

CT/NG Test for *Chlamydia trachomatis*

CTA

FOR *IN VITRO* DIAGNOSTIC USE

Order Information	AMPLICOR® CT/NG Specimen Preparation Kit	CT/NG PREP	100 Tests	P/N: 20759414 ART: 07 5941 4 US: 83315
	AMPLICOR CT/NG Amplification Kit	CT/NG AMP	96 Tests	P/N: 20759902 ART: 07 5990 2 US: 83319
	COBAS AMPLICOR <i>Chlamydia trachomatis</i> Detection Kit	CT DK	100 Tests	P/N: 20757497 ART: 07 5749 7 US: 83277
	COBAS AMPLICOR Detection Reagents Kit	DK	100 Tests	P/N: 20757470 ART: 07 5747 0 US: 83276
	COBAS AMPLICOR Conjugate Detection Reagent	CN4	200 Tests	P/N: 20764213 ART: 07 6421 3 US: 83305
	COBAS AMPLICOR Wash Buffer	WB	500 Tests	P/N: 20759899 ART: 07 5989 9 US: 83314
	<p><i>The following kit can be used to detect the CT/NG Internal Control amplified using the COBAS AMPLICOR CT/NG Amplification Kit. Detection of the CT/NG Internal Control is a user option.</i></p>			
	COBAS AMPLICOR Internal Control Detection Kit	IC DK	100 Tests	P/N: 20757608 ART: 07 5760 8 US: 83281

Intended Use

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is a qualitative *in vitro* test for the detection of *C. trachomatis* plasmid DNA in urine from males and females, in endocervical swab specimens, and in male urethral swab specimens as evidence of symptomatic or asymptomatic infection with *C. trachomatis*. *C. trachomatis* DNA is detected by Polymerase Chain Reaction (PCR) amplification of target DNA and by hybridization capture of amplified target using the COBAS AMPLICOR Analyzer.

Summary and Explanation of the Test

Chlamydia are gram-negative, non-motile bacteria that exist as obligate intracellular parasites of eukaryotic cells due to their inability to synthesize ATP. The genus *Chlamydia* consists of three reported species: *C. trachomatis*, *C. psittaci*, and *C. pneumoniae* (TWAR). *C. psittaci* is primarily an animal pathogen¹⁻³.

C. trachomatis infections are now recognized as the leading cause of sexually transmitted diseases (STD) in the United States, where more than 4,000,000 cases occur per year⁴. In Europe, approximately 3,000,000 cases occur annually⁵. *C. trachomatis* is known to cause cervicitis, pelvic inflammatory disease (PID), infant conjunctivitis, infant pneumonia, urethritis, epididymitis and proctitis^{1,6}. *C. trachomatis* is also the most frequent cause of non-gonococcal urethritis (NGU) in men (approximately 25-55% of cases). Among women, the consequences of chlamydial infections are severe if left untreated. Since approximately half of these infections are asymptomatic, many cases go undetected and untreated, leading to additional problems particularly with pregnant women. Babies born to chlamydia-infected mothers are at high risk of developing inclusion conjunctivitis and pneumonia.

Several methods are available for the detection of *C. trachomatis* in clinical specimens. These methods include direct Giemsa staining of infected tissue, detection of chlamydial inclusion bodies in infected culture cells using fluorescent antibody stain, direct antigen detection using fluorescent antibody stain and nucleic acid probes^{1,7-9}. Culture is highly specific but is less than 100% sensitive when applied in routine clinical practice. Because chlamydial culture is not 100% sensitive, it has been suggested that multiple non-culture tests be used to identify infected specimens that are missed by culture¹⁰. Non-culture testing methods are used to identify organism components (protein or nucleic acid) that are presumed to correlate with infectious organisms. These methods may detect additional positive specimens missed by culture. Because these methods are less than 100% specific, verification of *C. trachomatis* antigen or nucleic acid by an alternate method has been recommended (1993 CDC recommended action)⁴.

Principles of the Procedure

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is based on four major processes: specimen preparation; PCR amplification^{11,12} of target DNA using CT specific complementary primers; hybridization of the amplified DNA to oligonucleotide probes specific to the target(s); and detection of the probe-bound amplified DNA by colorimetric determination.

The development of a PCR assay involves identifying the particular region of the target DNA to be amplified and synthesizing two short biotinylated oligonucleotide

primers that are complementary to the regions flanking the target sequence. These biotinylated primers bind to the complementary flanking region, and the DNA polymerase extends the sequence in the 5' to 3' direction utilizing excess deoxynucleotide triphosphates (dNTPs) in the reaction mixture thereby creating a biotinylated, complementary DNA sequence termed an amplicon. An oligonucleotide probe, specific for the amplicon, is bound to a solid support (microparticles) and is used for hybridization (capture) of the amplicon. The assay detection system utilizes an avidin-horseradish peroxidase conjugate (HRP) that binds to the biotinylated amplicon captured on the microparticles. A hydrogen peroxide (H₂O₂) substrate and tetramethylbenzidine (TMB) chromophore are used for color formation. The assay detection system uses an avidin-horseradish peroxidase conjugate (HRP) that binds to biotinylated amplicon captured on the microparticles.

Specimen Preparation

Urogenital epithelial cells collected on swabs or pelleted from urine are treated with a detergent solution to release the chlamydial DNA contained in the chlamydial reticulate bodies. A second detergent solution is then added to prepare the lysed specimen for amplification.

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*^{13,14}. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* uses the biotinylated primers CP24 and CP27 to define a sequence of approximately 207 nucleotides within the cryptic plasmid DNA of *C. trachomatis*.

Target Amplification

Processed specimens are added to the amplification mixture in amplification tubes (A-tubes) in which PCR amplification occurs. The reaction mixture is heated to denature the double-stranded DNA helix and expose the specific primer target sequences on the *C. trachomatis* cryptic plasmid. As the mixture cools, the biotinylated primers CP24 and CP27 anneal to the target DNA. The thermostable *Thermus aquaticus* DNA polymerase (*Taq* pol), in the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine (in place of thymidine) triphosphates, extends the annealed primers along the target templates to produce a 207-base pair double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for 35 cycles, each cycle theoretically doubling the amount of amplicon DNA.

Internal Control Amplification

In enzyme-based amplification processes such as PCR, efficiency can be reduced by inhibitors that may be present in the clinical specimen. The CT/NG Internal Control permits the optional identification of processed specimens containing substances that may interfere with PCR amplification. The CT/NG Internal Control is a non-infectious, recombinant plasmid DNA with primer binding regions identical to those of the *C. trachomatis* target sequence, a randomized internal sequence of similar length and base composition as the *C. trachomatis* target sequence, and a unique probe binding region that differentiates the CT/NG Internal Control from target amplicon. These features were selected to ensure equivalent amplification of both the CT/NG Internal Control and the *C. trachomatis* target DNA. The CT/NG Internal Control Reagent is included in the

COBAS AMPLICOR CT/NG Amplification Kit and is introduced into each amplification reaction to be co-amplified with target DNA from the clinical specimen. The optional COBAS AMPLICOR Internal Control Detection Kit contains an IC-specific oligonucleotide capture probe that can be used to identify a positive IC signal in the reaction mixture. The CT/NG Internal Control is designed to ensure that specimens do not contain inhibitors that would interfere with the amplification and detection of 20 or more copies of *C. trachomatis* target nucleic acid as determined by Poisson analysis.

Selective Amplification

Selective amplification of target nucleic acid from the clinical specimen is achieved in the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* by the use of AmpErase® (uracil-N-glycosylase) and deoxyuridine triphosphate (dUTP). AmpErase recognizes and catalyzes the destruction of DNA containing deoxyuridine¹⁵, but not DNA strands containing thymidine. 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 prior to amplification of the target DNA. AmpErase, 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 the Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of any target amplicon. AmpErase in the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* has been demonstrated to inactivate at least 10³ copies of deoxyuridine-containing *C. trachomatis* amplicon per PCR.

Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds Denaturation Solution to the A-tubes to chemically denature the CT amplicon and the CT/NG Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for *C. trachomatis* (or Internal Control, at the user's option) is added to the individual D-cups. The biotin-labeled CT and CT/NG Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. The hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles in the D-cups to remove unbound material and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase Conjugate binds to the biotin-labeled amplicon hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) to each D-cup. In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyses the oxidation of TMB to form a colored complex, the absorbance of which is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

Reagents

AMPLICOR CT/NG Specimen Preparation Kit	CT/NG PREP	100 Tests	P/N: 20759414 ART: 07 5941 4 US: 83315
CT/NG URINE WASH (CT/NG Urine Wash Buffer)			1 x 50 mL
Tris-HCl buffer			
300 mM Sodium chloride			
< 0.1% Detergent			
0.09% Sodium azide			
CT/NG LYS (CT/NG Lysis Reagent)			1 x 25 mL
Tris-HCl buffer			
< 1% Solubilizer			
0.09% Sodium azide			
CT/NG DIL (CT/NG Specimen Diluent)			2 x 50 mL
Tris-HCl buffer			
6 mM Magnesium chloride			
< 25% Detergent			
0.05% Sodium azide			
AMPLICOR CT/NG Amplification Kit	CT/NG AMP	96 Tests	P/N: 20759902 ART: 07 5990 2 US: 83319
CT/NG MMX (CT/NG Master Mix)			3 x 1.8 mL
Tris-HCl buffer			
EDTA			
100 mM KCl			
Glycerol			
< 0.016% dUTP			
< 0.01% AmpliTaq® (<i>Taq</i> DNA Polymerase, microbial)			
< 0.005% dATP, dCTP, and dGTP			
< 0.01% AmpErase uracil-N-glycosylase (microbial)			
< 0.0004% CP24 and CP27 primers, biotinylated			
0.05% Sodium azide			
CT/NG IC (CT/NG Internal Control)			3 x 0.1 mL
Tris-HCl buffer			
8 copies/μL of non-infectious plasmid DNA (microbial)			
containing CT primer binding sequences and a unique probe			
binding region equivalent to approximately 21 IC copies/test			
< 0.005% Poly rA RNA (synthetic)			
EDTA			
Amaranth dye			
0.05% Sodium azide			


CT (+) C 1 x 0.8 mL
 [*C. trachomatis* (+) Control]
 Tris-HCl buffer
 8.6 copies/μL of non-infectious plasmid DNA (synthetic)
 containing *C. trachomatis* sequences equivalent to
 approximately 20 copies/test
 < 0.005% Poly rA RNA (synthetic)
 < 0.5% Detergent
 EDTA
 0.05% Sodium azide

NG (+) C 1 x 0.8 mL
 [*C. trachomatis* (-) Control]
 Tris-HCl buffer
 8.6 copies/μL of non-infectious plasmid DNA (synthetic)
 containing *N. gonorrhoeae* sequences equivalent to
 approximately 20 copies/test
 < 0.005% Poly rA RNA (synthetic)
 < 0.5% Detergent
 EDTA
 0.05% Sodium azide

COBAS AMPLICOR CT DK 100 Tests P/N: 20757497
Chlamydia trachomatis ART: 07 5749 7
Detection Kit US: 83277

CT PS1 1 x 100 Tests
 (CT Probe Suspension 1)
 MES buffer
 < 0.3% Suspension of Dynabeads® (paramagnetic particles)
 coated with *C. trachomatis*-specific oligonucleotide capture probe
 0.9% Sodium azide


CT4 1 x 100 Tests
 (CT Probe Suspension 2)
 Sodium phosphate buffer
 25% Sodium thiocyanate
 < 0.2% Detergent

Xn  25% (w/w) Sodium thiocyanate
 Harmful

COBAS AMPLICOR IC DK 100 Tests P/N: 20757608
Internal Control Detection Kit ART: 07 5760 8
 US: 83281


IC PS1 1 x 100 Tests
 (IC Probe Suspension 1)
 < 0.35% Suspension of Dynabeads (paramagnetic particles)
 coated with Internal Control-specific oligonucleotide capture probe
 0.9% Sodium azide

IC4 1 x 100 Tests
 (IC Probe Suspension 2)
 Sodium phosphate buffer
 25% Sodium thiocyanate
 < 0.2% Detergent

Xn  25% (w/w) Sodium thiocyanate
 Harmful

COBAS AMPLICOR DK 100 Tests P/N: 20757470
Detection Reagents Kit ART: 07 5747 0
 US: 83276

DN4 1 x 100 Tests
 (Denaturation Solution)
 1.6% Sodium hydroxide
 EDTA
 Thymol blue

Xi  1.6% (w/w) Sodium hydroxide
 Irritant

CN4 1 x 100 Tests
 (Avidin-Horseradish Peroxidase Conjugate)
 Tris-HCl buffer
 < 0.001% Avidin-horseradish peroxidase conjugate
 Bovine serum albumin (mammalian)
 Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
 0.1% Phenol
 1% ProClin® 150

SB3 5 x 75 Tests
 (Substrate A)
 Citrate solution
 0.01% Hydrogen peroxide
 0.1% ProClin 150

SB 5 x 5 mL

(Substrate B)
 0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)
 40% Dimethylformamide (DMF)

T  40% (w/w) Dimethylformamide (DMF)

Toxic

R: 61-20/21-36 May cause harm to the unborn child.
 Harmful by inhalation and in contact with skin. Irritating to eyes.

S: 53-45 Avoid exposure – obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

COBAS AMPLICOR CN4 200 Tests P/N: 20764213
Conjugate Detection Reagent ART: 07 6421 3
 US: 83305

CN4 2 x 100 Tests

(Avidin-Horseradish Peroxidase Conjugate)
 Tris-HCl buffer
 < 0.001% Avidin-horseradish peroxidase conjugate
 Bovine serum albumin (mammalian)
 Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
 0.1% Phenol
 1% ProClin 150

COBAS AMPLICOR WB 500 Tests P/N: 20759899
Wash Buffer ART: 07 5989 9
 US: 83314

WB 2 x 250 Tests

(10X-Wash Concentrate)
 < 2% Phosphate buffer
 < 9% Sodium chloride
 EDTA
 < 2% Detergent
 0.5% ProClin 300

Warnings and Precautions

For in vitro diagnostic use.

The use of the term copy in this method manual refers to 1 copy of *C. trachomatis* target nucleic acid. One (1) copy is equivalent to the smallest amount of *C. trachomatis* target nucleic acid that would generate a positive PCR test result.

This test is for use only with endocervical, urethral, and urine specimens. This test is not intended for use with throat, rectal, or other types of specimens.

Do not pipet by mouth.

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 kit reagents.

Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipets and pipet tips is recommended.

Do not pool reagents from different lots or from different bottles of the same lot.

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

Do not use a kit after its expiration date.

Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.

Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*¹⁶ and in the NCCLS Document M29-A¹⁷. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized water.

Note

Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

For specimens transported in **Chlamydia culture transport media**, swabs should be left in the culture transport tube to provide visual evidence of specimen inoculation. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was evaluated using specimens transported with the swab in the Chlamydia culture transport medium (CTM) tube. CTM specimens transported without swabs have not been evaluated and are not recommended for use with this test.

Storage of urine specimens at room temperature for more than 24 hours may result in specimen degradation. Urine specimens stored for longer than 24 hours at room temperature should not be used for testing.

CT/NG URINE WASH, CT/NG LYS, CT/NG DIL, CT/NG MMX, CT/NG IC, CT (+) C, NG (+) C, CT PSI and IC PSI 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 water to prevent azide buildup.

Wear eye protection, laboratory coats and disposable gloves when handling **DN4**, **CN4**, **SB3**, **SB**, and Working Substrate (mixed **SB3** and **SB** reagent). 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 of these reagents occur, dilute with water before wiping dry.

Avoid contact between the skin or mucous membranes and **SB** or the Working Substrate. If skin contact occurs, wash immediately with large amounts of water.

SB and Working Substrate contain dimethylformamide which has been reported to be toxic in high oral doses and may be harmful to the unborn child. Skin contact, inhalation of fumes and ingestion must be avoided. If skin contact occurs, wash thoroughly with soap and water and seek medical advice immediately.

Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. *Do not use snap cap tubes.*

Storage and Handling Requirements

Do not freeze reagents.

Store **CT/NG LYS** at 2-25°C. Store **CT/NG URINE WASH** and **CT/NG DIL** at 2-8°C. If a precipitate forms in any of these reagents during storage, warm to ambient temperature and mix thoroughly prior to use. These reagents are stable until the expiration date indicated.

Store **CT/NG MMX** and **CT/NG IC** at 2-8°C. These reagents are stable until the expiration date indicated. Working Master Mix (prepared by the addition of **CT/NG IC** to **CT/NG MMX**) must be stored at 2-8°C and is stable for 4 weeks.

Store **CT (+) C** and **NG (+) C** at 2-8°C. These reagents are stable until the expiration date indicated.

Store **CT PS1** and **CT4** at 2-8°C. These reagents are stable until the expiration date indicated. Once **CT PS1** and **CT4** are mixed, the Working Reagent is stable for 30 days at 2-8°C. This Working Reagent can be used for a maximum of 6 instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **IC PS1** and **IC4** at 2-8°C. These reagents are stable until the expiration date indicated. Once **IC PS1** and **IC4** are mixed, the Working Reagent is stable for 21 days at 2-8°C. This Working Reagent can be used for a maximum of 6 instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **DN4** at 2-25°C. **DN4** is stable until the expiration date indicated. Once opened, **DN4** is stable for 30 days at 2-8°C or until the expiration date, whichever comes first. **DN4** can be used for a maximum of 6 instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **CN4** at 2-8°C. **CN4** is stable until the expiration date indicated. Once opened, **CN4** reagent is stable for 30 days at 2-8°C or until the expiration date, whichever comes first. **CN4** can be used for a maximum of 6 instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **SB3** and **SB** at 2-8°C. Unopened, these reagents are stable until the expiration dates indicated. Working Substrate must be prepared each day by mixing **SB3** with **SB**. The Working Substrate is stable on the COBAS AMPLICOR Analyzer for 16 hours. Do not expose **SB3**, **SB**, or Working Substrate to metals, oxidizing agents or direct light.

Store **WB** at 2-25°C. **WB** is stable until the expiration date indicated. Working Wash Buffer (1X), prepared by diluting **WB** 1:10 with distilled or deionized water, must be stored at 2-25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation. Do not top off the COBAS AMPLICOR Wash Buffer Reservoir.

Store partially used detection reagents at 2-8°C between instrument runs. Check expiration date of opened or Working Reagents prior to loading on the COBAS AMPLICOR Analyzer.

Materials Provided

**COBAS AMPLICOR
CT/NG Test for
*Chlamydia trachomatis***

**AMPLICOR CT/NG
Specimen Preparation Kit**

CT/NG PREP

P/N: 20759414
ART: 07 5941 4
US: 83315

CT/NG URINE WASH
(CT/NG Urine Wash Buffer)

CT/NG LYS
(CT/NG Lysis Reagent)

CT/NG DIL
(CT/NG Specimen Diluent)

**AMPLICOR CT/NG
Amplification Kit**

CT/NG AMP

P/N: 20759902
ART: 07 5990 2
US: 83319

CT/NG MMX
(CT/NG Master Mix)

CT/NG IC
(CT/NG Internal Control)

CT (+) C
[*C. trachomatis* (+) Control]

NG (+) C
[*C. trachomatis* (-) Control]

COBAS AMPLICOR
***Chlamydia trachomatis* Detection Kit**

CT DK

P/N: 20757497
ART: 07 5749 7
US: 83277**CT PS1**
(CT Probe Suspension 1)**CT4**
(CT Probe Suspension 2)**COBAS AMPLICOR**
Internal Control Detection Kit

IC DK

P/N: 20757608
ART: 07 5760 8
US: 83281**IC PS1**
(IC Probe Suspension 1)**IC4**
(IC Probe Suspension 2)**COBAS AMPLICOR**
Detection Reagents Kit

DK

P/N: 20757470
ART: 07 5747 0
US: 83276**DN4**
(Denaturation Solution)**CN4**
(Avidin-Horseradish Peroxidase Conjugate)**SB3**
(Substrate A)**SB**
(Substrate B)**COBAS AMPLICOR**
Conjugate Detection Reagent

CN4

P/N: 20764213
ART: 07 6421 3
US: 83305**CN4**
(Avidin-Horseradish Peroxidase Conjugate)**COBAS AMPLICOR**
Wash Buffer

WB

P/N: 20759899
ART: 07 5989 9
US: 83314**WB**
(10X-Wash Concentrate)

Materials Required but Not Provided

Specimen Collection

- Endocervical and urethral specimen collection swabs - use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts
- Chlamydia Culture Transport Media (CTM) - use only 2SP CTM, Bartels ChlamTrans™ CTM (Bartels, Inc.), SPG CTM, or M4 CTM (MicroTest, Inc.)
- Polypropylene, preservative-free urine collection cups

Pre-Amplification – Reagent Preparation Area

- COBAS AMPLICOR A-ring fitted with 12 A-tubes (ART: 10 4563 6)
- COBAS AMPLICOR A-ring holder
- Plastic resealable bag
- Eppendorf® Repeater® pipet with 1.25 mL Eppendorf Combitip® Reservoir (sterile, individually wrapped)
- Pipettors (capacity 100 µL)* with aerosol barrier or positive displacement tips
- Vortex mixer
- Disposable gloves, powderless

Pre-Amplification – Specimen and Control Preparation Area

- 2.0 mL polypropylene screw-cap tubes, sterile, non-siliconized, conical (Sarstedt 72.693.105 or equivalent)**
- Tube racks (Sarstedt 93.1428 or equivalent)
- Pipettors (capacity 50 µL, 100 µL, 200 µL, 250 µL, 500 µL and 1000 µL)* with aerosol barrier or positive displacement tips
- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent

- Extended aerosol barrier tips (Matrix 7055 or equivalent) for use with specimens transported in M4 Culture Transport Medium or Bartels ChlamTrans Chlamydial Transport Medium
- 37°C ± 2°C heating block
- Vortex mixer
- Absorbent paper
- Disposable gloves, powderless

Post-Amplification – Amplification/Detection Area

- COBAS AMPLICOR Analyzer and printer
- *Operator's Manual* for the COBAS AMPLICOR Analyzer
- COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* Method Manual
- Racks of D-cups (ART: 10 4564 4)
- Distilled or deionized water
- 5 mL serological pipets
- Graduated cylinder (minimum 1 Liter)
- Vortex mixer
- Disposable gloves, powderless

* Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement tips must be used where specified to prevent specimen and amplicon cross-contamination.

** Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. *Do not use snap cap tubes.*

Specimen Collection, Transport and Storage

Note

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

The only acceptable specimens are:

1. Urine specimens (male and female) transported in clean polypropylene containers. **Do not use urine specimens collected in containers containing preservatives.**
2. Endocervical and urethral swab specimens collected and transported in 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), SPG CTM, or M4 Culture Transport Medium (MicroTest, Inc.). **Media lots must be qualified for use in each laboratory (see “Quality Control” section for details).**

For reliable test results, follow instructions below for proper specimen collection. This test is not intended for use with throat, rectal, or types of specimens other than those indicated.

In order to ensure the delivery of high quality specimens to the laboratory for testing, urine and urogenital swab specimens should be transported to the laboratory in as short a time as practical. Do not allow specimens to be transported without controlled temperature conditions.

Specimen Collection

Urine Specimens (Male and Female)

Note

Patient must not have urinated during the previous 2 hours.

1. Collect 10 to 50 mL of first catch urine (the first part of the stream) into a clean polypropylene container without preservatives.
2. Seal the specimen container and label appropriately. Follow the laboratory's collection and transport procedure. The specimen may be transported to the test site at room temperature 18-25°C.

Swab Specimens (Male and Female) Collected in Culture Transport Media

1. Endocervical and urethral swab specimens can be collected and transported in 1 to 3 mL 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), SPG Culture Transport Medium, or M4 Culture Transport Medium (MicroTest, Inc.). Use recommended methods to sample columnar and squamo-columnar cells after removing cervical mucus¹.
2. Use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts. Do not use collection swabs with wooden or aluminum shafts.
3. Leave swabs in the transport media. Seal the specimen container and label appropriately. Follow the laboratory's collection and transport procedure. Refrigerate swab specimens if transport to the laboratory is delayed for more than one hour from the time of collection.

Specimen Transport

Urine Specimens (Male and Female)

1. Urine specimens may be transported to the test site at 18 - 25°C. Urine specimens are stable for 24 hours at 18 - 25°C.
2. Urine specimens that require shipment to off-site test centers must be shipped via overnight delivery with guaranteed arrival within 24 hours; shipment can be at 18 - 25°C. If urine specimens are shipped at 18 - 25°C, they should be stored at 2-8°C until time of shipment to ensure that the period of 18 - 25°C storage does not exceed 24 hours. Specimens must be shipped in compliance with all applicable local, state and country regulations for the transport of etiologic agents¹⁸.

Swab Specimens (Male and Female) Collected in Culture Transport Media

1. Swab specimens may be transported to the test site at 18 - 25°C provided that the total time of storage and transport at 18 - 25°C is less than 1 hour. Refrigerate swab specimens if transport to the laboratory is delayed for more than one hour from the time of collection. If used for culture, specimens should be handled using recommendations for culture (-60° C if not cultured within 24 hr).
2. Swab specimens that require shipment to off-site laboratories should be shipped at refrigerated temperature as soon as possible after collection according to the laboratory's procedures for the transport of chlamydial culture specimens. Specimens must be shipped in compliance with all applicable local, state and country regulations for the transport of etiologic agents¹⁸.

Specimen Storage

Note *Routine freezing or prolonged storage of specimens may affect performance.*

Urine Specimens (Male and Female)

Urine specimens that will not be processed within 24 hours of collection must be stored at 2-8°C and must be processed within 7 days of collection. Urine specimens that can not be processed within 7 days of collection can be stored at -20°C or lower for up to 2 months.

Swab Specimens (Male and Female) Collected in Culture Transport Media

Store swab specimens that are not tested upon receipt at the testing laboratory at 2-8°C and process within 7 days. Swab specimens that cannot be processed within 7 days of collection must be stored at -20°C or colder and tested within 30 days of collection.

Instructions For Use

Note *For detailed operating instructions, refer to the Operator's Manual for the COBAS AMPLICOR Analyzer.*

Note *All reagents must be at ambient temperature before use. Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to ensure selective amplification.*

Note *Urine and swab specimens must be at ambient temperature before use. Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to ensure selective amplification.*

Note *Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. Do not use snap cap tubes.*

Run Size Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one replicate of the *C. trachomatis* (+) Control and one replicate of the *C. trachomatis* (-) Control must be included in each test run (see "Quality Control" section).

The Specimen Preparation Reagents are packaged in single 100-test bottles. The *C. trachomatis* (+) Controls and *C. trachomatis* (-) Controls are supplied in single bottles containing enough material to prepare 8 sets of processed controls. The CT/NG Master Mix and the CT/NG Internal Control are provided in three bottles each containing enough material to perform 32 specimen runs. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.

Workflow The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* can be completed in one day or over two days. If the testing is to be completed in a single workday, follow the instructions in *Reagent Preparation, Specimen Preparation, Control Preparation, Amplification and Detection* in order. Testing can be completed over 2 days by performing Specimen Preparation and Control Preparation on Day 1, followed by Reagent Preparation, Amplification and Detection on Day 2.

Reagent Preparation**Performed in: Pre-Amplification – Reagent Preparation Area**

1. Determine the appropriate number of A-rings needed for patient specimen and control testing. Place the A-ring(s) in the A-ring holder(s).

Note

Even if CT/NG Internal Control detection will not be performed, CT/NG IC must be added to the Master Mix.

2. Prepare Working Master Mix by adding 100 µL of CT/NG IC to one vial of CT/NG MMX. Mix well by inverting 10-15 times. Working Master Mix must be stored at 2-8°C and used within 4 weeks of preparation.
3. Add 50 µL of Working Master Mix into each A-tube using a repeat pipettor or a pipettor with an aerosol barrier tip or a positive displacement tip. Do not close the covers of the A-tube(s) at this time.
4. Place the A-ring(s) containing Working Master Mix in a resealable plastic bag and seal the plastic bag securely. Move the A-ring(s) to the Pre-Amplification Specimen Preparation Area. Store the A-ring(s) containing Working Master Mix at 2-8°C in the Pre-Amplification–Specimen Preparation Area until specimen and control preparation is complete. Working Master Mix is stable for 48 hours at 2-8°C in A-tubes sealed in the plastic bag.

Specimen Preparation**Performed in: Pre-Amplification – Specimen Preparation Area****Urine Specimens (Male and Female)**

1. Label one 2.0 mL screw-cap tube for each patient specimen.
2. Add 500 µL of CT/NG URINE WASH to each of the labeled tubes.
3. Vortex urine thoroughly (3-10 seconds). If using frozen specimens, thaw the specimens at room temperature before vortexing (*volumes greater than 2 mL must be thawed overnight at 2-8°C*); continue processing even if a precipitate is present. Carefully remove caps from urine specimen containers. Take care to avoid contaminating gloves with urine in the cap. If contamination should occur, replace gloves with a clean pair before proceeding to the next specimen.
4. Add 500 µL of each well-mixed patient urine to the appropriately labeled tube containing CT/NG URINE WASH. Use a new aerosol barrier tip for each specimen. Recap the tubes and mix well by vortexing.
5. Incubate at 37°C for 15 minutes.
6. Centrifuge at $\geq 12,500 \times g$ for 5 minutes.
7. Pour off supernatant and blot each tube on a separate sheet of absorbent paper.
8. Using a new aerosol barrier pipet tip for each specimen, add 250 µL of CT/NG LYS to each tube. Recap tubes and mix well by vortexing.
9. Incubate tubes for 15 minutes at room temperature.

10. Using a new aerosol barrier pipet tip for each specimen, add 250 µL of **CT/NG DIL** to each tube. Recap tubes and mix well by vortexing.
11. Centrifuge tubes for 10 minutes at $\geq 12,500 \times g$.
12. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to the A-tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the A-tubes within 2 hours. Processed specimens stored at 2-8°C must be tested within 7 days.
13. If processed specimens were stored at 2-8°C, they must be warmed to room temperature and mixed well by vortexing prior to amplification. After vortexing, centrifuge processed specimens for 10 minutes at $\geq 12,500 \times g$.
14. Using a pipettor with an aerosol barrier tip, transfer 50 µL of the supernatant to the appropriate A-tube. Use a new aerosol barrier tip for each specimen. Be careful not to disturb the pellet (pellet may not be clearly visible). Record the positions of the patient specimens on the A-ring map. Cap the A-tubes.
15. Store the remainder of each processed specimen at 2-8°C in the event retesting is required. Any retesting must be performed within 7 days of specimen processing.

Swab Specimens (Male and Female)

Note

The COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis has been evaluated with 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), SPG CTM and M4 Culture Transport Medium (MicroTest, Inc.). The use of alternative transport media must be evaluated by the laboratory.

1. **Check that the culture transport media tube contains a swab.** Swabs should be left in the culture transport media tube to avoid mishandling. The presence of a swab in the culture transport media tube does not assure adequate specimen collection.
2. Label one 2.0 mL screw-cap tube for each patient specimen.
3. Add 100 µL of **CT/NG LYS** to the appropriately labeled 2 mL polypropylene tubes. **Do not use snap cap tubes.** Label each tube.
4. Mix specimens by vortexing. If specimens were stored frozen, thaw at room temperature before vortexing. Carefully remove caps from specimen tubes. Take care to avoid contamination of gloves. If contamination should occur, replace gloves with a clean pair before proceeding to the next specimen.
5. Using a pipettor with a new aerosol barrier tip, add 100 µL of well-mixed specimen to the appropriate tube containing **CT/NG LYS**. Use a new aerosol barrier tip for each specimen. Recap the tube and mix well by vortexing.
6. Incubate at room temperature for 10 minutes.

7. Using a new aerosol barrier pipet tip for each sample, add 200 μ L of **CT/NG DIL** to each tube. Recap the tube and mix well by vortexing.
8. Incubate at room temperature for 10 minutes.
9. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to A-tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the A-tubes within 2 hours. Processed specimens stored at 2-8°C must be tested within 7 days.
10. If processed specimens were stored at 2-8°C, they must be warmed to room temperature and mixed well by vortexing prior to amplification.
11. Using a pipettor with an aerosol barrier tip, transfer 50 μ L of each processed specimen to the appropriate A-tube. Use a new aerosol barrier tip for each specimen. Record the positions of the patient specimens on the A-ring map. Cap the A-tubes.
12. Store the remainder of each processed specimen at 2-8°C in the event retesting is required. Any retesting must be performed within 7 days of specimen processing.

Control Preparation**Performed in: Pre-Amplification - Control Preparation Area****Note**

Working Controls must be prepared fresh each day the test is performed. Working Controls can be used to prepare multiple Processed Controls during the day, but must be discarded at the end of the day.

Note

The CT (+) Control serves as the positive control for the CT Test. The NG (+) Control serves as the negative control for the CT Test. If testing both swab and urine sample types, it is necessary to prepare one set of controls for each sample type.

A. Working Controls:**Prepare the following CT (+) and CT (-) Working Controls.**

1. Using a sterile pipet tip, add 1 mL of **CT/NG DIL** to each of two, 2-mL screw-cap polypropylene tubes. Label one tube "CT (+) Working Control" and label the other tube "CT (-) Working Control".
2. Vortex the **CT (+) C** and **NG (+) C** for 5 seconds at maximum speed. Carefully remove the caps from the tubes. Take care to avoid contamination of gloves. If contamination should occur, replace gloves with a clean pair before proceeding.
3. Using a pipettor with a new aerosol barrier tip, add 100 μ L of **CT (+) C** to the tube labeled "CT (+) Working Control".
4. Using a pipettor with a new aerosol barrier tip, add 100 μ L of **NG (+) C** to the tube labeled "CT (-) Working Control".
5. Recap the tubes and mix well by vortexing. Store at room temperature and discard at the end of the workday.

B. Urine specimens: Prepare the following CT (+) and CT (–) Processed Controls.

1. Using a sterile pipet tip, add 250 µL of **CT/NG LYS** into each of two 2-mL screw-cap polypropylene tubes. Label one tube "CT (+) Processed Control" and label the other tube "CT (–) Processed Control."
2. Using a pipettor with a new aerosol barrier tip, add 250 µL of the CT (+) Working Control to the tube labeled "CT (+) Processed Control".
3. Using a pipettor with a new aerosol barrier tip, add 250 µL of the CT (–) Working Control to the tube labeled "CT (–) Processed Control".
4. Re-cap the tubes and mix well by vortexing. Incubate for 10 minutes at room temperature. Store at room temperature and discard at the end of the workday.
5. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each Processed Control to the appropriate A-tube. Record the positions of the Controls on the A-ring map. Cap the A-tubes.
6. Move the prepared samples (patient specimens and controls) in the trays to the Post-Amplification Area. *These PCR-ready samples may be stored at 2-8°C for up to 16 hours.*

C. Swab specimens: Prepare the following CT (+) and CT (–) Processed Controls.

1. Using a sterile pipet tip, add 100 µL of **CT/NG LYS** to each of two, 2-mL screw-cap polypropylene tubes. Label one tube "CT (+) Processed Control" and label the other tube "CT (–) Processed Control."
2. Using a sterile pipet tip, add 100 µL of Culture Transport Medium to each of the tubes containing **CT/NG LYS**.
3. Re-cap the tubes and mix well by vortexing.
4. Using a pipettor with a new aerosol barrier tip, add 200 µL of the CT (+) Working Control to the tube labeled "CT (+) Processed Control"
5. Using a pipettor with a new aerosol barrier tip, add 200 µL of the CT (–) Control to the tube labeled "CT (–) Processed Control".
6. Re-cap the tubes and mix well by vortexing. Incubate for 10 minutes at room temperature. Store at room temperature and discard at the end of workday.
7. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each Processed Control to the appropriate A-tube. Record the positions of the Controls on the A-ring map. Cap the A-tubes.
8. Move the prepared samples (patient specimens and controls) in the trays to the Post-Amplification Area. *These PCR-ready samples may be stored at 2-8°C for up to 16 hours.*

**Amplification and
Detection**

Performed in: Post-Amplification – Amplification/Detection Area

Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

- Wipe initialization post with a lint-free moist cloth and dry
- Wipe D-cup handler tip with a lint-free moist cloth and dry
- Check Wash Buffer Reservoir and fill if necessary
- Prepare Working Wash Buffer (1X) by adding 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3-4 liters of Wash Buffer (1X) in the Wash Buffer Reservoir of the system at all times.
- Empty waste container
- Prime the system
- During the priming, check syringes and tubing
- During the priming, check transfer tip

Prior to each run:

- Check waste container and empty if necessary
- Check Wash Buffer Reservoir and add buffer if necessary
- Replace used D-cup racks
- Prime the system

Instrument Loading and System Operation

1. Examine the quantities of reagents on board the COBAS AMPLICOR Analyzer. Prepare enough reagent cassettes to complete the workload.
2. Mix **CT PS1** well by vortexing. Add 2.5 mL **CT PS1** to the **CT4** cassette. Place the cassette on the test specific reagent rack. Discard the used **CT PS1** vial. Record date of reagent preparation on the **CT4** cassette.
3. Mix **IC PS1** well by vortexing. Add 2.5 mL **IC PS1** to the cassette containing **IC4**. Place the cassette on the test specific reagent rack. Discard the used **IC PS1** vial. Record date of reagent preparation on the **IC4** cassette.
4. Prepare the Working Substrate by pipetting 5 mL of **SB** into one **SB3** cassette. Pipet up and down to mix. Discard the empty **SB** vial. Record the date of preparation on the **SB3** cassette.
5. Place the Working Substrate in the generic reagent rack.
6. Place **DN4** and **CN4** cassettes on the generic reagent rack. Record the date each cassette was opened on each cassette.
7. Identify the reagent racks as generic or test specific using the keypad, barcode scanner, or AMPLILINK™ software as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.

8. Configure the reagent racks by inputting reagent positions and lot numbers into the instrument using the keypad, barcode scanner, or AMPLILINK software as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
9. Load the reagent racks onto the instrument using the keypad, barcode scanner, or AMPLILINK software as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.
10. Place the D-cup rack on the D-cup platform. One D-cup is required for each detection of specimen or control and two D-cups are required for each cassette of Working Substrate to allow for blanking by the COBAS AMPLICOR Analyzer.
11. Place the A-ring(s) into the thermal cycler segment(s) of the COBAS AMPLICOR Analyzer.
12. Load the A-rings into the Analyzer using the keypad, barcode scanner, or AMPLILINK software as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
13. Create an A-ring Worklist as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
14. Tightly close the cover of the thermal cycler segment(s).
15. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.
16. Wait for Analyzer to indicate that the load check has passed.

Note

The COBAS AMPLICOR Analyzer permits up to 6 separate detections to be performed on the contents of each A-tube. The required quantity of each detection reagent is calculated by the Analyzer, and a load check performed at the start of each run determines if sufficient reagents are available for the requested test.

17. Amplification and detection are automatically performed by the COBAS AMPLICOR Analyzer. Qualitative results are expressed as positive, negative or equivocal based on the absorbance value at 660 nm compared to a Test specific, predefined absorbance cut-off.

Quality Control

At least one replicate of the CT (+) Processed Control, and one replicate of the CT (-) Processed Control must be included in each test run. As with any new laboratory procedure, new operators should consider the use of additional controls each time the test is performed until such time as a high degree of confidence is reached in their ability to perform the test correctly. Each laboratory may determine appropriate target values and limits using recommended methods, e.g., NCCLS C 24-A¹⁹. There are no recommendations regarding the order of controls and specimens in the A-ring(s).

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Negative Control

The absorbance of the CT (-) Control should be less than 0.20 at 660 nm. If the absorbance of the CT (-) Control is greater than or equal to 0.20, the entire run is invalid. Repeat the entire test process (specimen and control preparation, amplification, and detection). If the absorbance of the processed CT (-) Control is consistently greater than 0.20, contact your local Roche office for technical assistance. The CT (-) Control contains nonhomologous DNA (*N.gonorrhoeae* sequences) and is intended to monitor contamination of reagents or equipment with target DNA.

Positive Control

The absorbance of the CT (+) Control should be greater than or equal to 2.0 at 660 nm. If the absorbance of the CT (+) Control is less than 2.0, the entire run is invalid. Repeat the entire test process (specimen and control preparation, amplification, and detection). If the absorbance of the CT (+) Control is consistently less than 2.0, contact your local Roche office for technical assistance.

The CT (+) Control contains approximately 20 copies/test of a *C. trachomatis* plasmid DNA sequence. This is approximately four times the minimum detection level of the assay as determined by Poisson analysis. Amplification and detection of the CT (+) Control assures that amplification occurred. The CT (+) Control will not monitor amplification efficiency or the detection level of the Test.

Validation of Culture Transport Media and Specimen Collection Swabs

All new lots of specimen collection swabs and culture transport media (CTM) that are used to transport swab specimens to the laboratory for testing by the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* should be qualified for use with the Test to ensure that the swabs and media do not contain PCR interfering substances. Contact your local Roche office to obtain a copy of the Specimen Collection Swab and Transport Media Validation Procedure.

Specimen Processing Control

To test the effectiveness of sample processing, McCoys cells infected with *C. trachomatis* (available from the American Type Culture Collection) should be added to a tube of culture transport medium to a level of 10^3 - 10^4 cells/mL in the culture transport medium, and incubated for one hour at room temperature. The spiked culture transport medium should be processed and tested using the COBAS AMPLICOR CT/NG Swab Specimen Preparation Procedure described in this Method Manual. Properly processed specimens should give positive COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results with an absorbance greater than or equal to 2.0 at 660 nm.

Internal Control

The Internal Control is intended to identify specimens that contain polymerase inhibitors. The use of the Internal Control will not eliminate all false negative test results. In clinical studies the proportion of inhibited specimens was highest for urine from females (both symptomatic and asymptomatic) and specimens (both swab and urine) from symptomatic males. Use of the IC is an option for testing specimens routinely, for testing only negative specimens, or for designated patients/specimen types in conformance with laboratory practices.

Results

Interpretation of Results

Without Internal Control Detection

Note

Refer to the Operator's Manual for the COBAS AMPLICOR Analyzer for printing results and for the interpretation of flags and comments.

1. Check run printout for flags (FLG) and comments to ensure that the run is valid. If run is invalid, repeat the entire run (specimen and control preparation, amplification and detection).
2. For a valid run, specimen results are interpreted as follows:

A ₆₆₀	COBAS Flag	Interpretation
< 0.2	NEGATIVE	C. trachomatis DNA not detected. Specimen is presumptive negative for <i>C. trachomatis</i> . A negative result does not preclude <i>C. trachomatis</i> infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
≥ 2.0	POSITIVE	C. trachomatis DNA detected. <i>C. trachomatis</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 2.0	GZ 0.2 - 2.0	Equivocal. Results are inconclusive for <i>C. trachomatis</i> DNA. Repeat testing on a new specimen from the patient or additional testing by an alternate test procedure is recommended.

With Internal Control Detection

Note

Refer to the Operator's Manual for the COBAS AMPLICOR Analyzer for printing results and for the interpretation of flags and comments.

1. Check run printout for flags (FLG) and comments to ensure that the run is valid. If run is invalid, repeat the entire run (specimen and control preparation, amplification and detection).

2. For a valid run, specimen results are interpreted as follows:

CT Specimen Result		IC Specimen Result		Interpretation
A ₆₆₀	COBAS Flag	A ₆₆₀	COBAS Flag	
< 0.2	NEGATIVE	≥ 0.2	POSITIVE	C. trachomatis DNA not detected. Specimen is presumptive negative for <i>C. trachomatis</i> . A negative result does not preclude <i>C. trachomatis</i> infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
< 0.2	NEGATIVE	< 0.2	NEGATIVE	Inhibitory Specimen. <i>C. trachomatis</i> DNA, if present, would not be detectable. Process another aliquot of the original specimen and repeat the test. Inhibitors are often labile and specimens initially inhibitory may not be inhibited when repeated. If the original specimen is not available, a new specimen must be collected.
≥ 2.0	POSITIVE	ANY	ANY	C. trachomatis DNA detected. <i>C. trachomatis</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 2.0	GZ 0.2 - 2.0	ANY	ANY	Equivocal. Results are inconclusive for <i>C. trachomatis</i> DNA. Repeat testing on a new specimen from the patient or additional testing by an alternate test procedure is recommended.

Determination of CT and Internal Control Cutoff

The cutoffs for the CT specimen results and the IC specimen results were determined based on cumulative frequency distributions of absorbance values obtained with patient specimens (male urethral swab, female endocervical swab, male urine and female urine) tested during preclinical studies and were confirmed in the clinical studies performed for the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*. These studies show that the majority of the time, a specimen with CT test results ≥ 2.0 A₆₆₀ will indicate the presence of *C. trachomatis* as shown by culture or Direct Fluorescence Antibody (DFA). A specimen with CT test results < 0.2 A₆₆₀ correlates with negative *C. trachomatis* culture results the majority of the time. Specimens with CT test results ≥ 0.2 A₆₆₀ and < 2.0 A₆₆₀ are inconclusive for the presence of *C. trachomatis* and cannot be interpreted. Similarly, the majority of the time, a specimen with IC test results ≥ 0.2 A₆₆₀ and having a CT test result < 0.2 A₆₆₀ are negative for *C. trachomatis* by culture.

Procedural Precautions

Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each Pre-amplification activity and not used for other activities or moved between areas. Gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent prepa-

ration must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times.

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, extreme care should be taken in handling kit reagents or amplification mixtures to avoid contamination. Discard any reagents that may be suspect.

Procedural Limitations

Test only the indicated specimen types. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* has been evaluated using female endocervical swabs, male urethral swabs collected in 2SP, M4 and SPG culture transport media, and female and male urine collected without preservatives. Testing of other specimen types have not been evaluated for use and may result in false negative or false positive results.

Detection of *C. trachomatis* 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* strain.

False negative results may occur due to polymerase inhibition. The CT/NG Internal Control has been added to the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* to permit the identification of processed specimens containing substances that may interfere with PCR amplification of greater than 20 copies/test.

Prevalence of chlamydial infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection. See Table 2. Because the prevalence of *C. trachomatis* may be low in some populations or patient groups, a false positive rate of 4% to 5% can exceed the true positive rate so that the predictive value of a positive test is very low (see Table 2). Since some patients that are truly infected will not be identified by testing a single specimen for culture or DFA, the true rate of false positives cannot be determined or presumed from the clinical data. The rate of false positive test results may depend on training, operator ability, reagent and specimen handling or other such factors in each laboratory.

Reliable results are dependent on specimen collection, transport, storage and processing procedures. Variables due to storage have not been completely defined. In specimen stability studies, the performance for refrigerated and frozen specimens was similar. However, in clinical studies the sensitivity was lower for male urine specimens that had been frozen and higher for female urine specimens that had been frozen.

The clinical performance of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* on specimens from pregnant women has not been evaluated.

The addition of AmpErase to the Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Method Manual are necessary to avoid contamination of reagents.

Therapeutic success or failure cannot be determined using this test.

As with any diagnostic test, results from the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* should be interpreted with consideration of all clinical and laboratory findings.

Use of this product should be limited only to personnel trained in the techniques of PCR.

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* will not detect plasmid-free variants of *C. trachomatis*.

Specimen adequacy (for swab specimens) can only be assessed by microscopic visualization of columnar epithelial cells in the specimens.

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications.

Additional testing is recommended in any circumstance when false positive or false negative results could lead to adverse medical, social or psychological consequences.

Specimen storage recommendations are based on studies including 21 culture positive patient specimens (10 swabs and 11 urines) and 52 culture negative patient specimens.

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* provides qualitative results. No correlation can be drawn between the magnitude of a positive COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* absorbance signal and the number of *C. trachomatis* cells within an infected specimen. The test detects only *C. trachomatis*, not *C. psittaci* or *C. pneumoniae*.

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* for male and female urine testing is recommended to be performed on first catch random urine specimens (defined as the first 10 to 50 mL of the urine stream). The effects of other variables such as first-catch vs. mid-stream, post-douching, etc. have not been evaluated.

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

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is not intended to replace cervical exam and endocervical sampling 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.

Interfering Substances

The presence of PCR inhibitors may cause false negative results.

Interfering substances include, but are not limited to the following:

Replens® lubricant has been shown to inhibit PCR and may yield false negative results with this Test.

The presence of mucus in cervical samples may inhibit PCR and cause false negative test results. Mucus free samples are recommended for optimal test performance. Use a sponge or a large swab to remove cervical secretions and discharge before obtaining the sample⁴.

Samples containing greater than 5% (v/v) blood may give false positive results. In clinical studies, 689 of 2306 female swab specimens were noted to be bloody. False positive rates were not higher in these specimens.

EXPECTED VALUES

Prevalence

The rate of positive *C. trachomatis* test results in patient populations varies depending upon population characteristics such as age, sex, specimen type, risk factors and test methodology. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* clinical study was performed at 6 geographically diverse urban sites in the following states: Pennsylvania, Maryland, Louisiana, Indiana, Texas, California. The study included symptomatic and asymptomatic patients from the following populations: patients attending an STD Clinic, female patients at routine OB/GYN visits, female patients at prenatal care visits, patients attending adolescent health clinics, patients at family planning visits. The rate of positive COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results in the clinical study ranged from 2.4% to 31.3%. The prevalence data from the study are shown by site in Table 1.

Table 1
COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis
Prevalence Data

Site	Sex	Symptoms	Specimen	N	Culture and DFA Results			COBAS AMPLICOR Results (with IC)			
					No Culture Positive	No. Culture Negative DFA Positive	% Culture or DFA Positive	No. Initially Inhibitory	No. Equivocal	No. Positive	% Positive
1	Female	Symptomatic	Swab	277	30	0	10.8%	3	0	35	12.7%
			Urine	274				6	3	36	13.1%
		Asymptomatic	Swab	215	34	4	17.7%	1	3	37	17.5%
			Urine	212				4	2	36	17.0%
	Male	Symptomatic	Swab	519	83	7	17.3	0	5	106	20.8%
			Urine	515				13	4	120	23.3%
		Asymptomatic	Swab	290	30	0	10.3%	0	0	36	12.5%
			Urine	289				0	1	36	12.5%
2	Female	Symptomatic	Swab	120	12	0	10.0%	2	1	17	14.3%
			Urine	120				3	0	21	17.5%
		Asymptomatic	Swab	27	2	0	7.4%	4	0	7	25.9%
			Urine	27				3	0	5	18.5%
	Male	Symptomatic	Swab	100	2	0	2.0%	4	2	23	23.5%
			Urine	97				12	3	23	23.7%
		Asymptomatic	Swab	60	2	0	3.3%	0	2	5	8.6%
			Urine	59				0	0	11	18.6%
3	Female	Symptomatic	Swab	250	14	0	5.6%	0	0	11	4.4%
			Urine	248				6	2	11	4.4%
		Asymptomatic	Swab	245	8	1	3.7%	3	2	7	2.9%
			Urine	245				7	0	7	2.9%
	Male	Symptomatic	Swab	186	26	1	14.5%	2	0	31	16.7%
			Urine	185				6	1	33	17.8%
		Asymptomatic	Swab	62	3	0	4.8%	2	1	4	6.6%
			Urine	62				0	0	4	6.5%
4	Female	Symptomatic	Swab	248	14	0	5.6%	3	0	15	6.0%
			Urine	247				20	1	12	4.9%
		Asymptomatic	Swab	437	25	1	5.9%	0	1	26	6.0%
			Urine	432				24	2	24	5.6%
5	Female	Symptomatic	Swab	59	8	0	13.6%	0	0	8	13.6%
			Urine	59				1	0	9	15.3%
		Asymptomatic	Swab	132	9	0	6.8%	0	0	11	8.3%
			Urine	132				3	0	9	6.8%
	Male	Symptomatic	Swab	32	8	0	25.0%	4	0	11	34.4%
			Urine	31				5	1	10	32.2%
		Asymptomatic	Swab	168	32	0	19.0%	3	0	35	20.8%
			Urine	168				2	0	33	19.6%
6	Female	Symptomatic	Swab	215	23	3	12.1%	1	0	28	14.1%
			Urine	208				2	3	28	13.5%
		Asymptomatic	Swab	71	3	0	4.2%	3	0	4	6.5%
			Urine	71				1	0	7	9.9%
	Male	Symptomatic	Swab	459	60	15	16.3%	14	2	68	16.6%
			Urine	454				34	3	70	15.4%
		Asymptomatic	Swab	143	10	2	8.4%	1	0	10	7.8%
			Urine	142				0	0	11	7.7%

Predictive Values

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using sensitivity and specificity of 93.0% and 96.5%, respectively, are shown in Table 2.

Table 2
COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*
Hypothetical Predictive Values at Different Prevalence Rates

Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	93.0	96.5	21.0	99.9
5	93.0	96.5	58.0	99.6
10	93.0	96.5	74.5	99.2
20	93.0	96.5	86.8	98.2

Result Distribution

The distribution of COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* absorbance values for all male and female swab specimens, and all male and female urine specimens included in a multi-site clinical study for this product are shown in Figures 1 and 2, respectively. The A_{660} values ranged from 0.000 to 4.000 for each specimen type. The histograms show a distinct separation of positive and negative test results. Figures 3 and 4 show the results for the swab specimen Internal Control and urine specimen Internal Control, respectively. The inhibitory specimens are those with results below 0.2 A_{660} .

Figure 1
Swab Specimen Initial CT Absorbance Values
Combined Male and Female Data

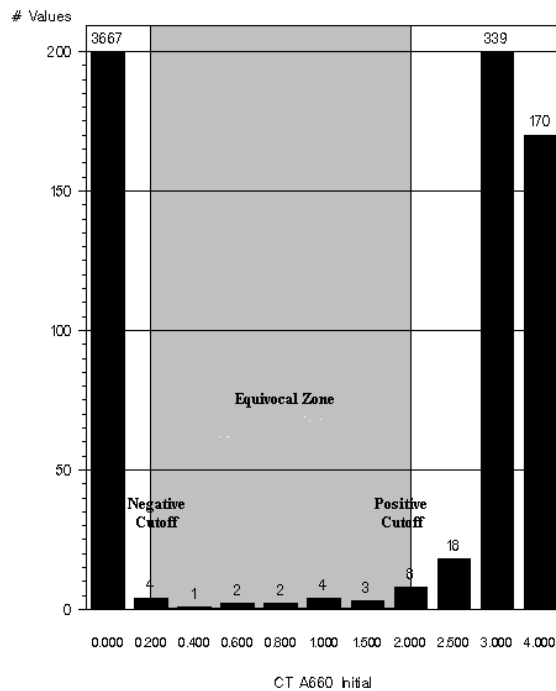


Figure 2
Urine Specimen Initial CT Absorbance Values
Combined Male and Female Data

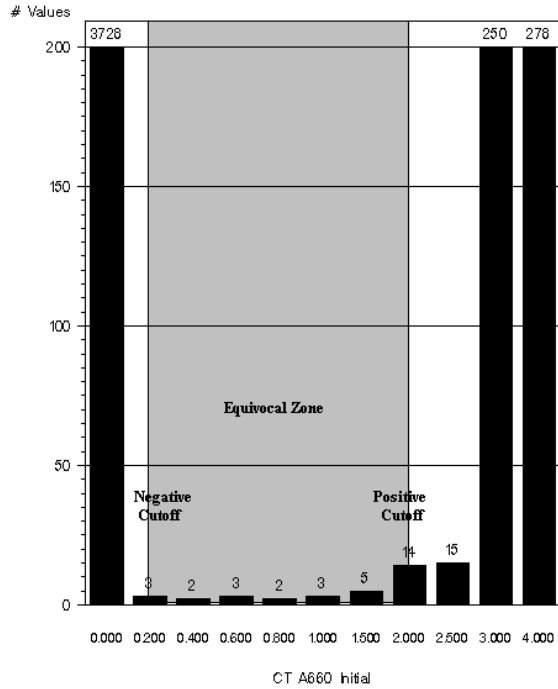


Figure 3
Swab Specimen Initial IC Absorbance Values
Combined Male and Female Data

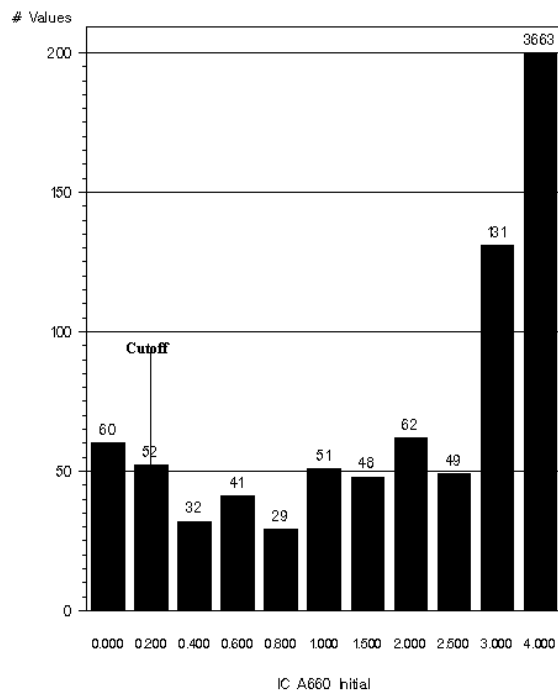
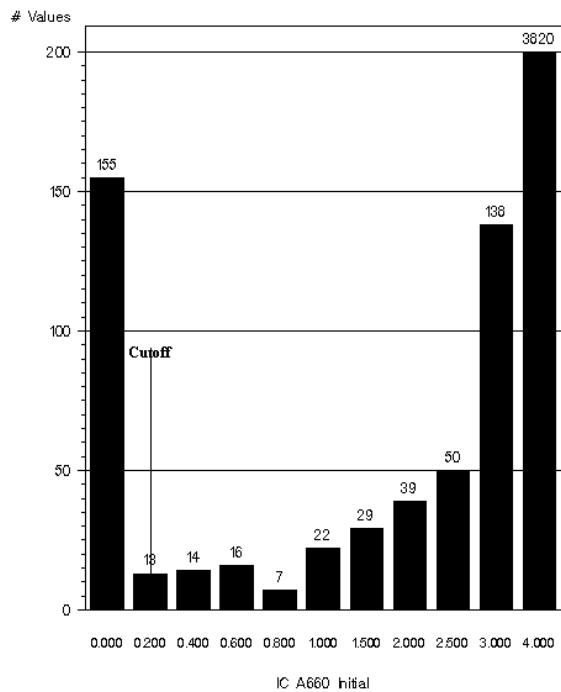


Figure 4
Urine Specimen Initial IC Absorbance Values
Combined Male and Female Data



Performance Characteristics

Analytical Specificity

The analytical specificity of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was tested against 132 bacteria, 6 fungal, 1 protozoan and 11 viral strains that may be isolated from the urogenital tract. Each isolate was added to culture transport media and normal human urine using at least 10^4 copies of genomic DNA per test (equivalent to 8×10^5 copies/mL in culture transport media and 4×10^5 copies/mL in urine specimens). The culture transport media and urine specimens were processed and tested using the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The following organisms and viruses (some of which had multiple strains tested) gave negative results by the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*:

<i>Achromobacter xerosis</i>	<i>Lactobacillus parabuchne</i>
<i>Acinetobacter calcoaceticus</i>	<i>Lactobacillus vaginalis</i>
<i>Acinetobacter genosp. 3</i>	<i>Lactococcus lactis cremoris</i>
<i>Acinetobacter lwoffii</i>	<i>Legionella bozemni</i>
<i>Actinomyces israelii</i>	<i>Legionella pneumophila</i>
<i>Aerococcus viridans</i>	<i>Leuconostoc paramesenteroides</i>
<i>Aeromonas hydrophila</i>	<i>Micrococcus luteus</i>
<i>Agrobacterium radiobacter</i>	<i>Mobiluncus curtsii subsp. curtsii</i>
<i>Alcaligenes faecalis</i>	<i>Mobiluncus curtsii subsp. homesi</i>
<i>Bacillus subtilis</i>	<i>Moraxella osloensis</i>
<i>Bacillus thuringiensis</i>	<i>Morganella morganii</i>
<i>Bacteroides caccae</i>	<i>Mycobacterium smegmatis</i>
<i>Bacterioides fragilis</i>	<i>Mycoplasma genitalium</i>
<i>Bacteroides gracilis</i>	<i>Mycoplasma hominis</i>
<i>Bifidobacillus longum</i>	<i>Mycoplasma pneumoniae</i>
<i>Bifidobacterium adolescentis</i>	<i>Neisseria cinerea</i>
<i>Branhamella catarrhalis</i>	<i>Neisseria elongata</i>
<i>Brevibacterium linens</i>	<i>Neisseria flavescens</i>
<i>Candida albicans</i>	<i>Neisseria gonorrhoeae</i>
<i>Candida glabrata</i>	<i>Neisseria kochi</i>
<i>Candida guilliermondi</i>	<i>Neisseria lactamica</i>
<i>Candida krusei</i>	<i>Neisseria meningitidis</i>
<i>Candida parapsilosis</i>	<i>Neisseria mucosa</i>
<i>Candida tropicalis</i>	<i>Neisseria perflava</i>
<i>Chlamydia pneumoniae</i>	<i>Neisseria polysaccharea</i>
<i>Chlamydia psittaci</i>	<i>Neisseria sicca</i>
<i>Chromobacter violaceum</i>	<i>Neisseria subflava</i>
<i>Citrobacter freundii</i>	<i>Paracoccus denitrificans</i>
<i>Clostridium innocuum</i>	<i>Pasteurella maltocida</i>
<i>Clostridium perfringens</i>	<i>Pediococcus acidilactica</i>
<i>Corynebacterium genitalium</i>	<i>Peptostreptococcus anaerobius</i>
<i>Corynebacterium xerosis</i>	<i>Peptostreptococcus magnus</i>
<i>Cryptococcus neoformans</i>	<i>Peptostreptococcus productus</i>
<i>Cytomegalovirus</i>	<i>Prevotella bivia</i>
<i>Deinococcus radiopugnans</i>	<i>Prevotella corporis</i>
<i>Derxia gummosa</i>	<i>Prevotella intermedia</i>
<i>Edwardsiella tarda</i>	<i>Propionibacterium acnes</i>
<i>Eikenella corrodens</i>	<i>Proteus mirabilis</i>
<i>Enterobacter cloacae</i>	<i>Providencia stuartii</i>
<i>Enterococcus avium</i>	<i>Pseudomonas aeruginosa</i>
<i>Enterococcus faecalis</i>	<i>Pseudomonas putida</i>
<i>Enterococcus faecium</i>	<i>Rahnella aquatilis</i>

<i>Epstein-Barr virus</i>	<i>Rhodospirillum rubrum</i>
<i>Erysipelothrix rhusiopathiae</i>	<i>Salmonella minnesota</i>
<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>
<i>Ewingella americana</i>	<i>Serratia marcescens</i>
<i>Flavobacterium meningosepticum</i>	<i>Staphylococcus aureus</i>
<i>Gemella haemolysans</i>	<i>Staphylococcus epidermidis</i>
<i>Gemella morbillorum</i>	<i>Streptococcus agalactiae</i>
<i>Gardnerella vaginalis</i>	<i>Streptococcus anginosus</i>
<i>Haemophilus ducreyi</i>	<i>Streptococcus bovis</i>
<i>Haemophilus influenzae</i>	<i>Streptococcus dysgalacti</i>
Herpes simplex virus 1	<i>Streptococcus mitis</i>
Herpes simplex virus 2	<i>Streptococcus equinus</i>
Human papilloma virus type 16	<i>Streptococcus mutans</i>
Human papilloma virus type 18	<i>Streptococcus pneumoniae</i>
<i>Kingella kingae</i>	<i>Streptococcus pyogenes</i>
<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	<i>Streptococcus salivarius</i>
<i>Lactobacillus acidophilus</i>	<i>Streptococcus sanguis</i>
<i>Lactobacillus brevis</i>	<i>Streptomyces griseinus</i>
<i>Lactobacillus crispus</i>	<i>Trichomonas vaginalis</i>
<i>Lactobacillus jensenii</i>	<i>Ureaplasma urealyticum</i>
<i>Lactobacillus lactis lactis</i>	<i>Vibrio parahaemolyticus</i>
<i>Lactobacillus oris</i>	<i>Yersinia enterocolitica</i>

Analytical Sensitivity

The analytical sensitivity of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was determined by testing all 15 *C. trachomatis* serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L₁, L₂, and L₃). Stock cultures of each serovar were diluted in culture transport media (CTM) and urine to prepare specimens that contained 20, 10, 5 and 1 IFU/test (equivalent to 1600, 800, 400, and 80 IFU/mL for swab specimens and 400, 200, 100, and 20 IFU/mL for urine specimens) after specimen processing. The specimens were amplified and detected according to the standard test protocol. Each processed specimen was amplified and then detected using the standard procedure. Serovars C and J were supplied at low titers that allowed testing only at 1 IFU/test. Serovar Ba was tested only at 1 and 5 IFU/test.

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* gave positive results for all serovars tested at 20, 10, 5 and 1 IFU/test. The analytical sensitivity (limit of detection) of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is 1 IFU/test (equivalent to 20 IFU/mL for urine specimens and 80 IFU/mL for CTM inoculated with a swab specimen).

Precision

A multi-operator study was performed to determine the qualitative precision of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The study was based upon the design suggested in the NCCLS document EP5A²⁰. Three independent operators at three different geographical sites tested a panel of unprocessed urine and swab specimens in duplicate, once a day, for three days. Each run consisted of specimen preparation, amplification and detection of the following specimens in duplicate (number of specimens in parenthesis): culture transport media specimens containing 0 (4), 1.25 (2), 3.75 (2) and 6.25 (2) *C. trachomatis* IFU/test; and urine specimens containing 0 (4), 1 (2), 3 (2) and 5 (2) *C. trachomatis* IFU/test. *N. gonorrhoeae* was added to some culture transport media and urine specimens to determine test performance in the presence of DNA from a non-specific analyte. Tables 3 and 4 summarize the results from this study. All qualitative results for each specimen at each site were correct.

Table 3
COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis
CTM Specimen Reproducibility

	0	<i>C. trachomatis</i> Spiked CTM (IFU/test)		
		1.25	3.75	6.25
Number of Replicates	72	36	36	36
% Correct Results	100	100	100	100
Median A ₆₆₀	0.003	3.17	3.08	3.12
Minimum A ₆₆₀	0.000	2.732	2.686	2.681
Maximum A ₆₆₀	0.018	4.000	3.610	3.962

Table 4
COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis
Urine Specimen Reproducibility

	0	<i>C. trachomatis</i> Spiked CTM (IFU/test)		
		1	3	5
Number of Replicates	72	36	36	36
% Correct Results	100	100	100	100
Median A ₆₆₀	0.003	3.454	3.294	3.391
Minimum A ₆₆₀	0.000	2.691	2.381	2.264
Maximum A ₆₆₀	0.023	4.000	4.000	4.000

Control Performance

A summary of the performance of the positive and negative kit controls in the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* tested during the clinical study is shown in Table 5. The study was performed over a 9 month period by multiple operators at 6 clinical laboratories. During the course of the study, there were a total of 49 invalid test runs due to out of range control values. There were 38 invalid results due to the CTM controls (9 positive control, 29 negative control) and 11 invalid results due to the Urine controls (5 positive control, 6 negative control).

Table 5
COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis
Control Results from Clinical Study

	<i>CTM</i>		<i>Urine</i>	
	<i>CT (+)</i>	<i>CT (-)</i>	<i>CT (+)</i>	<i>CT (-)</i>
Number of Results	643	623	628	627
Median A ₆₆₀	3.70	0.00	3.87	0.00
Mean A ₆₆₀	3.60	0.00	3.73	0.00
Minimum A ₆₆₀	2.00	0.00	2.26	0.00
Maximum A ₆₆₀	4.00	0.03	4.00	0.02
No. Invalid	9 (1.4%)	29 (4.4%)	5 (0.8%)	6 (0.9%)

Clinical Performance

The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was evaluated in a clinical study conducted at six geographically diverse sites. Swab (endocervical and urethral for females, urethral for males) and urine specimens were obtained from all patients entered into the study. Swab specimens were placed in culture transport media (CTM) used at each site. All swab specimens were tested by standard culture with cyclohexamide treated McCoy cells stained with fluorescein-labeled monoclonal antibody for *C. trachomatis*. Swab specimens that were culture negative but positive by the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* were tested by DFA for the presence of *C. trachomatis*. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was performed on all endocervical swab and urine specimens obtained from female patients, and all urethral swabs and urine specimens from male patients. The COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* was repeated for all specimens with initial results in the range of 0.2 to 0.8 A₆₆₀ and when IC results were inhibited (negative).

A total of 8523 specimens collected from 4277 patients met the criteria for inclusion in the clinical study (patient was not on antibiotics, a valid culture result was obtained, specimen met storage requirements etc.). Both a swab and urine specimen was entered into the study for 4201 patients; a urine specimen only was entered into the study from 76 patients. Of the 8523 specimens included in the study, 45 specimens gave initial test results in the Equivocal Range and were excluded from the data analyses. Two specimens that were initially inhibitory gave results in the Equivocal Range upon repeat testing. These specimens are excluded from the analyses when the Internal Control was used but included in the analyses when the Internal Control was not used. In addition, 79 specimens were repeatedly inhibitory and were excluded from the data analyses which include the use of the Internal Control because the results were not interpretable. Therefore, 8397 specimens were included in the analyses when the Internal Control result was used and a total of 8478 specimens were included in the analyses when the Internal Control results were not used.

The clinical performance of the test was evaluated by comparing the results of the 8478 swab and urine specimens to the composite results of the comparative tests (culture, sub-culture and DFA). Alternate PCR testing using oligonucleotide primers targeted for a region of the *C. trachomatis* MOMP gene was performed on COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive, culture/DFA negative specimens. The MOMP test results were not used to calculate the clinical performance characteristics of the test and are reported for information purposes only. Of the 266 COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive, culture/DFA negative specimens that were classified as false positive results in this study, 185 were positive for *C. trachomatis* when that specimen or the matching urine or swab specimen from that patient was tested by the MOMP assay. These data suggest that many specimens considered as false positive in the Clinical Data Performance Tables did contain *C. trachomatis* DNA.

The results from the clinical study are shown in Tables 6 and 7. Table 6 shows the clinical performance of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* in comparison to the endocervical culture/DFA results for female patients and to the urethral culture/DFA results for male patients. In this Table, True Positive (TP) represents the number of concordant positive culture or DFA and COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results. True Negative (TN) represents the number of concordant negative culture and COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results. False Negative (FN) represents the number of culture positive, COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* negative results. False Positive (FP) represents the number of culture and DFA negative, COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive results.

Table 7 shows the clinical performance of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* for testing both swab and urine specimens from female patients combined and separately, for each specimen type, in comparison to the patient infected status. Female patient infected status was determined by endocervical or urethral culture/DFA positive results. The data in Table 7 show that there is better concordance with culture/DFA positive patients when both swab and urine specimens are tested by the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The testing of both swab and urine specimens by the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* resulted in fewer unverified positive test results and higher assay sensitivity as compared to single specimen (swab or urine) testing only.

A summary of the test results obtained in the clinical study performed for the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* is contained in Tables 8 and 9. Table 8 summarizes the combinations of test results obtained for female patients; Table 9 summarizes the combinations of test results obtained for male patients. These tables show that patients with a positive result in both a urine and a swab specimen had a lower rate of unverified positivity (false positives relative to culture and DFA) than single positive specimen results. Testing of both specimen types may be useful for increasing the confidence in a positive result using the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*, particularly for low prevalence populations.

The clinical sensitivity and specificity of the COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* has not been reliably determined for detecting those patients with clinically active infection that can be transmitted to partners or cause Chlamydia-related sequelae. In the clinical study described here, 24.4% of COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive results were from patients with negative cultures and DFA tests. The significance of those results that were COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive, but culture and DFA negative is unknown. A proportion of these COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive specimens (63.8%) were also positive by an alternate target PCR assay; however, the performance of this alternate target assay has not been established.

Table 6
Clinical Performance of COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*
Including and Excluding the Internal Control¹

Sex	Specimen	Symptom	TP	TN	FP	FN	No. Inhib.	% Repeatedly Inhibitory	Total	Sensitivity (95% CI)	Specificity (95% CI)	MOMP+/FP
Female	CTM	Asymptomatic	75 (75)	1013 (1017)	17 (16)	4 (4)	2	0.20%	1111 (1112)	94.9% (93.6-96.2) (94.9%)	98.3% (97.6-99.1) (98.5%)	9/17 (10/16)
		Symptomatic	93 (93)	1025 (1031)	21 (21)	4 (4)	6	0.58%	1149 (1149)	95.9% (94.6-97.2) (95.9%)	98.0% (97.1-98.8) (98.0%)	11/21 (11/21)
Female	URINE	Asymptomatic	70 (70)	1011 (1023)	18 (18)	7 (8)	13	1.26%	1119 (1119)	90.9% (84.5-97.3) (89.7%)	98.3% (97.4-99.1) (98.3%)	13/18 (13/18)
		Symptomatic	84 (84)	1018 (1030)	33 (33)	9 (9)	12	1.15%	1156 (1156)	90.3% (84.3-96.3) (90.3%)	96.9% (95.8-97.9) (96.9%)	14/33 (14/33)
Total for Females			322 (322)	4067 (4101)	89 (88)	24 (25)	33	0.73%	4535 (4536)	93.1% (90.4-95.7) (92.8%)	97.9% (97.4-98.3) (97.9%)	47/89 (48/88)
.....												
Male	CTM	Asymptomatic	76 (76)	608 (612)	14 (14)	1 (1)	4	0.65%	703 (703)	98.7% (97.1-100) (98.7%)	97.7% (96.6-98.9) (97.8%)	5/14 (5/14)
		Symptomatic	183 (183)	977 (994)	56 (54)	6 (6)	14	1.40%	1236 (1237)	96.8% (94.3-99.3) (96.8%)	94.6% (93.2-96.0) (94.8%)	32/56 (32/54)
Male	URINE	Asymptomatic	71 (71)	616 (617)	24 (24)	8 (8)	1	0.16%	720 (720)	89.9% (83.2-96.5) (89.9%)	96.3% (94.8-97.7) (96.3%)	14/24 (14/24)
		Symptomatic	173 (168)	976 (1004)	83 (80)	23 (30)	27	2.63%	1282 (1282)	88.3% (83.8-92.8) (84.8%)	92.2% (90.5-93.8) (92.6%)	62/83 (60/80)
Total for Males			503 (498)	3177 (3227)	177 (172)	38 (45)	46	1.41%	3941 (3942)	93.0% (90.8-95.1) (91.7%)	94.7% (94.0-95.5) (94.9%)	113/177 (111/172)
.....												
Total for Males			503 (498)	3177 (3227)	177 (172)	38 (45)	46	1.41%	3941 (3942)	93.0% (90.8-95.1) (91.7%)	94.7% (94.0-95.5) (94.9%)	113/177 (111/172)

¹ Test results without the Internal Control shown in parentheses.

True Positive (TP) represents the number of concordant positive culture or DFA and COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results.

True Negative (TN) represents the number of concordant negative culture and COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* results.

False Negative (FN) represents the number of culture positive, COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* negative results.

False Positive (FP) represents the number of culture and DFA negative, COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive results.

Table 7
Performance of COBAS AMPLICOR CT/NG Test for Chlamydia trachomatis vs Patient Status¹ Female Patients Including and Excluding the Internal Control²

Specimen	Symptom	Total	% Inhibitory	No. Inhib.	Sensitivity	Specificity (95% CI)	MOMP+/FP
CTM + URINE	Asymp	1126 (1227)	0.00%	0	90.8% (84.7-96.9) (90.8%) (84.7-96.9)	97.4% (96.4-98.4) (97.7%) (96.9-98.6)	18/27 (18/26)
	Symptomatic	1169 (1169)	0.10%	1	95.2% (94.0-96.4) (95.2%) (94.0-96.4)	96.4% (95.3-97.5) (96.4%) (95.3-97.5)	15/38 (15/38)
CTM	Asymp	1111 (1112)	0.20%	2	87.2% (80.2-94.3) (87.2%) (80.2-94.3)	98.3% (97.6-99.1) (98.4%) (97.7-99.2)	11/17 (12/16)
	Symptomatic	1149 (1149)	0.58%	6	91.3% (85.8-96.7) (91.3%) (85.8-96.7)	98.1% (97.2-98.9) (98.1%) (97.3-98.9)	16/20 (12/20)
Totals - CTM		2260 (2261)	0.39%	8	89.4% (85.0-93.8) (89.4%) (85.0-93.8)	98.2% (97.6-98.8) (98.3%) (97.6-98.8)	27/37 (24/36)
.....							
URINE	Asymp	1119 (1119)	1.26%	13	85.7% (78.2-93.2) (84.7%) (77.1-92.4)	98.4% (97.7-99.2) (98.5%) (97.7-99.2)	13/16 (13/16)
	Symptomatic	1156 (1156)	1.15%	12	87.9% (81.4-94.3) (87.9%) (81.4-94.3)	97.1% (96.1-98.1) (97.2%) (96.2-98.2)	14/30 (14/30)
Totals - Urine		2275 (2275)	1.21	25	86.9% (82.0-91.8) (86.4%) (81.5-91.4)	97.8% (97.1-98.4) (97.8%) (97.2-98.4)	27/36 (27/36)

¹ Culture and DFA results in this table include endocervical and urethral results.

² Test results without the Internal Control shown in parentheses.

Table 8
COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*
Test Result Summary – Female Patients¹

No. Patients	Culture Status	Endocervical And Urethral Culture Results			DFA Results	COBAS AMPLICOR Results by Specimen Type	
		Endocervical Only	Urethral Only	Both Positive		Swab	Urine
146	+	85	2	59	N/A	+	+
11	+	8	0	3	N/A	+	-
7	+	2	4	1	N/A	-	+
11	+	1	8	2	N/A	-	-
6	-				+	+	+
2	-				+	+	-
1	-				+	-	-
18	-				-	+	+
18	-				-	+	-
26	-				-	-	+
1965	-				N/A	-	-

¹ Results from 85 patients without matched CTM and urine results are excluded from the table.

Table 9
COBAS AMPLICOR CT/NG Test for *Chlamydia trachomatis*
Test Result Summary – Male Patients¹

No. Patients	Urethral Culture Status	DFA Results	COBAS AMPLICOR Results By Specimen Type	
			Swab	Urine
215	+	N/A	+	+
20	+	N/A	+	-
4	+	N/A	-	-
16	-	+	+	+
3	-	+	+	-
48	-	-	+	+
17	-	-	+	-
51	-	-	-	+
2	-	-	Inhib	Inhib
1503	-	N/A	-	-

¹ Results from 140 patients without matched CTM and urine results are excluded from the table.

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