510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K110923

B. Purpose for Submission:

To determine substantial equivalence for the **cobas**® CT/NG Test for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA from self-collected vaginal swabs, and male urine.

C. Measurand:

Chlamydia trachomatis DNA

Neisseria gonorrhoeae DNA

D. Type of Test:

Qualitative in vitro diagnostic assay that utilizes amplification of target DNA by real-time Polymerase Chain Reaction.

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

Roche **cobas®** CT/NG Test and **cobas®** 4800 System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3120 Chlamydia serological reagents
   21 CFR 866.3390 Neisseria spp. direct serological test reagents
   21 CFR 862.2570 Instrumentation for Clinical Multiplex Systems

2. Classification:

   Class II

3. Product code:

MKZ: DNA Probe, Nucleic Acid Amplification, Chlamydia
LSL: DNA-Reagents, Neisseria
OOI: Real Time Nucleic Acid Amplification System

Panel:
Microbiology 083

H. Intended Use:

1. Intended use(s):

Assay:

The **cobas**® CT/NG Test is an *in vitro* nucleic acid amplification test that utilizes the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the qualitative detection of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) DNA to aid in the diagnosis of chlamydial and gonococcal disease. The test may be used with vaginal swab specimens self-collected in a clinical setting and male urine from both symptomatic and asymptomatic individuals. Specimens to be tested should be collected in **cobas**® PCR Media.

Ancillary Collection Kits:

The **cobas**® PCR Female Swab Sample Kit is used to collect and transport self-collected vaginal swab specimens in a clinical setting. The **cobas**® PCR Media serves as a nucleic acid stabilizing transport and storage medium for gynecological specimens. Use this collection kit only with the **cobas**® CT/NG Test. **NOTE: This collection kit should not be used for collection of alternative gynecological specimens.**

The cobas® PCR Urine Sample Kit is used to collect and transport male urine specimens. The cobas® PCR Media serves as a nucleic acid stabilizing transport and storage medium for urine specimens. Use this collection kit only with the cobas® CT/NG Test. **NOTE: This collection kit should not be used for collection of female urine specimens.**

2. Indication(s) for use:

Assay:

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

symptomatic and asymptomatic individuals. Specimens to be tested should be collected in **cobas**® PCR Media.

Ancillary Collection Kits:

The **cobas**® PCR Female Swab Sample Kit is used to collect and transport self-collected vaginal swab specimens in a clinical setting. The **cobas**® PCR Media serves as a nucleic acid stabilizing transport and storage medium for gynecological specimens. Use this collection kit only with the **cobas**® CT/NG Test. **NOTE: This collection kit should not be used for collection of alternative gynecological specimens.**

The cobas® PCR Urine Sample Kit is used to collect and transport male urine specimens. The cobas® PCR Media serves as a nucleic acid stabilizing transport and storage medium for urine specimens. Use this collection kit only with the cobas® CT/NG Test. **NOTE: This collection kit should not be used for collection of female urine specimens.**

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   cobas® 4800 System

I. Device Description

The **cobas**® CT/NG 4800 System is a multi-instrument platform that will perform qualitative in vitro nucleic acid amplification tests from self-collected vaginal specimens and male urine specimens. The system integrates automated total nucleic acid isolation, PCR setup, and real-time PCR. The **cobas**® CT/NG 4800 System consists of the **cobas x** 480 Instrument for specimen preparation, and the **cobas z** 480 Analyzer for amplification and detection. The **cobas®** 4800 system software integrates the sample preparation with nucleic acid amplification and detection to generate test results.

The Roche Molecular Systems (RMS) **cobas®** CT/NG Test consists of six reagent kits:

- **cobas®** 4800 System Sample Preparation Kit
- **cobas®** 4800 CT/NG Amplification/Detection Kit
- **cobas®** 4800 CT/NG Controls Kit
- **cobas®** 4800 System Wash Buffer Kit
- **cobas®** 4800 System Control Diluent Kit
- **cobas®** 4800 System Liquid Cytology Preparation Kit

Sample Collection Kits to be used with the **cobas**® CT/NG Test are:

- **cobas®** PCR Female Swab Sample Kit

• **cobas®** PCR Urine Sample Kit

J. Substantial Equivalence Information:

1. Predicate device name(s):

Gen-Probe APTIMA Combo 2 Assay
BD ProbeTec™ CT Q^x Amplified DNA Assay
BD ProbeTec™ GC Q^x Amplified DNA Assay

2. Predicate 510(k) number(s):

K060652
K091724
K091730

3. Comparison with predicate:

Similarities			
Item	**Roche cobas CT/NG Test**	**Gen-Probe Aptima Combo 2 Assay**	**BD ProbeTec CT Q^x and GC Q^x Assays**
General Intended Use	Qualitative in vitro diagnostic test for the direct qualitative detection of *Chlamydia trachomatis* and/or *Neisseria gonorrohoeae* in patient specimens	same	same
Subject Status	Asymptomatic and symptomatic	Asymptomatic and symptomatic	Asymptomatic and symptomatic
Sample Collection Devices	Urine collection kit Swab collection kit	Urine collection kit Swab collection kit	Urine collection kit Swab collection kit
Sample Preparation Procedure	Semi-automated	Semi-automated/automated	Manual/semi-automated

Differences			
Item	**Roche cobas CT/NG Test**	**Gen-Probe Aptima Combo 2 Assay**	**BD ProbeTec CT Q^x and GC Q^x Assays**
Specimen Types	Male urine Patient-collected vaginal swabs	Male urine Male urethral swabs Female urine Endocervical swabs Clinician-collected vaginal swabs Patient-collected vaginal swabs Cervical specimens in PreservCyt® media	Male urine Male urethral swabs Female urine Endocervical swabs Patient-collected vaginal swabs Cervical specimens in PreservCyt® and SurePath® media
CT Analyte Targets	CT cryptic plasmid DNA CT *omp*A gene	CT ribosomal RNA	CT cryptic plasmid DNA
NG Analyte Targets	NG genomic DNA	NG ribosomal RNA	NG genomic DNA
Amplification Technology	Real-time PCR	Ribosomal RNA transcription mediated amplification (TMA)	Strand displacement DNA amplification (SDA)
Detection Chemistry	Paired reporter and quencher fluorescence labeled probes (TaqMan Technology) using fluorescence resonance energy transfer (FRET)	Photon measurement from selectively hybridized chemiluminescent probes reported as Relative Light Units (RLU)	Fluorescent dye labeled probes using fluorescence resonance energy transfer (FRET)
Result Analysis	Based on PCR cycle threshold (Ct) analysis	Determined by a cut-off based on the total FLU and kinetic curve type	Determined by relating MOTA score (signal strength to pre-determined cutoff values)

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The **cobas**® CT/NG 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 Test is automated with the use of the **cobas x** 480 instrument. 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.

Target Selection

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

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

Target Amplification

Processed samples are added to the amplification mixture in a microwell plate, in which 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 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 Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, 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 Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing CT/NG amplicon per PCR.

Detection of PCR Products

The **cobas® CT/NG Test** utilizes real-time PCR technology. The use of fluorescent probes provides for 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision: In-house Precision was examined using a panel composed of CT and NG cultures diluted into **cobas**® PCR Media and **cobas**® PCR Media mixed with negative urine. The precision panel was designed to include members with either CT or NG at approximately the LOD for the panel matrix, members with both CT and NG at approximately the LOD and 2.5 x LOD for the panel matrix and a negative level. Testing was done with three unique lots of **cobas**® CT/NG 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 the table below. All positive panel levels yielded the anticipated hit rates. All negative panel levels tested negative throughout the study. An analysis of the variance of the Ct values from valid tests performed on positive panel members yielded overall CV (%) ranges from 1.1% to 1.8% for CT and from 1.2% to 1.9% for NG.

In-House Precision Study Hit Rate Analysis

Panel Number	Panel Matrix	Target Conc.		N Tested	N Pos CT	N Pos NG	Hit Rate	95% CI	
		CT	NG					Lower	Upper
1	**cobas**® PCR Media	Neg	Neg	144	0	0	0%	0.0	2.5
2	**cobas**® PCR Media	1 X LOD	Neg	144	144	0	100%	97.5	100.0
3	**cobas**® PCR Media	Neg	1 X LOD	144	0	144	100%	97.5	100.0
4	**cobas**® PCR Media	1 X LOD	2.5 X LOD	144	144	144	100%	97.5	100.0
5	**cobas**® PCR Media	2.5 X LOD	1 X LOD	144	144	144	100%	97.5	100.0
1	**cobas**® PCR Media + Urine	Neg	Neg	144	0	0	0%	0.0	2.5
2	**cobas**® PCR Media + Urine	1 X LOD	Neg	144	144	0	100%	97.5	100.0
3	**cobas**® PCR Media + Urine	Neg	1 X LOD	144	0	144	100%	97.5	100.0
4	**cobas**® PCR Media + Urine	1 X LOD	2.5 X LOD	144	144	144	100%	97.5	100.0
5	**cobas**® PCR Media + Urine	2.5 X LOD	1 X LOD	144	144	144	100%	97.5	100.0

b. Reproducibility: A Reproducibility Study was performed across lots, testing sites, operators, runs, and days for the **cobas**® 4800 CT/NG Test using 2 panels prepared from swabs and urine collected in **cobas** PCR Media. Testing was performed at two external sites as well as in-house at Roche Molecular Systems. A run for **cobas**® PCR Media (urine *or* swab) included 3 replicates of each of 5 panel members and 1 positive and 1 negative control (17

total tests). If **cobas®** PCR Media panels were combined in a run, only 1 positive and 1 negative control were included (32 total tests). The 2 operators at each site performed 2 runs per day, for a total of 3 days of testing per operator per panel type (6 days of testing total for each panel type and reagent lot). Testing was performed with 2 reagent lots (6 days of testing per lot). Overall, 74 runs were performed, and 72 valid runs were obtained for urine and swab panel types. The 2 invalid runs were due to instrument errors. A total of 1,080 tests were performed on the 5 panel members for each panel type. There was 1 invalid test result in the urine panel type, and 2 invalid test results in the swab panel type. These invalid 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 both panel types for negative panel members, thus giving negative percent agreement (NPA) of 100% for each analyte.

C. trachomatis Reproducibility results: For both matrix types, percent agreement for CT positive and negative samples was 100%. Analysis of variance components of the Ct values performed on positive panel members yielded overall CV (%) ranges from 1.1% to 1.5% for the urine panel type; 1.6% to 1.8% for the swab panel type.

***C. trachomatis:* Percent Agreement by Panel Member for Lot, Site/Instrument, and Day - PCR Media/Urine**

Panel Member	Ct SD	Ct CV %	Percent Agreement *								
			Lot			Site/ Instrument			Day		
Negative CT, Negative NG	n/a	n/a	2	100.0	108/108	1	100.0	71/71	1	100.0	72/72
			3	100.0	107/107	2	100.0	72/72	2	100.0	71/71
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, Negative NG	0.54	1.5	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
Negative CT, 1 X LOD NG	n/a	n/a	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, 2.5 X LOD NG	0.48	1.3	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
2.5 X LOD CT, 1 X LOD NG	0.40	1.1	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72

* For Negative samples, Percent Agreement = (number of negative results/total valid results);
For Positive samples, Percent Agreement = (number of positive results/total valid results)

C. trachomatis: **Percent Agreement by Panel Member for Lot, Site/Instrument, and Day - PCR Media/Swab**

Panel Member	Ct SD	Ct CV %	Percent Agreement *								
			Lot			Site/ Instrument			Day		
Negative CT, Negative NG	n/a	n/a	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, Negative NG	0.61	1.6	2	100.0	107/107	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	71/71	2	100.0	72/72
						3	100.0	72/72	3	100.0	71/71
Negative CT, 1 X LOD NG	n/a	n/a	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	107/107	2	100.0	71/71	2	100.0	71/71
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, 2.5 X LOD NG	0.66	1.8	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
2.5 X LOD CT, 1 X LOD NG	0.59	1.6	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72

* For Negative samples, Percent Agreement = (number of negative results/total valid results);
For Positive samples, Percent Agreement = (number of positive results/total valid results)

C. trachomatis **: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated**

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]													
Panel Member			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site/ Instrument		Total	
	n[1] / N	Mean Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
PCR Media/Urine																
1x LOD CT, Negative NG	216/216	36.14	0.51	1.4%	0.14	0.4%	0.00	0.0%	0.15	0.4%	0.00	0.0%	0.00	0.0%	0.54	1.5%
1x LOD CT, 2.5x LOD NG	216/216	36.06	0.43	1.2%	0.14	0.4%	0.00	0.0%	0.10	0.3%	0.00	0.0%	0.11	0.3%	0.48	1.3%

2.5x LOD CT, 1x LOD NG	216/216	35.01	0.36	1.0%	0.11	0.3%	0.08	0.2%	0.08	0.2%	0.06	0.2%	0.00	0.2%	0.40	1.1%
PCR Media/Swab																
1x LOD CT, Negative NG	215/215	37.19	0.50	1.3%	0.31	0.8%	0.05	0.1%	0.10	0.3%	0.13	0.3%	0.00	0.0%	0.61	1.6%
1x LOD CT, 2.5x OD NG	216/216	37.26	0.55	1.5%	0.02	0.1%	0.22	0.6%	0.09	0.2%	0.29	0.8%	0.00	0.0%	0.66	1.8%
2.5x LOD CT, 1x LOD NG	216/216	36.00	0.48	1.3%	0.26	0.7%	0.15	0.4%	0.04	0.1%	0.18	0.5%	0.08	0.2%	0.59	1.6%

[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.

N. gonorrhoeae Reproducibility results: The lowest overall PPA for positive panel members was 99.52% for the "Negative CT, 1 x LOD NG" panel member for PCR Media/Urine panel type. Analysis of variance components of the Ct values from valid tests performed on positive panel members yielded overall CV (%) ranges from 1.2% to 1.5% for the urine panel type and 1.4% to 1.9% for the swab panel type.

N. gonorrhoeae: **Percent Agreement by Panel Member for Lot, Site/Instrument, and Day - PCR Media/Urine**

Panel Member	Ct SD	Ct CV %	Percent Agreement [1]								
			Lot			Site/ Instrument			Day		
Negative CT, Negative NG	n/a	n/a	2	100.0	108/108	1	100.0	71/71	1	100.0	72/72
			3	100.0	107/107	2	100.0	72/72	2	100.0	71/71
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, Negative NG	n/a	n/a	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
Negative CT, 1 X LOD NG	0.53	1.5	2	99.1	107/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	98.6	71/72
						3	98.6	71/72	3	100.0	72/72

Panel Member	Ct SD	Ct CV %	Percent Agreement [1]								
			Lot			Site/ Instrument			Day		
1 X LOD CT, 2.5 X LOD NG	0.41	1.2	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
2.5 X LOD CT, 1 X LOD NG	0.54	1.5	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72

[1] For Negative samples, Percent Agreement = (number of negative results/total valid results);
For Positive samples, Percent Agreement = (number of positive results/total valid results)

N. gonorrhoeae: **Percent Agreement by Panel Member for Lot, Site/Instrument, and Day - PCR Media/Swab**

Panel Member	Ct SD	Ct CV %	Percent Agreement [1]								
			Lot			Site/ Instrument			Day		
Negative CT, Negative NG	n/a	n/a	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, Negative NG	n/a	n/a	2	100.0	107/107	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	71/71	2	100.0	72/72
						3	100.0	72/72	3	100.0	71/71
Negative CT, 1 X LOD NG	0.68	1.8	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	107/107	2	100.0	71/71	2	100.0	71/71
						3	100.0	72/72	3	100.0	72/72
1 X LOD CT, 2.5 X LOD NG	0.49	1.4	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72
2.5 X LOD CT, 1 X LOD NG	0.71	1.9	2	100.0	108/108	1	100.0	72/72	1	100.0	72/72
			3	100.0	108/108	2	100.0	72/72	2	100.0	72/72
						3	100.0	72/72	3	100.0	72/72

[1] For Negative samples, Percent Agreement = (number of negative results/total valid results);
For Positive samples, Percent Agreement = (number of positive results/total valid results)

***N. gonorrhoeae*: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated**

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]													
Panel Member			**Within-Run**		**Between-Run**		**Between-Day**		**Between-Operator**		**Between-Lot**		**Between-Site/ Instrument**		**Total**	
	n[1] / N	**Mean Ct**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
PCR Media/Urine																
Negative CT, 1x LOD NG	215/216	36.53	0.51	1.4%	0.07	0.2%	0.00	0.0%	0.05	0.1%	0.12	0.3%	0.00	0.0%	0.53	1.5%
1x LOD CT, 2.5x LOD NG	216/216	35.42	0.35	1.0%	0.12	0.3%	0.04	0.1%	0.12	0.3%	0.12	0.3%	0.05	0.1%	0.41	1.2%
2.5x LOD CT, 1x LOD NG	216/216	36.58	0.49	1.3%	0.12	0.3%	0.12	0.3%	0.12	0.3%	0.07	0.2%	0.00	0.0%	0.54	1.5%
PCR Media/Swab																
Negative CT, 1x LOD NG	215/215	37.26	0.64	1.7%	0.21	0.6%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.68	1.8%
1x LOD CT, 2.5x LOD NG	216/216	35.94	0.44	1.2%	0.09	0.3%	0.12	0.3%	0.08	0.2%	0.13	0.4%	0.00	0.0%	0.49	1.4%
2.5x LOD CT, 1x LOD NG	216/216	37.09	0.63	1.7%	0.00	0.0%	0.26	0.7%	0.11	0.3%	0.16	0.4%	0.00	0.0%	0.71	1.9%

[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:
A second study was conducted internally at Roche Molecular Systems to characterize the precision of test results at target levels below the analytical Limit of Detection of the assay. High-negative panels were prepared by spiking CT and NG cultures into urine and vaginal specimen stabilized in **cobas®** PCR Media to levels producing 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 two lots of reagents and three **cobas®** 4800 Systems. Five replicates of each panel member were tested in each run, generating up to 120 test results for each panel level. The high-negative panel testing yielded the anticipated hit rates.

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

Urine Stabilized in cobas® PCR Media								
Panel Level	**CT**				**NG**			
	Positive	**Valid**	**Hit Rate**	**95% CI**	**Positive**	**Valid**	**Hit Rate**	**95% CI**
1	0	120	0	0-2.5%	0	120	0	0-2.5%
2	55	120	45.8	36.7-55.2%	55	120	45.8	36.7-55.2%
Vaginal Swab Collected in cobas® PCR Media								
Panel Level	**CT**				**NG**			
	Positive	**Valid**	**Hit Rate**	**95% CI**	**Positive**	**Valid**	**Hit Rate**	**95% CI**
1	0	120	0	0-2.5%	0	120	0	0-2.5%
2	57	120	47.5	38.3-56.8%	81	120	67.5	58.3-75.8%

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

One set of **cobas® CT/NG 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 Test results from that run.

The CT/NG (+) Control contains non-infectious DNA plasmids of both *C. trachomatis* and *N. gonorrhoeae* sequences and is used as a run control to monitor the target capture, amplification, and detection steps of the test. The CT/NG (-) Control contains buffer with no nucleic acid.

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.

d. Detection limit:

The analytical sensitivity (Limit of Detection or LOD) for the **cobas®** CT/NG Test was determined by analyzing dilutions of quantified *Chlamydia trachomatis* and *Neisseria gonorrhoeae* cultures. CT and NG cultures were diluted into a matrix of negative vaginal swab specimen in **cobas®** PCR Media and into a matrix of negative urine specimen plus **cobas®** PCR Media to determine the LOD for vaginal swab and urine specimens. All levels were analyzed using the full **cobas®** CT/NG Test workflow across 3 unique lots of **cobas®** CT/NG Test reagents. LOD for this test is defined as the target concentration which can be detected as positive in ≥ 95% of the replicates tested. Since LOD evaluation is done with samples stabilized in **cobas®** PCR Media, the LOD for neat urine will be twice the level reported in the table below. The LOD for the CT serovar D culture and NG strain 19424 in vaginal swab specimens stabilized in **cobas®** PCR Media and urine specimens diluted into **cobas®** PCR Media are shown in the following table.

cobas® CT/NG Test Limit of Detection

Specimen Types	*C. trachomatis*			*N. gonorrhoeae*		
	Levels Tested	**Replicates/ Level**	**LOD (IFU/mL)**	**Levels Tested**	**Replicates/ Level**	**LOD (CFU/mL)**
Vaginal Swabs	5	192**	10.00	5	192**	100.00
Urine	7	192*	0.75	7	192*	2.25

*Testing included one negative level with 167-168 replicates
**Testing included one negative level with 82-84 replicates

e. Inclusivity

Inclusivity testing with the **cobas®** CT/NG Test was performed for 14 additional CT serovars, the Swedish new variant strain (nvCT) and an additional 44 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 culture and NG strain 19424. At least 49 replicates were tested for each panel level using one lot of **cobas®** CT/NG Test reagents. Results are shown in the tables below. In **cobas®** PCR Media plus urine, positive hit rates for the 14 CT serovars plus the nvCT variant (Table 18) were 100% for concentrations ranging from 0.13 to 0.75 IFU/ml. All CT serovars and the nvCT variant were tested at 10 IFU/mL in stabilized negative vaginal specimen, yielding 100% positive hit rates. All 44 NG strains were tested at 3.75 Colony Forming Units (CFU)/mL in **cobas®** PCR Media plus urine and at 100 CFU/mL in stabilized negative vaginal specimen. Positive hit rates ranged from 96 to 100%. In the table below, all NG strains with identical results are presented as a group, shown in the columns labeled "Numbers of NG Strains".

Summary of CT Serovars/Variant Inclusivity Verification Results

Serovar Type or Variant	Reactivity Results for *C. trachomatis*			
	Vaginal Swabs*		Urine	
	IFU/mL	% Pos	IFU/mL	% Pos
A	10.0	100%	0.13	100%
B	10.0	100%	0.75	100%
Ba	10.0	100%	0.75	100%
C	10.0	100%	0.75	100%
E	10.0	100%	0.75	100%
F	10.0	100%	0.75	100%
G	10.0	100%	0.75	100%
H	10.0	100%	0.75	100%
I	10.0	100%	0.75	100%
J	10.0	100%	0.13	100%
K	10.0	100%	0.75	100%
LV Type 1	10.0	100%	0.13	100%
LV Type 2	10.0	100%	0.13	100%
LV Type 3	10.0	100%	0.13	100%
nvCT	10.0	100%	0.75	100%

Summary of NG Strains Inclusivity Verification Results

Numbers of NG Strains	Inclusivity Results for Urine	
	CFU/mL	% Hit Rate
3	3.75	96%
4	3.75	98%
37	3.75	100%
Total = 44		
Numbers of NG Strains	**Inclusivity Results for Vaginal Swabs**	
	CFU/mL	**% Hit Rate**
Total = 44	100	100%

f. 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. cineria, N. flava N. lactamica, N. perflava* and *N. subflava* and other phylogenetically unrelated organisms, were tested with the **cobas®** CT/NG Test to assess analytical specificity. The organisms listed in the first table below were spiked at concentrations of 1 x 10^6 Units*/mL or higher .into **cobas®** PCR Media, pooled negative urine in **cobas®** PCR Media and pooled negative vaginal matrix in **cobas®** PCR Media. Organisms listed in the second table below 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 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.

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 dentrificans*	*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*
Branhamella 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*
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*	

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

Microorganism Tested	Concentration Tested in Listed Matrix*	
	Negative Vaginal Specimen	Negative Urine Specimen
Adenovirus	$8x10^5$ PFU /mL	
Chlamydophila pneumoniae	$1.1x10^4$ IFU /mL	
Gemella morbillorum	4.5×10^4 CFU /mL	
Hepatitis C virus (HCV)	5.6×10^4 IU /mL	
Human papillomavirus (HPV) type 16 (SiHa cells)	$1x10^4$ cells /mL	$5x10^4$ cells /mL
Human papillomavirus (HPV) type 18 (HeLa cells)	$1x10^4$ cells /mL	$1x10^4$ cells /mL
Neisseria cinerea 3307	$4x10^5$ CFU /mL	
Prevotella bivia	$9x10^4$ CFU /mL	
Prevotella corporis	$1.4x10^5$ CFU /mL	
Treponema pallidum	$1x10^5$ copies/mL	
Trichomonas vaginalis	$6.5x10^5$ cells/mL	

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

g. Interference Study

Interference testing was performed using **cobas**® PCR Media plus negative urine and negative vaginal swab specimen (stabilized in **cobas**® PCR Media) spiked with CT and NG cultures at ~ 3 x LOD for each target. Eighteen 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 in **cobas**® PCR Media plus negative urine, including clindamycin phosphate, estradiol, metronidazole and estrogen. Metronidazole vaginal gel was 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. The levels of whole blood, mucus and PBMC cells shown in the following table represent maximum allowable concentrations which will not interfere with **cobas**® CT/NG Test performance. Concentrations in urine samples were determined using total sample volume, including stabilizing media.

Results from Endogenous Interference Testing

	Blood (v/v)		PBMC (cells/mL)		Mucus	
	Conc. Tested	Interference Observed	Conc. Tested	Interference Observed	Conc. Tested	Interference Observed
cobas® PCR Media + Urine	0, 0.25%, 0.35%, 0.5%, 1%, 3%	> 0.35%	0, 1.0E+05, 1.0E+06, 1.0E+07	> 1x **10^5**	NT	NT
Vaginal Specimen stabilized in **cobas**® PCR Media	0, 1%, 3%, 5%, 10%	None	0, 1.0E+05, 1.0E+06, 1.0E+07	**> 1 x 10^5**	Routine level*	None

Varying 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 the table below, indicate no interference except for bilirubin at 0.5% or higher.

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

Substance Tested	Levels Tested	Interference Observed
Albumin	0%. 0.5%, 1%, 2%, and 5% (w/v)	None
Glucose	0%, 0.1% and 1% (w/v)	None
Bilirubin	0%, 0.1%, 0.2%, 0.5% and 1% (w/v)	≥ 0.5%
Acidic Condition	pH 4	None
Basic Condition	pH 9	None

h. Carry over/contamination

Sample-to-sample and run-to-run cross-contamination carryover studies were performed on the **cobas®** 4800 System using the CT/NG Workflow. Testing was performed on three instruments and included testing of five runs of high-positive and negative samples in a checkerboard configuration, followed by a sixth run of all negative samples. The sample-to-sample cross contamination rate for these studies was 1.24% and the run-to-run carry over rate was 0.0%.

i. Assay cut-off:

A preliminary Ct value cut-off for both CT and NG was initially verified using data from the Limit of Detection (LOD) studies. CT and NG spiked samples at, above and below the LOD of the test were used to identify the Ct value range for known positive samples at diminishingly low levels. The lowest level tested produced positive hit rates near 50%, which would correspond to approximately 1 copy of target per PCR based on a Poisson distribution. Samples without the addition of CT or NG were used to confirm that negative results did not generate Ct values. Next, data from the **cobas**® CT/NG clinical trial were compiled and analyzed to identify the distribution of CT and NG Ct values for all specimen types and to validate the appropriate separation between the highest Ct value observed in positive specimens and the assay cut-off.

j. Competitive Inhibition Studies

Panels were prepared by spiking CT and NG cultures into urine and vaginal specimens stabilized in **cobas®** PCR Media to various concentration levels to examine the potential for competitive inhibition. Panels were prepared with two strains each of CT and NG. Panels

were tested in one run per day over the course of 5 days. Two replicates of each panel member were tested in every run, generating a maximum of 10 test results for each level and CT and NG strain respectively. Average Ct values for each of the panel levels are summarized in the tables below. All CT and NG hit rates were 100% for all panel levels in both matrices. Competitive inhibition was not seen in any combination of CT and NG levels in either matrix.

Competitive Inhibition Study for CT and NG Cultures in Urine Stabilized in cobas® PCR Media (Ct Values)

Panel Level		Strain 1		Strain 2	
CT Level / IFU/mL	NG Level / (CFU/mL)	CT	NG	CT	NG
Low/2	Low/6	35.1	34.8	32.5	34.8
Low/2	Medium/ 24	34.9	33.1	32.6	33.0
Medium/ 8	Low/6	33.5	34.1	30.1	35.0
High/ 1.00E+05	Low/6	19.4	34.1	18.6	35.0
Low/2	High/ 1.00E+06	35.2	17.6	32.6	17.4
High/ 1.00E+05	High/ 1.00E+06	19.5	18.1	18.9	17.7

Competitive Inhibition Study for CT and NG Cultures in Vaginal Swabs Collected in cobas® PCR Media (Ct Values)

Panel Level		Strain 1		Strain 2	
CT Level/ IFU/mL	NG Level / (CFU/mL)	CT	NG	CT	NG
Low/25	Low/250	36.4	35.1	34.1	34.7
Low/25	Medium/ 1000	36.2	33.2	33.7	31.8
Medium/ 100	Low/250	34.5	35.1	32.2	34.4
High/ 1.00E+05	Low/250	23.9	33.8	22.9	33.8
Low/25	High/ 1.00E+06	35.7	22.8	33.8	22.0
High/ 1.00E+05	High/ 1.00E+06	23.7	23.2	21.9	22.1

2. Comparison studies:

   a. Method comparison with predicate devices:

   Not applicable

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   Specimen collection took place at 12 collection sites in the US, which included family planning and Obstetrics/Gynecology (OB/GYN) clinics, and sexually transmitted disease clinics. A total of 4 laboratory sites performed specimen testing. In the clinical study, a total of 12/286 (4.2%) runs were classified as invalid due to instrument or operator error. There were no invalid runs due to test controls failure. At collection sites, female subjects provided a self-collected vaginal specimen and male subjects provided a urine specimen in the respective **cobas**® media collection kits. To determine the patient infected status, female patients provided a urine specimen and clinician-collected endocervical swab specimen in collection media from two reference nucleic acid amplification tests (NAAT), and a cervical specimen in PreservCyt® Solution (Hologic Corporation, Bedford, MA) obtained with a spatula/cytobrush or a broom. To determine the patient infected status, male subjects provided urethral swab specimens and urine specimens in collection media from two reference nucleic acid amplification tests (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 Test and two reference nucleic acid amplification tests (NAAT). Testing with all devices followed the manufacturers' instructions. The clinical performance of the **cobas**® CT/NG 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 (reference devices).

Determination of 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
+/-	+/-	-	Infected (Urine) Non-Infected (Swabs)
+/- or -/+	-/-	+ or -	Non-Infected
+/+	-/-	+ or -	Non-Infected
-/-	+/+	+ or -	Non-Infected
-/-	+/- or -/+	+ or -	Non-Infected
-/-	-/-	+ or -	Non-Infected

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

Of the 2,985 subjects (2,195 females and 790 males) tested with the **cobas**® CT/NG Test, 3 were excluded from the analyses because they did not meet study entry criteria or because they withdrew consent; 131 were considered non-evaluable and 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 test results after initial testing and/or retesting. Therefore, of 2,982 subjects enrolled, 2,851 (95.6%) were evaluable for CT and NG primary analyses (2,083 females and 768 males). All performance calculations were based on valid **cobas**® CT/NG Test results from male urine specimens and self-collected vaginal swab specimens.

Chlamydia trachomatis (CT)

The following tables 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 131 females and 126 males were infected with CT. Symptoms were reported in 61.1% (80/131) of infected and 50.9% (993/1,952) of non-infected women. Similarly, symptoms were reported in 58.7% (74/126) of infected

and 34.6% (222/642) of non-infected men. Overall, the CT prevalence was 6.3% (131/2,083), 16.4% (126/768), and 9.0% (257/2,851) in women, men, and the entire study population, respectively.

CT: Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1		NAAT2			cobas CT/NG Test	Symptom Status[a]		Total
	SW	UR	SW	UR	PC Pre	VG	Symp	Asymp	
Infected	+	+	+	+	+	+	61	39	100
Infected	+	–	+	–	+	+	2	4	6
Infected	+	–	+	+	+	+	5	0	5
Infected	+	+	+	+	NA	+	2	1	3
Infected	–	+	+	+	+	+	2	1	3
Infected	+	+	+	–	+	+	1	1	2
Infected	+	+	–	+	+	+	1	1	2
Infected	+	+	+	+	+	–	1	0	1
Infected	+	+	+	+	–	+	0	1	1
Infected	+	+	+	–	–	–	0	1	1
Infected	+	+	–	+	–	+	1	0	1
Infected	+	–	+	+	+	–	1	0	1
Infected	+	–	+	–	+	–	1	0	1
Infected	+	–	+	–	–	–	0	1	1
Infected	–	+	+	+	+	–	1	0	1
Infected	–	+	+	+	–	–	1	0	1
Infected	–	+	–	+	NA	–	0	1	1
Total Infected							80	51	131
Non–Infected	–	–	–	–	–	–	957	911	1868
Non–Infected	–	–	–	–	NA	–	16	24	40
Non–Infected	–	–	+	–	–	–	8	3	11
Non–Infected	NA	NA	–	–	–	–	0	9	9
Non–Infected	–	–	+	+	–	–	3	0	3
Non–Infected	–	–	–	–	+	–	0	3	3
Non–Infected	–	–	–	–	–	+	2	1	3
Non–Infected	–	+	–	+	–	–	1	1	2
Non–Infected	–	+	–	–	–	–	0	2	2
Non–Infected	–	–	NA	–	–	–	0	2	2
Non–Infected	–	NA	–	–	–	–	2	0	2
Non–Infected	NA	–	–	–	–	–	2	0	2
Non–Infected	+	–	–	–	–	–	0	1	1
Non–Infected	–	–	+	+	+	+	0	1	1
Non–Infected	–	–	+	–	+	+	0	1	1
Non–Infected	–	–	–	–	+	+	1	0	1
Non–Infected	–	–	–	NA	–	–	1	0	1
Total Non-Infected							993	959	1952

[a] Symp = symptomatic; Asymp = asymptomatic.

Note: Subjects were designated as being infected with CT if at least 2 NAATs with different target regions gave positive results for the endocervical swab and/or urine specimen. However, females were 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 Test results were considered evaluable and are included in this summary table.

Note: + denotes Positive; – denotes Negative; NA indicates specimen was not obtained for testing or test result was missing/invalid.

Note: SW = endocervical swab; UR = urine; VG = vaginal swab; PC Pre = PreservCyt (pre–aliquot).

CT: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1		NAAT2		cobas CT/NG Test	Symptom Status[a]		
	SW	UR	SW	UR	UR	Symp	Asymp	Total
Infected	+	+	+	+	+	67	43	110
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
Infected	+	+	+	+	–	1	0	1
Total Infected						74	52	126
Non-Infected	–	–	–	–	–	218	412	630
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	–	–	+	+	+	0	1	1
Non-Infected	+	–	–	–	–	1	0	1
Non-Infected	+	+	–	–	+	1	0	1
Total Non-Infected						222	420	642

[a] Symp = symptomatic; Asymp = asymptomatic.

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

Note: Subjects with designated infection status and valid **cobas®** CT/NG Test results were considered evaluable and are 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 Test for CT defined by PIS are presented by gender, sample type, and symptom status in the following table. Sensitivity was 93.9% and 97.6%, respectively for self-collected vaginal swabs and male urine specimens. Sensitivity was similar by symptom status. Regardless of symptom status, specificity for CT ranged from 99.5%-99.7% in both females and males. With all data combined, PPV and NPV were 96.5% and 99.6%, respectively.

CT: 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									
VG	Symp	1073	93.8% (75/80)	(86.2%, 97.3%)	99.7% (990/993)	(99.1%, 99.9%)	7.5	96.2	99.5
	Asymp	1010	94.1% (48/51)	(84.1%, 98.0%)	99.7% (956/959)	(99.1%, 99.9%)	5.0	94.1	99.7
	Overall	2083	93.9% (123/131)	(88.4%, 96.9%)	99.7% (1946/1952)	(99.3%, 99.9%)	6.3	95.3	99.6
Male									
UR	Symp	296	97.3% (72/74)	(90.7%, 99.3%)	99.5% (221/222)	(97.5%, 99.9%)	25.0	98.6	99.1
	Asymp	472	98.1% (51/52)	(89.9%, 99.7%)	99.5% (418/420)	(98.3%, 99.9%)	11.0	96.2	99.8
	Overall	768	97.6% (123/126)	(93.2%, 99.2%)	99.5% (639/642)	(98.6%, 99.8%)	16.4	97.6	99.5
All Combined		2851	95.7% (246/257)	(92.5%, 97.6%)	99.7% (2585/2594)	(99.3%, 99.8%)	9.0	96.5	99.6

[a] VG-S = self-collected vaginal swab; UR = urine.
[b] Symp = symptomatic; Asymp = asymptomatic.
Note: Subjects were designated as being infected with CT if at least 2 NAATs with different target regions gave positive results for the endocervical swab (urethral swab for males) and/or the urine specimen. However, females were 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 Test results were 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.

Neisseria gonorrhoeae (NG)

The following tables 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 33 females and 71 males were infected with NG. Symptoms were reported in 69.7% (23/33) of infected and 51.2% (1,050/2,050) of non-infected women. Of the 768 male subjects enrolled, 296 (38.5%) were symptomatic and 472 (61.5%) were asymptomatic. Overall, the NG prevalence was 1.6% (33/2,083), 9.2% (71/768), and 3.6% (104/2,851), respectively, in women, men, and the entire study population.

NG Positive/Negative Analysis for Female Patient Infected Status

Patient Infected Status	NAAT1		NAAT2			cobas CT/NG Test	Symptom Status[a]		Total
	SW	UR	SW	UR	PC Pre	VG	Symp	Asymp	
Infected	+	+	+	+	+	+	16	9	25
Infected	+	-	+	-	+	+	3	0	3
Infected	-	+	+	+	+	+	1	1	2
Infected	+	+	+	+	+	-	1	0	1
Infected	+	+	+	+	-	+	1	0	1

Patient Infected Status	NAAT1		NAAT2			cobas CT/NG Test	Symptom Status[a]		Total
	SW	UR	SW	UR	PC Pre	VG	Symp	Asymp	
Infected	+	+	+	-	+	+	1	0	1
Total Infected							23	10	33
Non–Infected	-	-	-	-	-	-	1017	958	1975
Non–Infected	-	-	-	-	NA	-	18	26	44
Non–Infected	NA	NA	-	-	-	-	0	9	9
Non–Infected	+	-	-	-	-	-	4	1	5
Non–Infected	-	-	-	-	+	-	2	2	4
Non–Infected	-	-	NA	-	-	-	2	2	4
Non–Infected	-	-	-	+	-	-	1	1	2
Non–Infected	-	NA	-	-	-	-	2	0	2
Non–Infected	NA	-	-	-	-	-	2	0	2
Non–Infected	-	+	-	-	-	-	0	1	1
Non–Infected	-	-	-	-	-	+	1	0	1
Non–Infected	-	-	-	NA	-	-	1	0	1
Total Non-Infected							1050	1000	2050

[a] Symp = symptomatic; Asymp = asymptomatic.
Note: Subjects were designated as being infected with NG if at least 2 NAATs with different target regions gave positive results for the endocervical swab and/or the urine specimen.
Note: Subjects with designated infection status and valid **cobas®** CT/NG Test results were considered evaluable and are included in this summary table.
Note: + denotes Positive; – denotes Negative; NA indicates specimen was not obtained for testing or test result was missing/invalid.
Note: SW = endocervical swab; UR = urine; VG = vaginal swab; PC Pre = PreservCyt (pre-aliquot).

NG: Positive/Negative Analysis for Male Patient Infected Status

Patient Infected Status	NAAT1		NAAT2		cobas CT/NG Test	Symptom Status[a]		Total
	SW	UR	SW	UR	UR	Symp	Asymp	
Infected	+	+	+	+	+	63	7	70
Infected	+	+	–	+	+	1	0	1
Total Infected						64	7	71
Non-Infected	–	–	–	–	–	227	464	691
Non-Infected	–	–	–	–	+	2	0	2
Non-Infected	–	+	–	–	–	1	1	2
Non-Infected	–	–	+	–	–	1	0	1
Non-Infected	+	–	–	–	–	1	0	1
Total Non-Infected						232	465	697

[a] Symp = symptomatic; Asymp = asymptomatic.
Note: Subjects were designated as being infected with NG if at least 2 NAATs with different target regions gave positive results for the urethral swab and/or the urine specimen.
Note: Subjects with designated infection status and valid **cobas®** CT/NG Test results are considered evaluable and are 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 Test for NG as defined by PIS are shown by gender, sample type, and symptom status in the table below. Sensitivity ranged from 95.7%-100.0%. Overall specificity ranged from 99.1%-100.0% for both females and males. Performance estimates for NG detection were similar between symptomatic and asymptomatic

subjects for vaginal swab specimens and urine specimens. With all data combined, PPV and NPV were 97.2% and 100.0%, respectively.

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

Sample Type[a]	Symptom Status[b]	Total (n)	SENS	95% CI	SPEC	95% CI	PREV (%)	PPV (%)	NPV (%)
Female									
VG	Symp	1073	95.7% (22/23)	(79.0%, 99.2%)	99.9% (1049/1050)	(99.5%, 100.0%)	2.1	95.7	99.9
	Asymp	1010	100.0% (10/10)	(72.2%, 100.0%)	100.0% (1000/1000)	(99.6%, 100.0%)	1.0	100.0	100.0
	Overall	2083	97.0% (32/33)	(84.7%, 99.5%)	100.0% (2049/2050)	(99.7%, 100.0%)	1.6	97.0	100.0
Male									
UR	Symp	296	100.0% (64/64)	(94.3%, 100.0%)	99.1% (230/232)	(96.9%, 99.8%)	21.6	97.0	100.0
	Asymp	472	100.0% (7/7)	(64.6%, 100.0%)	100.0% (465/465)	(99.2%, 100.0%)	1.5	100.0	100.0
	Overall	768	100.0% (71/71)	(94.9%, 100.0%)	99.7% (695/697)	(99.0%, 99.9%)	9.2	97.3	100.0
All Combined		2851	99.0% (103/104)	(94.8%, 99.8%)	99.9% (2744/2747)	(99.7%, 100.0%)	3.6	97.2	100.0

[a]VG-S = self-collected vaginal swab; UR = urine.
[b] Symp = symptomatic; Asymp = asymptomatic.
Note: Subjects were designated as being infected with NG if at least 2 NAATs with different target regions gave positive results for the endocervical swab (urethral swab for males) and/or the urine specimen.
Note: Subjects with designated infection status and valid **cobas**® CT/NG Test results were 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.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:
   The prevalence of CT observed with the **cobas**® CT/NG Test during the clinical study ranged from 5.0% to 7.5% in females, and from 11.0% to 25.0% in males; the prevalence of NG ranged from 1.0% to 2.1% in females, and from 1.5% to 21.6% in males.

N. Instrument Name:
Roche **cobas**® 4800 System which consists of **cobas** 480 x and **cobas** 480 z instruments.

O. System Descriptions:

1. Modes of Operation:

   The Roche **cobas**® 4800 System operates in a batch mode with open sample tubes. The system may operate in full extraction and amplification mode or in PCR only mode with previously extracted samples.

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes __X____ or No _____

3. Specimen Identification:

   Specimens are identified using barcodes.

4. Specimen Sampling and Handling:

   Samples are placed on the instrument as open tubes.

5. Calibration:

   No calibration is required by the user. Roche technicians perform calibration periodically as required.

6. Quality Control:

   Positive and Negative Controls are included in every run. An Internal control is introduced for each Control and Specimen during sample preparation on the **cobas x** 480 instrument reaction.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.