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# Aptima Combo 2 Testing Detected Additional Cases of *Neisseria gonorrhoeae* Infection in Men and Women in Community Settings[▽]

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**Aptima Combo 2 (AC2) *Neisseria gonorrhoeae* testing of 81,405 patients who were tested by culture and 14,666 who were AC2 tested for *Chlamydia trachomatis* detected 142 extra infections and confirmed 106 culture-positive samples (the positivity rate increased from 0.13 in testing by culture to 0.26 in testing by AC2). Retrievable AC2 positive samples were confirmed (98.5%) by an alternate AGC test.**

Lower genital tract infections with *Neisseria gonorrhoeae* may be asymptomatic and accompanied by *Chlamydia trachomatis* infection(13). Efforts are needed to identify and treat lower tract infections to prevent upper tract complications, such as pelvic inflammatory disease, ectopic pregnancy, or tubal infertility in women and less commonly epididymitis or prostatitis in men, as well as transmission between asymptomatically infected patients and their uninfected partners. Attempts to culture *N. gonorrhoeae* from clinical specimens can be unsuccessful. Testing for *N. gonorrhoeae* with nucleic acid amplification tests (NAATs) has increased diagnostic sensitivity (3, 4, 9, 11, 18, 19) using traditional and less-invasive sampling. Although not all are FDA cleared, first-void urine (FVU), self-collected vaginal swabs (VS), and anal and oral swabs have been shown to yield positive results (1, 7, 15). The commercially available transcription-mediated amplification (TMA) test Aptima Combo 2 (AC2) is able to detect *N. gonorrhoeae* and *C. trachomatis* RNA in clinical specimens collected in specimen transportation media (STM), with no cross-reactions with non-*N. gonorrhoeae* strains. Positives can be confirmed in alternate individual TMA tests, Aptima GC (AGC) (for *N. gonorrhoeae*) and APTIMA CT (ACT) (for *C. trachomatis*) (2, 12).

Community physicians practicing in Southern Ontario who suspect that their patient may have an *N. gonorrhoeae* infection traditionally submit cervical swabs (CS) from women and urethral swabs (US) from men for culture. If a *C. trachomatis* infection is suspected, an NAAT will be ordered for a CS or FVU. If both infections are suspected, specimens are collected for *N. gonorrhoeae* culture and *C. trachomatis* nucleic acid amplification testing. We evaluated the utility of performing additional AC2 testing for *N. gonorrhoeae* on specimens submitted for *N. gonorrhoeae* culture and *C. trachomatis* TMA. The objectives were as follows: (i) to perform *N. gonorrhoeae* testing by AC2 on STM samples from patients receiving *N. gonorrhoeae* culture, (ii) to test STM samples submitted for *C. trachomatis* testing for *N. gonorrhoeae*, and (iii) to confirm AC2-GC positives using the AGC assay.

From March to August 2008, the microbiology laboratory at Gamma Dynacare Medical Laboratories in Brampton, Canada, received 96,071 urogenital samples from 961 men (FVU or US) and 95,110 women (FVU or CS) for *C. trachomatis* and *N. gonorrhoeae* testing or *C. trachomatis* testing only (Fig. 1). There were 81,405 patients in group A, whose physicians submitted a swab collected in an M40 Transystem specimen transport system (Copan Diagnostics Inc.) for *N. gonorrhoeae* culture and an additional sample collected in STM (GenProbe) for AC2 testing for *C. trachomatis*. Group B consisted of 14,666 men and women whose physicians collected FVU samples or swabs into STM for AC2 testing for *C. trachomatis* only. Specimens tested for *C. trachomatis* or *N. gonorrhoeae* RNA were processed on the semiautomated GenProbe DTS 1600 system or on an automated Tigris instrument. *N. gonorrhoeae* positives were confirmed using the AGC assay. Samples for *N. gonorrhoeae* culture were inoculated onto modified Martin-Lewis chocolate agar biplates (catalogue no. P4100; PML Microbiologicals). Cultures were confirmed with Vitek NH1 test cards and Gonogen serological tests.

Figure 1 shows that from group A, 106 of the patients with a positive *N. gonorrhoeae* culture (0.13% prevalence) were also positive by AC2 testing and an additional 67 were negative by culture but positive by AC2, an increase of 63%. There were no culture-positive, NAAT-negative findings. These findings are similar to those of a previous study (10) comparing Cobas Amplicor (AMP) PCR to culture of 3,023 clinical specimens from woman and men, which demonstrated an increase from 58 to 85 positives, an increase of 46% due to PCR testing. From group B, in the present study, the 14,666 samples yielded 75 *N. gonorrhoeae* positives. The total number of extra *N. gonorrhoeae* positives from AC2 testing in both groups was 142, more than doubling the number of *N. gonorrhoeae* positives by culture, for a total of 248 (0.26% prevalence). An examination of the gender and specimen types revealed the 248 *N. gonorrhoeae* positives to be distributed in 115 males (55 FVU and 60 US) and 133 females (28 FVU and 105 CS). For all patients,

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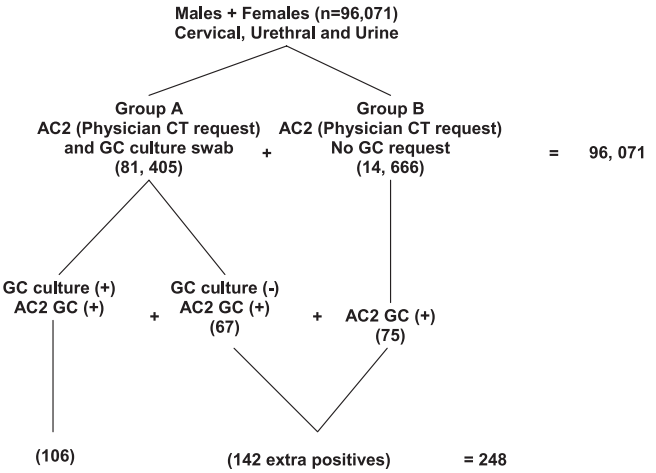


FIG. 1. Algorithm of testing shows 142 extra cases of *N. gonorrhoeae*.

the prevalence of *N. gonorrhoeae* infection by culture was 0.13% (106/81,405): in women it was 0.09% (73/80,590) by culture and 0.14% (133/95,110) by AC2 ($P < 0.001$). The prevalence in men by culture was 6% (49/815) and increased to 11.9% (115/961) ($P < 0.001$) when all were tested by AC2.

Of 142 extra positive samples, 65 were available for confirmatory testing by AGC and 64 (98.5%) were confirmed to be positive. Confirmatory testing of GC Amplicor (AMP)-positive results in a previous study (10) showed a 56.1% rate of confirmation (87/155) using a 16S rRNA *N. gonorrhoeae* PCR (Roche), and only 57 were culture positive. This lower rate of confirmation may have been due to the AMP PCR cross-reacting with nongonorrhoea *Neisseria* (NGN), a phenomenon which has been widely reported (6, 7, 17). In contrast, there have been no reports of AC2 or AGC assays falsely reacting with NGN (2, 12), but because the AC2 assays have such high analytical sensitivity (4), original low-level positives may not always repeat positive and confirm (12).

Several studies have examined populations for dual *N. gonorrhoeae*/*C. trachomatis* infections (5, 8, 14, 16), showing a wide range depending on the population examined. Although we were unable to examine our database for dual infections, a current 2-month determination showed similar prevalence rates due to dual combo testing (*C. trachomatis*, 2.9%; *N. gonorrhoeae*, 0.29%) with 48 patients infected with both. Thus, combination testing for both organisms provides data on dual and single infections, providing information preventing unnecessary antibiotic treatment for both infections in patients infected with only one pathogen, since current clinical guidelines are to treat both. Dual infections and culture failures for patients investigated in community referral settings suggest that Aptima Combo 2 testing of samples submitted for *C. trachomatis* testing can identify extra *N. gonorrhoeae*-positive patients who would benefit from treatment of the appropriate pathogen. This may also be a cost-beneficial strategy if testing for both *C. trachomatis* and *N. gonorrhoeae* costs the same as testing for *C. trachomatis* alone. However, until molecular methods are available for detection of antibiotic-resistant *N. gonorrhoeae* from samples positive by NAATs, some form of sentinel culturing will be required (19).

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