Confirming Positive Results of Nucleic Acid Amplification Tests (NAATs) for *Chlamydia trachomatis*: All NAATs Are Not Created Equal

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The Centers for Disease Control and Prevention recommended confirming positive screening tests for *Chlamydia trachomatis* when positive predictive values are <90%. It is accepted that less sensitive tests (i.e., culture and immunoassays) should not be used to confirm the results of more sensitive nucleic acid amplification tests (NAATs). We show that the same principle applies when NAATs are used for confirmation.

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen (4). It causes adverse reproductive consequences in women and conjunctivitis and pneumonia in infants exposed to the organism at birth. Screening women in family planning or in obstetrics and gynecology clinics and treatment of those infected with *C. trachomatis* prevents pelvic inflammatory disease and neonatal infections (7, 8). Because most genital chlamydial infections are asymptomatic, Centers for Disease Control and Prevention (CDC) guidelines call for routine screening of sexually active women between ages 15 and 24.

Nucleic acid amplification tests (NAATs) are the most sensitive tests for the screening and diagnosis of genital chlamydial infections (6). NAATs are more sensitive than previously available diagnostic tests (culture, antigen detection, or nucleic acid hybridization) by at least 20 to 30%. That NAATs can be used with noninvasively collected specimens, such as first-catch urine samples (FCU) from men or women and self- or clinician-collected vaginal swabs, improves our ability to screen for chlamydia.

As more screening for genital infections has been done by using NAATs, concerns have been raised about their specificity, particularly in screening low-prevalence populations. In some studies, positive NAAT results could not be reproduced (1–3, 5, 9). There are obvious concerns about the social ramifications of incorrectly informing individuals that they have a sexually transmitted disease based on false-positive screening test results. These concerns led the CDC to recommend confirmatory testing of positive test results when the positive predictive value is <90% (4). Several strategies for confirmation

were suggested. One was to perform a second NAAT, targeting a different nucleic acid sequence, with either the original specimen or a specimen collected in duplicate.

Gen-Probe, Inc., has introduced three assays based on its transcription-mediated amplification technology: the APTIMA CT assay (ACT), which detects *C. trachomatis*, the APTIMA GC assay (AGC), which detects *Neisseria gonorrhoeae*, and the APTIMA COMBO 2 assay (AC2), which detects both pathogens. The ACT and AGC oligonucleotide probes target rRNA sequences different from those for the AC2. These tests are thus each well suited for confirming results obtained with the other. During a clinical assessment of the performance of these assays, we had an opportunity to evaluate the confirmation of NAAT-positive results by using other NAATs.

The study population consisted of 1,465 females and 1,322 males who attended sexually transmitted disease, obstetrics and gynecology, teen, or family planning clinics in seven centers in North America. FCU and endocervical swabs (CS) from women and urethral swabs and FCU from males were collected and tested by ACT and AC2. The FCU and a duplicate swab from each subject were also tested with BDProbeTec ET System *C. trachomatis* assay (BD; Becton Dickinson and Co. Diagnostics, Sparks, Md.). With females, FCU were collected first, and then two randomized CS were collected. In males, two randomized urethral swabs were collected first, followed by the FCU. Thus, three NAATs, each having a different target, were used to test specimens from each subject. For this evaluation, the results from the BD, ACT, and AC2 were each considered as primary screening tests, and positive results for each test were then confirmed by the two other tests. The APTIMA assays do not have a control for inhibitors; the BD test does have such a control. All results presented here were valid (there were no indeterminate results due to inhibition).

Of all of the male and female swab specimens and FCU tested, there were 850 positive results with the AC2, 927 positive results with the ACT, and 720 positive results with the

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TABLE 1. Confirming positive results in nucleic acid amplification tests

Specimen type and test	Confirmatory test	No. tested and % positive for group indicated ^a		
		Male	Female	Total
FCU				
AC2		235	174	409
	ACT	96.6%	98.3%	97.3%
ACT	BD	86.8%	83.3%	85.3%
		248	191	439
BD	AC2	91.5%	89.5%	90.7%
	BD	80.6%	77.0%	79.0%
		212	155	367
	AC2	96.2%	94.2%	95.4%
	ACT	94.3%	92.9%	93.7%
Swab				
AC2		246	195	441
	ACT	98.4%	99.5%	98.9%
ACT	BD	78.0%	80.0%	78.9%
		264	224	488
BD	AC2	90.9%	86.6%	88.9%
	BD	72.7%	70.0%	71.5%
		194	159	353
	AC2	99.0%	98.1%	98.6%
	ACT	100.0%	98.7%	99.4%
Both specimen types				
AC2		481	369	850
	ACT	97.5%	98.9%	98.1%
ACT	BD	82.3%	81.6%	82.0%
		512	415	927
BD	AC2	91.2%	88.0%	89.8%
	BD	76.6%	73.3%	75.1%
		406	314	720
	AC2	97.5%	96.2%	96.9%
	ACT	97.0%	95.9%	96.9%

^a %, percent confirmed.

BD. Both the ACT and AC2 confirmed 96.9% of the positive results with the BD. Of the positive results with the AC2, 98.1% were positive with the ACT, but only 82% were positive with the BD. Of the positive results with the ACT, the AC2 confirmed 89.8%, but the BD confirmed only 75.1%. The positive results and confirmatory tests by sex and specimen type are shown in Table 1. There were no major differences in the observed patterns by sex or specimen type.

In the CDC guidelines, it was pointed out that less sensitive diagnostic tests, such as culture and enzyme immunoassays, should not be used to confirm positive results of the more sensitive NAATs for *C. trachomatis*. This guideline exists because 30% or more of specimens positive by NAATs will be negative by culture or enzyme immunoassay. Our study shows that the same principle applies when only NAATs are being used. Both APTIMA assays yielded more total and more confirmed-positive results than the BD assay. If reporting a positive result is based upon confirmation of the initial positive result, then using BD to confirm positive results by ACT or AC2 would result in the incorrect reporting of approximately 15% of confirmable positive results as negative (i.e., not confirmed).

In this evaluation, the ACT had the greatest number of positive results (927), and the BD had the least number of positive results (720). While a rule of thumb has been that the less sensitive test is more specific, that was not the case here (assuming that confirmation is a proxy for specificity). Approximately 97% of the 720 positive BD results were confirmed by the more sensitive AC2 and ACT. However, the AC2 had 850 positives, 98% of which were confirmed by ACT. The AC2 confirmed only about 90% of the positive results of the ACT. There are two obvious interpretations for the latter result. It may be that the ACT is less specific and that the AC2 failed to confirm positive results because they were false positives. The alternate explanation is that ACT is actually more sensitive than the other tests and that the inability to confirm its greater number of positive results reflects the lower sensitivity of the confirmatory assays. Further studies are needed to answer this question.

It is clear from our results that some NAATs cannot be used to confirm positive results from other NAATs. Even among NAATs, the assays employed should have equivalent sensitivities. The BD should not be used to confirm the APTIMA assays. If, however, the BD were used for the primary screening, the APTIMA assays could be used for confirmation (96.9% confirmed). Based on our results, we would recommend the AC2 for initial screening and the use of the ACT for confirmation, as 98% of AC2 positive results were confirmed by ACT. This strategy would identify more infected individuals.

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