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Comparison of the Abbott RealTi*m*e CT New Formulation Assay with Two Other Commercial Assays for Detection of Wild-Type and New Variant Strains of *Chlamydia trachomatis*▽

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In an analytical-method comparison study of clinical samples, the Abbott RealTi*m*e CT new formulation assay (*m*2000 real-time PCR), consisting of a duplex PCR targeting different parts of the cryptic plasmid in *Chlamydia trachomatis*, was compared both with version 2 of the Roche Cobas TaqMan CT assay, comprising a duplex PCR for a target in the cryptic plasmid and the *omp1* gene, and with the Gen-Probe Aptima Combo 2 assay (AC2) targeting the *C. trachomatis* 23S rRNA molecule. First-catch urine samples from Sweden were tested in Malmö, Sweden, for *C. trachomatis* with the *m*2000 real-time PCR assay and with an in-house PCR for the new variant *C. trachomatis* strain with a deletion in the cryptic plasmid. Aliquots of the urine samples were sent to Aarhus, Denmark, where they were further examined with the TaqMan CT and AC2 assays. A positive prevalence of 9.1% (148/1,632 urine samples examined) was detected according to the combined reference standard. The sensitivities and specificities of the three assays were as follows: for the Abbott *m*2000 assay, 95.3% (141/148) and 99.9% (1,483/1,485), respectively; for the Roche TaqMan assay, 82.4% (122/148) and 100.0% (1,485/1,485); and for the Gen-Probe AC2 assay, 99.3% (147/148) and 99.9% (1,484/1,485). The plasmid mutant strain was detected in 24% (36/148) of the *C. trachomatis*-positive samples. There is a difference in sensitivity between the new formulations of the Abbott and the Roche assays, but both assays detected the wild-type and new variant *C. trachomatis* strains equally well.

A new variant of *Chlamydia trachomatis* with a deletion in the cryptic plasmid has been described in Sweden (3, 4). In a previous study. we compared the Gen-Probe Aptima Combo 2 (AC2) and Aptima CT assays, directed against different rRNA targets of *C. trachomatis*, with the Abbott *m*2000 real-time PCR assay and the Roche Cobas Amplicor CT assay. The two latter assays both target a sequence within the area deleted in cryptic plasmid of the new variant (2). We used routine samples from females and males to confirm that the new variant strain was present in one-fourth of the *C. trachomatis*-positive samples from the Malmö area in Sweden and that the two assays targeting the cryptic plasmid were unable to detect the plasmid mutant (2). Modifications of the Abbott *m*2000 and Roche Cobas assays have now been released; each consists of a duplex PCR with different targets in *C. trachomatis*. The new formulation of the *m*2000 real-time PCR adds a different target region in the cryptic plasmid to the original plasmid PCR test, and the new version 2 of the Roche Cobas TaqMan CT assay combines the original plasmid PCR with a PCR targeting the *omp1* gene in *C. trachomatis*.

The aim of this study was to compare and assess the analytical performance of the Abbott RealTi*m*e CT new formulation assay (Abbott *m*2000 real-time PCR clinical diagnostic system) with those of version 2 of the Roche Cobas TaqMan CT test and the Gen-Probe Aptima Combo 2 assay for clinical samples from a population with a high rate of the plasmid mutant strain.

MATERIALS AND METHODS

Consecutive first-catch urine samples submitted to the Department of Clinical Microbiology, Malmö University Hospital, Malmö, Sweden, and received from Monday to Thursday, from May 2008 to June 2008, were included in the study. Samples (n = 1,632) 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. All samples were labeled with a bar code for identification.

Aliquots added to the Abbott collection devices were analyzed in the Malmö laboratory using the Abbott RealTi*m*e new formulation (*m*2000) assay. Aliquots for the Gen-Probe assays were sent to the Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark, where they were analyzed using the AC2 and Aptima CT (ACT) tests. Aliquots of the fresh urine samples were also sent to Aarhus, where they were analyzed using the Roche Cobas TaqMan CT assay, version 2.0 (RCT assay), according to the manufacturer's instructions, within 7 days from the time of sampling. Testing was performed according to the package inserts for the different kits. All *C. trachomatis*-positive samples were examined in Malmö for the new variant strain by a two-step procedure using in-house PCRs as previously described (2).

Inhibited samples were retested with the same test (except for the Gen-Probe AC2 and ACT assays, due to their lack of internal controls). The ACT assay was used as an arbiter test and was performed on all samples. We repeated all assays on samples for which only some of the primary test results were positive. A combined reference standard was used as the "gold standard" for evaluation of the analytical validity of the three primary assays examined (the AC2, *m*2000, and RCT assays). A sample was defined as true positive if any two of the four assays (ACT, AC2, *m*2000, and RCT assays) were positive. The final sensitivity and specificity were calculated for each assay using the combined reference standard.

RESULTS

We examined 1,632 urine samples. Female samples constituted 64% (1,044/1,632). The average ages of females and

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TABLE 1. Distribution of primary test results on 1,632 urine samples

No. of samples	Result[a] by the following assay:					
	Abbott m2000	Roche CT	Gen-Probe AC2	Arbiter test (ACT assay)	Reference standard[b]	In-house mutant plasmid PCR
28	+	+	+	+	+	+
91	+	+	+	+	+	− (3 NT)
7	+	−	+	+	+	+
15	+	−	+	+	+	−
1	−	+	+	+	+	+
1	−	+	+	+	+	NT
4	−	−	+	+	+	−
1	−	+	−	+	+	NT
2	+	−	−	−	−	ND
1	−	−	+	−	−	ND
1,481	−	−	−	−	−	ND

[a] +, positive, −, negative; ND, not done; NT, nontypeable.
[b] The reference standard is defined as positive results for a sample by any two of the four commercial assays.

males were 23.8 and 25.9 years, respectively. The overall prevalence of true-positive samples was 9.1% (148/1,632). More men than women were found positive for *C. trachomatis*: 79/588 (13.4%) and 69/1,044 (6.6%), respectively. The distribution of the primary test results with the three assays is shown in Table 1. The plasmid mutant strain was positively identified in 36 of the 148 positive samples (24%). For 5 of the 148 positive samples, the strain could not be typed by the in-house PCR. The proportion of mutant strains among *C. trachomatis*-positive samples was higher in men than in women: 23/79 (29.1%) versus 13/69 (18.8%), respectively. The difference was not statistically significant ($\chi^2 = 1.6$; $P = 0.2$).

The Gen-Probe AC2 assay detected all 36 plasmid mutant strains and 111 of 112 wild-type *C. trachomatis* strains. The Abbott *m*2000 assay found 35 (97.2%) mutant strains and 105 of 112 wild-type strains (94.6%). The Roche TaqMan CT assay found 29 of 36 mutant strains (80.5%) and 93 of 112 wild-type strains (83.0%). The ratio of the percentage of mutant strains detected to the percentage of wild-type *C. trachomatis* strains detected is 97.2% to 94.6% for the *m*2000 assay and 80.5% to 83.0% for the TaqMan CT assay. Thus, the difference in sensitivity for the detection of *C. trachomatis* between the *m*2000 and TaqMan CT assays is not caused by a difference between the two assays in detecting plasmid mutant and wild-type strains.

Discrepant analysis was performed on eight samples for which only one of the three assays was positive initially. The results are shown in Table 2. Four of the eight single-assay-positive samples were reproducibly positive by the same assay. Two samples reproducibly positive by the *m*2000 assay could not be found positive by repeat tests with any of the other three assays and are regarded as false-positive samples. Notably, these two samples were posttreatment samples from previous Abbott RealTime CT-positive patients. One TaqMan CT-positive sample was repeatedly negative by the other two assays but was confirmed as true positive by the arbiter test (ACT assay) (Table 2). Two repeatedly AC2 positive samples were found negative by the *m*2000 and RCT assays but were confirmed as true positive by the ACT test. In addition, three initially AC2 positive samples were not confirmed as positive by any of the three assays compared. However, two of these samples were confirmed positive by the ACT test, which may indicate samples containing very few *C. trachomatis* organisms. The last initially AC2 positive sample could not be confirmed positive by any other test. The sensitivities and specificities of the three independent assays compared are shown in Table 3, both for all samples and for samples broken out by gender.

Inhibition of the assay detected by the internal control was seen in the primary test in 3 of 1,632 (0.2%) urine samples with the Abbott *m*2000 assay and in 82 of 1,632 (5%) urine samples with the Roche TaqMan CT assay. All samples with inhibition were reexamined. The three inhibited Abbott *m*2000 assays were resolved by a repeat test after a 1:5 dilution of the urine sample. Not all inhibited Roche TaqMan assays could be resolved by dilution of the urine. Four of the 82 inhibited samples were classified as "true positive" by the reference standard. One sample became positive and two became negative by the retest; one could not be resolved but became positive using DNA purified from the urine sample by the *m*2000 assay. Among the 78 urine samples classified as "true-negative" samples, 19 became negative by the retest, but 59 could not be resolved by dilution of the sample.

Samples that repeatedly tested false negative by the regular TaqMan CT assay were reexamined using purified nucleic acids from the Abbott *m*2000 preparation instead of sample material prepared by the pretreatment washing step recommended by Roche. This procedure generated 13 additional

TABLE 2. Discrepant analysis

No. of samples	Result[a] by:							Conclusion
	Primary test			Repeat test			Arbiter test (ACT)	
	ART	RCT	AC2	ART	RCT	AC2		
2	+*	−	−	+/ND	−/ND	−/ND	−/−/ND	Negative
1	−	+	−	−/ND	−/−	−/ND	−/+/+	True positive
2	−	−	+	−/ND	−/ND	+/ND	+/+/ND	True positive
1	−	−	+	−/ND	−/ND	−/−	+/+/ND	True positive
1	−	−	+	−/ND	−/ND	−/−	−/+/+	True positive
1	−	−	+	−/ND	−/ND	−/−	−/−/ND	Negative

[a] +, positive; −, negative; ND, not done. ART, Abbott RealTime PCR; RCT, Roche Cobas TaqMan CT PCR; AC2, Gen-Probe Aptima Combo 2 assay; ACT, Gen-Probe Aptima CT assay. *, the two "false-positive" samples were posttreatment samples from previous Abbott RealTime-positive patients.

TABLE 3. Sensitivities and specificities of the *C. trachomatis* assays used on male and female urine samples

Urine sample group and assay	Sensitivity		Specificity	
	% (95% CI)	No. of positive samples/true positives	% (95% CI)	No. of true negatives/no. of negative samples
Male and female				
Abbott RealTi*m*e CT *m*2000	95.3 (90.6–97.7)	141/148	99.9 (99.5–100)	1,482/1,484
Roche Cobas TaqMan CT	83.0 (75.5–87.7)	122/148	100.0 (99.8–100)	1,484/1,484
Gen-Probe Aptima Combo 2	99.3 (96.3–99.8)	147/148	99.9 (99.6–100)	1,483/1,484
Male				
Abbott RealTi*m*e CT *m*2000	96.2 (89.4–98.6)	76/79	99.8 (98.9–100)	508/509
Roche COBAS TaqMan CT	83.5 (73.8–90.1)	66/79	100.0 (99.3–100)	509/509
Gen-Probe Aptima Combo 2	98.7 (93.3–99.7)	78/79	100.0 (99.3–100)	509/509
Female				
Abbott RealTi*m*e CT *m*2000	94.2 (86.0–97.6)	65/69	99.9 (99.4–100)	974/975
Roche Cobas TaqMan CT	81.2 (70.3–88.6)	56/69	100.0 (99.6–100)	975/975
Gen-Probe Aptima Combo 2	100.0 (94.9–100.0)	69/69	99.9 (99.4–100)	974/975

positive samples by the TaqMan CT assay. Four of the 13 samples (31%) had plasmid mutant strains.

DISCUSSION

The present study compares the analytical performances of two amended plasmid-based test methods with dual targeting of *C. trachomatis* with that of a third commercial nucleic acid amplification test for the detection of *C. trachomatis* in urine specimens in a population where a new deletion variant of *C. trachomatis* has recently been described (2–4). Using a combined reference standard to define true-positive samples, we found that 9.1% of the 1,632 consecutive male and female urine samples examined were positive for *C. trachomatis.* The percentage of positive samples is in accordance with the observed *Chlamydia*-positive sample rate in different areas of Sweden (1, 5) and with the findings of a previous study comprising samples from the same part of Sweden (2). The new variant strain constituted 24% of the *C. trachomatis*-positive samples. Although the new formulation of the Abbott *m*2000 RealTi*m*e assay had been used in Malmö for some time prior to the study period, the present proportion of plasmid mutant strains is almost unchanged from the proportion (27%) reported 1 year earlier from Malmö (2). Thus, the prevalence of the plasmid mutant strains seems stable in spite of the use of improved diagnostic assays. This stability was also observed by Hadad et al. (1), and it emphasizes the importance of using assays capable of targeting the Swedish plasmid mutant.

Both the new formulation of the *m*2000 assay and the AC2 assay proved to be highly sensitive in finding the true *Chlamydia*-positive samples, whereas a markedly lower sensitivity was seen with version 2 of the Roche Cobas TaqMan CT assay. We can only speculate as to whether or not the lower sensitivity observed for the TaqMan PCR test is caused by the lack of a specific nucleic acid extraction and purification step before the amplification of the dual targets in this commercial assay. When the urine samples found false negative by the Roche assay were reexamined by using purified DNA from the preanalytical preparation of samples in the Abbott *m*2000 assay as target material, the RCT test found 13 initially false negative samples positive. On the other hand, the possibility that the time delay in testing the refrigerated fresh urine samples by the Roche TaqMan assay compared to the Abbott *m*2000 assay may not be optimal for preserving and detecting DNA in urine samples cannot be excluded, although the test was performed within 7 days after collection, as stipulated in the manufacturer's instructions.

The specificities of the three assays examined were very similar, between 99.9 and 100.0% based on the primary test result. Discordant results consisting of samples positive by one assay only were seen for 8 of 1,632 samples examined. Based on the use of the Gen-Probe ACT assay as an arbiter test, we concluded that 5 of the 8 samples were true-positive samples. However, the remaining three samples may also be true-positive samples detected by the superior sensitivity of the single positive assay compared to the other assays. The variable patterns of results of the repeat tests and the arbiter test on some of the 8 discordant samples also seem to indicate that these samples may have had a very low number of *C. trachomatis* organisms. Notably, we cannot rule out the possibility that the Abbott *m*2000 assay was detecting residual, partially degraded DNA from nonviable organisms or a variant that none of the other assays could detect in the two posttreatment samples from previously positive patients, which were repeatedly positive by the *m*2000 assay only. The ACT and AC2 assays detect RNA and are expected to pick up living *Chlamydia* organisms.

A limitation of the study is the use of an arbiter test (the ACT test) based on the same technology as one of the primary tests (the AC2test). However, to minimize the method bias of the arbiter test, all samples, not just discordant samples, were tested. The arbiter (ACT) test became negative for all samples that tested concordantly negative by the three primary assays (Table 1). Therefore, we think that the sensitivities and specificities of the assays shown in Table 3 provide a fair estimate.

The three commercial assays examined represent different sample preparation methods, amplification principles, and nucleic acid targets in *C. trachomatis* (rRNA, plasmid DNA, and chromosomal DNA). However, a high concordance in clinical performance was observed between the Abbott and Gen-

Probe assays (99.4%) in our study. Less concordance (98.2%) between the Roche and the Gen-Probe assay may be explained by the lack of efficient preanalytical sample purification in the Roche assay but may also be caused partly by a lower analytical sensitivity of the TaqMan duplex PCR targeting the cryptic plasmid and the *omp1* gene. In a smaller study, the new formulation of the Abbott *m*2000 real-time PCR was found slightly, but not significantly, more sensitive than the BD ProbeTec ET assay (6). The Roche Cobas TaqMan CT test, version 2.0, was recently compared with a LightMix 480HT PCR (TIB Molbiol, Germany), and the two methods were found to detect wild-type and plasmid mutant strains of *C. trachomatis* in Sweden equally well (1).

In conclusion, the new formulations of the Abbott and Roche assays were each detecting wild-type and new variant *C. trachomatis* strains equally well in male and female urine samples. The sensitivities of the Abbott RealTi*m*e new CT formulation assay and the Gen-Probe Aptima Combo 2 assay were significantly higher than that of the new version 2 of the Roche Cobas TaqMan CT assay. However, the use of an automated nucleic acid extraction and purification step before the amplification step may improve the yield of the Cobas TaqMan CT assay.

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REFERENCES

1. **Hadad, R., H. Fredlund, and M. Unemo.** 2009. Evaluation of the new COBAS TaqMan CT Test v2.0 and the impact on the proportion of the new variant of Chlamydia trachomatis (nvCT) by introduction of diagnostics detecting nvCT (LightMix 480HT PCR) in Orebro county, Sweden. Sex. Transm. Infect. **85:**190–193.
2. **Møller, J. K., L. N. Pedersen, and K. Persson.** 2008. 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. J. Clin. Microbiol. **46:**3892–3895.
3. **Ripa, T., and P. Nilsson.** 2006. A variant of Chlamydia trachomatis with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill. **11:**E061109.2.
4. **Ripa, T., and P. A. Nilsson.** 2007. A Chlamydia trachomatis strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. Sex. Transm. Dis. **34:**255–256.
5. **Söderblom, T., A. Blaxhult, H. Fredlund, and B. Herrmann.** 2006. Impact of a genetic variant of Chlamydia trachomatis on national detection rates in Sweden. Euro Surveill. **11:**E061207.1.
6. **Walsh, A., F. O. Rourke, B. N. Laoi, and B. Crowley.** 2009. Evaluation of the Abbott RealTime CT assay with the BD ProbeTec ET assay for the detection of Chlamydia trachomatis in a clinical microbiology laboratory. Diagn. Microbiol. Infect. Dis. **64:**13–19.