cobas® CT/NG v2.0 Test

FOR *IN VITRO* DIAGNOSTIC USE.

Rx Only

cobas® 4800 System Sample Preparation Kit	**c4800 SMPL PREP**	**960 Tests** **240 Tests**	**P/N: 05235804190** **P/N: 05235782190**
cobas® 4800 System Liquid Cytology Preparation Kit	**c4800 LIQ CYT**	**960 Tests** **240 Tests**	**P/N: 05235839190** **P/N: 05235812190**
cobas® 4800 CT/NG v2.0 Amplification/Detection Kit	**c4800 CT/NG v2.0 AMP/DET**	**960 Tests** **240 Tests**	**P/N: 07102577190** **P/N: 07102569190**
cobas® 4800 CT/NG Controls Kit	**c4800 CT/NG CTLS**	**10 Sets**	**P/N: 05235928190**
cobas® 4800 System Wash Buffer Kit	**c4800 WB**	**960 Tests** **240 Tests**	**P/N: 05235871190** **P/N: 05235863190**
cobas® 4800 System Control Diluent Kit	**c4800 CDIL**	**10 Sets**	**P/N: 05235847190**

NOTICE: The purchase of this product allows the purchaser to use it for amplification and detection of nucleic acid sequences by polymerase chain reaction (PCR) and related processes for human in vitro diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

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INTENDED USE

The **cobas**® CT/NG v2.0 Test is an automated, *in vitro* nucleic acid amplification test for the qualitative detection of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) DNA in urogenital specimens. The Test utilizes the Polymerase Chain Reaction (PCR) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in male and female urine, 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.), 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

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[2] 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.[2] 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, 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 2011[3], corresponding to a rate of 104.2 cases per 100,000 population[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.[5] Only a small proportion of men remain asymptomatic without signs of urethritis.[5] 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.[6] Pelvic inflammatory disease can occur in 10%-20% of women, combined with endometritis, salpingitis, tubo ovarian abscess, pelvic peritonitis, and perihepatitis.[7] 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.[8] Infants from infected mothers can develop conjunctivitis.[8]

The intended targets for the **cobas**® CT/NG v2.0 Test include all major CT serovars, the Swedish *C. trachomatis* mutant (nvCT), variants that may harbor deletions in the cryptic plasmid or that have no cryptic plasmid at all, and both DR-9A and variant DR-9B sequences of NG.

PRINCIPLES OF THE PROCEDURE

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

Sample preparation for the **cobas**® CT/NG v2.0 Test is automated with the use of the **cobas** x 480 instrument, which is part of the **cobas**® 4800 System. Specimens are lysed in the collection device by the chaotropic agent in the **cobas**® PCR Media. Released nucleic acids, along with added CT/NG Internal Control DNA, are purified through binding to magnetic glass particles, washed, and finally separated from these particles making them ready for PCR amplification and detection.

The Master Mix reagent contains primer pairs and probes specific for the CT cryptic plasmid DNA, the CT genomic *ompA* gene DNA, NG genomic DNA sequences A and B within the DR-9 region, and CT and NG Internal Control DNA.

PCR Amplification

Target Selection

In addition to chromosomal DNA, *C. trachomatis* contains an approximately 7,500 base pair cryptic plasmid that is common to all serovars of *C. trachomatis*.[9,10] The **cobas**® CT/NG v2.0 Test uses the CT primers CP102 and CP103 to define a sequence of approximately 206 nucleotides within the cryptic plasmid DNA of *C. trachomatis*. In addition, the **cobas**® CT/NG v2.0 Test uses the CT primers CTMP101 and CTMP102 to define a sequence of approximately 182 nucleotides within the chromosomal DNA of *C. trachomatis*.

The *N. gonorrhoeae* target site is a highly conserved direct repeat region called DR-9. The **cobas**® CT/NG v2.0 Test uses the NG primers NG514 and NG519 to define a sequence of approximately 190 nucleotides (DR-9A) from this region. In addition, the **cobas**® CT/NG v2.0 Test uses another set of NG primers, NG552 and NG579, to define a second sequence of approximately 215 nucleotides (DR-9B) from this region.

Target Amplification

Processed samples are added to the amplification mixture in a microwell plate by the **cobas x** 480 instrument. The microwell plate is then transferred to the **cobas z** 480 analyzer, where PCR amplification occurs. The reaction mixture is heated to separate the isolated double-stranded DNA and expose the primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05 DNA polymerase, in the presence of Mn^{2+} and excess dNTPs, extends the annealed primers along the target templates to produce double-stranded DNA. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the target regions of the CT and/or NG DNA and the CT/NG Internal Control DNA. Repetition of this process results in the amplification of DNA between ´the primer target sequences, producing a double-stranded DNA molecule termed an amplicon. The **cobas z** 480 analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the **cobas**® 4800 Software. Amplification occurs only in the specific CT and/or NG targets between their respective primers; the entire CT cryptic plasmid or CT and/or NG genomes are not amplified.

Internal Control Amplification

The CT/NG Internal Control is a combination of two non-infectious recombinant plasmid DNAs, each with primer binding regions identical to those of either the *C. trachomatis* or the *N. gonorrhoeae* genomic target sequences. Both recombinant plasmid DNAs have an identical randomized internal target sequence, and a unique probe binding region that differentiates the CT/NG Internal Control from target amplicon. These features were selected to ensure independent detection of both the CT/NG Internal Control and the *C. trachomatis* and *N. gonorrhoeae* target DNAs. The CT/NG Internal Control Reagent is included in the **cobas**® CT/NG v2.0 Test and is introduced into each sample on the **cobas x** 480 instrument during sample processing.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® CT/NG v2.0 Test by the use of AmpErase® Uracil-N-Glycosylase (UNG) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[11], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase

enzyme is inactive at temperatures above 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. The **cobas**® CT/NG v2.0 Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing CT/NG amplicon per PCR.

Detection of PCR Products in the cobas® CT/NG v2.0 Test

The **cobas**® CT/NG v2.0 Test utilizes real-time[12,13] PCR technology. The use of fluorescent probes enables real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent dyes released during the amplification process. The probes include CT cryptic plasmid, CT *ompA*, NG DR-9A, NG DR-9B and CT/NG Internal Control-specific oligonucleotides, all labeled with a reporter dye and a quencher. When the fluorescent dye-labeled probes are intact, the reporter fluorescence is suppressed by the proximity of the quencher due to Förster-type energy transfer effects. During PCR, the probes hybridize to their respective target sequence and are cleaved by the 5´ to 3´ nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher are separated, quenching no longer occurs, and the fluorescent emission of the reporter dyes increases. The amplification of CT targets, NG targets and the CT/NG Internal Control are measured independently and at different wavelengths. This process is repeated for a designated number of cycles, each cycle increasing the emission intensity of the individual reporter dyes.

REAGENTS

cobas® 4800 System Sample Preparation Kit (P/N: 05235782190)	**c4800 SMPL PREP**	**240 Tests**
MGP (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol		10 x 4.5 mL
EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide		10 x 18 mL
cobas® 4800 System Sample Preparation Kit (P/N: 05235804190)	**c4800 SMPL PREP**	**960 Tests**
MGP (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol		10 x 13.5 mL
EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide		10 x 18 mL
cobas® 4800 System Wash Buffer Kit (P/N: 05235863190)	**c4800 WB**	**240 Tests**
WB (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl		10 x 55 mL
cobas® 4800 System Wash Buffer Kit (P/N: 05235871190)	**c4800 WB**	**960 Tests**
WB (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl		10 x 200 mL

cobas® 4800 System Liquid Cytology Preparation Kit | **c4800 LIQ CYT** | **240 Tests**

(P/N: 05235812190)

PK — 10 x 0.9 mL

(**cobas**® 4800 Proteinase K)

Tris buffer
EDTA
Glycerol
Calcium chloride
Calcium acetate
< 2% Proteinase K

SDS — 10 x 3 mL

(**cobas**® 4800 System SDS Reagent)

Tris buffer
0.2% SDS
0.09% Sodium azide

LYS — 10 x 10 mL

(**cobas**® 4800 System Lysis Buffer)

Tris buffer
37% (w/w) Guanidine HCl
< 5% polydocanol

cobas® 4800 System Liquid Cytology Preparation Kit | **c4800 LIQ CYT** | **960 Tests**

(P/N: 05235839190)

PK — 20 x 1.2 mL

(**cobas**® 4800 Proteinase K)

Tris buffer
EDTA
Glycerol
Calcium chloride
Calcium acetate
< 2% Proteinase K

SDS — 10 x 9 mL

(**cobas**® 4800 System SDS Reagent)

Tris buffer
0.2% SDS
0.09% Sodium azide

LYS — 10 x 36 mL

(**cobas**® 4800 System Lysis Buffer)

Tris buffer
37% (w/w) Guanidine HCl
< 5% polydocanol

cobas® 4800 CT/NG v2.0 Amplification/Detection Kit (P/N: 07102569190)

c4800 CT/NG v2.0 AMP/DET

240 Tests

CT/NG MMX (**cobas®** 4800 CT/NG Master Mix) — 10 x 0.5 mL

Tricine buffer
Potassium acetate
Potassium hydroxide
Glycerol
< 0.01 % dATP, dCTP, dGTP, dUTP
< 0.01 % Upstream and downstream CT and NG primers
< 0.01 % Fluorescent-labeled CT and NG probes
< 0.01 % Fluorescent-labeled Internal Control probes
< 0.01 % Oligonucleotide aptamer
< 0.10 % Z05 DNA polymerase (microbial)
< 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.09% Sodium azide

CT/NG Mn (**cobas®** 4800 CT/NG Manganese Solution) — 10 x 1.5 mL

< 1.0% Manganese acetate
< 0.02% Glacial acetic acid
0.09% Sodium azide

cobas® 4800 CT/NG v2.0 Amplification/Detection Kit (P/N: 07102577190)

c4800 CT/NG v2.0 AMP/DET

960 Tests

CT/NG MMX (**cobas®** 4800 CT/NG Master Mix) — 20 x 1.0 mL

Tricine buffer
Potassium acetate
Potassium hydroxide
Glycerol
< 0.01 % dATP, dCTP, dGTP, dUTP
< 0.01 % Upstream and downstream CT and NG primers
< 0.01 % Fluorescent-labeled CT and NG probes
< 0.01 % Fluorescent-labeled Internal Control probes
< 0.01 % Oligonucleotide aptamer
< 0.10 % Z05 DNA polymerase (microbial)
< 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.09% Sodium azide

CT/NG Mn (**cobas®** 4800 CT/NG Manganese Solution) — 10 x 1.5 mL

< 1.0% Manganese acetate
< 0.02% Glacial acetic acid
0.09% Sodium azide

cobas® 4800 System Control Diluent Kit (P/N: 05235847190)	**c4800 CDIL**	**10 Sets**
CDIL (**cobas®** 4800 Control Diluent) Tris buffer 37% (w/w) Guanidine HCl		10 x 4.3 mL
cobas® 4800 CT/NG Controls Kit (P/N: 05235928190)	**c4800 CT/NG CTLS**	**10 Sets**
CT/NG (+) C (**cobas®** 4800 CT/NG Positive Control) Tris buffer EDTA 0.05% Sodium azide < 0.002% Poly rA RNA (synthetic) < 0.01% Non-infectious plasmid DNA (microbial) containing *C. trachomatis* sequences < 0.01% Non-infectious plasmid DNA (microbial) containing *N. gonorrhoeae* sequences		10 x 0.5 mL
(–) C (**cobas®** 4800 System Negative Control Tris buffer EDTA 0.05% Sodium azide < 0.002% Poly rA RNA (synthetic)		10 x 0.5 mL
CT/NG IC (**cobas®** 4800 CT/NG Internal Control) Tris buffer EDTA 0.05% Sodium azide < 0.002 % Poly rA RNA (synthetic) < 0.01 % Non-infectious plasmid DNA (microbial) containing *C. trachomatis* primer binding sequences and a unique probe binding region < 0.01 % Non-infectious plasmid DNA (microbial) containing *N. gonorrhoeae* primer binding sequences and a unique probe binding region		10 x 0.3 mL

WARNINGS AND PRECAUTIONS

A. **FOR *IN VITRO* DIAGNOSTIC USE**

B. The performance of this test has been established with endocervical swab and vaginal swab specimens collected using the **cobas®** PCR Media Uni Swab Sample Kit and **cobas®** PCR Media Dual Swab Sample Kit, male and female urine collected using the **cobas®** PCR Urine Sample Kit, and cervical specimens collected in PreservCyt® Solution. Assay performance has not been validated for use with other liquid cytology collection media.

C. Do not pipette by mouth.

D. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

E. Avoid microbial and DNA contamination of reagents.

F. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

G. Do not use reagents after their expiration dates.

H. Do not pool reagents.

I. Safety Data Sheets (SDS) are available upon request from your local Roche office.

J. Gloves must be worn and must be changed between handling specimens and **cobas®** 4800 reagents to prevent contamination.

K. Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[14] and in the CLSI Document M29-A3[15].

L. **cobas®** PCR Media (from primary specimen tube) and **CDIL** contain 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.

M. **MGP** contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.

N. **EB**, **SDS**, **CT/NG MMX**, **CT/NG Mn**, **(–) C**, **CT/NG (+) C** and **CT/NG IC** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.

O. 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.

P. All disposable items are for one time use. Do not reuse.

Q. Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or **cobas z** 480 analyzer according to procedures described in the **cobas**® 4800 System - User Assistance.

R. For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System - User Assistance.

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents***.

B. Store the Sample Preparation Kit (**MGP**, **EB**), Liquid Cytology Preparation Kit (**PK**, **SDS**, **LYS)**, CT/NG Amplification/Detection Kit (**CT/NG MMX**, **CT/NG Mn**) and CT/NG Controls Kit (**CT/NG (+) C**, **(–) C** and **CT/NG IC**) at 2-8ºC. These reagents are stable until the expiration date indicated.

C. Store Wash Buffer Kit (**WB)** and Control Diluent Kit (**CDIL**) at 15-25°C. These reagents are stable until the expiration date indicated.

MATERIALS PROVIDED

A. cobas® 4800 System Sample Preparation Kit — **c4800 SMPL PREP** — **240 Tests**
(P/N: 05235782190)

MGP
(**cobas**® 4800 System Magnetic Glass Particles)

EB
(**cobas**® 4800 System Elution Buffer)

B. cobas® 4800 System Sample Preparation Kit — **c4800 SMPL PREP** — **960 Tests**
(P/N: 05235804190)

MGP
(**cobas**® 4800 System Magnetic Glass Particles)

EB
(**cobas**® 4800 System Elution Buffer)

C. cobas® 4800 System Wash Buffer Kit — **c4800 WB** — **240 Tests**
(P/N: 05235863190)

WB
(**cobas**® 4800 System Wash Buffer)

D. cobas® 4800 System Wash Buffer Kit — **c4800 WB** — **960 Tests**
(P/N: 05235871190)

WB
(**cobas**® 4800 System Wash Buffer)

E. cobas® 4800 System Liquid Cytology Preparation Kit — **c4800 LIQ CYT** — **240 Tests**
(P/N: 05235812190)

PK
(**cobas**® 4800 Proteinase K)

SDS
(**cobas**® 4800 System SDS Reagent)

LYS
(**cobas**® 4800 System Lysis Buffer)

F.	**cobas® 4800 System Liquid Cytology Preparation Kit** (P/N: 05235839190) **PK** (**cobas®** 4800 Proteinase K) **SDS** (**cobas®** 4800 System SDS Reagent) **LYS** (**cobas®** 4800 System Lysis Buffer)	**c4800 LIQ CYT**	**960 Tests**
G.	**cobas® 4800 CT/NG v2.0 Amplification/Detection Kit** (P/N: 07102569190) **CT/NG MMX** (**cobas®** 4800 CT/NG Master Mix) **CT/NG Mn** (**cobas®** 4800 CT/NG Mn Solution)	**c4800 CT/NG v2.0 AMP/DET**	**240 Tests**
H.	**cobas® 4800 CT/NG v2.0 Amplification/Detection Kit** (P/N: 07102577190) **CT/NG MMX** (**cobas®** 4800 CT/NG Master Mix) **CT/NG Mn** (**cobas®** 4800 CT/NG Mn Solution)	**c4800 CT/NG v2.0 AMP/DET**	**960 Tests**
I.	**cobas® 4800 CT/NG Controls Kit** (P/N: 05235928190) **CT/NG (+) C** (**cobas®** 4800 CT/NG Positive Control) **(–) C** (**cobas®** 4800 System Negative Control) **CT/NG IC** (**cobas®** 4800 CT/NG Internal Control)	**c4800 CT/NG CTLS**	**10 Sets**
J.	**cobas® 4800 System Control Diluent Kit** (P/N: 05235847190) **CDIL** (**cobas®** 4800 System Control Diluent)	**c4800 CDIL**	**10 Sets**

MATERIALS REQUIRED BUT NOT PROVIDED

Specimen and Reagent Handling

- **cobas**® PCR Media Uni Swab Sample Kit (Roche P/N 07958030190)
- **cobas**® PCR Media Dual Swab Sample Kit (Roche P/N 07958021190)
- **cobas**® PCR Media Disposable Tube Stand Kit (Roche P/N 07958064190) (optional)
- **cobas**® PCR Urine Sample Kit (Roche P/N 05170486190)
- CORE Tips, 1000 µL, rack of 96 (P/N: 04639642001 or Hamilton P/N: 235905)
- 50 mL Reagent Reservoir (P/N: 05232732001)
- 200 mL Reagent Reservoir (P/N: 05232759001)
- **cobas**® 4800 System Extraction (deep well) Plate (P/N: 05232716001)
- **cobas**® 4800 System AD (microwell) Plate 0.3 mL and Sealing Film (P/N: 05232724001)
- Solid waste Bag [Roche P/N: 05530873001 (small) or 04691989001 (large)]
- Hamilton STAR Plastic Chute (Roche P/N: 04639669001)
- Tubes 13 mL Round Base (Roche P/N: 07958048190) for use as secondary sample tubes
- Caps, neutral color (Roche P/N: 07958056190; for recapping post-run specimens in 13 mL Round Base tubes)
- Disposable gloves, powderless

Instrumentation and Software

- The **cobas**® 4800 System includes
  - **cobas x** 480 instrument
  - **cobas z** 480 analyzer
  - **cobas**® 4800 System control unit with System software version 2.2 or higher
  - **cobas**® 4800 System **cobas**® CT/NG v2.0 AP software version 2.1.0 or higher

OPTIONAL EQUIPMENT AND MATERIALS

- Pipettes: capable of delivering 1000 µL
- Aerosol barrier DNase-free tips: capable of delivering 1000 µL
- Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500
- Stand-alone magnetic plate (P/N: 05440777001)
- Vortex Mixer (single tube)
- Multi-tube vortexer (e.g. VWR P/N 58816-116)

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

A. Specimen Collection

Endocervical swab and vaginal swab specimens collected with the **cobas**® PCR Media Uni Swab Sample Kit and **cobas**® PCR Media Dual Swab Sample Kit, male and female urine collected with the **cobas**® PCR Urine Sample Kit, and cervical specimens collected in PreservCyt® Solution have been validated for use with the **cobas**® CT/NG v2.0Test. Follow the instructions for collecting endocervical swab, vaginal swab and urine specimens with the **cobas**® PCR Media Dual Swab Sample Kit, **cobas**® PCR Media Uni Swab Sample Kit and **cobas**® PCR Urine Sample Kit, respectively. Follow the manufacturer's instructions for collecting cervical specimens into PreservCyt® Solution.

B. Specimen Transport

Endocervical swab and vaginal swab specimens collected with the **cobas**® PCR Media Uni Swab Sample Kit and **cobas**® PCR Media Dual Swab Sample Kit, male and female urine collected with the **cobas**® PCR Urine Sample Kit, and cervical specimens collected in PreservCyt® Solution can be transported at 2-30ºC. 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[16].

C. Specimen Storage

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

INSTRUCTIONS FOR USE

NOTE: All reagents except CT/NG MMX and CT/NG Mn must be at ambient temperature prior to loading on the cobas x 480 instrument. The CT/NG MMX and CT/NG Mn may be taken directly from 2-8°C storage as they will equilibrate to ambient temperature on board the cobas x 480 instrument by the time they are used in the process.

NOTE: Specimens in cobas® PCR media and PreservCyt® Solution must be equilibrated to ambient temperature for at least 30 minutes before loading on the cobas® x 480 instrument.

NOTE: If transfer of specimens from their primary collection containers to properly barcoded secondary tubes is required, use pipettors with aerosol-barrier or positive-displacement tips to handle specimens. Exercise care to avoid contamination.

NOTE: Refer to the cobas® 4800 System – User Assistance for detailed operating instructions.

Run Size

The **cobas®** 4800 System is designed to support the **cobas®** CT/NG v2.0 Test with run sizes from 1 to 94 specimens plus controls (up to 96 tests per run). Each **cobas®** 4800 System Sample Preparation Kit, **cobas®** 4800 System Liquid Cytology Preparation Kit, and **cobas®** 4800 System Wash Buffer Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit). The **cobas®** 4800 CT/NG Amplification/Detection Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit); multiple 240 Test Kits can be used to optimize reagent usage for 48 or 72 tests. The **cobas®** 4800 System Control Diluent Kit and the **cobas®** 4800 CT/NG Controls Kit contain reagents sufficient for a total of 10 runs (10 sets per kit). The minimum run size on the **cobas®** 4800 System is 1 specimen plus controls. One replicate of the **cobas®** 4800 System Negative Control [**(–) C**] and one replicate of the **cobas®** 4800 CT/NG Positive Control [**CT/NG (+) C**] are required to perform each test run (see *"Quality Control"* section).

Workflow

NOTE: Although not an optimal use of reagents, a System Sample Preparation 960 Test Kit and System Liquid Cytology 960 Test Kit can be used for a 24 sample run and a CT/NG Amplification/Detection 960 Test Kit can be used for a 24, 48, or 72 sample run.

The **cobas®** CT/NG v2.0 Test can be run using either of two workflows, referred to as "full workflow" or "Recovery workflow" within the **cobas®** 4800 Software.

CT/NG Full Workflow

The "CT/NG full workflow" consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. Refer to the "Performing a Full Workflow" section below and the **cobas®** 4800 System - User Assistance for details.

CT/NG Recovery Workflow

The "CT/NG Recovery workflow" consists of manual PCR plate setup using eluate from the processed deep well plate followed by amplification/detection on the **cobas z** 480 analyzer. Refer to the "Performing a Recovery Workflow" section below and the **cobas®** 4800 System - User Assistance for details.

Specimens

The following specimen types have been validated using the **cobas®** CT/NG v2.0 Test: a) endocervical swab specimens in **cobas®** PCR media collected with the **cobas®** PCR Media Dual Swab sample kit, b) clinician collected and self-collected (in a clinical setting) vaginal swab specimens in **cobas®** PCR media collected with the **cobas®** PCR Media Uni Swab sample kit, c) male and female urine specimens stabilized in **cobas®** PCR media collected in the **cobas®** PCR Urine sample kit and d) cervical specimens collected in PreservCyt® Solution. Endocervical swab, vaginal swab and urine specimens must be in the **cobas®** PCR media tube containers with a proper barcode or aliquoted into a properly barcoded 13 mL round-based tube for processing on the **cobas x** 480 instrument. Cervical specimens must be in the PreservCyt® Solution primary container with a proper barcode or aliquoted into a properly barcoded 13 mL round-based tube for processing on the **cobas x** 480 instrument. Consult the **cobas®** CT/NG v2.0 Test Operator's Manual for proper barcoding procedures and the list of acceptable barcodes for the **cobas®** 4800 System.

Endocervical Swab and Vaginal Swab Specimens:

NOTE: The reagent kits required for processing endocervical swab and vaginal swab specimens on the cobas x 480 instrument include: cobas® 4800 System Sample Preparation Kit, cobas® 4800 System Control Diluent Kit, cobas® 4800 System Wash Buffer Kit, cobas® 4800 CT/NG v2.0 Amplification/Detection Kit and cobas® 4800 CT/NG Controls Kit.

NOTE: Use only the cobas® PCR Media Uni Swab Sample Kit or cobas® PCR Media Dual Swab Sample Kit to collect endocervical swab and vaginal swab specimens for the cobas® CT/NG v2.0 Test. The cobas® CT/NG v2.0 Test has not been validated with other swab collection devices or media types.

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: Endocervical swab and vaginal swab specimens containing a single swab in the cobas® PCR media tube can be directly processed on the cobas® 4800 System. If necessary, the swab may be removed before the specimen tube is loaded onto the instrument (see the cobas® 4800 System – User Assistance for details).

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 cobas x 480 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 two swabs have not been collected according to the instructions in the cobas® PCR Media Uni Swab Sample Kit and cobas® PCR Media Dual Swab Sample Kit 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 stabilized endocervical swab and vaginal swab specimens contain excessive mucus which may induce a pipetting error (e.g. clot or other obstruction) on the cobas x 480 instrument. 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: Endocervical swab and vaginal swab specimens can be assayed twice on the cobas x 480 instrument 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.

The **cobas**® PCR media tube containing the swab specimen can be uncapped and loaded directly onto the **cobas x** 480 instrument or an aliquot of at least **1.0 mL** of the specimen can be delivered into a properly barcoded 13 mL round-based tube and then loaded onto the **cobas x** 480 instrument.

NOTE: Use caution when transferring specimens from primary containers to 13 mL round-based secondary tubes. Mix primary specimens prior to transfer. Change pipet tips for each specimen.

Male and Female Urine Specimens:

NOTE: The reagent kits required for processing urine specimens on the cobas x 480 instrument include: cobas® 4800 System Sample Preparation Kit, cobas® 4800 System Control Diluent Kit, cobas® 4800 System Wash Buffer Kit, cobas® 4800 CT/NG v2.0 Amplification/Detection Kit and cobas® 4800 CT/NG Controls Kit.

NOTE: Use only the cobas® PCR Urine Sample Kit to collect urine specimens for the cobas® CT/NG v2.0 Test. The cobas CT/NG v2.0 Test has not been validated with other urine collection devices or media types.

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: Do not process urine specimens that appear bloody or have a dark brown color.

The **cobas**® PCR media tube containing the urine specimen can be uncapped and loaded directly onto the **cobas x** 480 instrument or an aliquot of at least **1.5 mL** of the specimen can be delivered into a properly barcoded 13 mL round-based tube and then loaded onto the **cobas x** 480 instrument.

NOTE: Use caution when transferring specimens from primary containers to 13 mL round-based secondary tubes. Mix primary specimens prior to transfer. Change pipet tips for each specimen.

A single run may have any combination of endocervical, vaginal and urine specimens and each specimen can be tested for CT or NG or both CT and NG.

Cervical Specimens in PreservCyt® Solution:

NOTE: The reagent kits required for processing cervical specimens on the cobas x 480 instrument include: cobas® 4800 System Sample Preparation Kit, cobas® 4800 System Liquid Cytology Preparation Kit, cobas® 4800 System Wash Buffer Kit, cobas® 4800 CT/NG v2.0 Amplification/Detection Kit and cobas® 4800 CT/NG Controls Kit.

NOTE: The cobas® CT/NG v2.0 Test is validated for cervical specimens collected in PreservCyt® Solution. The cobas® CT/NG v2.0 Test has not been validated for cervical specimens obtained in other media types. Using the cobas® CT/NG v2.0 Test with other media types may lead to false negative, false positive and/or invalid results.

NOTE: The cobas® CT/NG v2.0 Test is validated to test cervical specimens collected in PreservCyt® Solution either prior to cytology processing or after processing with the ThinPrep T2000 Processor only. The cobas® CT/NG v2.0 Test has not been validated for testing after cytology processing with the use of other cell processors. Testing of PreservCyt specimens after processing with devices other than the ThinPrep T2000 Processor may lead to false negative, false positive and/or invalid results.

NOTE: The cobas® 4800 System can process cervical specimens in both primary and secondary containers. When aliquoting specimens from primary containers into barcoded 13 mL round-based tubes for processing on the cobas x 480 instrument, 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 Materials Required but not Provided) should be used to recap these specimens after processing.

NOTE: Use caution when transferring specimens from primary containers to 13 mL round-based secondary tubes. Vortex primary specimens prior to transfer. Change pipetting tips after each specimen.

NOTE: Do not process specimens collected in PreservCyt® Solution that appear bloody or have a dark brown color.

For cervical specimens, the minimum volume required in the PreservCyt® Solution primary containers is **3.0 mL**. When using 13 mL round-based secondary tubes, fill to a minimum volume of **1.0 mL** and a maximum volume of **10 mL**.

A single run of cervical specimens may have any combination of primary or secondary container racks and each specimen can be tested for CT or NG or both CT and NG.

NOTE: Cervical specimens in PreservCyt® Solution cannot be processed with endocervical, vaginal or urine specimens in the same run. To maximize reagent use, run batches of 22 cervical specimen tests per run (plus one cobas® 4800 System negative control and one cobas® 4800 CT/NG positive control) using 240 Test Kits or batches of 94 cervical specimen tests per run (plus one cobas® 4800 System negative control and one cobas® 4800 CT/NG positive control) using 960 Test Kits.

Workflows

Performing a Full Workflow:

A. The **cobas**® CT/NG v2.0 Test may be used for runs of 1 to 94 specimens plus one **cobas**® 4800 System negative control and one **cobas**® 4800 CT/NG positive control.

B. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System - User Assistance.

C. Start a new run by clicking the “New run” button.

D. In the Select test window, select Workflow type “Full” and then select the Test “CT/NG v2.0”.

E. Enter a run name or leave as the default run name, then click “OK” to proceed.

F. Follow the software wizard guide to load specimens.

NOTE: Endocervical, vaginal and urine specimens can be loaded in barcoded primary or secondary tubes in any order.

NOTE: Cervical specimens in PreservCyt® Solution can be processed in racks of primary specimen vials or secondary tubes in the same run. If primary PreservCyt vials are used for processing, vortex each vial prior to loading.

G. Select a Specimen type for each specimen.
- Choose “Swab” for ordering endocervical swabs and vaginal swabs specimens
- Choose “Urine” for ordering urine specimens
- Choose “PC” for ordering PreservCyt specimens

H. Select the Requested result for each specimen.
- Choose Requested result “CT/NG” to report both CT and NG test results.
- Choose Requested result “CT” to report only CT test results.
- Choose Requested result “NG” to report only NG test results.

I. Follow the software wizard guide to load all consumables.

J. Follow the software wizard guide to load all reagents.

NOTE: Controls [CT/NG (+) C, CT/NG IC and (–) C] are not loaded together with specimens. They are loaded onto the reagent carrier during reagent loading. Two positions (A1 and B1) on each of the extraction plate and cobas® 4800 System AD (microwell) Plate are reserved for the CT/NG (+) and (–) controls, respectively.

NOTE: ***The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once the WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.***

NOTE: ***To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial prior to dispensing into the reagent reservoir.***

K. Load the sample preparation reagents (WB, MGP, EB, SDS, LYS) into the barcoded reagent reservoirs using the "scan-scan-pour-place" method:

- Scan the reagent bottle barcode.
- Scan the reagent reservoir barcode.
- Pour the reagent into the reservoir.
- Place the filled reagent reservoir into the designated position on the reagent carrier.

L. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the appropriate reagent reservoir sizes. The reagent reservoir barcodes must face to the right of the carrier.

NOTE: ***Amplification/detection reagents (CT/NG MMX and CT/NG Mn), Controls [CT/NG (+) C, CT/NG IC and (–) C] and Control Diluent (CDIL) are loaded directly onto the reagent carrier and scanned by the cobas x 480 instrument automatically.***

NOTE: ***All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs. The software also verifies that sufficient reagents are loaded on the instrument.***

M. Start sample preparation by clicking on "Start Run".

NOTE: ***Do not open the* cobas *x 480 instrument front or side doors during the run***.

N. After successful completion of sample preparation, click **'Unload' to unload the plate carrier from the **cobas x** 480 instrument.

** The status of sample preparation can be reviewed at this point, prior to clicking "Unload". See the **cobas®** 4800 System - User Assistance.

O. Follow the instructions in the **cobas®** 4800 System - User Assistance to seal the microwell plate, transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

NOTE: ***The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to working master mix. Amplification and detection should be started as soon as possible but no later than 90 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.***

P. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.

Q. Follow the instructions in the **cobas®** 4800 System - User Assistance to review and accept results.

Performing a Recovery Workflow

NOTE: ***The Recovery Workflow is available as a recovery option in the event that the full workflow cannot be completed due to circumstances beyond the user's control (e.g. power failure during amplification/detection run).***

NOTE: ***Only samples successfully processed on the cobas x 480 instrument can be amplified/detected using the Recovery Workflow. System surveillance for reagents and consumables is limited during the Recovery Workflow. No sample position tracking is provided when using the Recovery Workflow – the end user must ensure that the actual position of a sample on the microwell plate corresponds to the one designated in the Recovery Plate Layout Report. Extreme care must be exercised while preparing the microwell plate to ensure proper PCR set-up and to avoid contamination.***

NOTE: ***Samples processed on the cobas x 480 instrument have limited stability. They must be amplified/detected using the Recovery Workflow within 24 hours if stored at 2°C to 30°C.***

A. Start a Recovery run by clicking the "New run" button.

B. In the Select test window, select the Workflow type "Recovery" then select the Test "CT/NG v2.0".

C. Enter a run name or leave as the default run name, then click "OK" to proceed.

D. Select a run to recover.

E. If using CT/NG v2.0 AP software version 2.2 or higher, scan the original DWP ID from the full workflow.

F. Enter the new MWP ID.

G. Enter the Master Mix and Metal Ions IDs for all Amplification/Detection reagent vials in the kit.

H. Prepare the **cobas®** 4800 CT/NG working master mix:

1. For a 240 Test Kit, add 240 µL of **CT/NG Mn** to one vial of **CT/NG MMX** (0.5 mL vial from 240 Test Kit).
2. For a 960 Test Kit, add 450 µL of **CT/NG Mn** to each of two vials of **CT/NG MMX** (1.0 mL vials from 960 Test Kit).

NOTE: ***The Recovery run must be started within 90 minutes of addition of CT/NG Mn to the CT/NG MMX. The system does not monitor the length of time after addition of the prepared samples to working master mix in the Recovery workflow. The end user must ensure that amplification and detection is started within the allotted time.***

I. Thoroughly mix working master mix by carefully inverting the vial(s). Do not vortex the working master mix.

J. Transfer 25 µL of working master mix to each of the required wells in the microwell plate.

K. Place the extraction plate from the run to be repeated onto the stand-alone magnetic plate.

L. Manually transfer 25 µL of eluate from the extraction plate wells to the corresponding wells in the microwell plate. Ensure that well positions are maintained (e.g. eluate in A1 well in extraction plate is transferred to A1 on the microwell plate). Ensure that no MGP is carried over to the microwell plate.

M. Follow the instructions in the **cobas**® 4800 System - User Assistance to seal the microwell plate.

N. Centrifuge the microwell plate using a swinging bucket rotor for at least 5 seconds at 1500 RCF.

O. Transfer the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

P. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.

Q. Follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.

Quality Control

One set of **cobas**® CT/NG v2.0 Test Positive and Negative Controls are included in each run. For any run, valid results must be obtained for both the Positive and Negative Control for the **cobas**® 4800 Software to display the reportable **cobas**® CT/NG v2.0 Test results from that run. Users may choose to include external quality control testing in conformance with local, state, and federal regulations or accreditation organizations and their laboratory's standard quality control procedures.

Positive Control

The CT/NG (+) Control result must be 'Valid'. The CT/NG (+) Control contains non-infectious DNA plasmids of both *C. trachomatis* and *N. gonorrhoeae* sequences. The CT/NG (+) Control is used as a run control to monitor the target capture, amplification, and detection steps of the test. If the CT/NG (+) Control results are consistently invalid, contact your local Roche office for technical assistance.

Negative Control

The (–) Control result must be 'Valid'. If the (–) Control results are consistently invalid, contact your local Roche office for technical assistance.

Internal Control

The CT/NG Internal Control is a combination of two non-infectious recombinant plasmid DNAs, each with primer binding regions identical to those of either the *C. trachomatis* or the *N. gonorrhoeae* genomic target sequences. The Internal Control is added to all specimens and the Positive and Negative Controls during sample preparation on the **cobas x** 480 instrument. The Internal Control confirms the reliability of test specimens by monitoring for the presence of PCR inhibitors. The Internal Control is also required for validation of the run controls.

Interpretation of Results

NOTE: All assay and run validation is determined by the cobas® 4800 Software.

NOTE: A valid run may include both valid and invalid specimen results.

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

Table 1
Result Interpretation of the cobas® CT/NG v2.0 Test

cobas® CT/NG v2.0 Test Result (displayed in Test Report)	**Interpretation**
Requested Result "CT/NG":	
POS CT, POS NG	**CT Positive, NG Positive.** Specimen is positive for the presence of both CT and NG DNA.
NEG CT, NEG NG	**CT Negative*, NG Negative*.** Neither CT nor NG DNA, if present, could be detected.
POS CT, NEG NG	**CT Positive, NG Negative*.** Specimen is positive for the presence of CT DNA. NG DNA, if present, could not be detected.
POS CT, Invalid NG	**CT Positive, NG Invalid.** Specimen is positive for the presence of CT DNA. NG result is Invalid. Original specimen should be re-tested no more than two times to obtain valid NG results. If the results are still invalid a new specimen should be obtained.
NEG CT, POS NG	**CT Negative*, NG Positive.** CT DNA, if present, could not be detected. Specimen is positive for the presence of NG DNA.
Invalid CT, POS NG	**CT Invalid, NG Positive.** CT result is Invalid. Original specimen should be re-tested no more than two times to obtain valid CT results. If the results are still invalid a new specimen should be obtained. Specimen is positive for the presence of NG DNA.
Invalid CT, NEG NG	**CT Invalid, NG Negative*.** CT result is Invalid. Original specimen should be re-tested no more than two times to obtain valid CT results. If the results are still invalid a new specimen should be obtained. NG DNA, if present, could not be detected.
NEG CT, Invalid NG	**CT Negative*, NG Invalid.** CT DNA, if present, could not be detected. NG result is Invalid. Original specimen should be re-tested no more than two times to obtain valid NG results. If the results are still invalid a new specimen should be obtained.
Invalid**	**CT Invalid, NG Invalid.** Both CT and NG results are Invalid. Original specimen should be re-tested no more than two times to obtain valid CT and NG results. If the results are still invalid a new specimen should be obtained.

cobas® CT/NG v2.0 Test Result (displayed in Test Report)	**Interpretation**
Failed***	**No Result for Specimen** Consult the **cobas®** 4800 System - User Assistance for instructions to review run flags and recommended actions. Original specimen should be re-tested no more than two times to obtain valid CT and NG results. If the results are still invalid a new specimen should be obtained.
Requested Result "CT":	
POS CT	**CT Positive.** Specimen is positive for the presence of CT DNA.
NEG CT	**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 results. If the results are still invalid a new specimen should be obtained.
Failed***	**No Result for Specimen** Consult the **cobas®** 4800 System - User Assistance for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid CT results.
Requested Result "NG":	
POS NG	**NG Positive.** Specimen is positive for the presence of NG DNA.
NEG NG	**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 results. If the results are still invalid a new specimen should be obtained.
Failed***	**No Result for Specimen** Consult the **cobas®** 4800 System - User Assistance for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid NG results.

* 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.

** Tests are classified as "Invalid" when the Positive Control, Negative Control, or Internal Control is not within their specified ranges.

*** Tests are classified as "Failed" when a specimen does not complete the sample preparation or PCR cycling process.

LIST OF RESULT FLAGS

The following table lists flags which are relevant for result interpretation.

Table 2
List of flags for cobas® CT/NG v2.0 Test

Flag code	Description	Recommended action
R20	Positive control is invalid.	Positive control values were invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R21	Negative control is invalid.	Negative control values were invalid. To avoid carryover, use Good Laboratory Practice. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
X3	Error: Clot was detected Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. If a collection device is present, remove it from the sample tube. Recap and vortex. 3. Rerun the sample.

Flag code	Description	Recommended action
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. If a collection device is present, remove it from the sample tube. 3. Check whether the tip eject plate is placed correctly. 4. Rerun the sample.

PROCEDURAL PRECAUTIONS

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

1. The **cobas**® CT/NG v2.0 Test has only been validated for use with male and female urine, 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.) and cervical specimens collected in PreservCyt® Solution. Assay performance has not been validated for use with other liquid cytology collection media. The performance of the **cobas**® CT/NG v2.0 Test has not been evaluated with PreservCyt® specimens processed with instruments other than the ThinPrep 2000 processor.
2. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
3. The **cobas**® CT/NG v2.0 Test is not intended to replace other exams or tests for diagnosis of urogenital infection. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.
4. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
5. The predictive value of an assay depends on the prevalence of the disease in any particular population. See Tables 3 and 4 for hypothetical predictive values when testing varied populations.
6. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Package Insert, Package Inserts for the **cobas**® PCR Media collection kits and the **cobas**® 4800 System - User Assistance.
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.
8. The **cobas**® CT/NG v2.0 Test is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications.
9. The **cobas**® CT/NG v2.0 Test should not be used to determine therapeutic success as nucleic acids may be present after antimicrobial therapy.
10. The **cobas**® CT/NG v2.0 Test for urine testing is recommended to be performed on first catch urine specimens (defined as the first 10 to 50 mL of the urine stream). The effects of other variables such as first-catch vs. mid-stream, post-douching, etc. have not been evaluated.
11. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
12. The **cobas**® CT/NG v2.0 Test has not been evaluated with patients who were currently being treated with antimicrobial agents active against CT or NG as well as patients with a history of hysterectomy.
13. Though rare, mutations within the highly conserved regions of the cryptic plasmid or genomic DNA of *C. trachomatis* or the genomic DNA of *N. gonorrhoeae* covered by the **cobas**® CT/NG v2.0 Test's primers and/or probes may result in failure to detect the presence of the bacterium.
14. 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.
15. False negative or invalid results may occur due to polymerase inhibition. The CT/NG Internal Control is included in the **cobas**® CT/NG v2.0 Test to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
16. Interfering substances include, but are not limited to the following:
    - The presence of mucus in endocervical and cervical specimens may inhibit PCR and cause false negative test results. Mucus free specimens are required for optimal test performance. Use the additional swab from the **cobas**® PCR Media Dual Swab Sample Kit to remove cervical secretions and discharge before obtaining the specimen.
    - Urine specimens stabilized in **cobas**® PCR Media containing greater than 0.25% (v/v) whole blood may give false negative results.
    - Endocervical swab specimens and vaginal swab specimens, each containing up to 10% (v/v) whole blood exhibited no interference effects. Whole blood levels above 10% (v/v) may give invalid or false negative results.
    - Cervical specimens in PreservCyt® Solution containing up to 3% (v/v) whole blood exhibited no interference effects. Whole blood levels above 3% (v/v) may give invalid or false negative results.
    - Urine specimens stabilized in **cobas**® PCR Media containing greater than 1 x 10^5 Peripheral Blood Mononuclear Cells/mL (PBMC), endocervical and vaginal swab specimens collected in **cobas**® PCR Media containing greater than 1 x 10^6 PBMC cells/mL, and cervical specimens collected in PreservCyt® solution containing greater than 1 x 10^7 PBMC cells/mL may give invalid or false negative results.

- Urine specimens stabilized in **cobas**® PCR Media containing Metronidazole Vaginal Gel, Replens®, RepHresh™ Odor Eliminating Vaginal Gel, RepHresh™ Clean Balance and Vagisil Satin products may give invalid or false negative results.
- Endocervical specimens containing Replens®, RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance product may give invalid or false negative results.
- See Interference results (Tables 28 and 29) for further details.

17. The addition of AmpErase enzyme into the **cobas**® 4800 CT/NG Master Mix 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.
18. The **cobas**® CT/NG v2.0 Test has not been validated for use with vaginal swab specimens collected by patients at home. The patient-collected vaginal swab specimen application is limited to health care facilities where support/counseling is available to explain the procedures and precautions.
19. The **cobas**® CT/NG v2.0 Test has not been evaluated in patients younger than 14 years of age.

PERFORMANCE CHARACTERISTICS

Clinical Performance

The clinical performance characteristics of the **cobas**® CT/NG v2.0 Test were established in three multi-center clinical investigations conducted in the United States. One study evaluated the reproducibility at one internal and two external testing sites and two studies evaluated the sensitivity, specificity, and predictive values of the **cobas**® CT/NG v2.0 Test on clinical specimens tested at 7 external testing sites.

One clinical investigation used archived endocervical specimens, self-collected and clinician collected vaginal specimens, endocervical specimens in PreservCyt® Solution and male and female urine specimens, from symptomatic and asymptomatic males and females, collected during the clinical evaluation of the **cobas**® CT/NG Test (archived samples). A second investigation was performed using prospectively collected endocervical specimens, clinician-collected vaginal specimens, female urine specimens, and cervical specimens in PreservCyt® Solution from asymptomatic women (prospective fresh samples). Specimen collection for these studies took place at 18 collection sites in the US, which included family planning and Obstetrics/Gynecology (OB/GYN) clinics, and sexually transmitted disease clinics.

At collection sites, each female subject provided a urine specimen, a self-collected or clinician-collected vaginal swab specimen, a clinician-collected endocervical swab specimen, and a cervical specimen in PreservCyt® Solution obtained with a spatula/cytobrush or a broom. For determination of Patient Infected Status (PIS) an aliquot of each urine, aliquots of each cervical specimen in PreservCyt® Solution, and an additional endocervical specimen, were collected in the appropriate transport media for two commercially available nucleic acid amplification tests (NAAT).

Male subjects provided a urine specimen in **cobas**® PCR Media and for the determination of PIS, urine and urethral swab specimens in collection media from two commercially available NAAT.

Subjects were classified as symptomatic if they reported symptoms indicative of CT or NG infection, as listed below.

- Dysuria/pain during urination, coital pain/difficulty/bleeding, discharge, or pelvic pain
- Abnormal vaginal discharge
- Pelvic/uterine/ovarian pain
- Urethral discharge, testicular pain/scrotal pain/swelling

Subjects were classified as asymptomatic if they did not report these symptoms.

Samples were tested for CT and NG using the **cobas**® CT/NG v2.0 Test and two commercially available nucleic acid amplification tests (NAAT). Testing with all devices followed the manufacturers' instructions.

The clinical performance of the **cobas**® CT/NG v2.0 Test was evaluated by comparing the results from collected sample types to a pre-specified PIS (Patient Infected Status) algorithm as determined by combined results from 2 commercially available nucleic acid amplification tests. The PIS algorithms for Female and Male patients are shown in Table 3A and Table 3B, respectively.

Table 3A
Determination of Female Patient Infected Status

NAAT1 Urine/Endocervical	NAAT2 Urine/Endocervical	NAAT2 Cervical Swab in PreservCyt® Solution	Patient Infected Status (PIS)
+/+	+/+	+ or -	Infected
+/+	+/- or -/+	+ or -	Infected
+/- or -/+	+/+	+ or -	Infected
+/-	-/+	+ or -	Infected
-/+	+/-	+ or -	Infected
-/+	-/+	+ or -	Infected
+/-	+/-	+	Infected
+/-	+/-	-	**CT:** Infected (Urine) Non-Infected (Swabs) **NG**: Infected (Urine and Swabs)
+/- or -/+	-/-	+ or -	Non-Infected
+/+	-/-	+ or -	Non-Infected
-/-	+/+	+ or -	Non-Infected
-/-	+/- or -/+	+ or -	Non-Infected
-/-	-/-	+ or -	Non-Infected

Table 3B
Determination of Male Patient Infected Status

NAAT1 Urethral Swab/Urine	NAAT2 Urethral Swab/Urine	Patient Infected Status (PIS)
+/+	+/+	Infected
+/+	+/- or -/+	Infected
+/- or -/+	+/+	Infected
+/-	-/+	Infected
-/+	+/-	Infected
-/+	-/+	Infected
+/-	+/-	Infected
+/- or -/+	-/-	Non-Infected
+/+	-/-	Non-Infected
-/-	+/+	Non-Infected
-/-	+/- or -/+	Non-Infected
-/-	-/-	Non-Infected

For the primary objective, sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV) of the **cobas**® CT/NG v2.0 Test were calculated separately for detection of CT or NG using PIS as the reference standard and evaluated by gender, sample type, and symptom status. In addition, the predictive values were calculated based on sensitivity and specificity with all data combined for a range of hypothetical prevalence values.

Results

Of the 6,045 subjects enrolled (5,306 females and 739 males), 10 were excluded from the analyses because they did not meet study entry criteria or because they withdrew consent; 31 were considered non-evaluable and were excluded from all statistical analyses because of errors in specimen collection, transport, and storage; unknown PIS for both CT and NG; or invalid **cobas**® CT/NG v2.0 Test results after initial testing and/or retesting. Therefore, of 6,035 total eligible subjects enrolled, 6,004 (99.5%) were evaluable for CT and/or NG primary analyses (5,266 females and 738 males). Results obtained from 1,011 prospective female asymptomatic subjects were analyzed combined with results obtained in the re-testing of all available samples (archived specimens) collected in the previous clinical study (4,255 females and 738 males).

In the clinical study, there was 1/385 (0.3%) invalid runs and 15/385 (3.9%) failed runs due to instrument errors. Of the 26,283 specimens tested with the **cobas**® CT/NG v2.0 Test, 0.28% and 0.23% were initially invalid for CT and NG respectively and 1.38% initially had failed results for both CT and NG. Following retesting up to two more times, 0.10% and 0.13% had final results of invalid for CT and NG respectively and 0.14% had final results of failed for both CT and NG.

Chlamydia trachomatis (CT)

Table 4 and Table 5 summarize the results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT (females and males, respectively) according to the PIS algorithm. A total of 365 females and 122 males were infected with CT. Symptoms were reported in 44.4% (162/365) of infected and 37.0% (1,814/4,900) of non-infected women. Similarly, symptoms were reported in 57.4% (70/122) of infected and 33.8% (208/616) of non-infected men. Overall, the CT prevalence was 6.9% (365/5,265), 16.5% (122/738), and 8.1% (487/6,003) in women, men, and the entire study population, respectively.

Table 4

CT: Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	+	+	+	+	+	+	+	+	+	+	113	144	257
Infected	+	+	+	+	+	NA	+	+	+	+	3	7	10
Infected	+	-	+	-	+	+	-	+	+	+	3	2	5
Infected	-	+	-	+	-	-	+	-	-	-	2	3	5
Infected	+	+	+	+	+	+	+	+	+	NA	3	1	4
Infected	+	+	+	-	+	+	+	+	+	+	2	2	4
Infected	+	-	+	+	+	+	+	+	+	+	4	0	4
Infected	+	+	+	+	+	+	+	+	-	+	2	1	3
Infected	+	-	+	+	+	+	-	+	+	+	1	2	3
Infected	+	+	+	+	+	+	+	+	-	-	1	1	2
Infected	+	+	+	+	+	+	+	+	NA	NA	1	1	2
Infected	+	+	+	+	+	+	-	+	+	+	1	1	2
Infected	+	+	+	+	+	NA	+	NA	+	+	0	2	2
Infected	+	+	+	+	NA	+	+	+	NA	NA	1	1	2
Infected	+	-	+	-	-	+	-	-	-	-	0	2	2
Infected	-	+	+	+	+	+	+	+	+	+	0	2	2
Infected	-	+	+	+	+	-	+	+	-	-	1	1	2
Infected	-	+	+	+	-	-	+	+	-	-	2	0	2
Infected	-	+	-	+	-	-	+	+	-	-	1	1	2
Infected	+	+	+	+	+	+	+	+	NA	+	0	1	1
Infected	+	+	+	+	+	+	+	NA	+	+	1	0	1
Infected	+	+	+	+	+	+	-	+	+	-	0	1	1
Infected	+	+	+	+	+	+	NA	+	+	+	1	0	1
Infected	+	+	+	+	+	-	+	+	+	+	1	0	1
Infected	+	+	+	+	+	-	+	+	+	-	0	1	1
Infected	+	+	+	+	+	-	+	+	-	-	0	1	1
Infected	+	+	+	+	-	+	+	+	-	+	0	1	1
Infected	+	+	+	+	NA	NA	+	+	NA	NA	1	0	1
Infected	+	+	-	+	+	+	+	+	+	+	0	1	1
Infected	+	+	-	+	+	-	+	+	+	+	1	0	1
Infected	+	+	-	+	+	-	+	+	+	-	0	1	1
Infected	+	+	-	+	+	-	+	+	-	-	0	1	1
Infected	+	+	-	+	-	+	+	+	+	NA	1	0	1
Infected	+	+	NA	+	NA	NA	NA	NA	NA	+	0	1	1
Infected	+	+	+	-	+	+	+	+	NA	+	1	0	1
Infected	+	+	+	-	+	+	+	-	+	+	0	1	1

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Infected	+	+	+	-	+	+	-	+	+	+	0	1	1
Infected	+	+	+	-	+	-	+	+	-	-	0	1	1
Infected	+	+	+	-	+	NA	+	+	+	+	0	1	1
Infected	+	+	+	-	-	+	+	+	-	-	0	1	1
Infected	+	+	+	-	-	-	+	NA	-	NA	0	1	1
Infected	+	+	+	NA	+	+	+	+	+	+	1	0	1
Infected	+	-	+	+	+	+	+	+	+	-	0	1	1
Infected	+	-	+	+	+	+	+	+	+	NA	1	0	1
Infected	+	-	+	+	+	+	+	-	+	+	0	1	1
Infected	+	-	+	+	+	+	-	-	+	+	1	0	1
Infected	+	-	+	+	+	NA	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	+	+	+	+	0	1	1
Infected	+	-	+	-	+	+	-	+	+	-	0	1	1
Infected	+	-	+	-	+	+	-	-	+	+	1	0	1
Infected	+	-	+	-	+	-	-	+	-	-	0	1	1
Infected	+	-	+	-	-	+	-	+	+	+	0	1	1
Infected	+	-	+	-	-	-	-	NA	-	-	0	1	1
Infected	+	-	+	-	NA	+	+	+	-	-	0	1	1
Infected	+	-	+	NA	+	+	-	-	-	-	1	0	1
Infected	+	NA	+	NA	+	+	NA	+	NA	NA	1	0	1
Infected	-	+	+	+	+	+	+	+	-	+	1	0	1
Infected	-	+	+	+	+	-	+	+	+	+	1	0	1
Infected	-	+	+	+	+	-	+	+	-	+	0	1	1
Infected	-	+	+	+	+	-	+	-	-	-	1	0	1
Infected	-	+	+	+	-	-	+	+	+	-	0	1	1
Infected	-	+	+	+	-	-	-	+	+	-	1	0	1
Infected	-	+	-	+	+	+	+	+	+	-	1	0	1
Infected	-	+	-	+	-	+	+	+	-	+	0	1	1
Infected	-	+	-	+	-	-	+	+	+	+	1	0	1
Infected	-	+	-	+	-	-	+	+	-	+	1	0	1
Infected	-	+	-	+	NA	-	+	-	NA	NA	0	1	1
Infected	-	+	NA	+	-	+	+	+	-	-	0	1	1
Infected	-	+	+	-	-	-	-	+	-	-	0	1	1
Total Infected											**162**	**203**	**365**

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Non-Infected	-	-	-	-	-	-	-	-	-	-	1575	2561	4136
Non-Infected	-	-	-	-	-	-	-	-	-	NA	57	178	235
Non-Infected	-	-	-	-	-	NA	-	-	-	-	27	30	57
Non-Infected	-	-	-	-	-	-	-	NA	-	-	26	27	53
Non-Infected	-	-	-	-	-	-	NA	-	-	-	23	26	49
Non-Infected	-	-	-	-	NA	-	-	-	NA	NA	17	28	45
Non-Infected	-	-	-	-	-	-	NA	-	-	NA	3	37	40
Non-Infected	-	-	-	-	-	-	-	-	NA	-	5	32	37
Non-Infected	-	-	-	-	-	-	-	-	NA	NA	10	18	28
Non-Infected	-	-	-	-	-	NA	NA	-	-	-	1	18	19
Non-Infected	NA	NA	-	-	-	NA	-	-	-	-	0	16	16
Non-Infected	-	-	-	-	-	NA	-	NA	-	-	2	13	15
Non-Infected	-	-	+	-	-	-	-	-	-	-	8	6	14
Non-Infected	-	-	-	-	-	NA	-	-	-	NA	1	12	13
Non-Infected	-	-	-	-	-	-	-	+	-	-	7	5	12
Non-Infected	-	-	-	-	-	-	+	-	-	-	4	7	11
Non-Infected	-	-	-	-	-	NA	NA	-	-	NA	1	9	10
Non-Infected	NA	-	-	-	-	-	-	-	-	-	3	7	10
Non-Infected	-	-	-	-	-	+	-	-	-	-	7	2	9
Non-Infected	+	-	-	-	-	-	-	-	-	-	2	5	7
Non-Infected	-	-	-	+	-	-	-	-	-	-	4	3	7
Non-Infected	-	-	-	-	-	-	-	-	-	+	1	6	7
Non-Infected	-	-	-	-	+	-	-	-	-	-	1	5	6
Non-Infected	-	-	-	-	-	NA	NA	NA	-	-	3	3	6
Non-Infected	-	-	-	-	-	-	-	-	+	-	2	3	5
Non-Infected	-	+	-	-	-	-	-	-	-	-	0	4	4
Non-Infected	-	-	-	-	-	-	NA	-	NA	NA	1	2	3
Non-Infected	-	-	-	NA	-	-	-	-	-	-	2	1	3
Non-Infected	-	-	-	-	+	-	-	-	+	-	0	2	2
Non-Infected	-	-	NA	-	-	-	-	-	-	-	0	2	2
Non-Infected	-	NA	-	-	-	-	-	-	-	-	1	1	2
Non-Infected	NA	NA	-	-	-	NA	-	-	NA	-	0	2	2
Non-Infected	+	+	-	-	-	+	-	+	-	+	1	0	1
Non-Infected	+	-	-	-	+	+	-	+	+	+	1	0	1
Non-Infected	+	-	-	-	+	-	-	+	+	+	1	0	1
Non-Infected	+	-	-	-	-	+	-	+	-	-	0	1	1
Non-Infected	+	-	-	-	-	+	-	-	-	-	1	0	1
Non-Infected	-	+	-	-	-	-	NA	-	-	-	1	0	1
Non-Infected	-	-	+	+	+	+	+	+	+	+	0	1	1
Non-Infected	-	-	+	+	-	+	+	+	-	-	1	0	1
Non-Infected	-	-	+	+	-	-	-	+	-	-	1	0	1
Non-Infected	-	-	+	+	-	-	-	-	+	-	0	1	1
Non-Infected	-	-	-	+	+	-	+	+	+	-	0	1	1
Non-Infected	-	-	-	+	-	-	+	-	-	-	1	0	1

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		Total
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	
Non-Infected	-	-	+	-	+	-	-	+	+	+	0	1	1
Non-Infected	-	-	+	-	-	-	-	+	-	-	1	0	1
Non-Infected	-	-	+	-	-	-	-	-	-	+	1	0	1
Non-Infected	-	-	+	-	-	-	NA	NA	-	-	1	0	1
Non-Infected	-	-	-	-	+	+	-	-	-	+	1	0	1
Non-Infected	-	-	-	-	+	-	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	+	-	-	NA	-	-	0	1	1
Non-Infected	-	-	-	-	-	+	+	-	-	-	0	1	1
Non-Infected	-	-	-	-	-	+	-	+	-	-	1	0	1
Non-Infected	-	-	-	-	-	-	-	+	+	-	0	1	1
Non-Infected	-	-	-	-	-	-	-	+	-	NA	1	0	1
Non-Infected	-	-	-	-	-	-	-	NA	-	+	0	1	1
Non-Infected	-	-	-	-	-	-	-	NA	-	NA	0	1	1
Non-Infected	-	-	-	-	-	-	NA	-	+	NA	0	1	1
Non-Infected	-	-	-	-	-	-	NA	NA	-	-	0	1	1
Non-Infected	-	-	-	-	-	NA	-	NA	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	NA	-	NA	1	0	1
Non-Infected	-	-	NA	-	NA	-	-	-	NA	NA	1	0	1
Non-Infected	-	-	-	NA	NA	NA	NA	NA	-	-	1	0	1
Non-Infected	-	NA	-	-	-	-	-	-	-	NA	1	0	1
Non-Infected	NA	NA	-	-	-	-	-	-	-	-	0	1	1
Total Non-Infected											**1814**	**3086**	**4900**

[a] NAAT1 and NAAT2 = Commercially available CT/NG NAAT assays [b] Symp = symptomatic, Asymp = asymptomatic.

Note: Subjects are designated as being infected with CT if at least 2 predicate NAATs with different target regions give positive results in the endocervical swab and/or the urine specimen. However, females are categorized as non-infected for any swab specimen if the swab specimens and the PreservCyt specimen (NAAT2) were negative and the urine specimens were positive.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: + denotes Positive; - denotes Negative; NA indicates specimen was not obtained or available for testing.

Note: SW = endocervical swab, UR = urine, VG = vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).

Table 5

CT: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]		cobas® CT/NG v2.0 Test	Symptom Status[b]		Total
	SW	UR	SW	UR	UR	Symp	Asymp	
Infected	+	+	+	+	+	64	43	107
Infected	-	+	-	+	+	3	3	6
Infected	-	+	+	+	+	0	3	3
Infected	+	+	+	-	+	1	1	2
Infected	+	-	+	-	-	0	1	1
Infected	+	-	+	+	+	0	1	1
Infected	-	+	+	-	+	1	0	1
Infected	-	+	-	+	-	1	0	1
Total Infected						**70**	**52**	**122**
Non-Infected	-	-	-	-	-	203	399	602
Non-Infected	-	-	-	-	+	1	2	3
Non-Infected	-	-	+	-	-	1	1	2
Non-Infected	-	-	-	+	-	1	1	2
Non-Infected	-	-	+	+	-	0	2	2
Non-Infected	-	+	-	-	-	0	2	2
Non-Infected	-	-	+	+	+	0	1	1
Non-Infected	+	-	-	-	-	1	0	1
Non-Infected	+	+	-	-	+	1	0	1
Total Non-Infected						**208**	**408**	**616**

[a] NAAT1 and NAAT2 = Commercially available CT/NG NAAT assays [b] Symp = symptomatic, Asymp = asymptomatic.

Note: Subjects are designated as being infected with CT if at least 2 predicate NAATs with different target regions give positive results in the urethral swab and/or the urine specimen.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: + denotes Positive; - denotes Negative.

Note: SW = urethral swab, UR= urine.

Sensitivity, specificity, and predictive values of the **cobas**® CT/NG v2.0 Test for CT as defined by PIS are presented by gender, sample type, and symptom status in Table 6. Overall Sensitivity ranged from 93.7% to 98.4%. Overall specificity ranged from 98.8% to 99.8% in both females and males. Performance estimates for CT detection were similar between symptomatic and asymptomatic subjects (Table 6).

Table 6

CT: Clinical Performance Compared With Patient Infected Status by Gender and Sample Type, and Symptom Status

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
Female									
SW	Symp[c]	1932	94.7% (144/152)	(90.0%, 97.3%)	99.3% (1767/1780)	(98.8%, 99.6%)	7.9	91.7	99.5
	Asymp[d]	994	95.3% (81/85)	(88.5%, 98.2%)	99.6% (905/909)	(98.9%, 99.8%)	8.6	95.3	99.6
	Overall	2926	94.9% (225/237)	(91.4%, 97.1%)	99.4% (2672/2689)	(99.0%, 99.6%)	8.1	93.0	99.6
UR	Symp[c]	1937	94.4% (151/160)	(89.7%, 97.0%)	99.7% (1771/1777)	(99.3%, 99.8%)	8.3	96.2	99.5
	Asymp[d]	1008	93.3% (84/90)	(86.2%, 96.9%)	99.5% (913/918)	(98.7%, 99.8%)	8.9	94.4	99.3
	Overall	2945	94.0% (235/250)	(90.3%, 96.3%)	99.6% (2684/2695)	(99.3%, 99.8%)	8.5	95.5	99.4
VG-C	Symp[c]	899	96.2% (76/79)	(89.4%, 98.7%)	98.8% (810/820)	(97.8%, 99.3%)	8.8	88.4	99.6
	Asymp[d]	1003	100.0% (89/89)	(95.9%, 100.0%)	99.5% (909/914)	(98.7%, 99.8%)	8.9	94.7	100.0
	Overall	1902	98.2% (165/168)	(94.9%, 99.4%)	99.1% (1719/1734)	(98.6%, 99.5%)	8.8	91.7	99.8
VG-S	Symp[c]	1041	98.7% (76/77)	(93.0%, 99.8%)	99.2% (956/964)	(98.4%, 99.6%)	7.4	90.5	99.9
	Asymp[c]	996	96.0% (48/50)	(86.5%, 98.9%)	99.4% (940/946)	(98.6%, 99.7%)	5.0	88.9	99.8
	Overall	2037	97.6% (124/127)	(93.3%, 99.2%)	99.3% (1896/1910)	(98.8%, 99.6%)	6.2	89.9	99.8
PC Pre	Symp[c]	1935	94.1% (143/152)	(89.1%, 96.9%)	99.7% (1778/1783)	(99.3%, 99.9%)	7.9	96.6	99.5
	Asymp[d]	1002	94.3% (83/88)	(87.4%, 97.5%)	99.8% (912/914)	(99.2%, 99.9%)	8.8	97.6	99.5
	Overall	2937	94.2% (226/240)	(90.4%, 96.5%)	99.7% (2690/2697)	(99.5%, 99.9%)	8.2	97.0	99.5
PC Post	Symp[c]	1871	93.9% (139/148)	(88.8%, 96.8%)	99.5% (1715/1723)	(99.1%, 99.8%)	7.9	94.6	99.5
	Asymp[d]	1007	93.3% (83/89)	(86.1%, 96.9%)	99.5% (913/918)	(98.7%, 99.8%)	8.8	94.3	99.3
	Overall	2878	93.7% (222/237)	(89.8%, 96.1%)	99.5% (2628/2641)	(99.2%, 99.7%)	8.2	94.5	99.4
Male									
UR	Symp[c]	278	98.6% (69/70)	(92.3%, 99.7%)	99.0% (206/208)	(96.6%, 99.7%)	25.2	97.2	99.5
	Asymp[c]	460	98.1% (51/52)	(89.9%, 99.7%)	99.3% (405/408)	(97.9%, 99.7%)	11.3	94.4	99.8
	Overall	738	98.4% (120/122)	(94.2%, 99.5%)	99.2% (611/616)	(98.1%, 99.7%)	16.5	96.0	99.7

[a] SW = endocervical swab, UR = urine, VG-C = clinician-collected vaginal swab, VG-S = self-collected vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).

[b] Symp = symptomatic, Asymp = asymptomatic.

[c] **cobas**® CT/NG v2.0 Test results from archived specimens.

[d] **cobas**® CT/NG v2.0 Test results from prospectively collected specimens.

Note: Subjects are designated as being infected with CT if at least 2 NAATs with different target regions give positive results in the endocervical swab (urethral swab for males) and/or the urine specimen. However, females are categorized as non-infected for any swab specimen if the swab specimens and the PreservCyt specimen (NAAT2) were negative and the urine specimens were positive.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: CI = (score) confidence interval, PREV = prevalence, SENS = sensitivity, SPEC = specificity, PPV = positive predictive value, NPV = negative predictive value.

For archived specimens from female asymptomatic patients, sensitivity for CT was 91.2% (93/102), 92.9% (104/112), 94.4% (51/54), 88.6% (93/105), and 87.5% (91/104), respectively for endocervical swabs, female urine specimens, clinician-collected vaginal swabs, and PreservCyt specimens (pre- and post-ThinPrep processing), with specificity for CT of 99.9% (2,076/2,078), 99.8% (2,065/2,070), 99.9% (1,183/1,184), 99.6% (2,085/2,094), and 99.7% (1,881/1,886) respectively for these sample types.

Neisseria gonorrhoeae (NG)

Table 7 and Table 8 summarize the results from symptomatic and asymptomatic subjects designated as infected or non-infected with NG (females and males, respectively) according to the PIS algorithm. A total of 92 females and 67 males were infected with NG. Symptoms were reported in 46.7% (43/92) of infected and 37.4% (1,932/5,171) of non-infected women. Similarly, symptoms were reported in 89.6% (60/67) of infected and 32.5% (218/671) of non-infected men. Overall, the NG prevalence was 1.7% (92/5,263), 9.1% (67/738), and 2.6% (159/6,001), respectively, in women, men, and the entire study population.

Table 7
NG: Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	Total
Infected	+	+	+	+	+	+	+	+	+	+	29	37	66
Infected	+	+	+	+	+	+	+	NA	+	+	1	2	3
Infected	+	+	+	-	+	+	+	+	+	+	1	2	3
Infected	+	-	+	-	+	+	-	+	+	+	3	0	3
Infected	+	+	+	+	+	NA	+	+	+	+	1	1	2
Infected	+	+	+	+	-	+	+	+	+	+	0	2	2
Infected	+	+	+	+	+	+	+	+	+	-	1	0	1
Infected	+	+	+	+	+	+	+	+	-	-	0	1	1
Infected	+	+	+	+	+	+	+	-	+	+	1	0	1
Infected	+	+	+	+	+	+	-	+	+	+	1	0	1
Infected	+	+	+	+	+	+	NA	+	+	+	1	0	1
Infected	+	+	+	+	+	NA	NA	NA	+	+	0	1	1
Infected	+	+	+	+	-	-	+	+	+	-	1	0	1
Infected	+	-	+	-	+	+	+	+	+	+	1	0	1
Infected	+	-	+	-	-	+	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	+	+	+	+	0	1	1
Infected	-	+	+	+	+	+	+	+	-	NA	0	1	1
Infected	-	+	+	+	+	-	+	+	+	+	1	0	1
Infected	-	+	+	+	+	-	+	+	-	-	0	1	1
Total Infected											**43**	**49**	**92**
Non-Infected	-	-	-	-	-	-	-	-	-	-	1704	2709	4413
Non-Infected	-	-	-	-	-	-	-	-	-	NA	62	177	239
Non-Infected	-	-	-	-	-	NA	-	-	-	-	30	36	66
Non-Infected	-	-	-	-	-	-	-	NA	-	-	26	29	55
Non-Infected	-	-	-	-	-	-	NA	-	-	-	24	25	49
Non-Infected	-	-	-	-	NA	-	-	-	NA	NA	18	30	48
Non-Infected	-	-	-	-	-	-	-	-	NA	-	7	37	44
Non-Infected	-	-	-	-	-	-	NA	-	-	NA	3	38	41
Non-Infected	-	-	-	-	-	-	-	-	NA	NA	10	19	29
Non-Infected	-	-	-	-	-	NA	NA	-	-	-	1	18	19
Non-Infected	-	-	-	-	-	NA	-	NA	-	-	2	15	17
Non-Infected	NA	NA	-	-	-	NA	-	-	-	-	0	16	16
Non-Infected	-	-	-	-	-	-	+	-	-	-	5	9	14
Non-Infected	-	-	-	-	-	NA	-	-	-	NA	1	12	13
Non-Infected	+	-	-	-	-	-	-	-	-	-	5	6	11
Non-Infected	-	-	-	-	-	NA	NA	-	-	NA	1	9	10
Non-Infected	NA	-	-	-	-	-	-	-	-	-	3	7	10
Non-Infected	-	+	-	-	-	-	-	-	-	-	0	9	9
Non-Infected	-	-	-	-	-	-	-	-	-	+	3	4	7
Non-Infected	-	-	-	-	-	NA	NA	NA	-	-	3	2	5

Patient Infected Status	NAAT1[a]		NAAT2[a]			cobas® CT/NG v2.0 Test					Symptom Status[b]		
	SW	UR	SW	UR	PC Pre	SW	UR	VG	PC Pre	PC Post	Symp	Asymp	Total
Non-Infected	-	-	-	+	-	-	-	-	-	-	2	2	4
Non-Infected	-	-	-	-	-	-	-	+	-	-	2	2	4
Non-Infected	-	-	NA	-	-	-	-	-	-	-	2	2	4
Non-Infected	-	-	-	-	+	-	-	-	-	-	1	2	3
Non-Infected	-	-	-	-	-	-	-	-	+	-	1	2	3
Non-Infected	-	-	-	-	-	-	NA	-	NA	NA	1	2	3
Non-Infected	-	-	-	NA	-	-	-	-	-	-	2	1	3
Non-Infected	-	-	+	-	-	-	-	-	-	-	1	1	2
Non-Infected	-	-	-	-	-	-	-	+	-	+	0	2	2
Non-Infected	-	-	-	-	-	-	-	NA	-	NA	0	2	2
Non-Infected	-	-	-	-	-	-	NA	NA	-	-	1	1	2
Non-Infected	-	NA	-	-	-	-	-	-	-	-	1	1	2
Non-Infected	NA	NA	-	-	-	NA	-	-	NA	-	0	2	2
Non-Infected	+	+	-	-	+	+	+	+	+	+	0	1	1
Non-Infected	+	+	-	-	+	NA	+	+	+	+	0	1	1
Non-Infected	+	+	-	-	-	-	-	-	-	-	0	1	1
Non-Infected	+	-	-	-	-	+	NA	-	-	-	0	1	1
Non-Infected	-	+	-	-	-	-	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	+	-	-	-	+	-	0	1	1
Non-Infected	-	-	-	-	-	+	-	-	-	-	1	0	1
Non-Infected	-	-	-	-	-	+	-	-	-	NA	0	1	1
Non-Infected	-	-	-	-	-	+	-	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	-	+	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	-	-	+	-	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	-	-	+	+	1	0	1
Non-Infected	-	-	-	-	-	NA	-	NA	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	-	0	1	1
Non-Infected	-	-	-	-	-	NA	NA	-	NA	NA	1	0	1
Non-Infected	-	-	-	-	-	NA	NA	NA	-	NA	1	0	1
Non-Infected	-	-	-	-	NA	NA	-	-	NA	NA	1	0	1
Non-Infected	-	-	-	NA	NA	NA	NA	NA	-	-	1	0	1
Non-Infected	-	NA	-	-	-	-	-	-	-	NA	1	0	1
Non-Infected	NA	NA	-	-	-	-	-	-	-	-	0	1	1
Total Non-Infected											**1932**	**3239**	**5171**

[a] NAAT1 and NAAT2 = Commercially available CT/NG NAAT assays [b] Symp = symptomatic, Asymp = asymptomatic.

Note: Subjects are designated as being infected with NG if at least 2 predicate NAATs with different target regions give positive results in the endocervical swab and/or the urine specimen.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: + denotes Positive; - denotes Negative; NA indicates specimen was not obtained or available for testing.

Note: SW = endocervical swab, UR = urine, VG = vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).

Table 8

NG: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1[a]		NAAT2[a]		cobas® CT/NG v2.0 Test	Symptom Status[b]		Total
	SW	UR	SW	UR	UR	Symp	Asymp	
Infected	+	+	+	+	+	59	7	66
Infected	+	+	-	+	+	1	0	1
Total Infected						**60**	**7**	**67**
Non-Infected	-	-	-	-	-	213	449	662
Non-Infected	-	-	-	-	+	2	3	5
Non-Infected	-	+	-	-	-	1	1	2
Non-Infected	-	-	+	-	-	1	0	1
Non-Infected	+	-	-	-	-	1	0	1
Total Non-Infected						**218**	**453**	**671**

[a] NAAT1 and NAAT2 = Commercially available CT/NG NAAT assays [b] Symp = symptomatic, Asymp = asymptomatic.

Note: Subjects are designated as being infected with NG if at least 2 predicate NAATs with different target regions give positive results in the urethral swab and/or the urine specimen.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: + denotes Positive; - denotes Negative.

Note: SW = urethral swab, UR= urine.

Sensitivity, specificity, and predictive values of the **cobas**® CT/NG v2.0 Test for NG as defined by PIS are shown by gender, sample type, and symptom status in Table 9. Overall sensitivity ranged from 95.6% to 100.0%. Overall specificity ranged from 99.1% to 100.0% for both females and males. Performance estimates for NG detection were similar between symptomatic and asymptomatic subjects.

Table 9

NG: Clinical Performance Compared With Patient Infected Status by Gender, Sample Type, and Symptom Status

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
Female									
SW	Symp[c]	1930	95.2% (40/42)	(84.2%, 98.7%)	99.9% (1886/1888)	(99.6%, 100.0%)	2.2	95.2	99.9
	Asymp[d]	3174	97.9% (46/47)	(88.9%, 99.6%)	99.9% (3124/3127)	(99.7%, 100.0%)	1.5	93.9	100.0
	Overall	5104	96.6% (86/89)	(90.6%, 98.8%)	99.9% (5010/5015)	(99.8%, 100.0%)	1.7	94.5	99.9
UR	Symp[c]	1937	90.5% (38/42)	(77.9%, 96.2%)	99.7% (1890/1895)	(99.4%, 99.9%)	2.2	88.4	99.8
	Asymp[d]	3190	100.0% (48/48)	(92.6%, 100.0%)	99.6% (3130/3142)	(99.3%, 99.8%)	1.5	80.0	100.0
	Overall	5127	95.6% (86/90)	(89.1%, 98.3%)	99.7% (5020/5037)	(99.5%, 99.8%)	1.8	83.5	99.9
VG-C	Symp[c]	898	100.0% (21/21)	(84.5%, 100.0%)	99.7% (874/877)	(99.0%, 99.9%)	2.3	87.5	100.0
	Asymp[d]	2240	100.0% (37/37)	(90.6%, 100.0%)	99.7% (2197/2203)	(99.4%, 99.9%)	1.7	86.0	100.0
	Overall	3138	100.0% (58/58)	(93.8%, 100.0%)	99.7% (3071/3080)	(99.4%, 99.8%)	1.8	86.6	100.0
VG-S	Symp[c]	1041	95.2% (20/21)	(77.3%, 99.2%)	100.0% (1020/1020)	(99.6%, 100.0%)	2.0	100.0	99.9
	Asymp[c]	996	100.0% (9/9)	(70.1%, 100.0%)	100.0% (987/987)	(99.6%, 100.0%)	0.9	100.0	100.0
	Overall	2037	96.7% (29/30)	(83.3%, 99.4%)	100.0% (2007/2007)	(99.8%, 100.0%)	1.5	100.0	100.0
PC Pre	Symp[c]	1935	100.0% (43/43)	(91.8%, 100.0%)	99.9% (1890/1892)	(99.6%, 100.0%)	2.2	95.6	100.0
	Asymp[d]	3196	93.9% (46/49)	(83.5%, 97.9%)	99.8% (3142/3147)	(99.6%, 99.9%)	1.5	90.2	99.9
	Overall	5131	96.7% (89/92)	(90.8%, 98.9%)	99.9% (5032/5039)	(99.7%, 99.9%)	1.8	92.7	99.9
PC Post	Symp[c]	1872	95.3% (41/43)	(84.5%, 98.7%)	99.8% (1825/1829)	(99.4%, 99.9%)	2.3	91.1	99.9
	Asymp[d]	2996	95.8% (46/48)	(86.0%, 98.8%)	99.7% (2940/2948)	(99.5%, 99.9%)	1.6	85.2	99.9
	Overall	4868	95.6% (87/91)	(89.2%, 98.3%)	99.7% (4765/4777)	(99.6%, 99.9%)	1.9	87.9	99.9
Male									
UR	Symp[c]	278	100.0% (60/60)	(94.0%, 100.0%)	99.1% (216/218)	(96.7%, 99.7%)	21.6	96.8	100.0
	Asymp[c]	460	100.0% (7/7)	(64.6%, 100.0%)	99.3% (450/453)	(98.1%, 99.8%)	1.5	70.0	100.0
	Overall	738	100.0% (67/67)	(94.6%, 100.0%)	99.3% (666/671)	(98.3%, 99.7%)	9.1	93.1	100.0

[a] SW = endocervical swab, UR = urine, VG-C = clinician-collected vaginal swab, VG-S = self-collected vaginal swab, PC Pre = PreservCyt (pre-aliquot), PC Post = PreservCyt (post-aliquot).

[b] Symp = symptomatic, Asymp = asymptomatic.

[c] **cobas**® CT/NG v2.0 Test results from archived specimens.

[d] **cobas**® CT/NG v2.0 Test results from archived and prospectively collected specimens.

Note: Subjects are designated as being infected with NG if at least 2 predicate NAATs with different target regions give positive results in the endocervical swab (urethral swab for males) and/or the urine specimen.

Note: Subjects with designated infection status and valid **cobas**® CT/NG v2.0 Test results are considered evaluable and included in this summary table.

Note: CI = (score) confidence interval, PREV = prevalence, SENS = sensitivity, SPEC = specificity, PPV = positive predictive value, NPV = negative predictive value.

Expected Values

Prevalence

The prevalence of CT and NG in patient populations depends upon a variety of factors including age, gender, the presence of symptoms, clinic type, and test method. The prevalence of CT observed with the **cobas**® CT/NG v2.0 Test during a multi-center clinical trial ranged from 5.0% to 8.9% in females, and from 11.3% to 25.2% in males (Table 6); the prevalence of NG ranged from 0.9% to 2.3% in females, and from 1.5% to 21.6% in males (Table 9).

Positive and Negative Predictive Value

Hypothetical positive and negative predictive values (PPV & NPV) derived from disease prevalence of 1 to 50% for the **cobas**® CT/NG v2.0 Test are shown in Table 10 and Table 11. The overall sensitivity and specificity (compared with PIS) were 94.1% and 99.6% respectively, for CT; and 97.1% and 99.8%, respectively, for NG.

Table10
Positive and Negative Predictive Values for Hypothetical CT Prevalence

Prevalence (%)	Sensitivity* (%)	Specificity* (%)	PPV (%)	NPV (%)
1	94.1	99.6	69.0	99.9
3	94.1	99.6	87.2	99.8
5	94.1	99.6	92.0	99.7
10	94.1	99.6	96.1	99.3
15	94.1	99.6	97.5	99.0
20	94.1	99.6	98.2	98.5
30	94.1	99.6	98.9	97.5
50	94.1	99.6	99.5	94.4

* Overall sensitivity and specificity were estimated by comparing the **cobas**® CT/NG v2.0 Test results to patient infected status across all sample types in both female and male subjects.
Note: PPV = positive predictive value; NPV = negative predictive value.

Table 11
Positive and Negative Predictive Values for Hypothetical NG Prevalence

Prevalence (%)	Sensitivity* (%)	Specificity* (%)	PPV (%)	NPV (%)
1	97.1	99.8	82.0	100.0
3	97.1	99.8	93.3	99.9
5	97.1	99.8	96.0	99.8
10	97.1	99.8	98.0	99.7
15	97.1	99.8	98.8	99.5
20	97.1	99.8	99.1	99.3
30	97.1	99.8	99.5	98.8
50	97.1	99.8	99.8	97.2

* Overall sensitivity and specificity were estimated by comparing the **cobas**® CT/NG v2.0 Test results to patient infected status across all sample types in both female and male subjects.
Note: PPV = positive predictive value; NPV = negative predictive value.

Ct Frequency Distribution

A total of 1853 specimens (combined female and male) were positive for CT and a total of 557 specimens (combined female and male) were positive for NG. The frequency distribution of **cobas**® CT/NG v2.0 Test positive results for CT and NG infected specimens are shown in Figures 1 and 2, respectively.

Figure 1
Ct Distribution of CT Positive Specimens

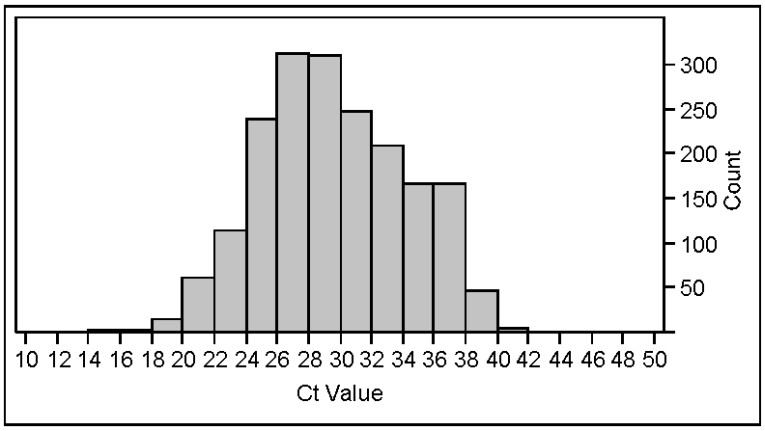
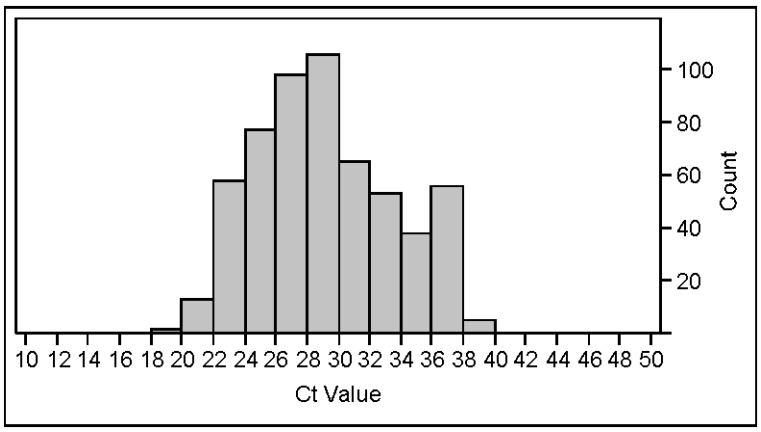


Figure 2
Ct Distribution of NG Positive Specimens



Reproducibility

A Reproducibility Study was performed across testing sites, operators, runs, and days for the **cobas**® CT/NG v2.0 Test using 3 panels prepared from negative vaginal swabs collected in **cobas**® PCR Media, negative urine stabilized in **cobas**® PCR Media and negative cervical specimens collected in PreservCyt® Solution. The 5 panel members for each matrix type contained single target levels of CT and NG at 1 x LOD and 3 x LOD plus one CT/NG negative level. Testing was performed at two external sites and one in-house site. A run for **cobas**® PCR Media (urine and swab) included 3 replicates of each of 5 panel members and 1 positive and 1 negative control (32 total tests). A run for the PreservCyt® Solution panel included 3 replicates of each of 5 panel members and 1 positive and 1 negative control (17 total tests). The 2 operators at each site performed 1 run per day each, for a total of 5 days of testing per operator per panel type (10 days of testing total for each panel type). Testing was performed with 1 reagent lot.

Overall, 127 runs were performed: 62 were for urine and swab panels (which were run together) and 65 were for PreservCyt panels. Sixty valid runs were obtained for each media type. Two failed runs occurred for the urine and swab panels, and 5 failed runs occurred for the PreservCyt panels. Failed runs were attributed to protocol deviations and instrument errors. A total of 900 tests were performed on the 5 panel members for each panel type. There was 1 invalid test result in the PreservCyt panel type, and 1 failed test result each in the swab, urine and PreservCyt panels. These failed tests were due to instrument errors.

All valid test results were included in the analyses of the percent agreement for CT and NG for each panel type separately. There were no false positive results for either analyte (CT and NG) for all panel types for negative panel members, thus giving negative percent agreement (NPA) of 100% for each analyte.

C. trachomatis (Tables 12, 13, 14, 15)

Table 12 below presents the total standard deviation (SD) and total percent coefficient of variation (CV [%]) for each panel type, respectively. Across all panel types, the total CV (%) ranged from 1.4% to 2.6%.

Percent agreement for the CT-positive panel members was 100% for 3 X LOD CT for all 3 panel types and also for the 1 X LOD CT of urine panel type CT Positive/NG Negative column). For the remaining 1 X LOD CT, percent agreement was 96.7% for swab and 98.9% for PreservCyt panel types (Tables 13-15).

Table 12

***C. trachomatis*: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated From Valid Samples of Positive Sample Type Panel Members**

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Site/ Instrument		Total	
Panel Member	n[1]/ N[1]	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCR Media/Urine														
1 X LOD CT, Negative NG	180/180	37.11	0.54	1.5%	0.00	0.0%	0.23	0.6%	0.13	0.4%	0.22	0.6%	0.64	1.7%
3 X LOD CT, Negative NG	180/180	35.77	0.38	1.1%	0.18	0.5%	0.15	0.4%	0.00	0.0%	0.21	0.6%	0.50	1.4%
PCR Media/Swab														
1 X LOD CT, Negative NG	174/180	36.99	0.82	2.2%	0.17	0.5%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.84	2.3%
3 X LOD CT, Negative NG	180/180	36.11	0.42	1.2%	0.24	0.7%	0.14	0.4%	0.14	0.4%	0.00	0.0%	0.53	1.5%
PreservCyt® Solution														
1 X LOD CT, Negative NG	177/179	35.21	0.88	2.5%	0.00	0.0%	0.28	0.8%	0.00	0.0%	0.00	0.0%	0.93	2.6%
3 X LOD CT, Negative NG	180/180	33.81	0.68	2.0%	0.03	0.1%	0.18	0.5%	0.15	0.4%	0.00	0.0%	0.72	2.1%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Table 13
C. trachomatis: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PCR Media/Urine

Panel Member	Ct SD	Ct CV %	Percent Agreement*											
			Overall		Site / Instrument			Operator			Day			
1 X LOD CT, Negative NG	0.64	1.7	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
3 X LOD CT, Negative NG	0.50	1.4	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 3 X LOD NG	n/a	n/a	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	35/35
								4	100.0	29/29	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; n/a = not applicable; CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

Table 14

C. trachomatis: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PCR Media/Swab

Panel Member	Ct SD	Ct CV %	Percent Agreement *											
			Overall		Site / Instrument			Operator			Day			
1 X LOD CT, Negative NG	0.84	2.3	96.7	174/180	1	98.3	59/60	1	96.7	29/30	1	100.0	36/36
					2	95.0	57/60	2	100.0	30/30	2	97.2	35/36
					3	96.7	58/60	3	96.7	29/30	3	97.2	35/36
								4	93.3	28/30	4	94.4	34/36
								5	96.7	29/30	5	94.4	34/36
								6	96.7	29/30			
3 X LOD CT, Negative NG	0.53	1.5	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 3 X LOD NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	99.4	178/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	59/59	2	100.0	30/30	2	100.0	35/35
					3	98.3	59/60	3	100.0	29/29	3	97.2	35/36
								4	100.0	30/30	4	100.0	36/36
								5	96.7	29/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; n/a = not applicable; CT = C. trachomatis; NG = N. gonorrhoeae; n/a = not applicable.

Table 15
C. trachomatis: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PreservCyt

Panel Member	Ct SD	Ct CV %	Percent Agreement *										
			Overall		Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	0.93	2.6	98.9	177/179	1	98.3	59/60	1	96.7	29/30	1	100.0	36/36
					2	98.3	58/59	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	96.7	29/30	3	97.2	35/36
								4	100.0	29/29	4	100.0	35/35
								5	100.0	30/30	5	97.2	35/36
								6	100.0	30/30			
3 X LOD CT, Negative NG	0.72	2.1	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	n/a	n/a	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	35/35
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	59/59	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	29/29	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 3 X LOD NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

N. gonorrhoeae (Tables 16, 17, 18, 19)

Table 16 below presents the total standard deviation (SD) and total percent coefficient of variation (CV [%]) for each panel type, respectively. Across all panel types, the total CV (%) ranged from 1.6% to 2.6%.

Percent agreement for the NG positive panel members was 99.4% for the 3 X LOD NG urine panel and 100% for the 3 X LOD NG swab and PreservCyt panel types. For the 1 X LOD NG, percent agreement was 95% for the urine panel type, 89.4% for the swab panel type and 99.4% for the PreservCyt panel type (Tables 17-19, CT Negative/NG Positive column).

There were no false positive results for NG negative panel members across all 3 panel types (Tables 17-19, CT Positive/NG Negative column), thus yielding an analytical specificity of 100% for each panel type.

Table 16
N. gonorrhoeae: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated From Valid Samples of Positive Sample Type Panel Members

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Site/ Instrument		Total	
Panel Member	n[1]/ N[1]	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCR Media/Urine														
Negative CT, 1 X LOD NG	171/180	38.00	0.58	1.5%	0.26	0.7%	0.00	0.0%	0.17	0.5%	0.16	0.4%	0.67	1.8%
Negative CT, 3 X LOD NG	178/179	36.93	0.52	1.4%	0.18	0.5%	0.17	0.5%	0.02	0.1%	0.26	0.7%	0.63	1.7%
PCR Media/Swab														
Negative CT, 1 X LOD NG	161/180	37.97	0.58	1.5%	0.24	0.6%	0.05	0.1%	0.27	0.7%	0.00	0.0%	0.68	1.8%
Negative CT, 3 X LOD NG	180/180	37.31	0.56	1.5%	0.12	0.3%	0.00	0.0%	0.08	0.2%	0.15	0.4%	0.60	1.6%
PreservCyt® Solution														
Negative CT, 1 X LOD NG	178/179	35.22	0.92	2.6%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.92	2.6%
Negative CT, 3 X LOD NG	180/180	33.72	0.70	2.1%	0.19	0.6%	0.00	0.0%	0.21	0.6%	0.10	0.3%	0.76	2.3%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Table 17
N. gonorrhoeae: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PCR Media/Urine

Panel Member	Ct SD	Ct CV %	Percent Agreement *										
			Overall		Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	0.67	1.8	95.0	171/180	1	96.7	58/60	1	96.7	29/30	1	91.7	33/36
					2	96.7	58/60	2	96.7	29/30	2	94.4	34/36
					3	91.7	55/60	3	96.7	29/30	3	100.0	36/36
								4	96.7	29/30	4	91.7	33/36
								5	96.7	29/30	5	97.2	35/36
								6	86.7	26/30			
Negative CT, 3 X LOD NG	0.63	1.7	99.4	178/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
					3	98.3	59/60	3	100.0	30/30	3	100.0	35/35
								4	100.0	29/29	4	100.0	36/36
								5	100.0	30/30	5	97.2	35/36
								6	96.7	29/30			
Negative CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

Table 18
N. gonorrhoeae: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PCR Media/Swab

Panel Member	Ct SD	Ct CV %	Percent Agreement *										
			Overall		Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	0.68	1.8	89.4	161/180	1	91.7	55/60	1	93.3	28/30	1	91.7	33/36
					2	85.0	51/60	2	90.0	27/30	2	94.4	34/36
					3	91.7	55/60	3	73.3	22/30	3	88.9	32/36
								4	96.7	29/30	4	86.1	31/36
								5	93.3	28/30	5	86.1	31/36
								6	90.0	27/30			
Negative CT, 3 X LOD NG	0.60	1.6	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	59/59	2	100.0	30/30	2	100.0	35/35
					3	100.0	60/60	3	100.0	29/29	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

Table 19

N. gonorrhoeae: Percent Agreement by Panel Member Overall and for Site/Instrument, Operator, and Day – PreservCyt

Panel Member	Ct SD	Ct CV %	Percent Agreement *										
			Overall		Site / Instrument			Operator			Day		
1 X LOD CT, Negative NG	n/a	n/a	100.0	179/179	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	59/59	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	29/29	4	100.0	35/35
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
3 X LOD CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 1 X LOD NG	0.92	2.6	99.4	178/179	1	100.0	60/60	1	100.0	30/30	1	100.0	35/35
					2	98.3	59/60	2	100.0	30/30	2	97.2	35/36
					3	100.0	59/59	3	96.7	29/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	29/29	5	100.0	36/36
								6	100.0	30/30			
Negative CT, 3 X LOD NG	0.76	2.3	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			
Negative CT, Negative NG	n/a	n/a	100.0	180/180	1	100.0	60/60	1	100.0	30/30	1	100.0	36/36
					2	100.0	60/60	2	100.0	30/30	2	100.0	36/36
					3	100.0	60/60	3	100.0	30/30	3	100.0	36/36
								4	100.0	30/30	4	100.0	36/36
								5	100.0	30/30	5	100.0	36/36
								6	100.0	30/30			

* For negative samples, percent agreement = (number of negative results/total valid results) x 100; for positive samples, percent agreement = (number of positive results/total valid results) x 100.

Ct = cycle threshold; SD = standard deviation; CV = coefficient of variation; LOD = limit of detection; CT = *C. trachomatis*; NG = *N. gonorrhoeae*; n/a = not applicable.

Analytical Performance

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® CT/NG v2.0 Test was determined by analyzing dilutions of quantified *Chlamydia trachomatis* (serovars D and I) and *Neisseria gonorrhoeae* (isolates 2948 and 6693) cultures. CT and NG cultures were diluted into a matrix of pooled negative endocervical swab specimen in **cobas**® PCR Media, a matrix of pooled negative vaginal swab specimen in **cobas**® PCR Media, negative male and female urine specimen **cobas**® PCR Media and a matrix of pooled negative cervical specimen in PreservCyt® Solution to determine the LOD for endocervical swab, vaginal swab, urine and PreservCyt specimens, respectively. All levels were analyzed using the **cobas**® CT/NG v2.0 Test across 3 unique lots of **cobas**® CT/NG v2.0 Test reagents. LOD is shown as the target concentration which can be detected as positive in ≥ 95% of the replicates tested. Since the LOD evaluation is done with samples stabilized in **cobas**® PCR Media, the LOD for neat urine will be twice the level reported in Table 20.

The LOD for the CT serovar D and I cultures and NG strains 2948 and 6693 in vaginal and endocervical swab specimens stabilized in **cobas**® PCR Media, urine specimens diluted into **cobas**® PCR Media and cervical specimens collected in PreservCyt® Solution are shown in Table 20. All CT serovars and NG strains were tested at 5 positive levels (60 replicates for each level), and one negative level (24 replicates).

Table 20
cobas® CT/NG v2.0 Test Limit of Detection

Specimen Types	*C. trachomatis*		*N. gonorrhoeae*	
	Serovar D	**Serovar I**	**Strain 2948**	**Strain 6693**
	LOD (EB/mL)	**LOD (EB/mL)**	**LOD (CFU/mL)**	**LOD (CFU/mL)**
Endocervical Swabs	200	100	2.0	2.0
Vaginal Swabs	300	70	3.0	1.5
Male Urine	40	20	0.2	0.6
Female Urine	40	10	0.2	0.4
PreservCyt Cervical	200	50	1.0	1.0

EB = Elementary Body; CFU = Colony Forming Units

Inclusivity Verification

Inclusivity testing with the **cobas**® CT/NG v2.0 Test was performed for 13 additional CT serovars, the Swedish new variant strain (nvCT) and an additional 43 independently isolated strains of NG. Testing was done to demonstrate that these targets can be detected around the LOD levels determined during analytical sensitivity testing for the CT serovar D and I culture and NG strains 2948 and 6693. At least 49 replicates were tested for each panel level using one lot of **cobas**® CT/NG v2.0 Test reagents. Testing was performed using CT and NG cultures diluted into a pool of negative endocervical swab specimen matrix in **cobas**® PCR Media, a pool of negative vaginal swab specimen matrix in **cobas**® PCR Media, a pool of negative urine specimen matrix plus **cobas**® PCR Media and a pool of negative cervical specimen in PreservCyt® Solution. Results are shown in Tables 21 and 22 for CT serovars and NG strains, respectively. In Table 22, all NG strains with identical results are presented as a group, shown in the columns labeled “Numbers of NG Strains”.

Table 21
Summary of CT Serovars/Variant Inclusivity Verification Results

Serovar Type or Variant	Endocervical Swabs		Vaginal Swabs		Urine		PreservCyt	
	EB/mL	% Pos	EB/mL	% Pos	EB/mL	% Pos	EB/mL	% Pos
A	100	100	150	100	20	100	100	100
B	100	100	150	100	20	100	100	100
Ba	100	100	150	100	20	100	100	100
C	100	100	150	100	20	100	100	100
E	100	100	150	100	20	100	100	100
F	100	100	150	100	20	100	100	100
G	100	95	150	100	20	100	100	100
H	100	95	150	100	20	100	100	100
J	100	100	150	100	20	100	100	100
K	100	100	150	100	20	100	100	100
LGV Type 1	100	100	150	100	20	100	100	100
LGV Type 2	100	100	150	100	20	100	100	100
LGV Type 3	100	100	150	100	20	100	100	100
nvCT	300	100	150	95	60	100	100	100

Table 22
Summary of NG Strains Inclusivity Verification Results

Numbers of NG Strains	Inclusivity Results for Endocervical Specimens	
	CFU/mL	% Hit Rate
39	3.0	≥95
4	10.0	100
Total = 43		
Numbers of NG Strains	**Inclusivity Results for Vaginal Specimens**	
	CFU/mL	**% Hit Rate**
42	4.5	≥95
1	10.0	100
Total = 43		
Numbers of NG Strains	**Inclusivity Results for Urine**	
	CFU/mL	**% Hit Rate**
34	0.3	≥95
9	1.0	100
Total = 43		
Numbers of NG Strains	**Inclusivity Results for PreservCyt Specimens**	
	CFU/mL	**% Hit Rate**
40	1.5	100
3	5.0	100
Total = 43		

Precision

In-house Precision was examined using a panel composed of CT and NG cultures diluted into a pool of negative vaginal 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. The precision panel was designed to include members with CT and NG at approximately the LOD and at approximately 3 x LOD for the panel matrix. Testing was performed with three unique lots of **cobas**® CT/NG v2.0 Test reagents and three instruments for a total of 24 runs. A description of the precision panels and the study performance hit rate is shown in Table 23. All positive panel members yielded the anticipated hit rates. 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 Tables 24A and 24B) yielded overall CV (%) ranges from 1% to 3% for CT and from 1% to 2% for NG.

Table 23
In-House Precision Study Hit Rate Analysis

		CT					NG				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
Vaginal	1	0	0	48	0	0 - 7.4%	0	0	48	0	0 - 7.4%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	3	3 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	4	1 x LOD	47	48	98	88.9 - 99.9%	3 x LOD	48	48	100	92.6 - 100%
		CT					**NG**				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
Urine	1	0	0	48	0	0 - 7.4%	0	0	48	0	0 - 7.4%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	3	3 x LOD	48	48	100	92.6 - 100%	1 x LOD	48	48	100	92.6 - 100%
	4	1 x LOD	48	48	100	92.6 - 100%	3 x LOD	48	48	100	92.6 - 100%
		CT					**NG**				
Panel Matrix	**Panel Member**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**	**Target Level**	**Positive**	**Valid**	**% Hit Rate**	**95% CI**
PreservCyt	1	0	0	47	0	0 - 7.5%	0	0	47	0	0 - 7.5%
	2	1 x LOD	48	48	100	92.6 - 100%	1 x LOD	47	48	98	88.9 - 99.9%
	3	3 x LOD	47	47	100	92.5 - 100%	1 x LOD	47	47	100	92.5 - 100%
	4	1 x LOD	48	48	100	92.6 - 100%	3 x LOD	48	48	100	92.6 - 100%

Table 24A
Overall Mean, Standard Deviations and Coefficients of Variation (%) for Cycle Threshold, Estimated CT Precision Panel Members

Panel Member	n[1]/N[1]	Mean Ct	SD Components / CV%						
			System	Lot	Operator	Day	Run	Random	Total
Vaginal Swab Specimen in cobas® PCR Media									
2	48/48	36.5	0.000	0.247	0.000	0.095	0.000	0.398	0.478
			0%	1%	0%	0%	0%	1%	1%
3	48/48	35.8	0.192	0.000	0.000	0.000	0.250	0.310	0.442
			1%	0%	0%	0%	1%	1%	1%
4	47/48	36.7	0.000	0.067	0.000	0.000	0.000	0.674	0.678
			0%	0%	0%	0%	0%	2%	2%
Urine Plus cobas® PCR Media									
2	48/48	35.5	0.147	0.058	0.000	0.000	0.000	0.335	0.370
			0%	0%	0%	0%	0%	1%	1%
3	48/48	34.6	0.100	0.077	0.062	0.000	0.000	0.184	0.232
			0%	0%	0%	0%	0%	1%	1%
4	48/48	35.4	0.145	0.161	0.000	0.000	0.243	0.295	0.439
			0%	0%	0%	0%	1%	1%	1%
Cervical Specimen in PreservCyt®									
2	48/48	35.0	0.289	0.365	0.000	0.000	0.539	0.815	1.082
			1%	1%	0%	0%	2%	2%	3%
3	47/47	33.7	0.234	0.000	0.000	0.000	0.385	0.420	0.616
			1%	0%	0%	0%	1%	1%	2%
4	48/48	34.7	0.000	0.000	0.000	0.420	0.287	0.655	0.830
			0%	0%	0%	1%	1%	2%	2%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Table 24B
Overall Mean, Standard Deviations and Coefficients of Variation (%) for Cycle Threshold, Estimated NG Precision Panel Members

Panel Member	n[1]/N[1]	Mean Ct	SD Components / CV%						
			System	Lot	Operator	Day	Run	Random	Total
Vaginal Swab Specimen in cobas® PCR Media									
2	48/48	37.1	0.000	0.211	0.000	0.000	0.000	0.583	0.620
			0%	1%	0%	0%	0%	2%	2%
3	48/48	37.2	0.252	0.185	0.000	0.052	0.000	0.509	0.600
			1%	0%	0%	0%	0%	1%	2%
4	48/48	36.5	0.152	0.000	0.000	0.000	0.000	0.417	0.444
			0%	0%	0%	0%	0%	1%	1%
Urine Plus cobas® PCR Media									
2	48/48	36.2	0.000	0.181	0.000	0.000	0.000	0.456	0.491
			0%	0%	0%	0%	0%	1%	1%
3	48/48	36.1	0.000	0.159	0.052	0.000	0.134	0.337	0.400
			0%	0%	0%	0%	0%	1%	1%
4	48/48	35.1	0.143	0.229	0.000	0.000	0.182	0.281	0.430
			0%	1%	0%	0%	0%	1%	1%
Cervical Specimen in PreservCyt®									
2	47/48	34.7	0.000	0.311	0.000	0.000	0.000	0.797	0.855
			0%	1%	0%	0%	0%	2%	2%
3	47/47	34.8	0.000	0.000	0.000	0.000	0.587	0.606	0.844
			0%	0%	0%	0%	2%	2%	2%
4	48/48	33.5	0.124	0.000	0.000	0.000	0.540	0.618	0.830
			0%	0%	0%	0%	2%	2%	2%

[1] n is the number of positive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Precision of High-Negative CT and NG Samples

High-negative panels were prepared by spiking CT and NG cultures into a pool of vaginal specimen matrix collected in **cobas**® PCR Media, a pool of urine matrix stabilized in **cobas**® PCR Media and a pool of cervical specimen matrix collected in PreservCyt® Solution to organism levels below the Limit of Detection and producing approximately from 20 to 80% negative results. Negative panel members were also prepared for each matrix.

For each sample matrix, panels were tested over the course of 12 days by two operators using three lots of reagents and two **cobas**® 4800 Systems. Two replicates of each panel member were tested in each run, generating up to 48 test results for each panel level. The high-negative panel testing yielded the anticipated hit rates (see Table 25).

Table 25
In-House Precision Study Hit Rate Analysis for High –Negative Levels

Vaginal Swab Specimen Collected in cobas® PCR Media										
Panel	**Levels[a]**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate**	**95% CI**	**Positive**	**Valid**	**Hit Rate**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	48	0	0 - 7.4	29	48	60	45.3 - 74.2%
3	HNeg	Neg	22	48	46	31.4 - 60.8%	0	48	0	0 - 7.4
Urine Stabilized in cobas® PCR Media										
Panel	**Levels[a]**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate**	**95% CI**	**Positive**	**Valid**	**Hit Rate**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	48	0	0 - 7.4	32	48	67	51.6 - 79.6%
3	HNeg	Neg	40	48	83	69.8 - 92.5%	0	48	0	0 - 7.4
Cervical Specimen Collected in PreservCyt® Solution										
Panel	**Levels[a]**		**CT**				**NG**			
	CT	**NG**	**Positive**	**Valid**	**Hit Rate**	**95% CI**	**Positive**	**Valid**	**Hit Rate**	**95% CI**
1	Neg	Neg	0	48	0	0 - 7.4	0	48	0	0 - 7.4
2	Neg	HNeg	0	47	0	0 - 7.5	26	47	55	40.1 - 69.8%
3	HNeg	Neg	26	47	55	40.1 - 69.8%	0	47	0	0 - 7.5

[a] Neg = negative level; HNeg = high negative level

Competitive Inhibition

Panels were prepared by spiking CT and NG cultures into a pool of vaginal specimen matrix collected in **cobas**® PCR Media, a pool of urine matrix stabilized in **cobas**® PCR Media and a pool of cervical specimen matrix collected in PreservCyt® Solution at various concentration levels to examine the potential for competitive inhibition. Panels were tested in two runs per day over the course of 12 days. Two replicates of each panel member were tested in every run, generating a maximum of 48 test results for each level and CT and NG strain respectively. Average Ct values for each of the panel levels are summarized in Table 26. All CT and NG hit rates were 100% for all panel levels in all sample matrices tested. Competitive inhibition was not seen in any combination of CT and NG levels in any sample matrix.

Table 26
Competitive Inhibition Study for CT and NG Cultures

Panel Level		**CT Mean Ct**	**NG Mean Ct**
CT Level (EB/mL)	**NG Level (CFU/mL)**		
Vaginal Swab Specimen Collected in cobas® PCR Media			
Low (300)	**High (1.0E+06)**	36.6	18.6
High (1.0E+07)	**Low (3)**	21.6	36.8
High (1.0E+07)	**High (1.0E+06)**	21.2	18.1
Urine Stabilized in cobas® PCR Media			
Low (40)	**High (1.0E+05)**	36.1	18.4
High (1.0E+07)	**Low (0.2)**	18.0	36.7
High (1.0E+07)	**High (1.0E+05)**	18.3	18.0
Cervical Specimen Collected in PreservCyt® Solution			
Low (200)	**High (1.0E+05)**	34.8	18.9
High (1.0E+07)	**Low (1)**	18.9	34.3
High (1.0E+07)	**High (1.0E+05)**	18.8	17.7

Analytical Specificity

A panel of 184 bacteria, fungi and viruses, including those commonly found in the female urogenital tract, as well as representatives of *N. cinerea, N. flava N. lactamica, N. perflava* and *N. subflava* and other phylogenetically unrelated organisms, were tested with the **cobas**® CT/NG v2.0 Test to assess analytical specificity. The organisms listed in Table 27 were spiked at concentrations of 1 x 10^6 Units*/mL or higher into **cobas**® PCR Media, pooled negative urine matrix in **cobas**® PCR Media and pooled negative vaginal specimen matrix in **cobas**® PCR Media. Organisms listed in Table 28 were tested at varying concentrations below 1 x 10^6 Units*/mL. Testing was performed with each potential interfering organism alone as well as with each organism mixed with CT and NG cultures at 3 times the limit of detection. 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 pneumoniae* as Inclusion Forming Units (IFU). *Treponema pallidum* and HBV were quantified as DNA copies. Adenovirus was quantified as Plaque Forming Units (PFU). CMV, EBV, HSV-1 and HSV-2 were quantified as Viral Particles (VP). HCV and HIV-1 were quantified in International Units (IU). *Trichomonas vaginalis*, HPV16 and HPV18 were quantified as cells/mL.

Table 27
Microorganisms Tested for Analytical Specificity

Achromobacter xerosis	*Helicobacter pylori*	*Neisseria sicca*
Acinetobacter calcoaceticus	Hepatitis B virus (HBV)	*Neisseria subflava*
Acinetobacter lwoffi	Hepatitis C virus (HCV)	*Neisseria subflava 6458*
Acinetobacter sp. genospecies 3	Human immunodeficiency virus	*Neisseria subflava 6617*
Actinomyces israelii	Human papillomavirus type 16 (CaSki cells)	*Neisseria subflava 6618*
Actinomyces pyogenes	Human papillomavirus type 18 (HeLa cells)	*Neisseria subflava 7441*
Adenovirus	Herpes Simplex Virus (HSV-1)	*Neisseria subflava 7452*
Aerococcus viridans	Herpes Simplex Virus (HSV-2)	*Neisseria weaverii*
Aeromonas hydrophila	*Kingella denitrificans*	*Pantoea agglomerans*
Alcaligenes faecalis	*Kingella kingae*	*Paracoccus denitrificans*
Bacillus subtilis	*Klebsiella oxytoca*	*Pasteurella maltocida*
Bacillus thuringiensis	*Klebsiella pneumoniae ss ozaenae*	*Pediococcus acidilactica*
Bacteroides caccae	*Lactobacillus acidophillus*	*Peptostreptococcus anaerobius*
Bacteroides fragilis	*Lactobacillus brevis*	*Peptostreptococcus asacharolyticus*
Bacteroides ureolyticus	*Lactobacillus crispatus*	*Peptostreptococcus magnus*
Bifidobacterium adolescentis	*Lactobacillus delbrueckii subsp. lactis*	*Plesiomonas shigelloides*
Bifidobacterium breve	*Lactobacillus jensenii*	*Prevotella bivia*
Bifidobacterium longum	*Lactobacillus lactis*	*Prevotella corporis*
Moraxella catarrhalis	*Lactobacillus oris*	*Prevotella intermedia*
Brevibacterium linens	*Lactobacillus parabuchnerri*	*Propionibacterium acnes*
Campylobacter gracilis	*Lactobacillus vaginalis*	*Proteus mirabilis*
Campylobacter jejuni	*Lactococcus lactis cremoris*	*Proteus vulgaris*
Candida albicans	*Legionella bozemnii*	*Providencia stuartii*
Candida glabrata	*Legionella pneumophila*	*Pseudomonas aeruginosa*
Candida guilliermondi	*Listeria monocytogenes*	*Pseudomonas fluorescens*
Candida krusei	*Micrococcus luteus*	*Pseudomonas putida*
Candida parapsilosis	*Mobiluncus curtisii subsp. curtisii*	*Rahnella aquatilis*
Candida tropicalis	*Mobiluncus curtisii subsp. holmesii*	*Rhizobium radiobacter*
Chlamydophila pneumoniae	*Mobiluncus mulieris*	*Rhodospirillum rubrum*
Chromobacter violaceum	*Moraxella catarrhalis*	*Ruminococcus productus*
Chryseobacterium meningosepticum	*Moraxella lacunata*	*Saccharomyces cerevisiae*
Citrobacter braakii	*Moraxella osloensis*	*Salmonella* Choleraesuis
Citrobacter freundii	*Morganella morganii*	*Salmonella* Minnesota
Clostridium innocuum	*Mycobacterium avium*	*Salmonella* Typhimurium
Clostridium perfringens	*Mycobacterium gordonae*	*Serratia denitrificans*
Clostridium sporogenes	*Mycobacterium smegmatis*	*Serratia marcescens*
Corynebacterium genitalium	*Mycoplasma genitalium*	*Staphylococcus aureus*
Corynebacterium renale	*Mycoplasma hominis*	*Staphylococcus epidermidis*

Table 27 (continued)
Microorganisms Tested for Analytical Specificity

Corynebacterium xerosis	*Mycoplasma pneumoniae*	*Staphylococcus saprophyticus*
Cryptococcus neoformans	*Neisseria cinerea 832*	*Streptococcus agalactiae*
Cytomegalovirus	*Neisseria cinerea 3306*	*Streptococcus anginosus*
Deinococcus radiodurans	*Neisseria cinerea 3307*	*Streptococcus bovis*
Deinococcus radiopugnans	*Neisseria cinerea 3308*	*Streptococcus dysgalactiae*
Derxia gummosa	*Neisseria cinerea 6317*	*Streptococcus equinis*
Edwardsiella tarda	*Neisseria dentrificans*	*Streptococcus mitis*
Eikenella corrodens	*Neisseria elongata subsp. niroreducans*	*Streptococcus mutans*
Enterobacter aerogenes	*Neisseria flava*	*Streptococcus pneumoniae*
Enterobacter cloacae	*Neisseria flavescens*	*Streptococcus pyogenes*
Enterococcus avium	*Neisseria kochi*	*Streptococcus salivarius*
Enterococcus faecalis	*Neisseria lactamica*	*Streptococcus sanguis*
Enterococcus faecium	*Neisseria meningitidis 135*	*Streptomyces griseinus*
Epstein Barr Virus	*Neisseria meningitidis Serogroup A*	*Treponema pallidum*
Erwinia herbicola	*Neisseria meningitidis Serogroup B*	*Trichomonas vaginalis*
Erysipelothrix rhusiopathiae	*Neisseria meningitidis Serogroup C*	*Ureaplasma urealyticum*
Escherichia coli	*Neisseria meningitidis Serogroup D*	*Veillonela parvula*
Ewingella americana	*Neisseria meningitidesSerogroup Y*	*Vibrio parahaemolyticus*
Flavobacterium meningosepticum	*Neisseria mucosa*	*Weissella paramesenteroides*
Fusobacterium nucleatum	*Neisseria perflava 837*	*Yersinia enterocolitica*
Gardnerella vaginalis	*Neisseria perflava 911*	
Gemella haemolysans	*Neisseria perflava 6339*	
Gemella morbillorum	*Neisseria perflava 6340*	
Haemophilus ducreyi	*Neisseria perflava 6341*	
Haemophilus influenzae	*Neisseria polysaccharea*	

Table 28
List of Microorganisms Tested at less than 1 x 10^6 Units/mL for Analytical Specificity

Microorganism Tested	Concentration Tested in Listed Matrix*			
	cobas® PCR Media	**Negative Urine Specimen**	**Negative Vaginal Specimen**	**Negative PreservCyt Specimen**
Adenovirus		$8x10^5$ PFU/mL	$8x10^5$ PFU/mL	$8x10^5$ PFU/mL
Cytomegalovirus (CMV)	$1x10^4$ VP/mL			
Chlamydophila pneumoniae	$1x10^5$ IFU/mL	$1.1x10^4$ IFU/mL	$1.1x10^4$ IFU/mL	$1.1x10^4$ IFU/mL
Gemella morbillorum		4.5×10^4 CFU/mL	4.5×10^4 CFU/mL	4.5×10^4 CFU/mL
Hepatitis C virus (HCV)		5.6×10^4 IU/mL	5.6×10^4 IU/mL	5.6×10^4 IU/mL
Human papillomavirus (HPV) type 16 (SiHa cells)		$1x10^4$ cells/mL	$1x10^4$ cells/mL	$1x10^4$ cells/mL
Human papillomavirus (HPV) type 18 (HeLa cells)		$1x10^4$ cells/mL	$1x10^4$ cells/mL	$1x10^4$ cells/mL
Neisseria cinerea 3307			$4x10^5$ CFU/mL	$4x10^5$ CFU/mL
Prevotella bivia		$9x10^4$ CFU/mL	$9x10^4$ CFU/mL	$9x10^4$ CFU/mL
Prevotella corporis			$1.4x10^5$ CFU/mL	$1.4x10^5$ CFU/mL
Treponema pallidum	Not Tested	$1x10^5$ copies/mL	$1x10^5$ copies/mL	$1x10^5$ copies/mL
Trichomonas vaginalis			$6.5x10^5$ cells/mL	$6.5x10^5$ cells/mL

*Gray cells indicate concentration tested was ≥ 1 x 10^6 Units/mL in that matrix

Interference

Interference testing was performed using a pool of negative endocervical swab specimen matrix collected in **cobas**® PCR Media, a pool of negative vaginal swab specimen matrix collected in **cobas**® PCR Media, a pool of negative urine specimen matrix plus **cobas**® PCR Media and a pool of negative cervical specimen matrix collected in PreservCyt® Solution spiked with CT and NG cultures at ~ 3 x LOD for each target. Twenty-three over-the-counter (OTC) products, including contraceptive jelly, lubricants, feminine sprays, anti-fungal cream and anti-itch cream, as well as whole blood, cervical mucus and PBMC cells were tested for interference. In addition, several prescription drugs were tested, including clindamycin phosphate, estradiol, metronidazole and estrogen. Metronidazole vaginal gel and Vagisil Silk were found to produce invalid and/or false negative

results in **cobas**® PCR Media plus negative urine spiked with CT and NG cultures at ~ 3 x LOD for each target. Replens was found to produce invalid and/or false negative results in spiked urine stabilized in **cobas**® PCR Media and in spiked endocervical specimen in **cobas**® PCR Media. RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance contain a similar formulation to Replens® vaginal moisturizer and could be expected to produce invalid and/or false negative results in urine samples.

The levels of whole blood, mucus and PBMC cells shown in Table 29 represent maximum allowable concentrations which will not interfere with **cobas**® CT/NG v2.0 Test performance. Concentrations in urine samples were determined using total sample volume, including stabilizing media.

Table 29
Results from Endogenous Interference Testing

	Blood		**PBMC (cells/mL)**		**Mucus**	
	Conc. Tested	**Interference Observed**	**Conc. Tested**	**Interference Observed**	**Conc. Tested**	**Interference Observed**
Urine stabilized in **cobas**® PCR Media	0, 0.1%, 0.25%, 0.5%, 1%	> 0.25%	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1 x 10^5	NT	NT
Vaginal Specimen collected in **cobas**® PCR Media	0, 3%, 5%, 10%	None	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1 x 10^6	Routine level*	None
Endocervical Specimen collected in **cobas**® PCR Media	0, 3%, 5%, 10%	None	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1 x 10^6	Routine level*	None
Cervical Specimens collected in PreservCyt® Solution	0, 3%, 5%, 10%	> 3%	0, 1.0E+05, 1.0E+06, 1.0E+07	None	Routine level*	None

NT = Not Tested.

*Routine level = Quantity of cervical mucus equivalent to amount normally removed prior to sampling

The levels of albumin, glucose, bilirubin, low pH and high pH were also tested in **cobas**® PCR Media plus negative urine spiked with CT and NG cultures at ~ 3 x LOD for each target. Results, shown in Table 30, indicate no interference was detected.

Table 30
Results from Additional Endogenous Interference Testing in Urine Stabilized in cobas® PCR Media

Substance Tested	**Levels Tested**	**Interference Observed**
Albumin	0%, 1%, 2%, and 5% (w/v)	None
Glucose	0%, 0.5% and 1% (w/v)	None
Bilirubin	0%, 0.05%, 0.1%, 0.25% and 0.5% (w/v)	None
Acidic Condition	pH 4	None
Basic Condition	pH 9	None

Cross-Contamination

Studies were performed to evaluate potential cross-contamination on the **cobas**® 4800 System using the **cobas**® CT/NG v2.0 Test. Sample-to-sample cross-contamination and run-to-run carryover on the **cobas**® 4800 System were evaluated using the CT/NG Workflow with samples in **cobas**® PCR media, and in urine stabilized in **cobas**® PCR Media and the CT/NG Cytology Workflow with samples in PreservCyt® Solution. CT positive, NG positive, and CT/NG negative samples were processed in a checkerboard configuration using three **cobas**® 4800 Systems. CT positive [CT(+)] and NG positive [NG(+)] samples were prepared by spiking very high concentrations of *Chlamydia trachomatis* or *Neisseria gonorrhoeae* into **cobas**® PCR Media, urine stabilized with **cobas**® PCR Media or PreservCyt® Solution to generate a signal that covers ≥ 95% of target signal found in specimens of infected patients in the intended use population. In this study, extreme testing conditions were utilized to challenge the sample-to-sample cross-contamination rate and run-to-run carry over rate of the system. Two checkerboard runs were performed on each of the three **cobas**® 4800 Systems with each media type. Results from testing in **cobas**® PCR Media and urine stabilized in **cobas**® PCR Media are analyzed together to represent the CT/NG Workflow. PreservCyt testing represented the CT/NG Cytology Workflow. The CT/NG Workflow gave a sample-to-sample cross-contamination rate of 1.07% (6/562) and a run-to-run carry over rate of 0.18% (1/563). The CT/NG Cytology Workflow gave a sample-to-sample cross-contamination rate of 1.18% (5/423) and a run-to-run carry over rate of 0.0% (0/280).

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

Ancillary Software

Authorized representative in the European community

Batch code


Biological risks

Catalogue number


Consult instructions for use


Contains sufficient for <*n*> tests

Contents of kit

Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.

Distributed by

In Vitro diagnostic medical device


Manufacturer


Store in the dark


Temperature limit

Test Definition File


Use-by date

Global Trade Item Number

US Customer Technical Support 1-800-526-1247

Document Revision Information	
Doc Rev. 11.0 01/2019	Added **cobas**® 4800 System - User Assistance. Removed reference to **cobas**® 4800 System Operator's Manual. Removed reference to **cobas**® CT/NG v2.0 Test Operator's Manual. Removed reference to Female Swab Sample Kit (Roche P/N 05170516190). Changed "Tris-HCl buffer" to "Tris buffer" as a reagent component Added Table of result flags. Added a Procedural Limitation that 100% agreement between results should not be expected in correlation studies. Added a Procedural Limitation and note in Interference section to state that RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance may give invalid or false negative results. Updated reference to IATA Dangerous Goods Edition. Updated descriptions of and added Rx Only symbol and description to the harmonized symbol page at the end of the package insert. Please contact your local Roche Representative if you have any questions.

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01/2019
Doc Rev. 11.0

07127553001-11