cobas® CT/NG

Qualitative nucleic acid test for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

cobas® CT/NG	P/N: 07460066190
cobas® CT/NG Positive Control Kit	P/N: 07460082190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190

Table of contents

Intended use

cobas® CT/NG for use on the **cobas®** 6800/8800 Systems is an automated, qualitative *in vitro* 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, clinician-collected vaginal swab specimens, endocervical swab specimens, oropharyngeal (throat) swab specimens, and anorectal swab specimens, all collected in **cobas®** PCR Media (Roche Molecular Systems, Inc.), and cervical specimens collected in PreservCyt® Solution. This test is intended as an aid in the diagnosis of chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

Summary and explanation of the test

Background

Infection with CT is the leading bacterial cause of sexually transmitted diseases worldwide, with approximately 89.1 million cases occurring annually.[1] *C. trachomatis* is the most frequently reported bacterial sexually transmitted disease (STD) in the United States[1,2] and prevalence is highest in persons aged ≤ 24 years.[3] In 2013, a total of 1,401,906 cases of *C. trachomatis* infection were reported to the CDC corresponding to a rate of 446.6 cases per 100,000 population.[3] CT is a gram-negative, nonmotile, obligate intracellular bacterium with a unique biphasic lifecycle.[1] CT causes a variety of infections including 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.[1] Many infections remain asymptomatic, and high numbers of infected patients may not seek care.[4] 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.[1]

The diagnosis of *C. trachomatis* urogenital infection in women is made by testing first-catch urine or collecting swab specimens from the endocervix or vagina. Diagnosis of *C. trachomatis* urethral infection in men can be made by testing a urethral swab or first-catch urine specimen. Nucleic acid amplification tests (NAATs) are the most sensitive tests for these specimens and therefore are recommended for detecting *C. trachomatis* infection.[5] Anorectal and oropharyngeal *C. trachomatis* infection in persons engaging in receptive anal or oral intercourse can be diagnosed by testing at the anatomic site of exposure.

Annual screening for CT of all sexually active women aged < 25 years is recommended and screening of older women is recommended in the presence of increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection).[6] Chlamydia screening programs have been demonstrated to reduce the rates of PID in women.[7,8] Although the evidence to support routine screening for CT in sexually active young men is insufficient, due to the relative lack of feasibility, efficacy, and cost-effectiveness studies, the screening of sexually active young men should be considered in clinical settings with a high prevalence of chlamydia (e.g., adolescent clinics, correctional facilities, and STD clinics) or in populations with high burden of infection (e.g., MSM).[2,6] The primary focus of chlamydia screening efforts among women should be to detect chlamydia, prevent complications, and test and treat their partners, whereas targeted chlamydia screening in men should only be considered when resources permit, prevalence is high, and such screening does not hinder chlamydia screening efforts in women.[9,10] More frequent screening for some women (e.g., adolescents) or certain men (e.g., MSM) might be indicated.[2]

NG is the etiologic agent of gonorrhea. NG are cytochrome oxidase-positive, non-motile, non-spore forming gram-negative diplococci. In the United States, an estimated 820,000 new *N. gonorrhoeae* infections occur each year.[11] Gonorrhea is the second most commonly reported communicable disease.[3] Clinical manifestations of NG infections are numerous.[4] 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.[12] 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.[13] Pelvic inflammatory disease (PID) can occur in 10%-20% of women, combined with endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis, and perihepatitis.[14] PID can result in tubal scarring that can lead to infertility and ectopic pregnancy. 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.

Annual screening for *N. gonorrhoeae* infection is recommended for all sexually active women aged < 25 years and for older women at increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI).[6] Additional risk factors include inconsistent condom use among persons with multiple sex partners, previous or coexisting sexually transmitted infections, and exchanging sex for money or drugs.[2] In addition to urethral infections, the CDC also recommends the use of NAATs for routine annual screening for men who have sex with men (MSM) for anorectal or oral infection.[5]

Rationale for CT/NG testing

NAATs are the recommended method for CT and NG screening.[15] 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, a urethral swab is also acceptable. In addition, the CDC recommends at least annual screening for CT from urethral or anorectal specimens and for NG from urethral, anorectal or oral specimens in MSM.[2]

cobas® CT/NG for use on the **cobas®** 6800/8800 Systems (referred to as **cobas®** CT/NG thoughout the remainder of this document) is an automated, qualitative real-time PCR test designed to detect CT and NG DNA in urogenital, oropharyngeal and anorectal 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.

Explanation of the test

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, oropharyngeal, anorectal, urine and cervical specimens of infected female patients and oropharyngeal, anorectal and urine specimens in 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. 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.

Principles of the procedure

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, external controls 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.

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 amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step.[16] However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

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.

Reagents and materials

cobas® CT/NG reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® CT/NG

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

Table 2 cobas® CT/NG Positive Control Kit

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)

Table 3 **cobas®** 6800/8800 Buffer Negative Control Kit

cobas® 6800/8800 Buffer Negative Control Kit Store at 2-8°C (P/N 07002238190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
cobas® 6800/8800 Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1mL)

cobas omni reagents for sample preparation

Table 4 **cobas omni** reagents for sample preparation*

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 H318: Causes serious 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 eye protection/ face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. 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. P501: Dispose of contents/ container to an approved waste disposal plant.
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2L	Not applicable

* These reagents are not included in the **cobas**® CT/NG kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas®** 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

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

Reagents loaded onto the **cobas®** 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas®** 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas®** 6800/8800 Systems

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 20 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.

Additional materials required

Table 7 Materials and consumables for use on **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

Instrumentation and software required

The **cobas®** 6800/8800 software and **cobas®** CT/NG analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 8 Instrumentation

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

cobas® CT/NG accepts the primary tube used for all **cobas®** PCR CT/NG swab and urine specimen types. Refer to the **cobas®** 6800/8800 Systems Operator's Manual for additional information for primary and secondary sample tubes accepted on the instruments.

MPA racks are required to use **cobas®** CT/NG. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Table 9 Specimen collection kits used with **cobas**® CT/NG

Collection Kit	P/N
cobas® PCR Media Kit	06466281190
cobas® PCR Urine Sample Kit	05170486190
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190
ThinPrep Pap Test Physician's Kit	70136-001 (500 vials & Broom-like collection devices) 70136-002 (500 vials & Cytobrush/spatula collection devices)

Precautions and handling requirements

Warnings and precautions

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.

- For *in vitro* diagnostic use only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[19,20] Only personnel proficient in handling infectious materials and the use of **cobas**® CT/NG and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- Do not freeze any samples stored in primary or secondary tubes.
- Use only supplied or specified required consumables to ensure established test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- 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.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples, reagents, or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Expended control kits contain pierced vials with residual reagent; special care should be taken during disposal to avoid spills and contact.

- **cobas**® CT/NG kit, **cobas**® CT/NG Positive Control kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Avoid contaminating gloves when handling samples and controls. Gloves must be changed between handling samples and **cobas**® CT/NG kit, **cobas**® CT/NG Positive Control kit, **cobas**® 6800/8800 Buffer Negative Control **kit,** and **cobas omni** reagents to prevent contamination.
- Wash hands thoroughly after handling samples and reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces 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.
- If spills occur on the **cobas**® 6800/8800 Systems, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Specimen collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Specimen collection

Endocervical swab specimens collected with the **cobas**® PCR Dual Swab Collection Kit, vaginal swab specimens, anorectal swab specimens and oropharyngeal swab specimens collected with the **cobas**® PCR Uni Swab Collection Kit, male and female urine collected with the **cobas**® PCR Urine Collection Kit and cervical specimens collected in PreservCyt® Solution have been validated for use with **cobas**® CT/NG (see Table 9 for a list of all collection kits). Follow the instructions for collecting all swab and urine specimens in their respective collection kit IFU. Follow the manufacturer's instructions for collecting cervical specimens into PreservCyt® Solution.

Specimen transport

Endocervical swab specimens collected with the **cobas**® PCR Dual Swab Collection Kit, vaginal swab specimens, anorectal swab specimens and oropharyngeal swab specimens collected with the **cobas**® PCR Uni Swab Collection Kit, male and female urine collected with the **cobas**® PCR Urine Collection Kit and cervical specimens collected in PreservCyt® Solution can be transported at 2-30°C. Transportation of CT/NG specimens in **cobas**® PCR Media and PreservCyt® Solution must comply with country, federal, state and local regulations for the transport of etiologic agents.[12]

Specimen storage

Endocervical swab specimens collected with the **cobas**® PCR Dual Swab Collection Kit, vaginal swab specimens, anorectal swab specimens and oropharyngeal swab specimens collected with the **cobas**® PCR Uni Swab Collection Kit and male and female urine collected with the **cobas**® PCR Urine Collection Kit may be stored at 2-30°C for up to 3 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 3 months.

Male and female urine specimens

- Use only the **cobas**® PCR Urine Sample Kit to collect urine specimens for **cobas**® CT/NG. **cobas**® CT/NG has not been validated for use with other urine collection devices or media types. Using **cobas**® CT/NG with other urine collection devices or other media types may lead to false negative, false positive, and/or invalid results.
- To avoid cross contamination of processed specimens, additional caps for **cobas**® PCR Media tubes in an alternate color (neutral; see **Additional materials required**) should be used to recap specimens after processing.
- 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.
- If additional testing is required, ensure that there is at least 1.2 mL of specimen remaining the in **cobas**® PCR Media tube.

Endocervical, vaginal, anorectal and oropharyngeal specimens

- The presence of mucus in endocervical and cervical 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 Collection Kit or an equivalent device to remove cervical secretions and discharge before obtaining the endocervical or cervical specimen.
- Use only the flocked swab in the **cobas**® PCR Dual Swab Collection Kit to collect endocervical specimens. Use only the woven polyester swab in either the **cobas**® PCR Uni Swab Collection Kit or the **cobas**® PCR Dual Swab Collection Kit to collect vaginal, anorectal, and oropharyngeal 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.
- To avoid cross contamination of processed specimens, additional caps for **cobas**® PCR Media tubes in an alternate color (neutral; see **Additional materials required**) should be used to recap specimens after processing.
- All swab specimens containing a single swab in the **cobas**® PCR Media tube can be directly processed on the **cobas**® 6800/8800 Systems. If desired, the swab may be removed before the specimen tube is loaded onto the instrument, however utmost care must be exercised to avoid cross contamination.
- A properly collected swab specimen should have a single swab with the shaft broken at the scoreline. Swab shafts which are broken above the score line will appear longer than normal and may also be bent over to fit into the **cobas**® PCR Media tube. This can create an obstruction to the pipetting system which may cause the loss of sample, test results and/or mechanical damage to the instrument. In the event that a swab specimen has an improperly broken shaft, remove the swab prior to sample processing on the **cobas**® 6800/8800 Systems. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other surfaces during disposal to prevent contamination.
- Incoming primary swab specimen tubes with no swabs or with two swabs have not been collected according to the instructions in their respective collective kit IFU and should not be tested.
- 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.

- 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 and the remaining fluid must have a minimum volume of 1.0 mL.

Cervical specimens in PreservCyt® Solution

- **cobas**®CT/NG is validated for use with cervical specimens collected in PreservCyt® Solution. **cobas**®CT/NG has not been validated for use with cervical specimens obtained in other media types. Using **cobas**®CT/NG with other media types may lead to false negative, false positive, and/or invalid results.
- When aliquoting cervical specimens from primary containers into barcoded 13 mL round-based **cobas**®PCR Media Secondary tubes for processing on the **cobas**®6800/8800 Systems, use pipettors with aerosol-barrier or positive-displacement tips to handle specimens. To avoid cross contamination, additional caps for these tubes in an alternate color (neutral; see **Additional materials required**) should be used to recap these specimens after processing.
- Use caution when transferring specimens from primary containers to 13 mL round-based **cobas**®PCR Media Secondary tubes. **Vortex primary specimens prior to transfer.** Change pipetting tips after each specimen.
- Aliquots of the primary specimen must contain a minimum volume of 1.0 mL.

Instructions for use

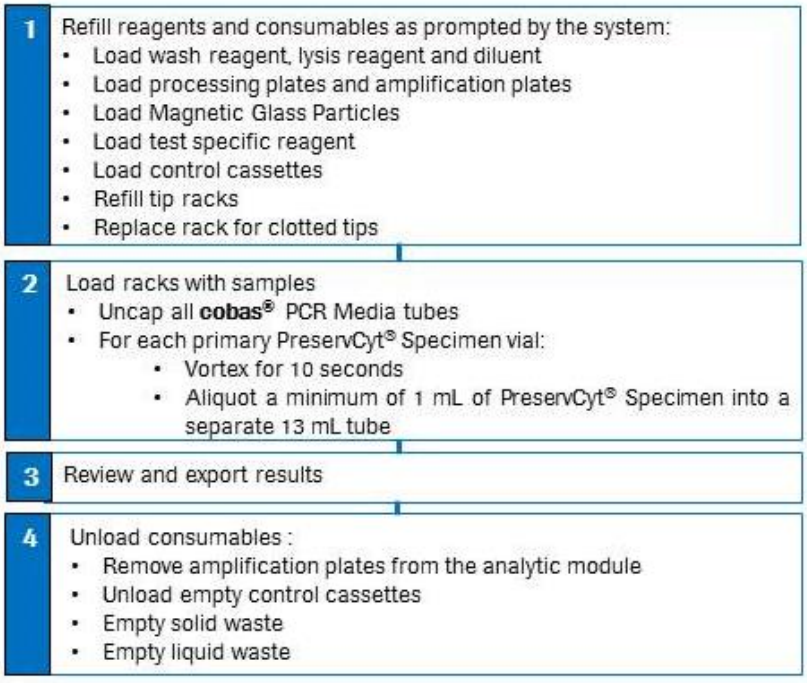
Procedural notes

- Do not use **cobas**® CT/NG, **cobas**® CT/NG Positive Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of MPA sample racks. Refer to the **cobas**® 6800/8800 Systems Operator's Manual for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Running cobas® CT/NG

cobas® CT/NG can be run with a minimum required sample volume of 1.0 mL for swab and PreservCyt® specimens, and 1.2 mL for urine specimens. The operation of the instrument is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual. Figure 1 below summarizes the procedure.

Figure 1 **cobas**® CT/NG procedure



Results

cobas® CT/NG automatically detects and discriminates CT and/or NG DNA simultaneously for samples and controls, displaying test validity, overall results, as well as individual target results.

Figure 2 Example of **cobas**® CT/NG results display

Test	Sample ID ▲	Valid	Flags	Sample type	Overall result	Target 1	Target 2
CT/NG	C161420284084196207422	Yes		CT/NG (+) C	**Valid**	Valid	Valid
CT/NG	C161420284090419545972	Yes		(-) Ctrl	**Valid**	Valid	Valid
CT/NG 400 ul	CTNG_PC1	Yes		PreservCyt®	**Positive**	CT Positive	NG Positive
CT/NG 400 ul	CTNG_PC2	Yes		PreservCyt®	**Reactive**	CT Negative	NG Positive
CT/NG 400 ul	CTNG_Swab1	Yes		Swab	**Negative**	CT Negative	NG Negative
CT/NG 400 ul	CTNG_Swab2	Yes		Swab	**Positive**	CT Positive	NG Positive
CT/NG 850 ul	CTNG_Urine1	Yes		Urine	**Reactive**	CT Positive	NG Negative
CT/NG 850 ul	CTNG_Urine2	Yes		Urine	**Negative**	CT Negative	NG Negative
CT/NG 850 ul	CTNG_Urine3	No	Y40T	Urine	**Invalid**	Invalid	Invalid

Quality control and validity of results

- One negative control and one positive control 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.
- The batch is valid if no flags appear for all controls.

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

Control flags

Table 10 Control flags for negative and positive controls

Negative Control	Flag	Overall Result	Interpretation
(–) Ctrl	Q01	Invalid	An invalid result or a target channel result for the negative control is not negative.
	C02H1	Invalid	Data for target 1 cannot be analyzed
	C02H2	Invalid	Data for target 2 cannot be analyzed
Positive Control	**Flag**	**Overall Result**	**Interpretation**
CT/NG (+) C	Q02	Invalid	An invalid result or a target channel result for the positive control is not within the assigned range.
	C02H1	Invalid	Data for target 1 cannot be analyzed
	C02H2	Invalid	Data for target 2 cannot be analyzed

If the batch is invalid, repeat testing of the entire batch.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**®6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values in "Overall Result" column for individual samples should be interpreted as follows:
  - Positive - All requested results are positive
  - Reactive – One of the requested results is positive and the other negative
  - Negative – All requested results are negative
  - Invalid – At least one requested result is invalid
- Reported target results for individual samples are valid unless indicated otherwise.

Results and their corresponding interpretation for detecting CT and NG (Table 11), CT only (Table 12) and NG only (Table 13) are shown below.

Table 11 cobas® CT/NG results and interpretation for the CT/NG result request

Valid	Overall Result	Target 1	Target 2	Interpretation
Yes	**Positive**	CT Positive	NG Positive	All requested results were valid. Target signal detected for CT and NG DNA.
Yes	**Reactive**	CT Positive	NG Negative	All requested results were valid. Target signal detected for CT DNA. No target signal detected for NG DNA.
Yes	**Reactive**	CT Negative	NG Positive	All requested results were valid. No target signal detected for CT DNA. Target signal detected for NG DNA.
Yes	**Negative**	CT Negative	NG Negative	All requested results were valid. No target signal detected for CT or NG DNA.
No	**Invalid**	CT Positive	Invalid	Not all requested results were valid. Target signal detected for CT DNA. NG result is invalid. Original specimen should be re-tested to obtain valid NG results. If the result is still invalid, a new specimen should be obtained.
No	**Invalid**	Invalid	NG Positive	Not all requested results were valid. CT result is invalid. Original specimen should be re-tested to obtain valid CT results. If the result is still invalid, a new specimen should be obtained. Target signal detected for NG DNA.
No	**Invalid**	CT Negative	Invalid	Not all requested results were valid. No target signal detected for CT DNA. NG result is invalid. Original specimen should be re-tested to obtain valid NG results. If the result is still invalid, a new specimen should be obtained.
No	**Invalid**	Invalid	NG Negative	Not all requested results were valid. CT result is invalid. Original specimen should be re-tested to obtain valid CT results. If the result is still invalid, a new specimen should be obtained. No target signal detected for NG DNA.
No	**Invalid**	Invalid	Invalid	Both CT and NG results are invalid. Original specimen should be re-tested to obtain valid CT and NG results.* If the results are still invalid, a new specimen should be obtained.

*Invalid results for swab specimens could result from processing errors due to clots (flagged P02T). Refer to the retesting instructions for swab specimens found in the section "**Endocervical, vaginal, anorectal and oropharyngeal specimens**."

Table 12 cobas® CT/NG results and interpretation for the CT result request

Valid	Overall Result	Target 1	Target 2	Interpretation
Yes	**Positive**	CT Positive		The requested result was valid. Target signal detected for CT DNA.
Yes	**Negative**	CT Negative		The requested result was valid. No target signal detected for CT DNA
No	**Invalid**	Invalid		CT result is invalid. Original specimen should be re-tested to obtain valid CT results.* If the result is still invalid, a new specimen should be obtained.

*Invalid results for swab specimens could result from processing errors due to clots (flagged P02T). Refer to the retesting instructions for swab specimens found in the section "**Endocervical, vaginal, anorectal and oropharyngeal specimens**."

Table 13 cobas® CT/NG results and interpretation for the NG result request

Valid	Overall Result	Target 1	Target 2	Interpretation
Yes	**Positive**		NG Positive	The requested result was valid. Target signal detected for NG DNA.
Yes	**Negative**		NG Negative	The requested result was valid. No target signal detected for NG DNA
No	**Invalid**		Invalid	NG result is invalid. Original specimen should be re-tested to obtain valid NG results.* If the result is still invalid, a new specimen should be obtained.

*Invalid results for swab specimens could result from processing errors due to clots (flagged P02T). Refer to the retesting instructions for swab specimens found in the section "**Endocervical, vaginal, anorectal and oropharyngeal specimens**."

Procedural limitations

- **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.
- Reliable results depend on proper sample collection, storage and handling procedures.
- **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, anorectal swab specimens, oropharyngeal 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.
- 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.
- 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.
- *Neisseria gonorrhoeae* may occasionally exchange genetic material with commensal bacteria commonly found in the normal microflora of the mouth and throat. It is possible that this exchange may include isolated DNA sequences which could, on rare occasion, produce a positive signal with this assay.[21]
- 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. Users should follow their own specific policies/procedures.

- **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.
- **cobas**® CT/NG is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications.
- **cobas**® CT/NG should not be used to determine therapeutic success as nucleic acids may be present after antimicrobial therapy.
- **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.
- The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
- **cobas**® CT/NG 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.
- 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.
- 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.
- **cobas**® CT/NG has not been evaluated in patients younger than 14 years of age.
- Urogenital specimens from patients who have used the over-the-counter products Replens® Vaginal Moisturizer, RepHresh Odor Eliminating Vaginal Gel and RepHresh Clean Balance or used Metronidazole Vaginal Gel may generate invalid or false negative results. See Interference results (Table 18) for further details.

Performance evaluation

Key performance characteristics

Limit of Detection (LoD)

The *Chlamydia trachomatis* analytical sensitivity claim for the assay is 40 Elementary Bodies (EB) per mL for all serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2, L3) as well as for the Swedish variant nvCT, in all claimed specimen types. However, dilutions of some serovars below 40 EB/mL have tested positive using **cobas**® CT/NG for use on the **cobas**® 6800/8800 Systems.

The *N. gonorrhoeae* analytical sensitivity claim for the assay is 1.0 Colony Forming Units (CFU) per mL (45 gonorrhoeae strains tested) in all claimed specimen types. However, dilutions of gonorrhoeae strains below 1.0 CFU/mL have tested positive using **cobas**® CT/NG for use on the **cobas**® 6800/8800 Systems.

Precision

In-house precision was examined using a panel composed of CT and NG cultures diluted into a pool of negative endocervical swab specimen matrix collected in **cobas**® PCR Media, a pool of negative urine matrix plus **cobas**® PCR Media and a pool of negative cervical specimen matrix collected in PreservCyt® Solution. Endocervical swabs were intended to represent all swab samples collected in **cobas**® PCR Media (endocervical, vaginal, oropharyngeal, and anorectal). Four levels were tested using CT serovar D and NG strain 2948 as the target organisms.

The precision panel was designed to include members with very low, low and medium concentrations of CT and NG (≤ 0.7EB/mL and ≤ 0.07CFU/mL, ≤ 4EB/mL and ≤ 0.4CFU/mL and ≤ 12EB/mL and ≤ 1.2CFU/mL) for each panel matrix. Testing was performed with three lots of **cobas**® CT/NG reagents and two instruments for a total of 24 runs. A description of the precision panels and the study performance hit rate is shown in Table 14. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation of the Ct values from valid tests performed on positive panel members (see Table 15 and Table 16) yielded overall CV (%) ranges from 1.62% to 4.05% for CT and from 1.17% to 3.55% for NG.

Table 14 Summary of within laboratory precision

Target Concentration		N Tested	N positive CT	N positive NG	Hit Rate		95% CI CT		95% CI NG	
CT	**NG**				**CT**	**NG**	**LL**	**UL**	**LL**	**UL**
Endocervical Swab in **cobas**® PCR Media										
Neg	Neg	72	0	0	0%	0%	0.0	5.0	0.0	5.0
0.7 EB/mL	0.07 CFU/mL	72	51	32	71%	44%	59	81	33	57
2 EB/mL	0.4 CFU/mL	72	69	68	96%	94%	88	99	86	98
6 EB/mL	1.2 CFU/mL	72	72	72	100%	100%	95	100	95	100
cobas® PCR Media with Urine										
Neg	Neg	72	0	0	0%	0%	0.0	5.0	0.0	5.0
0.3 EB/mL	0.05 CFU/mL	72	38	47	53%	65%	66	87	66	87
1 EB/mL	0.2 CFU/mL	72	72	69	100%	96%	92	100	95	100
3 EB/mL	0.6 CFU/mL	72	72	72	100%	100%	95	100	95	100
Cervical samples collected into PreservCyt® Solution										
Neg	Neg	72	0	0	0%	0%	0.0	5.0	0.0	5.0
0.7 EB/mL	0.07 CFU/mL	72	56	56	78%	78%	41	65	53	76
4 EB/mL	0.2 CFU/mL	72	71	72	99%	100%	95	100	88	99
12 EB/mL	0.6 CFU/mL	72	72	72	100%	100%	95	100	95	100

Table 15 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, CT panel members 2, 3, and 4

Hit Rate	Mean Ct	Within run		Between run		Between day		Between instrument		Between lot		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Endocervical Swab in **cobas**® PCR Media													
71%	39.7	0.00	0.00	0.00	0.00	1.27	3.21	0.00	0.00	0.34	0.85	1.32	3.32
96%	38.5	0.00	0.00	0.04	0.10	1.14	2.96	0.00	0.00	0.48	1.25	1.24	3.22
100%	36.9	0.00	0.00	0.25	0.69	0.54	1.45	0.07	0.18	0.00	0.00	0.60	1.62
Cervical Samples collected into PreservCyt® Solution													
53%	38.3	0.60	1.57	0.52	1.37	1.12	2.92	0.00	0.00	0.00	0.00	1.37	3.58
100%	36.9	0.21	0.56	0.28	0.76	0.68	1.85	0.00	0.00	0.00	0.00	0.77	2.08
100%	35.6	0.00	0.00	0.20	0.56	0.52	1.46	0.09	0.24	0.02	0.05	0.56	1.59
cobas® PCR Media with Urine													
78%	38.9	0.00	0.00	0.12	0.30	1.25	3.22	0.39	1.01	0.00	0.00	1.32	3.39
99%	38.3	0.11	0.28	0.00	0.00	1.52	3.97	0.00	0.00	0.29	0.77	1.55	4.05
100%	37.1	0.00	0.00	0.00	0.00	1.05	2.84	0.00	0.00	0.28	0.77	1.09	2.94

Table 16 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, NG panel members 2, 3, and 4

Hit Rate	Mean Ct	Within run		Between run		Between day		Between instrument		Between lot		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Endocervical Swab in **cobas**® PCR Media													
44%	39.1	0.00	0.00	0.31	0.79	0.84	2.14	0.72	1.85	0.57	1.46	1.28	3.28
94%	38.1	0.00	0.00	0.00	0.00	1.27	3.34	0.00	0.00	0.00	0.00	1.27	3.34
100%	36.5	0.00	0.00	0.24	0.67	0.69	1.89	0.00	0.00	0.15	0.40	0.74	2.04
Cervical Samples collected into PreservCyt® Solution													
65%	39.0	0.34	0.87	0.00	0.00	1.11	2.85	0.08	0.20	0.45	1.16	1.25	3.21
96%	38.0	0.00	0.00	0.00	0.00	1.25	3.28	0.00	0.00	0.00	0.00	1.25	3.28
100%	35.8	0.00	0.00	0.28	0.78	0.76	2.13	0.00	0.00	0.00	0.00	0.81	2.27
cobas® PCR Media with Urine													
78%	39.1	0.00	0.00	0.26	0.66	1.35	3.46	0.00	0.00	0.18	0.45	1.39	3.55
100%	36.7	0.14	0.38	0.16	0.42	0.71	1.92	0.00	0.00	0.00	0.00	0.74	2.00
100%	34.9	0.00	0.00	0.16	0.47	0.37	1.06	0.06	0.18	0.00	0.00	0.41	1.17

Analytical specificity/cross reactivity

A panel of 151 bacteria, fungi and viruses, including those commonly found in the male and female urogenital tract, 17 representatives of non-*gonorrhoeae Neisseria* strains and other phylogenetically unrelated organisms, were tested with **cobas®** CT/NG to assess analytical specificity. The organisms listed in Table 17 were spiked at concentrations of approximately 1 x 10^6 units*/mL for bacteria and approximately 1 x 10^5 units*/mL for viruses into pools of negative swab specimens in **cobas®** PCR Media (endocervical, oropharyngeal, and anorectal), urine stabilized in **cobas®** PCR Media and cervical specimens in PreservCyt® Solution. Testing was performed with each potential interfering organism alone as well as with each organism mixed with CT and NG cultures at ≤ 12EB/mL and ≤ 1.2CFU/mL. Results indicated that none of these organisms interfered with the detection of CT and NG or produced false positive results in the CT/NG negative matrices.

*All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydophila pneumonia* and *Chlamydophila psittaci* as Inclusion Forming Units (IFU). All viruses were quantified as units/mL as determined by $TCID_{50}$ Endpoint Dilution Assay. *Trichomonas vaginalis* and HPV16 were quantified as cells/mL.

Table 17 Microorganisms tested for analytical specificity/cross reactivity

Achromobacter xerosis	*Haemophilus ducreyi*	*Neisseria polysaccharea*
Acinetobacter calcoaceticus	*Haemophilus influenzae*	*Neisseria sicca*
Acinetobacter lwoffi	*Helicobacter pylori*	*Neisseria subflava*
Actinomyces israelii	HPV 16	*Neisseria weaverii*
Actinomyces pyogenes	HSV-1	*Paracoccus denitrificans*
Aerococcus viridans	HSV-2	*Peptostreptococcus anaerobius*
Aeromonas hydrophila	Human Adenovirus 40	*Peptostreptococcus asaccharolyticus*
Alcaligenes faecalis	Human Enterovirus 71	*Peptostreptococcus magnus*
Bacillus subtilis	Human Rotavirus	*Plesiomonas shigelloides*
Bacteriodes fragilis	*Kingella dentrificans*	*Propionibacterium acnes*
Bacteroides caccae	*Kingella kingae*	*Proteus mirabilis*
Bacteroides ureolyticus	*Klebsiella oxytoca*	*Proteus penneri*
Bifidobacterium adolescentis	*Klebsiella pneumoniae*	*Proteus vulgaris*
Bifidobacterium breve	*Lactobacillus acidophillus*	*Providencia rettgeri*
Bifidobacterium longum	*Lactobacillus brevis*	*Providencia stuartii*
Blautia producta	*Lactobacillus crispatus*	*Pseudomonas aeruginosa*
Branhamella catarrhalis	*Lactobacillus delbrueckii subsp. lactis*	*Pseudomonas fluorescens*
Brevibacterium linens	*Lactobacillus jensenii*	*Pseudomonas putida*
Campylobacter coli	*Lactobacillus lactis*	*Rahnella aquatilis*
Campylobacter jejuni	*Lactobacillus oris*	*Rhizobium radiobacter*
Candida albicans	*Lactobacillus parabuchnerri*	*Rhodospirillum rubrum*
Candida glabrata	*Lactobacillus reuteri*	*Saccharomyces cerevisiae*
Candida parapsilosis	*Lactobacillus vaginalis*	*Salmonella choleraesuis*
Candida tropicalis	*Lactococcus lactis cremoris*	*Salmonella minnesota*
Chlamydia psittaci	*Legionella pneumophila*	*Salmonella typhimurium*
Chlamydophila pneumoniae	*Leuconostoc paramensenteroides aka.*	*Serratia denitrificans*
Chromobacter violaceum	*Listeria monocytogenes*	*Serratia marcescens*
Citrobacter freundii	*Micrococcus luteus*	*Shigella dysenteriae*
Clostridium difficile (Serogroup B)	*Moraxella lacunata*	*Staphylococcus aureus*
Clostridium perfringens	*Moraxella osloensis*	*Staphylococcus epidermidis*

Corynebacterium genitalium	*Morganella morganii*	*Staphylococcus saprophyticus*
Corynebacterium xerosis	*Mycobacterium smegmatis*	*Streptococcus agalactiae*
Cryptococcus neoformans	*Mycoplasma genitalium*	*Streptococcus anginosus*
Cytomegalovirus	*Mycoplasma hominis*	*Streptococcus bovis*
Deinococcus radiodurans	*Neisseria cinerea*	*Streptococcus dysgalactiae*
Derxia gummosa	*Neisseria dentrificans*	*Streptococcus equinis*
Eikenella corrodens	*Neisseria elongata subsp. elongata*	*Streptococcus mitis*
Enterobacter aerogenes	*Neisseria elongata subsp. niroreducans*	*Streptococcus mutans*
Enterobacter cloacae	*Neisseria flava*	*Streptococcus pneumoniae*
Enterococcus avium	*Neisseria flavescens*	*Streptococcus pyogenes*
Enterococcus casseliflavus	*Neisseria kochi*	*Streptococcus salivarius*
Enterococcus faecalis	*Neisseria lactamica*	*Streptococcus sanguis*
Enterococcus faecium	*Neisseria macacae*	*Streptomyces griseinus*
Erwinia herbicola	*Neisseria meningitidis Serogroup A*	*Trichomonas vaginalis*
Erysipelothrix rhusiopathiae	*Neisseria meningitidis Serogroup B*	*Ureaplasma urealyticum*
Escherichia coli	*Neisseria meningitidis Serogroup C*	*Veillonela parvula*
Escherichia fergusonii	*Neisseria meningitidis Serogroup D*	*Vibrio cholerae*
Flavobacterium meningosepticum	*Neisseria meningitidis Serogroup W135*	*Vibrio parahaemolyticus*
Fusobacterium nucleatum	*Neisseria meningitidis Serogroup Y*	*Yersinia enterocolitica*
Gardnerella vaginalis	*Neisseria mucosa*	
Gemella haemolysans	*Neisseria perflava*	

Interference

The effect of over-the-counter or prescription feminine products that may be present in urogenital specimens (Table 18), over-the-counter oral hygiene products that may be present in oropharyngeal specimens (Table 19) and of hygiene and prescription products that may be present in anorectal specimens (Table 20) were evaluated. Testing was done using pooled clinical specimens (vaginal swab, urine and PreservCyt® specimens were used to represent urogenital) with spiking of potential interfents at levels expected from normal patient usage. Interferents were tested in CT/NG negative specimen pools as well as in specimen pools with CT/NG at $\leq$ 120EB/mL and $\leq$ 1.2CFU/mL, depending on the specimen type tested. CT serovars D and I and NG strains 2948 and 891 were used in this study.

Of the over-the-counter (OTC) feminine hygiene and prescription products tested in urogenital specimens, Metronidazole, Replens, RepHresh Odor Eliminating Vaginal Gel and RepHresh Clean Balance produced false negative or invalid results. None of the OTC oral hygiene products tested in oropharyngeal swabs or the OTC anorectal hygiene and prescription products tested in anorectal swabs produced interference to the test when examined at concentrations expected through typical product use.

Table 18 List of substances tested for interference in urogenital specimens

Product Name	
Clindamycin Phosphate Vaginal Cream	Norforms Suppositories
CVS Tioconazole 1 (Equate tioconazole 1)	Premarin
Equate Vagicaine Anti-Itch Cream	Replens Long-Lasting Vaginal Moisturizer*
Estrace	Summer's Eve Feminine Deodorant Spray
K-Y Ultra Gel (Replaces K-Y Silk E)	VCF - Vaginal Contraceptive Foam
Metronidazole Vaginal Gel *	Yeast Gard Advanced
Monistat 3 Vaginal Antifungal Combination Pack	Azo Standard* (urine only)
Monistat Complete Care Itch Relief Cream	RepHresh Odor Eliminating Vaginal Gel*‡
Gyne-Lotrimin 7	RepHresh Clean Balance*‡

* Metronidazole, Replens and RepHresh showed interference at levels that may potentially be present in clinical specimens

‡ RepHresh products were tested using simulated swab specimen

Table 19 List of substances tested for interference in oropharyngeal swab specimens

Product Name
Cepacol Maximum Strength Throat Drop Lozenges
Colgate Total Toothpaste
Robitussin Cough / Chest Congestion Cough Syrup
Listerine Ultra Clean Antiseptic Mouthwash
Scope Mouthwash
Sucrets Complete Lozenges
Vicks - Chloraseptic Sore Throat Spray Menthol
Zicam Oral Mist

Table 20 List of substances tested for interference in anorectal swab specimens

Product Name
ANUSOL® Plus Ointment
CB Fleet® Mineral Oil Enema
Doproct Suppositories/ Hemorrhoidal Treatment
K-Y Jelly
Lotrimin Antifungal Cream
Preparation H Hemorrhoidal Ointment
PREPARATION H Hemorrhoidal Suppositories
Driminate Generic for Dramamine Motion Sickness - Major Pharmaceuticals
Target - Triple Paste Diaper Rash Ointment
Tucks Medicated Cooling Hemorrhoidal Pads
Vaseline Original Petroleum Jelly

Endogenous substances that may be present in urogenital, oropharyngeal and anorectal specimens were tested for interference. Testing was done using pooled clinical specimens (endocervical swab, urine and PreservCyt® specimens were used to represent urogenital) with spiking of potential endogenous interfents. Interferents were tested in CT/NG negative specimen pools as well as in the presence of CT/NG at $\leq$ 120EB/mL and $\leq$ 1.2CFU/mL, depending on the specimen type tested. CT serovars D and I and NG strains 2948 and 891 were used in this study.

Interference was noted with whole blood at 10% for urine and PreservCyt® specimens, with stool at 0.4% in anorectal specimens and with cervical mucus at 1% in endocervical specimens. Levels of endogenous substances tolerated by the assay for all specimen types are shown in Table 21.

Table 21 Summary of endogenous substance concentrations that do not show interference

Interferent	Endocervical Swab	Anorectal Swab	Oropharyngeal Swab	PreservCyt®	Urine
Albumin (% w/v)	N/A	N/A	N/A	N/A	5%
Bilirubin (% w/v)	N/A	N/A	N/A	N/A	0.5%
Mucus (% w/v)	0.5%	1.0%	1.0%	1.0%	0.5%
Glucose (% w/v)	N/A	N/A	N/A	N/A	1.0%
Peripheral Blood Mononuclear Cells (PBMCs as cells/mL)	1.0E+06	1.0E+06	1.0E+06	1.0E+06	1.0E+06
pH (acidic and alkaline)	N/A	N/A	N/A	N/A	pH 4 and pH 9
Saliva (% w/v)	N/A	N/A	2.0%	N/A	N/A
Semen (% w/v)	1.5%	N/A	N/A	1.5%	N/A
Stool (% w/v)	N/A	0.3%	N/A	N/A	N/A
Whole Blood (% v/v)	10%	10%	10%	5%	5%

Whole system failure

The samples tested in the whole system failure study were pooled CT and NG negative clinical cervical specimens collected in PreservCyt® Solution, vaginal swab specimens collected in **cobas®** PCR Media and urine specimens stabilized in **cobas®** PCR Media. Each pool of clinical specimens was spiked with cultures of CT, serovar D (D-UW3) (CT) and NG 2948 (ATCC 19424) (NG) to a concentration of ≤ 12 EB/mL and ≤ 1.2 CFU/mL, depending on the sample type. The results of this study determined that all replicates were valid and positive for CT/NG, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.6% for the upper bound [0%: 3.6%].

Cross contamination

Studies were performed to evaluate potential cross contamination on the **cobas®** 6800/8800 Systems using **cobas®** CT/NG. Cross-contamination can cause false positive results. In this performance study the sample to sample cross-contamination rate of **cobas®** CT/NG has been determined to be 0.5% (2/432) when alternating very high positive and negative samples were tested over multiple runs. Run to run cross-contamination has not been observed (0/282). Testing was done using samples prepared with **cobas®** PCR Media, urine stabilized in **cobas®** PCR Media and with PreservCyt® Solution. High positive samples in the study were prepared to generate a Ct value that exceeds 95% or more of signal obtained from specimens of infected patients in the intended use population. The likelihood of encountering such specimens in the routine use of **cobas®** CT/NG is proportional to CT and NG prevalence in the testing population. Therefore the sample to sample cross-contamination rate in routine use of **cobas®** CT/NG will likely be less than 0.5% x 5% x CT prevalence in the testing population. Even at a maximum prevalence of 100%, the cross-contamination rate would be 0.5% x 5% x 100% = 0.025%.

Method correlation

The performance of **cobas®** CT/NG and the **cobas®** 4800 CT/NG Test were compared by analysis of the following specimen types:

- Endocervical swabs in **cobas®** PCR Media
- Vaginal swabs (clinician-collected) in **cobas®** PCR Media
- Vaginal swabs (self-collected) in **cobas®** PCR Media
- Oropharyngeal swabs in **cobas®** PCR Media
- Anorectal swabs in **cobas®** PCR Media
- Male and female urine mixed with **cobas®** PCR Media
- Cervical specimens collected in PreservCyt® Solution

A total of 6,318 subjects were recruited from 19 clinical sites in Germany and the US, producing 13,433 valid CT results and 13,398 valid NG results which were used for the correlation study analysis. The correlation results for all specimen types are shown in Table 22 and the calculated PPA, NPA, OPA with 95% Confidence Intervals are shown in Table 23. Across all specimen types, there were 125 discrepant specimens for *Chlamydia trachomatis*; of which 120 were positive on the 6800/8800 Systems and 5 were positive on the 4800 System. Also across all specimen types, there were 42 discrepant specimens for *Neisseria gonorrhoeae*, of which 40 were positive on the 6800/8800 Systems and 2 were positive on the **cobas®** 4800 System.

Correlation analysis between cobas® CT/NG and the cobas® 4800 CT/NG Test shows Positive Percent Agreements (PPA) greater than 95% for both CT and NG in all specimen types with the majority of specimen types having a PPA of 100% for both CT and NG. Negative and Overall Percent Agreements were greater than 98% for both CT and NG in all specimen types.

Table 22 Results summary for correlation of **cobas®** CT/NG and the **cobas®** 4800 CT/NG Test

Specimen Type	Chlamydia trachomatis				Neisseria gonorrhoeae			
	Con +	Con -	68+ /48 -	68 - /48 +	Con +	Con -	68+ /48 -	68 - /48 +
Endocervical Swab	114	1778	15	0	22	1883	1	1
Vaginal Swab	87	1040	15	0	20	1111	1	0
SC-Vaginal Swab	90	1028	14	0	18	1100	3	0
Oropharyngeal Swab	37	1915	14	0	74	1864	22	0
Anorectal Swab	100	1871	30	0	71	1923	8	0
Female Urine	272	2083	18	0	23	2340	4	0
Male Urine	114	717	3	0	30	803	0	1
PreservCyt®	157	1905	11	5	25	2049	1	0
All Specimens Total	971	12337	120	5	283	13073	40	2

Con = Concordant; + = Positive; - = Negative; SC = Self-Collected

Table 23 Agreement calculations for correlation of **cobas**® CT/NG and the **cobas**® 4800 CT/NG Test

Specimen Type	Chlamydia trachomatis			Neisseria gonorrhoeae		
	Result (%)		95% CI	Result		95% CI
Endocervical Swab	PPA	100%	96.8%-100%	PPA	95.7%	78.1%-99.9%
	NPA	99.2%	98.6%-99.5%	NPA	99.9%	99.7%-100%
	OPA	99.2%	98.7%-99.6%	OPA	99.9%	99.6%-100%
Vaginal Swab	PPA	100%	95.8%-100%	PPA	100%	83.2%-100%
	NPA	98.6%	97.7%-99.2%	NPA	99.9%	99.5%-100%
	OPA	98.7%	97.8%-99.3%	OPA	99.9%	99.5%-100%
SC-Vaginal Swab	PPA	100%	96.0%-100%	PPA	100%	81.5%-100%
	NPA	98.7%	97.8%-99.3%	NPA	99.7%	99.2%-99.9%
	OPA	98.8%	97.9%-99.3%	OPA	99.7%	99.2%-99.9%
Oropharyngeal Swab	PPA	100%	90.5%-100%	PPA	100%	95.1%-100%
	NPA	99.3%	98.8%-99.6%	NPA	98.8%	98.2%-99.3%
	OPA	99.3%	98.8%-99.6%	OPA	98.9%	98.3%-99.3%
Anorectal Swab	PPA	100%	96.4%-100%	PPA	100%	94.9%-100%
	NPA	98.4%	97.8%-98.9%	NPA	99.6%	99.2%-99.8%
	OPA	98.5%	97.9%-99.0%	OPA	99.6%	99.2%-99.8%
Female Urine	PPA	100%	98.7%-100%	PPA	100%	85.2%-100%
	NPA	99.1%	98.6%-99.5%	NPA	99.8%	99.6%-100%
	OPA	99.2%	98.8%-99.5%	OPA	99.8%	99.6%-100%
Male Urine	PPA	100%	96.8%-100%	PPA	96.8%	83.3%-99.9%
	NPA	99.6%	98.8%-99.9%	NPA	100%	99.5%-100%
	OPA	99.6%	99.0%-99.9%	OPA	99.9%	99.3%-100%
PreservCyt®	PPA	96.9%	92.9%-99.0%	PPA	100%	86.3%-100%
	NPA	99.4%	99.0%-99.7%	NPA	99.9%	99.7%-100%
	OPA	99.2%	98.8%-99.6%	OPA	99.9%	99.7%-100%
All Specimens Total	PPA	99.5%	98.8%-99.8%	PPA	99.3%	97.5%-99.9%
	NPA	99.0%	98.8%-99.2%	NPA	99.7%	99.6%-99.8%
	OPA	99.1%	98.9%-99.2%	OPA	99.7%	99.6%-99.8%

PPA = Positive Percent Agreement; NPA = Negative Percent Agreement; OPA = Overall Percent Agreement SC = Self-Collected

Additional information

Key assay features

Sample types	• Endocervical swab collected in **cobas®** PCR Media • Vaginal swab collected in **cobas®** PCR Media • Self-collected Vaginal swab collected in **cobas®** PCR Media • Oropharyngeal swab collected in **cobas®** PCR Media • Anorectal swab collected in **cobas®** PCR Media • Male and female urine stabilized in **cobas®** PCR Media • Cervical specimen collected in PreservCyt® Solution
Amount of sample required	• 400 µL for all swab samples • 400 µL for PreservCyt® samples • 850 µL for urine samples
Test duration	• < 3.5 hours to first result

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 24 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		In Vitro Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains Sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number
	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 25 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

Trademarks and patents

See http://www.roche-diagnostics.us/patents

Copyright

©2016 Roche Molecular Systems, Inc.

References

1. Bebear C, de Barbeyrac B. Genital Chlamydia trachomatis infections. Clinical Microbial Infect. 2009; 15:4-10.
2. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015; 64:1-137.
3. CDC. Sexually Transmitted Disease Surveillance 2013.
4. Haggerty CL, Ness RB. Epidemiology, pathogenesis and treatment of pelvic inflammatory disease. Expert Rev Anti Infect Ther. 2006; 4:235-47.
5. Papp JR, Schachter, J, Gaydos CA, Van Der Pol B. Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* - 2014. MMWR Recomm Rep. 2014; 63:1-19.
6. LeFevre ML. Screening for Chlamydia and gonorrhea: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2014; 161:902-10.
7. Scholes D, Stergachis A, Heidrich FE, Andrilla H, Holmes KK, Stamm WE. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. N Engl J Med. 1996; 334:1362-1366.
8. Kamwendo F, Forslin L, Bodin L, Danielsson D. Decreasing incidences of gonorrhea- and chlamydia-associated acute pelvic inflammatory disease. A 25-year study from an urban area of central Sweden. Sex Transm Dis. 1996; 23:384-91.
9. Gift TL, Blake DR, Gaydos CA, Marrazzo JM. The cost-effectiveness of screening men for Chlamydia trachomatis: a review of the literature. Sex Transm Dis. 2008; 35 (11 Suppl):S51-60.
10. Gift TL, Gaydos CA, Kent CK, Marrazzo JM, Rietmeijer CA, Schillinger JA, et al. The program cost and cost-effectiveness of screening men for Chlamydia to prevent pelvic inflammatory disease in women. Sex Transm Dis. 2008; 35 (11 Suppl):S66-75.
11. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, Ocfemia MC, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis. 2013; 40:187-93.
12. Handsfield HH, Lipman TO, Harnisch JP, Tronca E, Holmes KK. Asymptomatic gonorrhea in men. Diagnosis, natural course, prevalence and significance. N Engl J Med. 1974; 290:117-23.
13. McCormack WM, Stumacher RJ, Johnson K, Donner A. Clinical spectrum of gonococcal infection in women. Lancet. 1977; 1:1182-5.
14. Ross JD. An update on pelvic inflammatory disease. Sex Transm Infect. 2002; 78:18-9.
15. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep. 2010; 59:1-110.
16. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990; 93:125-8.
17. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Bio/Technology. 1992; 10:413-7.
18. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Research. 1996; 6:986-94.
19. Center for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
20. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.
21. Obergfell KP, Seifert H. Mobile DNA in the Pathogenic *Neisseria*. Microbiology Spectrum. 2014; 3:1-18.

Document revision

Document Revision Information	
Doc Rev. 1.0 11/2016	First Publishing.