APTIMA Combo 2 Testing Detected Additional Cases of *Neisseria gonorrhoeae* in Men and Women in Community Settings

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ABSTRACT

APTIMA Combo 2 [AC2] N. gonorrhoeae testing of 81,405 patients who were cultured and 14,666 who were AC2-tested for C. trachomatis, detected 142 extra infections and confirmed 106 culture-positive samples (positivity rate increased from 0.13 by culture to 0.26 by AC2). Retrievable AC2-positives confirmed [98.5%] by an alternate AGC test.
Lower genital tract infections with *Neisseria gonorrhoeae* [GC] may be asymptomatic and accompanied by *Chlamydia trachomatis* [CT] (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; and transmission between asymptptomatically infected patients to their uninfected partners. Attempts to culture GC from clinical specimens can be unsuccessful. Testing for GC with nucleic acid amplification tests [NAAT] 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], anal and oral swabs have been shown to yield positive patients (1,7,15). The commercially available transcription mediated amplification [TMA] test, APTIMA Combo 2 [AC2] is able to detect GC and CT RNA in clinical specimens collected into specimen transportation media (STM), with no cross reactions with non-GC strains. Positives can be confirmed in alternate individual TMA tests, APTIMA GC [AGC] and APTIMA CT [ACT] (2,12).

Community physicians practising in Southern Ontario suspecting that their patient may have a GC infection, traditionally will submit cervical swabs [CS] from women and urethral swabs [US] from men for culture. If a CT infection is suspected an NAA test will be ordered for a CS or FVU. If both infections are suspected, specimens are collected for GC-culture and CT NAA testing. We evaluated the utility of performing additional AC2 testing for GC on specimens submitted for GC-culture and CT-TMA. The objectives were as follows: [a] to
perform GC testing by AC2 on STM samples from patients receiving GC-culture; [b] to GC-test STM samples submitted for CT-testing; and [c] to confirm AC2-GC positives using the AGC assay.

From March to August 2008 the Microbiology laboratory at Gamma Dynacare Medical Laboratories in Brampton, Ontario received 96,071 urogenital samples from 961 men [FVU or US] and 95,110 women [FVU or CS] for CT and GC testing or CT only [Figure 1]. There were 81,405 patients in Group A whose physicians submitted a swab collected into an M40 Transystem specimen transport system [Copan Diagnostics Inc] for GC culture and an additional sample collected into STM (GenProbe) for AC2 testing for CT. Group B consisted of 14,666 men and women whose physicians collected FVU samples or swabs into STM for AC2 testing for CT only. Specimens tested for CT or GC RNA were processed on the semi-automated GenProbe DTS 1600 system or on an automated TIGRIS instrument. GC-positives were confirmed using the AGC assay. Samples for GC culture were inoculated onto Modified Martin-Lewis chocolate agar biplates [catalogue #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 GC 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 positives, 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 GC-positives. The total number of extra GC-positives from AC2 testing in both groups was 142, more than doubling the number of GC-positives by culture, for a total of 248 [0.26% prevalence]. An examination of the gender and specimen types revealed the 248 GC positives to be distributed in 115 males [55 FVU and 60 US] and 133 females [28 FVU and 105 CS]. For all patients, the prevalence of GC infection by culture was 0.13% [106/81,405]: in women it was 0.09% [73/80,590] by culture and 0.14% by AC2 [133/95,110] (p<0.001). 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 positives 65 were available for confirmatory testing by AGC and 64 [98.5%] were confirmed as 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 non-gonorrhoea *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 GC/CT 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 (CT 2.9% and GC 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 as current clinical guidelines are to treat both. Dual infections and culture failures from patients investigated in community referral settings suggest that APTIMA Combo 2 testing of samples submitted for CT testing can identify extra GC-positive patients who would benefit from treatment of the appropriate pathogen. This may also be a cost beneficial strategy if testing for both CT and GC costs the same as testing for CT alone. However, until molecular methods are available for detection of antibiotic resistant-GC from samples positive by NAATs, some form of sentinel culturing will be required (19).
References


Figure 1: Algorithm of testing shows 142 extra cases of GC.