Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine, Endocervical, Vaginal and Urethral Specimens

Background Information

Sexually transmitted diseases (STDs) continue to be a major cause of deteriorating reproductive health throughout the world. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* remain as two of the most common causes of STDs in the United States.[1] *C. trachomatis* infections have comprised the largest proportion of all STDs reported to the CDC since 1994 with a reported 1,244,180 cases in 2009 for a rate of 409.2/100,000 population. This was a 2.8% increase in rate from that reported in 2008.[1,2] The increase in reported chlamydial infections during the last 20 years reflects the expansion of chlamydia screening activities and the use of increasingly sensitive assays for the detection of *C. trachomatis*. The CDC recommends annual chlamydia screening of all sexually active women younger than 25 years of age.[3]

In 2009, there were 301,174 cases of *N. gonorrhoeae* infections reported for a rate of 99.1/100,000 population. This rate was a 10.5% decrease since 2008. The national gonorrhea rate declined by 74% between 1975 and 1997 following implementation of a national gonorrhea control program in the mid-1970s. However, since 1997, these rates have reached a plateau and are not continuing to decline.[1,2] Infections due to both *Chlamydia trachomatis* and *N. gonorrhoeae* are a major cause of pelvic inflammatory disease (PID) in the U.S. and both have been shown to facilitate the transmission of HIV as well.

More rapid and sensitive methods for the laboratory diagnosis of these two agents have been developed and make it reasonable to test for both agents simultaneously when the diagnosis of an STD is being considered.[2] The estimate of mixed infections with both agents can be as high as 40%, making it important to consider ordering both agents when sending material off to the laboratory for testing. Nucleic acid amplification tests (NAAT) are recommended for detection of reproductive tract infections caused by *C. trachomatis* and *N. gonorrhoeae infections* in men and women with and without symptoms. NAAT should be used for diagnosing both *C. trachomatis* and *N. gonorrhoeae* in women with cervicitis; testing can be performed on vaginal, cervical or urine samples. In men with urethritis, NAAT testing of urine or urethral swabs is recommended.[3]

Clinical Indications

Both *C. trachomatis* and *N. gonorrhoeae* cause urethritis in the male and cervicitis in the female. A significant number of cases, however, remain asymptomatic in both males and females. In addition, both can cause epididymitis and rectal infections in the male and PID in the female. Neonates, who contract chlamydial infection during birth, can develop inclusion conjunctivitis and/or pneumoniae; pregnant women can infect their newborns causing ophthalmia neonatorum; gonorrheal infections can produce joint infections, pharyngitis and disseminated disease.

Cleveland Clinic Laboratories offers a target amplification nucleic acid probe, APTIMA, Gen-Probe, Inc, San Diego, CA) for the laboratory diagnosis of *C. trachomatis* and *N. gonorrhoeae* from urethral and urine specimens from males suspected of these infections and in cervical, vaginal and urine samples from females suspected of the infection. Numerous articles have been published demonstrating the excellent performance of NAAT testing for diagnosis of both of these STD agents.[4-8]

Methodology

The laboratory diagnosis of *Neisseria gonorrhoeae* can include culture of urethral or cervical specimens, gram stain of the urethral secretions in symptomatic males, detection by specific nucleic acid gene probes and amplification of *N. gonorrhoeae* nucleic acids. Amplification of *N. gonorrhoeae* nucleic acids has been shown to be a very sensitive and specific method of detection.[4,5] The sensitivity is equivalent

to culture, but it is not fraught by the problem of organism fragility that can easily occur with delays in transport. Although culture or the use of a nucleic acids probe can be employed for the detection of *C. trachomatis*, nucleic acid amplification is the most sensitive method with studies indicating that it may be up to 40% more sensitive than culture. The same assay that detects *Chlamydia trachomatis* nucleic acids is also used to detect *Neisseria gonorrhoeae* nucleic acids in Cleveland Clinic Laboratories, thus providing a convenient approach to dual detection.

Specimen Collection and Transport

Acceptable specimens include urine, vaginal, urethral and endocervical swabs. A vaginal swab is optimal for screening asymptomatic females, while a first-catch urine is optimal for screening asymptomatic men. Collect swabs using the appropriate Aptima collection kits [APTIMA vaginal swab #389073; APTIMA urine specimen collection kit #150663; APTIMA unisex (urethral or endocervical) swab collection kit #150664]. Swabs in viral transport media (M4) are acceptable, but may reduce test sensitivity. Specimen collection/transport using APTIMA devices is preferred.

The APTIMA Unisex Swab Specimen collection kit for urethral or endocervical specimens contains a white cleaning swab to be used for removing excess mucus. The blue swab must be used for collection of specimens that are submitted for testing. For urethral specimens, patients should not urinate within 1 hour prior to specimen collection. Insert the blue shaft swab 2 to 4 cm into the urethra. Gently rotate swab clockwise for 2 to 3 seconds and withdraw carefully. For endocervical specimens, remove excess mucus using cleaning swab and then insert blue shaft swab into the endocervical canal. Rotate swab for 10-30 seconds in the endocervical canal to ensure adequate sampling and withdraw carefully (avoid contact with vaginal mucosa). Place swab in transport tube and carefully break at score line. Use care to avoid splashing contents. Discard top portion of swab shaft and recap transport tube tightly. Maintain specimen at 2ºC to 30ºC.

When collecting vaginal specimens, hold the swab with forefinger and thumb covering the score line. Do not hold the shaft below the score line. Carefully insert the swab into the vagina about 2 inches and gently rotate the swab for 10-30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin. While holding the swab in the same hand, unscrew the cap from the tube being careful not to spill contents of the tube. Immediately place the swab in the transport tube and carefully break swab shaft at score line against side of tube. Discard the top portion of swab shaft. Tightly screw cap onto tube. Maintain specimen at 2ºC to 30ºC.

If a urine specimen is collected, the patient should not have urinated within one hour of collection. Collect the first catch urine (approximately 20-30 ml of initial urine stream; collection of larger volumes of urine will reduce test sensitivity). Within 24 hours of collection, transfer 2 ml of urine into the APTMA urine transport tube using the disposable pipette provided in the collection kit. The correct volume of urine has been added when the fluid level is between the black lines on the transport tube label. Maintain urine specimen at 2ºC to 30ºC.

Alternatively, if a ThinPrep vial is being used for Liquid Cytology PAP Test, the same sample can be submitted for detection of *C. trachomatis* and *N. gonorrhoeae* as well.[9] The assay can only be performed on Thin Preps if 1 ml Cytyc PreservCyt Solution is transferred to the APTIMA Specimen Transfer Tube (#367648) before the specimen is processed in Cytology for a PAP test.

Interpretation

Amplification is performed Monday through Friday. Internal controls are run with each specimen in order to detect any inhibitors in the sample.

Results will be reported as “positive for *C. trachomatis* and/or *N. gonorrhoeae* by amplification” when the relative light unit (RLU) result is above our positive cutoff value.

Within a narrow range of RLU results, as determined by the assay manufacturer, an “equivocal result” will be reported with a request that a repeat specimen be submitted.

If the internal control indicates inhibition and the result is negative for *C. trachomatis* and/or *N. gonorrhoeae*, the report will be: “inhibition detected; *N. gonorrhoeae*, and/or *C. trachomatis*, if present, would not be detectable. Please send an additional specimen.”

All results for *N. gonorrhoeae* and/or *C. trachomatis* that are lower than the lab's derived positive cutoff, but within the instrument derived positive results, will be confirmed with a repeat amplification assay before reports are released. This is done to avoid any problems with false positive results that might occur with low positive results.[10]

Limitations of the Assay

There is currently no FDA clearance for use of amplification assays on specimens outside of the genitourinary tract. Culture is recommended for testing specimens from the throat, eye or rectal area. However, a laboratory can validate use of NAAT for rectal and pharyngeal specimens. In addition, for specimens obtained from infants and children, or if the information from the laboratory is to be used for legal purposes, culture is the preferred method.[7] Since NAAT is more sensitive, it may be run in conjunction with culture for purposes of treatment decision-making. Although "test-of-cure" samples are not recommended from patients in whom the diagnosis has previously been made within the last 4-6 weeks, if required, a culture is the preferred request.

If a culture is needed for any of these purposes, the collection swab for *Neisseria gonorrhoeae* needs to be placed into a culturette and **NOT** the Aptima transport tube and a specific request for culture should be made.

References

1. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2009. Atlanta, GA: U.S. Department of Health and Human Services; November 2010. Printed copies and the on-line version of this report can be obtained at the following website: http://www.cdc.gov/std/pubs.
2. http://www.cdc.gov/std/stats09/chlamydia.htm
3. Centers for Disease Control and Prevention. Sexually transmitted Diseases treatment Guidelines. MMWR. 2010;59(RR#2):1-110.
4. Chernesky M, Martin DH, Hook EW *et al*. Ability of Aptima CT and Aptima GC assays to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male urine and urethral swabs. *J Clin Microbiol*. 2005;43:127-31.
5. Gaydos CA, Quinn TC, Willis D, Weissfeld A, Hook EW, Martin DH, Ferrero DV, Schachter J. Performance of the APTIMA Combo 2 assay for the multiplex detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. *J Clin Microbiol.* 2003;41:304-309.
6. Fang J, Husman C, Dasilva L *et al*. Evaluation of self-collected vaginal swab, first void urine, and endocervical swabs for the detection of *C. trachomatis* and *N. gonorrhoeae* in adolescent females. *J Pediatr Adolesc Gynecol*. 2008;21:355-60.
7. Blake DR, Maldeis N, Barnes MR *et al*. Cost-effectiveness of screening strategies for *C. trachomatis* using cervical swabs, urine, and self-obtained vaginal swabs in a sexually transmitted disease clinic setting. *Sex Transm Dis*. 2008;35:649-55.
8. Schachter J, Chernesky MA, Willis DE, *et al*. Vaginal swabs are the specimens of choice when screening for *C. trachomatis* and *N. gonorrhoeae*: results from a multicenter evaluation of the Aptima assays for both infections. *Sex Transm Dis*. 2005;32:725-8.
9. Chernesky M, Jang D, Smieja M *et al*. Validation of the Aptima Combo 2 assay for detection of *C. trachomatis* and *N. gonorrhoeae* in Sure-Path liquid-based pap test samples taken with different collection devices. *Sex Transm Dis*. 2009;36:581-2.
10. Farrell, DJ. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using cppB nested PCR and 16S rRNA PCR. *J Clin Microbiology*. 1999;37:386-90.

Cleveland Clinic

Cleveland Clinic Laboratories

Test Overview

Test Name	*Chlamydia trachomatis* and *Neisseria gonorrhoeae* amplification		
Ordering Mnemonic	GCCT, CT, GT		
Reference Range	Negative		
Patient Preparation	Use the larger cotton swab for removal of excess mucus from endocervix, vagina and/or urethra; discard this swab.		
Methodology	Target amplification nucleic acid probe, qualitative		
Acceptable Specimens	Vaginal swab (optimal for screening of asymptomatic females), first-catch urine (optimal for screening of asymptomatic men), male urethral swab, endocervical swab. Collect in appropriate Aptima transport media [APTIMA vaginal swab #389073; APTIMA urine specimen collection kit #150663; APTIMA unisex (urethral or endocervical) swab collection kit #150664]. Swabs in viral transport media (M4) are acceptable, but may reduce test sensitivity. Specimen collection/transport using APTIMA devices is preferred.		
Specimen Preparation	Transfer urine (2 ml) or swab to respective APTIMA specimen transport tube. Urine fluid level must be between the black lines indicated as fill area. The transport solutions release the rRNA targets and protect them from degradation. Note: A vaginal swab is the optimal specimen for screening women, but ThinPrep PreservCyt may also be used for cervical collections. Prior to cytology testing, transfer 1 ml aliquot to GenProbe APTIMA Specimen Transfer Tube (APTIMA Specimen Transfer Kit #367648) within 30 days of collection when stored at 2 to 30ºC.		
Stability	Unpreserved urine (ambient or refrigerated): 24 h Swab in APTIMA transport media (vaginal #389073; urethral #150664), ambient or refrigerated: 60 days (12 months if frozen) Swab in viral transport media, ambient 1 week; refrigerated 1 month (3 months if frozen) Urine in APTIMA transport media (#150663), ambient or refrigerated: 30 days (12 months if frozen) ThinPrep PreservCyt solution, unprocessed, ambient or refrigerated: 30 days ThinPrep PreservCyt solution in APTIMA transfer tube (#367648), refrigerated: 30 days (12 months if frozen; 14 days if ambient) Unacceptable conditions: Unpreserved urine received >24 h after collection, overfilled or underfilled urine APTIMA tube, swab not received in correct transport media.		
Test Ordering Information	**GC/Chlamydia Amplification** GCCT	**Chlamydia Amplification** CT	**GC Amplification** GC
Billing Code	79830	79809	79810
CPT Code(s)	87491; 87591	87491	87591
Related Tests	*Chlamydia trachomatis* amplification		

Technical Information Contacts:

Colleen Starkey, MT(ASCP)
216.444.8792
starkec@ccf.org

Scientific Information Contacts:

Sandra S. Richter, MD
216.444.6519
richtes@ccf.org

Gary W. Procop, MD
216.444.5879
procopg@ccf.org