



TECHNICAL NOTICE

THE MEDICAL FOUNDATION

Chlamydia trachomatis and Neisseria gonorrhoeae by Nucleic Acid Amplification (NAA) using Roche cobas® CT/NG v2.0 Test

Effective Date: August 1, 2016

Performing Department: Molecular Pathology

Clinical Significance: Sexually transmitted bacterial infection by *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) is common. CT is the most frequently reported infectious disease in the United States, and prevalence is highest in persons aged ≤ 24 years. In addition to causing urethritis in both sexes and cervicitis in women, serious sequelae may result from CT infection in women, including pelvic inflammatory disease, ectopic pregnancy, and involuntary infertility. Additionally, an estimated 820,000 new NG infections occur each year in the US, causing similar disease processes. Unfortunately, infection by either CT or NG may also be asymptomatic, increasing the potential for spread.

Method: The **cobas® CT/NG v2.0 Test** for CT and NG is an FDA approved assay that is based on 2 major processes: (1) automated sample preparation to obtain nucleic acids, including CT and NG DNA; (2) simultaneous PCR amplification of target DNA sequences using both CT and NG specific primer pairs and real-time detection of cleaved fluorescent-labeled CT and NG specific oligonucleotide detection probes. An Internal Control, containing CT and NG DNA, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

This test employs specific primers to define target sequences of approximately 206 nucleotides within CT cryptic plasmid DNA, and approximately 182 nucleotides within CT chromosomal DNA. The NG target site is a highly conserved direct repeat region called DR-9. NG primers define sequences of approximately 190 and 215 nucleotides from this region.

Use: This test is used for the qualitative detection of CT and/or NG DNA in urogenital specimens from patients suspected to have CT and/or NG infection. Additionally, this test may be used for routine screening to reduce the burden of CT and NG infections per CDC testing recommendations.

Limitations of Test:

1. A Negative result does not preclude presence of CT and/or NG infection because accurate results depend on adequate specimen collection, absence of inhibitors, and sufficient microbial DNA present to be detected.
2. This test is NOT recommended for evaluation of suspected sexual abuse and/or other medicolegal applications. In such instances, culture may be more appropriate.
3. This test should NOT be used to determine therapeutic success as microbial nucleic acids can be identified following antimicrobial therapy.
4. This test has not been evaluated in patients younger than 14 years of age.
5. Some substances may interfere with this test, including: Metronidazole Vaginal Gel, Replens, Vagisil Satin products, and excessive white blood cells, endocervical mucus, or whole blood.

Reference Range: Each specimen result will be reported as “Positive” for CT and/or NG, indicating that organism-specific DNA was detected and “Negative” when no organism-specific DNA was detected by the assay. Test results will be reported as “Indeterminate” when amplification fails in two independent runs. This may be due to inhibitory substances in the specimen, inadequate specimen collection, or insufficient sample volume.

SPECIMEN REQUIREMENTS AND COLLECTION:

SPECIMEN TYPE:

1. Endocervical and Vaginal Swabs: The **cobas® PCR Female Swab Sample Kit** (SBMF # S1034) is used to collect and transport endocervical and vaginal swab specimens. Vaginal specimens may be provider-collected or self-collected. Self-collected vaginal swabs are an option for screening women when a pelvic examination is not otherwise indicated.
2. Urine (men and women): The **cobas® PCR Urine Sample Kit** (SBMF # S1035) is used to collect and transport urine specimens. In men, the first catch urine sample is equivalent to, and in some situations superior to, urethral swabs, and is the preferred specimen. **There is no validated collection system for this test that uses male urethral swabs.**
3. Liquid-based cytology specimens, either Thin-Prep® or SurePath®.

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COLLECTION: See specimen collection instructions below.

STORAGE: Endocervical and vaginal swab specimens collected with the **cobas®** PCR Female Swab Sample Kit and male and female urine collected with the **cobas®** PCR Urine Sample Kit may be stored at 2-30°C for up to 12 months once the specimens have been stabilized in **cobas®** PCR Media. Cervical specimens collected in PreservCyt Solution may be stored at 2-30°C for up to 12 months. Aliquots (≥ 1 mL) of cervical specimens collected in PreservCyt Solution may be stored in 13 mL round-based Sarstedt tubes for up to 4 weeks at 2-30°C.

TRANSPORT: Specimens collected with the various **cobas®** PCR Sample Kits, and liquid cervical specimens collected in PreservCyt Solution, can be transported at 2-30°C.

CAUSES FOR REJECTION:

1. Specimens collected using a collection device not validated for this platform will be rejected (e.g., E-swab).
2. Incoming primary endocervical and vaginal specimen tubes with no swabs or with two swabs have not been collected according to the instructions in the **cobas®** PCR Female Swab Sample Kit and will not be tested.
3. Endocervical and vaginal swab specimens that appear bloody or have a dark brown color will not be tested.
4. Untested urine specimens must show the top of the liquid level between the two black lines on the **cobas®** PCR Media tube label window. If the liquid level is above or below these lines, the specimen has not been collected properly and will be rejected.
5. Urine specimens that appear bloody or have a dark brown color will not be tested.

Testing Schedule: Monday thru Friday

Estimated Turnaround Time: 48 to 72 hours of test run

Order:

- Test Name: Chlamydia trachomatis and Neisseria gonorrhoeae by NAA, SurePath®
SBMF Number: 36380 CPT Code: 87491; 87591
- Test Name: Chlamydia trachomatis and Neisseria gonorrhoeae by NAA, Swab
SBMF Number: 36350 CPT Code: 87491; 87591
- Test Name: Chlamydia trachomatis and Neisseria gonorrhoeae by NAA, ThinPrep®
SBMF Number: 36370 CPT Code: 87491; 87591
- Test Name: Chlamydia trachomatis and Neisseria gonorrhoeae by NAA, Urine
SBMF Number: 36360 CPT Code: 87491; 87591
- Test Name: Chlamydia trachomatis by NAA, SurePath®
SBMF Number: 36381 CPT Code: 87491
- Test Name: Chlamydia trachomatis by NAA, Swab
SBMF Number: 36351 CPT Code: 87491
- Test Name: Chlamydia trachomatis by NAA, ThinPrep®
SBMF Number: 36371 CPT Code: 87491
- Test Name: Chlamydia trachomatis by NAA, Urine
SBMF Number: 36362 CPT Code: 87491
- Test Name: Neisseria gonorrhoeae by NAA, SurePath®
SBMF Number: 36382 CPT Code: 87591
- Test Name: Neisseria gonorrhoeae by NAA, Swab
SBMF Number: 36352 CPT Code: 87591
- Test Name: Neisseria gonorrhoeae by NAA, ThinPrep®
SBMF Number: 36372 CPT Code: 87591
- Test Name: Neisseria gonorrhoeae by NAA, Urine
SBMF Number: 36361 CPT Code: 87591

For additional information contact Bobbie C. Sutton, M.D., Ph.D. (bsutton@sbmf.org) at 574-236-1392, or Kevin Maggert (kmaggert@sbmf.org) at 574-234-4176 extension 61538.

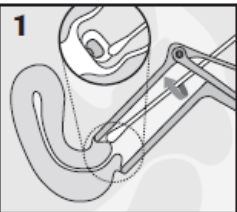
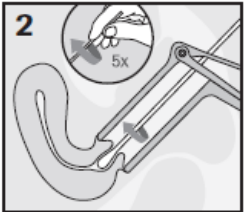
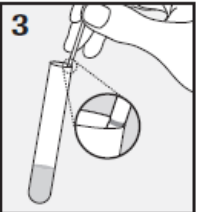
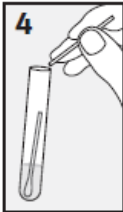
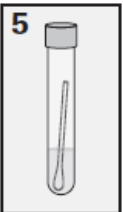
See following pages for specimen collection instructions

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ENDOCERVICAL SWAB SPECIMEN COLLECTION

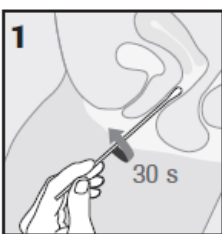
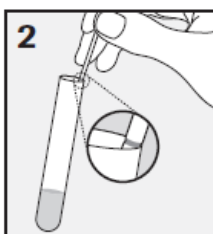

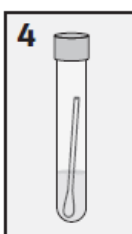
WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

 <p>1. CLEAN: Using one of the swabs (provided), remove excess mucus from the cervical os and surrounding mucosa. Discard the swab after use. NOTE: Cleaning excess mucus from the cervical os is required to assure an adequate sample is obtained for processing. A large-tipped cleaning swab, such as Puritan 25-808 1PR (not provided) can be used.</p>	 <p>2. COLLECT: To collect the specimen, insert the other provided swab into the endocervical canal. Gently rotate the swab 5 times in one direction in the endocervical canal. Do not over-rotate. Carefully withdraw the swab, avoiding any contact with the vaginal mucosa.</p>	 <p>3. ALIGN: Remove the cap from the cobas® PCR Media tube and lower the swab specimen into the tube until the visible dark line on the swab shaft is aligned with the tube rim. The tip of the swab should be just above the media surface near the hexagonal Roche logo.</p>	 <p>4. BREAK: Carefully leverage the swab against the tube rim to break the swab shaft at the dark line; discard the top portion of the swab.</p>	 <p>5. CLOSE: Tightly re-cap the cobas® PCR Media tube. The specimen is now ready for transport.</p>
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VAGINAL SWAB SPECIMEN – CLINICIAN COLLECTION

NOTE: The following instructions are for the doctor, nurse or care provider to perform a vaginal swab specimen collection.

WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

 <p>1. COLLECT: To collect the specimen, insert the swab about 5 cm (2 inches) into the vaginal opening. Gently turn the swab for about 30 seconds while rubbing the swab against the walls of the vagina. Withdraw the swab carefully. Do not let the swab touch any surface before placing it into the collection tube.</p>	 <p>2. ALIGN: Remove the cap from the cobas® PCR Media tube and lower the swab specimen into the tube until the visible dark line on the swab shaft is aligned with the tube rim. The tip of the swab should be just above the media surface near the hexagonal Roche logo.</p>	 <p>3. BREAK: Carefully leverage the swab against the tube rim to break the swab shaft at the dark line; discard the top portion of the swab.</p>	 <p>4. CLOSE: Tightly re-cap the cobas® PCR Media tube. The specimen is now ready for transport.</p>
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How To Self-Collect a vaginal swab sample:

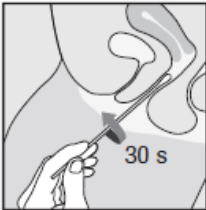
Handling Precautions

The collection tube media can cause irritation if contacted with skin or other body parts. Handle the collection tube carefully.

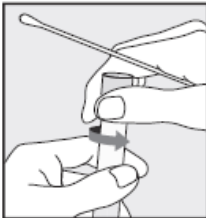
- **Do NOT** pre-wet collection swab with the collection media or any other liquid before obtaining the vaginal sample.
- Use care to avoid splashing contents of the tube. If the contents of the tube are spilled on your skin, wash the affected area with soap and water. If the contents of the tube splash into your eyes, flush them with water immediately. Always notify your healthcare provider. **NOTE: In case the contents of the tube are accidentally spilled, do not attempt to clean up. Immediately notify your healthcare provider for appropriate action.**



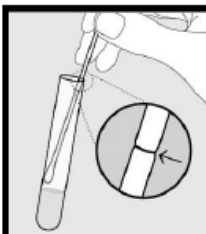
1. POSITION: Hold the swab in one hand and with the other hand separate the folds of skin around the vaginal opening (labia).



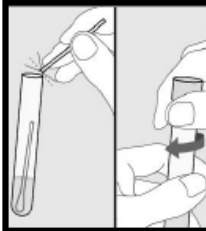
2. COLLECT: Insert the swab about 5 cm (2 inches) into the vaginal opening. Gently turn the swab for about 30 seconds while rubbing the swab against the wall of the vagina. Remove the swab carefully. Do not touch the swab to any surface before placing it into the collection tube.



3. OPEN TUBE: While holding the swab in the same hand remove the cap from the tube as shown in the diagram.



4. ALIGN: Lower the swab into the tube until the visible dark line on the swab shaft is lined up with the tube rim. The tip of the swab should be just above the liquid in the tube.



5. BREAK: Carefully lean the swab against the tube rim to break the swab shaft at the dark line; discard the top portion of the swab.

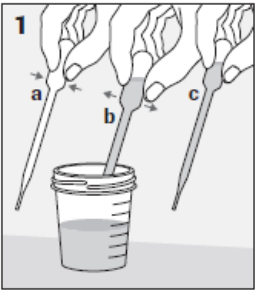
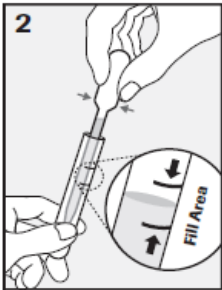
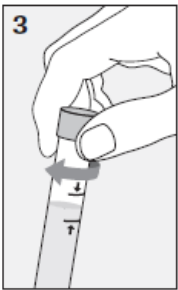
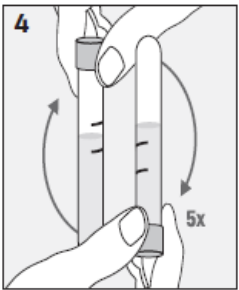
6. CLOSE: Tightly close the **cobas**[®] PCR Media tube. Return the sample to your healthcare provider as instructed.

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SPECIMEN COLLECTION URINE

COLLECT: Prior to sampling, the patient should not have urinated for at least one hour. Given that collection of larger volumes of urine may reduce test sensitivity, please direct patient to provide first-catch urine (approximately 10 to 50 mL of the initial urine stream) into a urine collection cup (not provided).

 <p>1. PIPETTE: Immediately transfer the urine into the cobas® PCR Media tube using the provided disposable pipette.</p> <p>NOTE: If the urine specimen cannot be transferred immediately, it can be stored at 2°C to 30°C for up to 24 hours.</p>	 <p>2. TRANSFER: The correct volume of urine has been added when the fluid level is between the two black lines on the tube label.</p>	 <p>3. CAP: Tightly re-cap the cobas® PCR Media tube.</p>	 <p>4. MIX: Invert the tube 5 times to mix. The specimen is now ready for transport.</p>
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