



AMPLICOR® CT/NG Test for *Neisseria gonorrhoeae*

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 <i>Neisseria gonorrhoeae</i> Detection Kit	NG MWP DK	96 Tests	P/N: 20759406 018 ART: 07 5940 6 US: 83071

The following kit can also be used to detect *Chlamydia trachomatis* in specimens amplified using the AMPLICOR CT/NG Amplification Kit. Detection of *Chlamydia trachomatis* is a user option.

AMPLICOR <i>Chlamydia trachomatis</i> Detection Kit	CT MWP DK	96 Tests	P/N: 20759392 018 ART: 07 5939 2 US: 83070
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The following kit can be used to detect CT/NG Internal Control amplified using the AMPLICOR CT/NG Amplification Kit. Detection of 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
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INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

Neisseria gonorrhoeae (gonococci) is the causative agent of gonorrhea. *N. gonorrhoeae* are gram-negative diplococci, cytochrome oxidase positive, non-motile and non-spore forming¹⁻³. *N. gonorrhoeae* is closely related genetically to *N. meningitidis* (meningococci), the causative agent of one type of bacterial meningitis, and slightly less related to *N. lactamica*, an occasional human pathogen. Both *N. gonorrhoeae* and *N. meningitidis* infect humans only. There are several additional species of *Neisseria* that may be considered normal flora in humans including *N. cinerea*, *N. elongata*, *N. flavescens*, *N. mucosa*, *N. sicca*, and *N. subflava*^{1,2}. Gonorrhea is one of the most frequently reported bacterial infections in the world. In the United States alone, 325,883 cases of gonorrhea were reported in 1996⁴. The total number of new cases of gonorrhea each year in the United States is estimated to be 600,000⁵. In men, most infections are symptomatic and are associated with anterior urethritis accompanied by purulent exudate. Gonococcal infections in women are often asymptomatic, and most often found in the cervix, but the vagina and uterus may also be infected^{2,6}.

Presumptive diagnosis of gonorrhea is based on: (1) observation of gram-negative intracellular diplococci in gram-stained smears of urethral discharges from men and of endocervical secretions from women; (2) growth of *N. gonorrhoeae* from the urethra (men) or endocervix on selective culture media followed by demonstration of typical colonial morphology, positive oxidase activity, and typical gram-negative diplococcal morphology; and/or (3) detection of *N. gonorrhoeae* with nonculture laboratory tests. A definitive diagnosis of gonorrhea requires (1) isolation of *Neisseria gonorrhoeae* from the sites of exposure by culture (48-72 hour cultures on selective medium), demonstration of typical colonial morphology, positive oxidase test, typical gram-negative morphology, and (2) confirmation of *N. gonorrhoeae* culture isolates by specific identification methods (acid production from carbohydrates, rapid enzyme tests, serologic assays, tests for specific nucleic acid)^{1-3,6-8}. Culture is required for determination of antimicrobial susceptibility.

PRINCIPLES OF THE PROCEDURE

The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is based on four major processes: specimen preparation; PCR amplification^{9,10} of target DNA using NG 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 (H₂O₂) substrate and tetramethylbenzidine (TMB) chromophore are used for color formation.

The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is a multiplex assay that permits the simultaneous amplification of *N. gonorrhoeae* target DNA, *C. trachomatis* target DNA, and CT/NG Internal Control (CT/NG IC) DNA. The Master Mix reagent contains biotinylated primer pairs specific for *N. gonorrhoeae* and *C. trachomatis*. The CT/NG Internal Control contains identical primer binding sequences as the *C. trachomatis* target DNA and uses the CT primers for amplification. The detection reactions are performed independently for *N. gonorrhoeae*, *C. trachomatis* and the CT/NG Internal Control.

Specimen Preparation

Urogenital epithelial cells, leukocytes and associated *N. gonorrhoeae* cells are collected on swabs or pelleted from urine. Specimens are treated with a detergent solution to lyse cells and release gonococcal DNA. A second detergent solution is then added to to prepare the specimen for amplification.

PCR Amplification

Target Selection

Neisseria gonorrhoeae contain a highly-conserved, DNA sequence (M-Ngo PII) that, based on sequence homology, apparently encodes a cytosine DNA methyltransferase, which inhibits the digestion of chromosomal DNA by *Hae* III restriction endonuclease. The M-Ngo PII gene sequence (approximately 1044 base pairs) is present in the different strains of *N. gonorrhoeae*, and not found in most other, non-gonococcal *Neisseria* species¹¹. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* uses the biotinylated primers SS01 and SS02 to define a sequence of approximately 201 nucleotides within the M-Ngo PII gene of *N. gonorrhoeae*.

Target Amplification

Processed specimens are added to the amplification mixture reaction tubes containing master mix 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 *N. gonorrhoeae* M-Ngo PII gene. As the mixture cools, the biotinylated primers anneal to the complementary sequence of *N. gonorrhoeae* 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 201-base pair double-stranded DNA molecule termed an amplicon. This process is repeated for a number of cycles, each cycle effectively doubling the amount of amplicon DNA. The AMPLICOR CT/NG Master Mix also contains a second set of biotinylated primers that co-amplify *Chlamydia trachomatis* (CT) DNA in the specimen and the CT/NG Internal Control using an analogous process to that described above for *N. gonorrhoeae*.

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 optimal 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 NG and CT target sequences, and a unique probe binding region distinct from the target amplicon. These features were selected to ensure equivalent amplification of the CT/NG Internal Control and CT/NG 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 *N. gonorrhoeae* target nucleic acid as determined by Poisson analysis. The CT/NG Internal Control is added to the Master Mix and is co-amplified with target DNA from the clinical specimen.

Selective Amplification

Selective amplification of target DNA from the clinical specimen in the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is achieved by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine¹², 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 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 *Neisseria gonorrhoeae* has been demonstrated to inactivate at least 10³ copies of deoxyuridine containing amplicon per PCR.

Hybridization Reaction

Following PCR amplification, Denaturation Solution is added to the reaction mixture to chemically denature the NG amplicon and the CT/NG Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon are added to wells of microwell plates (MWP) coated with an oligonucleotide probe specific for *N. gonorrhoeae* (SS06T5), or at the user's option, to separate wells coated with CT/NG Internal Control probe (SK535). The biotin labeled NG and CT/NG Internal Control amplicon are hybridized to the target-specific oligonucleotide probe bound microwells for each target. This hybridization of amplicon to the target-specific probe increases the overall specificity of the test. This complementary specific DNA hybridization of amplicon to target-specific probe enhances the overall specificity of the test.

Detection Reaction

Following the hybridization reaction, the MWP is washed to remove unbound reactants, 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 plate-bound, target-specific oligonucleotide probe for *N. gonorrhoeae* (or Internal Control, at the user's option). The MWP is washed again to remove unbound conjugate and Working Substrate (Substrate A mixed with Substrate B) containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide 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. The sample absorbance is compared to a specific, predefined cutoff for the detection of *N. gonorrhoeae* DNA. Separate detection reactions are performed for *N. gonorrhoeae* and the CT/NG Internal Control.

REAGENTS

AMPLICOR CT/NG Specimen Preparation Kit
(P/N: 20759414 122; ART: 07 5941 4; US: 83315)

CT/NG PREP

100 Tests

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
(P/N: 20759902 122; ART: 07 5990 2; US: 83319)

CT/NG AMP

96 Tests

CT/NG MMX
(CT/NG Master Mix)

3 x 1.8 mL

Tris-HCl buffer
EDTA
Glycerol
100 mM Potassium chloride
< 0.01% AmpliTaq[®] (*Taq* DNA Polymerase, microbial)
< 0.005% dATP, dCTP, dGTP
< 0.016% dUTP
< 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)
< 0.0004% SS01, SS02, 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 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

NG (+) C
[*N. gonorrhoeae* (+) Control]

1 x 0.8 mL

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% Non-specific carrier DNA (mammalian)
< 0.5% Detergent
EDTA
0.05% Sodium azide

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

1 x 0.8 mL

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% Non-specific carrier DNA (mammalian)
< 0.5% Detergent
EDTA
0.05% Sodium azide

AMPLICOR *Neisseria gonorrhoeae* Detection Kit
(P/N: 20759406 018; ART: 07 5940 6; US: 83071)

NG MWP DK

96 Tests

NG MWP
(NG Microwell Plate)

1 x 96 Tests

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

[1] DN
(Denaturation Solution)

1 x 12 mL

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

OPTIONAL REAGENTS

AMPLICOR Internal Control Detection Kit
(P/N: 20751952 018; ART: 07 5195 2; US: 83068)

IC MWP DK

96 Tests

IC MWP

(Internal Control Microwell Plate)

1 x 96 Tests

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

[3] AV-HRP

(Avidin-Horseradish Peroxidase Conjugate)

1 x 12 mL

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

(Substrate A)

1 x 12 mL

Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

[4B] SUB B

(Substrate B)

1 x 3 mL

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

(Stop Reagent)

1 x 12 mL

4.9% Sulfuric acid

10X WB

(10X-Wash Concentrate)

2 x 90 mL

< 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 *N. gonorrhoeae* target nucleic acid. One (1) copy is equivalent to the smallest amount of *N. gonorrhoeae* target nucleic acid that would generate a positive PCR test result.
- C. This test is for use only with endocervical specimens, male urethral specimens from symptomatic patients, and male urine specimens. This test is not intended for use with male urethral specimens from asymptomatic patients, female urine specimens, and throat, rectal, or other types of specimens.

- D. Specimens are stable for the timepoints indicated in the “Specimen Collection, Transport and Storage” section of this product insert. However, testing of all specimens at the earliest interval following collection will help ensure the most accurate test results. Multiple variable effects of storage times and variations associated with specimen shipment have not been assessed.
- E. Do not pipet by mouth.
- F. 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.
- G. Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipets and pipet tips is recommended.
- H. Do not pool reagents from different lots or from different bottles of the same lot.
- I. Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- J. Do not use a kit after its expiration date.
- K. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.
- L. 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.
- M. Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*¹³ and in the NCCLS Document M29-A¹⁴. 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.

- N. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may react with *N. subflava* and *N. cinerea*. These organisms may be common components of normal throat flora. Avoid contaminating specimen processing reagents, amplification reagents and patient specimens with respiratory aerosols.
- O. 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 *Neisseria gonorrhoeae* 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.
- P. 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.
- Q. Specimens stored frozen prior to testing with the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may result in variable performance. For male urine specimens from symptomatic patients, sensitivity of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results for specimens frozen prior to testing was reduced at one clinical site relative to sensitivity of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* testing for specimens that were stored at 2-8°C prior to testing.
- R. **CT/NG URINE WASH, CT/NG LYS, CT/NG DIL, CT/NG MMX, CT/NG IC, NG (+) C and CT (+) 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
- S. 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.
- T. Avoid contact between the skin or mucous membranes and **[4B] SUB B** or the Working Substrate. If skin contact occurs, wash immediately with large amounts of water.

- U. **[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.
- V. 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

- 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 **NG (+) C** and **CT (+) 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 **[1] DN**, **[2] CT/NG HYB** and **[5] STOP** at 2-25°C. These reagents are stable until the expiration date indicated.
- F. Store **NG MWP** at 2-8°C in the foil pouch provided. **NG MWP** is stable in the unopened pouch until the expiration date indicated. Once opened, **NG MWP** are stable for 3 months (or until the expiration date, whichever comes first) in the resealed pouch containing desiccant.
- G. Store **[3] AV-HRP**, **[4A] SUB A** and **[4B] SUB B** at 2-8°C. Unopened, 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 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. Examine the **10X WB**, and if necessary, warm at 30-37°C to redissolve any precipitate. Working Wash Solution (1X), prepared by adding 1 volume of **10X WB** to 9 volumes of deionized or distilled 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 *Neisseria gonorrhoeae*

- A. **AMPLICOR CT/NG Specimen Preparation Kit**
(P/N: 20759414 122; ART: 07 5941 4; US: 83315)

CT/NG PREP

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**
(P/N: 20759902 122; ART: 07 5990 2; US: 83319)

CT/NG AMP

CT/NG MMX
(CT/NG Master Mix)

CT/NG IC
(CT/NG Internal Control)

NG (+) C
[*N. gonorrhoeae* (+) Control]

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

C. **AMPLICOR *Neisseria gonorrhoeae* Detection Kit**
(P/N: 20759406 018; ART: 07 5940 6; US: 83071)

NG MWP DK

NG MWP

(*N. gonorrhoeae* 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)

OPTIONAL MATERIALS NOT PROVIDED

A. **AMPLICOR Internal Control Detection Kit**
(P/N: 20751952 018; ART: 07 5195 2; US: 83068)

IC MWP DK

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 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

- 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 cyclers, 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 cyclers, 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 mL polypropylene screw-cap tubes, sterile, non-siliconized, conical (e.g., 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 (e.g., 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 and 9700 thermal cyclers, Applied Biosystems GeneAmp PCR System 2400 thermal cycler or Applied Biosystems GeneAmp PCR System 9700 thermal cycler
- Microwell Plate Washer***
- Microwell Plate Reader and printer****
- 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 cylinder (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. **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 A₄₅₀; Repeatability ≤ 1%; Accuracy ≤ 3% from 0 to 2.00 A₄₅₀; Drift ≤ 0.01 A₄₅₀ 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) Male urine specimens transported in clean polypropylene containers without preservatives. **Do not use urine specimens collected in containers containing preservatives.**
- (2) Endocervical specimens from asymptomatic or symptomatic female patients and urethral swab specimens from symptomatic male patients. Specimens must be collected and transported in 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), SPG Culture Transport Medium 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 specimen types 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

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. The specimen may be transported to the test site at room temperature (18-30°C).

Swab Specimens Collected in Culture Transport Media (CTM)

1. Endocervical and male 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 for obtaining swab specimens after removing cervical mucus¹⁶.
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.

B. Specimen Transport

Urine Specimens

1. Urine specimens may be transported to the test site at 18-30°C. Urine specimens are stable for 24 hours at room temperature (18-30°C). 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 and tested within 30 days of the date of collection.
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 room temperature (18-30°C). If urine specimens are shipped at room temperature, they should be stored at 2-8°C until time of shipment to ensure that the period of room temperature 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 agents¹⁵.

Swab Specimens Collected in Culture Transport Media (CTM)

1. Refrigerate swab specimens if transport to the laboratory or if specimen processing is delayed for more than one hour from the time of collection. Swab specimens that require shipment to off-site laboratories should be shipped as soon as possible after collection according to the laboratory's procedures for the transport of Chlamydial culture specimens.
2. 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 the date of collection.

C. Specimen Storage

NOTE: Routine freezing or prolonged storage of specimens may affect performance.

Urine Specimens

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 30 days.

Specimens Collected in Culture Transport Media (CTM)

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 the date 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 one replicate of the AMPLICOR *N. gonorrhoeae* (+) Control and one replicate of the AMPLICOR *C. trachomatis* (+) Control must be included in each test run (see "Quality Control" section).

NOTE: The *C. trachomatis* (+) Control serves as the *N. gonorrhoeae* (-) Control for the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*.

The Specimen Preparation Reagents are packaged to perform 100-tests. The *N. gonorrhoeae* (+) Control and *C. trachomatis* (+) Control 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 *Neisseria gonorrhoeae* 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 on Day 1, followed by Reagent Preparation, Control Preparation, Amplification, and Detection on Day 2. Alternatively, perform Reagent Preparation, Specimen Preparation and Control Preparation; and add prepared specimens and controls to Master Mix on Day 1, followed by Amplification and Detection 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 detection of the CT/NG IC will not be performed, the Internal Control 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 (the mixture is sufficient for 32 amplifications).
3. Add 50 µL of 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 and Control Preparation Area. Store the tray(s) containing Working Master Mix at 2-8°C in the Pre-Amplification – Specimen and Control 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 and Control Preparation Area

B.1. Urine Specimens (Male)

1. Label one 2.0 mL screw-cap tube for each patient specimen. **Do not use snap cap tubes.**
2. Add 500 µL **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 on the cap. If contamination occurs, replace with a clean pair of gloves before proceeding to the next specimen.

4. Add 500 µL of each well-mixed patient urine to the appropriate 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 **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 **CT/NG DIL** to each tube. Recap the 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 reaction tubes containing Working Master Mix. Store processed specimens at 2-8°C if amplification will not be started 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. Add 50 µL of each processed specimen to the appropriately labeled reaction tube containing Working Master Mix, using a pipettor with an aerosol barrier or positive displacement tip. Use a new aerosol barrier tip for each specimen. **Be careful not to disturb the pellet (pellet may not be clearly visible)**. Cap the tubes. Record the positions of the patient specimens on a tray map.
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 *Neisseria gonorrhoeae* has been evaluated for use with 2-SP Culture Transport Medium, SPG Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), and M4 Culture Transport Medium (MicroTest, Inc.). The use of alternative transport media should be evaluated by the laboratory.

1. **Check that the Culture Transport Media tube contains a swab.** Performance specifications have not been developed for this test for specimens that do not contain swabs.
2. Label one 2.0 mL screw-cap tube for each patient specimen. **Do not use snap cap tubes.**
3. Add 100 µL **CT/NG LYS** to the appropriate labeled 2.0 mL polypropylene 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 an 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 tubes and mix well by vortexing.
6. Incubate 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 at room temperature for 10 minutes.
9. 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 amplification will not be started 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 containing Working Master Mix. Use a new aerosol barrier tip for each specimen. Cap the tubes and record the positions of the patient specimens on a tray map.
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 and 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 NG (+) C serves as the positive control for the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* and as the negative control for the AMPLICOR CT/NG Test for *Chlamydia trachomatis*. The CT (+) C serves as the negative control for the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* and as positive control for the AMPLICOR CT/NG Test for *Chlamydia trachomatis*. Therefore, when running both the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* and the AMPLICOR CT/NG Test for *Chlamydia trachomatis* on a sample, it is only necessary to prepare one set of the appropriate controls.

NOTE: If testing both swab and urine sample types, it is necessary to prepare one set of controls for each sample type.

1. Prepare the following NG (+) and NG (-) Working Controls.
 - a. Using a sterile pipet tip, add 1 mL **CT/NG DIL** to each of two, 2.0 mL screw-cap polypropylene tubes. Label one tube “NG (+) Working Control” and label the other tube “NG (-) Working Control”.
 - b. Vortex the **NG (+) C** and **CT (+) 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 **NG (+) C** to the tube labeled “NG (+) Working Control”.
 - d. Using a pipettor with a new aerosol barrier tip, add 100 µL **CT (+) C** to the tube labeled “NG (-) 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 specimens, prepare the following NG (+) and NG (-) Processed Controls.
 - a. Using a sterile pipet tip, add 250 µL **CT/NG LYS** into each of two 2.0 mL screw-cap polypropylene tubes. Label one tube “NG (+) Processed Control” and label the other tube “NG (-) Processed Control”.
 - b. Using a pipettor with a new aerosol barrier tip, add 250 µL NG (+) Working Control to the tube labeled “NG (+) Processed Control”.
 - c. Using a pipettor with a new aerosol barrier tip, add 250 µL NG (-) Working Control to the tube labeled “NG (-) 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 containing Working Master Mix. Cap the tubes and record the positions of the Controls on the tray map.
 - f. Move the prepared samples (patient specimens and controls) in the trays to the Post-Amplification – Amplification/Detection Area. **These PCR-ready samples may be stored at 2-8°C for 16 hours.**
3. When testing swab specimens, prepare the following NG (+) and NG (-) Processed Controls.
 - a. Using a sterile pipet tip, add 100 µL **CT/NG LYS** to each of two, 2.0 mL screw-cap polypropylene tubes. Label one tube “NG (+) Processed Control” and label the other tube “NG (-) 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 NG (+) Working Control to the tube labeled “NG (+) Processed Control”.
 - e. Using a pipettor with a new aerosol barrier tip, add 200 µL NG (-) Control to the tube labeled “NG (-) 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 containing Working Master Mix. Cap the tubes and record the positions of the Controls on the tray map.

- h. Move the prepared samples (patient specimens and controls) in the trays to the Post-Amplification – Amplification/Detection Area. **These PCR-ready samples may be stored at 2-8°C for 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 for *Neisseria gonorrhoeae* 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 FOREVER (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 *Neisseria gonorrhoeae* 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 Volume 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 the detection reaction. 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 NG amplicon and CT/NG Internal Control amplicon. Use NG MWP and IC MWP, as appropriate, for the detection reaction. Use CT/NG HYB supplied in the AMPLICOR Neisseria gonorrhoeae Detection Kit for IC MWP.

1. Warm all reagents to room temperature.
2. Prepare Working Wash Solution as follows. Examine **10X WB**, and if necessary, warm at 30-37°C to redissolve any precipitate. Add 1 volume of **10X WB** to 9 volumes of distilled or deionized water. Mix well. For manual washing, prepare 40 mL of 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 clean, closed plastic container and is stable for 2 weeks from the date of preparation.
3. Allow **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. 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 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 one replicate of the NG (+) Control and one replicate of the NG (-) Control must be included in each test run. The positive control will monitor for reagent failure and for correct operation of essential procedural elements (amplification, hybridization and detection). The negative control will monitor for reagent or environmental contamination. 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 C24-A¹⁷.

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

Conventional microwell plate reader and photometer absorbance measurements greater than 3.0 at 450 nm are nonlinear and cannot be used for precision calculations.

Negative Control

The absorbance value for the NG (-) Control should be less than 0.2 at 450 nm. If the absorbance of the NG (-) 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 NG (-) Control is consistently greater than 0.2, contact your local Roche office for technical assistance. The NG (-) Control contains nonhomologous DNA (*C. trachomatis* sequences) and is intended to monitor contamination of reagents or equipment with target DNA.

Positive Control

The absorbance value for the NG (+) Control should be greater than or equal to 1.5 at 450 nm. If the absorbance of the NG (+) Control is less than 1.5, the entire run is invalid. Repeat the entire test process (specimen and control preparation, amplification, and detection). If the absorbance of the NG (+) Control is consistently less than 1.5 at 450 nm, contact your local Roche office for technical assistance.

The NG (+) Control contains approximately 20 copies/test of a *N. gonorrhoeae* 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 NG (+) Control assures that amplification occurred. The AMPLICOR NG (+) 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 Culture Transport Media (CTM) that are used to transport swab specimens to the laboratory for testing by the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* must be qualified for use with the Test to ensure that the media do not contain PCR interfering substances. Contact your local Roche office to obtain a copy of the Transport Media Validation Procedure.

Specimen Processing Control

To test the effectiveness of sample processing, 10⁴ CFU *N. gonorrhoeae* (available from the American Type Culture Collection) should be added to a tube of validated Culture Transport Medium and incubated for 1 hour at room temperature. The spiked specimen should be processed and tested using the AMPLICOR CT/NG Swab Preparation Procedure as described in this Package Insert. Properly processed specimens should give positive AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results with an absorbance value greater than or equal to 3.5 at 450 nm.

Internal Control

The CT/NG Internal Control is intended to identify specimens that contain polymerase inhibitors and that do not support minimal amplification and detection (20 copies) of an exogenous target sequence. Effects of reduced amplification and detection of the IC (A_{450} values < 3.5) have not been determined. High levels of *Chlamydia trachomatis* target DNA (2.5×10^5 copies/Test) have been shown to cause a decrease in the amplification of the Internal Control target and result in reduced A_{450} values for the IC. 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 swab specimens from females (both symptomatic and asymptomatic) and urine specimens from symptomatic males. Use of the IC is recommended in order to obtain adequate performance with urine specimens from male patients. Use of the IC is an option for testing swab specimens from symptomatic and asymptomatic females and from symptomatic males, for testing only negative specimens, and for testing designated patients/specimen types in conformance with laboratory practices.

RESULTS

NOTE: The magnitude of the measured absorbance above the cutoff is not indicative of organism load.

Interpretation of Results – Without Internal Control Detection

1. Ensure that the control values for the run are valid. If run is invalid, repeat the entire run (specimen and control preparation, amplification and detection).
2. For a valid run, specimens results are interpreted as follows:

A ₄₅₀	INTERPRETATION
< 0.2	<i>N. gonorrhoeae</i> DNA not detected. Specimen is presumptive negative for <i>N. gonorrhoeae</i> . A negative result does not preclude <i>N. gonorrhoeae</i> infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
≥ 3.5	<i>N. gonorrhoeae</i> DNA detected. <i>N. gonorrhoeae</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 3.5	Equivocal. Results are inconclusive for <i>N. gonorrhoeae</i> DNA and additional patient testing is necessary. Process another aliquot of the original specimen and repeat the test in duplicate. Interpret results based on all three results (initial and duplicate repeats).* Alternatively, culture may be performed on the patient specimen.

* The final test interpretation of these specimens should be determined using 2.0 A₄₅₀ as the cutoff. A specimen yielding an initial equivocal result (GZ 0.2-3.5) must have duplicate repeat testing performed on another aliquot of the original specimen prior to reporting the final result. Specimens with at least 2 of 3 results (initial and duplicate repeats) with A₄₅₀ greater than or equal to 2.0 should be considered positive for *N. gonorrhoeae*. If 2 of 3 results have an A₄₅₀ less than 2.0, the specimen is presumptive negative for *N. gonorrhoeae*.

Interpretation of Results – With Internal Control Detection

1. Ensure that the control values for the run are valid. If run is invalid, repeat the entire run (specimen and control preparation, amplification and detection).
2. For a valid run, specimens are interpreted as follows:

NG Result A ₄₅₀	IC Result A ₄₅₀	INTERPRETATION
< 0.2	≥ 0.2	<i>N. gonorrhoeae</i> DNA not detected. Specimen is presumptive negative for <i>N. gonorrhoeae</i> . A negative result does not preclude <i>Neisseria gonorrhoeae</i> infection because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
< 0.2	< 0.2	Inhibitory Specimen. <i>N. gonorrhoeae</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.
≥ 3.5	ANY	<i>N. gonorrhoeae</i> DNA detected. <i>N. gonorrhoeae</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
≥ 0.2, < 3.5	ANY	Equivocal. Results are inconclusive for <i>N. gonorrhoeae</i> DNA and additional patient testing is necessary. Process another aliquot of the original specimen and repeat the test in duplicate. Interpret results based on all three test results (initial and duplicate repeats) as described in Step 3 below. Alternatively, culture may be performed on the patient specimen.

- For a **valid** run, specimens with an equivocal result ($A_{450} \geq 0.2, < 3.5$) must have duplicate repeat testing performed on another aliquot of the original specimen, regardless of the IC result. The final test interpretation of these specimens should be determined using an absorbance value of 2.0 at 450 nm as the cutoff. Interpretation of repeat test results must be done according to the following tables:

Step 1 - Determine whether each repeat result is valid

Repeat NG Test Result	Repeat IC Test Result	Interpretation of Assay Result
NG Result ≥ 0.2	Any IC Result	VALID
NG Result < 0.2	IC Result ≥ 0.2	VALID
NG Result < 0.2	IC Result < 0.2	INVALID

Step 2 - Determine specimen result

Initial NG Test Result and Repeat NG Test Results	Interpretation of Specimen Result
2 or more VALID test results ≥ 2.0	<i>N. gonorrhoeae</i> DNA detected.
2 or more VALID test results < 2.0	<i>N. gonorrhoeae</i> DNA not detected.
a) one VALID result ≥ 2.0 , one VALID result < 2.0 and one INVALID test result. OR b) 2 or more INVALID test results.	Results are inconclusive for <i>N. gonorrhoeae</i> DNA.

Determination of NG and Internal Control Cutoff

The cutoffs for the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* specimen results and the IC specimen results were determined based on cumulative frequency distributions of absorbance values obtained with patient specimens (male urethral swabs from symptomatic males, female endocervical swabs, male urine). The majority of the time, a specimen with AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results $\geq 3.5 A_{450}$ indicates the presence of *N. gonorrhoeae* as shown by culture. A specimen with NG test results $< 0.2 A_{450}$ on initial test correlates with negative *N. gonorrhoeae* culture results the majority of the time. Results from supplemental testing have shown that specimens with values $\geq 0.2 A_{450}$ and $< 3.5 A_{450}$ have a decreased likelihood of being true positive compared to results above $\geq 3.5 A_{450}$.

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 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.
- 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.
- 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.
- The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may detect non-pathogenic isolates of *N. subflava* and *N. cinerea*. These organisms may be a component of normal throat flora. Avoid contaminating specimen processing reagents, amplification reagents, and patient specimens with respiratory aerosols.

PROCEDURAL LIMITATIONS

1. Performance has only been established for the indicated specimen types (symptomatic male swabs, male urine, female endocervical swab specimens). Although evaluated with urine from females and swabs from asymptomatic males, the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* had reduced performance and is not recommended for testing female urine specimens or asymptomatic male swab specimens.
2. The interpretation of AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results for asymptomatic males requires caution due to the low prevalence of infection in this patient group and the short duration of asymptomatic infection¹⁸. Predictive values for a positive result will be lower in this group; the confidence of the sensitivity estimate for this group is reduced due to the low number of culture positive asymptomatic men sampled (1.6%, 11/689).
3. Prevalence of gonococcal 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 *N. gonorrhoeae* 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, 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. Culturing should be considered for confirmation and is required for antimicrobial susceptibility testing and retention for medico-legal purposes.
4. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* 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.
5. Oropharyngeal aerosols or other sources of oropharyngeal contamination have a high probability of causing false-positive results for *N. gonorrhoeae*. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may detect non-pathogenic isolates of *N. subflava* and *N. cinerea*. These organisms may be a component of normal throat flora. Avoid contaminating specimen processing reagents, amplification reagents, and patient specimens with respiratory aerosols.
6. Detection of *N. gonorrhoeae* is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, history of STD, presence of symptoms), stage of infection and/or infecting *Neisseria gonorrhoeae* strain.
7. False negative results may occur due to polymerase inhibition. The CT/NG IC has been added to the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* to permit the identification of processed specimens containing substances that may interfere with PCR amplification of greater than 20 copies/test.
8. 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 Package Insert are necessary to avoid contamination of reagents.
9. Therapeutic success or failure cannot be determined using this test.
10. As with any diagnostic test, results from the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* should be interpreted with consideration of all clinical and laboratory findings.
11. Use of this product should be limited only to personnel trained in the techniques of PCR.
12. Specimen storage recommendations are based on studies including 21 culture positive patient specimens (10 swabs and 11 urines) and 52 culture negative patient specimens.
13. Population and site variables, as well as testing frozen specimens may affect performance. Performance may vary according to practices in each laboratory and characteristics of the patient population. No differences in the performance of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* were noted when 20 culture positive specimens were stored at different temperatures including at -20°C or below. However, in clinical studies reduced sensitivity of AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results was observed for frozen urines from symptomatic males. Since the majority of the frozen specimens were tested at one site, it is not known if this observation is due to a site variable or a difference in AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* performance between testing fresh and frozen specimens. Table 6 contains overall performance (results from all sites and for specimens tested after freezing are pooled). Footnotes show the one specimen type where there may have been a site or storage difference (95% Confidence Intervals overlap, but point estimates not included in the overlap).
14. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* provides qualitative results. No correlation can be drawn between the magnitude of a positive AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* absorbance signal and the number of *N. gonorrhoeae* cells within an infected specimen.

15. The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* for male 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 have not been evaluated.
16. The type and volume of Culture Transport Media used to transport swab specimens to the laboratory for testing by the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may cause variable effects on the performance of the Test.

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® and Astroglide™ lubricants and talcum powder have 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.

Table 1
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Prevalence Data

Site	Sex	Symptoms	Specimen	N	No. Culture Positive [‡]	AMPLICOR Results (with IC)			
						No. Initially Inhibitory	No. Equivocal	No. Positive	% Positive
1	Female	Symptomatic	Swab	277	33	5	7	34	12.4%
		Asymptomatic		214	36	6	12	39	18.3%
	Male	Symptomatic	Swab	519	158	0	4	158	30.7%
		Asymptomatic	Urine	519	5	2	6	159	30.6%
2	Female	Symptomatic	Swab	120	7	6	6	10	8.3%
		Asymptomatic		27	2	1	1	4	14.8%
	Male	Symptomatic	Swab	100	33	0	1	33	33.0%
		Asymptomatic	Urine	100	3	2	2	32	32.2%
3	Female	Symptomatic	Swab	240	5	1	0	5	2.1%
		Asymptomatic		230	2	0	1	2	0.9%
	Male	Symptomatic	Swab	182	18	0	1	20	11.0%
		Asymptomatic	Urine	182	0	1	1	18	9.9%
4	Female	Symptomatic	Swab	244	3	4	4	4	1.6%
		Asymptomatic		434	6	0	6	9	2.1%
	Male	Symptomatic	Swab	64	2	0	5	3	4.7%
		Asymptomatic		136	1	0	7	3	2.2%
5	Female	Symptomatic	Swab	33	14	1	19	17	51.5%
		Asymptomatic		33	2	0	4	3	1.8%
	Male	Symptomatic	Swab	33	2	0	4	3	1.8%
		Asymptomatic	Urine	167	2	0	4	3	1.8%
6	Female	Symptomatic	Swab	211	26	5	18	29	14.9%
		Asymptomatic		68	3	0	2	4	6.7%
	Male	Symptomatic	Swab	458	137	1	51	150	36.3%
		Asymptomatic	Urine	370	1	22	56	111	29.7%
6	Male	Symptomatic	Urine	112	1	0	16	4	3.6%
		Asymptomatic		112	1	0	16	4	3.6%

[‡] For symptomatic males, swab culture results are applicable to AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results with both swab and urine specimens. For asymptomatic males, swab culture results are listed with AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results for urine, since there are no corresponding AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* swab results.

Predictive Values

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using sensitivity and specificity of 96.4% and 97.9%, respectively, are shown in Table 2.

Table 2
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Hypothetical Predictive Values at Different Prevalence Rates

Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	96.4	97.9	31.7	99.9
5	96.4	97.9	70.8	99.8
10	96.4	97.9	83.7	99.6
20	96.4	97.9	92.0	99.1

Result Distribution

The distribution of AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* absorbance values from combined symptomatic and asymptomatic female swab specimens and symptomatic male swab specimens, and symptomatic and asymptomatic male urine specimens are shown in Figures 1 and 2, respectively. The A_{450} values observed for 3481 swab specimens and 1893 urine specimens tested in the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* clinical study 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 values below 0.2 A_{450} .

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

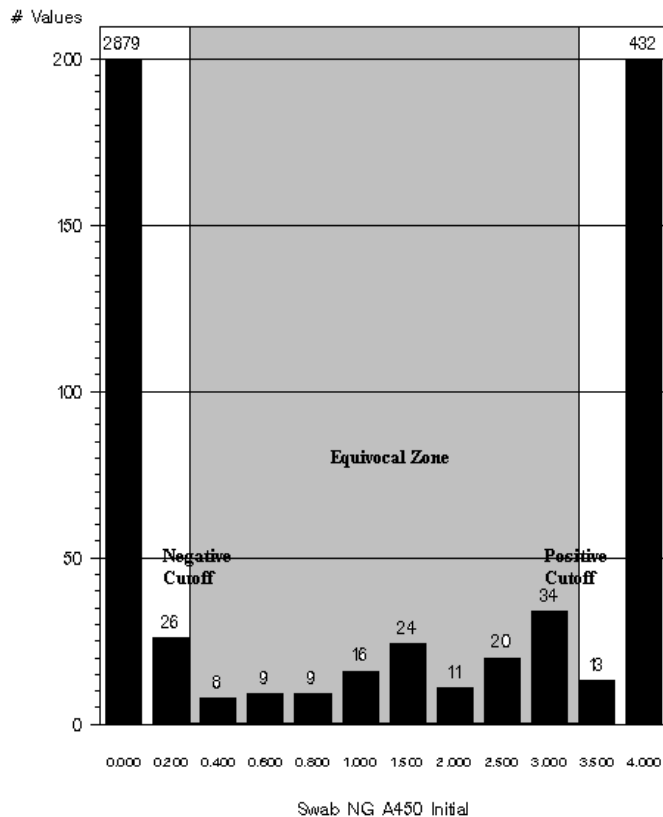


Figure 2
Urine Specimen Initial NG Absorbance Values
Male Data

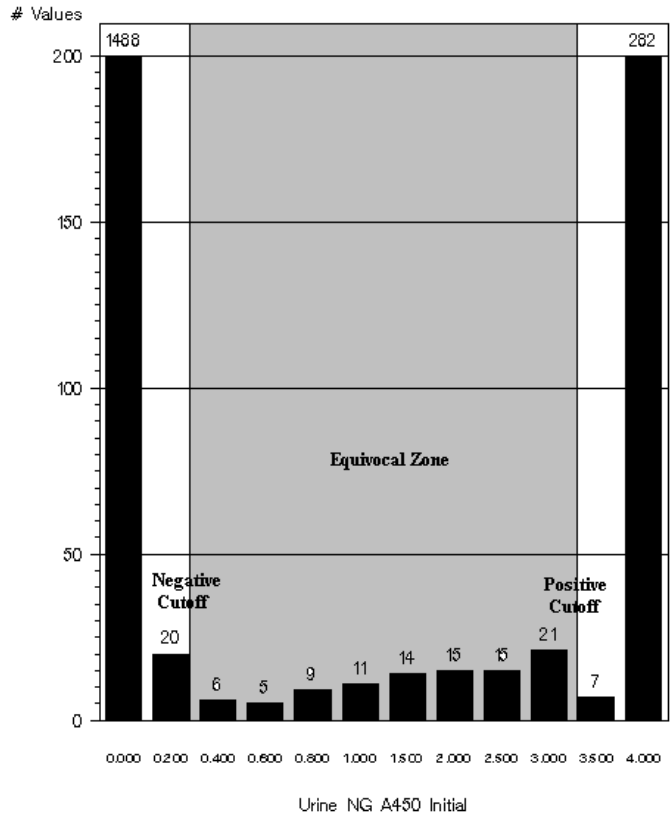


Figure 3
Swab Specimen Initial IC Absorbance Values
Combined Male and Female Data

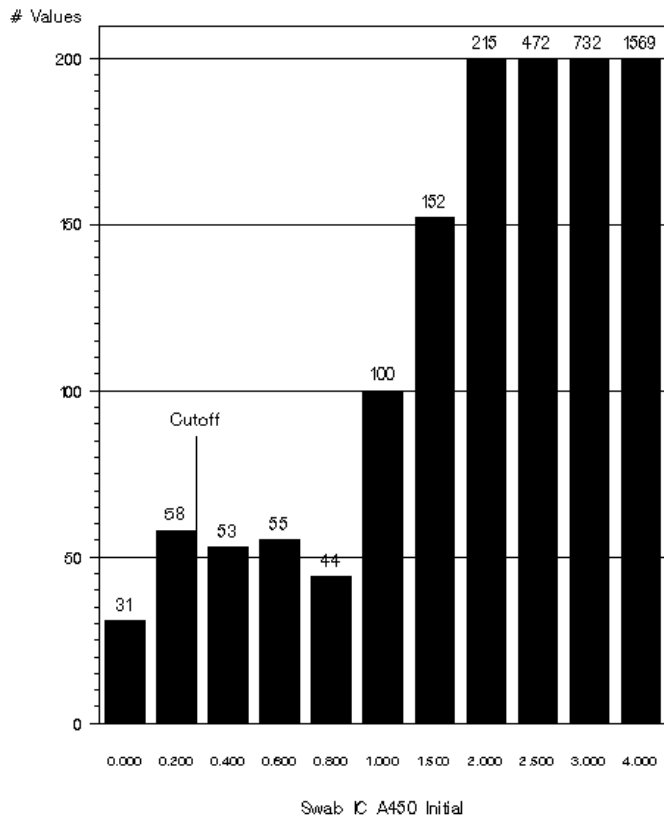
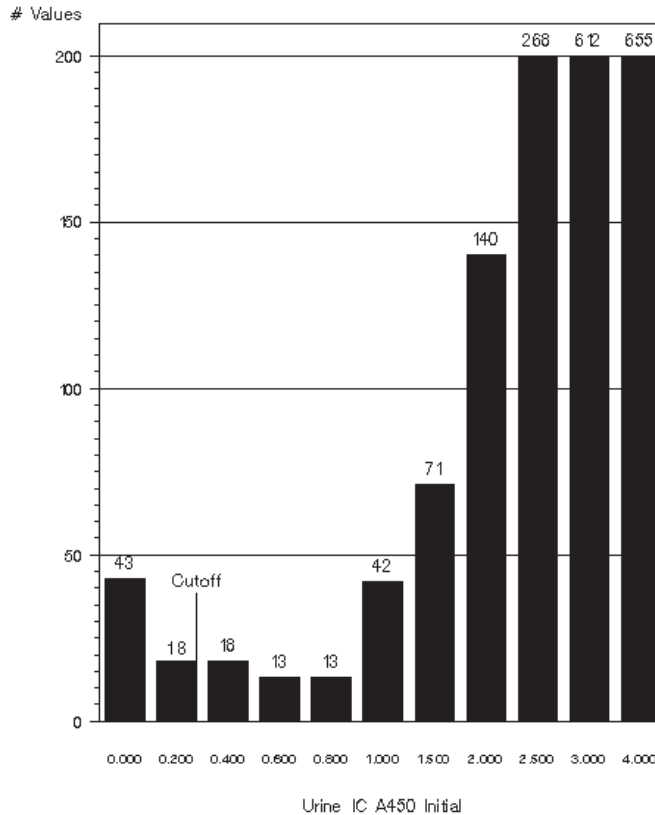


Figure 4
Urine Specimen Initial IC Absorbance Values
Male Data



PERFORMANCE CHARACTERISTICS

A. Analytical Specificity

The analytical specificity of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was tested against 132 bacteria, 6 fungi, 1 protozoan and 11 virus isolates that are commonly isolated from the urogenital tract. Each isolate was added to CTM and normal human urine at approximately 10⁴ copies of genomic DNA per test. CTM and urine samples were processed and tested using the standard AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* procedure. Multiple isolates of *Neisseria subflava* and *Neisseria cinerea* obtained from the American Type Culture Collection and other sources were tested. Four of the *Neisseria subflava* isolates and one *Neisseria cinerea* isolate gave false positive test results.

The following organisms were tested and all gave negative results with the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*.

- | | | |
|--|-----------------------------------|---------------------------------------|
| <i>Achromobacter xerosis</i> | <i>Candida glabrata</i> | <i>Eikenella corrodens</i> |
| <i>Acinetobacter calcoaceticus</i> | <i>Candida guilliermondi</i> | <i>Enterobacter cloacae</i> |
| <i>Acinetobacter sp. genospecies 3</i> | <i>Candida krusei</i> | <i>Enterococcus avium</i> |
| <i>Acinetobacter Iwoffii</i> | <i>Candida parapsilosis</i> | <i>Enterococcus faecalis</i> |
| <i>Actinomyces israelii</i> | <i>Candida tropicalis</i> | <i>Enterococcus faecium</i> |
| <i>Aerococcus viridans</i> | <i>Chlamydia pneumoniae</i> | Epstein Barr Virus |
| <i>Aeromonas hydrophila</i> | <i>Chlamydia psittaci</i> | <i>Erysipelothrix rhusiopathiae</i> |
| <i>Agrobacterium radiobacter</i> | <i>Chlamydia trachomatis</i> | <i>Escherichia coli</i> |
| <i>Alcalligenes faecalis</i> | <i>Chromobacter violaceum</i> | <i>Ewingella americana</i> |
| <i>Bacillus subtilis</i> | <i>Citrobacter freundii</i> | <i>Flavobacterium meningosepticum</i> |
| <i>Bacillus thuringiensis</i> | <i>Clostridium innocuum</i> | <i>Gamella haemolysans</i> |
| <i>Bacteriodes caccae</i> | <i>Clostridium perfringens</i> | <i>Gamella morbillorum</i> |
| <i>Bacteroides fragilis</i> | <i>Corynebacterium genitalium</i> | <i>Gardnerella vaginalis</i> |
| <i>Bacteroides gracilis</i> | <i>Corynebacterium xerosis</i> | <i>Haemophilus ducreyi</i> |
| <i>Bifidobacillus longum</i> | <i>Cryptococcus neoformans</i> | <i>Haemophilus influenzae</i> |
| <i>Bifidobacterium adolescentis</i> | Cytomegalovirus | Herpes simplex virus 1 |
| <i>Branhamella catarrhalis</i> | <i>Deinococcus radiopugnans</i> | Herpes simplex virus 2 |
| <i>Brevibacterium linens</i> | <i>Derxia gummosa</i> | Human papilloma virus type 16 |
| <i>Candida albicans</i> | <i>Edwardsiella tarda</i> | Human papilloma virus type 18 |

<i>Kingella kingae</i>	<i>Neisseria flavescens</i>	<i>Rhodospirillum rubrum</i>
<i>Klebsiella pneumoniae</i> ss <i>ozaenae</i>	<i>Neisseria kochi</i>	<i>Salmonella minnesota</i>
<i>Lactobacillus acidophilus</i>	<i>Neisseria lactamica</i>	<i>Salmonella typhimurium</i>
<i>Lactobacillus brevis</i>	<i>Neisseria meningitidis</i> 135	<i>Serratia marscence</i>
<i>Lactobacillus crispus</i>	<i>Neisseria mucosa</i>	<i>Staphylococcus aureus</i>
<i>Lactobacillus jensenii</i>	<i>Neisseria perflava</i>	<i>Staphylococcus epidermidis</i>
<i>Lactobacillus lactis lactis</i>	<i>Neisseria polysacchara</i>	<i>Streptococcus agalactiae</i>
<i>Lactobacillus oris</i>	<i>Neisseria polysaccharia</i>	<i>Streptococcus anginosus</i>
<i>Lactobacillus parabuchnerri</i>	<i>Neisseria sicca</i>	<i>Streptococcus bovis</i>
<i>Lactobacillus vaginalis</i>	<i>Paracoccus denitrificans</i>	<i>Streptococcus dysgalati</i>
<i>Lactococcus lactis cremoris</i>	<i>Pasteurella maltocida</i>	<i>Streptococcus equinus</i>
<i>Legionella bozemannii</i>	<i>Pediococcus acidilactica</i>	<i>Streptococcus mitis</i>
<i>Legionella pneumophila</i>	<i>Peptostreptococcus anaerobius</i>	<i>Streptococcus mutans</i>
<i>Leuconostoc paramesenteroides</i>	<i>Peptostreptococcus magnus</i>	<i>Streptococcus pneumoniae</i>
<i>Micrococcus luteus</i>	<i>Peptostreptococcus productus</i>	<i>Streptococcus pyogenes</i>
<i>Mobiluncus curtisii</i> subsp. <i>curtisii</i>	<i>Previttia bivia</i>	<i>Streptococcus salivarius</i>
<i>Mobiluncus curtisii</i> subsp. <i>homesi</i>	<i>Previttia corporis</i>	<i>Streptococcus sanguis</i>
<i>Moraxella osloensis</i>	<i>Prevotella intermedia</i>	<i>Streptomyces griseus</i>
<i>Morganella morganii</i>	<i>Propionibacterium acnes</i>	<i>Treponema pallidum</i> *
<i>Mycobacterium smegmatis</i>	<i>Proteus mirabilis</i>	<i>Trichomonas vaginalis</i>
<i>Mycoplasma genitalium</i>	<i>Providencia stuartii</i>	<i>Ureaplasma urealyticum</i>
<i>Mycoplasma hominis</i>	<i>Pseudomonas aeruginosa</i>	<i>Vibrio parahaemolyticus</i>
<i>Mycoplasma pneumoniae</i>	<i>Pseudomonas putida</i>	<i>Yersinia enterocolitica</i>
<i>Neisseria elongata</i>	<i>Rahnella aquatilis</i>	

* Purified DNA was added to processed CTM and urine.

Some isolates from the following organisms may give false positive test results with the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*.

- *Neisseria cinerea*
- *Neisseria subflava*

B. Analytical Sensitivity

The analytical sensitivity of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was determined using fifteen stock cultures, each representing distinct gonococcal strains. Stock cultures of each strain were diluted in Culture Transport Media and urine to prepare samples that contained 20, 10, 5, 2 and 1 CFU/test after specimen processing. For each isolate, three aliquots at each dilution were independently processed and tested using the standard test procedure.

The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* gave positive results for all strains tested at 20, 10 and 5 CFU/test. At 1 CFU/test, the Test gave positive results for at least one replicate of all 15 strains and positive results for all three replicates for 14 of the 15 strains at 2 CFU/test. The analytical sensitivity (limit of detection) of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is 5 CFU/test (equivalent to 100 CFU/mL for urine specimens and 400 CFU/mL for CTM inoculated with a swab specimen) for Culture Transport Media specimens and urine specimens.

C. Precision

A multi-operator study was performed to determine the qualitative precision of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*. The study was based upon the design suggested in the NCCLS document EP5-A¹⁹. Three independent operators at three different geographical sites tested a panel of unprocessed urine and swab samples in duplicate, once a day, for three days. Each run consisted of specimen preparation, amplification and detection of the following samples in duplicate (number of specimens in parenthesis): Culture Transport Media samples containing 0 (4), 12.5 (2), 37.5 (2) and 62.5 (2) *Neisseria gonorrhoeae* CFU/test and urine samples containing 0 (4), 10 (2), 30 (2) and 50 (2) *Neisseria gonorrhoeae* CFU/test. *C. trachomatis* was added to some CTM and urine specimens to determine test performance in the presence of non-specific analyte. Tables 3 and 4 summarize the results from this study.

Table 3
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
CTM Reproducibility Study Results

	<i>N. gonorrhoeae</i> Spiked CTM (CFU/test)			
	0	12.5	37.5	62.5
Number of Replicates	72	36	36	36
% Correct Results*	72 (100)	36 (100)	36 (100)	33 (100)
No. Equivocal A ₄₅₀ 0.2-1.999	0	0	0	3
No. Equivocal A ₄₅₀ 2.0-3.499	0	14	16	16
Median A ₄₅₀	0.056	4.000	4.000	2.889
Minimum A ₄₅₀	0.044	2.208	2.054	1.634
Maximum A ₄₅₀	0.177	4.000	4.000	4.000

* Specimens with initial equivocal results 0.2-1.999 were considered non-reportable, and were excluded from the calculations of % correct results. Specimens with equivocal results 2.0-3.499 were considered positive in the calculations.

Table 4
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Urine Reproducibility Study Results

	<i>N. gonorrhoeae</i> Spiked Urine (CFU/test)			
	0	10	30	50
Number of Replicates	72	36	36	36
% Correct Results*	72 (100)	27 (90.0) [†]	29 (100)	32 (100)
No. Equivocal A ₄₅₀ 0.2-1.999	0	6	7	4
No. Equivocal A ₄₅₀ 2.0-3.499	0	12	5	7
Median A ₄₅₀	0.055	2.754	4.000	4.000
Minimum A ₄₅₀	0.047	1.097 [§]	1.535	1.395
Maximum A ₄₅₀	0.106	4.000	4.000	4.000

* Specimens with initial equivocal results 0.2-1.999 were considered non-reportable, and were excluded from the calculations of % correct results. Specimens with equivocal results 2.0-3.499 were considered positive in the calculations.

[†] One sample gave negative results in three of six initial replicates at one site. The sample was repeat tested in duplicate at the site and correct results were obtained for all replicates. % Correct results based on 27 correct results out of 30 tests.

[§] Minimum absorbance excluding the three initial negative tests.

D. Control Performance

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

Table 5
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Control Results from Clinical Study

	CTM		Urine	
	NG (+)	NG (-)	NG (+)	NG (-)
Number of Results	286	285	282	281
Median A ₄₅₀	4.000	0.056	4.000	0.056
Mean A ₄₅₀	3.772	0.058	3.721	0.057
Minimum A ₄₅₀	2.010	0.042	2.095	0.034
Maximum A ₄₅₀	4.000	0.144	4.000	0.199

E. Clinical Performance

The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was evaluated in a clinical study conducted at six geographically diverse sites. Swab (endocervical for females, urethral for males) and urine specimens were obtained from each patient entered into the study. Swab specimens were placed in Culture Transport Media (CTM) used at each site. All swab specimens were isolated by routinely used procedures (Modified Thayer Martin (MTM) plates, 4-10% CO₂) and identified with biochemical (acid production, substrate utilization) and serological (fluorescent antibody, coagglutination, monoclonal antibody-colloidal gold) methods. The performance of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was assessed with endocervical swab specimens obtained from female patients, urethral swabs from symptomatic male patients and urine specimens from symptomatic and asymptomatic male patients. AMPLICOR testing was repeated for all specimens with initial values in the range of 0.0 to 0.199 A₄₅₀ and that IC results were inhibited (negative), and for all specimens with initial values in the range of 0.2 to 3.5 A₄₅₀.

The clinical performance of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was evaluated by comparing the results of the 5374 swab and urine specimens to the *Neisseria gonorrhoeae* culture results. Specimens with discrepant results were also tested by an alternate primer (16S rRNA) PCR test. Analyses were also performed including and excluding the use of the Internal Control result. The alternate primer PCR test results were not used to calculate the clinical performance characteristics of the test and are reported for information purposes only.

When the Internal Control result was used in the analysis, specimens with repeatedly negative Internal Control test results were excluded because the results were not interpretable. Of the 5374 specimens collected and tested in the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* clinical study, 33 were repeatedly inhibitory. Therefore, a total of 5341 specimens were used in the analyses when the Internal Control result was used. Table 6 shows the results from the clinical study. Table 7 provides a detailed summary of the performance of the Internal Control and includes initial and repeat inhibitory results by culture, patient sex, and specimen type.

The clinical sensitivity and specificity of the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* has not been reliably determined for detecting those patients with clinically active infection that can be transmitted to partners or cause gonorrhea-related sequelae. In the clinical study described here, 10.9% of all positive results (19.2% for females) were from patients with negative cultures. The significance of results that were positive, but culture negative, is unknown. A proportion of these AMPLICOR positive culture negative specimens (54.7%) were also positive by an alternate target PCR assay; however, the performance of this alternate target assay has not been established.

The AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* clinical data presented for females includes testing of specimens stored at 2-8°C (n=1707) and specimens stored frozen (n=531) prior to testing. There were no differences between the AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results with either fresh or frozen specimens. For males, specimens that were frozen are not included in the overall data summary but are noted with each table.

Table 6
Clinical Performance of AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Including and Excluding the Internal Control¹

Sex	Specimen	Symptom	TP	TN	FP	FN	No. Inhib.	% Repeat Inhibitory	Total	Sensitivity (95% CI)	Specificity (95% CI)	16s+/FP ²
Female	CTM	Asymptomatic	49 (49)	1032 (1038)	12 (12)	1 (1)	6	0.58%	1100 (1100)	98.0% (89.3-99.9) (98.0%) (89.3-99.9)	98.9% (98.2-99.5) (98.9%) (98.2-99.5)	4/12 (4/12)
		Symptomatic	69 (68)	1034 (1048)	16 (16)	4 (6)	15	1.42%	1138 (1138)	94.5% (86.6-98.5) (91.9%) (85.7-98.1)	98.5% (97.7-99.2) (98.5%) (97.8-99.2)	10/16 (10/16)
Total for Females			118 (117)	2066 (2086)	28 (28)	5 (7)	21	1.00%	2238 (2238)	95.9% (90.8-98.7) (94.4%) (90.3-98.4)	98.7% (98.2-99.2) (98.7%) (98.2-99.2)	14/28 (14/28)
Male	CTM	Symptomatic	340 (340)	858 (860)	38 (38)	5 (5)	2	0.23%	1243 (1243)	98.6% (96.6-99.5) (98.6%) (96.6-99.5)	95.8% ³ (94.4-97.1) (95.8%) (94.5-97.1)	21/38 (21/38)
	URINE	Asymptomatic	8 (8)	672 (674)	4 (4)	3 (3)	2	0.30%	689 (689)	72.7% (39.0-94.0) (72.7%) (39.0-94.0)	99.4% (98.5-99.8) (99.4%) (98.5-99.8)	2/4 (2/4)
		Symptomatic	308 (296)	847 (848)	25 (24)	16 (36)	8	0.92%	1204 (1204)	95.1% ⁴ (92.7-97.4) (89.2%) (85.8-92.5)	97.1% (96.0-98.2) (97.2%) (96.2-98.3)	15/25 (14/24)
Total for Males			656 (644)	2377 (2382)	67 (66)	24 (44)	12	0.50%	3136 (3136)	96.5% (95.1-97.9) (93.6%) (91.8-95.4)	97.3% (96.6-97.9) (97.3%) (96.7-97.9)	38/67 (37/66)

- 1 Numbers in parenthesis show the performance results when the Internal Control was not used.
- 2 Number of apparent AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* false positive results that were positive by alternate primer pair PCR/Total number of apparent AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* false positive results.
- 3 For site #6, specificity was 89.5% (95% CI 86.0 – 93.0) for CTM specimens. CTM specimens (n = 412) at this site were frozen prior to AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* testing. At all other sites specimens were not frozen prior to AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* testing (n = 733) and Test specificity was 99.1% (95% CI 97.9 – 99.7).
- 4 For site #6 sensitivity was 79.6% (95% CI 68.3 – 90.9) for AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* testing frozen urine specimens (n = 161) and 96.2% (95% CI 87.0 – 99.5) for AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* testing fresh urine specimens (n = 209). At all other sites sensitivity was 98.1% (95% CI 95.1 – 99.5) for both fresh (n = 778) or frozen (n = 56) specimens.

Table 7
AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Internal Control Performance Data from the Clinical Study

Culture Results	Specimen Group			AMPLICOR Results			
	Sex	Symptomatic	Specimen	N	Initial Inhibitory (%)	Repeat Inhibitory (%)	Initial Equivocal, Repeat Inhibitory
Negative	Female	Asymptomatic	CTM	1050	7 (0.67)	6 (0.57)	0
		Symptomatic	CTM	1064	19 (1.79)	13 (1.22)	1 (0.09)
	Male	Symptomatic	CTM	898	2 (0.22)	0	2 (0.22)
		Asymptomatic	Urine	678	1 (0.15)	1 (0.15)	1 (0.15)
		Symptomatic	Urine	872	8 (0.92)	0	0
Total Males and Females, Culture Negative				4562	37 (0.81)	20 (0.44)	4 (0.09)
Positive	Female	Asymptomatic	CTM	50	0	0	0
		Symptomatic	CTM	74	2 (2.70)	1 (1.35)	0
	Male	Symptomatic	CTM	345	0	0	0
		Asymptomatic	Urine	11	0	0	0
		Symptomatic	Urine	332	21 (6.33)	7 (2.11)	1 (0.30)
Total Males and Females, Culture Positive				812	23 (2.83)	8 (0.99)	1 (0.12)
Total All Results				5374	60 (1.12)	28 (0.52)	5 (0.09)

EXPECTED VALUES

Prevalence

The rate of positive *N. gonorrhoeae* 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 *Neisseria gonorrhoeae* 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 AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* results in the clinical study ranged from 0% to 51.5%. The prevalence data from the study are shown by site in Table 1.

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