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Analytical Evaluation of the Abbott RealTime CT/NG Assay for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae in Rectal and Pharyngeal Swabs

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**Chlamydia trachomatis** and **Neisseria gonorrhoeae** are the two most commonly reported sexually transmitted infections in the United States, and the number of cases is increasing.\(^1\) The rectum and pharynx are important extragenital reservoirs of infection because rectal infections are associated with increased risk of HIV acquisition,\(^3\) and oropharyngeal infections are hypothesized to be a source of gonococcal antimicrobial resistance due to decreased tissue penetration of antimicrobial agents.\(^4\) Rectal and pharyngeal infections are typically asymptomatic; thus, detection and treatment of infections at these sites rely on effective screening tests. The US Centers for Disease Control and Prevention (CDC) recommends screening of all sexually active men who have sex with men (MSM) for rectal **C. trachomatis** and **N. gonorrhoeae** infections and pharyngeal **N. gonorrhoeae** at least annually.\(^1\)

Because of their high sensitivity and specificity, nucleic acid amplification tests have become the recommended method for detecting **N. gonorrhoeae** and **C. trachomatis** infections of the urogenital tract.\(^3\) Until recently, no commercial nucleic acid amplification test was approved by the US Food and Drug Administration (FDA) for use on pharyngeal or rectal specimens. However, a recent clinical trial performed by the authors evaluated the performance of three commercially available assays for detection of **C. trachomatis** and **N. gonorrhoeae** in pharyngeal and rectal specimens (ClinicalTrials.gov Identifier: NCT02870101). The results of that study were used to support FDA clearance for the detection of **C. trachomatis** and **N. gonorrhoeae** in two of the three assays for rectal and pharyngeal swabs.\(^6\)

The Abbott RealTime CT/NG assay was included in the aforementioned study and used to create the anatomic site reference standard, described below. Prior interference studies for FDA approval in urogenital specimens were performed previously and not repeated in the current study (Abbott Molecular Inc. package insert: Abbott RealTime Chlamydia trachomatis/Neisseria gonorrhoeae Assay. Des Plaines, IL). The current study reports on the analytical performance of the Abbott Realtime CT/NG assay for detection of **C. trachomatis** and **N. gonorrhoeae** in rectal and pharyngeal specimens.

**Materials and Methods**

All tests were performed on the Abbott m2000 RealTime system (Abbott Laboratories, Abbott Park, IL). Testing was performed by the Alameda County Department of Public Health Laboratory (Oakland, CA), except those tests performed as part of the verification process, described below. Prior interference studies for FDA approval in urogenital specimens were performed previously and not repeated in the current study (Abbott Molecular Inc. package insert: Abbott RealTime Chlamydia trachomatis/Neisseria gonorrhoeae Assay. Des Plaines, IL). All tests were performed using rectal and pharyngeal matrices purchased from a commercial laboratory (BioCollections, Miami, FL); they were obtained from asymptomatic individuals and had documentation of negative test results for **C. trachomatis** and **N. gonorrhoeae** by commercially available assays that the FDA approved for urogenital specimens. The matrices were provided in M2000 multicollection vials that were frozen at −45°C. The specimens were subsequently thawed and pooled into 50-mL volumes for testing.

**Limit of Detection**
The limit of detection for **C. trachomatis** was determined by testing dilutions of **C. trachomatis** (Serovar LGV-II, 434 Strain, Advanced Biotechnologies Inc., Eldersburg, MD) in clinical rectal swab and pharyngeal swab matrices. The quantified stock (320 elementary bodies (EB)/mL) was obtained from a commercial laboratory (Advanced Biotechnologies Inc., Eldersburg, MD), and the elementary bodies and the DNA copy number of the stock were quantified by electron microscopy and quantitative PCR. This strain was chosen as the reference standard because it had been extensively characterized by a number of methods: PCR (genomically) quantified, the EBs were quantified by electron microscopy, and the strain is validated and known to be inactivated. The stock was subsequently diluted in a series of 10 fivefold dilutions, resulting in a range of 64 EB/mL to \(3.3 \times 10^{-5}\) EB/mL. To estimate the limit of detection, each dilution was tested in duplicate. Subsequently, six of the fivefold dilutions were identified across the apparent limit of detection (three above and three below) and assessed in replicates of 20 by the Abbott RealTime PCR for CT/NG on the M2000 system. The highest dilution of EB detectable in ≥95% (19 of 20) of the replicates was defined as the limit of detection.

The limit of detection for **N. gonorrhoeae** was determined using plate counts for quantification. **N. gonorrhoeae** ATCC 49226 was first streaked for purity on chocolate agar. A single colony was isolated and diluted in isotonic nutrient broth in a series of 10-fold dilutions. Each dilution was split into two aliquots, with one aliquot being heat inactivated at 95°C for 15 minutes and 100 μL of the other aliquot simultaneously plated on chocolate agar plates and cultured overnight. Plate counts were then assessed in colony-forming units per milliliter (CFU/mL) to quantify the concentrations of organisms in the heat inactivated aliquot. The heat inactivated aliquot of **N. gonorrhoeae** corresponding to a plate count of 16 CFU/mL was used to make a series of fivefold dilutions of heat inactivated **N. gonorrhoeae**, ranging from 16 to 0.000205 CFU/mL, in rectal or pharyngeal matrices. Those heat inactivated aliquots of **N. gonorrhoeae** ATCC 49226 were evaluated on the Abbott M2000 system in replicates of at least 20. The highest dilution detectable at ≥95% frequency was defined as the limit of detection and reported in CFU/mL.
Results

Limit of Detection

The limit of detection of the Abbott RealTime CT/NG assay for *C. trachomatis* was 2.56 EB/mL in pharyngeal specimens, with all 20 specimens testing positive at this level (100%; 95% CI, 83.2%–100%), and 12.8 EB/mL in rectal specimens, with all 20 specimens testing positive at this level (100%; 95% CI, 83.2%–100%). The limit of detection for *N. gonorrhoeae* was 0.0256 CFU/mL for both pharyngeal and rectal specimens, with 19 pharyngeal specimens (95%; 95% CI, 75.1%–99.9%) and 20 rectal specimens (100%; 95% CI, 83.2%–100%) testing positive at this level (Table 1).

Inclusivity

The inclusivity for *C. trachomatis* was 100% because all 12 *C. trachomatis* serovars tested at four times the limit of detection were detected in 100% (20 of 20) rectal and pharyngeal matrices. The inclusivity for *N. gonorrhoeae* also achieved 100% (20 of 20) inclusivity for detection of each of the seven strains tested.

Analytical Specificity

In total, there were 28 microorganisms spiked into both pharyngeal and rectal specimens and tested by the Abbott RealTime CT/NG assay. There was no cross-reactivity observed, corresponding to an analytical specificity of 100% (Table 2).

Assay Verification

In total, there were 80 specimens tested by the second laboratory. All 40 rectal and pharyngeal specimens spiked with *C. trachomatis* and all 40 rectal and pharyngeal specimens spiked with *N. gonorrhoeae* tested positive. *C. trachomatis* was detected in 0 of the 20 *N. gonorrhoeae*–spiked samples from each anatomic site and *N. gonorrhoeae* was detected in 0 of the 20 *C. trachomatis*–spiked samples from each anatomic site. For the precision testing, all 10 specimens spiked with *C. trachomatis* (100%) tested positive; all 10 specimens spiked with *N. gonorrhoeae* also tested positive by the external laboratory.

Discussion

This study reports the findings of an analytical evaluation of the Abbott RealTime CT/NG assay for detection of *C. trachomatis* and *N. gonorrhoeae* in rectal and pharyngeal specimens. The findings indicate that the assay had 100% inclusivity and specificity among the serotypes and microorganisms tested and that it performed well in the verification study, with 100% accuracy and precision among
In addition, the assay was able to detect low bacterial loads. The limits of detection claim for the Abbott RealTime CT/NG assay in urogenital specimens is 320 copies of C. trachomatis target DNA and 320 copies of N. gonorrhoeae target DNA per assay, corresponding to approximately 30 to 40 organisms per assay (Abbott Molecular Inc. package insert: Abbott RealTime Chlamydia trachomatis/Neisseria gonorrhoeae Assay. Des Plaines, IL). Results of the current study showed the limit of detection for C. trachomatis to be 12.8 EB/mL in rectal specimens, 2.56 EB/mL in pharyngeal specimens, and 0.0256 CFU/mL in both rectal and pharyngeal specimens for N. gonorrhoeae. Although it is difficult to compare precisely copies of DNA with EB or CFU, these findings suggest that the limits of detection for C. trachomatis and N. gonorrhoeae in rectal and pharyngeal specimens are similar or slightly lower than the reported claims for the Abbott RealTime CT/NG assay in urogenital specimens.

The limits of detection of the Abbott RealTime CT/NG assay for C. trachomatis in both rectal and pharyngeal specimens were somewhat lower than those reported by another PCR-based assay, the Xpert CT/NG assay (Cepheid, Sunnyvale, CA), an FDA-approved assay for rectal and pharyngeal specimens. The Xpert CT/NG assay limit of detection was 88 to 161 EB/mL in rectal and 161 to 225 EB/mL in pharyngeal specimens by probit analysis (Cepheid package insert: Xpert CT/NG. Sunnyvale, CA). For N. gonorrhoeae, the limit of detection on the Abbott RealTime CT/NG assay was estimated to be 0.0256 CFU/mL in both rectal and pharyngeal specimens, which also compared favorably to the Xpert CT/NG assay, with limits of detection reported to be 4.9 to 5.3 CFU/mL in rectal specimens and 6.4 to 7.1 CFU/mL in pharyngeal specimens tested. In addition, the assay was able to detect low bacterial loads.

Table 1 Serial Dilutions of Chlamydia trachomatis and Neisseria gonorrhoeae in Rectal and Pharyngeal Specimens Used to Determine the Limit of Detection of the Abbott RealTime CT/NG Assay

| Assay type | Source | Concentration* | No. of positive samples | No. of negative samples | Percentage of positive samples (95% CI) |
|------------|--------|---------------|-------------------------|------------------------|--------------------------------------|
| Chlamydia trachomatis (EB/mL) | Pharyngeal | 320 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 64 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 12.8 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 2.56 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 0.512 | 17 | 3 | 85.0 (62.1—96.8) |
| | | 0.1024 | 0 | 20 | 0.0 (0.0—16.8) |
| | | 0.02048 | 0 | 22 | 0.0 (0.0—15.4) |
| | | 0.004096 | 0 | 19 | 0.0 (0.0—17.7) |
| | Rectal | 320 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 64 | 21 | 0 | 100.0 (83.9—100.0) |
| | | 12.8 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 2.56 | 18 | 2 | 90.0 (68.3—98.8) |
| | | 0.512 | 16 | 4 | 80.0 (56.3—94.3) |
| | | 0.1024 | 5 | 15 | 25.0 (8.7—49.1) |
| | | 0.02048 | 3 | 17 | 15.0 (3.2—37.9) |
| | | 0.004096 | 0 | 20 | 0.0 (0.0—16.8) |
| | | 0.0008192 | 0 | 20 | 0.0 (0.0—16.8) |
| Neisseria gonorrhoeae [CFU/mL] | Pharyngeal | 16 | 6 | 0 | 100.0 (54.1—100.0) |
| | | 3.2 | 22 | 0 | 100.0 (84.6—100.0) |
| | | 0.64 | 21 | 0 | 100.0 (83.9—100.0) |
| | | 0.128 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 0.0256 | 19 | 1 | 95.0 (75.1—99.9) |
| | | 0.00512 | 4 | 16 | 20.0 (5.7—43.7) |
| | | 0.000124 | 0 | 20 | 0.0 (0.0—16.8) |
| | | 0.0000205 | 0 | 20 | 0.0 (0.0—16.8) |
| | Rectal | 16 | 6 | 0 | 100.0 (54.1—100.0) |
| | | 3.2 | 21 | 0 | 100.0 (83.9—100.0) |
| | | 0.64 | 21 | 0 | 100.0 (83.9—100.0) |
| | | 0.128 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 0.0256 | 20 | 0 | 100.0 (83.2—100.0) |
| | | 0.00512 | 2 | 18 | 10.0 (1.2—31.7) |
| | | 0.000124 | 1 | 19 | 5.0 (0.1—24.9) |
| | | 0.0000205 | 0 | 20 | 0.0 (0.0—16.8) |

Bold values indicate the limit of detection, where ≥95% of the replicates were detected.

* Concentrations are given in elementary bodies per milliliter (EB/mL) for C. trachomatis and colony-forming units per milliliter (CFU/mL) for N. gonorrhoeae.
specimens by probit analysis (Cepheid package insert: Xpert CT/NG. Sunnyvale, CA). The limits of detection for both assays were performed on heat inactivated specimens corresponding to a CFU/mL broth dilution. The genetic targets of the assay exist in numerous copies within each cell and are released at heat inactivation, thus making it possible to detect >1 CFU/mL, which is a method for quantification of viability. In addition, limit of detection data from the Abbott RealTime CT/NG assay also compare favorably with other PCR platforms for the detection of C. trachomatis and N. gonorrhoeae in vaginal and urine specimens. For example, the FDA-approved BD Max CT/GC/TV assay has a limit of detection as low as 5 to 100 EB/mL in urine specimens for C. trachomatis and as low as 60 to 117 cells/mL in vaginal specimens for N. gonorrhoeae (Becton Dickinson and Company Life Sciences package insert: BD MAX CT/GC/TV Assay. Sparks, MD).

Previous studies have evaluated the bacterial loads associated with N. gonorrhoeae and C. trachomatis infections at rectal and pharyngeal sites. In 2013, Bissessor et al\textsuperscript{9} found that bacterial loads of N. gonorrhoeae obtained from MSM were higher in the rectum (median, 18,960 copies per swab) compared with the pharynx (median, 2100 copies per swab) and highest among men with symptoms of proctitis (median, 278,000 copies per swab), whereas Chow et al\textsuperscript{10} reported a median 170,493 copies per swab among men testing positive by nucleic acid amplification tests. In a systematic review that assessed bacterial loads for C. trachomatis, Vodstrcil et al\textsuperscript{11} found considerable variation of bacterial loads by anatomic site, sex, and quantification technique, which limits comparisons. However, more recently, Phillips et al\textsuperscript{12} evaluated bacterial loads of C. trachomatis in MSM and found a median of 1204 copies per swab in the posterior oropharynx. Two studies that evaluated C. trachomatis bacterial loads in rectal infections found similar results, with a median log of 3.4 copies/mL (2512) and mean log of 3.5 copies/mL (3162) C. trachomatis among women\textsuperscript{13} and men\textsuperscript{14}.

One recent large study found higher bacterial loads in rectal infections compared with pharyngeal infections and no differences between men and women tested but reported only cycle threshold levels.\textsuperscript{15} Although it is difficult to define the precise number of bacteria needed to cause infections at these sites, the limit of detection for the Abbott RealTime CT/NG assay reported here would be expected to detect infections for the bacterial loads in those reports.

Table 2  Analytical Specificity of Abbott RealTime CT/NG Assay against 28 Bacteria and Viruses in Rectal and Pharyngeal Matrices

| Microorganism                      | Concentration          | Pharyngeal specimens | Rectal specimens |
|------------------------------------|------------------------|----------------------|-----------------|
|                                    |                        | Chlamydia trachomatis| Neisseria gonorrhoeae | Chlamydia trachomatis| Neisseria gonorrhoeae |
| Acinetobacter baumannii            | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Arcanobacterium hemolyticum        | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Adenovirus                         | $2.5 \times 10^6$ DNA copies/assay | Negative | Negative | Negative | Negative |
| Bacteroides oralis                 | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Bordetella pertussis               | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Burkholderia cepacia               | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Campylobacter rectus               | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Citrobacter koseri                 | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Clostridium difficile              | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Corynebacterium diphtheriae        | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Corynebacterium pseudodiphtheriticum | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Enterovirus                        | $2.5 \times 10^6$ DNA copies/assay | Negative | Negative | Negative | Negative |
| Epstein-Barr virus                 | $2.5 \times 10^6$ genomic copies/assay | Negative | Negative | Negative | Negative |
| Fusobacterium necrophorum          | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Hemophilus parahaemolyticus        | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Hemophilus parainfluenzae          | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Helicobacter pylori                | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Legionella micdadei                | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Neisseria meningitidis            | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Norovirus                          | $3.0 \times 10^6$ RNA copies/assay | Negative | Negative | Negative | Negative |
| Peptostreptococcus micros          | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Providencia alcalifaciens          | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Staphylococcus hemolyticus         | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Stenotrophomonas maltophilia       | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Streptococcus anginosus group       | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Streptococcus bovis                 | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Streptococcus mitis                | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
| Veillonella parvula                | $1.0 \times 10^6$ cells/assay | Negative | Negative | Negative | Negative |
The clinical performance of the Abbott RealTime CT/NG in rectal and pharyngeal specimens was recently evaluated in a multicenter study of 2598 symptomatic and asymptomatic patients, alongside two other assays that have now been FDA approved for detection of *C. trachomatis* and *N. gonorrhoeae* at rectal and pharyngeal sites. The negative predictive value of the Abbott RealTime CT/NG assay was high: 98.7% and 99.0% for *N. gonorrhoeae* at pharyngeal and rectal sites, respectively, and 99.7% and 98.3% for *C. trachomatis* at pharyngeal and rectal sites, respectively. Those estimates were similar to the other two assays tested and correspond to high sensitivity of the assays. The limit of detection of the assays support the high sensitivity of the test.

The data presented herein demonstrate that the Abbott CT/NG RealTime assay is an accurate, sensitive, specific, and reliable assay in laboratory-derived rectal and pharyngeal specimens. These findings, alongside clinical performance data, support potential claims of use of the assay for detection of *C. trachomatis* and *N. gonorrhoeae* infections in rectal and pharyngeal specimens.

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