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Comparison of Gen-Probe Transcription-Mediated Amplification, Abbott PCR, and Roche PCR Assays for Detection of Wild-Type and Mutant Plasmid Strains of *Chlamydia trachomatis* in Sweden[▽]

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The clinical performance of two nucleic acid amplification assays targeting the cryptic plasmid and two assays targeting rRNA molecules in *Chlamydia trachomatis* was examined. First-catch urine samples from Malmoe, Sweden, were tested for *C. trachomatis* with the Abbott real-time PCR assay m2000 and an in-house PCR for the new variant strain of *C. trachomatis* with a deletion in the cryptic plasmid. Aliquots of the urine samples were sent to Aarhus, Denmark, and further examined with the Roche COBAS Amplicor CT (RCA) PCR, the Gen-Probe Aptima Combo 2 assay (AC2) targeting the *C. trachomatis* 23S rRNA, and the Aptima *C. trachomatis* assay (ACT) targeting the 16S rRNA molecule. A positive prevalence of 9% (163/1,808 urine samples examined) was detected according to the combined reference standard. The clinical sensitivity and specificity of the four assays were as follows: for ACT, 100% (163/163) and 99.9% (1,643/1,645), respectively; for AC2, 100% (163/163) and 99.6% (1,640/1,645); for m2000, 68.7% (112/163) and 99.9% (1,644/1,645); for RCA, 63.8% (104/163) and 99.9% (1,643/1,645). The two Gen-Probe assays detected all mutant strains characterized by the in-house PCR as having the deletion in the cryptic plasmid, whereas the Roche and the Abbott PCRs targeting the plasmid were both unable to detect the plasmid mutant. The difference in clinical sensitivity between the plasmid PCR assays m2000 and RCA, on the one hand, and the rRNA assays AC2 and ACT, on the other, could be attributed almost exclusively to the presence of the plasmid mutant in about one-quarter of the *Chlamydia*-positive samples examined.

Urogenital infections with *Chlamydia trachomatis* are endemic in young adults between the ages of 15 and 25 years. Extensive diagnostic screening and treatment of these infections with antibiotics have been performed for more than a decade (3, 8). In Scandinavia, the prevalence and presumably also the true incidence of genital *Chlamydia* infections have been increasing in recent years after a transient decrease (12). Nucleic acid amplification tests (NAATs) are sensitive and specific for screening and diagnosis of urogenital infections with *C. trachomatis* (5). Commercial nucleic acid amplification assays are often based on the detection of a target in the cryptic *Chlamydia* plasmid or the chromosome (MOMP gene). Plasmid-free variants of *C. trachomatis* have been reported (2, 6, 13), but these variants have not spread extensively, suggesting that the cryptic plasmid may play a role in the infectivity of the pathogen. The Gen-Probe Aptima Combo 2 (AC2) assay targets the 23S rRNA of *C. trachomatis* and the 16S rRNA of *Neisseria gonorrhoeae* and thus is independent of the cryptic plasmid of *C. trachomatis*. The Aptima *C. trachomatis* (ACT) assay detects *C. trachomatis* by targeting a different rRNA molecule (16S rRNA). The high number of rRNA molecules in *C. trachomatis* compared to the copy number of the cryptic plasmid tends to increase the sensitivities of the AC2 and ACT assays compared to NAATs based on a plasmid or a chromosome target.

A new variant of *C. trachomatis* with a deletion in the cryptic plasmid has recently been detected in Sweden (10, 11). Once this new variant was characterized, it became immediately clear that some of the commercially available *C. trachomatis* assays based on a plasmid target were unable to detect strains with the deletion in the plasmid. The Abbott m2000 real-time PCR assay has recently been launched in Europe, and there are limited clinical studies of its clinical performance. The m2000 assay and the Roche Cobas Amplicor (RCA) assay both target a sequence within the area deleted in the new variant (7) and are therefore unable to detect such strains.

The aim of this study was to compare the clinical performances of the Gen-Probe AC2 and ACT assays with those of the Abbott m2000 and RCA assays in a population with a high rate of the new variant strain of *C. trachomatis*. The difference between positive results by the AC2 and ACT assays, on the one hand, and the m2000 and RCA assays, on the other, would presumably reflect the prevalence of the deletion variant.

MATERIALS AND METHODS

Consecutive first-catch urine samples submitted to the Department of Clinical Microbiology in Malmoe, Sweden, and received on Monday to Thursday from November 2006 to February 2007 were included in the study. Samples (n = 1,808) were from male and female patients undergoing routine screening for *C. trachomatis*. Upon arrival, aliquots were transferred to the Gen-Probe and Abbott collection devices according to the respective package inserts. For some female urine samples, vaginal swabs were included in the urine tubes as part of the routine setup for *Chlamydia* testing in Malmoe. The presence of a vaginal

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TABLE 1. Distribution of primary test results on 1,808 urine samples

No. of samples	Result[a] by the following method:					
	Reference standard	Abbott m2000	Roche RCA	Gen-Probe		In-house PCR for the plasmid mutant
				AC2	ACT	
102	+	+	+	+	+	ND
10	+	+	−	+	+	ND
2	+	−	+	+	+	ND
5	+	−	−	+	+	−
44	+	−	−	+	+	+
5	−	−	−	+	−	ND
1	−	+	−	−	−	ND
2	−	−	+	−	−	ND
2	−	−	−	−	+	ND
1,635	−	−[b]	−	−	−	ND

[a] +, positive; −, negative; ND, not done.
[b] Two m2000 borderline-positive samples were both negative by retest and were interpreted as negative samples according to the Abbott package insert.

TABLE 2. Proportions of wild-type and plasmid mutant strains in female (n = 1,231) and male (n = 577) samples

Group	Rate of positivity (% [no. of positive samples/no. of samples examined]) for:		
	Female urine samples without vaginal swabs	Female urine samples with vaginal swabs	Male urine samples
All *C. trachomatis* strains[a]	6.0 (59/978)	8.3 (21/253)	14.4 (83/577)
Plasmid mutants among all *C. trachomatis* strains[b]	28.8 (17/59)	28.6 (6/21)	25.3 (21/83)

[a] $\chi^2 = 1.35$ and $P = 0.25$ for the difference in the rate of positivity between female urine samples with and without a vaginal swab.
[b] $\chi^2 = 0.01$ and $P = 0.92$ for the difference in the rate of positivity between female urine samples with and without a vaginal swab.

swab was recorded, swabs were removed, and aliquots of urine were distributed as described above. All samples were labeled with a bar code for identification.

Aliquots added to the Abbott collection devices were analyzed in the Malmoe laboratory using the Abbott m2000 real-time PCR assay. Aliquots for the Gen-Probe assays were sent to the Department of Clinical Microbiology in Aarhus, Denmark, and were analyzed using the AC2 and ACT tests. Aliquots of the fresh urine samples were also sent to Aarhus and analyzed using the RCA assay within 7 days of the time of sampling according to the instructions of the manufacturer. Testing was performed according to the package inserts for the different kits.

A combined reference standard was used as the "gold standard" for a positive sample. A sample was defined as true positive if at least two of four tests were positive (9). Discrepant analysis was performed when only one test was positive. Such samples were retested by the same test and, if positive again, retested by the other three tests. Sensitivity and specificity were calculated for each assay using the initial test results. Clinical performance was calculated separately for the urine samples containing a vaginal swab.

Samples positive by the m2000 or RCA assay were a priori considered positive for the wild-type strain, since both assays targeted the deletion area of the plasmid.

The new variant *C. trachomatis* strain was detected by a two-step procedure. Samples that were negative by both plasmid PCR assays (m2000 and RCA) were retested by an in-house real-time PCR targeting a sequence of the plasmid outside the deletion. The primers were Ct_{forw} (5′-CCG CTC AAG GAC CAG CAA) and Ct_{rev} (5′-AGA AGC ATT GGT TGA TGG ATT A), and the probe was Ct(5′–3′) (6-carboxyfluorescein–AA TCC TTG GGA CAA CAT CAA CAC CTG TCG–6-carboxytetramethylrhodamine). TaqMan fast universal PCR master mix was used in a 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA). The sensitivity of the in-house real-time PCR compared to that of the m2000 assay was 95% for wild-type *C. trachomatis* (unpublished data). Samples positive by the real-time in-house PCR were then tested by a seminested PCR, which could positively identify the new variant strain, because the primer sets spanned the deletion. The primers were nvCt-1 (5′-CGA TTT CTA AGC AGG AAT GGA C), nvCt-2 (5′-TTG AAG CGC TCC GGA TAG T), and nvCt-3 (5′-CCG CTG TGA CGG AGT ACA). The primer combinations used for the seminested PCR were nvCt1–nvCt3 in the first PCR and nvCt2–nvCt3 in the second PCR.

RESULTS

A total of 1,808 urine samples were examined. Female urine samples constituted 68% (1,231/1,808) of the total. The average ages of females and males were 25 and 26 years, respectively. Twenty-one percent (253/1,231) of the female urine samples also contained a vaginal swab. The overall prevalence of true-positive samples was 9% (163/1,808). The distribution of the primary test results with the four assays is shown in Table 1.

Discrepant analysis was performed on the 12 samples for which only one of the four assays was positive initially. Five AC2-positive samples were repeatedly negative upon retesting by the AC2 assay. Two m2000 borderline-positive samples were confirmed negative upon retesting by the m2000 assay, and one sample remained borderline positive after retesting. Two samples initially positive by the RCA assay were confirmed negative at retesting, and one of two ACT-positive samples was repeatedly positive by the ACT assay. The two samples with a repeatedly positive m2000 or ACT test result, respectively, were not evaluated further.

The new variant strain of *C. trachomatis* was positively identified in 44 cases. Another five samples, which were negative by both the m2000 and RCA assays, were also negative by the specific test for the plasmid mutant and therefore could not be typed (Table 1). The new variant strain of *C. trachomatis* was thus demonstrated in 44/163 of the positive samples (27%).

The two rRNA assays, AC2 and ACT, both detected all true-positive samples. The difference in clinical sensitivity between the Abbott m2000 real-time PCR and the RCA PCR test, on the one hand, and the Gen-Probe AC2 and ACT assays, on the other, could be attributed almost exclusively to the presence of the cryptic plasmid with a 377-bp deletion, which was found in about one-quarter of the *Chlamydia*-positive samples examined. The specificities of the four assays were almost equal. If the 44 plasmid mutant strains identified were excluded from the comparison, the positivity rates of the four assays—the AC2, ACT, m2000, and RCA assays—were 100.0% (119/119), 100.0% (119/119), 94.1% (112/119), and 87.4% (104/119), respectively. The difference between the positivity rates of the m2000 and AC2 assays was not statistically significant ($\chi^2 = 3.34$; $P = 0.067$), as opposed to the difference between those of the RCA and AC2 assays ($\chi^2 = 13.95$; $P = 0.0001$).

The prevalences of *C. trachomatis* in male and female urine specimens were 14.4% (83/577) and 6.0% (59/978), respectively (Table 2). *C. trachomatis* tended to be more commonly detected in combined samples of urine and a vaginal swab (8.3% [21/253]) than in urine samples alone for women. However, the difference in the positivity rate between female urine

TABLE 3. Sensitivities of the various *Chlamydia* assays for female (n = 1,231) and male (n = 577) samples

Assay	Sensitivity (% [no. of samples in which *C. trachomatis* was detected/no. of true-positive samples]) for:		
	Female urine samples without vaginal swabs	Female urine samples with vaginal swabs	Male urine samples
AC2	100.0 (59/59)	100.0 (21/21)	100.0 (83/83)
ACT	100.0 (59/59)	100.0 (21/21)	100.0 (83/83)
m2000[a]	64.4 (38/59)	71.4 (15/21)	71.1 (59/83)
RCA[b]	55.9 (33/59)	66.7 (14/21)	68.7 (57/83)

[a] $\chi^2 = 0.1$ and $P = 0.75$ for the difference in the rate of positivity between female urine samples with and without a vaginal swab.
[b] $\chi^2 = 0.36$ and $P = 0.55$ for the difference in the rate of positivity between female urine samples with and without a vaginal swab.

samples with and without a vaginal swab did not reach statistical significance ($\chi^2 = 1.35$; $P = 0.25$). The mutant strains were uniformly distributed among female and male samples. The differences in the sensitivities of the m2000 and RCA assays for female urine samples with and without a vaginal swab were not statistically significant (Table 3).

DISCUSSION

The present study compares the performances of four different commercial NAATs for the detection of *C. trachomatis* in urine specimens in a population where a new deletion variant of *C. trachomatis* has recently been reported (11). Using the combined reference standard to define true-positive samples, we found that 9% of the 1,808 consecutive male and female urine samples examined were positive for *C. trachomatis*. This is in accordance with the observed *Chlamydia*-positive sample rate in different areas of Sweden (12). The prevalence of *C. trachomatis* was higher in male urine samples than in female urine samples. This is usually the case and merely reflects different testing opportunities for males and females. Males are more likely to present with symptoms or a history of presumptive exposure to a sexually transmitted infection (unpublished data).

For females, a higher rate of *C. trachomatis* was found in urine samples containing a vaginal swab than in urine samples only. The difference was not found to be statistically significant. However, cervical swabs in urine samples have been shown to significantly improve yield over that with urine only (1). Chan et al. noted a similar slight increase in sensitivity over that with the urine specimen alone when cervical cells were added to the urine and a NAAT was used for detection (4).

A marked difference in sensitivity was seen between the Gen-Probe rRNA assays (ACT and AC2) and the plasmid-based PCR assays from Abbott (m2000) and Roche (RCA). Both the AC2 and the ACT assay proved to be highly sensitive in finding all the true *Chlamydia*-positive samples. When the samples with a confirmed new variant were excluded from the comparison, however, the difference in sensitivity between the AC2 and ACT assays, on the one hand, and the m2000 assay, on the other, was no longer statistically significant.

Five true-positive samples were negative by the m2000 and RCA assays after retesting and were also negative by all in-house tests for the plasmid mutant strain. Since at least one-quarter of all the positive samples were found to have the new variant, some of these negative samples could be expected to contain the new variant, assuming that the calculated sensitivity of the in-house plasmid mutant PCR is about 95%. Therefore, the number of plasmid mutant strains may have been underestimated in our comparison.

The specificities of the assays examined were very similar, between 99.6 and 99.9% based on the primary test result, reflecting a high concordance (96%) between the four NAATs evaluated. The high specificity of the NAATs is in accordance with the literature (5). Discordant results in which samples were positive by one assay were seen only for 12 of 1,808 samples examined. After retesting, only two samples remained positive by one assay only. These were considered false-positive results, although superior sensitivity compared to the other tests cannot be ruled out.

The finding of a mutant strain of *C. trachomatis* with a deletion in the cryptic plasmid that serves as the target of two commercial PCR assays raises several important questions, not only for the detection of *Chlamydia* but for the detection of microorganisms by NAATs in general. The plasmid mutant strain was recognized only by coincidence during an assessment of a new test for *C. trachomatis* in a comparative study (11). At the time of discovery, 13% of all positive samples in Halmstad already contained the plasmid mutant strain. The strain had probably been present for some time by then and had been able to spread uncontrolled despite a screening program for *C. trachomatis*. It is important for different test methods to be available and in use in order to avoid the exploitation of a common niche by a microorganism with a new mutation or nucleic acid rearrangement.

The cryptic plasmid in *C. trachomatis* may not be important for the survival of a *Chlamydia* strain. Yet it is remarkable that plasmid-free strains have not spread widely. This is in contrast to the new variant strain with the 377-bp deletion in the cryptic plasmid, which has established itself firmly in Sweden. Assays have now been modified and are able to detect the new variant. Still, constant vigilance is warranted to discover strains with new genetic makeups.

In conclusion, the new variant of *C. trachomatis* may spread quickly, and the situation will be aggravated if the diagnostic tests are unable to detect such strains. Preferably, routine detection methods for urogenital *Chlamydia* infections should be highly sensitive and specific but should also use target areas in *C. trachomatis* that are robust toward mutational changes.

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