitalium* at any site by the TMA assay and at any site by PCR in our study, the relative specificity of detection of *M. genitalium* for all sites by both assays was >99%. Supplementing the testing of vaginal swab specimens with testing of urine or cervical swab specimens would increase the sensitivity of detection by 2 to 11%; but this would increase the cost of testing twofold, unless these two specimen types were combined in the same assay. Although the PCR procedures of Jensen et al. (17) were different from ours and they did not test vaginal swab specimens, Jensen et al. (17) also noted differences in the rates of detection of *M. genitalium* in different genital specimen types (88% in urine specimens, 71% in cervical swab specimens, and 57% in urethral swab specimens) among women positive at any site.

Our finding that vaginal swab specimens are relatively more sensitive than cervical swab or urine specimens for the detection of *M. genitalium* in women is consistent with the findings of studies that have ranked the optimal specimen type for the detection of *C. trachomatis* and *N. gonorrhoeae*, other genital pathogens with disease spectrums similar to those of *M. genitalium*. Schachter et al. (33) found that the rates of detection of *C. trachomatis* by ligase chain reaction, PCR, and TMA assays were the highest with vaginal swab specimens (93%) and endocervical swab specimens (91%) but were lower with first-void urine specimens from women (81%); the specificities obtained with all specimens with either assay were above 99%. Similarly higher sensitivities have been observed with vaginal swab specimens than with urine and/or cervical swab specimens for the detection of *C. trachomatis* in other studies (32, 34, 40) and for the detection of *N. gonorrhoeae* (32, 34). Many studies have shown that self-collection of vaginal swab specimens is well accepted by women (12, 15, 28) and that the results obtained with such specimens are comparably sensitive to those obtained with clinician-obtained vaginal specimens, making them the specimen type of choice for laboratory testing (5, 12, 15, 32, 40). The use of self-obtained vaginal swabs also allows women to be screened in nontraditional clinical settings, such as at street clinics, community sites, and home, thereby increasing the number of patients who can be tested (11, 31). These self-obtained specimens, which can be collected without the assistance of a clinician and mailed to laboratories for analysis, would facilitate programs for screening for this emerging pathogen.

The differential detection of *M. genitalium* by specimen type may reflect differences in the bacterial load between vaginal swab, cervical swab, and urine specimens. Although we do not know the true preferred site of infection or the relative density of *M. genitalium* at different genital sites in women, Blaylock et al. (4) determined that *M. genitalium* is associated with vaginal epithelial cells and resides intracellularly, supporting colonization at this site. Several studies have shown an association of *M. genitalium* with cervicitis (1, 25, 30, 39) and urethritis in women (1), yet the interaction of *M. genitalium* at these sites, as well as the bacterial loads among women with and without these syndromes, has not yet been determined. Other factors specific to the specimen type might affect the sensitivity of detection. For example, to collect cervical swab specimens, clinicians typically remove the overlying mucus before collecting the underlying epithelium for testing by PCR. This procedure was originally designed for optimal culture of *C. trachomatis* but has an unknown effect on the detection of *M. genitalium*. In addition, in many studies multiple cervical swab specimens are collected from each patient, likely reducing the amount of sample and, possibly, the sensitivity of detection per specimen. In our study, the cervix was sampled for the detection of *M. genitalium* after specimens were collected for testing for patient management, including testing by Gram stain, culture, and/or NAAT for *N. gonorrhoeae* and *C. trachomatis*. These multiple samplings may have limited the quantity of the material for *M. genitalium* detection and thus the sensitivity of *M. genitalium* detection in cervical specimens. Factors affecting the sensitivity of detection in urine specimens include the time since last urination and the patient's ability to collect the first-void urine specimen (the first 30 ml). The detection of *M. genitalium* in female urine specimens may be dependent upon contamination with vaginal or cervical secretions or may be enhanced by urethral infection in a subset of women (1), which may increase the organism load and therefore the ability to detect the organism.

Specimen handling before testing, specimen preparation procedures, and the volume of original specimen used in the assay are additional factors that might affect the sensitivities of NAATs. In our study, we stored the patient specimens at 4°C before testing within 1 week to avoid freeze-thaw cycles and to remain consistent with the methods used for testing for this organism in specimens obtained through the "ordinary mail" in Denmark (18); however, this storage method may have limited our sensitivity of detection of the organism in female urine specimens. Because *M. genitalium* lacks a cell wall, it may be especially sensitive to lysis in urine and the subsequent degradation of the NAAT targets. While TMA transport media are designed to stabilize RNA during shipment and storage, potentially circumventing this problem, these collection media were not used in our study until after one freeze-thaw cycle, since this was a retrospective analysis of previously collected specimens. Finally, the amount of original specimen analyzed by NAAT undoubtedly affects the sensitivity of the assay and limits the ability to concentrate the specimen without intro-

ducing inhibition. In our study, the amount of the original specimen used for PCR was 62.5 μl of the 30-ml urine specimen requested, 62.5 μl of the 1-ml vaginal swab specimen, and 12.5 μl of the 1-ml cervical swab specimen, whereas the amounts were 200 μl for urine and 100 μl for the vaginal and cervical swab specimens for analysis by the TMA assay. Thus 0.2%, 6.25%, and 1.25% of the urine, vaginal swab, and cervical swab specimens, respectively, were used in the PCR assay. Similarly, 0.67%, 10%, and 10% of the urine, vaginal swab, and cervical swab specimens, respectively, were used in the TMA assay.

While the TMA and PCR assays demonstrated remarkably similar detection abilities with all specimen types, it is possible that the TMA assay might have performed even better had the specimens been placed directly into APTIMA transport medium to stabilize the RNA, as recommended by the manufacturer. Both the TMA and the PCR assays were performed with frozen-thawed specimens, which could have resulted in a lower sensitivity of detection for both assays. Even without the potential advantages of direct inoculation of specimens into APTIMA medium, the TMA assay method proved to be an efficient tool for the identification of *M. genitalium* infection. The TMA assay is partially automated, which allows the processing, amplification, and detection of up to 100 specimens in 1 day by one technician.

Future studies are required to investigate the effects of specimen variables, as outlined above. Such studies include those investigating whether alternate specimen-handling protocols affect the sensitivity of detection of *M. genitalium*; whether men and women carry different loads of this organism; and whether women without signs of infection carry a lower organism load, similar to that of men, possibly increasing the magnitude of the association of *M. genitalium* with reproductive tract disease. The use of validated *M. genitalium*-specific NAATs, particularly the high-throughput TMA assay, as described above, should facilitate future studies of the epidemiologic significance and clinical associations of this emerging pathogen.

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ADDENDUM IN PROOF

Subsequent to the submission of the manuscript, another study was published in which an in-house multiplex PCR assay for *M. genitalium* was compared to the Gen-Probe TMA assay for detection of this organism (J. Hardick, J. Giles, A. Hardick, Y.-H. Hsieh, T. Quinn, and C. Gaydos, J. Clin Microbiol. **44:**1236–1240, 2006).

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