JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 400–405 Vol. 44, No. 2
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.400–405.2006

High Analytical Sensitivity and Low Rates of Inhibition May Contribute to Detection of *Chlamydia trachomatis* in Significantly More Women by the APTIMA Combo 2 Assay

Max Chernesky,[1]* Dan Jang,[1] Kathy Luinstra,[1] Sylvia Chong,[1] Marek Smieja,[1] Wenjie Cai,[1] Beth Hayhoe,[2] Eder Portillo,[1] Cindy MacRitchie,[3] Cheryl Main,[3] and Ruth Ewert[2]

McMaster University, Hamilton, Ontario, Canada[1]; Evergreen Health Centre, Toronto, Ontario, Canada[2]; and Sexual Health Awareness Centre, Hamilton, Ontario, Canada[3]

Received 8 September 2005/Returned for modification 12 October 2005/Accepted 9 November 2005

The clinical sensitivity of nucleic acid amplification tests may be determined by analytical sensitivity and inhibitors in patient samples. We established endpoints for detection of propagated *Chlamydia trachomatis* L2 434, diluted according to swab and urine protocols for APTIMA Combo 2 (AC2), ProbeTec ET (PT), and Amplicor (AMP) assays. AC2 was 1,000-fold more sensitive than PT and 10-fold more sensitive than AMP on mock swab specimens. For urine, AC2 analytical sensitivity was 100-fold greater than those of the other assays. Spiking an aliquot of each clinical-trial sample from 298 women demonstrated inhibition rates in first-void urine (FVU), cervical swabs (CS), and vaginal swabs (VS) of 12.1%, 12.8%, and 10.4% for AMP; 27.2%, 2%, and 2%, for PT; and 0.3%, 1.7%, and 1.3% for AC2. Inhibition of our *C. trachomatis* spike and the PT or AMP amplification controls from the manufacturers showed less than 50% correlation. Using an infected-patient reference standard (a specimen positive in at least two tests or a single test positive in two of three samples) in AC2, the VS identified 68/69 (98.6%) infected women compared to CS (89.9%) or FVU (81.2%). Significantly fewer women were identified by PT (65.2%, 63.8%, and 66.7%) or AMP (65.2%, 59.4%, and 56.5%) with the three specimens. By individual specimen type, AC2 confirmed virtually all PT- and AMP-positive specimens, but rates of AC2 confirmation by AMP or PT ranged from 62.9 to 80.3%. The AC2 test identified significantly more women infected with *C. trachomatis* ($P = 0.001$). Vaginal swabs appear to be the specimen of choice for screening.

Chlamydia trachomatis is one of the most common sexually transmitted infections in the United States and worldwide (17), largely due to high rates of asymptomatic infection in the lower genital tracts of women and men (24). Early diagnosis followed by treatment of this infection can prevent upper genital tract infection, such as pelvic inflammatory disease (21).

For the past 10 years, a great deal of research has led to the development and evaluation of sensitive and specific diagnostic tests for *C. trachomatis*. These newer nucleic acid amplification tests (NAATs) have been commercialized and are now in routine use in many parts of the world. The important aspect of these assays is their capacity to be used on less invasive specimens, such as vaginal swabs (VS) and first-void urine (FVU), which can be self-collected. Studies have shown that NAATs performed on these less invasive samples are able to detect as many or more infected patients than traditional swabs from the urethra or cervix (2–4, 6–10, 19–22, 25–27, 31).

The analytical sensitivity of each test and its susceptibility to inhibitors in different specimen types may determine the clinical sensitivity of each test. We compared detection thresholds and determined the inhibitor and infection rates in three different specimens from 298 women by using three commercial assays for *C. trachomatis*.

* Corresponding author. Mailing address: St. Joseph's Healthcare, 50 Charlton Avenue East, Hamilton, ON L8N 4A6, Canada. Phone: (905) 521-6021. Fax: (905) 521-6083. E-mail: chernesk@mcmaster.ca.

MATERIALS AND METHODS

Specimens. Three cervical swabs (CS), three VS, and the first 30 ml of voided urine (FVU) were collected from 298 nonpregnant women, who signed a consent form while they attended a street youth clinic or the Sexual Health Awareness Centre. The order of swab collection was randomized, and urine was collected before insertion of the speculum. The swabs were collected in specimen collection tubes specific for each assay and transferred to the laboratory on the same day, stored at 4°C, and then tested within 48 to 72 h.

Determination of *Chlamydia trachomatis* concentrations for spiking samples. *C. trachomatis* L2 434 was propagated in McCoy cells for 72 h. Serial 10-fold dilutions from 10^0 to 10^{-10} of an elementary-body (EB) preparation which had been purified by differential centrifugation (5) were made in phosphate-free saline (16), and the EBs were counted by direct fluorescent-antibody staining with monoclonal antibodies specific for major outer membrane proteins of *C. trachomatis* (Syva Behring Microtrak, San Jose, CA). The 10^{-5}, 10^{-7}, and 10^{-8} dilutions contained an average of 100, l, and 0.1 EB per 100 μl, respectively. A volume of 100 μl of each dilution was added to a swab-saline specimen and an uninfected, noninhibitory urine specimen collected in assay transport tubes. The spiked specimens were tested in replicates of 16 to determine a concentration closest to the cutoff value of the test. One hundred microliters of the last dilution for the swab specimens and the urine to yield a positive signal in an assay in all 16 replicates was added (spiked) to each respective specimen type collected for the study prior to amplification testing.

Amplification assays. For the APTIMA Combo 2 (AC2) assay (Gen-Probe Incorporated, San Diego, CA), 400 μl of the "spiked" (*C. trachomatis* in diluent) or "unspiked" (an equal volume of diluent) urine or swab specimen was added to 100 μl of Target Capture Reagent (solution B), mixed, and incubated at 62°C for 30 min. The mixture was incubated at room temperature for another 30 min, allowing released *C. trachomatis* rRNA to bind to coated magnetic beads. The rack of tubes containing the rRNA-bound beads was subsequently placed on a magnetic Target Capture System for a series of washes, during which the captured target molecules were pulled to the side of the reaction vessel and the supernatant fluid was removed. Seventy-five microliters of AC2 Amplification Reagent and 200 μl of oil was added to the reaction tubes and incubated at 62°C

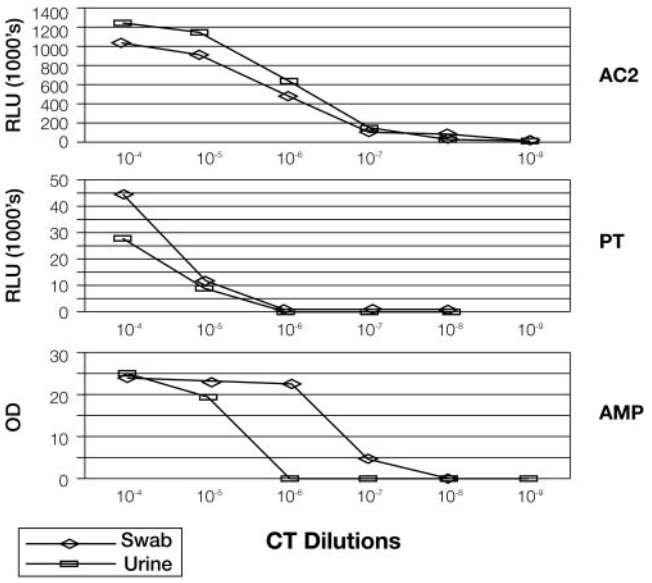

FIG. 1. RLU and optical densities (OD) of mock swab and urine specimens containing 10-fold dilutions of *C. trachomatis* (L2) tested by AC2, PT, and AMP.

for 10 min. The tubes were cooled to 42°C, and 25 μl of enzyme reagent was added prior to an incubation of 1 hour at 42°C for the transcription-mediated amplification reaction. Combo Probe Reagent (100 μl) was added, followed by an incubation of 20 min at 62°C. During this dual-kinetic-assay step, single-stranded chemiluminescent DNA probes labeled with acridinium ester molecules were combined with the amplicon to form stable RNA-DNA hybrids. Finally, 250 μl of selection reagent, which differentiates hybridized from unhybridized probes, was added, and the tubes were incubated at 62°C for another 10 min. After the tubes were allowed to cool to room temperature, the light emitted from the labeled RNA-DNA hybrids was measured as photon signals in the Leader HC+ Luminometer. The results were interpreted by computer software provided by the manufacturer.

For the Amplicor (AMP) assay (Roche Molecular Systems), 500 μl of urine wash buffer was added to the "spiked" and "unspiked" 500-μl urine aliquots and vortexed well, and then the aliquots were incubated at 37°C for 15 min. The samples were centrifuged for 5 min. The supernatant fluid was discarded, and the pellet was resuspended in 250 μl of lysis buffer. After a 15-min incubation at room temperature, 250 μl of specimen diluent was added. The sample was then centrifuged for 10 min. Five hundred microliters of specimen diluent was added to the 500 μl of the "spiked" and "unspiked" swab aliquots and vortexed well, and the mixture was incubated at room temperature for 10 min. Fifty microliters (each) of the prepared specimens was added to 50 μl of PCR Master Mix for amplification. Amplification and detection of the prepared specimens was carried out as outlined by the manufacturer with no modifications to the protocol.

For the ProbeTec assay (Becton Dickinson Bioscience), the urine-processing pouch was placed in the urine sample for at least 2 h. Two 4.0-ml aliquots (labeled unspiked and spiked) of each urine were pipetted into the tubes. The *C. trachomatis* spike (100 μl) was aliquoted into the spiked tubes, and diluent without the *C. trachomatis* spike was added to the unspiked tubes. All samples were processed and tested for *C. trachomatis* according to the manufacturer's package insert. Vaginal or cervical swabs were placed in the specimen diluent tubes, and material was expressed. A 1-ml sample of each swab was aliquoted into separate tubes as spiked or unspiked (100 μl). Both the unspiked and spiked samples were processed according to the cervical-swab-processing procedure from the package insert.

If a sample containing a *C. trachomatis* spike tested negative, it was considered inhibitory. The amplification controls for both the ProbeTec ET (PT) and the AMP assays were also performed according to the instructions in the package inserts.

Statistical analysis. Statistical analyses were performed using SPSS Software for Windows, version 11.5. The McNemar test was used for pairs of tests on the same specimens, and the Cochran Q test was used to compare three or more groups. A P value of <0.05 was deemed statistically significant.

RESULTS

Analytical sensitivity of each assay and determination of spiking concentrations. Figure 1 shows the relative light units (RLU) and optical densities of dilutions of *C. trachomatis* L2 (mock swab and urine specimens) in AC2, PT, and AMP. The endpoint of detection was 10^{-8} (0.1 EB) for swabs and 10^{-7} (1 EB) for urine in the AC2 test. In the PT assay, the endpoint for both sample types was 10^{-5} (100 EBs). The values for AMP were 10^{-5} (100 EBs) for urine and 10^{-7} (1 EB) for swabs. For swabs, the AC2 assay demonstrated 1,000-fold greater sensitivity than the PT test (10^{-8} versus 10^{-5}) and 10-fold greater sensitivity than the AMP test (10^{-8} versus 10^{-7}). For urine, the analytical sensitivity of AC2 was 100-fold greater than those of the other assays (10^{-7} versus 10^{-5}).

From these data, aliquots of CS and VS clinical samples were spiked with *C. trachomatis* L2 at a dilution of 10^{-7} (1 EB per 100 μl) for AC2, 10^{-4} (1,000 EBs) for PT, and 10^{-6} (10 EBs) for AMP testing. The spiking dilutions for aliquots of FVU from the enrolled patients were 10^{-6} (10 EBs) for AC2 and 10^{-4} (1,000 EBs) for PT and AMP testing.

Inhibitor rates in specimens. A total of 298 CS, VS, and FVUs were processed in each assay. To determine the inhibition rates of each specimen type for the three assays, we calculated the number of spiked specimens that recorded negative outcomes in the assays (Table 1). In the AC2 assay, low percent inhibition rates of 1.3, 1.7, and 0.3 were recorded for VS, CS, and FVU, respectively, compared to 2.0, 2.0, and 27.2 in the PT assay and 10.4, 12.8, and 12.1 in the AMP test. In a pairwise comparison (with the McNemar test), the percent inhibition rates in the VS and CS by the AMP assay were significantly different than in the other two assays ($P < 0.001$ and $P < 0.002$). For FVU, the AMP and PT assays showed more inhibition than the AC2 test ($P < 0.001$). In the PT assay, the percent inhibition in FVU was significantly higher than in the two swab types ($P < 0.001$). There were no statistically significant differences across specimen types for AC2 or AMP.

Comparison of inhibition rates determined by Amplicor and ProbeTec ET amplification controls and the *C. trachomatis* spike. Since both the PT and AMP assays provide amplification control reagents to detect amplification inhibitors in clinical specimens, an aliquot of each specimen was also tested with those reagents in the two tests. This allowed a comparison of the clinical samples determined to cause inhibition by the manufacturer's amplification control to our "in-house" L2 challenge. The results of the comparison of inhibitors in FVU in the PT assay are shown in Table 2. The data show the inhibition rates in both *C. trachomatis*-positive and -negative women. A total of 10 infected and 98 uninfected patients

TABLE 1. Number of VS, CS, and FVU found to have inhibitors to a *C. trachomatis* spike in AC2, PT, and AMP assays

Specimen (n = 298)	No. (%) positive by NAAT[a]			P
	AC2	PT	AMP	
VS	4 (1.3)	6 (2.0)	31 (10.4)	0.001
CS	5 (1.7)	6 (2.0)	38 (12.8)	0.002
FVU	1 (0.3)	81 (27.2)	36 (12.1)	0.001

[a] $P = 0.236$ for AC2, 0.001 for PT, and 0.036 for AMP.

TABLE 2. Inhibition rates in FVU determined by *C. trachomatis* spike and PTAC[a]

Control and/or spike used to test inhibition	No. (%) inhibited		
	C. trachomatis positive (n = 69)	*C. trachomatis* negative (n = 229)	Total (n = 298)
PTAC only	1 (1.4)	26 (11.3)	27 (9.1)
C. trachomatis spike only[b]	4 (5.8)	27 (11.8)	31 (10.4)
Both	5 (7.2)	45 (19.6)	50 (16.8)
Total	10 (14.5)	98 (42.8)	108 (36.2)

[a] PTAC, ProbeTec amplification control.
[b] An additional six vaginal swabs and six cervical swabs were found to be inhibitory only by the CT spike.

demonstrated inhibition in their FVU. Inhibition was found by both the *C. trachomatis* spike and the PT amplification control in 7.2% (5/69) of the infected and 19.7% (45/229) of the uninfected women, for a total of 16.8% (50/298). An additional 31 FVUs showed inhibition only by the *C. trachomatis* spike, and another 27 FVUs showed inhibition by the PT amplification control only. Thus, the agreement for FVU inhibition between the two amplification controls was only 46.3% (50/108). Urinary inhibitors were predominantly in uninfected women (42.8%), but 14.5% of FVUs from infected women were inhibitory and amplification control agreement in this subgroup was 50% (5/10). Examination of the comparisons in VS and CS revealed that six of each sample type were found to be inhibitory by the *C. trachomatis* spike but not the PT amplification control (data not shown).

A similar analysis of inhibition rates determined by the AMP amplification control and our *C. trachomatis* spike for all three specimen types from *C. trachomatis*-infected and uninfected women is shown in Table 3. In this comparison examination, agreements of inhibition determined by the two challenges to the three specimen types from 298 women were 46.5% (20/43) for FVU, 69.8% (30/43) for CS, and 15.2% (5/33) for VS. Proportionally, most of the inhibition appeared in specimens from uninfected women.

TABLE 3. Inhibition rates determined by a *C. trachomatis* spike and AMPAC[a]

Control and/or spike used to test inhibition	Sample	No. (%) inhibited		
		C. trachomatis positive (n = 69)	*C. trachomatis* negative (n = 229)	Total (n = 298)
AMPAC only	FVU	0 (0)	7 (3.0)	7 (2.3)
	CS	0 (0)	5 (2.2)	5 (1.7)
	VS	0 (0)	2 (0.9)	2 (0.7)
C. trachomatis spike only	FVU	2 (2.9)	14 (6.1)	16 (5.4)
	CS	0 (0)	8 (3.5)	8 (2.8)
	VS	1 (1.4)	25 (10.9)	26 (8.7)
Both	FVU	2 (2.8)	18 (7.8)	20 (6.7)
	CS	7 (10.1)	23 (10.0)	30 (10.0)
	VS	1 (1.4)	4 (1.7)	5 (1.6)
Total	FVU	4 (5.8)	39 (17.0)	43 (14.4)
	CS	7 (10.1)	36 (15.7)	43 (14.4)
	VS	2 (2.9)	31 (13.5)	33 (11.1)

[a] AMPAC, Amplicor amplification control.

TABLE 4. Positive samples according to assay for 69 *C. trachomatis*-positive women

Sample type(s) that yielded positive results:	No. positive by:		
	AC2	PT	AMP
VS, CS, and FVU	51	38	30
VS and CS	10	2	6
VS and FVU	4	3	5
CS and FVU	1	2	3
VS only	3	2	4
CS only	0	4	2
FVU only	0	3	1
Total	69	54	51

Clinical sensitivities of the assays performed on each specimen type. Performing AC2, PT, and AMP testing on VS, CS, and FVU from each woman allowed the declaration of an infected patient as having at least one specimen positive in more than one test or at least two specimens positive in a single test (two or more of nine specimen-test combinations). Table 4 shows the number of patients with positive results in the assays according to the number of different specimen types. A total of 51 patients had all three specimens positive by the AC2 assay, whereas 38 and 30 women had three samples positive by PT or AMP, respectively. In the three assays, there were 66 women by AC2, 45 by PT, and 44 by AMP who had at least two positive specimens. There were small numbers of infected patients with a single specimen positive (three VS by AC2; two VS, four CS, and three FVU by PT; and four VS, two CS, and one FVU by AMP). Thus, if a woman was infected with *C. trachomatis*, most of her specimen types were positive.

Table 5 summarizes the ability of each assay to detect infected women according to the clinical sample tested. The AC2 assay detected 98.6% (68/69) of the infected women by testing the VS, and the rates were 89.9% for CS and 81.2% for FVU testing. Comparatively, the detection rates for VS, CS, and FVU were 65.2%, 63.8%, and 66.7% by PT and 65.2%, 59.4%, and 56.5% by AMP. For each assay, the VS detected as many or more infected patients than the CS or FVU, except for the PT assay, where the FVU detected one more positive patient than the VS. All three specimens tested by AC2 identified more infected patients than either of the other two assays performed on any of the specimen types.

Examination of the abilities of the assays to confirm each other's positive results. Confirmation of VS, CS, and FVU positive results by other tests is shown in Table 6. For the 68 positive VS results by AC2, PT or AMP confirmed 45 (66.2%)

TABLE 5. Numbers[b] of women identified as infected with *C. trachomatis* by testing VS, CS, and FVU in AC2, PT, or AMP assay

Assay[a]	No. (%) detected by testing:			P
	VS	CS	FVU	
AC2	68 (98.6)	62 (89.9)	56 (81.2)	0.076
PT	45 (65.2)	44 (63.8)	46 (66.7)	0.946
AMP	45 (65.2)	41 (59.4)	39 (56.5)	0.385

[a] P = 0.001 for VS, CS, and FVU.
[b] A total of 69 women were infected, and percentages were calculated on this basis.

TABLE 6. Confirmation of positive VS, CS, and FVU by AC2, PT, and AMP

Specimen	Original positive		No. (%) confirmed positive by:		
	Test	*n*	AC2	PT	AMP
VS	AC2	68		45 (66.2)	45 (66.2)
	PT	45	45 (100)		42 (93.3)
	AMP	45	45 (100)	41 (91.1)	
CS	AC2	62		44 (70.9)	39 (62.9)
	PT	44	44 (100)		40 (90.9)
	AMP	41	41 (100)	40 (97.6)	
FVU	AC2	56		45 (80.3)	39 (69.6)
	PT	46	45 (97.8)		37 (80.4)
	AMP	39	39 (100)	36 (92.3)	

as positive. AC2 confirmed all PT- and AMP-positive VS. AMP confirmed 93.3% (42/45) of the PT-positive VS, and PT confirmed 91.1% (41/45) of the AMP-positive VS. Similar trends of confirmation were demonstrated for CS and FVU. AC2 failed to confirm one PT-positive FVU. PT was slightly better at confirming AMP-positive CS and FVU than was AMP at confirming PT positives.

Specificities of the assays. There were six VS, six CS, and five FVUs by AC2 and one CS by AMP which did not meet the criteria for positivity. If these are false positives, the specificities based on 229 true "negative" patients were 100% for AMP on VS and FVU and PT on all specimen types. The rates were 97.4% (223/229) for VS, 97.4% for CS, and 97.8% (224/229) for FVU in the AC2 test. The specificity of the AMP test on CS was 99.6% (228/229). Sufficient volumes for 14 of the 17 AC2 samples were available for retesting, and six repeated positive. Examination of the RLU values (data not shown) revealed that all were low positives.

DISCUSSION

By collecting multiple specimens from each patient and testing all of them in three different assays, we were able to create a reference standard of an infected patient and compare the ability of each assay to detect an infection according to the type of specimen tested. This type of study maneuver usually lowers the sensitivity of single specimen evaluations (13, 14). We elected to declare a patient infected if two or more samples were positive in at least one of the assays or if at least one sample was positive in more than one assay. Most of the women (66 by AC2, 45 by PT, and 44 by AMP) had two or more specimens positive, and most of those had all three specimens positive (Table 4). In the AC2 and AMP assays, the VS identified more infected women than the CS or FVU specimens tested by the respective assays, whereas the PT testing of VS detected the same number of infections as PT-tested CS. These observations are similar to published data (18, 19, 22) comparing VS to CS and FVU using AMP, ligase chain reaction, and AMP *C. trachomatis*, a first-generation transcription-mediated amplification test. Using a protocol for testing CS adapted to VS, as we did for the PT assay, Consentino et al. (6) showed, as we have, that VS identified the same number of *C. trachomatis*-positive patients as PT-tested CS.

Comparing the performance of each assay for each specimen type in our study (Table 5) showed that for VS, AC2 detected 23 more infected women than PT or AMP; for CS, AC2 detected 18 more than PT and 21 more than AMP. PT detected three more infections than AMP with CS. Urine testing showed AC2 detecting 17 more cases than AMP and 10 more cases than PT, which detected 7 more cases than AMP. These are significant and clinically important differences not observed in previous published studies, which may be explained by several factors. Most clinical comparisons have used two or three different assays on one or two specimen types (3, 8, 13–15, 26–29). Our study had the unique design of collecting three VS, three CS, and FVU from each patient and enabled us to expand the reference standard. This may account for some of the lower sensitivities seen for PT and AMP for the three specimen types. Moncada et al. (14) have shown that the infected-patient standard does reduce the sensitivity of single-anatomical-site evaluation. The performance of PT compared to AMP is similar to published data (3, 29), where AC2 results were not included.

Another factor which may have contributed to differences in clinical sensitivity is the analytical sensitivity of each assay. Serial 10-fold dilutions of *C. trachomatis* EBs showed that the AC2 test was able to detect positive swab samples which were 10- to 1,000-fold more dilute than those detected by PT or AMP. Ikeda-Dantsuji et al. (11) found similar large differences in *C. trachomatis* endpoint detection rates between AC2 and AMP. Chong et al. (5) showed that the AC2 test was able to detect *C. trachomatis* diluted to 0.01 EBs (10^{-9} dilution) compared to 12 EBs (10^{-5} dilution) detected by an LCx assay. The higher sensitivity rates for AC2 may be due to the increased levels of rRNA target by AC2 compared to the DNA target measured by AMP and PT (1). There are approximately 2,000 copies of rRNA in each EB, but approximately 10 copies of plasmids and fewer chromosomal DNA copies. Schachter et al. (18) have published similar findings in which AC2 detected more positive patients than PT by testing FVU and CS or urethral swabs, indicating a difference in clinical sensitivity between these assays.

The third factor which may have contributed to the large differences in clinical sensitivity could be the significant differences among the tests for inhibitory specimens. This study and a previous one (5) showed that the inhibitory rates of CS and FVU in AC2 were very low. We also now report that very few VS are inhibitory for the AC2 test. This outcome was expected, as the role of the target capture step in the AC2 assay is to purify the rRNA prior to amplification. Using the appropriately diluted spike to detect inhibitors in the three clinical-specimen types showed inhibitor rates of 2.0% in VS and CS but 27.2% in FVU tested by PT. Becton Dickinson (the manufacturer of PT) was undergoing difficulties with some lot numbers of the urine pouch used to concentrate *C. trachomatis* in urine. Our records indicated that we were not using those lot numbers, but further studies will be needed to confirm or contradict our finding of high inhibitor rates in FVU tested by PT. All three specimen types showed relatively high rates of inhibition for the AMP test. These findings are similar to the rates reported previously in CS and FVU in the AMP test (12).

By including approved amplification controls for the AMP and PT assays, we were able to compare inhibitors determined by these commercially available amplification controls and our *C. trachomatis* spike. The PT amplification control indicated an

inhibitor rate in FVU of 25.8% (77/298), and the rate was 27.2% (81/298) by our *C. trachomatis* spike. Only 46.3% (50/108) of the samples which were recorded as inhibitory by both reagents were the same specimens (Table 2). There were six VS and six CS which were inhibitory with our *C. trachomatis* spike but not by the PT amplification control. These differences are to be expected, since the PT amplification control is not specific for *C. trachomatis* inhibition. Our VS inhibitor rate with the PT reagent was 2% compared to 25% reported by Consentino et al. (6) in an earlier study examining the relevance of the PT amplification control for use with VS, and it may be due to methodological differences between the two studies or an improvement of the PT assay to avoid serious inhibition in VS.

There were significant rates of AMP inhibitors in different specimen types with our spike (Table 1). The inhibition rates of our *C. trachomatis* spike and the AMP amplification control were higher in the uninfected patients for all specimen types (Table 3). For all 298 women, the inhibition rates were lower by the AMP amplification control than by our *C. trachomatis* spike, with the most dramatic difference seen for VS. This may be due to the fact that our protocol for VS was experimental, and although our *C. trachomatis* spike was optimized, the AMP amplification control was not optimized for VS. The differences may also be due to the fact that our *C. trachomatis* spike was a dilution of EBs but the AMP amplification control was a plasmid DNA with primer regions identical to those of the *C. trachomatis* target, scrambled internal sequence, and a unique probe region (according to the AMP package insert). The data suggest that the concentration of plasmid DNA in the AMP amplification control may be slightly higher than optimal, as fewer inhibitors were shown by the AMP amplification control only ($n = 14$) than by spike only ($n = 50$). This phenomenon has been demonstrated previously (5, 12, 30).

Our calculations of specificity in this study revealed that, using the reference standard specified, the PT and AMP assays were 100% specific, or close to it. There were 18 women who were positive by AC2 in only one sample type. Sufficient volumes were available for retesting 14 by AC2, and 6 repeated positive. These were all originally reactive near the test cutoff for positivity and may have been true positives demonstrating an inability to repeat as positive according to the distribution of targets in the aliquot sampled (23). Another approach would have been to test these samples with alternate primers (1) if sufficient material had been available.

The concept of repeating all positives in one NAAT with another NAAT was examined in our study. For each specimen type the AC2 test was able to confirm most, and sometimes all, of the PT- and AMP-positive samples, whereas the less sensitive PT and AMP assays confirmed only 62.9% to 80.3% of the AC2-positive samples. The PT and AMP assays confirmed 80.4% to 97.6% of each other's positive samples, depending upon the specimen type. These findings partially confirm those reported by Schachter et al. (18). The dilemma is that sensitivity differences of the NAATs make it unclear whether the most sensitive test should be used for screening and treating patients, knowing that some positive results cannot be confirmed by the less sensitive tests, or whether screening should be done with less sensitive assays to ensure confirmation, at the risk of decreasing the effectiveness of screening programs by missing many infections.

In summary, using an infected patient as the reference standard, determined by collection of multiple specimens which were tested in several different assays, revealed that the greatest number of women infected with *C. trachomatis* were identified by performing an AC2 test on a vaginal swab and slightly fewer were identified by performing the AC2 test on a CS or an FVU. All three sample types tested individually by PT or AMP failed to identify more than 66.7% of the infections. These differences in clinical sensitivity are probably due to the superior analytical sensitivity of the AC2 assay and its target capture mechanism, rendering it virtually immune to amplification inhibitors.

REFERENCES

1. **Boyadzhyan, B., T. Yashina, J. H. Yatabe, M. Patnaik, and C. S. Hill.** 2004. Comparison of the APTIMA CT and GC assays with the APTIMA combo 2 assay, the Abbott LCx assay, and direct fluorescent-antibody and culture assays for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**3089–3093.
2. **Carder, C., A. J. Robinson, C. Broughton, J. M. Stephenson, and G. L. Ridgway.** 1999. Evaluation of self-taken samples for the presence of genital *Chlamydia trachomatis* infection in women using the ligase chain reaction assay. Int. J. STD AIDS **10:**776–779.
3. **Chan, E. L., K. Brandt, K. Olienus, N. Antonishyn, and G. B. Horsman.** 2000. Performance characteristics of the Becton Dickinson ProbeTec System for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male and female urine specimens in comparison with the Roche Cobas System. Arch. Pathol. Lab. Med. **124:**1649–1652.
4. **Chernesky, M., D. Jang, H. Lee, J. D. Burczak, H. Hu, J. Sellors, S. J. Tomazic-Allen, and J. B. Mahony.** 1994. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J. Clin. Microbiol. **32:**2682–2685.
5. **Chong, S., D. Jang, X. Song, J. Mahony, A. Petrich, P. Barriga, and M. Chernesky.** 2003. Specimen processing and concentration of *Chlamydia trachomatis* added can influence false-negative rates in the LCx assay but not in the APTIMA Combo 2 assay when testing for inhibitors. J. Clin. Microbiol. **41:**778–782.
6. **Consentino, L. A., D. V. Langers, and S. L. Hillier.** 2003. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by strand displacement amplification and relevance of the amplification control for use with vaginal swab specimens. J. Clin. Microbiol. **41:**3592–3596.
7. **Gaydos, C. A., A. Crotchfelt, N. Sha, M. Tennant, T. C. Quinn, J. C. Gaydos, K. T. McKee, Jr., and A. M. Rompalo.** 2002. Evaluation of dry and wet transported intravaginal swabs in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in female soldiers by PCR. J. Clin. Microbiol. **40:**758–761.
8. **Gaydos, C. A., M. Theodore, N. Dalesio, B. J. Wood, and T. C. Quinn.** 2004. Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. J. Clin. Microbiol. **42:**3041–3045.
9. **Gray, R. H., M. J. Wawer, J. Girdner, N. K. Sewankambo, D. Serwadda, M. Meehan, C. Gaydos, C. Li, and T. Quinn.** 1998. Use of self-collected vaginal swabs for detection of *Chlamydia trachomatis* infection. Sex. Transm. Dis. **25:**450.
10. **Hook, E. W., III, K. Smith, C. Mullen, J. Stephens, L. Rinehardt, M. S. Pate, and H. H. Lee.** 1997. Diagnosis of genitourinary *Chlamydia trachomatis* infections by using the ligase chain reaction on patient-obtained vaginal swabs. J. Clin. Microbiol. **35:**2133–2135.
11. **Ikeda-Dantsuji, Y., I. Konomi, and A. Nagayama.** 2005. *In vitro* assessment of the APTIMA Combo 2 assay for the detection of *Chlamydia trachomatis* using highly purified elementary bodies. J. Med. Microbiol. **54:**357–360.
12. **Mahony, J. B., S. Chong, D. Jang, K. Luinstra, M. Faught, D. Dalby, J. Sellors, and M. A. Chernesky.** 1998. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. J. Clin. Microbiol. **36:**3122–3126.
13. **Martin, D. H., M. Nsuami, J. Schachter, E. W. Hook III, D. Ferrero, T. C. Quinn, and C. Gaydos.** 2004. Use of multiple nucleic acid amplification tests to define the infected-patient "gold standard" in clinical trials of new diagnostic tests for *Chlamydia trachomatis* infections. J. Clin. Microbiol. **42:**4749–4758.
14. **Moncada, J., J. Schachter, E. W. Hook III, D. Ferrero, C. Gaydos, T. C. Quinn, D. Willis, A. Weissfeld, and D. H. Martin.** 2004. The effect of urine testing in evaluations of the sensitivity of the Gen-Probe Aptima Combo 2 assay on endocervical swabs for *Chlamydia trachomatis* and *Neisseria gonor-*

rhoeae: the infected patient standard reduces sensitivity of single site evaluation. Sex. Transm. Dis. **31:**273–277.
15. **Morré, S. A., I. G. M. VanValkengoed, R. M. Moes, A. J. P. Boeka, C. J. L. M. Meijer, and A. J. C. Van den Brule.** 1999. Determination of *Chlamydia trachomatis* prevalence in an asymptomatic screening population: performances of the LCx and COBAS Amplicor tests with urine specimens. J. Clin. Microbiol. **37:**3092–3096.
16. **Notomi, T., Y. Ikeda, A. Okadome, and A. Nagayama.** 1998. The inhibitory effect of phosphate on the ligase chain reaction used for detecting *Chlamydia trachomatis*. J. Clin. Pathol. **51:**306–308.
17. **Peipert, J. F.** 2003. Genital chlamydial infections. N. Engl. J. Med. **349:**2424–2430.
18. **Schachter, J., E. W. Hook, D. H. Martin, D. Willis, P. Fine, D. Fuller, J. Jordan, W. M. Janda, and M. Chernesky.** 2005. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: all NAATs are not created equal. J. Clin. Microbiol. **43:**1372–1373.
19. **Schachter, J., W. M. McCormack, M. A. Chernesky, D. H. Martin, P. B. Van Der, P. A. Rice, E. W. Hook III, W. E. Stamm, T. C. Quinn, and J. M. Chow.** 2003. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. J. Clin. Microbiol. **41:**3784–3789.
20. **Schachter, J., J. Moncada, R. Whidden, H. Shaw, G. Bolan, J. D. Burczak, and H. H. Lee.** 1995. Noninvasive tests for diagnosis of *Chlamydia trachomatis* infection: application of ligase chain reaction to first-catch urine specimens of women. J. Infect. Dis. **172:**1411–1414.
21. **Scholes, D., A. Stergachis, F. E. Heidrich, H. Andrilla, K. K. Holmes, and W. E. Stamm.** 1996. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. N. Engl. J. Med. **334:**1362–1366.
22. **Shafer, M.-A., J. Moncada, C. B. Boyer, K. Betsinger, S. D. Flinn, and J. Schachter.** 2003. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. J. Clin. Microbiol. **41:**4395–4399.
23. **Smieja, M., J. B. Mahony, C. H. Goldsmith, S. Chong, A. Petrich, and M. Chernesky.** 2001. Replicate PCR testing and probit analysis for detection and quantitation of *Chlamydia pneumoniae* in clinical specimens. J. Clin. Microbiol. **39:**1796–1801.
24. **Stamm, W. E.** 1999. *Chlamydia trachomatis* infections of the adult, p. 407–422. *In* K. K. Holmes, P.-A. Mardh, P. F. Sparling, et al. (ed.), Sexually transmitted diseases, 3rd ed. McGraw-Hill, New York, N.Y.
25. **Stary, A., B. Najim, and H. H. Lee.** 1997. Vulval swabs as alternative specimens for ligase chain reaction detection of genital chlamydial infection in women. J. Clin. Microbiol. **35:**836–838.
26. **Van der Pol, B., T. C. Quinn, C. A. Gaydos, K. Crotcheft, J. Schachter, J. Moncada, D. Jungkind, D. H. Martin, B. Turner, C. Peyton, and R. B. Jones.** 2000. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **38:**1105–1112.
27. **Van Der Pol, B., D. V. Ferrero, L. Buck-Barrington, E. Hook III, C. Lenderman, T. Quinn, C. A. Gaydos, J. Lovchik, J. Schachter, J. Moncada, G. Hall, M. J. Tuohy, and R. B. Jones.** 2001. Multicenter evaluation of the BDProbeTec ET system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. J. Clin. Microbiol. **39:**1008–1016.
28. **van Doornum, G. J. J., L. M. Schouls, A. Pijl, I. Cairo, M. Buimer, and S. Bruisten.** 2001. Comparison between the LCx probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. J. Clin. Microbiol. **39:**829–835.
29. **Van Dyck, E., M. Ieven, S. Pattyn, L. Van Damme, and M. Laga.** 2001. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by enzyme immunoassay, culture, and three nucleic acid amplification tests. J. Clin. Microbiol. **39:**1751–1756.
30. **Verkooyen, R. P., A. Luijendijk, W. M. Huisman, W. H. F. Goessens, J. A. J. W. Kluytmans, J. H. van Rijsoort-Vos, and H. A. Verbrugh.** 1996. Detection of PCR inhibitors in cervical specimens by using the AMPLICOR *Chlamydia trachomatis* assay. J. Clin. Microbiol. **34:**3072–3074.
31. **Wiesenfeld, H. C., R. P. Heine, A. Rideout, I. Macio, F. DiBiasi, and R. L. Sweet.** 1996. The vaginal introitus: a novel site for *Chlamydia trachomatis* testing in women. Am. J. Obstet. Gynecol. **174:**1542–1546.