

Nucleic Acid Amplification Tests in the Diagnosis of Chlamydial and Gonococcal Infections of the Oropharynx and Rectum in Men Who Have Sex With Men

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Background: Several nucleic acid amplification tests (NAATs) are US Food and Drug Administration-cleared for detecting urogenital *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) infection, but they have not been adequately evaluated for the relatively common oropharyngeal or rectal CT and GC infections in men who have sex with men (MSM).

Methods: Multiple swabs were collected from the oropharynx and rectum of MSM attending a city sexually transmitted disease clinic. The specimens were tested by standard culture and the following NAATs: Roche's Amplicor (PCR), Becton Dickinson's ProbeTec (SDA), and Gen-Probe's APTIMA Combo 2 (AC2) for the detection of CT and GC. Confirmatory testing of specimens with discrepant results was done by NAATs using alternate primers.

Results: A total of 1110 MSM were enrolled. Based on initial findings on 205 MSM, PCR had a 78.9% GC specificity with oropharyngeal swabs. Thus, we discontinued PCR testing for the rest of the study. For oropharyngeal GC (89 infections detected), sensitivities were 41% for culture, 72% for SDA, and 84% for AC2. For rectal GC (88 infections detected), sensitivities were 43% for culture, 78% for SDA and 93% for AC2. For oropharyngeal CT (9 infections detected), sensitivities were 44% for culture, 67% for SDA, and 100% for AC2. For rectal CT (68 infections detected), sensitivities were 27% for culture, 63% for SDA, and 93% for AC2. Specificities of SDA and AC2 were $\geq 99.4\%$ for both organisms and anatomical sites.

Conclusions: AC2 and SDA were far superior to culture for the detection of CT or GC from the oropharynx and rectum with AC2 detecting twice as many infections as culture. Further analyses with larger pharyngeal samples are needed, but clearly NAATs can improve our ability to diagnose rectal and oropharyngeal infection with CT or GC in MSM.

MEN WHO HAVE SEX WITH MEN (MSM) and engage in risky behavior (unprotected oral and rectal sex, multiple partners) have a high prevalence of sexually transmitted diseases (STD).¹ Early detection and treatment of STDs in MSM may prevent transmission to sexual partners, serious sequelae, or HIV transmission. Pharyngeal swabs are indicated for patients with pharyngitis or who practice fellatio, whereas rectal specimens are indicated for

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patients with proctitis or who have a history of receptive anal intercourse.^{2,3} The Centers for Disease Control and Prevention guidelines recommend routine pharyngeal and rectal screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in MSM to control these infections.⁴

Currently, the standard for testing in extragenital sites is culture. For CT, this means isolation in tissue culture (TC), a labor intensive, time consuming, and costly test that is not readily available. For GC, this is done by inoculation of solid media and subsequent identification of colonies as oxidase-positive Gram-negative diplococci, followed by confirmation (e.g., carbohydrate utilization tests). Specimen handling is also critical for culture. Optimum GC culture requires incubation (36°C) of a selective medium in CO₂ or a candle jar immediately after specimen collection, whereas maintenance of a cold chain and a sensitive cell line is essential for CT culture. Neither procedure is suited for mass screening, nor is the direct Gram stain for GC appropriate, as it is insensitive for these sites.⁵

Nucleic acid amplification tests (NAATs) are highly sensitive and specific for the detection of CT and GC using swabs from the genital tract, or first catch urine specimens.^{6–9} These tests are widely available and have been used routinely for screening men and women. Assuming similar performance, it would be advantageous to use NAATs on rectal and pharyngeal swabs but none are US Food and Drug Administration cleared for use with extragenital specimens. Thus, to adhere to Clinical Laboratory Improvement Amendments regulations, laboratories must perform in-house validations to use NAATs with pharyngeal and rectal specimens. Previous studies have shown ligase chain reaction (Abbott Laboratories, North Chicago, IL, no longer manufactured) was sensitive and specific for GC from the pharynx and CT from the rectum.^{10,11} However, of the currently available NAATs, there are just a few anecdotal reports of testing Roche's Amplicor PCR (Branchburg, NJ) and Becton Dickinson's ProbeTec, (Sparks, MD) on extra genital sites,^{10,12–15} and none using Gen-Probe's APTIMA Combo 2 (San Diego CA). Extensive clinical evaluations have not been performed. Theoretically, NAATs should work on these specimen types. Recently, Lister et al.¹⁶ evaluated the specificity of the PCR

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assay and found that 94% (49/52) of COBAS positive Amplicor CT pharyngeal and rectal specimens collected from MSM were confirmed as positive by repeat PCR testing using the *omp1* gene as target.

We initially planned to evaluate the performance profile of Roche's Amplicor CT/NG Test (PCR), Becton Dickinson's ProbeTec Assay (SDA), and Gen-Probe's APTIMA Combo 2 Assay (AC2), on oropharyngeal and rectal swabs collected from MSM. Results were compared with standard culture and a combination of positive NAATs. Apparent NAAT false positives were resolved by further testing with another NAAT targeting alternate primers. After the first 205 MSM were evaluated, we discontinued the use of PCR because of poor GC specificity obtained with oropharyngeal specimens. The study was continued, to evaluate the SDA and AC2 assays.

Materials and Methods

Patient Population

Subjects consisted of 1110 MSM attending the San Francisco City STD Clinic. Patients on antibiotic therapy within the last 21 days were excluded from participation. The Committee on Human Research at the University of California San Francisco approved this study and verbal consent was obtained from all patients. MSM with positive tests for CT were treated orally with 1 g of azithromycin and those positive for gonorrhea were treated orally with 400 mg of cefpodoxime. Patients with Gram stain evidence of GC on their urethral smear were treated for both GC and CT. The clinic recommends repeat screening at 3 months; however, most fail to return and only return if symptoms worsen. There is no routine "test of cure" as none are recommended by Centers for Disease Control and Prevention or others.

Specimen Collection

We obtained oropharyngeal and rectal specimens from each patient. Swabs were collected according to a block randomization schedule. From each anatomical site, a dacron swab for CT isolation and NAATs was placed into 3 mL of M4 medium (Remel, Lenexa, KS) which contains vancomycin, amphotericin B, and colistin, and a cotton swab for GC culture was streaked onto a Thayer-Martin plate (Remel, Lenexa, KS). Inoculated M4 tubes were held at 4°C and transported in an ice chest within 24 hours of collection to the University of California San Francisco Chlamydia Laboratory, where CT culture and all NAATs were performed. GC plates were immediately placed into candle jars and incubated at 36°C. At the end of each day, GC cultures were transported to the San Francisco Public Health laboratory for final identification.

C. trachomatis Tissue Culture

CT isolation was carried out in cycloheximide-treated McCoy cells in one-dram shell vials using a modification of the procedure of Ripa and Mardh.¹⁷ M4 tubes were vortexed for 2 minutes, and then an aliquot was removed for NAATs. To reduce bacterial contamination in TC, rectal specimens were diluted 1 to 2 and 1 to 10 for inoculation onto McCoy cells. The cells were inoculated by centrifugation of inoculum at 3000g for 1 hour and incubated for 72 hours at 36°C in 5% CO₂ before monolayers were stained to detect chlamydial inclusions. We used the MicroTrak *C. trachomatis* Culture Confirmation Reagent, a species-specific fluorescent antibody stain (Trinity Biotech Plc, Wicklow, Ireland). A blind passage was performed 4 days post inoculation, and we read coverslips from that second pass 3 days post inoculation.

N. gonorrhoeae Isolation

Inoculated Thayer-Martin plates were incubated at 36°C in 5% CO₂ for 48 hours. Presumptive GC colonies were Gram stained, oxidase tested, and subcultured onto chocolate agar. Pure cultures were confirmed by carbohydrate reaction tests (api NH, bioMérieux, Marcy l'Etoile, France).

Standard NAATs

Before the study, the M4 media was validated for in-house use with each of the NAATs. For the PCR assay, 100 µL of the M4 sample was directly processed. For the SDA assay, 100 µL of the M4 sample was inoculated into a ProbeTec specimen transport tube and then processed. For the AC2 assay, 100 µL of the M4 sample was inoculated into an AC2 specimen transport tube and then processed. We followed the swab processing protocols in the individual NAAT package insert instructions. Technologists performing the NAATs were masked to any of the other results.

Alternate NAATs for Resolution

Specimens that were uniquely positive in 1 NAAT (negative by culture and other NAATs) received additional testing by a NAAT targeting alternate primers. For apparent false positive GC specimens, either a PCR or SDA targeting the pilin gene of *N. gonorrhoeae* or the APTIMA GC assay, which detects a different region of the 16s rRNA from AC2, was performed.¹⁸ For apparent false positive CT specimens, either a PCR or SDA targeting the *omp1* gene of *C. trachomatis* or the APTIMA CT assay, which detects the 16s rRNA, was performed.¹⁸ This testing was performed blind. The APTIMA GC assay and APTIMA CT assay were done in-house, whereas the alternate amplification with SDA and PCR were done by the manufacturers. They were supplied coded specimens with 2 negative specimens for each potentially positive specimen.

Definition of a True Positive

For CT, true positives were defined as culture positive or 2 positive NAATs at that anatomical site or a single NAAT positive confirmed by an alternate NAAT assay. For GC, true positives were defined as culture positive or AC2/PCR positive or AC2/SDA positive or a single NAAT positive confirmed by an alternate NAAT assay. Because GC false positives are known to occur with the PCR and SDA (both assays may cross react with other *Neisseria* spp.), a PCR/SDA only positive was not considered a true positive result.¹⁹⁻²¹

Results

We enrolled 1110 MSM in the study. The median age was 35.4 years, 91% identified as homosexual, 8% bisexual; 25% were HIV-positive. There were 672 (60.5%) symptomatic and 438 (39.5%) asymptomatic men. Of the 672 men with symptoms, 407 (60.6%) reported dysuria or urethral discharge, 14 (2.1%) rectal ulcers, 21 (3.1%) rectal discharge, 17 (2.5%) rectal warts, and 50 (7.4%) had a sore throat. The remaining 163 (24.3%) had other symptoms (rash, abdominal pain, genital irritation, etc.) More gonococcal infections were detected at both anatomical sites than chlamydial infections. There were 4 MSM infected with CT at both sites and 27 MSM infected with GC at both sites. Our preliminary evaluation (Table 1) of the first 205 MSM found that SDA and AC2 had good performance profiles, and were more sensitive than culture. However, PCR results obtained with the oropharyngeal swabs showed a 78.9% GC specificity. This result was unacceptable, as 39

TABLE 1. Preliminary Performance Profile of Culture and NAATs for the Detection of *N. gonorrhoeae* and *C. trachomatis* in Oropharyngeal and Rectal Specimens (n = 205)

| | Culture | SDA | AC2 | PCR |
|----------------------|------------------|------------------|------------------|------------------|
| Oropharyngeal | | | | |
| GC sensitivity | 60.0% (12/20) | 75.0% (15/20) | 95.0% (19/20) | 60.0% (12/20) |
| GC specificity | 100.0% (185/185) | 99.5% (184/185) | 100.0% (185/185) | 78.9% (146/185)* |
| CT sensitivity | 66.7% (2/3) | 33.3% (1/3) | 100.0% (3/3) | 33.3% (1/3) |
| CT specificity | 100.0% (200/200) | 100.0% (200/200) | 99.5% (199/200) | 100.0% (200/200) |
| Rectal | | | | |
| GC sensitivity | 50.0% (9/18) | 77.8% (14/18) | 100.0% (18/18) | 44.4% (8/18) |
| GC specificity | 100.0% (187/187) | 100.0% (187/187) | 99.5% (186/187) | 99.5% (186/187) |
| CT sensitivity | 35.3% (6/17) | 76.5% (13/17) | 100.0% (17/17) | 64.7% (11/17) |
| CT specificity | 100.0% (186/186) | 100.0% (186/186) | 100.0% (186/186) | 100.0% (186/186) |

CT true positives defined as culture positive or 2 positive NAATs at that anatomic site or a single NAAT positive confirmed by an alternate NAAT assay. GC true positives defined as culture positive or AC2/PCR positive or AC2/SDA positive or a single NAAT positive confirmed by an alternate NAAT assay.

* $P < 0.05$ for comparison with culture.

of 51 (76.5%) of the PCR positives were false positives. Thus, we discontinued the use of PCR for the rest of the evaluation.

By culture, prevalence of GC and CT was 3.3% (36/1077) and 0.4% (4/1110) in the pharynx, and 3.5% (38/1077) and 1.6% (18/1110) in the rectum, respectively. Use of NAATs for GC identified an additional 53 oropharyngeal and 50 rectal positive specimens, thereby increasing the prevalence to 8.3% and 8.2%. For CT, 5 more oropharyngeal and 50 rectal positive specimens were detected, increasing the prevalence to 0.8% and 6.1% by NAAT testing.

Table 2 shows the performance profiles of culture, SDA and AC2 for the detection of *N. gonorrhoeae*. There were 13 (7 oropharynx and 6 rectum) GC culture positives that were NAAT negative. Discrepant analysis on apparent false positives (solely positive by 1 NAAT) confirmed an additional 23 more pharyngeal and 10 more rectal GC infections. Resolved SDA and AC2 sensitivities were 71.9% and 84.3% for the pharynx, and 78.4% and 93.2% for the rectum. After the uniquely AC2 positive specimens were confirmed as true positives the AC2 detected more GC infections than any of the other tests. GC specificities of the AC2 and SDA assays were $\geq 99.4\%$ for both specimen types. Table 3 shows the performance profiles of culture and the NAATs for the detection of *C. trachomatis*. There were too few oropharyngeal positive (only 9) specimens to allow conclusions as to performance. We had 2 rectal CT culture positives that were NAAT

negative. With rectal specimens, SDA and AC2 were more sensitive than TC. Following confirmatory testing, again AC2 detected more infections than SDA or culture. Confirmatory testing of unique positive NAATs identified 22 more (2 in SDA, 20 in AC2) rectal CT infections. Resolved SDA and AC2 sensitivities were 63.2% and 92.7% for the rectum. CT specificities were $\geq 99.6\%$ with both oropharyngeal and rectal specimens.

Table 4 shows the resolution of all positive results. Using an alternate amplification, AC2 confirmed more specimens than SDA. There was no inhibition detected with the internal controls for the SDA and PCR assays with either specimen type.

Figure 1 shows prevalence of infection by symptomatic and asymptomatic status. More chlamydial rectal infection was seen in asymptomatic than symptomatic MSM. However, the opposite was seen with GC. We found more gonococcal infections (oropharynx and rectum) in symptomatic MSM. Table 5 shows NAAT sensitivities were better than culture regardless of symptoms for GC and CT detection. In general, culture performed better on symptomatic MSM compared to asymptomatic MSM.

Discussion

We evaluated all 3 commercially available NAATs for the detection of CT and GC with oropharyngeal and rectal specimens

TABLE 2. Final Performance Profile of Culture and NAATs for the Detection of *N. gonorrhoeae* in Oropharyngeal and Rectal Specimens (n = 1077)

| | Culture | SDA | AC2 |
|--------------------------|--------------------|----------------------------|----------------------------|
| Oropharyngeal | | | |
| Sensitivity* | 54.5% (36/66) | 87.9% (58/66) [†] | 87.9% (58/66) [†] |
| Specificity* | 100.0% (1011/1011) | 98.9% (1000/1011) | 97.7% (988/1011) |
| Sensitivity [‡] | 40.5% (36/89) | 71.9% (64/89) [†] | 84.3% (75/89) [†] |
| Specificity [‡] | 100.0% (988/988) | 99.5% (983/988) | 99.4% (982/988) |
| Rectal | | | |
| Sensitivity* | 48.7% (38/78) | 88.5% (69/78) [†] | 92.3% (72/78) [†] |
| Specificity* | 100.0% (999/999) | 99.9% (998/999) | 98.7% (986/999) |
| Sensitivity [‡] | 43.2% (38/88) | 78.4% (69/88) [†] | 93.2% (82/88) [†] |
| Specificity [‡] | 100.0% (989/989) | 99.9% (988/989) | 99.7% (986/989) |

*Compared to culture positive or 2 positive NAATs.

[†] $P < 0.05$ for comparison with culture.

[‡]Following confirmation of uniquely positive NAAT result using an alternate primer.

TABLE 3. Final Performance Profile of Culture and NAATs for the Detection of *C. trachomatis* in Oropharyngeal and Rectal Specimens (n = 1110)

| | Culture | SDA | AC2 |
|----------------------|--------------------|--------------------|-------------------|
| Oropharyngeal | | | |
| Sensitivity* | 57.1% (4/7) | 85.7% (6/7) | 100.0% (7/7) |
| Specificity* | 100.0% (1103/1103) | 100.0% (1103/1103) | 99.6% (1099/1103) |
| Sensitivity† | 44.4% (4/9) | 66.7% (6/9) | 100.0% (9/9)‡ |
| Specificity† | 100.0% (1101/1101) | 100.0% (1101/1101) | 99.8% (1099/1101) |
| Rectal | | | |
| Sensitivity* | 39.1% (18/46) | 89.1% (41/46) | 93.5% (43/46)‡ |
| Specificity* | 100.0% (1064/1064) | 99.8% (1062/1064) | 97.7% (1040/1064) |
| Sensitivity† | 26.5% (18/68) | 63.2% (43/68) | 92.7% (63/68)‡ |
| Specificity† | 100.0% (1042/1042) | 100.0% (1042/1042) | 99.6% (1038/1042) |

*Compared to culture positive or 2 positive NAATs.

†Following confirmation of uniquely positive NAAT result using an alternate primer.

‡P < 0.05 for comparison with culture.

in MSM. Based on 205 NAAT comparisons, PCR had a GC specificity of 78.9% with oropharyngeal swabs which would preclude its use with this specimen type. This result is probably because of the detection of genes shared with other *Neisseria* species. It is known that false positive results can occur with SDA and PCR as their target may cross react with several different species of *Neisseria* (*N. cinerea*, *N. flavescens*, *N. lactamica*, *N. subflava*, and *N. sicca*).^{19–21} However, this was not the case with rectal specimens where PCR specificity was 99.5%, and SDA specificity was 100%. Table 1 shows PCR had lower sensitivities compared with the other NAATs, but was equal to GC culture and better than CT culture. The internal control was performed with PCR; missed positives were not because of inhibition. That result may be in part because of the amount of processed specimen amplified. Test sensitivity may have been adversely affected because PCR uses the smallest volume, only 50 μ L, whereas AC2 and SDA use 400 μ L and 150 μ L, respectively. Our results indicated that PCR would have limited application in a MSM population, as GC and CT should be tested for simultaneously with both oropharyngeal and rectal specimens. We therefore discontinued PCR testing after these 205 MSM were evaluated.

NAATs identified more oropharyngeal and rectal GC infections than did culture in our total sample of 1110 MSM (Table 2). Our findings are similar to results found at this same clinic in which

twice as many pharyngeal *N. gonorrhoeae* infections were detected with ligase chain reaction compared with culture.¹¹ However, not all NAATs have comparable performance profiles. Clearly, confirmatory testing gives a more accurate picture of test performance.^{22,23} Using an alternate amplification assay on sole positives (Table 4), the majority of AC2 apparent false positives (17 oropharyngeal and 10 rectal) were confirmed as true positives. Although 6 additional oropharyngeal positives were identified and confirmed by SDA, AC2 still detected more infections than SDA. Other studies using urogenital specimens have shown that AC2 is more sensitive and specific compared with other NAATs.^{7,18} Thus, these results are not surprising. Overall, AC2 had the best performance for GC detection at both anatomical sites. It should be noted that we used Thayer-Martin plates (containing vancomycin) for the isolation of GC; and there are reports of *N. gonorrhoeae* strains that are inhibited by vancomycin.^{24,25} Thus, if there were any vancomycin susceptible GC strains in our study group, these strains would probably be missed by culture and detected by NAAT. Because of the higher bacterial (and yeast) load from these anatomical sites compared with the male urethral, the use of a selective medium was necessary.

Prevalence of CT by culture was 0.4% and 1.6% in the oropharynx and rectum, respectively. Using NAATs with the same specimen types resulted in a 2 \times increase of positives with oropharyngeal

TABLE 4. Distribution *N. gonorrhoeae* and *C. trachomatis* Positives by Specimen Type and Test

| Organism (n)* | Test | Specimen | Positive | Concordant† | Confirmed‡ | TP | FP |
|---------------|---------|------------|----------|-------------|------------|----|----|
| GC (89): | Culture | Oropharynx | 36 | 29 | NA | 36 | 0 |
| GC (89): | SDA | Oropharynx | 69 | 58 | 6 | 64 | 5 |
| GC (89): | AC2 | Oropharynx | 81 | 58 | 17 | 75 | 6 |
| GC (88): | Culture | Rectum | 38 | 32 | NA | 38 | 0 |
| GC (88): | SDA | Rectum | 70 | 69 | 0 | 69 | 1 |
| GC (88): | AC2 | Rectum | 85 | 72 | 10 | 82 | 3 |
| CT (9): | Culture | Oropharynx | 4 | 4 | NA | 4 | 0 |
| CT (9): | SDA | Oropharynx | 6 | 6 | NA | 6 | 0 |
| CT (9): | AC2 | Oropharynx | 11 | 7 | 2 | 9 | 2 |
| CT (68): | Culture | Rectum | 18 | 16 | NA | 18 | 0 |
| CT (68): | SDA | Rectum | 43 | 41 | 2 | 43 | 0 |
| CT (68): | AC2 | Rectum | 67 | 43 | 20 | 63 | 4 |

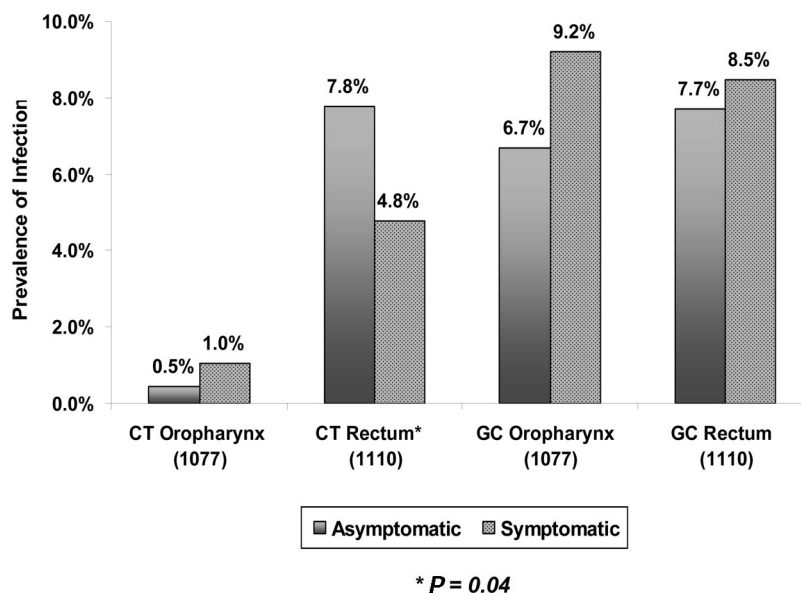
*Infections detected.

†With 1 or 2 of the other tests.

‡Confirmed by another NAAT using an alternate primer.

TP indicates true positive; FP, false positive; NA, not applicable.

Fig. 1. Prevalence of chlamydial and gonococcal infection in symptomatic versus asymptomatic men who have sex with men by anatomical site.



specimens (4 to 9) and almost a 4× increase with rectal specimens (18 to 68). The large increase seen, especially with the rectal specimens, may in part be because these specimens are heavily contaminated with bacteria and may be toxic to the cells in TC, thus inhibiting isolation in cell culture. Only 9 true positive oropharyngeal CT specimens were identified. This number is too small to discuss NAAT sensitivities. NAAT CT specificities were $\geq 99.8\%$ for the oropharynx. With rectal CT specimens (Table 3), AC2 had the best performance profile (sensitivity of 92.7%) compared with SDA (sensitivity of 63.2%). Here AC2 detected an additional 20 rectal positives following discrepant analysis (Table 4). Both NAATs were more sensitive than rectal cultures and were highly specific ($\geq 99.6\%$).

In Tables 2 and 3 we present the performance profiles of the NAATs with and without the use of discrepant analysis. That analytic approach has been criticized as upwardly biased, increasing both sensitivity and specificity.²⁶ Unfortunately lacking a true gold standard, there are few options as to analytic methods. If discrepant analysis is not performed, the “excess” positives seen with any more sensitive test will be considered to be false positives, as the other tests will not provide concordant positive results.

Thus, there is a trade off here: one accepts the misclassification bias and considers confirmable unique results as false positives, or one accepts the bias inherent in discrepant analysis. The distribution of results that we obtained was predictable, given earlier studies that show the higher sensitivity of the AC2 assay, when compared with SDA.^{7,18}

Although more CT rectal infections were seen in asymptomatic than symptomatic MSM, a similar pattern was not seen with GC infections (Fig. 1). Kent et al.¹³ also had comparable findings, her study found a higher amount of chlamydia infection in the rectum of asymptomatic MSM. As expected, culture performed better on symptomatic MSM, but NAAT performances varied by specimen type and symptom status. Regardless of whether MSM had symptoms or not, NAATs were more sensitive than culture for the detection of CT and GC with oropharyngeal and rectal specimens (Table 5).

In conclusion, it is feasible to use oropharyngeal and rectal specimens for the identification of CT and GC by AC2 or SDA. Performance profiles of NAATs do vary and there are limitations with the PCR assay; it is unsuitable for GC detection with the oropharyngeal swab. Introduction of more specific primers for

TABLE 5. Sensitivities of Culture and NAATs for *C. trachomatis* and *N. gonorrhoeae* Detection in Symptomatic vs. Asymptomatic Men Who Have Sex With Men

| | GC Sensitivity* | | CT Sensitivity* Rectum |
|------------------|----------------------------|----------------------------|----------------------------|
| | Oropharynx | Rectum | |
| Symptomatic MSM | | | |
| Culture | 43.5% (27/62) | 49.1% (28/57) | 29.4% (10/34) |
| SDA | 77.4% (48/62) [†] | 77.2% (44/57) [†] | 76.5% (26/34) [†] |
| AC2 | 87.1% (54/62) [†] | 93.0% (53/57) [†] | 91.2% (31/34) [†] |
| Asymptomatic MSM | | | |
| Culture | 33.3% (9/27) | 32.3% (10/31) | 23.5% (8/34) |
| SDA | 59.3% (16/27) | 80.6% (25/31) [†] | 50.0% (17/34) [†] |
| AC2 | 77.8% (21/27) [†] | 93.5% (29/31) [†] | 94.1% (32/34) [†] |

*Following confirmation of apparent false positives by NAAT using an alternate primer.

[†] $P < 0.05$ for comparison with culture.

MSM indicates men who have sex with men.

confirmation (or primary detection) would be useful. Only a small number of positive CT oropharyngeal specimens were evaluated; further analyses with a larger sample size are warranted to obtain more precise performance estimates. It is clear from this study that NAATs are useful with these specimens, and that many more CT and GC infections will be detected than can be identified with culture. However, 100% NAAT sensitivities were not obtained. It is possible that optimal NAAT performance may only be achieved with specimens collected in the transport medium designed for each specific NAAT, and this may have resulted in some of our false negative NAATs. Even so the AC2 test detected twice as many CT and GC infections from both anatomical sites as were found by culture. It would be an important advance in the control of STDs, if manufacturers would seek US Food and Drug Administration-clearance for testing samples from these anatomical sites.

References

- Klausner JD, Wong W. Sexually transmitted diseases in men who have sex with men: A clinical review. *Curr Infect Dis Rep* 2003; 5:135–144.
- Kim AA, Kent CK, Klausner JD. Risk factors for rectal gonococcal infection amidst resurgence in HIV transmission. *Sex Transm Dis* 2003; 30:813–817.
- Rietmeijer CA, Patnaik JL, Judson FN, et al. Increases in gonorrhea and sexual risk behaviors among men who have sex with men: A 12-year trend analysis at the Denver Metro Health Clinic. *Sex Transm Dis* 2003; 30:562–567.
- Centers for Disease Control and Prevention. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections—2002. *MMWR* 2002; 51(RR15):1–27.
- Janda WM, Gaydos CA. *Neisseria*. In: Murray PR, Baron EJ, Jorgensen JH, et al. eds. *Manual of Clinical Microbiology*, 9th ed. Washington, DC: ASM Press, 2007:601–621.
- Martin DH, Cammarata C, Van Der Pol B, et al. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae*. *J Clin Microbiol* 2000; 38:3544–3549.
- Moncada J, Schachter J, Hook EW III, et al. The effect of urine testing in evaluations of the sensitivity of the Gen-Probe APTIMAO Combo 2 Assay on endocervical swabs for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: The infected patient standard reduces sensitivity of single site evaluation. *Sex Transm Dis* 2004; 31:273–277.
- Van Der Pol B, Ferrero D, Buck-Barrington L, et al. Multicenter evaluation of the BDProbeTec™ system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs and male urethral swabs. *J Clin Microbiol* 2001; 39:1008–1016.
- Van Der Pol B, Quinn TC, Gaydos CA, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 2000; 38:1105–1112.
- Golden MR, Astete SG, Galvan R, et al. Pilot study of COBAS PCR and ligase chain reaction for detection of rectal infections due to *Chlamydia trachomatis*. *J Clin Microbiol* 2003; 41:2174–2175.
- Page-Shafer K, Graves A, Kent C, et al. Increased sensitivity of DNA amplification testing for the detection of pharyngeal gonorrhea in men who have sex with men. *Clin Infect Dis* 2002; 34:173–176.
- Cook RL, St George K, Silvestre AJ, et al. Prevalence of chlamydia and gonorrhoea among a population of men who have sex with men. *Sex Transm Infect* 2002; 78:190–193.
- Kent CK, Chaw JK, Wong W, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. *Clin Infect Dis* 2005; 41:67–74.
- Lister NA, Smith A, Tabrizi S, et al. Screening for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in men who have sex with men at male-only saunas. *Sex Transm Dis* 2003; 30:886–889.
- Morris SR, Klausner JD, Buchbinder SP, et al. Prevalence and incidence of pharyngeal gonorrhea in a longitudinal sample of men who have sex with men: The EXPLORE study. *Clin Infect Dis* 2006; 43:1284–1289.
- Lister NA, Tabrizi SN, Fairley CK, et al. Validation of Roche COBAS Amplicor assay for detection of *Chlamydia trachomatis* in rectal and pharyngeal specimens by an *omp1* PCR assay. *J Clin Microbiol* 2004; 42:239–241.
- Ripa KT, Mardh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977; 6:328–331.
- Chernesky MA, Martin DH, Hook EW III, et al. Ability of new APTIMA CT and APTIMA GC assays to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male urine and urethral swabs. *J Clin Microbiol* 2005; 43:127–131.
- Becton, Dickinson and Co. Package insert—BDProbeTec™ *Chlamydia trachomatis* and *Neisseria gonorrhoeae* amplified DNA assays. Sparks, MD: Becton, Dickinson and Co., 2001. Cat No. 440450.
- Farrell DJ. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using *cppB* nested PCR and 16S rRNA PCR. *J Clin Microbiol* 1999; 37:386–390.
- Van Der Pol B, Martin DH, Schachter J, et al. Enhancing the specificity of the COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae* by retesting specimens with equivocal results. *J Clin Microbiol* 2001; 39:3092–3098.
- Schachter J, Chow JM, Bolan G, et al. Detection of *Chlamydia trachomatis* by nucleic acid amplification testing: Our evaluation suggests that CDC-recommended approaches for confirmatory testing are ill-advised. *J Clin Microbiol* 2006; 44:2512–2517.
- Schachter J, Hook EW III, Martin DH, et al. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: All NAATs are not created equal. *J Clin Microbiol* 2005; 43:1372–1373.
- Brorson JE, Holmberg I, Nygren B, et al. Vancomycin-sensitive strains of *Neisseria gonorrhoeae*. A problem for the diagnostic laboratory. *Br J Vener Dis* 1973; 49:452–453.
- Mirrett S, Reller LB, Knapp JS. *Neisseria gonorrhoeae* strains inhibited by vancomycin in selective media and correlation with auxotype. *J Clin Microbiol* 1981; 14:94–99.
- Hadgu A. Bias in the evaluation of DNA-amplification tests for detecting *Chlamydia trachomatis*. *Stat Med* 1997; 16:1391–1399.