AMPLICOR® CT/NG Test for *Chlamydia trachomatis*

FOR *IN VITRO* DIAGNOSTIC USE.

AMPLICOR CT/NG Specimen Preparation Kit	CT/NG PREP	100 Tests	P/N: 20759414 122 ART: 07 5941 4 US: 83315
AMPLICOR CT/NG Amplification Kit	CT/NG AMP	96 Tests	P/N: 20759902 122 ART: 07 5990 2 US: 83319
AMPLICOR *Chlamydia trachomatis* Detection Kit	CT MWP DK	96 Tests	P/N: 20759392 018 ART: 07 5939 2 US: 83070

The following kit can be used to detect CT/NG Internal Control amplified using the AMPLICOR CT/NG Amplification Kit. Detection of the CT/NG Internal Control is a user option.

AMPLICOR Internal Control Detection Kit	IC MWP DK	96 Tests	P/N: 20751952 018 ART: 07 5195 2 US: 83068

INTENDED USE

The 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 the amplified target.

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 pathogen[1-3].

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 year[4]. In Europe, approximately 3,000,000 cases occur annually[5]. *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 culture[10]. 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 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 (microwell plate) and is used for hybridization (capture) of the amplicon. The assay detection system uses an avidin-horseradish peroxidase conjugate (HRP) that binds to biotinylated amplicon captured on the microwell plate. A hydrogen peroxide (H_2O_2) substrate and tetramethylbenzidine (TMB) chromophore are used for color formation.

Specimen Preparation

Urogenital epithelial cells, collected on swabs or pelleted from urine, are treated with a detergent solution to release 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 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 reaction 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 DNA polymerase, *Thermus aquaticus* DNA polymerase (*Taq* pol), in the presence of excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and 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. This process is repeated 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 (IC) 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 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 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[15], but not DNA strands 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 the 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 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 enzyme in the AMPLICOR CT/NG Test for *Chlamydia trachomatis* has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing *C. trachomatis* amplicon per PCR.

Hybridization Reaction

Following PCR amplification, Denaturation Solution is added to the reaction tubes to chemically denature the CT amplicon and the CT/NG Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are added to individual wells of microwell plates (MWP) coated with an oligonucleotide probe specific for *C. trachomatis* (or Internal Control, at the user's option). The biotin-labeled CT and CT/NG Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the MWP. The hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

Detection Reaction

Following the hybridization reaction, the MWP is washed to remove unbound material, and Avidin-Horseradish Peroxidase Conjugate is added to each well of the MWP. The Avidin-horseradish peroxidase conjugate binds to biotinylated amplicon hybridized to the target-specific oligonucleotide probes bound to the wells of the MWP. The MWP is washed again to remove unbound conjugate and a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added to the wells. In the presence of hydrogen peroxide, the bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The reaction is stopped by the addition of a weak acid, and the optical density at 450 nm is measured using an automated microwell plate reader.

REAGENTS

AMPLICOR CT/NG Specimen Preparation Kit — CT/NG PREP — **100 Tests**
(P/N: 20759414 122, ART: 07 5941 4; US: 83315)

CT/NG URINE WASH — 1 x 50 mL
(CT/NG Urine Wash Buffer)

Tris-HCl buffer
300 mM Sodium chloride
< 0.1% Detergent
0.09% Sodium azide

CT/NG LYS — 1 x 25 mL
(CT/NG Lysis Reagent)

Tris-HCl buffer
< 1% Solubilizer
0.09% Sodium azide

CT/NG DIL — 2 x 50 mL
(CT/NG Specimen Diluent)

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 122, ART: 07 5990 2; US: 83319)

CT/NG MMX — 3 x 1.8 mL
(CT/NG Master Mix)

Tris-HCl buffer
EDTA
100 mM Potassium chloride
Glycerol
< 0.01% AmpliTaq® (*Taq* DNA Polymerase, microbial)
< 0.016% dUTP
< 0.005% dATP, dCTP, dGTP
< 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)
< 0.0004% SS01, SS02, CP24 and CP27 primers, biotinylated
0.05% Sodium azide

CT/NG IC 3 x 0.1 mL
(CT/NG Internal Control)

Tris-HCl buffer
8 copies/µL of non-infectious plasmid DNA (microbial) containing
C. trachomatis 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 (microbial) containing
C. trachomatis sequences equivalent to approximately 20 copies/test
< 0.005% Non-specific carrier DNA (mammalian)
< 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 (microbial) containing
N. gonorrhoeae sequences equivalent to approximately 20 copies/test
< 0.005% Non-specific carrier DNA (mammalian)
< 0.5% Detergent
EDTA
0.05% Sodium azide

AMPLICOR *Chlamydia trachomatis* Detection Kit CT MWP DK **96 Tests**
(P/N: 20759392 018 ART: 07 5939 2; US: 83070)

CT MWP 1 x 96 Tests
(*C. trachomatis* Microwell Plate)

Microwell plate coated with CT-specific DNA probe
Twelve, 8-well strips in one resealable pouch with desiccant

[1] DN 1 x 12 mL
(Denaturation Solution)

1.6% Sodium hydroxide
EDTA
Thymol blue

Xi  1.6% (w/w) Sodium hydroxide

Irritant

[2] CT/NG HYB 1 x 20 mL
(CT/NG Hybridization Buffer)

Sodium phosphate solution
< 0.2% Solubilizer
< 35% Sodium thiocyanate

Xn  < 35% (w/w) Sodium thiocyanate

Harmful

[3] AV-HRP 1 x 12 mL
(Avidin-Horseradish Peroxidase Conjugate)

Tris-HCl buffer
< 0.001% Avidin-horseradish peroxidase conjugate
Bovine gamma globulin (mammalian)
Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
0.1% Phenol
1% ProClin® 150

[4A] SUB A 1 x 12 mL
(Substrate A)

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

[4B] SUB B 1 x 3 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).

[5] STOP 1 x 12 mL
(Stop Reagent)

4.9% Sulfuric acid

10X WB 2 x 90 mL
(10X-Wash Concentrate)

< 2% Phosphate buffer
< 9% Sodium chloride
EDTA
< 2% Detergent
0.5% ProClin 300

AMPLICOR Internal Control Detection Kit IC MWP DK **96 Tests**
(P/N: 20751952 018, ART: 07 5195 2; US: 83068)

IC MWP 1 x 96 Tests
(Internal Control Microwell Plate)

Microwell plate coated with IC-specific DNA probe
Twelve, 8-well strips in one resealable pouch with desiccant

[3] AV-HRP 1 x 12 mL
(Avidin-Horseradish Peroxidase Conjugate)

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

[4A] SUB A 1 x 12 mL
(Substrate A)

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

[4B] SUB B 1 x 3 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).

[5] STOP 1 x 12 mL
(Stop Reagent)

4.9% Sulfuric acid

10X WB 2 x 90 mL
(10X-Wash Concentrate)

< 2% Phosphate buffer
< 9% Sodium chloride
EDTA
< 2% Detergent
0.5% ProClin 300

WARNINGS AND PRECAUTIONS

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

B. The use of the term copy in this package insert 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.

C. 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.

D. Do not pipet by mouth.

E. 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 reagents.

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

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

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

I. Do not use a kit after its expiration date.

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

K. 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 to the Post-Amplification Area at all times.

L. 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-A[17]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled 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.

M. 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 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.

N. 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.

O. **CT/NG URINE WASH, CT/NG LYS, CT/NG DIL, CT/NG MMX, CT/NG IC, CT (+) C** and **NG (+) C** 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.

P. Wear eye protection, laboratory coats and disposable gloves when handling **[1] DN, [3] AV-HRP, [4A] SUB A, [4B] SUB B** and Working Substrate (mixed **[4A] SUB A** and **[4B] SUB B** 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.

Q. Avoid contact between the skin or mucous membranes **[4B] SUB B** and or Working Substrate. If skin contact occurs, wash immediately with large amounts of water.

R. **[4B] SUB B** 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.

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

STORAGE AND HANDLING REQUIREMENTS

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

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

C. Store **CT (+) C** and **NG (+) C** at 2-8°C. These reagents are stable until the expiration date indicated. Working Controls should be prepared fresh for use each day.

D. 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 either of these reagents during storage, warm to ambient temperature and mix thoroughly prior to use. These reagents are stable until the expiration date indicated.

E. Store **CT MWP** at 2-8°C in the foil pouch provided. **CT MWP** is stable in the unopened pouch until the expiration date indicated. Once opened, **CT MWP** is stable for 3 months (or until the expiration date, whichever comes first) in the resealed pouch containing desiccant.

F. Store **[1] DN, [2] CT/NG HYB** and **[5] STOP** at 2-25°C. These reagents are stable until the expiration date indicated.

G. Store **[3] AV-HRP, [4A] SUB A** and **[4B] SUB B** at 2-8°C. These reagents are stable until the expiration date indicated. Once opened, these reagents are stable for 3 months (or until the expiration date, whichever comes first).

H. Working Substrate must be freshly prepared each day by mixing **[4A] SUB A** with **[4B] SUB B** and is stable for 3 hours at ambient temperature when protected from light. Do not expose **[4A] SUB A, [4B] SUB B** or Working Substrate to metals, oxidizing agents or direct light.

I. Store **10X WB** at 2-25°C. **10X WB** is stable until the expiration date indicated. Working Wash Solution (1X), prepared by diluting **10X WB** 1:10 with distilled or deionized water, must be stored at 2-25°C in a clean, closed plastic container and is stable for 2 weeks from date of preparation.

MATERIALS PROVIDED

AMPLICOR CT/NG Test for *Chlamydia trachomatis*

A. **AMPLICOR CT/NG Specimen Preparation Kit** — CT/NG PREP
(P/N: 20759414 122, 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)

B. **AMPLICOR CT/NG Amplification Kit** — CT/NG AMP
(P/N: 20759902 122, 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]

C. **AMPLICOR *Chlamydia trachomatis* Detection Kit** — CT MWP DK
(P/N: 20759392 018, ART: 07 5939 2; US: 83070)

CT MWP
(*C. trachomatis* Microwell Plate)

[1] DN
(Denaturation Solution)

[2] CT/NG HYB
(CT/NG Hybridization Buffer)

[3] AV-HRP
(Avidin-Horseradish Peroxidase Conjugate)

[4A] SUB A
(Substrate A)

[4B] SUB B
(Substrate B)

[5] STOP
(Stop Reagent)

10X WB
(10X-Wash Concentrate)

D. **AMPLICOR Internal Control Detection Kit** — IC MWP DK
(P/N: 20751952 018, ART: 07 5195 2; US: 83068)

IC MWP
(Internal Control Microwell Plate)

[3] AV-HRP
(Avidin-Horseradish Peroxidase Conjugate)

[4A] SUB A
(Substrate A)

[4B] SUB B
(Substrate B)

[5] STOP
(Stop Reagent)

10X 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 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

- For Applied Biosystems GeneAmp® PCR System 9600 and 9700 thermal cyclers, use MicroAmp® Reaction Tubes (AB# N801-0533), Caps (AB# N801-0535), Tray/Retainers (AB# 403081) and Base (AB# N801-0531)
- For Applied Biosystems GeneAmp PCR System 2400 thermal cycler, use MicroAmp Reaction Tubes (AB# N801-0533), Caps (AB# N801-0535), Tray/Retainers (AB# N801-5530) and Base (AB# N801-5531)
- For Applied Biosystems 96-well GeneAmp PCR System 9700 thermal cycler, use MicroAmp Reaction Tubes (AB# N801-0533), Caps (AB# N801-0535), Tray/Retainers (AB# 403081) and Base (AB# N801-0531)
- 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
- 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

- Multichannel pipettor (capacity 25 µL and 100 µL) or electronic pipettor (Impact® or AMPLICOR)
- Aerosol barrier pipet tips (25 µL and 100 µL) and barrier-free tips (100 µL)*
- Applied Biosystems GeneAmp PCR System 9600, Applied Biosystems GeneAmp PCR System 2400 thermal cycler or Applied Biosystems GeneAmp PCR System 9700
- Microwell Plate Washer***
- Microwell Plate Reader***
- Disposable Reagent Reservoirs
- Microwell plate lid
- 96-well strip ejector, Costar® #2578
- Incubator 37°C ± 2°C
- Distilled or deionized water
- 5 mL Serological pipets
- Graduated vessels (minimum 1 L)

- 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 preparation to prevent splashing and potential cross-contamination of specimens and controls. ***Do not use snap cap tubes.***

*** Capable of washing 12 x 8 microwell format with 250-300 µL of Wash Solution per well at 30 second timed intervals.

**** Microwell Reader Specifications: Bandwidth = 10 nm ± 3 nm; Absorbance Range = 0 to ≥ 3.00 A_{450}; Repeatability ≤ 1%; Accuracy ≤ 3% from 0 to 2.00 A_{450}; Drift ≤ 0.01 A_{450} per hour.

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 the instructions below for proper specimen collection. This test is not intended for use with throat, rectal or other 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.

A. Specimen Collection

Urine Specimens (Male and Female)

NOTE: Patient must <u>not</u> 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 laboratories' 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 mucus[1].
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 laboratories collection and transport procedure. Refrigerate swab specimens if transport to the laboratory is delayed for more than one hour from the time of collection.

B. 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. Transportation of specimens must comply with country, federal, state and local regulations for the transport of etiologic agents[18].

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 laboratories procedures for the transport of chlamydial culture specimens. Transportation of specimens must comply with country, federal, state and local regulations for the transport of etiologic agents[18].

C. 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: All reagents must be at ambient temperature before use. Visually examine all reagents for sufficient reagent volume before beginning the test procedure. 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 two replicates of the AMPLICOR *C. trachomatis* (+) Control and two replicates of the AMPLICOR *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 process 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 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 (Parts A-E). If the testing is to be completed over 2 days, the procedure may be stopped after *Specimen Preparation* (Part B) or after *Amplification* (Part D).

- To perform specimen preparation on Day 1 and control preparation, amplification and detection on Day 2, perform Steps B.1.1 through B.1.11 for Urine Specimens or Steps B.2.1 through B.2.8 for Swab Specimens. Store the processed urine specimen as indicated in Step B.1.12 and the processed swab specimen as indicated in Step B.2.9. On Day 2, begin with *Reagent Preparation* (Part A), then equilibrate processed specimens to room temperature and continue with either Step B.1.13 or Step B.2.10.
- To complete specimen preparation, control preparation and amplification on Day 1 and detection on Day 2, perform *Reagent Preparation* (Part A), *Specimen Preparation* (Part B), *Control Preparation* (Part C) *Amplification* (Part D) on Day 1 and store the denatured amplicon as indicated in Step D.6. Continue with *Detection* (Part E) on Day 2.

A. Reagent Preparation

Performed in: Pre-Amplification – Reagent Preparation Area

1. Determine appropriate number of reaction tubes needed for patient specimen and control testing. Place tubes in the MicroAmp tray and lock in place with retainer.

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

2. Prepare Working Master Mix by adding 100 µL **CT/NG IC** to one vial **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 Working Master Mix into each reaction tube using a repeater pipettor or a pipettor with an aerosol barrier or positive displacement tip. Do not cap the reaction tubes at this time.
4. Place the tray containing Working Master Mix and the appropriate number of reaction tube caps in a resealable plastic bag and seal the plastic bag securely. Move to the Pre-Amplification – Specimen Preparation Area. Store the tray(s) containing Working Master Mix at 2-8°C in the Pre-Amplification – Specimen Preparation Area until specimen and control preparation is completed. Working Master Mix is stable for 48 hours at 2-8°C in reaction tubes sealed in the plastic bag.

B. Specimen Preparation

Performed in: Pre-Amplification – Specimen Preparation Area

B.1. Urine Specimens (Male and Female)

1. Label one 2.0 mL screw-cap tube for each patient specimen.
2. Add 500 µL **CT/NG URINE WASH** into 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 specimen 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 the specimen tubes at 37°C for 15 minutes.
6. Centrifuge the tubes at ≥ 12,500 x 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 **CT/NG LYS** to each tube. Recap the tubes and mix well by vortexing.
9. Incubate the tubes for 15 minutes at room temperature.
10. Using a new aerosol barrier pipet tip for each specimen, add 250 µL **CT/NG DIL** to each tube. Recap the tubes and mix well by vortexing.
11. Centrifuge tubes for 10 minutes at ≥ 12,500 x g.
12. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to the reaction tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the reaction 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 ≥ 12,500 x g.
14. Using a pipettor with an aerosol barrier tip, transfer 50 µL of the supernatant to the appropriately labeled reaction 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 a tray map. Cap the 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.

B.2. Swab Specimens (Male and Female)

NOTE: The AMPLICOR CT/NG Test for Chlamydia trachomatis has been evaluated with 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), SPG 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 **CT/NG LYS** to the appropriately labeled 2 mL polypropylene tubes. ***Do not use snap cap tubes.***

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 the specimen tubes at room temperature for 10 minutes.
7. Using a new aerosol barrier pipet tip for each specimen, add 200 µL **CT/NG DIL** to each tube. Recap the tubes and mix well by vortexing.
8. Incubate the tubes for 10 minutes at room temperature.
9. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to reaction tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the reaction 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 µL of each processed specimen to the appropriate reaction tube. Use a new aerosol barrier tip for each specimen. Record the positions of the patient specimens on a tray map. Cap the 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.

C. Control Preparation

Performed in: Pre-Amplification – Specimen 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, prepare one set of controls for each sample type.

1. Prepare the following CT (+) and CT (–) Working Controls.
   a. Using a pipettor with a sterile pipet tip, add 1 mL **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”.
   b. 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.
   c. Using a pipettor with a new aerosol barrier tip, add 100 µL **CT (+) C** to the tube labeled “CT (+) Working Control”.
   d. Using a pipettor with a new aerosol barrier tip, add 100 µL **NG (+) C** to the tube labeled “CT (–) Working Control”.
   e. Recap the tubes and mix well by vortexing. Store at room temperature and discard at the end of the workday.
2. When testing urine, prepare the following CT (+) and CT (–) Processed Controls.
   a. Using a pipettor with a sterile pipet tip, add 250 µL **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”.
   b. Using a pipettor with a new aerosol barrier tip, add 250 µL CT (+) Working Control to the tube labeled “CT (+) Processed Control”.
   c. Using a pipettor with a new aerosol barrier tip, add 250 µL CT (–) Working Control to the tube labeled “CT (–) Processed Control”.
   d. 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.
   e. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each Processed Control to the appropriately labeled reaction tube. Record the positions of the Controls on the tray map. Cap the tubes.

f. 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.***

3. When testing swab specimens, prepare the following CT (+) and CT (–) Processed Controls.

   a. Using a sterile pipet tip, add 100 µL **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."

   b. Using a sterile pipet tip, add 100 µL Culture Transport Medium to each of the tubes containing **CT/NG LYS**.

   c. Re-cap the tubes and mix well by vortexing.

   d. Using a pipettor with a new aerosol barrier tip, add 200 µL CT (+) Working Control to the tube labeled "CT (+) Processed Control".

   e. Using a pipettor with a new aerosol barrier tip, add 200 µL CT (–) Working Control to the tube labeled "CT (–) Processed Control".

   f. 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.

   g. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each Processed Control to the appropriately labeled reaction tube. Record the positions of the Controls on the tray map. Cap the tubes.

   h. 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.***

D. Amplification

Performed in: Post-Amplification – Amplification/Detection Area

NOTE: Turn on the Applied Biosystems GeneAmp PCR System 9600, GeneAmp PCR System 2400 or GeneAmp PCR System 9700 thermal cycler at least 30 minutes prior to beginning the amplification.

1. Place the Tray/Retainer assembly into the thermal cycler sample block.

2. Program the Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400 thermal cycler for the AMPLICOR CT/NG Test as follows:

HOLD Program:	2 min 50°C
HOLD Program:	5 min 95°C
CYCLE Program (35 cycles):	10 sec 91°C; 50 sec 62°C; 35 sec 72°C
HOLD Program:	5 min 72°C
HOLD Program:	72°C (**NOT TO EXCEED 24 HOURS**)

   In the CYCLE programs, the ramp times should be left at the default setting (0:00), which is the maximum rate, and the allowed setpoint error at the default setting (2°C).

   Link the 5 programs together into a METHOD program.

   Consult either the Applied Biosystems GeneAmp PCR System 9600 or GeneAmp PCR System 2400 User's Manual for additional information on programming and operation of the thermal cycler.

3. Program the Applied Biosystems GeneAmp PCR System 9700 thermal cycler for the AMPLICOR CT/NG Test for *Chlamydia trachomatis* by creating a Method as follows:

HOLD Program:	2 min 50°C
HOLD Program:	5 min 94°C
CYCLE Program (36 cycles):	20 sec 93°C; 60 sec 61°C; 40 sec 71°C
HOLD Program:	5 min 72°C
HOLD Program:	72°C FOREVER (**NOT TO EXCEED 24 HOURS**)

   In the CYCLE program, all of the up and down ramp rates must be adjusted to 50% of the default setting of 100%. From the Create screen, select the CYCLE Program with the cursor and press the **More** function to access the Modify screen. Press **Modify** from the Modify screen to open the Select Modification screen. Press **Ramp** to access the Ramp Rate Modification screen. Use the circular key to select a ramp to modify and select **50% Slower**. Repeat this setting for each of the up and down ramp rates in the CYCLE Program.

   During run set-up, set the Reaction Volume to **100 µL** by first changing the Ramp Speed from the 9600 Mode to the Max Mode. To do this, cursor down to Ramp Speed and select **Max**. Then

cursor back up to Reaction Voume and key in **100 µL**.

Consult the Applied Biosystems GeneAmp PCR System 9700 User's Manual for additional information on programming and operation of the thermal cycler.

4. Start the METHOD program. The program runs approximately 2 hours. Specimens and controls must be removed within 24 hours during the final HOLD Program.

5. Remove the tray from the thermal cycler at anytime during the final HOLD program, place in the MicroAmp Base and continue with Step 5. ***DO NOT BRING AMPLIFIED DNA INTO THE PRE-AMPLIFICATION AREA. THE AMPLIFIED CONTROLS AND SPECIMENS SHOULD BE CONSIDERED A MAJOR SOURCE OF CONTAMINATION.***

6. Remove reaction tube caps carefully to avoid aerosolizing the contents of the reaction tubes. Immediately pipet 100 µL **[1] DN** to the first column (or row) of reaction tubes using a multichannel pipettor with aerosol barrier tips and mix by pipetting up and down (AMPLICOR Electronic Pipettor, Program 1). For each column (or row), repeat this procedure using a fresh set of tips. Incubate for 10 minutes at room temperature to allow complete denaturation.

7. The denatured amplicon can be held at room temperature for no more than 2 hours before proceeding to Detection (Part D). If the detection reaction can not be performed within 2 hours, re-cap the tube and store the denatured amplicon at 2-8°C for up to one week.

E. Detection

Performed in: Post-Amplification – Amplification/Detection Area

NOTE: Follow this procedure for the detection of CT and CT/NG Internal Control amplicon. Use CT MWP and IC MWP, as appropriate, for the detection reaction. Use CT/NG HYB supplied in the AMPLICOR Chlamydia trachomatis Detection Kit for IC MWP.

1. Warm all reagents to room temperature.

2. Prepare Working Wash Solution by adding 1 volume **10X WB** to 9 volumes of distilled or deionized water. Mix well. For manual washing, prepare 40 mL Working Wash Solution for each 8-well MWP strip. For automated washing, prepare amount according to MWP washer model being used. Working Wash Solution should be stored at 2-25°C in a closed plastic container and is stable for 2 weeks from the date of preparation.

3. Allow **CT/NG MWP** and **IC MWP** to warm to room temperature before removing from the foil pouch(es). Remove the appropriate number of 8-well MWP strips from the foil package(s) and set into the MWP frame. Return unused strips to pouch and reseal making sure that the desiccant remains in the pouch. ***NOTE: MWP strips must be handled carefully to avoid breakage.*** To remove strips from the frame, center the MWP on top of the Costar 96-well strip ejector and press down evenly on the corners of the frame. To lock strips in place, place the Costar 96-well strip ejector on top of the strips and press uniformly against the strips.

4. Add 100 µL **[2] CT/NG HYB** to each well on the MWP to be tested (AMPLICOR Electronic Pipettor, Program 2).

5. If the denatured amplicon were stored at 2-8°C, incubate at 37°C for 2-4 minutes in order to reduce viscosity.

6. Using aerosol barrier tips, pipet 25 µL of denatured amplicon to the appropriate well(s) of the MWP (AMPLICOR Electronic Pipettor, Program 4). Gently tap the plate approximately 10-15 times until the color changes from blue to light yellow (this color change indicates sufficient mixing has occurred).

7. Cover MWP with MWP lid, incubate for 1 hour at 37°C ± 2°C.

8. Wash the MWP 5 times manually or by using an automated MWP washer using the Working Wash Solution.

   For manual washing:

   a. Empty contents of plate and tap dry on paper towels.
   b. Pipet Working Wash Solution to fill each well to top (250-300 µL). Let soak for 30 seconds. Empty out contents and tap dry.
   c. Repeat Step (b) 4 additional times.

   For automated washing, program washer to:

   a. Aspirate contents of wells.
   b. Fill each well to top with Working Wash Solution (approximately 250-300 µL depending on plate washer), soak for 30 seconds and aspirate dry.
   c. Repeat Step (b) 4 additional times.
   d. After automated washing is completed, tap the plate dry.

9. Add 100 µL **[3] AV-HRP** to each well (AMPLICOR Electronic Pipettor, Program 2). Cover MWP and incubate for 15 minutes at 37°C ± 2°C.

10. Wash MWP as described in Step 8.
11. Prepare Working Substrate by mixing 2.0 mL **[4A] SUB A** and 0.5 mL **[4B] SUB B** for each multiple of two 8-well microwell plate strips (16 tests). Prepare Working Substrate no more than 3 hours before use. Store at room temperature and protect from exposure to direct light.
12. Add 100 µL of Working Substrate into each well being tested (AMPLICOR Electronic Pipettor, Program 2).
13. Allow color to develop for 10 minutes at room temperature (20-25°C), in the dark.
14. Add 100 µL **[5] STOP** to each well (AMPLICOR Electronic Pipettor, Program 2).
15. Measure the absorbance at 450 nm within 1 hour of adding the **[5] STOP**. Record the absorbance value for each patient specimen and control tested.

QUALITY CONTROL

At least two replicates of the CT (+) Processed Control and two replicates of the CT (–) Processed Control must be included in each test run. When testing both swab specimens and urine specimens in the same run, test one replicate of the CT (+) Processed Control and one replicate of the CT (–) Processed Control for each specimen type. As with any new laboratory procedure, new operators should consider the use of additional controls each time the test is performed until such time that a high degree of confidence is reached in their ability to perform the test procedure correctly. Each laboratory may determine appropriate target values and limits using recommended methods, e.g., NCCLS C24-A[19]. There are no recommendations regarding the order of the controls and specimens in the MicroAmp tray.

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 0.2 at 450 nm. If the absorbance of the CT (–) Control value is greater than or equal to 0.2, 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 greater than 0.2, contact your local Roche office for technical assistance. The CT (–) Control contains nonhomologous NG 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 450 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 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 AMPLICOR CT/NG Swab Specimen Preparation Procedure described in this Package Insert. Properly processed specimens should give positive AMPLICOR CT/NG Test results for *Chlamydia trachomatis* with an absorbance greater than or equal to 2.0 at 450 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 for specimens (both swab and urine) from symptomatic males. Use of the Internal Control 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

1. Check control values to ensure that the run is valid (refer to section *"Quality Control"*). If the 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_{450}	INTERPRETATION
< 0.2	***C. trachomatis* DNA not detected.** Specimen is presumptive negative for *C. trachomatis*. A negative result does not preclude *C. trachomatis* infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
≥ 2.0	***C. trachomatis* DNA detected.** *C. trachomatis* organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 2.0	**Equivocal.** Results are inconclusive for *C. trachomatis* DNA. Repeat testing on a new specimen from the patient or additional testing by an alternate test procedure is recommended.

Interpretation of Results – With Internal Control Detection

1. Check control values to ensure that the run is valid (refer to section *"Quality Control"*). 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 Result A_{450}	IC Result A_{450}	INTERPRETATION
< 0.2	≥ 0.2	***C. trachomatis* DNA not detected.** Specimen is presumptive negative for *C. trachomatis*. A negative result does not preclude *C. trachomatis* infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
< 0.2	< 0.2	**Inhibitory Specimen.** *C. trachomatis* 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	ANY	***C. trachomatis* DNA detected.** *C. trachomatis* organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 2.0	ANY	**Equivocal.** Results are inconclusive for *C. trachomatis* 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis*. These studies show that the majority of the time, a specimen with CT test results ≥ 2.0 A_{450} will indicate the presence of *C. trachomatis* as shown by culture or Direct Fluorescence Antibody (DFA). A specimen with CT test results < 0.2 A_{450} correlates with negative *C. trachomatis* culture results the majority of the time. Specimens with CT test results ≥ 0.2 A_{450} and < 2.0 A_{450} 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 A_{450} and having a CT test result < 0.2 A_{450} are negative for *C. trachomatis* by culture.

PROCEDURAL PRECAUTIONS

1. 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.
2. 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 to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.
3. Avoid pipetting solid or particulate matter from swab specimens into the reaction tubes. The presence of solid material in the reaction tubes may cause erroneous results.

PROCEDURAL LIMITATIONS

1. Test only the indicated specimen types. The 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.
2. 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.
3. False negative results may occur due to polymerase inhibition. The CT/NG Internal Control has been added to the 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.
4. 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.
5. 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.
6. The clinical performance of the AMPLICOR CT/NG Test for *Chlamydia trachomatis* on specimens from pregnant women has not been evaluated.
7. The addition of AmpErase enzyme 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.
8. Therapeutic success or failure cannot be determined using this test.
9. As with any diagnostic test, results from the AMPLICOR CT/NG Test for *Chlamydia trachomatis* should be interpreted with consideration of all clinical and laboratory findings.
10. Use of this product should be limited only to personnel trained in the techniques of PCR.
11. The AMPLICOR CT/NG Test for *Chlamydia trachomatis* will not detect plasmid-free variants of *C. trachomatis*.
12. Specimen adequacy (for swab specimens) can only be assessed by microscopic visualization of columnar epithelial cells in the specimens.
13. The AMPLICOR CT/NG Test for *Chlamydia trachomatis* is not recommended for evaluation of suspected sexual abuse and for other medico-legal indications.

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

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

16. The AMPLICOR CT/NG Test for *Chlamydia trachomatis* provides qualitative results. No correlation can be drawn between the magnitude of a positive 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*.

17. The 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.

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

19. The 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

1. The presence of PCR inhibitors may cause false negative results.

2. 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 sample[4].

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 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 and 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* results in the clinical study ranged from 2.4% to 35.5%. The prevalence data from the study are shown by site in Table 1.

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

Site	Sex	Symptoms	Specimen	N	Culture and DFA Results			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	28	1	10.5%`	2	2	31	11.4%
			Urine	273				5	4	30	11.0%
		Asymptomatic	Swab	215	32	4	16.7%	0	2	37	17.5%
			Urine	213				4	1	36	16.9%
	Male	Symptomatic	Swab	519	83	7	17.3%	1	10	109	21.6%
			Urine	513				8	6	123	24.0%
		Asymptomatic	Swab	290	30	0	10.3%	0	2	38	13.3%
			Urine	288				0	2	42	14.6%
2	Female	Symptomatic	Swab	120	12	0	10.0%	1	2	21	17.8%
			Urine	117				2	3	19	16.2%
		Asymptomatic	Swab	27	2	0	7.4%	0	0	6	22.2%
			Urine	27				0	0	5	18.5%
	Male	Symptomatic	Swab	100	2	0	2.0%	1	3	17	17.5%
			Urine	98				4	2	27	27.6%
		Asymptomatic	Swab	60	2	0	3.3%	0	2	5	8.6%
			Urine	58				0	1	10	17.2%
3	Female	Symptomatic	Swab	250	11	0	4.4%	0	2	11	4.4%
			Urine	250				10	0	13	5.2%
		Asymptomatic	Swab	244	5	1	2.4%	0	0	6	2.5%
			Urine	245				13	1	6	2.4%
	Male	Symptomatic	Swab	186	26	1	14.5%	1	1	32	17.3%
			Urine	186				1	0	32	17.2%
		Asymptomatic	Swab	62	3	0	4.8%	1	0	3	4.8%
			Urine	62				0	0	4	6.5%
4	Female	Symptomatic	Swab	248	13	0	5.2%	3	0	14	5.6%
			Urine	247				14	1	12	4.9%
		Asymptomatic	Swab	437	23	1	5.5%	0	0	26	5.9%
			Urine	431				25	3	23	5.3%
5	Female	Symptomatic	Swab	60	7	0	11.7%	0	2	7	12.1%
			Urine	60				0	0	8	13.3%
		Asymptomatic	Swab	131	9	0	6.9%	0	1	12	9.2%
			Urine	131				0	1	9	6.9%
	Male	Symptomatic	Swab	33	8	0	24.2	2	2	11	35.5%
			Urine	33				4	0	10	30.3%
		Asymptomatic	Swab	167	32	0	19.2%	2	3	33	20.2%
			Urine	167				0	0	33	19.8%
6	Female	Symptomatic	Swab	215	23	3	12.1%	3	2	25	12.8%
			Urine	197				2	14	24	12.2%
		Asymptomatic	Swab	71	3	0	4.2%	0	0	3	4.8%
			Urine	64				3	7	3	4.7%
	Male	Symptomatic	Swab	459	60	15	16.3%	13	7	61	15.1%
			Urine	419				38	38	55	13.1%
		Asymptomatic	Swab	143	10	2	8.4%	3	3	10	7.9%
			Urine	130				1	12	7	5.4%

Predictive Values

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using sensitivity and specificity of 93.4% and 96.7%, respectively, are shown in Table 2.

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

Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	93.4	96.7	22.5	99.9
5	93.4	96.7	60.2	99.6
10	93.4	96.7	76.1	99.2
20	93.4	96.7	87.8	98.3

Result Distribution

The distribution of 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_{450} 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_{450}.

Figure 1
Swab Specimen Initial C. trachomatis Absorbance Values
Combined Male and Female Data

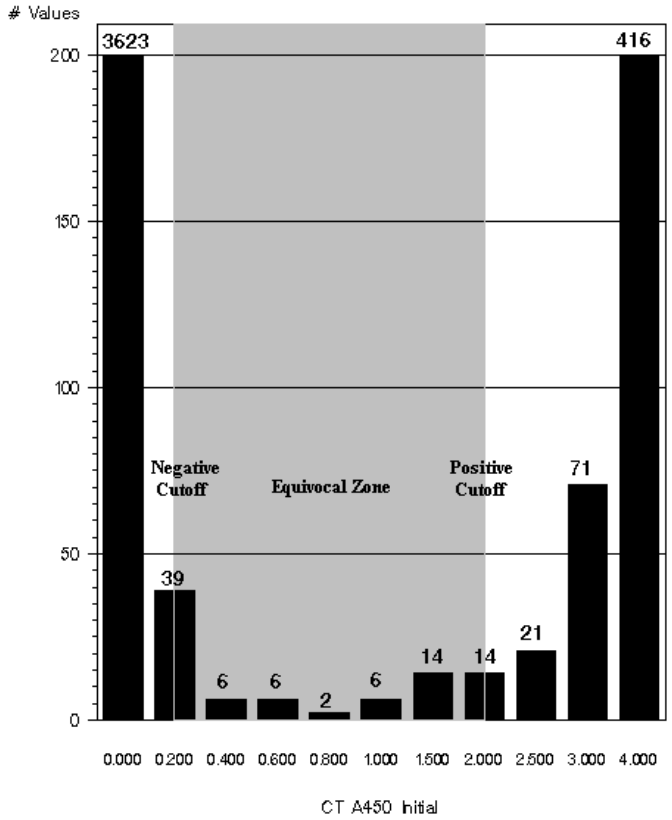


Figure 2
Urine Specimen Initial C. trachomatis Absorbance Values
Combined Male and Female Data

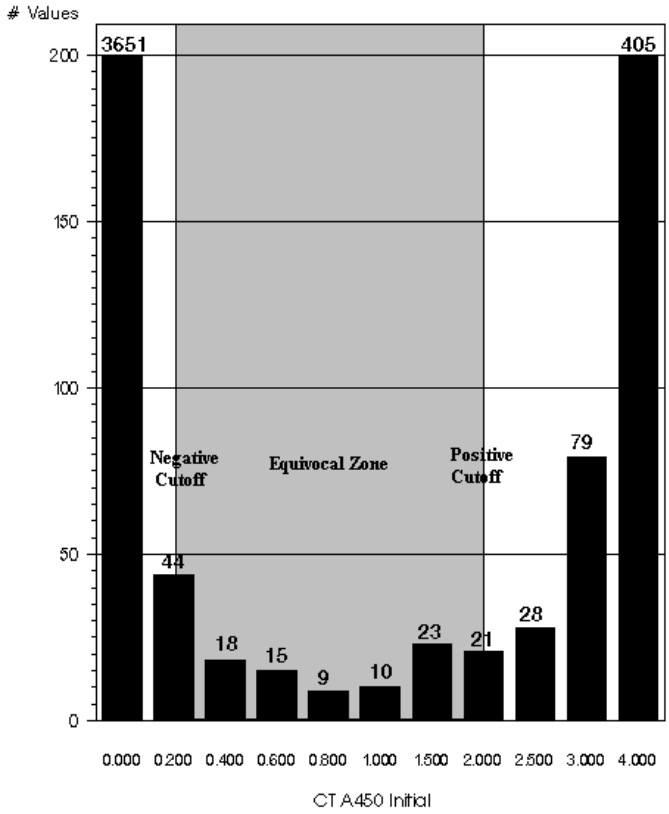


Figure 3
Swab Specimen Initial Internal Control Absorbance Values
Combined Male and Female Data

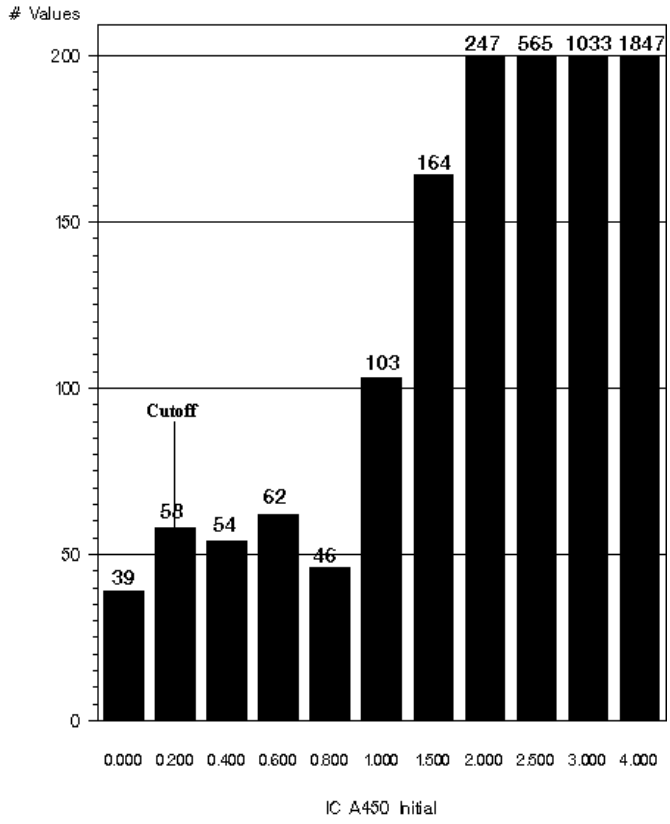
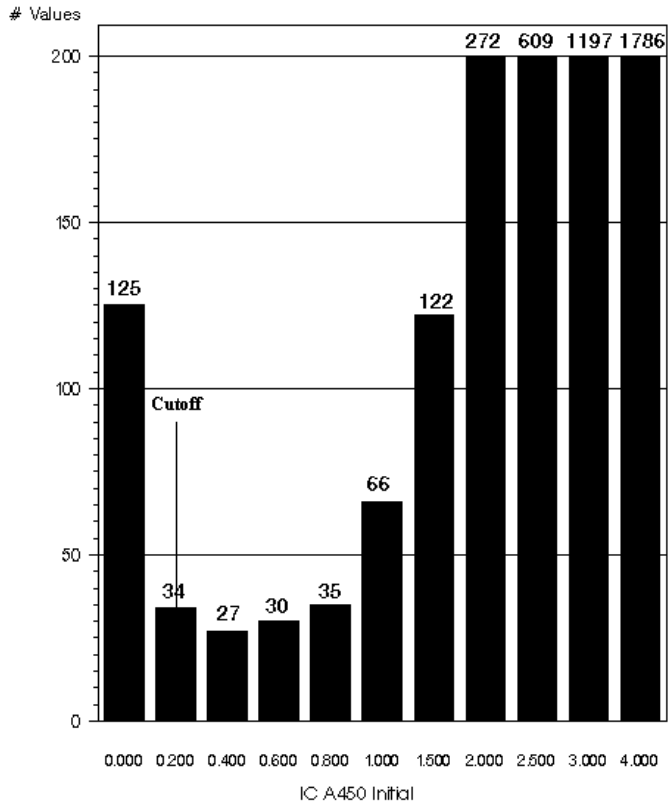


Figure 4
Urine Specimen Initial Internal Control Absorbance Values
Combined Male and Female Data



PERFORMANCE CHARACTERISTICS

A. Specificity

The analytical specificity of the AMPLICOR CT/NG Test for *Chlamydia trachomatis* was tested against 133 bacteria, 6 fungal, 1 protozoon 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 $8x10^5$ copies/mL in culture transport media and $4x10^5$ copies/mL in urine specimens). The culture transport media and urine specimens were processed and tested using the AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The following organisms and viruses (some of which had multiple strains tested) gave negative results by the AMPLICOR CT/NG Test for *Chlamydia trachomatis*:

Achromobacter xerosis
Acinetobacter calcoaceticus
Acinetobacter sp. genospecies 3
Acinetobacter lwoffi
Actinomyces israelii
Aerococcus viridans
Aeromonas hydrophila
Agrobacterium radiobacter
Alcaligenes faecalis
Bacillus subtilis
Bacillus thuringiensis
Bacteroides caccae
Bacteriodes fragilis
Bacteroides gracilis
Bifidobacillus longum
Bifidobacterium adolescentis
Branhamella catarrhalis
Brevibacterium linens
Candida albicans
Candida glabrata
Candida guilliermondi
Candida krusei
Candida parapsilosis
Candida tropicalis
Chlamydia pneumonae
Chlamydia psittaci
Chromobacter violaceum
Citrobacter freundii
Clostridium innocuum
Clostridium perfringens
Corynebacterium genitalium
Corynobacterium xerosis
Cryptococcus neoformans
Cytomegalovirus
Deinococcus radiopugnans
Derxia gummosa
Edwardsiella tarda
Eikenella corrodens
Enterobacter cloacae
Enterococcus avium
Enterococcus faecalis
Enterococcus faecium
Epstein-Barr Virus
Erysipelothrix rhusiopathiae
Escherichia coli
Ewingella americana
Flavobacterium meningosepticum
Gemella haemolysans
Gemella morbillorum
Gardnerella vaginalis
Haemophilus ducreyi
Haemophilus influenzae
Herpes simplex virus 1
Herpes simplex virus 2
Human papilloma virus type 16
Human papilloma virus type 18
Kingella kingae
Klebsiella pneumoniae ss ozaenae
Lactobacillus acidophillus
Lactobacillus brevis
Lactobacillus crisptus
Lactobacillus jensenii
Lactobacillus lactis lactis

Lactobacillus oris
Lactobacillus parabuchne
Lactobacillus vaginalis
Lactococcus lactis cremoris
Legionella bozemnii
Legionella pneumophila
Leuconostocc paramesenteroides
Micrococcus luteus
Mobiluncus curtsil ss curtsii
Mobiluncus curtsil ss homesi
Moraxella osloensis
Morganella morganii
Mycobacteruim smegmatis
Mycoplasma genitalium
Mycoplasma hominis
Mycoplasma pneumoniae
Neisseria cinerea
Neisseria elongata
Neisseria flavescens
Neisseria gonorrhoeae
Neisseria kochi
Neisseria lactamica
Neisseria meningitidis W135
Neisseria mucosa
Neisseria perflava
Neisseria polysaccharea
Neisseria sicca
Neisseria subflava
Paracoccus denitrificans
Pasteurella maltocida
Pediococcus acidilactica
Peptostreptococcus anaerobius
Peptostreptococcus magnus
Peptostreptococcus productus
Prevotella bivia
Prevotella corporis
Prevotella intermedia
Propionibacterium acnes
Proteus mirabilis
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas putida
Rahnella aquatilis
Rhodospirillum rubrum
Salmonella minnesota
Salmonella typhimurium
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus dysgalacti
Streptococcus equinis
Streptococcus mitis
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius
Streptococcus sanguis
Streptomyces griseinus
*Treponema pallidum**
Trichomonas vaginalis
Ureaplasma urealyticum
Vibrio parahaemolyticus
Yersinia enterocolitica

* Purified DNA was added to processed CTM and urine.

B. Analytical Sensitivity

The analytical sensitivity of the 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, L_1, L_2, and L_3). 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 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 of the AMPLICOR CT/NG Test for *Chlamydia trachomatis* in both CTM and urine specimens is 1 IFU/test (equivalent to 20 IFU/mL for urine specimens and 80 IFU/mL for CTM inoculated with a swab specimen).

C. Precision

A multi-operator study was performed to determine the qualitative precision of the AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The study was based upon the design suggested in the NCCLS document EP5-T2[20]. 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) *Chlamydia trachomatis* IFU/test; and urine specimens containing 0 (4), 1 (2), 3 (2) and 5 (2) *Chlamydia 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.

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

	C. trachomatis Spiked CTM (IFU/test)			
	0	***1.25***	***3.75***	***6.25***
Number of Replicates	72	36	36	36
% Correct Results	100	100	100	100
Median A_{450}	0.056	4.000	4.000	4.000
Minimum A_{450}	0.049	4.000	4.000	4.000
Maximum A_{450}	0.073	4.000	4.000	4.000

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

	C. trachomatis Spiked CTM (IFU/test)			
	0	***1***	***3***	***5***
Number of Replicates	72	36	36	36
% Correct Results	100	100†	100‡	100
Median A_{450}	0.057	4.000	4.000	4.000
Minimum A_{450}	0.049	1.491	1.893	2.538
Maximum A_{450}	0.083	4.000	4.000	4.000

† - One of 36 (2.8%) test results was in the equivocal zone

‡ - Two of 36 (5.6%) test results were in the equivocal zone

D. Control Performance

A summary of the performance of the positive and negative kit controls in the AMPLICOR CT/NG Test for *Chlamydia trachomatis* 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 8 invalid test runs due to out of range control values. There were 4 invalid results due to the CTM controls (2 positive control, 2 negative control) and 5 invalid results due to the Urine controls (3 positive control, 1 negative control).

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

	CTM		***Urine***	
	CT (+)	***CT (–)***	***CT (+)***	***CT (–)***
Number of Results	289	289	285	287
Median A_{450}	4.000	0.057	4.000	0.057
Mean A_{450}	3.878	0.060	3.844	0.058
Minimum A_{450}	2.011	0.035	2.467	0.037
Maximum A_{450}	4.000	0.189	4.000	0.103

E. Clinical Performance

The 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* were tested by DFA for the presence of *C. trachomatis*. The 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* was repeated for all specimens with initial results in the range of 0.2 to 0.8 A_{450} and when Internal Control results were inhibited (negative).

A total of 8520 specimens collected from 4208 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). Of the 8520 specimens included in the study, 142 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, 69 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, 8309 specimens were included in the analyses when the Internal Control result was used and a total of 8378 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 8378 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 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 242 AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive, culture/DFA negative specimens that were classified as false positive results in this study, 153 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 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* results. True Negative (TN) represents the number of concordant negative culture and AMPLICOR CT/NG Test for *Chlamydia trachomatis* results. False Negative (FN) represents the number of culture positive, AMPLICOR CT/NG Test for *Chlamydia trachomatis* negative results. False Positive (FP) represents the number of culture and DFA negative, AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive results.

Table 7 shows the clinical performance of the 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The testing of both swab and urine specimens by the 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 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis*, particularly for low prevalence populations.

The clinical sensitivity and specificity of the 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, 23.1% of 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 AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive, but culture and DFA negative is unknown. A proportion of these AMPLICOR CT/NG Test for *Chlamydia trachomatis* positive specimens (63.2%) 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 AMPLICOR CT/NG Test for Chlamydia trachomatis Including and Excluding the Internal Control[1]

Sex	Specimen	Symptom	TP	TN	FP	FN	No. Inhib.	% Repeat Inhibitory	Total	Sensitivity (95% CI)	Specificity (95% CI)	MOMP+/FP
Female	CTM	Asymptomatic	76 (76)	1019 (1019)	14 (14)	4 (4)	0	0.00%	1113 (1113)	95.0% (93.7-96.3) (95.0%) (93.7-96.3)	98.6% (97.9-99.3) (98.6%) (97.9-99.3)	10/14 (10/14)
		Symptomatic	94 (94)	1026 (1031)	15 (15)	1 (1)	5	0.48%	1141 (1141)	98.9% (97.6-100.0) (98.9%) (97.6-100.0)	98.6% (97.8-99.3) (98.6%) (97.8-99.3)	10/15 (10/15)
	URINE	Asymptomatic	68 (67)	1004 (1018)	14 (14)	8 (10)	15	1.46%	1109 (1109)	89.5% (82.6-96.4) (87.0%) (79.5-94.5)	98.6% (97.9-99.3) (98.6%) (97.9-99.3)	10/14 (10/14)
		Symptomatic	82 (82)	1021 (1032)	24 (23)	7 (7)	10	0.96%	1144 (1144)	92.1% (86.5-97.7) (92.1%) (86.5-97.7)	97.7% (96.8-98.6) (97.8%) (96.9-98.7)	14/24 (13/23)
Total for Females			320 (319)	4070 (4100)	67 (66)	20 (22)	30	0.73%	4507 (4507)	94.1% (91.6-96.6) (93.5%) (90.9-96.2)	98.4% (98.0-98.8) (98.4%) (98.0-98.8)	44//67 (43/66)
Male	CTM	Asymptomatic	75 (75)	598 (604)	14 (14)	1 (1)	6	0.99%	694 (694)	98.7% (97.1-100.0) (98.7%) (97.1-100.0)	97.7% (96.5-98.9) (97.7%) (96.6-98.9)	4/14 (4/14)
		Symptomatic	181 (180)	974 (986)	49 (49)	7 (8)	12	1.21%	1223 (1223)	96.3% (93.6-99.0) (95.7%) (92.9-98.6)	95.2% (93.9-96.5) (95.3%) (94.0-96.6)	31/49 (31/49)
	URINE	Asymptomatic	69 (69)	603 (603)	27 (27)	6 (6)	0	0.00%	705 (705)	92.0% (85.9-98.1) (92.0%) (85.9-98.1)	95.7% (94.1-97.3) (95.7%) (94.1-97.3)	14/27 (14/27)
		Symptomatic	162 (160)	958 (981)	85 (83)	23 (25)	21	2.10%	1249 (1249)	87.6% (82.8-92.3) (86.5%) (81.6-91.4)	91.9% (90.2-93.5) (92.2%) (90.6-93.8)	60/85 (58/83)
Total for Males			487 (484)	3133 (3174)	175 (173)	37 (40)	39	1.22%	3871 (3871)	92.9% (90.7-95.1) (92.4%) (90.1-94.6)	94.7% (93.9-95.5) (94.8%) (94.1-95.6)	109/175 (107/173)

[1] Test results without the Internal Control shown in parentheses.

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

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

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

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

Table 7
Performance of AMPLICOR CT/NG Test for Chlamydia trachomatis vs Female Patient Status Including and Excluding the Internal Control[1]

Sex	Specimen	Symptom	Total	% Inhibitory	No. Inhib.	Sensitivity (95% CI)	Specificity (95% CI)	MOMP+/FP
Female	CTM + URINE	Asymptomatic	1123 (1121)	0.00%	0	93.1% (87.8-98.4) (93.1%) (87.8-98.4)	98.0% (97.1-98.8) (98.0%) (97.1-98.8)	16/21 (16/21)
		Symptomatic	1172 (1170)	0.19%	2	94.3% (89.9-98.7) (95.2%) (94.0-96.4)	97.7% (96.7-98.6) (97.7%) (96.7-98.6)	14/25 (14/25)
	CTM	Asymptomatic	1113 (1113)	0.00%	0	87.4% (80.4-94.3) (87.4%) (80.4-94.3)	98.6% (97.9-99.3) (98.6%) (97.9-99.3)	11/14 (11/14)
		Symptomatic	1141 (1141)	0.48%	5	94.0% (89.3-98.7) (94.0%) (89.3-98.7)	98.6% (97.8-99.3) 98.6%) (97.8-99.3)	11/15 (11/15)
	Total CTM		2254 (2254)	0.24%	5	90.9% (86.8-95.0) (90.9%) (86.8-95.0)	98.6% (98.1-99.1) (98.6%) (98.1-99.1)	22/29 (22/29)
	URINE	Asymptomatic	1109 (1109)	1.46%	15	84.3% (76.5-92.2) (82.1%) (74.0-90.3)	98.8% (98.1-99.5) (98.8%) (98.2-99.5)	10/12 (10/12)
		Symptomatic	1144 (1144)	0.96%	10	89.5% (83.3-95.6) (88.4%) (82.0-94.9)	98.0% (97.1-98.8) (98.0%) (97.2-98.8)	14/21 (14/21)
	Total Urine		2253 (2253)	1.21	25	87.1% (82.2-92.0) (85.5%) (80.3-90.6)	98.4% (97.8-98.9) (98.4%) (97.9-98.9)	24/33 24/33

[1] Test results without the Internal Control shown in parentheses.

Table 8
AMPLICOR CT/NG Test for Chlamydia trachomatis
Test Result Summary - Female Patients[1]

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
140	Positive	81	0	59	N/A	Pos	Pos
11	Positive	8	0	3	N/A	Pos	Neg
8	Positive	3	4	1	N/A	Neg	Pos
9	Positive		8	1	N/A	Neg	Neg
1	Positive		1		Pos	Pos	Pos
5	Negative				Pos	Pos	Pos
2	Negative				Pos	Pos	Neg
16	Negative				Neg	Pos	Pos
11	Negative				Neg	Pos	Neg
15	Negative				Neg	Neg	Pos
1970	Negative				N/A	Neg	Neg

[1] Results from 108 patients without matched CTM and urine results are excluded from the table.

Table 9
AMPLICOR CT/NG Test for Chlamydia trachomatis
Test Result Summary - Male Patients[1]

No. Patients	Urethral Culture Status	DFA Results	COBAS AMPLICOR Results By Specimen Type	
			Swab	Urine
208	Positive	N/A	Pos	Pos
13	Positive	N/A	Pos	Neg
6	Positive	N/A	Neg	Neg
14	Negative	Pos	Pos	Pos
5	Negative	Pos	Pos	Neg
1	Negative	Pos	Neg	Pos
44	Negative	Neg	Pos	Pos
18	Negative	Neg	Pos	Neg
55[2]	Negative	Neg	Neg	Pos
1461	Negative	N/A	Neg	Neg

[1] Results from 194 patients without matched CTM and urine results are excluded from the table.

[2] 27 of the 55 specimens were positive by alternate primer (MOMP) PCR testing.

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