

COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*

NGA

FOR *IN VITRO* DIAGNOSTIC USE

Order Information	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
	COBAS AMPLICOR <i>Neisseria gonorrhoeae</i> Detection Kit	NG DK	100 Tests	P/N: 20757535 122 ART: 07 5753 5 US: 83278
	COBAS AMPLICOR Detection Reagents Kit	DK	100 Tests	P/N: 20757470 122 ART: 07 5747 0 US: 83276
	COBAS AMPLICOR Conjugate Detection Reagent	CN4	200 Tests	P/N: 20764213 123 ART: 07 6421 3 US: 83305
	COBAS AMPLICOR Wash Buffer Kit	WB	500 Tests	P/N: 20759899 123 ART: 07 5989 9 US: 83314

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

COBAS AMPLICOR <i>Chlamydia trachomatis</i> Detection Kit	CT DK	100 Tests	P/N: 20757497 122 ART: 07 5749 7 US: 83277
---	-------	-----------	--

The following kit can be used to detect the CT/NG Internal Control amplified using the COBAS AMPLICOR CT/NG Amplification Kit.

COBAS AMPLICOR Internal Control Detection Kit	IC DK	100 Tests	P/N: 20757608 122 ART: 07 5760 8 US: 83281
--	-------	-----------	--

P/N: 03003981 018

Intended Use

The COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is a qualitative *in vitro* test for the detection of *N. 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 using the COBAS AMPLICOR™ Analyzer.

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 the third most common cause of Sexually Transmitted Diseases (STD's) with 62.2 million cases of gonorrhea being reported worldwide⁴. In the United States alone, 351,852 cases of gonorrhea were reported in 2002⁵. The total number of new cases of gonorrhea each year in the United States is estimated to be 700,000⁶. In men, this disease generally results in anterior urethritis accompanied by purulent exudate. In women, the disease is most often found in the cervix, but the vagina and uterus may also be infected.^{2,7}

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)^{1-3,7-9}. Culture is required for determination of antimicrobial susceptibility.

Principles of the Procedure

The COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* is based on four major processes: specimen preparation; PCR amplification^{10,11} of target DNA using NG specific complementary biotinylated primers; hybridization of the amplified DNA to oligonucleotide probes specific to the target(s); and detection of probe-bound amplified DNA by colorimetric determination.

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

The COBAS AMPLICOR CT/NG Test is a multiplex assay that permits the simultaneous amplification of *N. gonorrhoeae* target DNA, *C. trachomatis* target DNA and CT/NG Internal Control DNA. The Master Mix reagent contains biotinylated primer pairs specific for *C. trachomatis* and *N. gonorrhoeae*. The CT/NG Internal Control contains identical primer binding sequences as the *C. trachomatis* target DNA and uses the *C. trachomatis* primers for amplification. The detection reactions are performed independently for *C. trachomatis*, *N. gonorrhoeae* 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 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 HaeIII 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* species¹². The COBAS 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 in amplification tubes (A-tubes) in which PCR amplification occurs. The COBAS AMPLICOR Analyzer thermal cycler heats the reaction mixture to denature the double-stranded DNA and expose the specific primer target sequences on the *Neisseria gonorrhoeae* M-*Ngo* PII gene. As the mixture cools, the biotinylated primers SS01 and SS02 anneal to the complementary sequence of *N. gonorrhoeae* DNA. The thermostable *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 template to produce a 201-base pair double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for a designated 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) target DNA in the specimen and the CT/NG Internal Control using an analogous process to that described for *Neisseria 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 optional identification of processed specimens containing substances that may interfere with PCR amplification. The CT/NG Internal Control is a non-infectious recombinant plasmid DNA with primer binding regions identical to those of the *C. trachomatis* target sequence, a randomized internal sequence of similar length and base composition as the 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 COBAS AMPLICOR Internal Control Detection Kit contains an IC-specific oligonucleotide capture probe that can be used to identify a positive IC signal in the reaction mixture. The CT/NG Internal Control is designed to ensure that specimens do not contain inhibitors that would interfere with the amplification and detection of 20 or more copies of *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 COBAS AMPLICOR CT/NG Test is achieved by the use of AmpErase (uracil-N-glycosylase) enzyme (microbial) and deoxyuridine triphosphate (dUTP). AmpErase enzyme recognizes and catalyzes the destruction of DNA containing deoxyuridine¹³, 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 COBAS 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, the COBAS AMPLICOR Analyzer automatically adds Denaturation Solution to the reaction mixture in each A-tube to chemically denature the NG amplicon and the CT/NG Internal Control amplicon to form single-stranded DNA. Aliquots of denatured amplicon from each A-tube are then transferred by the Analyzer to individual detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for *N. gonorrhoeae* (or Internal Control, at the user's option) is added to individual D-cups. The biotin labeled NG and CT/NG Internal Control amplicon are hybridized to the target-specific oligonucleotide probe-bound magnetic particles. The hybridization of amplicon to target-specific probe enhances the overall specificity of the test.

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

Reagents

AMPLICOR CT/NG Specimen Preparation Kit	CT/NG PREP	100 Tests	P/N: 20759414 122 ART: 07 5941 4 US: 83315
--	------------	-----------	--

CT/NG URINE WASH (CT/NG Urine Wash Buffer)	1 x 50 mL
Tris-HCl buffer	
300 mM NaCl	
< 0.1% Detergent	
0.09% Sodium azide	

CT/NG LYS (CT/NG Lysis Reagent)	1 x 25 mL
Tris-HCl buffer	
< 1% Solubilizer	
0.09% Sodium azide	

CT/NG DIL (CT/NG Specimen Diluent)	2 x 50 mL
Tris-HCl buffer	
6 mM Magnesium chloride	
< 25% Detergent	
0.05% Sodium azide	

**AMPLICOR CT/NG
Amplification Kit**

CT/NG AMP

96 Tests P/N: 20759902 122
ART: 07 5990 2
US: 83319**CT/NG MMX**

3 x 1.8 mL

(CT/NG Master Mix)

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

CT/NG IC

3 x 0.1 mL

(CT/NG Internal Control)

Tris-HCl buffer
8 Copies/ μ L non-infectious plasmid DNA (microbial) containing
CT primer binding sequences and a unique probe binding region
(equivalent to approx. 21 IC copies/test)
< 0.005% Poly rA RNA (synthetic)
EDTA
Amaranth dye
0.05% Sodium azide

NG (+) C

1 x 0.8 mL

[*N. gonorrhoeae* (+) Control]

Tris-HCl buffer
8.6 Copies/ μ L 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

1 x 0.8 mL

[*N. gonorrhoeae* (-) Control]

Tris-HCl buffer
8.6 Copies/ μ L 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

COBAS AMPLICOR
Neisseria gonorrhoeae
Detection Kit

NG DK

100 Tests P/N: 20757535 122
ART: 07 5753 5
US: 83278

NG PS1

1 x 100 Tests

(NG Probe Suspension 1)

MES buffer

< 0.3% Suspension of Dynabeads® (paramagnetic particles) coated
with *N. gonorrhoeae*-specific oligonucleotide capture probe

0.09% Sodium azide

NG4

1 x 100 Tests

(NG Probe Suspension 2)

Sodium phosphate buffer

37% Sodium thiocyanate

< 0.2% Detergent

Xn



Harmful

37% (w/w) Sodium thiocyanate

COBAS AMPLICOR
Internal Control Detection Kit

IC DK

100 Tests P/N: 20757608 122
ART: 07 5760 8
US: 83281

IC PS1

1 x 100 Tests

(IC Probe Suspension 1)

MES buffer

< 0.35% Suspension of Dynabeads (paramagnetic particles) coated
with Internal Control-specific oligonucleotide capture probe

0.09% Sodium azide

IC4

1 x 100 Tests

(IC Probe Suspension 2)

Sodium phosphate buffer

25% Sodium thiocyanate

< 0.2% Detergent

Xn



Harmful

25% (w/w) Sodium thiocyanate

**COBAS AMPLICOR
Detection Reagents Kit**

DK

100 Tests P/N: 20757470 122
ART: 07 5747 0
US: 83276**DN4**


1 x 100 Tests

(Denaturation Solution)

1.6% Sodium hydroxide

EDTA

Thymol blue

Xi  1.6% (w/w) Sodium hydroxide

Irritant

CN4

1 x 100 Tests

(Avidin-Horseradish Peroxidase Conjugate)

Tris-HCl buffer

< 0.001% Avidin-horseradish peroxidase conjugate

Bovine serum albumin (mammalian)

Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)

0.1% Phenol

1% ProClin® 150

SB3

5 x 75 Tests

(Substrate A)

Citrate solution

0.01% Hydrogen peroxide

0.1% ProClin 150

SB

5 x 5 mL

(Substrate B)

0.1% 3,3',5,5'-Tetramethylbenzidine (TMB)

40% Dimethylformamide (DMF)

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

Toxic

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

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

COBAS AMPLICOR CN4 200 Tests P/N: 20764213 123
Conjugate Detection Reagent ART: 07 6421 3
 US: 83305

CN4 2 x 100 Tests

(Avidin-Horseradish Peroxidase Conjugate)

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

COBAS AMPLICOR WB 500 Tests P/N: 20759899 123
Wash Buffer ART: 07 5989 9
 US: 83314

WB 2 x 250 Tests

(10X-Wash Concentrate)

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

Warnings and Precautions

For in vitro diagnostic use.

The use of the term copy in this method manual refers to 1 copy of *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.

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.

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.

Do not pipet by mouth.

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

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

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

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

Do not use a kit after its expiration date.

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

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

Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*¹⁴ and in the NCCLS Document M29-A¹⁵. 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.

The COBAS AMPLICOR CT/NG Test for *N. 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.

For specimens transported in **Chlamydia culture transport media**, swabs should be left in the culture transport tube to provide visual evidence of specimen inoculation. The COBAS AMPLICOR CT/NG Test for *N. 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.

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

CT/NG URINE WASH, CT/NG LYS, CT/NG DIL, CT/NG MMX, CT/NG IC, NG (+) C, CT (+) C, NG PS1 and IC PS1 contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

Wear eye protection, laboratory coats and disposable gloves when handling **DN4**, **CN4**, **SB3**, **SB**, and Working Substrate (mixed **SB3** and **SB** reagent). Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.

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

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

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

Storage and Handling Requirements

Do not freeze reagents.

Store **CT/NG LYS** at 2-25°C. Store **CT/NG URINE WASH** and **CT/NG DIL** at 2-8°C. If a precipitate forms in 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.

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

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

Store **NG PS1** and **NG4** at 2-8°C. These reagents are stable until the expiration date indicated. Once **NG PS1** and **NG4** are mixed, the Working Reagent is stable for 30 days at 2-8°C. This Working Reagent can be used for a maximum of 6 instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

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

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

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

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

Store **WB** at 2-30°C. **WB** is stable until the expiration date indicated. Examine the **WB**, and if necessary, warm at 30-37°C to redissolve any precipitate. Working Wash Buffer (1X), prepared by diluting **WB** 1:10 with distilled or deionized water must be stored at 2-25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation.

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

Materials Provided

**AMPLICOR CT/NG
Specimen Preparation Kit**

CT/NG PREP

P/N: 20759414 122
ART: 07 5941 4
US: 83315

CT/NG URINE WASH
(CT/NG Urine Wash Buffer)
CT/NG LYS
(CT/NG Lysis Reagent)
CT/NG DIL
(CT/NG Specimen Diluent)

**AMPLICOR CT/NG
Amplification Kit**

CT/NG AMP

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

CT/NG MMX
(CT/NG Master Mix)
CT/NG IC
(CT/NG Internal Control)
NG (+) C
[*N. gonorrhoeae* (+) Control]
CT (+) C
[*C. trachomatis* (+) Control]

**COBAS AMPLICOR
Neisseria gonorrhoeae
Detection Kit**

NG DK

P/N: 20757535 122
ART: 07 5753 5
US: 83278

NG PS1
(NG Probe Suspension 1)
NG4
(NG Probe Suspension 2)

COBAS AMPLICOR IC DK P/N: 20757608 122
Internal Control Detection Kit ART: 07 5760 8
US: 83281

IC PS1
(IC Probe Suspension 1)
IC4
(IC Probe Suspension 2)

COBAS AMPLICOR DK P/N: 20757470 122
Detection Reagents Kit ART: 07 5747 0
US: 83276

DN4
(Denaturation Solution)
CN4
(Avidin-Horseradish Peroxidase Conjugate)
SB3
(Substrate A)
SB
(Substrate B)

COBAS AMPLICOR CN4 P/N: 20764213 123
Conjugate Detection Reagent ART: 07 6421 3
US: 83305

CN4
(Avidin-Horseradish Peroxidase Conjugate)

COBAS AMPLICOR WB P/N: 20757497 122
Wash Buffer ART: 07 5989 9
US: 83314

WB
(10X-Wash Concentrate)

Materials Required But Not Provided

Specimen Collection

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

Pre-Amplification - Reagent Preparation Area

- COBAS AMPLICOR A-ring fitted with 12 A-tubes (ART: 10 4563 6)
- COBAS AMPLICOR A-ring holder
- Eppendorf® Repeater™ pipet with 1.25 mL Combitip® Reservoir (sterile, individually wrapped)

- Pipettors (capacity 100 µL)* with aerosol barrier or positive displacement tips
- Vortex mixer
- Disposable gloves, powderless
- Plastic resealable bag

Pre-Amplification - Specimen and Control Preparation Area

- 2.0 mL polypropylene screw-cap tubes, sterile, non-siliconized, conical (Sarstedt 72.693.105 or equivalent)**
- Tube racks (Sarstedt 93.1428 or equivalent)
- Pipettors (capacity 50 µL, 100 µL, 200 µL, 250 µL, 500 µL and 1000 µL)* with aerosol barrier or positive displacement tips
- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent
- Extended aerosol barrier tips (Matrix 7055 or equivalent) for use with specimens transported in M4 Culture Transport Medium or Bartels ChlamTrans Chlamydial Transport Medium
- 37°C ± 2°C heating block
- Vortex mixer
- Absorbent paper
- Disposable gloves, powderless

Post-Amplification - Amplification/Detection Area

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

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

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

Specimen Collection, Transport and Storage

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

The only acceptable specimens are:

1. 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 patients and male urethral swab specimens from symptomatic patients. Specimens must be collected and transported in 2SP Culture Transport Medium, Bartels ChlamTrans Chlamydial Transport Medium (Bartels, Inc.), or M4 Culture Transport Medium (MicroTest, Inc.). **Media lots should 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 other types of specimens other than those indicated.

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

Specimen Collection

Male 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 mucus¹⁷.
2. Use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts. Do not use collection swabs with wooden or aluminum shafts
3. Leave swabs in the transport media. Seal the specimen container and label appropriately. Follow the laboratory's collection and transport procedure. Refrigerate swab specimens if transport to the laboratory is delayed for more than one hour from the time of collection.

Specimen Transport**Male 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. 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 agents¹⁶.

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.

Specimen Storage**Note**

Routine freezing or prolonged storage of specimens may affect performance.

Male Urine Specimens

1. 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.

Swab Specimens Collected in Culture Transport Media (CTM)

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

Instructions For Use

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

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

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

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

Run Size Each kit contains reagents sufficient for eight 12-specimen runs, which may be performed separately or simultaneously. At least one replicate of the COBAS AMPLICOR *N. gonorrhoeae* (+) Control and one replicate of the COBAS 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 COBAS AMPLICOR CT/NG Test for Neisseria gonorrhoeae.*

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

Workflow The COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoea* 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.

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

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

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

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

Specimen Preparation **Performed in: Pre-Amplification – Specimen Preparation**

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 of **CT/NG URINE WASH** to each of the labeled tubes.
3. Vortex urine thoroughly (3-10 seconds). If using frozen specimens, thaw the specimens at room temperature before vortexing (**volumes greater than 2 mL must be thawed overnight at 2-8°C**); continue processing even if a precipitate is present. Carefully remove caps from urine specimen containers. Take care to avoid contaminating gloves with urine in the cap. If contamination occurs, replace with a clean pair 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 of **CT/NG LYS** to each tube. Recap tubes and mix well by vortexing.
9. Incubate tubes for 15 minutes at room temperature.
10. Using a new aerosol barrier pipet tip for each specimen, add 250 µL of **CT/NG DIL** to each tube. Recap tubes and mix well by vortexing.
11. Centrifuge tubes for 10 minutes at $\geq 12,500 \times g$.
12. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to the A-tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the A-tubes within 2 hours. Processed specimens stored at 2-8°C must be tested within 7 days.

13. If processed specimens were stored at 2-8°C, they must be warmed to room temperature and mixed well by vortexing prior to amplification. After vortexing, centrifuge processed specimens for 10 minutes at $\geq 12,500 \times g$.
14. Using a pipettor with an aerosol barrier tip, transfer 50 μL of the supernatant to the appropriate A-tube. Use a new aerosol barrier tip for each specimen. **Be careful not to disturb the pellet (pellet may not be clearly visible).** Record the positions of the patient specimens on the A-ring map. Cap the A-tubes.
15. Store the remainder of each processed specimen at 2-8°C in the event retesting is required. Any retesting must be performed within 7 days of specimen processing.

Swab Specimens (Male and Female)

Note

The COBAS AMPLICOR CT/NG Test for Neisseria gonorrhoeae has been evaluated for use with 2SP 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 of **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 tube and mix well by vortexing.
6. Incubate at room temperature for 10 minutes.
7. Using a new aerosol barrier pipet tip for each sample, add 200 μL of **CT/NG DIL** to each tube. Recap the tube and mix well by vortexing.
8. Incubate at room temperature for 10 minutes.
9. Processed specimens may be kept at room temperature for up to 2 hours before transferring aliquots to A-tubes containing Working Master Mix. Store processed specimens at 2-8°C if aliquots will not be added to the A-tubes within 2 hours. Processed specimens stored at 2-8°C must be tested within 7 days.

10. If processed specimens were stored at 2-8°C, they must be warmed to room temperature and mixed well by vortexing prior to amplification.
11. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each processed specimen to the appropriate A-tube. Use a new aerosol barrier tip for each specimen. Record the positions of the patient specimens on the A-ring map. Cap the A-tubes.
12. Store the remainder of each processed specimen at 2-8°C in the event retesting is required. Any retesting must be performed within 7 days of specimen processing.

Control Preparation**Performed in: Pre-Amplification – Specimen and Control 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 NG Test and the negative control for the CT Test. The CT (+) C serves as the negative control for the NG Test and the positive control for the CT Test. Therefore, when running both the NG and CT Tests on a sample, it is only necessary to prepare one set of the appropriate controls. If testing both swab and urine sample types, it is necessary to prepare one set of controls for each sample type.

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

1. Using a sterile pipet tip, add 1 mL of **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".
2. 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.
3. Using a pipettor with a new aerosol barrier tip, add 100 µL of the **NG (+) C** to the tube labeled "NG (+) Working Control".
4. Using a pipettor with a new aerosol barrier tip, add 100 µL of the **CT (+) C** to the tube labeled "NG (-) Working Control".
5. Recap the tubes and mix well by vortexing. Store at room temperature and discard at the end of the workday.

B. Urine Specimens:**Prepare the following NG (+) and NG (-) Processed Controls.**

1. 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".
2. Using a pipettor with a new aerosol barrier tip, add 250 µL of the **NG (+) Working Control** to the tube labeled "NG (+) Processed Control".
3. Using a pipettor with a new aerosol barrier tip, add 250 µL of the **NG (-) Working Control** to the tube labeled "NG (-) Processed Control".

4. Re-cap the tubes and mix well by vortexing. Incubate for 10 minutes at room temperature. Store at room temperature and discard at the end of the workday.
5. Using a pipettor with an aerosol barrier tip, transfer 50 µL of each Processed Control to the appropriate A-tube. Cap the A-tubes and record the positions of the processed specimens and controls on the A-ring map.
6. Move the prepared samples (patient specimens and controls) in the A-rings to the Post-Amplification Area. ***These PCR-ready samples may be stored at 2-8°C for 16 hours.***

C. Swab Specimens: Prepare the following NG (+) and NG (-) Processed Controls.

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

Amplification and Detection

Performed in: Post-Amplification Area - Amplification/Detection

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

- Wipe initialization post with a lint-free moist cloth and dry
- Wipe D-cup handler tip with a lint-free moist cloth and dry
- Check Wash Buffer Reservoir and fill if necessary

- Prepare Working Wash Buffer (1X) as follows. Examine **WB** and if necessary, warm at 30-37°C to redissolve any precipitate. Add 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3-4 liters of Wash Buffer (1X) in the Wash Buffer Reservoir of the system at all times.
- Empty waste container
- Prime the system
- During the priming, check syringes and tubing
- During the priming, check transfer tip

Prior to each run:

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

Instrument Loading and System Operation

1. Examine the quantities of reagents on board the COBAS AMPLICOR Analyzer. Prepare enough reagent cassettes to complete the workload.
2. Mix **NG PS1** well by vortexing. Add 2.5 mL **NG PS1** to the **NG4** cassette. Place the cassette on the test specific reagent rack. Discard the used **NG PS1** vial. Record date of reagent preparation on the **NG4** cassette.
3. Mix **IC PS1** well by vortexing. Add 2.5 mL **IC PS1** to the cassette containing **IC4**. Place the cassette on the test specific reagent rack. Discard the used **IC PS1** vial. Record date of reagent preparation on the **IC4** cassette.
4. Prepare the Working Substrate by pipetting 5 mL of **SB** into one **SB3** cassette. Pipet up and down to mix. Discard the empty **SB** vial. Record the date of preparation on the **SB3** cassette.
5. Place the Working Substrate in the generic reagent rack.
6. Place **DN4** and **CN4** cassettes on the generic reagent rack. Record the date each cassette was opened on cassette.
7. Identify the reagent racks as generic or test specific using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or by using the AMPLILINK® software as described in the *Operator's Manual* for the AMPLILINK software.
8. Configure the reagent racks by inputting reagent positions and lot numbers into the instrument using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or by using the AMPLILINK software as described in the *Operator's Manual* for the AMPLILINK software.
9. Load the reagent racks onto the instrument using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or by using the AMPLILINK software as described in the *Operator's Manual* for the AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.

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

Note

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

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

Quality Control

At least one replicate of the NG (+) Processed Control and one replicate of the NG (-) Processed Control must be included in each test run. As with any new laboratory procedure, new operators should consider the use of additional controls each time the test is performed until such time as a high degree of confidence is reached in their ability to perform the test correctly. Each laboratory may determine appropriate target values and limits using recommended methods, e.g., NCCLS C 24-A¹⁸. There are no recommendations regarding the order of controls and specimens in the A-ring(s).

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

Negative Control

The absorbance of the NG (-) Control should be less than 0.2 at 660 nm. If the absorbance of the NG (-) Control 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 processed 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 of the NG (+) Control should be greater than or equal to 2.0 at 660 nm. If the absorbance of the NG (+) Control is less than 2.0, the entire run is invalid. Repeat the entire test process (specimen and control preparation, amplification, and detection). If the absorbance of the NG (+) Control is consistently less than 2.0, 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 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 COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* should 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 Specimen Collection Swab and Transport Media Validation Procedure.

Specimen Processing Control

To test the effectiveness of sample processing, 10^4 *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 COBAS AMPLICOR CT/NG Swab Preparation Procedure as described in this Method Manual. Properly processed specimens should give positive COBAS AMPLICOR CT/NG Test results for *Neisseria gonorrhoeae* with an absorbance value greater than or equal to 3.5 at 660 nm.

Internal Control

The Internal Control is intended to identify specimens that contain polymerase inhibitors. The use of the Internal Control will not eliminate all false negative test results. In clinical studies, the proportion of inhibited specimens was highest for 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

Interpretation of Results Without Internal Control Detection

Note

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

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

A ₆₆₀	COBAS Flag	Interpretation
< 0.2	NEGATIVE	<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	POSITIVE	<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	GZ 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 A₆₆₀ 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 A₆₆₀ greater than or equal to 2.0 should be considered positive for *N. gonorrhoeae*. If 2 of 3 results have an A₆₆₀ less than 2.0, the specimen is presumptive negative for *N. gonorrhoeae*.

Interpretation of Results With Internal Control Detection

Note

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

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

NG Specimen Result		IC Specimen Result		Interpretation
A ₆₆₀	COBAS Flag	A ₆₆₀	COBAS Flag	
< 0.2	NEGATIVE	≥ 0.2	POSITIVE	N. gonorrhoeae 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	NEGATIVE	< 0.2	NEGATIVE	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	POSITIVE	ANY	ANY	N. gonorrhoeae 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	GZ 0.2 - 3.5	ANY	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). Alternatively, culture may be performed on the patient specimen.

3. For a **valid** run, specimens with a test result in the Grey Zone (≥ 0.2 , < 3.5 at 660 nm) 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 660 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 NG 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) tested during preclinical studies and were confirmed in the clinical studies performed for the COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*. These studies show that the majority of the time, a specimen with NG test results $\geq 3.5 A_{660}$ on the initial test will indicate the presence of *N. gonorrhoeae* as shown by culture. A specimen with NG test results $< 0.2 A_{660}$ on the initial test correlates with negative *N. gonorrhoeae* culture results the majority of the time. Specimens with NG test results $\geq 0.2 A_{660}$ and $< 3.5 A_{660}$ on the initial test are inconclusive for the presence of *N. gonorrhoeae* and require retesting. Upon repeat testing, specimens with two of the three cumulative test results $\geq 2.0 A_{660}$ a majority of the time indicate the presence of culturable *N. gonorrhoeae*. Specimens with two of the three cumulative test results $< 2.0 A_{660}$ a majority of the time are negative for *N. gonorrhoeae* by culture. Similarly, the majority of the time, a specimen with IC test results $> 0.2 A_{660}$ and having a NG test result $< 0.2 A_{660}$ is negative for *N. gonorrhoeae* by culture.

Procedural Precautions

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

The COBAS 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

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 COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* had reduced performance and is not recommended for testing female urine specimens or asymptomatic male swab specimens.

The interpretation of COBAS 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 infection¹⁹. 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 (1.7%, 12/719).

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.

The COBAS 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.

Oropharyngeal aerosols or other sources of oropharyngeal contamination have a high probability of causing false-positive results for *N. gonorrhoeae*. The COBAS 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.

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.

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

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

Therapeutic success or failure cannot be determined using this test.

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

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

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

The COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* provides qualitative results. No correlation can be drawn between the magnitude of a positive COBAS Test absorbance signal and the number of *N. gonorrhoeae* cells within an infected specimen.

The COBAS 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.

The type and volume of Culture Transport Media used to transport swab specimens to the laboratory for testing by the COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* may cause variable effects on the performance of the Test.

Interfering Substances

The presence of PCR inhibitors may cause false negative results.

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

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

The presence of mucus in cervical samples may inhibit PCR and cause false negative test results. Mucus free samples are recommended for optimal test performance. Use a sponge or a large swab to remove cervical secretions and discharge before obtaining the sample⁵.

Samples containing greater than 5% (v/v) blood may give false positive results. In Clinical studies, 681 of 2265 female swab specimens were noted to be bloody. False positive rates were not higher in these specimens.

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 COBAS AMPLICOR CT/NG Test for *N. 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 COBAS AMPLICOR CT/NG Test for *N. gonorrhoeae* results in the clinical study ranged from 0% to 46.9%. The prevalence data from the study are shown by site in Table 1.

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

Site	Sex	Symptoms	Specimen	N	No. Culture Positive	COBAS AMPLICOR Results (with IC)			
						No. Initially Inhibitory	No. Equivocal	No. Positive	% Positive
1	Female	Symptomatic	Swab	277	33	5	4	35	12.7%
		Asymptomatic		214	36	7	3	38	17.8%
	Male	Symptomatic	Swab	519	158	0	12	158	30.7%
		Symptomatic	Urine	519		6	16	160	30.8%
		Asymptomatic		290	5	0	6	2	0.7%
2	Female	Symptomatic	Swab	120	7	6	9	8	6.7%
		Asymptomatic		27	2	3	5	4	14.8%
	Male	Symptomatic	Swab	100	33	0	7	33	33.0%
		Symptomatic	Urine	100	33	6	4	33	33.0%
		Asymptomatic		59	3	0	2	3	5.1%
3	Female	Symptomatic	Swab	240	5	1	1	5	2.1%
		Asymptomatic		230	2	3	1	3	1.3%
	Male	Symptomatic	Swab	182	18	0	3	20	11.0%
		Symptomatic	Urine	182		4	2	17	9.3%
		Asymptomatic		61	0	0	0	0	0%
4	Female	Symptomatic	Swab	244	3	4	1	4	1.6%
		Asymptomatic		434	6	0	2	9	2.1%
5	Female	Symptomatic	Swab	63	2	1	1	3	4.8%
		Asymptomatic		137	1	3	7	3	2.2%
	Male	Symptomatic	Swab	32	13	0	2	15	46.9%
		Symptomatic	Urine	32		3	2	12	37.5%
		Asymptomatic		168	3	2	4	4	2.4%
6	Female	Symptomatic	Swab	211	26	6	7	28	14.4%
		Asymptomatic		68	3	3	0	5	8.3%
	Male	Symptomatic	Swab	458	137	2	33	150	36.3%
		Symptomatic	Urine	454		24	18	136	30.0%
		Asymptomatic		141	1	1	1	3	2.1%

Predictive Values

The hypothetical positive and negative predictive values (PPV and NPV) for different prevalence rates using sensitivity and specificity of 97.1% and 98.1%, respectively, are shown in Table 2.

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

Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	97.1	98.1	34.5	99.9
5	97.1	98.1	73.3	99.8
10	97.1	98.1	85.3	99.7
20	97.1	98.1	92.9	99.3

Result Distribution

The distribution of COBAS 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_{660} values observed for 3480 swab specimens and 2008 urine specimens tested in the COBAS 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_{660} .

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

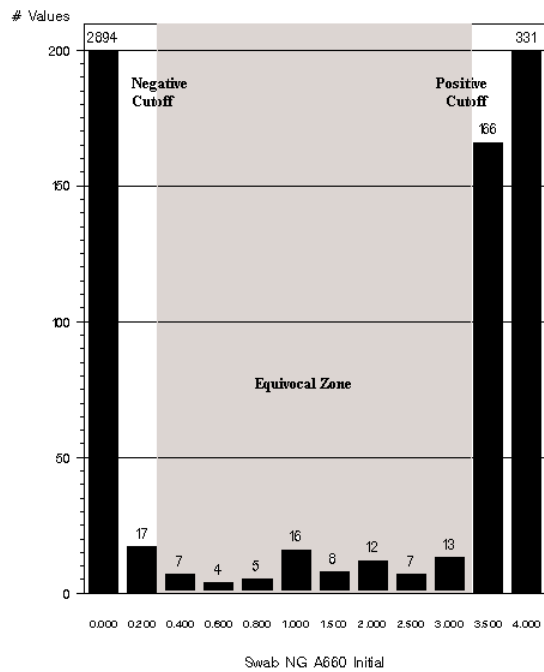


Figure 2
Urine Specimen Initial NG Absorbance Values
Male Data

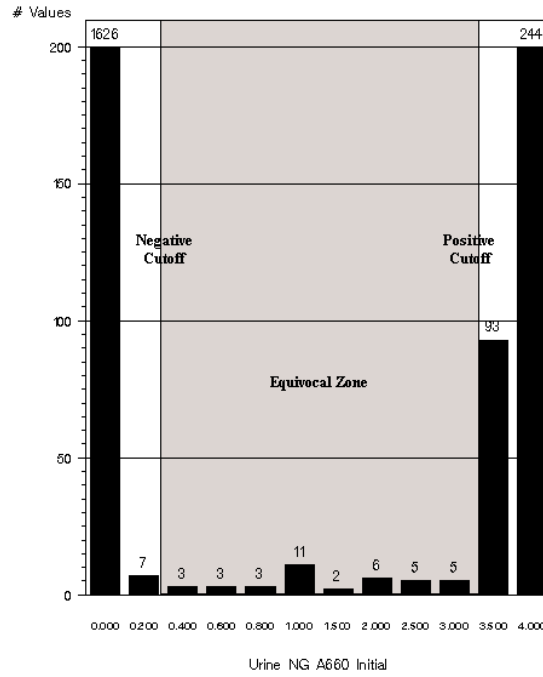


Figure 3
Swab Specimen Initial IC Absorbance Values
Combined Male and Female Data

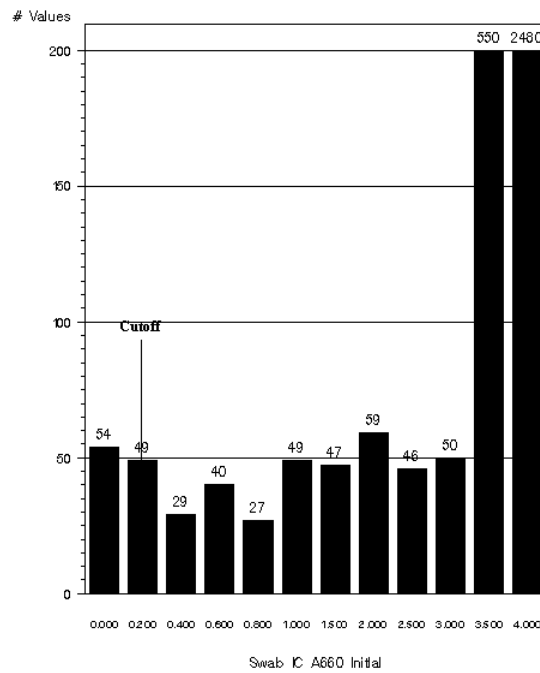
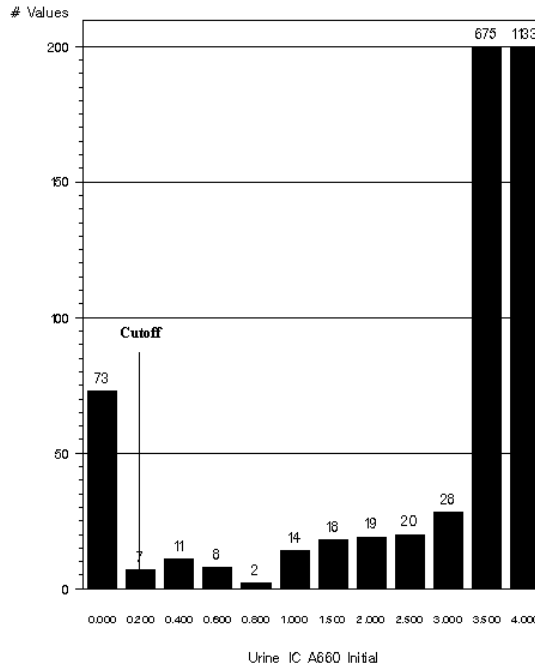


Figure 4
Urine Specimen Initial IC Absorbance Values
Male Data



Performance Characteristics

Analytical Specificity

The analytical specificity of the COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was tested against 133 bacterial, 6 fungal, 1 protozoan and 11 viral strains that may be isolated from the urogenital tract. Each isolate was added to culture transport media and normal human urine using at least 10^4 copies of genomic DNA per test (equivalent to 8×10^5 copies/mL in culture transport media and 4×10^5 copies/mL in urine specimens). The culture transport media and urine specimens were processed and tested using the standard COBAS AMPLICOR CT/NG Test procedure. Multiple isolates of *Neisseria subflava* and *Neisseria cinerea* obtained from the American Type Culture Collection and other sources were tested. Two 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 COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*.

<i>Achromobacter xerosis</i>	<i>Erysipelothrix rhusiopathiae</i>
<i>Acinetobacter calcoaceticus</i>	<i>Escherichia coli</i>
<i>Acinetobacter genosp. 3</i>	<i>Ewingella americana</i>
<i>Acinetobacter lwoffii</i>	<i>Flavobacterium meningosepticum</i>
<i>Actinomyces israelii</i>	<i>Gemella haemolysans</i>
<i>Aerococcus viridans</i>	<i>Gemella morbillorum</i>
<i>Aeromonas hydrophila</i>	<i>Gardnerella vaginalis</i>
<i>Agrobacterium radiobacter</i>	<i>Haemophilus ducreyi</i>
<i>Alcaligenes faecalis</i>	<i>Haemophilus influenzae</i>
<i>Bacillus subtilis</i>	Herpes simplex virus 1
<i>Bacillus thuringiensis</i>	Herpes simplex virus 2

<i>Bacteroides caccae</i>	Human papilloma virus type 16
<i>Bacteriodes fragilis</i>	Human papilloma virus type 18
<i>Bacteroides gracilis</i>	<i>Kingella kingae</i>
<i>Bifidobacillus longum</i>	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>
<i>Bifidobacterium adolescentis</i>	<i>Lactobacillus acidophilus</i>
<i>Branhamella catarrhalis</i>	<i>Lactobacillus brevis</i>
<i>Brevibacterium linens</i>	<i>Lactobacillus crispus</i>
<i>Candida albicans</i>	<i>Lactobacillus jensenii</i>
<i>Candida glabrata</i>	<i>Lactobacillus lactis lactis</i>
<i>Candida guilliermondii</i>	<i>Lactobacillus oris</i>
<i>Candida krusei</i>	<i>Lactobacillus parabuchne</i>
<i>Candida parapsilosis</i>	<i>Lactobacillus vaginalis</i>
<i>Candida tropicalis</i>	<i>Lactococcus lactis cremoris</i>
<i>Chlamydia pneumoniae</i>	<i>Legionella bozemni</i>
<i>Chlamydia psittaci</i>	<i>Legionella pneumophila</i>
<i>Chromobacter violaceum</i>	<i>Leuconostoc paramesenteroides</i>
<i>Citrobacter freundii</i>	<i>Micrococcus luteus</i>
<i>Clostridium innocuum</i>	<i>Mobiluncus curtsii</i> subsp. <i>curtsii</i>
<i>Clostridium perfringens</i>	<i>Mobiluncus curtsii</i> subsp. <i>homiesi</i>
<i>Corynebacterium genitalium</i>	<i>Moraxella osloensis</i>
<i>Corynebacterium xerosis</i>	<i>Morganella morganii</i>
<i>Cryptococcus neoformans</i>	<i>Mycobacterium smegmatis</i>
<i>Cytomegalovirus</i>	<i>Mycoplasma genitalium</i>
<i>Deinococcus radiopugnans</i>	<i>Mycoplasma hominis</i>
<i>Dexia gummosa</i>	<i>Mycoplasma hominis</i>
<i>Edwardsiella tarda</i>	<i>Mycoplasma pneumoniae</i>
<i>Eikenella corrodens</i>	<i>Neisseria elongata</i>
<i>Enterobacter cloacae</i>	<i>Neisseria flavescens</i>
<i>Enterococcus avium</i>	<i>Neisseria kochi</i>
<i>Enterococcus faecalis</i>	<i>Neisseria lactamica</i>
<i>Enterococcus faecium</i>	<i>Rhodospirillum rubrum</i>
Epstein-Barr Virus	<i>Salmonella minnesota</i>
<i>Neisseria meningitidis</i>	<i>Salmonella typhimurium</i>
<i>Neisseria mucosa</i>	<i>Serratia marcescens</i>
<i>Neisseria perflava</i>	<i>Staphylococcus aureus</i>
<i>Neisseria polysaccharea</i>	<i>Staphylococcus epidermidis</i>
<i>Neisseria sicca</i>	<i>Streptococcus agalactiae</i>
<i>Paracoccus denitrificans</i>	<i>Streptococcus anginosus</i>
<i>Pasteurella maltocida</i>	<i>Streptococcus bovis</i>
<i>Pediococcus acidilactica</i>	<i>Streptococcus dysgalacti</i>
<i>Peptostreptococcus anaerobius</i>	<i>Streptococcus mitis</i>
<i>Peptostreptococcus magnus</i>	<i>Streptococcus equinus</i>
<i>Peptostreptococcus productus</i>	<i>Streptococcus mutans</i>
<i>Prevotella bivia</i>	<i>Streptococcus pneumoniae</i>
<i>Prevotella corporis</i>	<i>Streptococcus pyogenes</i>
<i>Prevotella intermedia</i>	<i>Streptococcus salivarius</i>
<i>Propionibacterium acnes</i>	<i>Streptococcus sanguis</i>
<i>Proteus mirabilis</i>	<i>Streptomyces griseinus</i>
<i>Providencia stuartii</i>	<i>Trichomonas vaginalis</i>
<i>Pseudomonas aeruginosa</i>	<i>Ureaplasma urealyticum</i>
<i>Pseudomonas putida</i>	<i>Vibrio parahaemolyticus</i>
<i>Rahnella aquatilis</i>	<i>Yersinia enterocolitica</i>

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

Neisseria cinerea

Neisseria subflava

Analytical Sensitivity

The analytical sensitivity of the COBAS 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 COBAS 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 10 of the 15 strains tested. The analytical sensitivity (limit of detection) of the COBAS 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.

Precision

A multi-operator study was performed to determine the qualitative precision of the COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*. The study was based upon the design suggested in the NCCLS document EP5-A²⁰. 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. *Chlamydia trachomatis* was added to some culture transport media and urine specimens to determine test performance in the presence of a non-specific analyte. Tables 3 and 4 summarize the results from this study.

Table 3
COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
CTM Specimen Reproducibility

	<i>N. gonorrhoeae</i> Spiked CTM (CFU/test)			
	0	12.5	37.5	62.5
Number of Replicates	72	36	36	36
No. Correct Results (%) [*]	70 (100)	35 (100)	36 (100)	36 (100)
No. Equivocal A ₆₆₀ 0.2-1.999	2 [†]	1	0	0
No. Equivocal A ₆₆₀ 2.0-3.499	0	11	10	8
Median A ₆₆₀	0.002	3.932	3.984	3.968
Minimum A ₆₆₀	0.000	1.554	2.925	3.266
Maximum A ₆₆₀	0.023 [‡]	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.

[†] Both samples gave negative results when repeated in duplicate at the test site.

[‡] Maximum absorbance excluding the two initial equivocal tests.

Table 4
COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae*
Urine Specimen Reproducibility

	N. gonorrhoeae Spiked Urine (CFU/test)			
	0	10	30	50
Number of Replicates	72	36	36	36
No. Correct Results (%)*	72 (100)	26 (86.7) [†]	32 (100)	34 (100)
No. Equivocal A ₆₆₀ 0.2-1.999	0	6	4	2
No. Equivocal A ₆₆₀ 2.0-3.499	0	5	8	11
Median A ₆₆₀	0.003	3.759	3.966	3.967
Minimum A ₆₆₀	0.000	0.820 [§]	0.771	1.518
Maximum A ₆₆₀	0.024	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.

[†] Two samples tested at one site gave a total of four negative test results. The specimens gave positive test results when repeated in duplicate at the site. % Correct calculation based on 26 correct results out of 30 tests.

[§] The minimum absorbance excluding the four initial negative tests.

Control Performance

A summary of the performance of the positive and negative kit controls in the COBAS 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 19 invalid results due to the CTM controls (8 positive control, 11 negative control) and 10 invalid results due to the Urine controls (4 positive control, 6 negative control).

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

	CTM		Urine	
	NG (+)	NG (-)	NG (+)	NG (-)
Number of Results	713	710	692	690
Median A ₆₆₀	4.000	0.003	4.000	0.003
Mean A ₆₆₀	3.930	0.006	3.930	0.005
Minimum A ₆₆₀	2.011	0.000	2.055	0.000
Maximum A ₆₆₀	4.000	0.194	4.000	0.173

Clinical Performance

The COBAS 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 all patients 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 COBAS 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. Testing was repeated for all specimens with initial values in the range of 0.0 to 0.199 A₆₆₀ when IC results were inhibited (negative), and for all specimens with initial values in the range of 0.2 to 3.5 A₆₆₀.

The clinical performance of the COBAS AMPLICOR CT/NG Test for *Neisseria gonorrhoeae* was evaluated by comparing the results of the 5486 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 5486 specimens collected and tested in the COBAS AMPLICOR CT/NG Test clinical study, 44 were repeatedly inhibitory. Therefore, a total of 5442 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 COBAS 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, 9.7% of all positive results (18.6% for females) were from patients with negative cultures. The significance of those results that were positive, but culture negative is unknown. A proportion of these positive specimens (61.6%) were also positive by an alternate target PCR assay; however, the performance of this alternate target assay has not been established.

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

Sex	Specimen	Symptom	TP	TN	FP	FN	No. Inhib.	% Repeatedly Inhibitory	Total	Sensitivity (95% CI)	Specificity (95% CI)	16S+/FP*
Female	CTM	Asymptomatic	49 (49)	1026 (1039)	13 (12)	1 (1)	12	1.15%	1101 (1101)	98.0% (89.3-99.9) (98.0%) (89.3-99.9)	98.7% (98.1-99.4) (98.9%) (98.2-99.5)	5/13 (5/12)
		Symptomatic	69 (69)	1032 (1050)	14 (13)	4 (5)	18	1.71%	1137 (1137)	94.5% (86.6-98.5) (93.2%) (84.9-97.8)	98.7% (98.0-99.4) (98.8%) (98.1-99.4)	9/14 (9/13)
	Total for Females		118 (118)	2058 (2089)	27 (25)	5 (6)	30	1.43%	2238 (2238)	95.9% (90.8-98.7) (95.2%) (91.4-98.9)	98.7% (98.2-99.2) (98.8%) (98.4-99.3)	14/27 (14/25)
Male	CTM	Symptomatic	342 (342)	862 (865)	34 (33)	2 (2)	2	0.23%	1242 (1242)	99.4% (97.9-99.9) (99.4%) (97.9-99.9)	96.2% (95.0-97.5) (96.3%) (95.1-97.6)	19/34 (19/33)
		Asymptomatic	9 (8)	703 (704)	3 (3)	3 (4)	1	0.14%	719 (719)	75.0% (42.8-94.5) (66.7%) (34.9-90.1)	99.6% (98.8-99.9) (99.6%) (98.8-99.9)	2/3 (2/3)
	URINE	Symptomatic	336 (310)	904 (907)	22 (21)	14 (49)	11	1.18%	1287 (1287)	96.0% (93.9-98.1) (86.4%) (82.8-89.9)	97.6% (96.6-98.6) (97.7%) (96.8-98.7)	18/22 (17/21)
		Asymptomatic	9 (8)	703 (704)	3 (3)	3 (4)	1	0.14%	719 (719)	75.0% (42.8-94.5) (66.7%) (34.9-90.1)	99.6% (98.8-99.9) (99.6%) (98.8-99.9)	2/3 (2/3)
Total for Males		687 (660)	2469 (2476)	59 (57)	19 (55)	14	0.56%	3248 (3248)	97.3% (96.1-98.5) (92.3%) (90.4-94.3)	97.7% (97.1-98.3) (97.7%) (97.2-98.3)	39/59 (39/57)	

¹ Numbers in parenthesis show the performance results when the Internal Control was not used.

* Number of apparent false positive COBAS AMPLICOR Test results that were positive by alternate primer pair PCR/Total number of apparent COBAS AMPLICOR false positive results.

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

Culture Results	Specimen Group			COBAS AMPLICOR Results			
	Sex	Symptomatic	Specimen	N	Initial Inhibitory (%)	Repeat Inhibitory (%)	Initial Equivocal, Repeat Inhibitory
Negative	Female	Asymptomatic	CTM	1051	19 (1.81%)	11 (1.05%)	1 (0.10%)
		Symptomatic	CTM	1063	22 (2.07%)	17 (1.60%)	0
	Male	Symptomatic	CTM	898	2 (0.22%)	1 (0.11%)	1 (0.11%)
		Asymptomatic	Urine	707	2 (0.28%)	1 (0.14%)	0
		Symptomatic	Urine	928	10 (1.08%)	2 (0.22%)	0
Total Males and Females, Culture Negative				4647	55 (1.18%)	32 (0.69%)	2 (0.04%)
Positive	Female	Asymptomatic	CTM	50	0	0	0
		Symptomatic	CTM	74	1 (1.35%)	1 (1.35%)	0
	Male	Symptomatic	CTM	344	0	0	0
		Asymptomatic	Urine	12	1 (8.33%)	0	0
		Symptomatic	Urine	359	33 (9.19%)	7 (1.95%)	2 (0.56%)
Total Males and Females, Culture Positive				839	35 (4.17%)	8 (0.95%)	2 (0.24%)
Total All Results				5486	90 (1.64%)	40 (0.73%)	4 (0.07%)

References

1. Sparling, P.F. 1990. Biology of *Neisseria gonorrhoeae*, in Sexually Transmitted Diseases (Holmes, K.K., Mardh, P-A, Sparling, P.F. and Wiesner, P.J., ed.) Second Edition, McGraw-Hill, New York, 131-147.
2. Knapp, J.S. and Koumans, E.H. 1999. *Neisseria* and *Branhamelia*, in Manual of Clinical Microbiology (P.R. Murray, ed.) 7th Edition, ASM Press, Washington, D.C., 586-603.
3. Mardh, P-A and Danielson, D. 1990. *Neisseria gonorrhoeae*, in Sexually Transmitted Diseases (Holmes, K.K., Mardh, P-A, Sparling, P.F., and Wiesner, P.J., ed.) 2nd Edition, McGraw-Hill, New York, 131-147.
4. Gerbase, A., Rowley J.T., and Mertens, T.E. 1998. Global epidemiology of sexually transmitted diseases. *Lancet* **351**:(S3) 2-4.
5. Centers for Disease Control and Prevention. Sexually transmitted disease surveillance, 2002. Division of STD/HIV Prevention, Centers for Disease Control and Prevention, Atlanta, GA.
6. Centers for Disease Control and Prevention. STD Facts-Gonorrhea, 2004. National Center for HIV, STD and TB Prevention. Division of Sexually Transmitted Diseases, Atlanta, GA.
7. Hook III, E.W. and Handsfield, H.H. 1990. Gonococcal infections in the adult, in Sexually Transmitted Diseases. (Holmes, K.K., Mardh, P-A, Sparling, P.F., and Wiesner, P.J., ed.) Second Edition, McGraw-Hill, New York, 131-147.
8. Guide for the Diagnosis of Gonorrhea Using Culture and Gram Stained Smear. 1991. US Department of Health and Human Services, Public Health Service, Atlanta, GA 30333.
9. Laboratory Methods for the Diagnosis of Sexually Transmitted Diseases. 1991. American Public Health Association.
10. Saiki, R.K., Scharf, S., Faloona, F. et al. 1985. Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**: 1350-1354.
11. Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA *in-vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* **155**: 335-350.
12. Miyada, C.G. and Born, T.L. 1991. A DNA sequence for the discrimination of *Neisseria gonorrhoeae* from other *Neisseria* species. *Molecular and Cellular Probes* **5**: 327-335.
13. Longo, M.C., Berninger, M.S. and Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* **93**: 125-128.

14. Richmond, J.Y. and McKinney, R.W. (eds.). 1999. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication Number (CDC) 93-8395.
15. National Committee for Clinical Laboratory Standards. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline. NCCLS Document M29-A Villanova, PA:NCCLS, 1997.
16. International Air Transport Association. Dangerous Goods Regulations, 41st Edition. 2000. 704 pp.
17. Evangelista, A.T. and Bilstein, H.R. 1993. Cumitech 4A: Laboratory Diagnosis of Gonorrhea. (C. Abramson, ed.) ASM Press, Washington, D.C.
18. National Committee for Clinical Laboratory Standards. Internal Quality Control Testing: Principles and Definitions; Approved Guideline. NCCLS Document C24-A Villanova, PA: NCCLS, 1991.
19. Hook, E.W. and Zenilman, J.M. 1998. Gonorrhea, in Infectious Diseases , (Gorbach, S.L., Bartlett, J.G., and Blacklow, N.R., ed.) Second Edition, W.B. Saunders, Philadelphia PA, 969-974).
20. National Committee for Clinical Laboratory Standards. Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition; Approved Guideline. NCCLS Document EP5-A Villanova, PA: NCCLS, 1999.



Distributed by

Roche Molecular Systems, Inc., Branchburg, NJ 08876 USA
A Member of the Roche Group

Roche Diagnostics
Indianapolis, IN 46256 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800 526 1247)

Roche Diagnostics
H7V 4A2 Laval, Quebec
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877 273 3433)

Roche Diagnostics (Schweiz) AG
CH-6343 Rotkreuz

Roche Diagnostics
F-38240 Meylan

Roche Diagnostics GmbH
D-68298 Mannheim, Germany

Distributore in Italia:
Roche Diagnostics SpA
Piazza Durante 11
I-20131 Milano

Roche Diagnostics S.L.
E-08006 Barcelona

Distribuidor em Portugal:
Roche Farmacêutica Química, Lda
P-2700 Amadora

ROCHE, COBAS, AMPLICOR, AMPLITAQ, AMPLILINK and AMPERASE are
trademarks of a member of the Roche Group.

ROCHE RESPONSE CENTER is a service mark of a member of the Roche Group.

ChlamTrans™ is a trademark of Bartels, Inc.

Dynabeads® paramagnetic particles are licensed under patents owned by Dynal Biotech
ASA, Oslo, Norway. Dynabeads® is a registered trademark of Dynal Biotech ASA,
Oslo, Norway, licensed to Roche Diagnostics Corporation, Indianapolis, Indiana.

Eppendorf® and Eppendorf Combitip® are registered trademarks of Eppendorf-Netheler-
Hinz GmbH, Hamburg, Germany.

Pipetman® is a registered trademark of Gilson Medical Electronics, Inc.

ProClin® is a registered trademark of Rohm and Haas Company.

Repeater® is a registered trademark of Brinkmann Instruments, Inc.

Replens® is a registered trademark of Columbia Laboratories Inc.

Copyright 2004, Roche Molecular Systems, Inc.
All rights reserved.

10/2004
00058003358-04