Evaluation of the Abbott RealTime™ CT/NG assay in comparison to the Roche COBAS AMPLICOR CT/NG assay

Running Title: Evaluation of the Abbott RealTime™ CT/NG assay

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Abstract

Several commercial methods exist for molecular detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in clinical samples. Here we evaluated the performance characteristics of the newly FDA-cleared, Abbott RealTime CT/NG assay that uses the automated m2000 molecular platform. Results were compared to the Roche COBAS AMPLICOR CT/NG assay. 926 cervical swab, 45 female urine, 6 male urethral swab and 407 male urine specimens were examined from 1384 patients. After resolving all Roche NG positive results with two additional real time PCR assays, we found agreement between the assays was excellent. For urine samples, there was 99.6% positive and 97.7% negative agreement for CT, and for male urine samples, there was 100% positive and 99.7% negative agreement for NG. For cervical swab samples, there was 98.8% positive and 98.5% negative agreement for CT, and 96.6% positive and 99.8% negative agreement for NG. In limiting dilution analysis, we found that the Abbott assay was more sensitive than the Roche assay for both CT and NG. In addition, there appeared to be an enhanced ability of the Abbott assay to detect dual infections especially in the presence of large amounts of NG and small amounts of CT. In summary, we conclude that the Abbott RealTime CT/NG assay is an accurate and automated, new addition to the available testing options for CT/NG.
Introduction

The incidence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infection continues to increase globally. A more intensive screening effort has been advocated by the U.S. Preventive Services Task Force (17), Centers for Disease Control and Prevention (24), other public health agencies, and medical societies to bring this emerging epidemic under control. This includes yearly screening of sexually active women under the age of twenty-five for *C. trachomatis*. The goal of screening is to prevent transmission and the severe sequelae of unrecognized infection, such as pelvic inflammatory disease and associated infertility.

Rapid, automated and sensitive nucleic acid amplification testing methods are needed to respond optimally to this public health mandate. In this regard, there has been limited published evaluation of the recently FDA-cleared Abbott RealTime CT/NG assay (9). There was recent publication of clinical trial data comparing the Abbott Assay to Gen-Probe Aptima Combo 2 (AC2), BD ProbeTec™ ET CT/GC, and GC culture (6). There was also one prior study from Canada using a CE-marked Abbott kit with different cutoff and interpretation algorithms (8), and a U.S. study performed by Abbott Laboratories (Des Plaines, IL) using a prototype version of the now FDA-cleared assay (9). This last study included only a small number of NG positive samples. Taken together, these studies provide only minimal data regarding performance of the Abbott assay in comparison to the well-established, FDA-cleared, Roche COBAS AMPLICOR CT/NG method.

In order to obtain further insight into the performance characteristics of this new commercial method, we performed a comparative evaluation with several
interdependent goals. The first was to assess the Abbott RealTime CT/NG method’s clinical and analytical performance in comparison with the well-established Roche COBAS AMPLICOR CT/NG assay in current use in our laboratory. The second was to examine the ability of the Abbott method to avoid false positive NG results, observed with some commercially available nucleic acid amplification test (NAAT) methods (22). These false positive results have previously been linked to spurious detection of non-pathogenic Neisseria species. The third was to examine the effect of specimen transport media on the analytic performance of the Abbott method. The Abbott test recommends collection of specimens in assay specific transport media containing the denaturant, guanidinium thiocyanate. In contrast, the Roche method recommends collection of swab specimens in M4-RT media (MicroTest, Inc., Lilburn, GA), a general purpose transport medium also used for culture of viruses and Chlamydia. During the study period, swab specimens were transported in M4-RT. Therefore we sought to determine whether the use of M4-RT vs. Abbott transport medium affected the analytical performance of the Abbott RealTime CT/NG assay. Results would confirm the reasonableness of comparing clinical test performance on samples collected in M4-RT medium.

Materials and Methods

Clinical samples and testing. During the study, 926 cervical swabs, 45 female urines, 6 male urethral swabs, and 407 male urines were collected from 1384 patients for clinical diagnosis. At our institution we receive relatively few female urine specimens, because of the lack of FDA-approval for Roche NG testing for this specimen
type. The prevalence of NG and CT at our institution is also relatively low at 0.2% and 2.2%, respectively. Therefore, in order to establish more confidently comparative performance on positive specimens, we selectively tested a larger percentage of positive specimens than we otherwise would have based on prevalence of CT/NG in our patient population. With this caveat, swab and first catch urine specimens were otherwise selected randomly for comparative testing by the Abbott method without knowledge of the sex of the patient.

Cervical and urethral swabs were transported in 3 ml of M4-RT medium. Urine was collected in sterile tubes with no additives. Testing was performed using two methods: the Roche COBAS AMPLICOR CT/NG used for clinical testing in our hospital system and the Abbott RealTime CT/NG assay. Specimens were tested on the Roche system, and either tested concurrently using the Abbott system or frozen at -20°C and later tested in batch runs by the Abbott method. For the Roche assay, 100 µl of swab sample and 500 µl of urine sample were extracted for testing. For the Abbott assay, 3 ml of each urine specimen were first mixed with 1.2 ml of Abbott transfer buffer. Then 400 µl of this urine mixture or 400 µl of swab specimens (collected in M4-RT) were extracted with the Abbott M2000 per manufacturer’s protocol. The Abbott method uses an automated nucleic acid extraction platform based on magnetic bead technology, and robotic PCR reaction set up, followed by manual transfer of PCR reactions to an m2000rt thermocycler for real time PCR amplification and reading. Testing was performed according to the manufacturer’s instructions including recommended procedures for resolution of indeterminate results. This study was approved by our institutional review board.
Confirmation of NG positive test results. Because of known NG false positivity resulting from cross reactivity of primer and probe sets of some commercial methods with non-pathogenic Neisseria (22), all Roche NG positives were confirmed in our laboratory by two previously described, hybridization probe-based, real time PCR tests that amplify alternative targets. Both tests were previously shown to have high specificity. The target of the first method, the porA pseudogene, is found only in N. gonorrhoeae and N. meningitides (5, 18, 20). Although both species yield positive amplification signals, they can be distinguished by their different melting curves, resulting from nucleotide polymorphisms that differentially affect the melting temperature of the hybridization probes. The second test targets the multicopy 16S rRNA gene, again distinguishing among the several Neisseria species amplified through melting temperature analysis (3). Both methods were previously described as confirmatory methods for the Roche NG assay used in this study (3, 5).

For NG positives samples, we extracted a portion of original patient sample with High Pure Columns (Roche Applied Sciences, Indianapolis, IN) as described previously (21). This differed from the Boom extraction cited in the 16S rRNA gene amplification method (3). Amplification was performed on a Roche Lightcycler 2.0 as described in the original publications with the exception that the cycling protocol for the porA pseudogene was used for both assays (i.e., incorporating a longer denaturation time of 10 minutes and 55 amplification cycles) (3, 21). Primers and probes were from Tib-Molbiol (Adelphia, NJ), and the Lightcycler FastStart DNA Master HybProbe master mix was from Roche Applied Sciences (Indianapolis, IN). Limiting dilution studies showed that the confirmatory methods (referred to going forward as “LC”) had slightly lower to
equivalent sensitivity than the primary Roche method (Cheng and Kirby, unpublished data). This likely resulted from a smaller volume of extracted sample being tested in the confirmatory real time PCR assays. Therefore, to ensure adequate sensitivity, Roche NG+, LC negative samples were then concentrated using ultrafiltration with a Micron filter (Millipore, MA) with a 100,000 kDa nominal molecular weight cutoff. This led to an estimated 10 to 50-fold concentration of sample based on volume. Real time PCR analysis of a limiting dilution series demonstrated that this concentration step increased sensitivity beyond that of unconcentrated samples tested by the Roche method. Therefore, the LC methodology combined with ultrafiltration could serve as a confirmatory test for samples with low levels of target.

As described in the results section, our initial clinical verification of the LC confirmatory methods in our laboratory also included testing of Roche NG+ samples using a second commercial method, specifically the ProbeTec™ ET Chlamydia trachomatis and Neisseria gonorrhoeae Amplified DNA Assay. ProbeTec™ testing was performed at the Cleveland Clinic Clinical Microbiology Laboratory, which had previously verified use of M4-RT based specimens with this assay.

**Testing algorithm and resolution of discrepancies.** As mentioned above, all Roche NG+ results were confirmed by LC. LC confirmed specimens were considered Roche NG+ true positives. Any LC non-confirmed specimen results were considered Roche false positives and are listed as NG- under Roche results in Tables 1 and 2. Otherwise, the test results are listed in Tables 1 and 2 as determined on initial testing by each method. Any discrepant samples underwent repeat testing by both Roche and Abbott methods. Furthermore, any Abbott NG+, Roche NG- was also tested by LC.
Lastly, when sufficient sample was available, de-identified, coded samples were also further tested using the Gen-Probe APTIMA COMBO 2 (AC2), DTS system. In this case, 1.5-2 ml of urine samples or 100 µl of swab samples in M4-RT were placed into an Aptima Urine Collection tubes or Aptima Unisex Swab Collection tube containing 2 and 2.9 ml of transport media respectively and further processed according to the manufacturer’s instructions. Testing was performed in the Indiana University School of Medicine, Infectious Diseases Department, which uses AC2 for clinical testing. Follow up testing results on discrepant samples are described in the results section.

Analysis of discrepant results also made use of quantitative data available from the Abbott assay. As described previously (16), the Abbott software calculates a measure called the FCN, the cycle number at which the change in signal from one amplification cycle to the next is greatest. The FCN is used as an alternative to cycle threshold. The software then calculates a Delta Cycle (DC), which is the difference in cycle number between the sample FCN and cutoff control, with an additional number of buffer cycles added to establish a cutoff for positivity (1). The DC value correlates in a positive, log-linear fashion with target concentration in the original sample.

**Limiting dilution studies in M4-RT media.** Serial ten-fold dilutions of AmpliTrol CT/GC (Bio-Rad Laboratories, Hercules, CA) were made in M4-RT and Abbott-specific CT/NG transport media in siliconized eppendorf tubes. The AmpliTrol reagent contains a mixture of CT LGV type II strain 434 elementary bodies and whole cell lysate of NG ATCC 19424 (2). Initially four replicates of each ten-fold dilution were tested to roughly define detection limits, followed by 20 replicates of a finer series of dilutions to define the 95% limit of detection (LOD). M4-RT dilutions were also tested in the Roche assay.
per the manufacturer’s instructions for swab-based specimens. Correlation analysis between samples diluted in M4-RT and Abbott transfer buffer, and tested by the Abbott method, was performed using data points from the initial ten-fold dilution series.

For LOD analysis, Roche NG test results with values in the expanded indeterminate zone (\(A_{660} 0.2\) to 3.5) were scored as positives. In clinical practices these samples would normally be retested in duplicate and resolved as positive if \(A_{660}\) values were above 2.0 in two of the three test runs (19). Conversely, the sample would be scored as negative if two of three results were less than this cutoff. The expanded indeterminate zone is used during clinical testing to reduce the frequency of false positive results due to low level cross reactivity with other *Neisseria* species (19). However, for our analysis, we made the conservative assumption that all signal above 0.2 was specific for NG, as cross-reacting species are presumably not present in the AmpliTrol control material. The Roche assay does not provide a procedure for resolving CT equivocal results (\(A_{660} \geq 0.2\) and \(\leq 2\)). As equivocal results would likely lead to further clinical follow up, they were conservatively scored as positive in the LOD analysis.

**Statistical analysis.** Statistical comparisons were performed using JMP Version 8.0.2 (SAS Institute, Cary, NC). DC and LOD data were compared using the Wilcoxon ranked sum test and Fisher’s exact test, respectively. A \(P\) value \(\leq 0.05\) was considered statistically significant. \(R^2\) correlation coefficients were determined in Excel 2008 (Microsoft, Redmond, Washington). Percent agreement and 95% score confidence intervals (listed as a range in parentheses following percent agreement) were calculated
Results

NG confirmatory assay. As the Roche COBAS NG test is known to yield false positive test results arising from cross reactivity with non-pathogenic Neisseria species (19, 22), positive Roche NG tests are confirmed as part of our standard laboratory practice through use, with minor modifications (see materials and methods section), of two previously described real time PCR assays for the alternative porA pseudogene and 16S rRNA gene targets (3, 21). Roche NG positives were considered true positives (and scored as positive in Tables 1 and 2), only if confirmed by both of these LightCycler™ (LC)-based assays.

To verify the ability of LC to distinguish between Roche NG true and false positive prior to comparison of Abbott and Roche methods, we retested all Roche NG positive samples during a one year time period using both LC and a second commercially available assay, the ProbeTec™ ET System (BD, Franklin Lakes, NJ). The latter assay detects the NG pilin gene-inverting protein homologue, in contrast to the NG cytosine methyltransferase gene detected by the Roche assay, and is known to cross react with a presumably, non-overlapping set of non-pathogenic Neisseria (22). Of 50 Roche “positive” swab samples evaluated, 25 were confirmed negative by both ProbeTec and LC. Similarly, of 28 Roche “positive” urine samples, 3 were confirmed negative by both ProbeTec and LC. All samples confirmed positive by ProbeTec were also confirmed positive by both LC assays, except for one ProbeTec positive sample,
which was confirmed by neither LC assay. Notably, in this sample, the 16S rRNA LC assay showed amplification, but with a low Tm, suggesting the presence of non-gonococcal *Neisseria*. Unfortunately, no sample was left for further investigation. However, this observation raises the concern that this one strain may have given false positive results in two commercial assays with different targets. Based upon this verification study and prior literature, we concluded that the two LC assays are both sensitive and specific for confirmation of Roche NG positivity (3, 14, 20, 21).

**Urine specimens.** After identifying Roche NG true positives by confirmatory LC assays, there was 99.6% (97.9%-99.9%, 95% score confidence interval) positive and 97.7% (94.6%-99.0%) negative agreement for CT; and 100% (93.2%-100%) positive and 99.7% (98.4%-100%) negative agreement for NG between Abbott and Roche methods, respectively. As shown in Table 1, there were only six discrepant CT results, five of which were Abbott CT positive, and Roche CT negative (n=3) or equivocal (n=2). On repeat Abbott testing, one of the five samples changed from CT positive to negative. Interestingly, all of these specimens were positive for NG by both methods. Four of the five discrepant samples were also tested by Gen-Probe APTIMA COMBO 2 (AC2). Two were confirmed as CT+/NG+, while only NG was detected in the remaining two samples. In addition, the median DC (a measure of cycle number difference between the specimen and negative control that varies in a positive log-linear fashion with target concentration) for CT was significantly lower ($P = 0.0007$) for discrepant (DC=2.7) than for non-discrepant samples (DC=9.4). Furthermore, DC values of NG were greater than CT for each discrepant sample (average ratio of NG:CT of 4, range 1.4 to 8.0). Taken together these observations suggested that at least for some samples the Roche assay
might not always detect dual infections especially when there is a small amount of CT and excess NG present. Lastly, there was a single Roche CT+/Abbott CT- sample. The sample was CT- by both methods on repeat testing.

Amongst NG discrepancies, there was one Abbott NG+/Roche NG- sample, which was CT+ by both methods. It initially tested Roche NG+, but was scored as NG- based on negative LC confirmatory testing. Notably, the Abbott NG+ DC value was barely above the cutoff for positivity (DC=0.05) and repeat Abbott NG was negative. CT signal in this sample was very strong (DC=11). AC2 only detected CT. It is possible that this sample also represented a dual infection with NG level near the threshold for detection.

**Swab specimens.** After identifying Roche NG true positives by confirmatory LC assays, there was 98.8% (97.5%-99.5%) positive and 98.5% (96.8%-99.3%) negative agreement for CT; and 96.6% (88.3%-99.1%) positive and 99.8% (99.2%-99.9%) negative agreement for NG between Abbott and Roche methods, respectively, amongst cervical swab specimens. As shown in Table 2, there were 12 discrepant CT results. Among the 6 Roche CT+, Abbott CT- samples, all were CT- on repeat Roche testing, and one was weakly CT+ on repeat Abbott testing (DC=1.43). Furthermore, all of these samples were CT- by AC2.

In addition there were 6 Roche CT-, Abbott CT+ samples. All of these were low level Abbott positives (average DC value of 1.8, range 0.9 - 3.6). One of four samples available for confirmatory testing by AC2 testing was CT+. Two additional samples were Roche CT-, Abbott CT equivocal, and NG- by both methods. However, there was insufficient sample for resolution of the equivocal Abbott CT results by repeat testing as
suggested in the manufacturer’s package insert. Therefore, these specimen results were not included in the CT tabulation.

In comparison to CT results, NG agreement was almost complete when Roche NG+ results were resolved by LC. Amongst the Abbott NG+, Roche NG- discrepants, one specimen was repeatedly weakly NG+ by Abbott (average DC=1.6). Notably the specimen was strongly CT+ by Abbott (DC=14.84), Roche, and AC2 (961 relative light units). The Roche internal amplification control was initially negative, however, it was positive on diluting the sample 1:10 and retesting. Nevertheless, NG remained negative and was also negative either testing a 1:10 dilution or neat sample on LC, or neat sample by AC2. A second discrepant sample was repeatedly Roche NG-, low Abbott NG+ (DC=1.9). The *porA* pseudogene LC test was negative. However, 16S rRNA test showed amplification with a melting temperature of 56.6°C (vs. the expected 61°C and/or 67°C), suggesting the potential presence of a non-gonococcal *Neisseria* (3). Unfortunately, no sample remained for further investigation of this possibility.

Amongst the two Roche NG+, Abbott NG- samples (Table 2), one sample was weakly Abbott NG+ on repeat testing (DC=0.43). Initially, only the 16S rRNA LC confirmatory test was positive; however on repeat testing both 16S rRNA and *porA* pseudogene LC assays were positive. The second sample was LC positive for the 16S rRNA target alone, only after ultra-filtration concentration of the extracted sample. The specimen was QNS for repeat Abbott and Roche testing. These results are consistent with samples having an amount of NG target near the detection threshold for all methods.
There were also six male swab specimens in the data set, all of which had concordant positive test results, 5 for NG and 1 for CT.

**Quantitative data analysis.** The inherent quantitative nature of real time PCR enabled quantitative data to be culled from the Abbott CT/NG qualitative assay. Interestingly, the median DC value for CT positive cervical swab specimens (14.7) was significantly greater than the median DC values for CT positive female (9.4) and male (9.3) urine specimens ($P <= 0.0002$ in pair-wise comparisons between cervical swabs, and male and female urine samples, respectively, see Fig. 1A). This is a difference of 5.3 cycles or an approximately 39-fold greater median amount of CT target in cervical swab specimens (assuming 100% PCR efficiency). In contrast, median DC values for NG positive cervical swab and male urine samples were not statistically different ($P = 0.18$). However, the median DC value for NG positive male swab specimens (16.4) was significantly greater than the median DC values for NG positive male urine (13.4) and cervical swab (12.5) samples ($P = 0.013$ and $P = 0.004$ in pairwise comparisons, respectively, Fig. 1B). There were no Abbott NG positive female urine specimens for comparison.

**Limiting dilution analysis.** The relative analytical sensitivity of the Roche and Abbott methods was also assessed by comparing the ability to detect CT and NG in serial dilutions of the commercially available, AmpliTrol™ CT/GC control material in M4- RT. Note, an assigned CT/NG target concentration is not available for the control material from the manufacturer, and therefore we were able to establish relative but not absolute sensitivities for each method. In this analysis (Table 3), we found that the Abbott method to have a lower LOD (detecting a greater dilution of control material) for
CT (Abbott: 1:2000 < LOD ≤ 1:500; Roche: 1:500 < LOD ≤ 1:100; \( P < 0.05 \) for difference in detection at 1:500 and 1:2000 dilutions). The Abbott method also had a lower LOD for NG (Abbot: LOD ≤ 1:5000; Roche: 1:2000 < LOD ≤ 1:500; \( P < 0.05 \) for difference in detection at 1:2000 and 1:5000 dilutions). To assess whether transport buffer might affect the analytical performance of the Abbott assay, control material was also diluted in parallel in Abbott transport media and tested by the Abbott method (Table 3). No statistically significant difference in detection was observed when performing Abbott testing on samples diluted in M4-RT verses Abbott transport media. Furthermore, DC values over the range of dilutions tested for these two transport media were highly correlated (data not shown, \( R^2 = 0.944 \) for CT and \( R^2 = 0.985 \) for NG). Therefore, the two transport media appeared to perform equivalently in analytical testing.

### Discussion

Agreement between the Abbott RealTime CT/NG test and the Roche COBAS AMLICOR CT/NG test was very high, after resolution of Roche NG positive results using alternative LC methods. Analysis of discrepant results, quantitative data from clinical samples, and limiting dilution studies led to several instructive observations.

First, our data suggested that the Roche method had difficulty detecting dual infections, generally in male urine specimens when there were relatively large amounts of NG and small amounts of CT target based on DC analysis. Notably, all of the Abbott CT+/Roche CT- or CT equivocal urine discrepents (Table 1) were NG+ by both methods. The corresponding CT DC values were low indicating only small amounts of CT in the samples, and the average ratio of NG:CT was approximately 16 fold (based...
on a DC ratio of 4). Two of the four urine specimens available for further analysis were confirmed as dual positives by AC2. Therefore, we infer that all of these discrepancies were true dual infections, below the CT detection threshold for the Roche method and potentially in some cases for AC2 as well. However, it should be noted that AC testing was performed after freeze-thaw of urine samples, and it is possible that sample target may therefore have suffered degradation prior to AC2 testing. Furthermore, we recognize that we cannot conclude with absolute certainty that all of the discrepant Abbott dual positive samples in our study should be resolved in Abbott’s favor – a previously noted limitation of discrepant analysis (11).

However, assuming the Abbott dual detection to be accurate, Roche failed to reliably detect CT in 5 of 13 (38%) of dually infected male urine specimens identified during our study. In contrast, it appeared to miss NG in only 1 of 22 (5%) of dually infected cervical swab samples. In this latter sample, Roche was NG-, CT+, and the Abbott CT:NG ratio was very high at 9 or a roughly 500-fold difference between CT and NG target concentration. We hypothesize that significantly more frequent observation of detection failures in dually infected male urine vs. cervical swab specimens ($P = 0.02$, Fisher’s exact test) relates to the relative amounts of NG and CT generally found in these specimen types. Specifically, there was a significantly lower median DC value for CT vs. NG in male urine specimens (DC of 9.3 vs. 13.4, respectively, $P < 0.0001$) vs. a significantly greater median DC value for CT vs. NG in cervical swab specimens (DC of 14.7 vs. 12.5 respectively, $P < 0.0001$). Moreover, the disparity between CT and NG was generally greater and the absolute amount of CT generally lower in male urine than cervical swabs specimens. Mechanistically, we therefore postulate that conditions were
more likely to occur in male urine specimens where a much greater amount of NG masks a small amount of CT and led to failure of the CT component of the assay.

Interestingly, in one previous study AC2 was also found to miss either NG or CT in approximately 14% of dual infections in a female study population (7). The percentage in fact may have been an underestimate as the comparators were Roche COBAS AMPLICOR, Abbott Ligase Chain Reaction (LCx), and/or culture, all potentially less sensitive methods for detection of dual infection. The majority of these presumed dual infection misses were from urine specimens, similar to observations in this study.

Notably, the 2006 CDC sexually transmitted diseases treatment guidelines recommend treating for NG alone, when NG is positive and CT was found to be negative by a nucleic acid amplification method (NAAT) (23). However, our data raises the concern that the Roche method and potentially AC2 as well may not adequately detect low level CT infection, especially in male urine samples from patients co-infected with CT and NG. Based on current treatment recommendations and extrapolating to patient populations in other healthcare settings, a significant number of dually infected patients may therefore receive inadequate treatment as a result of suboptimal ability of some NAAT tests to detect co-infection. It is therefore imperative that manufacturers optimize and clinical laboratories thoroughly evaluate multiplex PCR tests for adequacy in detecting dual infections. Furthermore, clinicians should be aware of the potential for inadequate detection of dual infection with some of the current NAAT methods. They therefore may potentially wish to either treat empirically for dual infection or screen patients at a suitable interval after treatment (e.g., after the 4 weeks generally needed...
Second, although the Abbott assay did not initially receive FDA clearance for detection of CT in cervical swab samples, its comparative clinical performance for CT detection from this sample type was excellent. Specifically, it was found that performance characteristics of the Abbott method on cervical swabs appeared at least as good as the Roche method. Amongst our data set, the six Roche CT+, Abbott CT-swab specimen discrepant were likely due to specimen degradation in cervical specimens, as the 6 specimens that were initially Roche CT+/Abbott CT- were all negative on repeat testing by Roche and AC2. Furthermore, the 6 Roche CT-, Abbott CT+ discrepant may have resulted from potentially superior detection by the Abbott method. Supporting this notion, all samples had low DC values suggesting low bacterial load, which likely stress tested the lower limits of detection of both Abbott and Roche assays. One sample was also NG+ and might have been a Roche dual infection detection failure, as noted above. Lastly, one of four discrepant specimens tested by AC2 was positive for CT. Although the remaining three samples were negative by AC2, it should be noted that cervical swab samples were diluted 30-fold in AC2 transport buffer prior to nucleic acid extraction thereby reducing AC2 sensitivity.

Furthermore, DC analysis indicated that cervical swab samples had a greater median level of CT target than urine samples (which initially were the only female sample type approved for use with the Abbott assay), and therefore should presumably allow enhanced detection of infection in patients with a very low level of organism. However, one potential limitation of DC analysis should be noted. It is possible, but we...
think unlikely, that much higher CT load observed in cervical swab samples may have been specific to our patient population, e.g., if patients screened clinically for CT alone using urine samples (based on the Roche test only being cleared for detection of CT and not NG in this specimen type) differed from those screened for both CT and NG in cervical swab samples. Furthermore, it is possible that first catch urine specimens were not always obtained, thereby leading to dilution of CT in an undefined subset of samples.

Third, we similarly noted a higher median DC value for NG in male swab vs. urine samples. However, in this case, it seemed plausible that only men with more symptomatic infection (e.g., obvious exudate) and therefore a higher burden of infection were likely to be tested via swab samples, helping account for these differences. Supporting this notion, all of the male swab samples analyzed during the course of this study were positive for NG or CT. However, the higher DC values are also consistent with prior observations with paired collections from asymptomatic men in which swab specimens were clinically more sensitive than urine specimens (10).

Fourth, in limiting dilution studies on a CT/NG control material, the Abbott method appeared substantially more sensitive than the Roche method in detection of NG. This difference may relate in part to detection of the multicopy Opa gene (1) (up to 11 copies per cell) by the former verses the single copy M.Ngo PI gene encoding cytosine DNA methyltransferase gene (12) by the latter assay, respectively. The apparent enhanced analytic sensitivity did not translate into observed differences in clinical detection where agreement between the two methods (after resolving Roche NG+ results with LC confirmatory testing) was excellent. However, it should be noted that we did not have
any Abbott NG positive female urine samples during the course of this study, likely because of the smaller number of female urine samples received coupled with the much lower prevalence of NG in our clinical practice. NG+ female urine specimens may have been a specimen type where differences in NG sensitivity would have been more obvious. Supporting this notion, the Roche assay was previously shown to have suboptimal sensitivity for detecting NG in female urine samples and was not FDA-approved for this indication (10). In contrast, the Roche and Abbott CT assays both target the same multi-copy cryptic plasmid present in approximately 5 copies per bacteria (15), and as expected had more similar analytic sensitivity.

Fifth, we also examined the effects of transport media on analytical performance of the Abbott method. Use of M4-RT media in place of the Abbott transport media appeared to have little if any effect on performance characteristics. Therefore, the M4-RT medium may serve as a useful alternative collection media for some laboratories that wish to simplify collection of specimens by use of a single transport medium that will also allow culture of viruses and Chlamydia. The one caveat is that we did observe several Roche CT+ specimens becoming negative on retesting by Roche, Abbott, and AC2 (Roche CT+, Abbott CT- specimens in Table 2), potentially related to degradation of CT positive swab specimens on storage. It is possible that the guanidinium thiocyanate-containing Abbott transfer buffer would have stabilized target in these specimens through denaturation of nucleases in the sample.

Finally, it should be noted that the Abbott assay provides benefit in terms of automation and throughput. On average, the Abbott assay required 1.9 minutes of hands on technologist time per patient sample vs. 2.9 minutes for the Roche assay,
assuming testing of the maximum number of patient samples per run. Furthermore, the Roche assay requires a manual extraction and PCR reaction set up. In contrast, these functions are automated on the Abbott platform. Total duration of a sample run on each instrument platform was 255 minutes for the Roche assay and 420 minutes for the Abbott assay. However, it should be noted that the latter includes time for both automated extraction and PCR reaction set up. Furthermore, the Abbott platform tests up to 93 patient samples per run, while the Roche platform only tests up to 22 patient samples per run.

In summary, the Abbott RealTime CT/NG assay appears to be robust, automated, and accurate in comparison to the well-established Roche COBAS AMPLICOR CT/NG assay. It did not appear to suffer to an appreciable degree from non-specific cross reactivity in the NG portion of the assay noted for some other assays. It also appears to show an enhanced ability to detect dual CT/NG infections. As such it is a welcome addition to the nucleic acid amplification testing options for CT/NG detection.
Acknowledgments. This study was supported in part by grant support received from Abbott Laboratories. Evaluation of the real time, NG confirmatory testing methods was performed as part of a verification of assays used in our clinical laboratory practice prior to and independent of the evaluation of the Abbott RealTime CT/NG method.
References


TABLE 1. Comparison of test results for urine specimens

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CT: 99.6% (97.7%-99.9%, 95% score confidence interval) positive and 97.7% (94.6%-99.0%) negative agreement.

NG: 100% (93.2%-100%) positive and 99.7% (98.4%-100%) negative agreement.

\(^a\)Roche NG test results were scored as positive in this table only if both porA and 16S rRNA real time PCR confirmatory (LC) assays were also positive. Accordingly, 11 Roche NG+ test results were considered false positives based on confirmatory LC testing, and are tabulated among the Roche NG- test results. 45 female urine samples were tested only for CT using the Roche method, and are therefore not included in the NG tabulation. All were Abbott NG-.

\(^b\)Three specimens were Roche CT-, two were Roche CT equivocal.
TABLE 2. Comparison of tests results for cervical swabs

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<td>CT+</td>
<td>CT-</td>
<td>NG+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG-</td>
</tr>
<tr>
<td>CT+</td>
<td>511</td>
<td>6</td>
<td>NG+</td>
<td>56</td>
</tr>
<tr>
<td>CT-</td>
<td>6</td>
<td>401</td>
<td>NG-</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbott

CT: 98.8% (97.5%-99.5%) positive and 98.5% (96.8%-99.3%) negative agreement.
NG: 96.6% (88.3%-99.1%) positive and 99.8% (99.2%-99.9%) negative agreement.

<sup>a</sup>Roche NG test results were only scored as positive if confirmed by LC assays. Accordingly, 185 initially Roche NG+ samples were considered false positives based on LC, and are tabulated as Roche NG- test results.
TABLE 3. Limit of detection (LOD) comparison between Roche COBAS AMPLICOR and Abbott RealTime CT/NG tests

<table>
<thead>
<tr>
<th>Dilution</th>
<th>CT</th>
<th>NG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roche</td>
<td>Abbott</td>
</tr>
<tr>
<td></td>
<td>M4-RT</td>
<td>M4-RT</td>
</tr>
<tr>
<td>1:100</td>
<td>100% (1)</td>
<td>ND</td>
</tr>
<tr>
<td>1:500</td>
<td>70% (1)</td>
<td>100%</td>
</tr>
<tr>
<td>1:2000</td>
<td>25%</td>
<td>65%</td>
</tr>
<tr>
<td>1:5000</td>
<td>15%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Values represent percentage of twenty replicates tested by Abbott and Roche methods that were positive at each dilution of AmpliTrol CT/GC control material. Samples tested by the Abbott method were diluted in either M4-RT or ATB (Abbott Transport Buffer). The number of test results in the Roche equivocal (CT) or expanded indeterminate range (NG) are indicated in parentheses. Note, these results were scored as positives for calculation of percentages presented in this table (see material and methods section for rationale). ND = Testing not done.
Figure Legend.

FIG 1. Plot of DC values for Abbott NG and CT amplification reactions from different specimen types. (A) DC values for CT amplification reactions. Box and whisker plots are shown. The outer edges of the boxes represent the 25 and 75% percentiles respectively, and the dividing line between them the median. The whiskers extend from the lower and upper quartiles to the lowest and highest data points, respectively, still within a region bounded by the interquartile range multiplied by 1.5. (B) DC values for NG amplification reactions.
A

CT

B

NG

cervical swab
female urine
male urine

cervical swab
male swab
male urine

DC Value

DC Value