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Nucleic Acid Amplification Tests for Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Rectal Infections▽

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It is uncertain which methods for the diagnosis of rectal gonococcal and chlamydial infection are optimal. This study evaluated the performance of culture and nucleic acid amplification tests (NAATs) for rectal chlamydial and gonococcal diagnosis. From July 2003 until February 2007, 441 rectal test sets were collected from individuals attending a sexually transmitted disease clinic and three HIV clinics who gave a history of anal intercourse or were women at high risk for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* infections. Rectal swab specimens were tested using culture and commercial NAATs employing transcription-mediated amplification (TMA), strand displacement amplification (SDA), and PCR amplification. Test performance was evaluated using a rotating standard by which patients were classified as infected if either two or three comparator tests were positive. Test sensitivities for the detection of *N. gonorrhoeae* ranged from 66.7% to 71.9% for culture to 100% for TMA. Specificities were 99.7% to 100% for culture and greater than 95.5% for all three NAATs. Test sensitivities for *C. trachomatis* ranged from 36.1% to 45.7% for culture and among NAATS from 91.4% to 95.8% for PCR to 100% for TMA. Specificities of the NAATs ranged from 95.6% to 98.5% (two-of-three standard) and from 88.8% to 91.8% (three-of-three standard). Over 60% and 80% of gonococcal and chlamydial infections, respectively, among men who have sex with men and over 20% of chlamydial infections in women would have been missed if the rectal site had not been tested. Currently available NAATs are more sensitive for the detection of chlamydial and gonococcal infection at the rectal site than is culture.

Historically, the focus of sexually transmitted disease (STD) diagnostic testing has been on the development and evaluation of tests for diagnosis of genital infections. However, a substantial proportion of the population engages in nongenital (e.g., oral or anal) sexual activity. For instance, a recent study conducted at three U.S. STD clinics found that 37% of heterosexual clients reported having practiced anal intercourse and over a quarter (28.9%) reported this activity with at least one of their last three sexual partners (4). These clinic-based findings are consistent with results of population-based studies indicating that 30% to 40% of U.S. men and women had ever engaged in anal sex with an opposite-sex partner (11, 13). In addition, among men who have sex with men (MSM), there is evidence that nongenital sites may serve as (often asymptomatic) reservoirs of gonococcal and chlamydial infection, with one study demonstrating infection rates of 7% for gonorrhea and 8% for chlamydia; among men with urethral, pharyngeal, and rectal exposures, 54% of chlamydial infections and 21% of gonococcal infections involved only the rectum (10). In an earlier study utilizing chlamydia culture techniques, Jones and colleagues found that 6.4% (24 of 373) of women with chlamydial infection harbored the organism at the rectal site only and therefore would have been missed in the absence of rectal chlamydia testing (9).

In recent years, culture testing for genital STDs has been largely supplanted by use of nonculture tests, yet no nonculture tests are currently approved for use at the rectal site. In addition, as outlined above, increased appreciation of the potential import of nongenital STDs has contributed to the need for sensitive and specific tests to diagnose rectal gonococcal and chlamydial infections. Nucleic acid amplification tests (NAATs) have been found to be more sensitive than culture for detection of genital gonococcal and chlamydial infections, but there are limited comparative data on the performance of commercially available NAATs for diagnosis of extragenital STDs. To help address these deficits, we conducted a study to compare the performances of culture and NAATS commercially available in the United States. Although these tests are not cleared by the U.S. Food and Drug Administration (FDA) for oral or rectal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* diagnosis, some clinical laboratories are utilizing these tests on rectal specimens after performing the limited in-house verification study required by the Clinical Laboratory Improvement Act (1). To help address deficits in the critical evaluation of the performance of NAATs for diagnosis of extragenital gonococcal and chlamydial infections, we conducted a study comparing the performances of culture and NAATs commercially available in the United States. The results for diagnosis of oral infections have been reported (2);

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this paper describes the results with respect to diagnosis of rectal gonorrhea and chlamydial infection.

(Part of these data were presented at the 2006 CDC STD Prevention Conference in Jacksonville, FL.)

MATERIALS AND METHODS

From July 2003 until February 2007, patients were recruited at four participating clinical sites, including a county STD clinic (males and females), a county hospital-based HIV clinic (males and females) in Birmingham, AL, and university hospital-based HIV clinics (males only) in Birmingham, AL, and Chicago, IL.

At each site participants were eligible for enrollment if they were over the age of 15, had engaged in receptive anal sex within the preceding 2 months, and were willing and able to sign written informed consent. Due to high rates of concurrent rectal infection in women with cervical gonococcal and chlamydial infections (15, 17), female participants were also eligible for enrollment in the absence of a history of anal sex if they presented to the clinic as a contact to sexual partners with *N. gonorrhoeae*, *C. trachomatis*, or nongonococcal urethritis (NGU) or if they presented for treatment for a previously collected but untreated positive gonorrhea or chlamydia test. Participants were ineligible if they had received antibiotics active against *N. gonorrhoeae* and/or *C. trachomatis* within 30 days of study enrollment.

After submitting a written informed consent, each participant answered a brief survey, and four rectal swabs were collected from each participant. Each rectal swab was inserted approximately 5 cm into the rectum, and rotated against the rectal wall several times. Swabs grossly contaminated with feces were discarded, and collection was repeated. Swabs used for specimen collection included the following: a cotton-tipped swab for inoculation onto gonorrhea culture media, a Dacron swab for chlamydia culture and PCR (Roche Cobas), and specimen collection swabs contained in the test kits for Gen-Probe Aptima Combo 2 (TMA) and BD ProbeTec ET (SDA) amplified DNA assays, respectively. Following collection, specimens were stored according to manufacturers' instructions and were transported to the University of Alabama at Birmingham STD Research Laboratory daily for testing. Swab order was rotated at 3-month intervals throughout the study period.

Patients recruited at the STD clinic underwent oral (if participants reported oral exposure) and routine genital testing on the day of enrollment according to clinic protocol. Genital testing was performed using a gonococcal culture transformation test (Gonostat; Sierra Diagnostics, Inc., Sonora, CA) and chlamydia cell culture or, in July 2005, a Gen-Probe APTIMA Combo 2 assay which tests for both *N. gonorrhoeae* and *C. trachomatis*. Oral testing was performed using gonococcal and chlamydial culture. Genital testing on participants recruited from HIV clinics was performed at their primary care provider's discretion. When genital testing did occur, a NAAT was utilized. Patients with one or more positive tests and who had not received appropriate therapy on the day of enrollment were asked to allow the study staff to recollect all four swabs at the time that they presented for treatment, though the recollected test sets were not included in the current analysis. All study procedures were approved by the Institutional Review Boards of the Centers for Disease Control and Prevention (CDC), the University of Alabama at Birmingham, the Cooper Green Hospital, the Northwestern University, and the Alabama Department of Public Health.

For *N. gonorrhoeae* culture, modified Thayer-Martin medium was directly inoculated and incubated at 35°C in 5% CO_2 within 30 min of collection, examined after overnight incubation, and, if negative, examined daily for another 2 days. Typical colonies containing Gram-negative diplococci and giving a positive oxidase reaction were presumptively identified as *N. gonorrhoeae* and subcultured onto chocolate agar. The identity of presumptive *N. gonorrhoeae* was confirmed using the NET (Remel) (3).

For chlamydial culture, swabs were placed directly into collection tubes containing 1.5 ml of 0.2 M sucrose-phosphate buffer transport medium (2-SP) containing 5% fetal bovine serum and antibiotics and stored at 4°C. Swabs for chlamydial rectal culture were placed in 2-SP transport medium with two times the vancomycin concentration used for specimens collected from other sites to prevent nonchlamydial bacterial overgrowth (Jeanne Moncada, UCSF Chlamydia Laboratory, personal communication). Chlamydial transport medium was held at 4°C until it was used to inoculate DEAE-dextran-treated McCoy cells for culture in a 96-well microtiter plate culture system as previously described (8).

Chlamydia cultures were set up in triplicate, and blind passage was performed on cultures that were negative after initial incubation. Cultures were read using commercially available monoclonal reagents. Specimens for PCR and tissue culture were placed in 2-SP transport medium from July 2003 until July 2005, when the laboratory switched to M4 transport medium following in-house validation studies that demonstrated 100% concordance of results between specimens processed from M4 and 2-SP transport media. Testing for *N. gonorrhoeae* and *C. trachomatis* was performed using TMA (Gen-Probe Inc. V 5.16, San Diego, CA), the Roche Cobas Amplicor assay (PCR; Roche Diagnostics Systems Inc. V 2.0, Pleasanton, CA), and SDA (Becton Dickinson and Co. V3.11B, Sparks, MD). SDA and PCR tests were performed with internal inhibition controls. TMA utilized a target capture step prior to amplification rather than an internal amplification control. Retests following initial equivocal or indeterminate results were performed on the original specimens as prescribed in the package inserts. For TMA and SDA, the test was repeated. If a second equivocal result was obtained, the final result was classified as unevaluable. For PCR, the test was repeated in duplicate following equivocal results. The final result was classified as positive, negative, or unevaluable based on all three test results (initial and duplicate repeats).

Questionnaire data were directly scanned into the study database by using TeleForm software (Cardiff v. 8.2, Vista, CA). Genital *N. gonorrhoeae* and *C. trachomatis* test results obtained through standard patient care were abstracted through medical record review. A rectal test set (for *N. gonorrhoeae* and/or *C. trachomatis*) included the following: culture, TMA, PCR, and SDA. Gonococcal and chlamydial rectal test sets were analyzed separately. Rectal test sets were eligible for inclusion in this analysis if they were complete and if all four tests results were either positive or negative. All test sets recollected within 30 days of enrollment were excluded as were test sets that were recollected when a participant returned for one or more untreated positive test results, even if the recollection occurred outside the 30-day window. Participants with negative test sets may have had more than one test set included in the analysis if the subsequent swab collection took place 30 days or more following the initial collection. Test sensitivity and specificity were calculated using a rotating standard which compared each test under evaluation with a performance standard that classified subjects as infected if two or more of the three remaining comparator tests were positive (2, 12). A second standard was applied in which each test was compared to a performance standard that classified subjects as infected only if all three of the remaining comparator tests were positive. The 95% confidence interval was calculated based on exact binomial distribution. All the statistical analyses were performed using SAS (SAS OnlineDoc 9.1.3; SAS Institute Inc., Cary, NC).

RESULTS

From July 2003 through February 2007, 441 test sets were collected from 377 individuals. Of these test sets, 375 gonococcal test sets were contributed by 334 eligible individuals, and 387 chlamydial test sets were contributed by 351 individuals that were evaluable for the purposes of analysis. Among the 66 excluded rectal gonorrhea test sets, 22 (33.3%) were excluded because one or more tests in the set contained a result that was neither positive nor negative (i.e., unevaluable results for PCR [17 sets], TMA [three sets], or SDA [two sets]), nine (13.6%) sets were excluded due to incomplete collection of the set, 12 (18.2%) were excluded because they were recollections immediately following a positive test, and 12 (18.2%) were excluded due to collection <30 days following a previously negative test set for gonorrhea at the rectal site. Among the 54 excluded rectal chlamydial test sets, 17 (31.5%) were recollections following a positive test, 12 (22.2%) were excluded because one or more tests in the set contained a result that was neither positive nor negative (i.e., nine sets due to unsatisfactory or inconclusive culture, and one set each for unevaluable PCR, TMA, and SDA results), eight (14.8%) sets were excluded due to incomplete collection of the set, and six (11.1%) were excluded due to collection <30 days following a previously negative test set for chlamydia at the rectal site. Eleven sets from both the gonococcal and chlamydial test sets were excluded due to lack of rectal exposure within the defined 2-month time frame (16.7% and 20.4%, respectively).

The study population (Table 1) differed significantly by gen-

TABLE 1. Study population (n = 396)

Characteristic[e]	Value(s) for[d]: Male (n = 297)	Female (n = 99)	P value
Age (median [range])	37.5 (16–76)	23 (16–44)	<0.0001
Race			<0.0001
Black	118 (39.7)	86 (86.9)	
White	172 (57.9)	12 (12.1)	
Other	5 (1.7)	1 (1.0)	
Missing	2 (0.7)	0 (0)	
Clinic type			<0.0001
HIV, university (AL)	153 (51.5)	0 (0)	
HIV, university (IL)	31 (10.4)	0 (0)	
HIV, county	41 (13.9)	5 (5.1)	
STD, county	72 (24.2)	94 (94.9)	
No. of partners during last 2 months (Median, range)	2 (1–99)	5 (1–99)	0.0011
Gender of partner during last 2 months			0.29
Female	2 (0.7)	1 (1.0)	
Male	283 (97.3)	93 (93.9)	
Both	5 (1.7)	5 (5.1)	
Missing	1 (0.3)	0 (0)	
Positive for rectal symptoms[a]	34 (11.6)	3 (3.0)	<0.0001
Reason for enrollment[b]			
Rectal exposure in last 2 months			<0.0001
Yes	297 (100.0)	40 (40.4)	
No	0 (0)	59 (59.6)	
Contact with[c]:	NA	26 (26.3)	NA
CT	NA	7 (26.9)	
GC	NA	14 (53.8)	
NGU	NA	6 (23.1)	
Untreated for:	NA	36 (36.4)	NA
CT	NA	26 (72.2)	
GC	NA	8 (22.2)	
CT and GC	NA	2 (5.6)	
Positive for:			
Genital CT	3/142 (2.0)	23/84 (27.4)	<0.0001
Genital GC	7/137 (5.1)	16/79 (20.3)	0.0005
Oral CT	4/225 (1.7)	1/52 (1.9)	0.94
Oral GC	18/213 (8.3)	4/49 (8.2)	0.98

[a] Five men were missing data.
[b] More than one category may have applied.
[c] More than one contact category may have applied.
[d] Values in fields are numbers of patients (%), unless otherwise indicated. NA, not applicable.
[e] CT, *C. trachomatis*; GC, *N. gonorrhoeae*.

der in terms of age, racial group, and recruitment site. As specified by enrollment criteria, all of the men reported rectal exposure. Among women, rectal exposure was reported by 40.4%. Among the 99 female patients, 26 (27.3%) reported contact with gonorrhea, chlamydia, or NGU, while 36 (36.4%) were enrolled because they presented to clinic with untreated gonorrhea and/or chlamydia (Table 1). Seven (25.9%) women reporting contact with STD, and two (7.4%) women presenting with untreated chlamydial infection also reported rectal sexual activity (data not shown). Symptoms consistent with rectal infection were reported in a minority of the study population (11.6% of men and 3% of women).

Test sensitivity was first calculated using a rotating standard which compared each test under evaluation with a performance standard defined as any two positive tests of three comparator tests (two-of-three standard). A second standard was applied in which each test was consecutively compared to a performance standard defined as three positive tests of three comparator tests (three-of-three standard). Based on a rotating gold standard of two of three comparator tests being positive, test sensitivity for the detection of *N. gonorrhoeae* was 66.7% (95% confidence interval [CI], 49.0% to 81.4%) for culture and, for the NAATs, ranged from 91.4% (95% CI, 76.9% to 98.2%) for PCR to 100% (95% CI, 89.4% to 100%) for TMA (Table 2). Specificities were high at 100% (95% CI, 98.9% to 100%) for culture and greater than 98% for all three NAATs. Using the three-of-three reference standard, test sensitivities were 71.9% (95% CI, 53.3% to 86.3%) for culture, 95.8% (95% CI, 78.9% to 99.9%) for PCR, and 100% (95% CI, 85.2% to 100%) for TMA and SDA. Specificities remained high—99.7% (95% CI, 98.4% to 100%) for culture—but were substantially lower—95.5% (95% CI, 92.7% to 97.4%) to 96.0% (95% CI, 93.4% to 97.8%)—for the NAATs (Table 2).

Similarly, when the performance for *C. trachomatis* detection was calculated, test sensitivity was only 36.1% (95% CI, 24.2 to 49.4%) for culture but, for NAATs, ranged from 80.7% (95% CI, 68.1% to 90.0%) for PCR to 100% (95% CI 92.5 to 100%) for TMA, utilizing the two-of-three reference standard, and, utilizing the three-of-three reference standard, was only 45.7% (95% CI, 30.9% to 61.0%) for culture but 95.5% (95% CI, 77.2% to 99.9%) for PCR and 100% (95% CI, 83.9% to 100%) for TMA and SDA. Specificities of the NAATs ranged from 95.6% (95% CI, 92.8% to 97.5%) to 98.5% (95% CI, 96.5% to 99.5%) when the two-of-three standard was utilized and from 88.8% (95% CI, 85.1% to 91.8%) to 91.8% (95% CI, 88.5% to 94.4%) for the three-of-three reference standard and were higher for culture with each standard (Table 2).

We calculated the prevalences of *N. gonorrhoeae* and *C. trachomatis* based on a composite test measure, with infection defined as a positive culture and/or two or more positive NAATs. Among men, all of whom reported rectal exposure, the prevalence of rectal *N. gonorrhoeae* infection was 7.9% (95% CI, 5.0 to 11.7%) based on the composite standard, and the prevalence of *C. trachomatis* infection was 10.3% (95% CI, 7.1 to 14.4%) (Table 3). Prevalence of *N. gonorrhoeae* in females ranged from 5.6% (95% CI, 0.7 to 18.7%) in women with rectal exposure to 19.2% (95% CI, 6.6 to 39.4%) in women reporting contact with an STD. *C. trachomatis* prevalence in women was 23.1% (95% CI, 11.1 to 39.3%) in women engaging in rectal sex and over 50% in women presenting for treatment for a previously diagnosed STD (Table 3).

One hundred fifty-one men and 84 women received chlamydial and/or gonococcal genital testing. With genital infection defined as a positive standard of care test and rectal infection defined as a positive culture and/or two or more positive NAATs, 19 gonococcal infections and 19 chlamydial infections were identified at the rectal and/or genital site in male participants (Table 4), with 63.2% of gonococcal infections and 84.2% of chlamydial infections present at the rectal site only. Among female participants with genital and rectal

TABLE 2. Estimates of SDA, PCR, TMA, and culture sensitivities and specificities for detection of *N. gonorrhoeae* or *C. trachomatis* by reference standard

Standard	Test	No. infected	% Sensitivity (95% CI)	No. uninfected	% Specificity (95% CI)
For *N. gonorrhoeae*					
Infected if any two of three comparator tests are positive; otherwise, uninfected	SDA	34	97.1 (84.7–99.9)	341	98.8 (97.0–99.7)
	PCR	35	91.4 (76.9–98.2)	340	98.5 (96.6–99.5)
	TMA	33	100.0 (89.4–100.0)	342	98.3 (96.2–99.4)
	Culture	36	66.7 (49.0–81.4)	339	100.0 (98.9–100.0)
Infected if all three comparator tests are positive; otherwise, uninfected	SDA	23	100.0 (85.2–100.0)	352	96.0 (93.4–97.8)
	PCR	24	95.8 (78.9–99.9)	351	96.0 (93.4–97.8)
	TMA	23	100.0 (85.2–100.0)	352	95.5 (92.7–97.4)
	Culture	32	71.9 (53.3–86.3)	343	99.7 (98.4–100.0)
For *C. trachomatis*					
Infected if any two of three comparator tests are positive; otherwise, uninfected	SDA	51	92.2 (81.1–97.8)	336	96.4 (93.8–98.1)
	PCR	57	80.7 (68.1–90.0)	330	98.5 (96.5–99.5)
	TMA	47	100.0 (92.5–100.0)	340	95.6 (92.8–97.5)
	Culture	61	36.1 (24.2–49.4)	326	99.7 (98.3–100.0)
Infected if all three comparator tests are positive; otherwise, uninfected	SDA	21	100.0 (83.9–100.0)	366	89.6 (86.0–92.6)
	PCR	22	95.5 (77.2–99.9)	365	91.8 (88.5–94.4)
	TMA	21	100.0 (83.9–100)	366	88.8 (85.1–91.8)
	Culture	46	45.7 (30.9–61.0)	341	99.4 (97.9–99.9)

test results available, 3 (15.8%) of the 19 with gonococcal infection and 7 (23.3%) of the 30 with chlamydial infection were infected only at the rectal site. Compared to females without rectal exposure, females reporting rectal sex were more frequently infected only at the rectal site. However, only 4 of the 19 females with gonococcal infection and 7 of the 30 females with chlamydial infection reported rectal exposure.

DISCUSSION

This study evaluating the performances of currently available NAATs and culture for detection of *N. gonorrhoeae* and *C. trachomatis* within the rectum demonstrates that each of the three NAATs is substantially more sensitive than is culture. Based on the standard of two of three comparator tests being positive, sensitivity for gonorrhea detection was only 66.7% for gonococcal culture but ranged from 91.4% to 100% for the NAATs. When the standard was three out of three comparator tests being positive, sensitivity was 71.9% for culture, but sensitivities ranged from 95.8% to 100% for the NAATs. The differences between test performances for chlamydia diagnosis were even more marked, in large part due to the low sensitivity of chlamydial culture. The low sensitivity of chlamydial culture found in this study (ranging from 36.1% to 45.7%) is consistent with the low chlamydial rectal culture sensitivities noted by Schachter and colleagues (26.5% to 39.1%, depending on the standard utilized) (14). These findings are also consistent with the superior sensitivity of NAATs documented at the genital site and support findings from Schachter and colleagues, who recently reported similar data using different methodology (14). They withdrew PCR early in their study due to a high oral false-positive rate for *N. gonorrhoeae* for oral specimens. The rectal specimen sensitivity estimates for PCR (44.4%) and SDA (77.8%) in their truncated sample (18 infected subjects) were lower than our estimates, which exceeded 91% for both tests. Much of the difference for SDA may have been due to their truncated sample size, since the final SDA sensitivity result for their full sample was 88.5% (78 infected subjects) when they used a gold standard that incorporated the evaluated tests and did not perform discrepant analysis (14). The truncated sample size for PCR and differences in gold standard probably account for some of the differences between our study and theirs in sensitivity estimates for PCR as well. While the specificities of PCR, SDA, and TMA in this study are slightly lower than the specificities of culture,

TABLE 3. Rectal *N. gonorrhoeae* and *C. trachomatis* prevalence by reason for enrollment

Test	No. of positive patients/total no. [%] (95% CI)			
	Male with rectal exposure	Female with rectal exposure	Female contact with STD	Female with untreated STD
Gonorrhea culture ± 2 or more positive NAATs	22/280 [7.9] (5.0–11.7)	2/36 [5.6] (0.7–18.7)	5/26 [19.2] (6.6–39.4)	6/36 [16.7] (6.4–32.8)
Chlamydial culture ± 2 or more positive NAATs	30/290 [10.3] (7.1–14.4)	9/39 [23.1] (11.1–39.3)	6/26 [23.1] (9.0–43.7)	19/35 [54.3] (36.7–71.2)

TABLE 4. Gonococcal or chlamydial infection by site among enrollees tested at both genital and rectal sites and infected at one or both sites

Site(s) positive for:	No. (%) of patients infected		
	Male with rectal exposure	Female	
		Without rectal exposure	With rectal exposure
Gonococcal or chlamydial infection			
Genital[a] and rectal[b] sites	2 (10)	9 (60.0)	1 (25)
Genital site only	5 (26.3)	4 (26.7)	2 (50)
Rectal site only	12 (63.2)	2 (13.3)	1 (25)
Chlamydial infection			
Genital[c] and rectal[d] sites	0 (0)	17 (73.9)	3 (42.9)
Genital site only	3 (15.8)	3 (13.0)	0 (0)
Rectal site only	16 (84.2)	3 (13.0)	4 (57.1)

[a] Genital infection defined as positive standard of care genital test (i.e., gonococcal culture, Gonostat, or NAAT). A total of 137 males and 79 females received genital and rectal testing for gonorrhea.
[b] Rectal infection defined as positive culture and/or two or more positive NAATs.
[c] Genital infection defined as positive NAAT. A total of 142 males and 84 females received genital and rectal testing for chlamydial infection.
[d] Rectal infection defined as positive culture and/or two or more positive NAATs.

they are still within an acceptable range for many clinical situations.

The specificities of PCR, SDA, and TMA that we obtained were lower than the specificities reported by Schachter and colleagues (14). These differences demonstrate the analytic challenges associated with the definition of "infection" in the absence of an agreed-upon gold standard and their impact on estimates of test sensitivity or specificity. Using any two positive of three comparator tests to define infection, culture detected only 66.7% and 36.1% of gonococcal and chlamydial infections, respectively, while when we applied the more rigorous standard requiring all three comparators to agree, the sensitivity of culture increased, but only to 71.9% (for gonorrhea) and 45.7% (for chlamydia). Estimated sensitivities for the NAATs also increased. At the same time, a consequence of using the more stringent definition of infection was a decline in estimated specificities. Had we chosen the least stringent definition of sensitivity, requiring only one of the three comparator tests to define infection, the estimated sensitivities of culture and the NAATs would have been still lower, while the estimated specificities would have been both higher and more stringently defined. Using the rotating standard for defining infection introduced similar biases into each estimate of sensitivity and specificity for each of the assays evaluated in our study. The higher specificity estimates obtained by Schachter and colleagues (14) resulted at least in part from their use of gold standards that incorporated the result of the evaluated test. Disagreement exists regarding the extent of the associated positive bias (5, 7). In the absence of an agreed-upon gold standard, it seems reasonable to presume that the true specificity of the NAATs falls between the estimates in this study and those in the study by Schachter and colleagues (14), which is acceptable for the screening and diagnostic testing of at-risk individuals. Importantly, in contrast to the case with pharyngeal specimens, neither study demonstrated the reduced specificity of Roche Cobas Amplicor PCR to detect *N. gonorrhoeae* in rectal specimens that had been observed previously with pharyngeal specimens (2, 14). Presumably, this difference can be attributed to the common occurrence of cross-reactive nongonococcal *Neisseria* bacteria in the pharynx and their uncommon occurrence in the rectum. Our study affirms the superiority of commercial NAATs for diagnosis of *N. gonorrhoeae* and *C. trachomatis* infections.

This study reinforces previous reports describing the high prevalence of rectal infection among MSM with rectal exposure, especially when tested by a NAAT (Table 3) (1, 6, 10). Moreover, as has been found in earlier studies (10), patients were not concordant for infection at all sites (Table 4). Over 60% of gonococcal infections and over 80% of chlamydial infections diagnosed in MSM study participants that had tests performed at both genital and rectal sites had positive rectal results only and would have been missed if the rectal site had not been tested. This study also leads to insights related to gonococcal and chlamydial prevalence among heterosexual women. The prevalences of rectal gonococcal infection were similar between male and female participants reporting rectal intercourse, while women enrolled because of contact with an STD or because of an untreated STD had the highest prevalence of rectal gonorrhea, a finding that may have been impacted by the fact that seven (25.9%) women reporting contact with STD and two (7.4%) women presenting with untreated chlamydial infection also reported rectal sexual activity and therefore resulted in some overlap in the indications for enrollment in the study (data not shown). The proportion of women infected with chlamydia at the rectal site was roughly two to over five times higher than men, depending on the enrollment group (Table 3). Interestingly, among females tested at both cervical and rectal sites, 15.8% of those with gonococcal infections and 23.3% of those with chlamydial infections had isolated positive rectal results, and among the small group of females reporting rectal sex, 25.0% of gonococcal infections and 57.1% of chlamydial infections would not have been identified if the rectal site had not been tested (Table 4). While the sample size of infected female participants in this study was limited and the application of several different enrollment criteria for the female population in this study may limit generalization of the findings, these data call for additional studies to evaluate the role of rectal gonococcal and chlamydial infections as a reservoir of undiagnosed STD in the female population. In addition, the findings from this study emphasize the importance for MSM of including questions about extragenital sexual activity in their sexual history and testing based on exposure. Since rectal infection in women is commonly a result of contiguous spreading from the genitalia, studies of women are needed that are designed to determine the contribution of rectal sexual exposure to the occurrence and transmission of isolated rectal infection.

Importantly, few individuals in this study population reported rectal symptoms. The absence of symptoms in the presence of rectal infection emphasizes the importance of screening based on a comprehensive history of sites of sexual exposure. If risk-based screening is not performed, it is only logical to conclude that many rectal infections will go undiagnosed and have the potential to serve as a reservoir of infection

for the community. While there are no specific guidelines to address rectal testing among female patients with exposure at this site, the 2006 CDC STD Treatment Guidelines currently recommend annual rectal gonococcal and chlamydial screening of HIV-infected and HIV-uninfected MSM who have practiced receptive anal sex within the preceding year, with more frequent screening for individuals who practice risky sexual behaviors (16). The data from this study not only reinforce the importance of these screening recommendations and the need for additional evaluation of screening at the rectal site in exposed females, but also emphasize the need to have more sensitive methodologies available for use when such screenings are conducted.

In conclusion, the rectum has the potential to be a significant reservoir for *N. gonorrhoeae* and *C. trachomatis* infection, and screening at this anatomic site is indicated in male and female patients exposed through sexual activity. The superior sensitivity and acceptable specificity of NAATs for the diagnosis of rectal chlamydial and gonococcal infections represent a significant step forward in the field of STD control, and therefore, FDA clearance should be sought for the use of these tests at the rectal site. In the meantime, clinical laboratories should consider conducting the necessary Clinical Laboratory Improvement Act verification studies to make these tests available for testing at-risk clients (1).

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