CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

Technical Bulletin
No. 98b

October 13, 2016

Chlamydia trachomatis and Neisseria gonorrhoeae Dual Target PCR Assay UPDATED Specimen Requirements

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Ordering Information:

TEST NUMBER	TEST NAME	
3009004	NG CERVICAL	
3009006	NG URINE	
3009008	NG VAGINAL	
3009010	NG CYTOLOGY	
3009012	CT CERVICAL	
3009014	CT URINE	
3009016	CT VAGINAL	
3009018	CT CYTOLOGY	
3009020	CTNGCER	**Ordering Group**
3009025	CTNGURN	**Ordering Group**
3009040	CTNGVAG	**Ordering Group**
3009050	CTNGCYT	**Ordering Group**

Effective Date: June 15, 2016

Performed: Set up Monday through Saturday. Reported daily.

UPDATED Specimen Requirements:
Specimen Types:
The following specimen types are acceptable for CT/NG PCR testing (see collection, transport, and storage instructions that follow below).

1. Endocervical swabs collected with the cobas® PCR Female Swab Sample Kit
2. Male and female urine collected in sterile containers
3. Physician-collected vaginal swab specimens collected with the cobas® PCR Female Swab Sample Kit
4. Self-Collected vaginal swab specimens collected with the cobas® PCR Female Swab Sample Kit
5. Female cervical specimens received in sterile collection tubes containing PreservCyt

Specimen Collection:

ENDOCERVICAL SWAB SPECIMEN COLLECTION:

IMPORTANT: Vaginal swabs may only be collected using cobas® PCR Female Swab Sample Kit. These kits are available from the laboratory.

1. Remove the cleaning swab from packaging.
2. Using cleaning swab, remove excess mucus from the cervical os.
3. Discard the used cleaning swab.
4. Remove the collection swab from packaging.
5. Insert the collection swab into the cervical canal and rotate for 15-30 seconds.
6. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
7. Uncap the CT/GC Diluent tube.
8. Fully insert the collection swab into the CT/GC Diluent tube.
9. Break the shaft of the swab at the score mark. Use care to avoid splashing of contents.
10. Tightly recap the tube.

URINE SPECIMEN COLLECTION:

1. The patient should not have urinated for at least 1 hr. prior to specimen collection.
2. Collect specimen in a sterile, plastic, preservative-free specimen collection cup.
3. The patient should collect the first 15-20 mL of voided urine (the first part of the stream, NOT midstream).

VAGINAL SWABS:

IMPORTANT: Vaginal swabs may only be collected using cobas® PCR Female Swab Sample Kit. These kits are available from the laboratory.

The vaginal swab specimen must be collected according to the instructions included with the collection kit. The cobas® CT/NG Test has not been validated for use with vaginal swab specimens collected by patients at home. The patient-collected vaginal swab specimen application is limited to health care facilities where support/counseling is available to explain the procedures and precautions.

ENDOCERVICAL THIN PREP SPECIMENS (PRE-QUOTTED):

Critical Specimen Processing Prior to Sending to CPAL:

An aliquot of the PreservCyt media should be removed from the ThinPrep vial prior to processing/preparation of the ThinPrep Slide. Following this process will greatly reduce opportunities for contamination and prevent delays in testing and resulting.

Note: Good laboratory practice should be followed to avoid introducing contaminants into either the PreservCyt sample vial or the aliquot. It is strongly recommended to use powder free gloves and a pipetting device with an aerosol barrier tip that is sized appropriately for the volume of the aliquot being withdrawn and dispensed. You should not use serological pipettes.

Note: Only one aliquot may be removed from the PreservCyt sample vial prior to performing the ThinPrep Test, regardless of the volume of the aliquot. (Maximum aliquot volume = 4 mL)

Procedure for Removing Aliquot:

1. Vortex the vial at high speed for 8 to 12 seconds.
   (Caution: The desired aliquot must be removed immediately after vortexing the vial to ensure homogeneity of the sample).
2. Carefully remove the vial cap.
3. Using a pipetting device, withdraw an aliquot of 4.0 mL from the vial. Take care to avoid contaminating gloves with solution. If gloves should become contaminated, replace with a clean pair before proceeding to the next specimen.

4. Dispense the aliquot into a suitably sized and labeled polypropylene tube and close tightly to prevent leakage/evaporation.
5. Using a new pipet tip, withdraw a quantity of unused PreservCyt solution from its container that is equal in volume to that of the aliquot removed from the vial in step 3. (i.e., if you removed 4.0 mL of specimen for testing, add 4.0 mL of unused PreservCyt to the specimen vial).

Note: The following steps will return the thin prep sample vial to its original volume:

6. Transfer the volume of unused PreservCyt solution to the vial from which the aliquot was removed in step 3.
7. Secure the vial cap (The line on the cap and the line on the vial should meet or slightly overlap).

While following the above pre-quotting process is preferred, samples that have already been processed using the ThinPrep 2000 Processor may be tested for Chlamydia trachomatis and Neisseria gonorrhoeae using the CT/NG Test. This should be an infrequent request. However, it will be accommodated to prevent the patient from having an additional sample collected should the request be missed or not made until after Thin Prep processing occurs. ***Note that ONLY the ThinPrep 2000 Processor is approved for add-on testing. Do not send samples after sampling on other processors.***

Specimen Storage and Transport:
All specimens must be transported refrigerated.

1. Neat male and female urines may be stored refrigerated for up to 7 days.
2. Endocervical swab specimens collected with the cobas® PCR Female Swab Sample Kit may be stored at 2°C – 30°C for up to 12 months.
3. Vaginal swab specimens collected with the cobas® PCR Female Swab Sample Kit may be stored at 2°C – 30°C for up to 12 months.
4. Endocervical specimens received in PreservCyt may be stored at 2°C – 30 °C for up to 12 months. Aliquots of cervical specimens collected in PreservCyt Solution may be stored for up to 4 weeks at 2-30°C.

Intended Use:
The cobas® CT/NG Test is an in vitro nucleic acid amplification test that utilizes the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the qualitative detection of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG) DNA to aid in the diagnosis of chlamydial and gonococcal disease. The test may be used with vaginal swab specimens self-collected in a clinical setting, male and female urine, endocervical swabs, and pre-quotted PreservCyt specimens.

Reference Ranges:
Negative

Method:
The cobas 4800 CT/NG assay is a multiplex real time PCR method that comprises reactions for Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and internal controls. Notably, the assay comprises two target sequences for detecting C. trachomatis, including the C. trachomatis cryptic plasmid and major outer membrane protein gene. The newly designed N. gonorrhoeae assay targets a direct repeat region called DR-9. This target region is repeated three times on the N gonorrhoeae genome and has two highly conserved sequence variations. The cobas 4800 CT/NG test uses two sets of NG primers and probes to detect any combination of both target variations. See PCR Amplification Target Selection section (below). The intended targets for the cobas® CT/NG Test include all major CT serovars, the Swedish C. trachomatis mutant (nvCT), variants that may harbor deletions in the cryptic plasmid or that have no cryptic plasmid at all, and both DR-9A and variant DR-9B sequences of NG.

PCR Amplification Target Selection:

In addition to chromosomal DNA, C. trachomatis contains an approximately 7,500 base pair cryptic plasmid that is common to all serovars of C. trachomatis. The cobas® CT/NG Test uses the CT primers CP102 and CP103 to define a sequence of approximately 206 nucleotides within the cryptic plasmid DNA of C. trachomatis. In addition, the cobas® CT/NG Test uses the CT primers CTMP101 and CTMP102 to define a sequence of approximately 182 nucleotides within the chromosomal DNA of C. trachomatis.
The N. gonorrhoeae target site is a highly conserved direct repeat region called DR-9. The cobas® CT/NG Test uses the NG primers NG514 and NG519 to define a sequence of approximately 190 nucleotides (DR-9A) from this region. In addition, the cobas® CT/NG Test uses another set of NG primers, NG552 and NG579, to define a second sequence of approximately 215 nucleotides (DR-9B) from this region.

Limitations:

1. Interfering substances include, but are not limited to the following:
    a. Urine specimens stabilized in cobas® PCR Media containing greater than 0.35% (v/v) blood may give false negative results.
    b. Urine specimens stabilized in cobas® PCR Media containing 0.5% or greater bilirubin may give false negative or invalid results.
    c. Vaginal swab specimens containing up to 10% (v/v) whole blood exhibited no interference effects. Whole blood levels above 10% (v/v) may give invalid or false negative results.
    d. Vaginal swab specimens and urine specimens, all stabilized in cobas® PCR Media and containing greater than 1 x 105 PBMC cells/mL may give invalid or false negative results.
2. Detection of C. trachomatis and N. gonorrhoeae is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, history of STD, presence of symptoms), stage of infection, and/or infecting C. trachomatis and N. gonorrhoeae strains.
3. The cobas® CT/NG Test should not be used to determine therapeutic success as nucleic acids may be present after antimicrobial therapy.
4. False negative or invalid results may occur due to polymerase inhibition. The CT/NG Internal Control is included in the cobas® CT/NG Test to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
5. The cobas® CT/NG Test is not recommended for evaluation of suspected sexual abuse or for other medico-legal indications.
6. The cobas® CT/NG Test for urine testing is recommended to be performed on first catch urine specimens (defined as the first 10 to 50 mL of the urine stream). The effects of other variables such as first-catch vs. mid-stream, post-douching, etc. have not been evaluated.
7. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
8. The cobas® CT/NG Test has not been evaluated with patients who were currently being treated with antimicrobial agents active against CT or NG as well as patients with a history of hysterectomy.
9. The cobas® CT/NG Test is not intended to replace other exams or tests for diagnosis of urogenital infection. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.
10. Though rare, mutations within the highly conserved regions of the cryptic plasmid or genomic DNA of C. trachomatis or the genomic DNA of N. gonorrhoeae covered by the cobas® CT/NG Test's primers and/or probes may result in failure to detect the presence of the bacterium.
11. The cobas® CT/NG Test has not been evaluated in patients younger than 14 years of age.

References:

1. cobas® CT/NG Test Package Insert. Roche Molecular Systems, Inc., Branchburg, NJ 08876 USA. Doc Rev 1.0; 01/2012. First publishing
2. cobas® 4800 CT/NG Test Package Insert. Copyright 2011 Roche Molecular Systems, Inc. Branchburg, NJ 08876 USA. Doc Rev 4.0; 8/2014
3. cobas® PCR Female Swab Sample Kit Package Insert. Roche Molecular Systems, Inc., Branchburg, NJ 08876 USA. Doc Rev 4.0; 11/2013.

4. Rockett, R. et al. Evaluation of the cobas 4800 CT/NG test for detecting Chlamydia trachomatis and Neisseria gonorrhoeae. Sex Transm Infect. 86: p 470-473. 2010.
5. Tabrizi SN, et al. Evaluation of Six Commercial Nucleic Acid Amplification Tests for Detection of Neisseria gonorrhoeae and other Neisseria Species. J. Clin. Microbiol. Vol 49. p. 3610-3615. 2011.
6. Fang, J. et al. Evaluation of Self-collected Vaginal swab, First Void Urine and Endocervical Swab Specimens for the Detection of Chlamydia Trachomatis and Neisseria Gonorrhoeae in Adolescent Females. J Pediatr Adolesc Gynecol. 21(6): p. 355-360. 2008.