Wake Forest Baptist Medical Center	GC/Chlamydia PCR COBAS 6800 MM-31	**Dept:**	Cl Micro
		Effective Date:	2/13/2020
		Revised Date:	
		Contact:	Microbiology Manager
Name & Title: Dr. Gregory Pomper		**Date:**	2/13/2020
Signature:			

1) General Procedure Statement:

a. **Purpose:** This procedure is to serve as a guide for trained personnel in the Clinical Microbiology Laboratory to perform the test described herein. This procedure should be used in conjunction with proper training and only by qualified technologists.

b. **Responsible Department/Scope:**
   i. Procedure owner/implementer: Dr. Elizabeth Palavecino
   ii. Procedure prepared by: Christopher Powers, MLS(ASCP)
   iii. Who performs procedure: Clinical Microbiology Laboratory personnel

2) Procedure:

1. Principle of Test

General Testing Principle

1.1 **Testing and Specimen Types**

The cobas® CT/NG for use on the cobas® 6800/8800 Systems is an automated, qualitative in vitro nucleic acid diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae(NG) DNA in male and female urine, clinician-instructed self-collected vaginal swab specimens (collected in a clinical setting), clinician-collected vaginal swab specimens, and endocervical swab specimens, all collected in cobas® PCR Media (Roche Molecular Systems, Inc.). This test is intended as an aid in the diagnosis of chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

1.2 **Instrumentation**

The cobas® CT/NG is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800/8800 software which assigns test results for all tests as positive,

negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report. Nucleic acid from patient samples and added internal control DNA (DNA-IC) molecules is simultaneously extracted. In summary, bacterial nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each cobas® CT/NG run.

1.3 Target Specificity

Cobas® CT/NG is a qualitative test performed on the cobas® 6800 System and cobas® 8800 System. cobas® CT/NG enables the detection of CT/NG DNA in endocervical, vaginal, urine and cervical specimens of infected female patients and urine specimens in infected male patients. Target-specific primers and two probes are used to detect but not discriminate between the CT cryptic plasmid and the ompA gene. Additionally, target-specific primers and two probes are used to detect but not discriminate between two conserved sequences in the NG DR-9 region.

1.4 Target Amplification

cobas® CT/NG is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800/8800 software which assigns test results for all tests as positive, negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added internal control DNA (DNA-IC) molecules is simultaneously extracted. In summary, bacterial nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each cobas® CT/NG run.

1.5 Internal Control

The DNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes a low titer positive and a negative control.

1.6 Selective Amplification

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers which are selected from highly conserved plasmid and genomic regions of CT and NG. A region on the CT cryptic plasmid and the

ompA gene (dual target) and two conserved sequences of the NG DR-9 region are amplified by cobas® CT/NG. Selective amplification of DNA-IC is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with either the CT or NG target regions. A thermostable DNA polymerase enzyme is used for PCR amplification. The target and DNA-IC sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

1.7 Detection Reaction

The cobas® CT/NG master mix contains two detection probes specific for the CT target sequences, two detection probes specific for the NG target sequences and one for the DNA-IC. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of CT targets, NG targets and DNA-IC in three different target channels.17,18 When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the CT and NG targets and DNA-IC, respectively.

2. Clinical Significance

2.1 Infection with *Chlamydia trachomatis* (CT) is the most frequently reported bacterial sexually transmitted disease (STD) in the United States.[1,2] CT is the leading bacterial cause of sexually transmitted diseases worldwide, with approximately 89.1 million cases occurring annually.[2] The Centers for Disease Control (CDC) Sexually Transmitted Disease Surveillance 2011 Supplement reports 1,412,791 CT infections in the U.S.[3]

CT is a gram-negative, nonmotile, obligate intracellular bacterium with a unique biphasic lifecycle and is the causative infectious agent for a variety of diseases. CT can cause urethritis, cervicitis, proctitis, conjunctivitis, endometritis, and salpingitis; if left untreated, the infection may ascend to the uterus, fallopian tubes, and ovaries causing pelvic inflammatory syndrome, ectopic pregnancy, and tubal factor infertility. Reiter's syndrome (urethritis, conjunctivitis, arthritis, and mucocutaneous lesions) has also been associated with genital CT infection. Many infections remain asymptomatic, and high numbers of infected patients may not seek care. Patients often become re-infected if their sexual partners are not treated. Infants born to infected mothers can develop

conjunctivitis, pharyngitis, and pneumonia. The predominant symptoms in men and women are increased discharge and dysuria; women may also present with irregular uterine bleeding.

2.2 *Neisseria gonorrhoeae* (NG) is the causative agent of gonorrhea. NG are gram-negative diplococci, cytochrome oxidase positive, non-motile and non-spore forming. A total of 321,849 cases of NG infection have been reported to the CDC in 20113, corresponding to a rate of 104.2 cases per 100,000 population. Clinical manifestations of NG infections are numerous. In men, acute urethritis presents itself after a 1-10 day incubation period with urethral discharge and dysuria. Only a small proportion of men remain asymptomatic without signs of urethritis. Acute epididymitis is the most common complication, especially in young men. In women, the primary site of infection is the endocervix. There is a high prevalence of coalescence of symptoms with CT, *Trichomonas vaginalis*, and vaginosis; many women remain asymptomatic and therefore do not seek medical care. In symptomatic women increased discharge, dysuria, and intermenstrual bleeding may be observed. Pelvic inflammatory disease can occur in 10%-20% of women, combined with endometritis, salpingitis, tubo ovarian abscess, pelvic peritonitis, and perihepatitis. Other gonococcal infected sites in men and women are the rectum, pharynx, conjunctiva, and to a lesser degree the disease presents itself as disseminated gonococcal infection. Infants from infected mothers can develop conjunctivitis.

NAATs are the recommended method for CT and NG screening. For women, a vaginal swab is the recommended sample type and first catch urine is recommended for men. Alternative acceptable sample types for women include an endocervical swab when a pelvic examination is indicated or a first catch urine sample, but a urine sample may detect up to 10% fewer infections when compared with vaginal and endocervical swabs. In addition to urine for men. Cobas® CT/NG for use on the cobas® 6800/8800 Systems (referred to as cobas® CT/NG throughout the remainder of this document) is an automated, qualitative real-time PCR test designed to detect CT and NG DNA in urogenital specimens from male and female patients and thus fulfills the medical need for a rapid, high throughput molecular screening test for use as an aid in the diagnosis of chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

3. Specimen Requirements

Specimen Collection

Note Handle all specimens as if they are capable of transmitting infectious agents.

3.1 Endocervical swab and vaginal swab specimens collected with the **cobas**® PCR Female Swab Sample Kit, male and female urine collected with the **cobas**® PCR Urine Sample Kit or urines collected in a sterile container. Follow the instructions for collecting endocervical swab, vaginal swab and urine specimens with the **cobas**® PCR Female Swab Sample Kit and **cobas**® PCR Urine Sample Kit, respectively.

3.2 Specimen collection supplies are located:

Swab collection kits and urine collection kits are stored in the Core Lab Storeroom area, in the shelving units marked COBAS 6800.

Specimen Transport

3.3 Endocervical swab and vaginal swab specimens collected with the **cobas**® PCR Female Swab Sample Kit, male and female urine collected with the **cobas**® PCR Urine Sample Kit or urines collected in a sterile container can be transported at 2-30ºC. Transportation of CT/NG specimens in **cobas**® PCR Media must comply with country, federal, state and local regulations for the transport of etiologic agents.

Specimen Storage

3.4 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. Urines collected in a sterile container are stable at 2-30 C for 24 hours.

3.5 Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[10] and in the CLSI Document M29-A3.[15]

*Urine specimens from CSA (Child Sexual Abuse) protocol patients should be received after collection in a urine container. Per Dr. Goodpasture, these urines should be from children 12 years of age or less. Any positive specimen from a patient 12 years of age or less, whether designated CSA or not, should be confirmed by another method. All unused urine should be saved until the test is completed. If the specimen is positive for either Chlamydia or Neisseria gonorrhea, an aliquot of the untested urine should be taken to Sendouts to be sent out for confirmation. The result should not be entered in the laboratory information system until confirmation is received from the referral lab, but Dr. Goodpasture should be called and notified of the potential positive so that followup can be initiated if necessary. Document the sendout & the notification of Dr. Goodpasture in the yellow section for lab comments. Comments made in this area are only available to lab personnel.

4. Reagents

4.1 Reagent Composition and Storage Requirments

The **cobas**® CT/NG Test includes the following reagents

cobas® CT/NG
Store at 2-8°C
480 test cassette (P/N 07460066190)

Kit components	Reagent ingredients	Quantity per kit 480 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% Proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin, 9014-01-1. May produce an allergic reaction.	38 mL
DNA Internal Control (DNA-IC)	Tris buffer, < 0.05% EDTA, < 0.001% non-CT/NG related DNA construct containing primer and probe specific sequence regions, < 0.1% Sodium azide	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% Methyl-4 hydroxibenzoate	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, Potassium hydroxide, < 0.1% Sodium azide	14.5 mL
CT/NG Master Mix Reagent 2 (CT/NG MMX-R2)	Tricine buffer, Potassium acetate, EDTA, Glycerol, < 18% Dimethyl sulfoxide, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.1% Tween 20, < 0.1% Sodium azide, < 0.1% Z05 DNA polymerase, < 0.10% AmpErase (uracil-N glycosylase) enzyme (microbial), < 0.01% Internal Control forward and reverse primers, < 0.01% Upstream and downstream CT/NG primers, < 0.01% Fluorescent-labeled oligonucleotide probes specific for CT, NG and the DNA Internal Control, < 0.01% Oligonucleotide aptamer	17.5 mL

cobas® CT/NG Positive Control Kit
Store at 2-8°C
(P/N 07460082190)

Kit components	Reagent ingredients	Quantity per kit
CT/NG Positive Control (CT/NG (+) C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, < 0.003% Poly rA, <0.01% Non-infectious plasmid DNA (microbial) containing *C. trachomatis*, <0.01% Non-infectious plasmid DNA (microbial) containing *N. gonorrhoeae*	16 mL (16 x 1mL)

cobas® Buffer Negative Control Kit
Store at 2-8°C
(P/N 07002238190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1mL)

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

Reagent	Storage temperature
cobas® CT/NG	2–8°C
cobas® CT/NG Positive Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® CT/NG	90 days from first usage	Max 20 runs	Max 20 hours
cobas® CT/NG Positive Control Kit	Not applicable	Not applicable	Max 10 hours
cobas® Buffer Negative Control Kit	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	30 days from loading*	Not applicable	Not applicable

* Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container	07094361001
cobas® PCR Media Secondary Tube Kit	07958048190
cobas® PCR Media Tube Replacement Cap Kit	07958056190
Replacement Caps for PreservCyt® Vials	08037230190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050[a,b]	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR[a,b]	11902997001

Collection Kit	P/N
cobas® PCR Urine Sample Kit	05170486190
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190

Equipment	P/N
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

4.2 Reagent Labeling and Preparation

All reagents are labeled by the manufacturer. The labeling includes contents, lot number, expiration date, and storage instructions.
All reagents are liquid, ready-to-use.

4.3 Proper Reagent Use

Note: The system set-up & workflow of the COBAS 6800 ensures that all new lot numbers & new shipments of reagents are verified in turn as they come in to use. Controls containing both CT & NG analytes are tested with every run. If any run fails for any reason, no results are produced. The run should be repeated, & if it fails again, Roche should be contacted for technical assistance. All lot numbers & expiration dates are recorded by the COBAS 6800 & are available on the instrument printout.

4.3.1 As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

4.3.2 For in vitro diagnostic use only.

4.3.3 All human-sourced materials should be considered potentially infectious and should be handled with universal precautions

4.3.4 Use only supplied or specified required consumables to ensure established test performance.

4.3.5 Safety Data Sheets (SDS) are available on request from your local Roche representative.

4.3.6 Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect established test performance.

4.3.7 False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

4.3.8 cobas® PCR Media (from primary specimen tube) contains guanidine hydrochloride. Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas. If liquid containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.

4.3.9 Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.

4.4 Reagents required to perform this assay are located:

Stored consumables & stored Wash Buffer used to perform the COBAS CT/NG assay are located in the Core Lab Storeroom on the shelving units marked COBAS 6800.

Stored reagents & controls marked "store at 2-8 degrees C" are located in the back walk-in refrigerator near where supplies are delivered.

Lot numbers & expiration dates of all reagents & controls are read & recorded by the COBAS 6800 itself & are shown on the instrument printout of results. The instrument will not run tests if any expiration dates are out of range.

5. Test Procedure

NOTE Refer to the cobas® 6800 System Operator's Manual for detailed operating instructions including instrument startup and shutdown.

NOTE Cobas omni Specimen Diluent and Lysis Reagent should be removed from refrigerator and allowed to reach room temperature (15-30°C) prior to loading on the instrument.

NOTE Do not freeze any patient samples

All patient samples should be handled as if infectious, using good laboratory procedures as outlined in MB53 General Policies and MB55 Laboratory Safety and Biohazard Prevention. Only personnel proficient in handling infectious materials and in the use of cobas® CT/NG and cobas® 6800/8800 Systems should perform this procedure. Thoroughly clean and disinfect all laboratory work surface with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Specimens

Specimen Collection and Requirements

Endocervical swab specimens collected with the cobas® PCR Media Dual Swab Sample Kit, vaginal swab specimens collected with either the cobas® PCR Media Uni Swab Sample Kit or cobas® PCR Media Dual Swab Sample Kit, male and female urine collected with the cobas® PCR Urine Sample Kit have been validated for use with cobas® CT/NG. Follow the instructions for collecting swab and urine specimens in their respective collection kit IFU.

All specimen types listed in the "Specimen collection" section above can be transported at 2-30°C. Transportation of CT/NG specimens in cobas® PCR Media must comply with country, federal, state and local regulations for the transport of etiologic agents.

NOTE To avoid cross-contamination of processed specimens, additional caps for cobas® PCR Media tubes in an alternate color (neutral; see Materials Required, but not Provided) should be used to recap specimens after processing.

NOTE 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 cannot be used for testing.

NOTE A properly collected endocervical swab or vaginal swab specimen should have a single swab with the shaft broken at the scoreline. Swab shafts which are broken above the scoreline will appear longer than normal and may also be bent over to fit into the cobas® PCR Media tube. This can produce an obstruction to the system which may cause the loss of test results. In the event that a swab specimen has an improperly broken shaft,

remove the swab prior to sample processing on the instrument. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other surfaces during disposal to prevent contamination.

NOTE Incoming primary endocervical and vaginal specimen tubes with no swabs or with multiple swabs have not been collected according to the instructions in their respective collective kit IFU and should not be tested.

NOTE Do not process endocervical swab and vaginal swab specimens that appear bloody or have a dark brown color.

NOTE Occasionally, incoming swab specimens contain excessive mucus which may induce a pipetting error (e.g., clot or other obstruction) on the cobas® 6800/8800 Systems. Prior to retesting of specimens that exhibited clots during initial processing, remove and discard the swab, then re-cap and vortex these specimens for 30 seconds to disperse the excess mucus.

NOTE Swab specimens can be assayed twice on the cobas® 6800/8800 Systems while the swab is in the collection tube. If additional testing is required, or if the first test fails due to specimen pipetting error (e.g., clot or other obstruction), the swab must be removed prior to testing and the remaining fluid must have a minimum volume of 1.0 mL.

NOTE Use only the flocked swab in the cobas® PCR Media Dual Swab Sample Kit to collect endocervical specimens. Use only the woven polyester swab in either the cobas® PCR Media Uni Swab Sample Kit or the cobas® PCR Media Dual Swab Sample Kit to collect vaginal swab specimens. cobas® CT/NG has not been validated for use with other swab collection devices or media types. Using cobas® CT/NG with other swab collection devices or media types may lead to false negative, false positive, and/or invalid results.

NOTE The presence of mucus in endocervical specimens may cause processing delays due to clotting. Mucus free specimens are required for optimal test performance. Use the large woven polyester swab in the cobas® PCR Dual Swab Sample Kit or an equivalent device to remove cervical secretions and discharge before obtaining the endocervical specimen.

Workflows

Performing a Full Workflow:

A.1 Swab and urine specimens must be uncapped and loaded directly onto racks for processing on the cobas® 6800/8800 Systems.

B.1 A single run can have any combination of specimens (Swab and Urine) and each specimen can be tested with either the CT/NG, CT, or NG ASAPs.

C.1 Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 6800 System Operator's Manual in the Operation section.

1	Log onto the system Press Start to prepare the system
	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette • Load control cassettes • Load pipette tips • Load processing plates • Load MGP Reagent • Load amplification plates • Refill Specimen Diluent • Refill Lysis Reagent • Refill Wash Reagent
3	Loading specimens onto the system • For each primary urine or swab in **cobas**® PCR Media o Uncap tube o Transfer tube directly to rack
	• Load sample rack and clot tip racks into the sample supply module • Confirm samples have been accepted into the transfer module
4	Start run
5	Review and export results
6	Remove sample tubes. If needed, cap any sample tubes meeting the minimum volume requirements for future use. Clean up instrument • Unload empty control cassettes • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

6. Reporting Results

Cobas® CT/NG automatically detects and discriminates CT and/or NG DNA simultaneously for each individually processed sample and control, displaying individual target results for samples as well as validity and overall results for controls.

Quality control and validity of results

One cobas® Buffer Negative Control [(-) Ctrl] and one CT/NG Positive Control [CT/NG (+) C] are processed with each batch of a requested result type.

In the cobas® 6800/8800 software and/or report, check for flags and their associated results to ensure batch validity.

All flags are described in the cobas® 6800/8800 Systems User Guide.

The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the cobas® 6800/8800 software based on negative and positive control performance.

NOTE The reference range of this test is defined as Negative.

NOTE All run and specimen validation is performed by the cobas®6800 software. As part of the built-in safety features of the COBAS 6800, the instrument will not give patient results if the QC fails. In addition the instrument will not start a run if expired controls or reagents are put on the instrument or if an incomplete control set is used.

NOTE A valid test run may include both valid and invalid sample results.

NOTE If a run fails (i.e. failure of any control), no patient results will be produced. The failure should be documented in the problem log. The run should be repeated. If the run fails a second time, call Roche for technical assistance.

6.1. For a valid run, specimen results are interpreted as shown in Table 1:

Table 1

Result Interpretation of the cobas® CT/NG

cobas® CT/NG Test	**Result Report and Interpretation**
SubTest CT/NG	
CT POS, NG POS	**CT Positive, NG Positive.** Specimen is positive for the presence of both CT and NG DNA.
CT NEG, NG NEG	**CT Negative, NG Negative.** Neither CT nor NG DNA, if present, could be detected.
CT POS, NG NEG	**CT Positive, NG Negative.** Specimen is positive for the presence of CT DNA. NG DNA, if present, could not be detected.
CT POS, NG Invalid	**CT Positive, NG Invalid.**

	Specimen is positive for the presence of CT DNA. NG result is Invalid. Original specimen should be re-tested once to obtain valid NG result. If results are still invalid, a new specimen must be obtained.
CT NEG, NG POS	**CT Negative, NG Positive.** CT DNA, if present, could not be detected. Specimen is positive for the presence of NG DNA.
CT Invalid, NG POS	**CT Invalid, NG Positive.** CT result is Invalid. Original specimen should be re-tested once to obtain valid CT result. Specimen is positive for the presence of NG DNA. If results are still invalid, a new specimen must be obtained.
CT Invalid, NG NEG	**CT Invalid, NG Negative.** CT result is Invalid. Original specimen should be re-tested once to obtain valid CT results. NG DNA, if present, could not be detected. If results are still invalid, a new specimen must be obtained.
CT NEG, NG Invalid	**CT Negative, NG Invalid.** CT DNA, if present, could not be detected. NG result is Invalid. Original specimen should be re-tested once to obtain valid NG result. If results are still invalid, a new specimen must be obtained.
CT Invalid, NG Invalid	**CT Invalid, NG Invalid.** Both CT and NG results are Invalid. Original specimen should be re-tested once to obtain valid CT and NG results. If results are still invalid, a new specimen must be obtained.

*** A negative result does not preclude the presence of CT and/or NG infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.**

Table 2

Result Interpretation of the cobas® CT/NG

cobas® CT/NG v2.0 Test	**Result Report and Interpretation**
SubTest CT	

CT POS	**CT Positive.** Specimen is positive for the presence of CT DNA.
CT NEG	**CT Negative.** CT DNA, if present, could not be detected.
Invalid	**CT Invalid.** CT result is Invalid. Original specimen should be re-tested no more than two times to obtain valid CT result. If results are still invalid, a new specimen must be obtained.
SubTest NG	
NG POS	**NG Positive.** Specimen is positive for the presence of NG DNA.
NG NEG	**NG Negative.** NG DNA, if present, could not be detected.
Invalid	**NG Invalid.** NG result is Invalid. Original specimen should be re-tested no more than two times to obtain valid NG result. If results are still invalid, a new specimen must be obtained.

7. Positivity Rate and Reporting Policy

The percentage of results that are positive for Chlamydia trachomatis and Neisseria gonorrhoeae are maintained and monitored on a monthly basis and are included in the monthly QA report.

The Positivity Rate is determined by: # positive / total # samples

(Do not include repeated positives from previous runs in this calculation.)

The threshold for GC is 3% and the threshold for Chlamydia is 10%.

If the positivity rate for a run of samples exceeds 3% for Neisseria gonorrhoeae or 10% for Chlamydia trachomatis, the positive results for that run should not be reported for the analyte that is above threshold. The positive samples for the analyte above threshold should be repeated on the next scheduled run. If all samples agree from the two runs, the results may be reported. If not, consult supervisor for the next course of action.

8. Quality Control

8.1 Quality control and validity of results

One cobas® Buffer Negative Control [(-) Ctrl] and one CT/NG Positive Control [CT/NG (+) C] are processed with each batch of a requested result type.

In the cobas® 6800/8800 software and/or report, check for flags and their associated results to ensure batch validity.

All flags are described in the cobas® 6800/8800 Systems User Guide.

The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the cobas® 6800/8800 software based on negative and positive control performance.

8.2 Store the **controls at** 2-8 °C. Store control diluent at room temperature. The controls & control diluent are stable until the expiration date indicated.

8.3 The quality control material is located:

Controls currently in use are stored on board the instrument. If the instrument will be shut down for an extended period of time controls should be removed and refrigerated at 2-8 °C. Stored controls are located in the back walk-in refrigerator near where supplies are delivered.

Recording Quality Control Results

8.4 All run and specimen validation is performed by the cobas®6800 software. Lot numbers & expiration dates of all reagents & controls are read & recorded by the COBAS 6800 itself & are shown on the instrument printout of results. QC results for both Negative & Positive Controls are shown on the instrument printout. As part of the built-in safety features of the COBAS 6800, the instrument will not give patient results if the QC fails. In addition, the instrument will not start a run if expired controls or reagents are put on the instrument or if an incomplete control set is used. If a run fails (i.e. failure of any control), no patient results will be produced, & the failure should be documented in the problem log kept on the shelf to right of the instrument. The run should be repeated. If the run fails a second time, call Roche for technical assistance.

Acceptable Limits

8.5 CT/NG (+) Control result must be 'Valid.' The (–) Control result must be 'Valid.'

Corrective Actions

8.6 If the CT/NG (+) Control results are consistently invalid, contact your local Roche office for technical assistance.

8.7 *If the (–) Control results are consistently invalid, contact your local Roche office for technical assistance.*

9. Procedural Notes

Procedural Precautions

9.1 As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

Procedural Limitations

9.2 cobas® CT/NG has been evaluated only for use in combination with the cobas® CT/NG Positive Control Kit, cobas® Buffer Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 6800/8800 Systems.

9.3 Reliable results depend on proper sample collection, storage and handling procedures.

9.4 cobas® CT/NG has only been validated for use with male and female urine, clinician-instructed self-collected vaginal swab specimens, clinician-collected vaginal swab specimens, and endocervical swab specimens, all collected in cobas® PCR Media (Roche Molecular Systems, Inc.) and cervical specimens collected in PreservCyt® Solution. Assay performance has not been validated for use with other collection media and/or specimen types.

9.5 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.

9.6 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 cobas® CT/NG primers and/or probes may result in failure to detect the presence of the bacterium.

9.7 Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.

9.8 cobas® CT/NG 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.

9.9 cobas® CT/NG is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications.

9.10 cobas® CT/NG should not be used to determine therapeutic success as nucleic acids may be present after antimicrobial therapy.

9.11 cobas® CT/NG 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.

9.12 The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.

9.13 cobas® CT/NG has not been evaluated with patients who are currently being treated with antimicrobial agents active against CT or NG as well as patients with a history of hysterectomy.

9.14 False negative or invalid results may occur due to polymerase inhibition. The CT/NG Internal Control is included in cobas® CT/NG to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.

9.15 The addition of AmpErase enzyme into the cobas® CT/NG Master Mix reagent enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.

9.16 Cobas® CT/NG has not been evaluated in patients younger than 14 years of age.

9.17 Urogenital specimens from patients who have used the over-the-counter products ReplensTM Long-Lasting Vaginal Moisturizer, RepHreshTM Odor Eliminating Vaginal Gel and RepHreshTM Clean Balance or used Metronidazole Vaginal Gel may generate invalid or false negative results. See Interference results (Table 22 in the product insert) for further details.

9.18 The presence of mucus (> 0.5% w/v) in endocervical specimens may cause false negative test results.

9.19 The presence of whole blood (> 5% v/v) in urine and cervical specimens collected in PreservCyt® Solution may cause false negative and/or invalid test results. Do not test specimens that appear bloody or have a dark brown color.

9.20 When C.trachomatis is present at very high concentration, (≥ 103 IFU/mL, corresponding to less than 5% of positive clinical samples), the detection of N.gonorrhoeae present at concentrations near the limit of detection (LoD) of cobas® CT/NG may be impacted.

For telephone technical assistance:

Roche Customer Support Center: 1-800-526-1247

3) Review/Revision/Implementation:

All procedures must be reviewed at least every 2 years.

- All new and procedures that have major revisions must be signed by the CLIA Laboratory Director.

- All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.

4) Related Procedures: n/a

5) References:

Roche Diagnostics: Cobas CT/NG Test Package Insert, Doc Rev 1.0. Indianapolis, IN, January 2020.

Roche Cobas 6800 Operator's Manual.

6) Attachments:

7) Revised/Reviewed Dates and Signatures:

Review/Revision Date	Signature