cobas® HPV Test

cobas

FOR *IN VITRO* DIAGNOSTIC USE

cobas® 4800 system Sample Preparation Kit	**c4800 SMPL PREP**	**960 Tests** **240 Tests**	**P/N: 05235804190** **P/N: 05235782190**
cobas® 4800 HPV Amplification/Detection Kit	**c4800 HPV AMP/DET**	**960 Tests** **240 Tests**	**P/N: 05235898190** **P/N: 05235880190**
cobas® 4800 HPV Controls Kit	**c4800 HPV CTLS**	**10 Sets**	**P/N: 05235855190**
cobas® 4800 system Liquid Cytology Preparation Kit	**c4800 LIQ CYT**	**960 Tests** **240 Tests**	**P/N: 05235839190** **P/N: 05235812190**
cobas® 4800 system Wash Buffer Kit	**c4800 WB**	**960 Tests** **240 Tests**	**P/N: 05235871190** **P/N: 05235863190**

NOTICE: The purchase of this product allows the purchaser to use it for amplification and detection of nucleic acid sequences by polymerase chain reaction (PCR) and related processes for human *in vitro* diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

TABLE OF CONTENTS

INTENDED USE

The **cobas**® HPV Test is a qualitative *in vitro* test for the detection of Human Papillomavirus (HPV) in patient specimens. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies types HPV 16 and HPV 18 while concurrently detecting the rest of the high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The **cobas**® HPV Test is indicated:

(a) To screen patients 21 years and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology test results to determine the need for referral to colposcopy

(b) To be used in patients 21 years and older with ASC-US cervical cytology results, to assess the presence or absence of high-risk HPV genotypes 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy

(c) In women 30 years and older, the **cobas**® HPV Test can be used with cervical cytology to adjunctively screen to assess the presence or absence of high risk HPV types. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

(d) In women 30 years and older, the **cobas**® HPV Test can be used to assess the presence or absence of HPV genotypes 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

Cervical specimens that may be tested with the cobas® HPV Test include the following liquid based collection media and collection device:

- ThinPrep® Pap Test™ PreservCyt® Solution
- Endocervical Brush/Spatula

WARNING

This test is not intended for use as a screening device for women under age 30 with normal cervical cytology.

The **cobas**® HPV Test is not intended to substitute for regular cervical cytology screening.

The **cobas**® HPV Test is not intended for use in determining the need for treatment (i.e. excisional or ablative treatment of the cervix) in the absence of high-grade cervical dysplasia. Patients who are HPV 16/18 positive should be monitored carefully for the development of high-grade cervical dysplasia according to current practice guidelines.

The use of this test has not been evaluated for the management of women with prior ablative or excisional therapy, hysterectomy, who are pregnant, or who have other risk factors (e.g. HIV+, immunocompromised, history of STI).

The **cobas**® HPV Test is designed to enhance existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.

SUMMARY AND EXPLANATION OF THE TEST

Persistent infection with human papillomavirus (HPV) is the principal cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN)[1-3]. The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide[3]. HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 118 different types of HPV[4,5], and approximately 40 different HPVs that can infect the human anogenital mucosa[6,7]. However, only a subset of approximately 14 of these types is considered high-risk for the development of cervical cancer and its precursor lesions[3,8-13]. In this document "HPV" means "high risk HPV," except where otherwise noted.

Although persistent infection with high-risk (HR) HPV is a necessary cause of cervical cancer and its precursor lesions, a very small percentage of infections progress to these disease states. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women experiencing exposure to HPV at some point[14]. However, almost all of infected women will mount an effective

immune response and clear the infection within 2 years without any long term health consequences[15-20]. An infection with any HPV type can produce cervical intraepithelial neoplasia (CIN) although this also usually resolves once the HPV infection has been cleared[21].

In developed countries with cervical cancer screening programs, the Pap smear has been used since the mid-1950s as the primary tool to detect early precursors to cervical cancer. Although it has decreased the death rates due to cervical cancer dramatically in those countries, the Pap smear and subsequent liquid based cytology methods require interpretation by highly trained cytopathologists and have a high rate of false negatives. Cytological abnormalities are primarily due to infection with HPV; however, various inflammatory or sampling variations can result in false positive cytology results. Triage of an abnormal cytology result involves repeat testing, colposcopy and biopsy. A histologically confirmed high-grade lesion must be surgically removed in order to prevent the development of invasive cervical cancer.

Papillomavirus is extremely difficult to culture *in vitro*, and not all patients infected with HPV have a demonstrable antibody response. Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV infection. Proper implementation of nucleic acid testing for HPV may increase the sensitivity of cervical cancer screening programs by detecting high-risk lesions earlier in women 30 years and older with NILM (negative for intraepithelial lesion or malignancy) cytology and reducing the need for unnecessary colposcopy and treatment in patients 21 and older with ASC-US cytology.

PRINCIPLES OF THE PROCEDURE

The **cobas**® HPV Test is based on two major processes: (1) automated specimen preparation to simultaneously extract HPV and cellular DNA; (2) PCR amplification[22] of target DNA sequences using both HPV and β-globin specific complementary primer pairs and real-time detection of cleaved fluorescent-labeled HPV and β-globin specific oligonucleotide detection probes. The concurrent extraction, amplification and detection of β-globin in the **cobas**® HPV Test monitors the entire test process.

The master mix reagent for the **cobas**® HPV Test contains primer pairs and probes specific for the 14 high-risk HPV types and β-globin DNA. The detection of amplified DNA (amplicon) is performed during thermal cycling using oligonucleotide probes labeled with four different fluorescent dyes. The amplified signal from 12 high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), is detected using the same fluorescent dye, while HPV 16, HPV 18 and β-globin signals are each detected with their own dedicated fluorescent dye.

Specimen Preparation

Specimen preparation for the **cobas**® HPV Test is automated with the use of the **cobas** x 480 instrument. Cervical specimens collected in PreservCyt solution are digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids, along with the β-globin DNA serving as process control, are purified through adsorption to magnetic glass particles, washed and finally separated from these particles, making them ready for PCR amplification and detection.

PCR Amplification

Target Selection

The **cobas**® HPV Test uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the master mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)[3,8-13,23]. Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers.

An additional primer pair and probe target the human β-globin gene (330 bp amplicon) to provide a process control.

Target Amplification

Eagle Z05® DNA Polymerase[24], a chemically modified version of *Thermus* species Z05 DNA polymerase[25], is utilized for "hot start" amplification of the HPV targets and the β-globin control. First, the PCR reaction mixture is heated to activate Eagle Z05® DNA Polymerase, to denature the viral DNA and genomic DNA and to expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The Eagle Z05® DNA Polymerase, in the presence of divalent metal ion and excess dNTPs, extends the primer(s), and a second DNA strand is synthesized. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the target region of the HPV genome and β-globin gene. The DNA Polymerase extends the annealed primers along the target templates to produce an approximately 200-base pair double-stranded HPV target DNA molecule or a 330 base pair β-globin DNA molecule termed an amplicon. This process is repeated for a number of cycles, each cycle effectively doubling the

amount of amplicon DNA. Amplification occurs only in the region of the HPV genome and/or β-globin gene between the appropriate primer pair. The entire genome is not amplified.

Automated Real-time Detection

The **cobas**® HPV Test utilizes real-time[27,28] PCR technology. Each oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher that quenches fluorescent emissions from the dye in an intact probe. As amplification progresses, probes that are complementary to the amplicon bind to specific single-stranded DNA sequences and are cleaved by the 5' to 3' nuclease activity of the Eagle Z05® DNA Polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, it emits fluorescence of a characteristic wavelength when excited by the proper spectrum of light. This characteristic wavelength for each dye allows HPV-16 amplicon, HPV-18 amplicon, other HR amplicons (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and the beta-globin control to be measured independently because the probes specific for these sequences are labeled with different dyes.

Selective Amplification

Selective amplification of target nucleic acid from the patient specimen is achieved in the **cobas**® HPV Test by the use of AmpErase enzyme (uracil-N-glycosylase) and deoxyuridine triphosphate (dUTP). AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[26], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the master mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, 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. AmpErase enzyme in the **cobas**® HPV Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing HPV amplicon per PCR.

REAGENTS

cobas® 4800 system Sample Preparation Kit		**240 Tests**
(P/N: 05235782190)	**c4800 SMPL PREP**	
MGP		10 x 4.5 mL
(**cobas**® 4800 system Magnetic Glass Particles)		
Magnetic Glass Particles 93% Isopropanol Xi, 93% (w/w) Isopropanol *(Irritant symbol)* F, 93% (w/w) Isopropanol *(Highly Flamable symbol)* R: 11-36-67, S: 7-16-24/25-26		
EB		10 x 18 mL
(**cobas**® 4800 system Elution Buffer)		
Tris-HCl buffer 0.09% Sodium azide		

cobas® 4800 system Sample Preparation Kit **960 Tests**
(P/N: 05235804190)

MGP 10 x 13.5 mL
(**cobas®** 4800 system Magnetic Glass Particles)

Magnetic Glass Particles
93% Isopropanol
Xi, 93% (w/w) Isopropanol
(Irritant symbol)
F, 93% (w/w) Isopropanol
(Highly Flamable symbol)
R: 11-36-67, S: 7-16-24/25-26

EB 10 x 18 mL
(**cobas®** 4800 system Elution Buffer)

Tris-HCl buffer
0.09% Sodium azide

cobas® 4800 system Wash Buffer Kit **c4800 WB** **240 Tests**
(P/N: 05235863190)

WB 10 x 55 mL
(**cobas®** 4800 system Wash Buffer)

Sodium citrate dihydrate
0.05% N-Methyl isothiazolone HCl

cobas® 4800 system Wash Buffer Kit **c4800 WB** **960 Tests**
(P/N: 05235871190)

WB 10 x 200 mL
(**cobas®** 4800 system Wash Buffer)

Sodium citrate dihydrate
0.05% N-Methyl isothiazolone HCl

cobas® 4800 system Liquid Cytology Preparation Kit **240 Tests**
(P/N: 05235812190)

P 10 x 0.9 mL
(**cobas®** 4800 Proteinase K)

Tris-HCl buffer
EDTA
Glycerol
Calcium chloride
Calcium acetate
< 2% Proteinase K
Xi, < 2% Proteinase K

(Irritant symbol)

SDS 10 x 3 mL
(**cobas®** 4800 system SDS Reagent)

Tris-HCl buffer
0.2% SDS
0.09% Sodium azide

LYS			10 x 10 mL
(**cobas**® 4800 system Lysis Buffer)			
Tris-HCl buffer			
37% (w/w) Guanidine HCl			
Xn, 37% (w/w) Guanidine HCl			
(Harmful symbol)			
R: 22-36/38, S: 13-26-36-46			
< 5 % Polydocanol			
(Environmental Hazard symbol)			
cobas® 4800 system Liquid Cytology Preparation Kit			**960 Tests**
(P/N: 05235839190			
PK			20 x 1.2 mL
(**cobas**® 4800 Proteinase K)			
Tris-HCl buffer			
EDTA			
Glycerol			
Calcium chloride			
Calcium acetate			
< 2% Proteinase K			
Xi, < 2% Proteinase K			
(Irritant symbol)			
SDS			10 x 9 mL
(**cobas**® 4800 system SDS Reagent)			
Tris-HCl buffer			
0.2% Sodium dodecyl sulfate			
0.09% Sodium azide			
LYS			10 x 36 mL
(**cobas**® 4800 system Lysis Buffer)			
Tris-HCl buffer			
37% (w/w) Guanidine HCl			
Xn, 37% (w/w) Guanidine HCl			
(Harmful symbol)			
R: 22-36/38, S: 13-26-36-46			
< 5 % Polydocanol			
(Environmental Hazard symbol)			
cobas® 4800 HPV Amplification/Detection Kit		**c4800 HPV AMP/DET**	**240 Tests**
(P/N: 05235880190)			
HPV MMX			10 x 0.5 mL
(**cobas**® 4800 HPV Master Mix)			
Tricine buffer			
Potassium acetate			
Potassium hydroxide			
Glycerol			
< 0.13 % dATP, dCTP, dGTP, dUTP			
< 0.01 %Upstream and downstream HPV primers			
< 0.01 %Upstream and downstream ß-Globin primers			
< 0.01 %Fluorescent-labeled HPV probes			
< 0.01 %Fluorescent-labeled ß-Globin probes			
< 0.10 % Eagle Z05® DNA polymerase (microbial)			
< 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)			
0.09% Sodium azide			

HPV Mg/Mn			10 x 1.0 mL
(**cobas**® 4800 HPV Mg/Mn Solution)			
Magnesium acetate			
Manganese acetate			
< 0.02% Glacial acetic acid			
0.09% Sodium azide			
cobas® 4800 HPV Amplification/Detection Kit		**c4800 HPV AMP/DET**	**960 Tests**
(P/N: 05235898190)			
HPV MMX			20 x 1.0 mL
(**cobas**® 4800 HPV Master Mix)			
Tricine buffer			
Potassium acetate			
Potassium hydroxide			
Glycerol			
< 0.13 % dATP, dCTP, dGTP, dUTP			
< 0.01 %Upstream and downstream HPV primers			
< 0.01 %Upstream and downstream ß-Globin primers			
< 0.01 %Fluorescent-labeled HPV probes			
< 0.01 %Fluorescent-labeled ß-Globin probes			
< 0.10 % Eagle Z05® DNA polymerase (microbial)			
< 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)			
0.09% Sodium azide			
HPV Mg/Mn			10 x 1.0 mL
(**cobas**® 4800 HPV Mg/Mn Solution)			
Magnesium acetate			
Manganese acetate			
< 0.02% Glacial acetic acid			
0.09% Sodium azide			
cobas® 4800 HPV Controls Kit		**c4800 HPV CTLS**	**10 Sets**
(P/N: 05235855190)			
HPV (+) C			10 x 0.5 mL
(**cobas**® 4800 HPV Positive Control)			
Tris-HCl buffer			
EDTA			
0.05% Sodium azide			
< 0.002% Poly rA RNA (synthetic)			
< 0.01% Non-infectious plasmid DNA (microbial) containing HPV-16, 18, 39 sequences			
< 0.01% Non-infectious plasmid DNA (microbial) containing ß-Globin sequences			
(–) C			10 x 0.5 mL
(**cobas**® 4800 system Negative Control			
Tris-HCl buffer			
EDTA			
0.05% Sodium azide			
< 0.002% Poly rA RNA (synthetic)			

WARNINGS AND PRECAUTIONS

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

B. Do not pipette by mouth.

C. 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 test reagents.

D. Avoid microbial and DNA contamination of reagents.

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

F. Do not use reagents after their expiration dates.

G. Do not pool reagents.

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

I. Gloves must be worn and must be changed between handling specimens and **cobas**® 4800 reagents to prevent contamination.

J. Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[29] and in the CLSI Document M29-A3[30].

K. **LYS** contains guanidine hydrochloride. **Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.** If liquid containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, **FIRST** clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.

L. **MGP** contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.

M. **EB**, **SDS**, **HPV MMX**, **HPV Mg/Mn**, **(–)C**, and **HPV (+)C** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.

N. Wear eye protection, laboratory coats and disposable gloves when handling any reagents. 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 occur, dilute with water before wiping dry.

O. All disposable items are for one time use. Do not reuse.

P. Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or **cobas z** 480 analyzer according to procedures described in the **cobas**® 4800 system Operator's Manual.

Q. For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 system Operator's Manual.

STORAGE AND HANDLING REQUIREMENTS

A. Do not freeze reagents.

B. Store MGP, EB, PK, SDS, LYS, HPV MMX, HPV Mg/Mn, HPV (+) C and (-) C at 2-8°C. These reagents are stable until the expiration date indicated.

C. Store WB at 15-25°C. This reagent is stable until the expiration date indicated.

MATERIALS PROVIDED

A. cobas® 4800 system Sample Preparation Kit (P/N: 05235782190) **MGP** (**cobas®** 4800 system Magnetic Glass Particles) **EB** (**cobas®** 4800 system Elution Buffer)	**c4800 SMPL PREP**	**240 Tests**
B. cobas® 4800 system Sample Preparation Kit (P/N: 05235804190) **MGP** (**cobas®** 4800 system Magnetic Glass Particles) **EB** (**cobas®** 4800 system Elution Buffer)	**c4800 SMPL PREP**	**960 Tests**
C. cobas® 4800 system Wash Buffer Kit (P/N: 05235863190) **WB** (**cobas®** 4800 system Wash Buffer)	**c4800 WB**	**240 Tests**
D. cobas® 4800 system Wash Buffer Kit **(P/N: 05235871190)** **WB** (**cobas®** 4800 system Wash Buffer)	**c4800 WB**	**960 Tests**
E. cobas® 4800 system Liquid Cytology Preparation Kit (P/N: 05235812190) **PK** (**cobas®** 4800 Proteinase K) **SDS** (**cobas®** 4800 system SDS Reagent) **LYS** (**cobas®** 4800 system Lysis Buffer)	**c4800 LIQ CYT**	**240 Tests**
F. cobas® 4800 system Liquid Cytology Preparation Kit (P/N: 05235839190) **PK** (**cobas®** 4800 Proteinase K) **SDS** (**cobas®** 4800 system SDS Reagent) **LYS** (**cobas®** 4800 system Lysis Buffer)	**c4800 LIQ CYT**	**960 Tests**
G. cobas® 4800 HPV Amplification/Detection Kit (P/N: 05235880190) **HPV MMX** (**cobas®** 4800 HPV Master Mix) **HPV Mg/Mn** (**cobas®** 4800 HPV Mg/Mn Solution)	**c4800 HPV AMP/DET**	**240 Tests**

H. cobas® 4800 HPV Amplification/Detection Kit | **c4800 HPV AMP/DET** | **960 Tests**
(P/N: 05235898190)

HPV MMX
(**cobas®** 4800 HPV Master Mix)

HPV Mg/Mn
(**cobas®** 4800 HPV Mg/Mn Solution)

I. cobas® 4800 HPV Controls Kit | **c4800 HPV CTLS** | **10 Sets**
(P/N: 05235855190)

HPV (+) C
(**cobas®** 4800 HPV Positive Control)

(–) C
(**cobas®** 4800 system Negative Control)

MATERIALS REQUIRED BUT NOT PROVIDED

Specimen and Reagent Handling

- CORE Tips, 1000 µL, rack of 96 (P/N: 04639642001 or Hamilton P/N: 235905)
- 50 mL Reagent Reservoir (P/N: 05232732001)
- 200 mL Reagent Reservoir (P/N: 05232759001)
- **cobas®** 4800 system Extraction (deep well) Plate (P/N: 05232716001)
- **cobas®** 4800 system AD (microwell) Plate and Sealing Film (P/N: 05232724001)
- Waste Bag [P/N: 05530873001 (small) or P/N:04691989001 (large)]
- Hamilton STAR Plastic Chute (P/N: 04639669001)
- Tubes 13 mL Round Base, (Sarstedt P/N: 540.500) for use as secondary sample tubes
- Caps, neutral color (Sarstedt: P/N 65.176; for recapping post-run specimens in 13 mL round base Sarstedt tubes)
- Vortex mixer
- Disposable gloves, powderless
- Pipettes: capable of delivering 1000 µL
- Aerosol barrier DNase-free tips: capable of delivering 1000 µL

Instrumentation and Software

- **cobas x** 480 instrument
- **cobas z** 480 analyzer
- **cobas®** 4800 system control unit with system software version 1.1 or higher
- **cobas®** 4800 Work Order Editor version 1.1.0.1016 or higher
- Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500 (optional, for PCR Only workflow)
- Stand-alone magnetic plate (P/N: 05440777001, optional, for PCR Only workflow)

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

A. Specimen Collection

Cervical specimens collected in PreservCyt solution using an endocervical brush/spatula have been validated for use with the **cobas®** HPV Test. Follow the manufacturer's instructions for collecting cervical specimens.

B. Specimen Transport

Cervical specimens collected in PreservCyt solution can be transported at 2-30°C. Transportation of HPV specimens must comply with country, federal, state and local regulations for the transport of etiologic agents[31].

C. Specimen Storage

Cervical specimens collected in PreservCyt solution may be stored at 2-30°C for up to 6 months after the date of collection prior to performing the **cobas®** HPV test. See PreservCyt solution labeling for storage requirements prior to cytology processing. PreservCyt specimens should not be frozen.

INSTRUCTIONS FOR USE

NOTE: All reagents except HPV MMX and HPV Mg/Mn must be at ambient temperature prior to loading on the cobas x 480 instrument. The HPV MMX and HPV Mg/Mn may be taken directly from 2-8°C storage as they will equilibrate to ambient temperature on board the cobas x 480 instrument by the time they are used in the process.

NOTE: Specimens in PreservCyt solution must be at ambient temperature before loading on the cobas x 480 instrument.

NOTE: Refer to the cobas® 4800 system Operator's Manual for detailed operating instructions.

Run Size

The **cobas®** 4800 system is designed to support the **cobas®** HPV Test with run sizes from 1 to 22 specimens plus controls (up to 24 tests per run) and from 1 to 94 specimens plus controls (up to 96 tests per run). Each **cobas®** 4800 system Sample Preparation Kit, **cobas®** 4800 system Liquid Cytology Preparation Kit, **cobas®** 4800 system Wash Buffer Kit and **cobas®** 4800 HPV Amplification/Detection Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit). The **cobas®** 4800 HPV Controls Kit contains reagents sufficient for a total of 10 runs of either 24 or 96 tests (10 sets per kit). The minimum run size on the **cobas®** 4800 system is 1 specimen plus controls. One replicate of the **cobas®** 4800 system Negative Control [**(–) C**] and one replicate of the **cobas®** 4800 HPV Positive Control [**HPV (+) C**] are required to perform each test run (see *"Quality Control"* section).

Workflow

NOTE: Although not an optimal use of reagents, a 960 Test Kit can be used for a 24 sample run.

The **cobas®** HPV Test can be run using either of two workflows, referred to as "full workflow" or "PCR only workflow" within the **cobas®** 4800 software.

HPV Full Workflow

The "HPV full workflow" consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. Run size can be a 24-test format (from 1 to 22 specimens plus 2 controls) or a 96-test format (from 1 to 94 specimens plus 2 controls). Refer to the "Performing a Full Workflow" section below and the **cobas** 4800 system Operator's Manual for details.

HPV PCR Only Workflow

The "HPV PCR Only workflow" consists of amplification/detection on the **cobas z** 480 analyzer. Run size can be from 1 to 94 specimens plus 2 controls. Refer to the "Performing a PCR Only Workflow" section below and the **cobas®** 4800 system Operator's Manual for details.

Specimens

PreservCyt solution specimens must be processed out of a properly barcoded 13 mL round-based secondary (Sarstedt) tube on the **cobas x** 480 instrument. Consult the **cobas®** 4800 system Operator's Manual for proper barcoding procedures and the list of acceptable

barcodes for the **cobas®** 4800 system. ThinPrep collection vials should not be placed directly on the cobas 4800 system for processing. The cobas HPV Test has been clinically validated only with PreservCyt specimens aliquotted into 13 mL round-based Sarstedt tubes.

Fill 13 mL round-based secondary tubes to a minimum volume of 1.0 mL and a maximum volume of 4 mL of PreservCyt specimen.

NOTE: Use only PreservCyt solution and an endocervical brush/spatula to collect cervical specimens for the cobas® HPV Test. The cobas® HPV Test has not been validated with other collection devices or media types. Using the cobas® HPV Test with other collection devices and/or media types may lead to false negative, false positive and/or invalid results.

NOTE It is necessary to aliquot specimens into barcoded 13 mL round-based secondary tubes for processing on the cobas x 480 instrument. Use pipettors with aerosol-barrier or positive-displacement tips to handle specimens. To avoid cross-contamination, additional caps for these tubes in an alternate color (neutral) should be used to recap these specimens after processing.

NOTE The cobas HPV Test should not be performed on PreservCyt specimens after cytology processing on a ThinPrep processor (i.e. on the TP2000 or TP3000). The cobas HPV Test has been clinically validated only with PreservCyt specimens aliquotted prior to performing a cytology test. A single aliquot of up to 4 ml may be removed from a ThinPrep vial prior to cytology processing.

NOTE Use caution when transferring specimens from primary containers to 13 mL round-based secondary tubes. Vortex primary specimens prior to transfer. Change pipetting tips after each specimen. See ThinPrep labeling for detailed instructions on aliquot removal.

NOTE: Do not process specimens which appear bloody or have a dark brown color.

Workflows

Performing a Full Workflow:

A. The **cobas®** HPV Test may be used for runs of 1 to 22 specimens plus one **cobas®** 4800 system negative control and one **cobas®** 4800 HPV positive control (24-test format) and for runs of 1 to 94 specimens plus one **cobas®** 4800 system negative control and one **cobas®** 4800 HPV positive control (96-test format).

B. Perform the system startup and maintenance procedures by following the instructions in the **cobas®** 4800 system Operator's Manual in the Operation section.

C. Create a Work Order file for a full run by following the instructions in the **cobas®** 4800 system Operator's Manual. A Work Order file is not required if an LIS is in use.

D. Select the test subtype and media type (PreservCyt) for each specimen.

- Choose test subtype "HPV High Risk Panel" to report High Risk HPV test results without separate reporting of HPV16 and HPV18 results.
- Choose test subtype "HPV High Risk Panel Plus Genotyping" to report High Risk HPV and separate HPV16 and HPV18 results.

G. Start the new run by following the software wizard guide. Select the test type as "HPV workflow".

H. Follow the software wizard guide to load specimens and the Work Order file.

NOTE: Specimens can be loaded in barcoded secondary tubes in any order as long as their barcodes match those in the Work Order.

I. Follow the software wizard guide to load all consumables.

J. Follow the software wizard guide to load all reagents.

NOTE: Controls [HPV (+) C and (–) C] are not loaded together with specimens. They are loaded onto the reagent carrier during reagent loading. Two positions (A1 and B1) on each of the deep well plate and microwell plate are reserved for the HPV (+) and (–) controls, respectively.

NOTE: ***The cobas® 4800 system has an internal clock to monitor the length of time the reagents are on-board. Once the WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.***

NOTE: ***To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial prior to pouring into the reagent reservoir.***

K. Load the sample preparation reagents (WB, MGP, EB, SDS and LYS) into the barcoded reagent reservoirs using the "scan-scan-pour-place" method:

- Scan the reagent bottle barcode
- Scan the reagent reservoir barcode
- Pour the reagent into the reservoir
- Place the filled reagent reservoir into the designated position on the reagent carrier

L. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the appropriate reagent reservoir sizes. The reagent reservoir barcodes must face to the right of the carrier.

NOTE: ***Amplification/detection reagents (HPV MMX and HPV Mg/Mn), Controls [HPV (+) C and (–) C] and PK are loaded directly onto the reagent carrier and scanned by the cobas x 480 instrument automatically.***

NOTE: ***All reagents and reagent reservoirs are bar-coded and designed for one time use. The cobas® 4800 software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs. The software also verifies that reagents from appropriately sized kits are loaded on the instrument, i.e. preventing 240 test kit reagents from being used in a run with more than 22 patient specimens.***

NOTE: ***The cobas® 4800 software tacks the expiration date of all reagents. Reagents that are beyond their expiration date will not be accepted for use on the cobas® 4800 system.***

M. Start sample preparation by clicking on "Start Run".

N. After successful completion of sample preparation, click **"Unload' to unload the plate carrier.

** The status of sample preparation can be reviewed at this point, prior to clicking "Unload". See the **cobas®** 4800 system Operator's Manual for details.

O. Follow the instructions in the **cobas®** 4800 system Operator's Manual to seal the microwell plate, transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

NOTE: ***The cobas® 4800 system has an internal clock to monitor the length of time after addition of the prepared samples to working master mix. Amplification and detection should be started as soon as possible but no later than 90 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.***

P When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.

Q. Follow the instructions in the **cobas®** 4800 system Operator's Manual to review and accept results.

Performing a PCR Only Workflow

NOTE: ***The PCR only run is available as a recovery option in the event that the full workflow cannot be completed due to circumstances beyond the user's control (e.g. power failure during amplification/detection run).***

NOTE: ***Only samples successfully processed on the cobas x 480 instrument can be amplified/detected using the PCR only run. System surveillance for reagents and consumables is limited during the PCR only run. No sample position tracking is provided when using the PCR only run – the end user must ensure that the actual position of a sample on the microwell plate corresponds to the one designated in the Work Order file. Extreme care must be exercised while preparing the microwell plate to ensure proper PCR set-up and to avoid contamination.***

NOTE: ***Samples processed on the cobas x 480 instrument have limited stability. They must be amplified/detected using the PCR only run within 24 hours if stored at 15°C to 30°C and within 7 days if stored at 2°C to 8°C.***

NOTE: Follow the instructions in the cobas® 4800 system Operator's Manual for renaming of Positive and Negative Control barcodes.

A. Create a Work Order file for a PCR only workflow run by following the instructions in the **cobas®** 4800 system Operator's Manual.

   a. Refer to the result printout or the result export file for sample barcodes, media types, sub-test types and positions in the **cobas®** 4800 deep well plate for the run which requires a repeat of the amplification/detection.

   b. For the positive and negative controls, edit the last 4 digits to identify a reuse of the control barcodes for amplification and detection only workflow by following the instructions in the **cobas®** 4800 system Operator's Manual.

B. Prepare the **cobas®** 4800 HPV working master mix:

   a. For a run of up to 24 tests, add 240 µL of HPV Mg/Mn to one vial of HPV MMX (0.5 mL vial from 240 Test Kit).

   b. For a run of up to 96 tests, add 450 µL of HPV Mg/Mn to each of the two vials of HPV MMX (1.0 mL vials from 960 Test Kit).

NOTE: The PCR Only run must be started within 90 minutes of addition of HPV Mg/Mn to the HPV MMX. The system does not monitor the length of time after addition of the prepared samples to working master mix in the PCR only workflow. The end user must ensure that amplification and detection is started within the allotted time.

C. Thoroughly mix working master mix by carefully inverting the vial(s). Do not vortex the working master mix.

D. Transfer 25 µL of working master mix to each of the required wells in the microwell plate.

E. Place the deep well plate from the run to be repeated onto the stand-alone magnetic plate.

F. Manually transfer 25 µL of eluate from the deep well plate wells to the corresponding wells in the microwell plate. Ensure that well positions are maintained (e.g. eluate in A1 well in deep well plate is transferred to A1 on the microwell plate). Ensure that no MGP is carried over to the microwell plate.

G. Follow the instructions in the **cobas®** 4800 system Operator's Manual to seal the microwell plate.

H. Centrifuge the microwell plate using a swinging bucket rotor for at least 5 seconds at 1500 RCF.

I. Transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

J. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.

K. Follow the instructions in the **cobas®** 4800 system Operator's Manual to review and accept results.

Interpretation of Results

NOTE: All assay and run validation is performed by the cobas® 4800 software.

NOTE: A valid run may include both valid and invalid specimen results.

For a valid run, specimen results are interpreted as shown in Tables 1 and 2:

612 ***Table 1***
613 ***Result Interpretation of the cobas® HPV Test for Presence of HPV DNA***

cobas® HPV Test	Result Report and Interpretation
SubTest "HPV High Risk Panel":	
HR HPV POS	**High Risk HPV Positive** Specimen is positive for the DNA of any one of, or combination of, the following high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
HR HPV NEG	**High Risk HPV Negative*** HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid	**High Risk HPV Invalid** Results are invalid. Original specimen should be re-tested to obtain valid result.
Failed	**No Result for Specimen** Consult the **cobas®** 4800 system Operator's Manual for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid result.
SubTest "HPV High Risk Panel Plus Genotyping"	
Other HR HPV POS, HPV16 POS, HPV18 POS	**Other High Risk HPV Positive, HPV16 Positive, HPV18 Positive.** Specimen is positive for HPV types 16 and 18 DNA and the DNA of any one of, or combination of, the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
Other HR HPV POS, HPV16 POS, HPV18 NEG	**Other High Risk HPV Positive, HPV16 Positive, HPV18 Negative*.** Specimen is positive for HPV type 16 DNA and the DNA of any one of, or combination of, the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. HPV type 18 DNA was undetectable or below the pre-set threshold.
Other HR HPV POS, HPV16 NEG, HPV18 POS	**Other High Risk HPV Positive, HPV16 Negative*, HPV18 Positive.** Specimen is positive for HPV type 18 DNA and the DNA of any one of, or combination of, the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. HPV type 16 DNA was undetectable or below the pre-set threshold.
Other HR HPV POS, HPV16 NEG, HPV18 NEG	**Other High Risk HPV Positive, HPV16 Negative*, HPV18 Negative*.** Specimen is positive for the DNA of any one of, or combination of, the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. HPV types 16 and 18 DNA were undetectable or below the pre-set threshold.
Other HR HPV NEG, HPV16 POS, HPV18 POS	**Other High Risk HPV Negative*, HPV16 Positive, HPV18 Positive.** HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold. Specimen is positive for HPV types 16 and 18 DNA.
Other HR HPV NEG, HPV16 NEG, HPV18 POS	**Other High Risk HPV Negative*, HPV16 Negative*, HPV18 Positive.** HPV types 16, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold. Specimen is positive for HPV type 18 DNA.
Other HR HPV NEG, HPV16 POS, HPV18 NEG	**Other High Risk HPV Negative*, HPV16 Positive, HPV18 Negative*.** HPV types 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold. Specimen is positive for HPV type 16 DNA.
Other HR HPV NEG, HPV16 NEG, HPV18 NEG	**Other High Risk HPV Negative*, HPV16 Negative*, HPV18 Negative*.** HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid	**Invalid.** The results are Invalid. Original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained.
Failed	**No Result for Specimen** Consult the **cobas®** 4800 system Operator's Manual for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid results.

*A negative result does not preclude the presence of HPV infection because results depend on adequate specimen collection, absence of inhibitors and sufficient DNA to be detected.

Table 2
Result Interpretation of the cobas® HPV Test*

Results	Interpretation for Patients with ASC-US cytology who are ≥21 years old	Interpretation for Patients with NILM cytology who are ≥30 years old
Other HR HPV** NEG, HPV16 NEG, HPV18 NEG	Very low likelihood of underlying ≥ CIN2;	Lowest likelihood of underlying ≥ CIN2.
Other HR HPV** POS, HPV16 NEG, HPV18 NEG	Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy.	Low likelihood of underlying ≥ CIN2.
HPV16 POS and/or HPV18 POS	Highest likelihood that underlying ≥ CIN2 will be detected at colposcopy [32, 33].	Increased likelihood of underlying ≥CIN2.

**Other HR HPV DNA includes the following types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
*According to the 2006 consensus guidelines, HPV testing should not be performed on women younger than 21 years of age. Also, women 21 years and older with greater than ASC-US cytology (including ASC-H, LSIL or above) should proceed to colposcopy regardless of their HPV test results.

NOTE: ***HPV negative results are not intended to prevent women from proceeding to colposcopy.***

NOTE: ***In addition to the results tabulated above, invalid results for one or more combinations are also possible. If such a result is obtained, for example:***

Other HR HPV NEG, HPV16 POS, HPV18 Invalid

The positive and negative results should be interpreted as shown in Table 1. In this example, HPV 18 results are invalid. The specimen should be re-tested to obtain valid results.

NOTE: ***Negative results indicate HPV DNA concentrations are undetectable or below the pre-set threshold.***

NOTE: ***Positive test results indicates the presence of any one or more of the high risk types, but since patients are often co-infected with low-risk types it does not rule out the presence of low-risk types in patients with mixed infections.***

NOTE: ***Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.***

QUALITY CONTROL

One set of **cobas®** 4800 HPV Test Positive and Negative Controls are included in each run. For any run, valid results must be obtained for both the Positive and Negative Control for the **cobas®** 4800 software to display the reportable **cobas®** HPV Test results from that run.

Positive Control

The HPV (+) Control result must be 'Valid'. If the HPV (+) Control results are consistently invalid, contact your local Roche office for technical assistance.

Negative Control

The (-) Control result must be 'Valid'. If the (-) Control results are consistently invalid, contact your local Roche office for technical assistance.

PROCEDURAL PRECAUTIONS

1. ThinPrep collection vials should not be placed directly on the cobas 4800 system for processing. The **cobas®** HPV Test has been clinically validated only with PreservCyt specimens aliquotted into 13 mL round-based Sarstedt tubes.
2. The **cobas®** HPV Test should not be performed on PreservCyt specimens after cytology processing on a ThinPrep processor (i.e. on the TP2000 or TP3000). The **cobas®** HPV Test has been clinically validated only with PreservCyt specimens aliquotted prior to performing a cytology test.
3. 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, care should be taken to keep reagents and amplification mixtures free of contamination.

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

PROCEDURAL LIMITATIONS

1. The **cobas**® 4800 HPV Test detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types[34].
2. The **cobas**® 4800 HPV Test for detection of human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is not recommended for evaluation of suspected sexual abuse.
3. The performance of the **cobas**® 4800 HPV Test has not been adequately established for HPV vaccinated individuals[35].
4. Test only the indicated specimen type. The **cobas**® HPV Test has only been validated for use with cervical specimens collected in PreservCyt solution using a Pap Perfect® plastic spatula and Cytobrush® plus GT gentle touch.
5. Detection of high-risk HPV is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances.
6. Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
7. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer.
8. A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.
9. ß-globin amplification and detection is included in the **cobas**® HPV Test to differentiate HPV negative specimens from those that do not exhibit HPV signal due to insufficient cell mass in the specimen. All HPV negative specimens must have a valid ß-globin signal within a pre-defined range to be identified as valid negatives by the **cobas**® 4800 system.
10. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Package Insert and the **cobas**® 4800 system Operator's Manual.
11. The addition of AmpErase enzyme into the **cobas**® 4800 HPV Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.
12. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 system.
13. The **cobas**® 4800 system includes the **cobas x** 480 instrument and **cobas z** 480 analyzer together with the control unit. This is the only configuration that has been validated for use with this product. No other sample preparation instrument or PCR system can be used with this product.
14. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.
15. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
16. Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the **cobas**® HPV Test's primers and/or probes may result in failure to detect the presence of the viral DNA.
17. The presence of PCR inhibitors may cause false negative or invalid results.
18. Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the **cobas**® 4800 system. If concentrations of whole blood exceeds 1.5% (dark red or brown coloration) in PreservCyt solution, there is a likelihood of obtaining a false-negative result. The **cobas**® HPV Test performance has not been validated with PreservCyt specimens which have been treated with glacial acetic acid for removal of red blood cells. Any such processing of PreservCyt specimens prior to HPV testing would invalidate the cobas HPV Test results.
19. Cross-contamination of samples can cause false positive results. The sample to sample cross-contamination rate of the **cobas**® HPV Test on the **cobas**® 4800 system has been determined in a non-clinical study to be 0.71%. Run to run cross-contamination has not been observed.

EXPECTED RESULTS

A total of 47,208 subjects were enrolled in the study across 61 collection sites, and cervical samples were tested at 5 testing sites in the US. Of these, 46,887 (99.3%) subjects were eligible to participate in the study. Eligible subjects were women ≥ 21 years that had signed informed consent, satisfied study inclusion/exclusion criteria, had not enrolled in the study previously, and had not withdrawn authorization before undergoing any study procedures.

The median age of the eligible subjects was 39 years, with ~25% subjects in age group 21-29 years, ~27% in age group 30-39 years, and ~48% subjects in age group ≥ 40 years. A total of 90.0% of subjects had NILM cytology, and 4.1% subjects had ASC-US cytology.

A total of 1,918 subjects (ASC-US population with age ≥ 21 years) were evaluable; evaluable subjects were those who had an ASC-US cytology result and had valid results from the IUO HR HPV Test, IUO HPV genotyping Test, and **cobas**® HPV Test.

A total of 32,260 subjects (NILM population ≥ 30 years) were evaluable; evaluable subjects were eligible subjects ≥ 30 years who had a NILM cytology result and had valid results from the IUO HR HPV Test, IUO HPV genotyping Test, and **cobas**® HPV Test.

Table 3 shows HPV prevalence by the **cobas**® HPV Test by testing site and study population. The HPV prevalence was 31.9% in the ASC-US (≥ 21 years) population and 6.7% in the NILM (≥ 30 years) population.

Table 3
Summary of HPV Prevalence by the cobas® HPV Test by Testing Sites and Study Population

Testing Site	cobas® HPV Test – HPV Prevalence	
	ASC-US Population (≥ 21 Years)	NILM Population (≥ 30 Years)
1	32.8% (165/503)	6.4% (572/8,925)
2	35.5% (99/279)	6.5% (395/6,041)
3	36.5% (74/203)	7.1% (309/4,370)
4	34.6% (106/306)	7.0% (387/5,539)
5	26.8% (168/627)	6.9% (507/7,385)
Overall	31.9% (612/1,918)	6.7% (2,170/32,260)

Table 4 shows HPV prevalence by **cobas**® HPV Test result by age and study population. In the ASC-US population, HPV prevalence dropped from 53.5% in 21-29 years to 29.7% in 30-39 years and remained relatively constant at 15-20% after 40 years old. In the NILM population, HPV prevalence was 9.0% in 30-39 years and remained ~5% in ≥ 40 years.

Table 4
Summary of HPV Prevalence by cobas® HPV Test Result by Age and Study Population

Age Group (Years)	ASC-US Population (≥ 21 Years)	NILM Population (≥ 30 Years)
21-29	53.5% (335/626)	N/A
30-39	29.7% (151/508)	9.0% (1,029/11,398)
40-49	15.0% (76/508)	5.7% (627/10,944)
50-59	19.3% (40/207)	5.3% (378/7,106)
60-69	17.3% (9/52)	4.9% (111/2,287)
≥70	5.9% (1/17)	4.8% (25/525)

The **cobas**® HPV Test results, stratified into four groups by age for the ASC-US population is presented in Table 5 and for the NILM population in Table 6. In both populations, the 12 Other HR HPV positive results were more frequent than HPV16 positive and HPV18 positive results in general and within age groups. HPV prevalence decreases with age in both populations.

Table 5
Summary of cobas® HPV Test Result (Four-groups) by Age Group for Evaluable ASC-US Subjects

Age Group (Years)	cobas® HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	Negative	
21-29	15.5% (97/626)	5.6% (35/626)	32.4% (203/626)	46.5% (291/626)	626
30-39	6.1% (31/508)	2.2% (11/508)	21.5% (109/508)	70.3% (357/508)	508
40-49	3.5% (18/508)	0.6% (3/508)	10.8% (55/508)	85.0% (432/508)	508
50-59	1.4% (3/207)	2.9% (6/207)	15.0% (31/207)	80.7% (167/207)	207
60-69	0.0% (0/52)	1.9% (1/52)	15.4% (8/52)	82.7% (43/52)	52
≥70	0.0% (0/17)	0.0% (0/17)	5.9% (1/17)	94.1% (16/17)	17

Note: HPV16 positive implies HPV16 positive, HPV18 positive or negative and 12 Other HR HPV positive or negative.
HPV18 positive implies HPV16 negative, HPV18 Positive and 12 Other HR HPV positive or negative.
12 Other HR HPV positive implies HPV16 negative, HPV18 negative and 12 Other HR HPV positive .

Table 6
Summary of Four-Category cobas® HPV Test Result by Age Group for Evaluable NILM Subjects

Age Group (Years)	cobas® HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	Negative	
30-39	1.6%(183/11,398)	0.7%(84/11,398)	6.7%(762/11,398)	91.0% (10,369/11,398)	11,398
40-49	0.7%(80/10,944)	0.4%(41/10,944)	4.6%(506/10,944)	94.3% (10,317/10,944)	10,944
50-59	0.6%(41/7,106)	0.4%(27/7,106)	4.4%(310/7,106)	94.7% (67,28/7,106)	7,106
60-69	0.7%(16/2,287)	0.2%(4/2,287)	4.0%(91/2,287)	95.1% (2,176/2,287)	2,287
≥70	0.8%(4/525)	0.2%(1/525)	3.8%(20/525)	95.2% (500/525)	525

Note: HPV16 positive implies HPV16 positive, HPV 18 positive or negative and 12 Other HR HPV positive or negative.
HPV18 positive implies HPV16 negative, HPV18 Positive and 12 Other HR HPV positive or negative.
12 Other HR HPV positive implies HPV16 negative, HPV18 negative and 12 other HR positive.

PERFORMANCE CHARACTERISTICS

Clinical Performance

Baseline Phase

A multicenter, prospective study (ATHENA Study) was conducted to evaluate the performance of the **cobas**® HPV Test as a triage test to stratify women with ASC-US cytology results for colposcopy, and also as an adjunctive test to cervical cytology to guide management decisions. The study consisted of a Baseline Phase, as well as a 3 year Follow-up Phase. In the Baseline Phase, Subjects ≥ 21 years old undergoing routine cervical cancer screening were invited to participate in the study. In total, 47,208 subjects were enrolled from May 2008 to August 2009 at 61 clinical sites in the Baseline Phase. Following written informed consent, demographic information and gynecologic histories were obtained. Two cervical samples were collected for HPV testing and ThinPrep liquid based cytology (LBC). HPV testing was performed at five different laboratories and LBC testing at four. Cytology samples were classified according to the criteria of the 2001 Bethesda System. The first cervical sample collected from each study participant was tested with the **cobas**® HPV Test as well as an investigational use only (IUO) HR HPV test and an IUO HPV genotyping test. For testing with the **cobas**® HPV Test, the first ~29,000 samples collected were stored and were within the window for sample stability at the time of testing. The remaining ~18,000 samples collected were tested prospectively, i.e., in "real time" by the testing sites at the time of cervical sample collection. The second sample collected from all subjects with ASC-US Pap test results was tested with an FDA-approved test according to the manufacturer's instructions[36].

Those subjects ≥ 21 years old with ASC-US cytology were invited to undergo colposcopy. In addition, all subjects ≥30 years old with NILM (negative for intraepithelial lesions or malignancy) cytology and a positive test result for HR HPV DNA (positive by the IUO HR HPV test and/or the IUO HPV genotyping test), as well as a randomly selected subset of subjects (approximately 1:35) with NILM cytology/negative HR HPV DNA (by both the IUO HR HPV and the IUO HPV genotyping test), were invited to proceed to colposcopy. In order to avoid bias, both study participants and colposcopists were blinded to all HPV tests and cytology results until after the colposcopy was completed. Colposcopy was conducted according to a standardized protocol in which biopsies were obtained on all visible lesions; endocervical curettage was performed in all patients in whom the squamocolumnar junction was not visualized and a single random cervical biopsy was obtained if no lesions were visible. All biopsies were examined by a Central Pathology Review Panel (CPR) consisting of three expert pathologists, and discordant results adjudicated according to a pre-defined protocol. For all analyses, the clinical performance of **cobas**® HPV Test was measured against CPR histology results. The analyses were performed for those subjects with histology ≥ CIN2 and ≥ CIN3 by CPR . Subjects with a CPR diagnosis of ≥ CIN2 by CPR exited the study. All subjects who had undergone colposcopy and biopsy, without a diagnosis of ≥ CIN2 by CPR were invited to proceed to the Follow-up Phase of the study.

Follow-Up Phase

All subjects who did not have histology ≥ CIN2 by CPR were invited to participate in a 3 year longitudinal study. Approximately 8,000 eligible subjects have entered the follow-up study. Subjects undergo annual visits for cervical sampling for cytology and HPV DNA testing (by **cobas**® HPV test). All subjects with ≥ ASC-US are invited to proceed to colposcopy. Colposcopy and biopsies are performed in a standardized manner as described above. All cervical biopsies are examined by the Central Pathology Review Panel. All subjects with ≥ CIN2 by CPR exit the study and those with < CIN2 by CPR are invited to proceed to the follow-up year visit. In order to maximize disease ascertainment, an exit colposcopy and endocervical curettage (ECC) will be offered to all subjects in Year 3.

STUDY DESIGN TO DEMONSTRATE CLINICAL SENSITIVITY AND SPECIFICITY FOR SCREENING PATIENTS WITH ASC-US CYTOLOGY RESULTS TO DETERMINE THE NEED FOR REFERRAL FOR COLPOSCOPY

Those subjects ≥ 21 years old with ASC-US cytology, regardless of HPV results, were invited to undergo colposcopy. Both study participants and colposcopists were blinded to all HPV tests and cytology results until after the colposcopy was completed. Colposcopy was conducted according to a standardized protocol and all biopsies were read by the CPR, as described above. The clinical performance of **cobas**® HPV Test was measured against histology results of ≥ CIN2 and ≥ CIN3 by CPR.

STUDY DESIGN TO DEMONSTRATE CLINICAL PERFORMANCE OF THE COBAS HPV TEST AS AN ADJUNCT TO CERVICAL CYTOLOGY IN WOMEN ≥30 YEARS

All subjects • 30 years old with NILM (negative for intraepithelial lesions or malignancy) cytology and a positive test result for HR HPV DNA (positive by the IUO HR HPV test and/or the IUO HPV genotyping test), as well as a randomly selected subset of subjects (approximately 1:35) with NILM cytology/negative HR HPV DNA (by both the IUO HR HPV and the IUO HPV genotyping test), were invited to proceed to colposcopy. The analyses were performed for histology results • •CIN2 and • •CIN3 by CPR. All subjects ≥ 30

years who were invited to colposcopy and did not have histology ≥ CIN2 by CPR were eligible to participate in a 3 year longitudinal study for the **cobas®** HPV Test. All subjects with follow-up cytology ≥ ASC-US are invited to proceed to colposcopy; colposcopy and biopsies are performed in a standardized manner as describe above. All cervical biopsies are examined by the CPR and all subjects with ≥ CIN2 exit the study. Exit colposcopy and ECC are offered to all subjects. The objectives of the follow-up phase of the study are to determine the 3-year risk (cumulative incidence rates, CIRs) of developing ≥ CIN2 and ≥ CIN3 in subjects ≥ 30 years with NILM cytology. Risk will be measured according to the baseline HPV status (as determined by the **cobas®** HPV Test) for: positive and negative for HR HPV DNA and positive for genotype 16 and/or 18, as well as 12 other HR types. As with the baseline study, the histology of ≥ CIN2 and ≥ CIN3 will be determined by CPR.

Performance Characteristics in the ASC-US Population (≥ 21 Years)

A total of 1,612 subjects with ASC-US cytology completed Study Visit 2 procedures. The results of the **cobas®** HPV Test reported as (HR HPV) Positive or (HR HPV) Negative together with the CPR diagnosis are presented in Table 7. In a total of 1,578 ASC-US subjects with valid CPR panel diagnoses, 80 subjects had a ≥CIN2 result (prevalence of ~5.1%), and 46 subjects had a ≥CIN3 result (prevalence of ~2.9%).

Table 7
Results of the cobas® HPV Test and Central Pathology Review Panel Diagnosis in the ASC-US Population (≥ 21 Years)

cobas® HPV Test Result	Central Pathology Review Panel Diagnosis					Total
	Undetermined	Normal	CIN1	CIN2	≥CIN3	
Positive	13	351	91	29	43	527
Negative	19	989	67	5	3	1,083
Invalid	0	2	0	0	0	2
Total	32	1,340	158	34	46	1,612

Note: The 32 Undetermined CPR results were due to biopsy sample(s) collected out of study visit window or biopsy sample(s) found to be inadequate for diagnosis. These were excluded from the analysis, resulting in 1578 valid biopsy results.

Percent of Invalid cobas® HPV Test results was 0.12% (2/1612) with 95% CI: 0.03% to 0.45%

Performance of the **cobas®** HPV Test in detecting high-grade cervical disease (≥ CIN2 and ≥ CIN3) is presented in Table 8. The sensitivity and the specificity of the test for detecting ≥ CIN2 histology were 90.0% ((72/80) with 95% CI: 81.5% to 94.8%) and 70.5% ((1,056/1,498) with 95% CI: 68.1% to 72.7%), respectively. The positive likelihood ratio (PLR) was estimated as 3.1, which implies a positive **cobas®** HPV Test result is 3.1 times more likely in subjects with ≥ CIN2 than in subjects with < CIN2. The negative likelihood ratio (NLR) was estimated as 0.1, which implies that a negative **cobas®** HPV Test result is 10 (1/0.1) times more likely in subjects with < CIN2 than in subjects with ≥ CIN2.

The sensitivity and specificity of the **cobas®** HPV Test for detecting ≥ CIN3 histology were 93.5% ((43/46) with 95% CI: 82.5% to 97.8%) and 69.3% ((1,061/1,532) with 95% CI: 66.9% to 71.5%), respectively.

Table 8
Performance of the cobas® HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population (≥ 21 Years)

Performance	CPR Panel Diagnosis ≥ CIN2		CPR Panel Diagnosis ≥ CIN3	
	Point Estimate	95% CI	Point Estimate	95% CI
Sensitivity (%)	90.0 (72/80)	(81.5, 94.8)	93.5 (43/46)	(82.5, 97.8)
Specificity (%)	70.5 (1,056 /1,498)	(68.1, 72.7)	69.3 (1,061/1,532)	(66.9, 71.5)
PLR	3.1 (72/80) (442/1,498)	(2.7, 3.4)	3.0 (43/46)/(471/1,532)	(2.7, 3.4)
NLR	0.1 (8/80)/(1,056/1,498)	(0.1, 0.3)	0.1 (3 46)/(1,061/1,532)	(0.0, 0.3)
PPV (%)	14.0 (72/514)	(12.8, 15.3)	8.4 (43/514)	(7.6, 9.2)
NPV (%)	99.2 (1,056/1,064)	(98.6, 99.6)	99.7 (1,061/1,064)	(99.2, 99.9)
Prevalence (%)	5.1 (80/1,578)	(4.1, 6.3)	2.9 (46/1,578)	(2.2, 3.9)

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value.
PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio.

The performance of the **cobas®** HPV Test in detecting high-grade cervical disease (≥ CIN2 and ≥ CIN3) and the performance of the FDA approved HPV Test are presented in Table 9.

The sensitivity for detecting ≥ CIN2 histology was 90.0% ((72/80) with 95% CI: 81.5% to 94.8%) for the **cobas®** HPV Test and 87.2% ((68/78) with 95% CI: 78.0% to 92.9%) for the FDA approved HPV Test. The specificity for detecting ≥ CIN2 histology was 70.5% (1,056/1,498) with 95% CI: 68.1% to 72.7%) for the **cobas®** HPV Test and 71.1% ((1,056/1,495) with 95% CI: 68.8% to 73.4%) for the FDA approved HPV Test.

The sensitivity for detecting ≥ CIN3 histology was 93.5% ((43/46) with 95% CI: 82.5% to 97.8%) for the **cobas®** HPV Test and 91.3% ((942/46) with 95% CI:79.7% to 96.6%) for the FDA approved HPV Test.. The specificity for detecting ≥ CIN3 histology was 69.3% ((1,053/1,517) with 95% CI:66.9% to 71.5%) for the **cobas®** HPV Test and 70.0% ((1,062/1,517) with 95% CI: 67.7% to 72.3%) for the FDA approved HPV Test.

Table 9
Comparison of the Performance of the cobas® HPV Test and an FDA approved HPV test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population

	cobas® HPV Test		FDA approved HPV Test	
	Point Estimate	**95% CI**	**Point Estimate**	**95% CI**
≥ CIN2				
Sensitivity (%)	90.0 (72/80)	(81.5, 94.8)	87.2 (68/78)[1]	(78.0, 92.9)
Specificity (%)	70.5 (1,056/1,498)	(68.1, 72.7)	71.1 (1,056/1,485)[2]	(68.8, 73.4)
PPV (%)	14.0 (72/514)	(12.8, 15.3)	13.7 (68/497)	(12.4, 15.1)
NPV (%)	99.2 (1,056/1,064)	(98.6, 99.6)	99.1 (1,056/1,066)	(98.3, 99.5)
Prevalence (%)	5.1 (80/1578)	(4.1, 6.3)	5.0 (78/1563)	(4.0, 6.2)
≥ CIN3				
Sensitivity (%)	93.5 (43/46)	(82.5, 97.8)	91.3 (42/46)	(79.7, 96.6)
Specificity (%)	69.3 (1,053/1,517)	(66.9, 71.5)	70.0 (1,062/1,517)	(67.7, 72.3)
PPV (%)	8.4 (43/514)	(7.6, 9.2)	8.5 (42/497)	(7.6, 9.4)
NPV (%)	99.7 (1,061/1,064)	(99.2, 99.9)	99.6 (1,062/1,066)	(99.0, 99.9)
Prevalence (%)	2.9 (43/1578)	(2.2, 3.9)	3.0 (46/1563)	(2.2, 3.9)

[1] Results for two subjects with a ≥ CIN2 diagnosis could not be determined by the FDA approved HPV Test due to insufficient volume resulting from repeated testing
[2] Results for thirteen subjects with a < CIN2 diagnosis could not be determined by the FDA approved HPV Test due to insufficient volume resulting from repeated testing.

Performance of the **cobas®** HPV Test in detecting ≥ CIN2 and ≥ CIN3 evaluated by age group is presented in Table 10. The sensitivity of the **cobas®** HPV Test for detecting ≥ CIN2 histology was 93.3% ((42/45) with 95% CI: 82.1% to 97.7%) in the 21-29 year age group, 100% ((20/20) with 95% CI: 83.9% to 100%) in the 30-39 year age group, and 66.7% ((10/15) with 95% CI: 41.7% to 84.8%) in the ≥ 40 years age group. The specificity of the test was highest in ≥ 40 years, with an estimate of 85.0% (95% CI: 82.0% to 87.6%).

The sensitivity in detecting ≥ CIN3 was 100% ((24/24) with 95% CI: 74.1% to 100%) in the 21-29 year age group, 100% ((11/11) with 95% CI: 86.2% to 100%) in the 30-39 year age group, and 72.7% ((8/11) with 95% CI: 43.4% to 90.3%) in the ≥ 40 years age group. The specificity of the test was highest in ≥ 40 years, with an estimate of 84.8% ((535/ 631) with 95% CI: 81.8% to 87.4%).

Table 10
Performance of the cobas® HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population by Age Group

Performance	21-29 Years	30-39 Years	≥ 40 Years
N	514	422	642
≥ CIN2			
Sensitivity (%)	93.3 (42/45)	100.0 (20/20)	66.7 (10/15)
95% CI (%)	(82.1, 97.7)	(83.9, 100.0)	(41.7, 84.8)
Specificity (%)	49.7 (233/469)	72.1 (290/402)	85.0 (533/627)
95% CI (%)	(45.2, 54.2)	(67.6, 76.3)	(82.0, 87.6)
PPV (%)	15.1 (42/278)	15.2 (20/132)	9.6 (10/104)
95% CI (%)	(13.6, 16.7)	(13.1, 17.5)	(6.6, 13.7)
NPV (%)	98.7 (233/236)	100.0 (290/290)	99.1 (533/538)
95% CI (%)	(96.3, 99.6)	(97.4, 100.0)	(98.1, 99.5)
≥ CIN2 prevalence	8.8% (45/514)	4.7% (20/422)	2.3% (15/642)
95% CI (%)	(6.6, 11.5)	(3.1, 7.2)	(1.4, 3.8)
≥ CIN3			
Sensitivity (%)	100.0 (24/24)	100.0 (11/11)	72.7 (8/11)
95% CI (%)	(86.2, 100.0)	(74.1, 100.0)	(43.4, 90.3)
Specificity (%)	48.2 (236/490)	70.6 (290/411)	84.8 (535/ 631)
95% CI (%)	(43.8, 52.6)	(66.0, 74.8)	(81.8, 87.4)
PPV (%)	8.6 (24/278)	8.3 (11/132)	7.7 (8/104)
95% CI (%)	(7.9, 9.5)	(7.0, 9.9)	(5.3, 11.1)
NPV (%)	100.0 (236/236)	100.0 (290/290)	99.4 (535/538)
95% CI (%)	(96.8, 100.0)	(97.5, 100.0)	(98.5, 99.8)
≥ CIN3 prevalence	4.7% (24/514)	2.6% (11/422)	1.7% (11/642)

Performance of the FDA approved HPV test in detecting ≥ CIN2 and ≥ CIN3 by age group is presented in Table 11.

Table 11
Performance of an FDA approved HPV test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population by Age Group

Performance	21-29 Years	30-39 Years	≥ 40 Years
N	506	417	640
≥ CIN2			
Sensitivity (%)	88.4 (38 / 43)	100.0 (20 / 20)	66.7 (10 / 15)
95% CI (%)	(75.5, 94.9)	(83.9, 100.0)	(41.7, 84.8)
Specificity (%)	50.1 (232 / 463)	73.6 (292 / 397)	85.1 (532 / 625)
95% CI (%)	(45.6, 54.6)	(69.0, 77.6)	(82.1, 87.7)
PPV (%)	14.1 (38 / 269)	16.0 (20 / 125)	9.7 (10 / 103)
95% CI (%)	(12.5, 15.9)	(13.8, 18.5)	(6.7, 13.9)
NPV (%)	97.9 (232 / 237)	100.0 (292 / 292)	99.1 (532 / 537)
95% CI (%)	(95.3, 99.1)	(97.4, 100.0)	(98.1, 99.5)
≥ CIN2 prevalence	8.5 (43/506)	4.8 (20/417)	2.3 (15/640)
95% CI (%)	(6.4, 11.3)	(3.1, 7.3)	(1.4, 3.8)
≥ CIN3			
Sensitivity (%)	95.8 (23 / 24)	100.0 (11 / 11)	72.7 (8 / 11)
95% CI (%)	(79.8, 99.3)	(74.1, 100.0)	(43.4, 90.3)
Specificity (%)	49.0 (236 / 482)	71.9 (292 / 406)	84.9 (534 / 629)
95% CI (%)	(44.5, 53.4)	(67.4, 76.1)	(81.9, 87.5)
PPV (%)	8.6 (23 / 269)	8.8 (11 / 125)	7.8 (8 / 103)
95% CI (%)	(7.7, 9.5)	(7.3, 10.5)	(5.3, 11.2)
NPV (%)	99.6 (236 / 237)	100.0 (292 / 292)	99.4 (534 / 537)
95% CI (%)	(97.2, 99.9)	(97.5, 100.0)	(98.5, 99.8)
≥ CIN3 prevalence	4.7 (24/506)	2.6 (11/417)	1.7 (11/640)
95% CI (%)	(3.2, 7.0)	(1.5, 4.7)	(1.0, 3.1)

ASC-US (≥ 21 Years) Population – Likelihood Ratios and Risk Estimates

Likelihood ratios (LRs) and the risks of disease (≥ CIN2 and ≥ CIN3) along with 95% CIs for **cobas®** HPV Test results (HR HPV 16 positive/18 positive, 12 Other HR, and HR HPV negative are presented in Table 12 for the ASC-US (≥21 years) population.

For the ≥ CIN2 histology, the estimate of the LR of HPV16 positive/18 positive was 6.1, indicating that an HPV16 positive/18 positive result is 6.1 times more likely to come from a subject with disease (≥ CIN2) than from a subject without disease (< CIN2). The risk of a ≥ CIN2 outcome for an ASC-US subject with an HPV16 positive/18 positive result was 24.4%. The LRs of 12 Other HR HPV positive was 1.8. Both LRs were significantly greater than 1.

The estimate of the LR of a negative **cobas®** HPV Test result was 0.1, indicating that a negative result was 10 times more likely to come from a subject without disease (< CIN2) than from a subject with disease (≥ CIN2).

The risk of disease (≥ CIN2) is the chance/probability of having the disease given an HPV test outcome. The risk of disease (≥ CIN2) was 5.1% in the ASC-US population regardless of the HPV test result (prevalence =5.1%). The risk of disease was significantly increased for the test results of HPV16 positive/18 positive and 12 Other HR HPV positive and significantly decreased for an HR HPV negative result.

For ≥ CIN3 histology, both LRs of HPV16 positive/18 positive and 12 Other HR HPV positive were statistically significantly greater than 1, and the LR of an HPV negative result was statistically significantly less than 1. The risk of the disease (≥ CIN3) was 2.9% in the ASC-US population (see Table 7). The risk of ≥ CIN3 was significantly increased for the HPV16 positive/18 positive and 12 Other HR HPV positive, and significantly decreased for an HPV negative result.

Table 12

Likelihood Ratios and Risk of Disease by cobas® HPV Test Result in Detecting ≥CIN2 and ≥CIN3 in the ASC-US Population

Diagnosis by CPR	cobas® HPVTest Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
≥CIN2	HPV 16 positive/18 positive	6.1 (4.7, 7.9)	24.4 (20.1, 29.7)
	12 Other HR HPV positive	1.8 (1.3, 2.4)	8.6 (6.6, 11.6)
	HPV Negative	0.1 (0.1, 0.2)	0.8 (0.3, 1.0)
	Prevalence	5.1%	
≥CIN3	HPV 16 positive/18 positive	6.3 (4.8, 8.3)	15.9 (12.5, 20.0)
	12 Other HR HPV positive	1.5 (1.0, 2.3)	4.4 (2.9, 6.5)
	HPV Negative	0.1 (0.0, 0.3)	0.3 (0.1, 0.9)
	Prevalence	2.9%	

ASC-US (≥ 21 Years) Population - Absolute and Relative Risk Estimates

Table 13 presents the CPR diagnosis by all possible **cobas®** HPV Test result in ASCUS population.

Table 13

Summary of cobas® HPV Test Result and Central Pathology Review Panel Diagnosis in the ASC-US Population (>=21 years)

	Central Pathology Review Diagnosis					
cobas® HPV Test Result	**Undetermined**	**Negative**	**CIN1**	**CIN2**	**>=CIN3**	**Total**
Other HR HPV NEG, HPV16 NEG, HPV18 NEG	19	989	67	5	3	1,083
Other HR HPV NEG, HPV16 NEG, HPV18 POS	1	21	3	0	1	26
Other HR HPV NEG, HPV16 POS, HPV18 NEG	0	40	8	13	12	73
Other HR HPV NEG, HPV16 POS, HPV18 POS	0	5	0	0	1	6
Other HR HPV POS, HPV16 NEG, HPV18 NEG	9	246	63	14	15	347
Other HR HPV POS, HPV16 NEG, HPV18 POS	2	12	8	0	1	23
Other HR HPV POS, HPV16 POS, HPV18 NEG	1	25	9	2	12	49
Other HR HPV POS, HPV16 POS, HPV18 POS	0	2	0	0	1	3
Invalid	0	2	0	0	0	0
Overall	32	1,342	158	34	46	1,612

	Central Pathology Review Diagnosis					
cobas® HPV Test Result	**Undetermined**	**Negative**	**CIN1**	**CIN2**	**>=CIN 3**	**Total**

Note1: Undetermined results include inadequate biopsy sample for diagnosis and sample collected outside the Study Visit window.

Note2: None of the subjects in the ASC-US population had a CPR diagnosis >CIN3

852

853 Table 14 presents the CPR diagnosis and the absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas®** HPV Test result. HPV16 positive/18
854 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3. In general, the absolute risks for both ≥ CIN2 and ≥ CIN3 were higher
855 in subjects with results of HPV positive, HPV16 positive/18 positive, or 12 Other HR positive than in subjects with an HPV negative result.

856 **Table 14**
857 **Central Pathology Review Diagnosis and Absolute Risk of ≥ CIN2 and ≥ CIN3 for**
858 **Different cobas® HPV Test Results in the ASC-US Population (≥ 21 Years)**

cobas® HPV Test Result	**Total**	**Central Pathology Review Diagnosis**					**Absolute Risk for ≥ CIN2 (%)**	**Absolute Risk for ≥ CIN3 (%)**
		Undetermined	**Normal**	**CIN1**	**CIN2**	**≥CIN3**		
HPV positive	527	13	351	91	29	43	14.0 (72/514)	8.4 (43/514)
HPV16 positive and/or HPV18 positive	180	4	105	28	15	28	24.4 (43/176)	15.9 (28/176)
HPV16 positive	131	1	72	17	15	26	31.5 (41/130)	20.0 (26/130)
HPV18 positive	49	3	33	11	0	2	4.4 (2/46)	4.3 (2/46)
12 Other HR HPV positive	347	9	246	63	14	15	8.6 (29/338)	4.3 (15/338)
HPV negative	1,083	19	989	67	5	3	0.8 (8/1,064)	0.3 (3/1,064)

Note1: Undetermined results include inadequate biopsy sample for diagnosis and sample collected outside the Study Visit window.
Note 2: HPV16 positive and/ or HPV18 positive include all subjects with either or both of these genotypes occurring with or without 12 other HR positive results
Note 3: 12 Other HR HPV positive include all subjects with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18 .

859

860 The relative risks (RRs) of disease (≥ CIN2 and ≥ CIN3) were calculated between subjects with different **cobas®** HPV Test results by RR
861 and its associated 95% CIs as presented in Table 15. The estimated RRs of ≥ CIN2 and of ≥ CIN3 for subjects with positive vs. negative
862 **cobas®** HPV Test results were 18.6 (95% CI: 9.0 to 38.4) and 29.7 (95% CI: 9.2 to 95.2), respectively, indicating that subjects with a
863 positive result were 18.6 times more likely to have ≥ CIN2 histology and 29.7 times more likely to have ≥ CIN3 histology than were
864 subjects with a negative test result.

865 Similarly, subjects who have HPV16 and/or HPV18 positive results from the **cobas®** HPV Test were significantly more likely to have ≥
866 CIN2 than the subjects with (a) a positive result for 12 Other HR HPV types, or (b) a negative result. Subjects with a positive result for 12
867 Other HR HPV types were significantly more likely to have ≥ CIN2 than the subjects with a negative result. Similar results were observed
868 for ≥ CIN3 histology.

869 **Table 15**
870 **Relative Risks of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the**
871 **ASC-US Population (≥ 21 Years)**

cobas® HPV Test Result	**CPR Diagnosis ≥ CIN2**		**CPR Diagnosis ≥ CIN3**	
	Relative Risk	**95% CI**	**Relative Risk**	**95% CI**
HPV Positive vs. Negative	18.6	(9.0, 38.4)	29.7	(9.2, 95.2)
HPV16 positive/18 positive vs. Negative	32.5	(15.5, 69.7)	56.4	(17.3, 183.6)
HPV16 positive /18 positive vs. 12 Other HR HPV positive	2.8	(1.8, 4.4)	3.6	(2.0, 6.5)
12 Other HR HPV positive vs. Negative	11.4	(5.3, 24.7)	15.7	(4.6, 54.0)
Prevalence	5.1%		2.9%	

cobas® HPV Test Result	CPR Diagnosis ≥ CIN2		CPR Diagnosis ≥ CIN3	
	Relative Risk	95% CI	Relative Risk	95% CI

Note 1: HPV16 positive and/ or HPV18 positive include all subjects with either or both of these genotypes occurring with or without 12 other HR positive results
Note 2: 12 other HR HPV positive include all subjects with positive results for 12 other HR genotypes with negative results for HPV16 and HPV18 .

The relative risks of disease (≥ CIN2 and ≥ CIN3) were calculated between subjects with different **cobas®** HPV Test results among different age groups and are presented in Table 16. The RRs of all comparisons were significantly greater than 1 for ≥ CIN2 histology, except for HPV 16 positive /18 positive vs. 12 Other HR HPV positive in ≥ 40 years.

Table 16
Relative Risks of ≥ CIN2 and ≥ CIN3 by cobas® HPV Test Result
Stratified by Age in the ASC-US Population

cobas® HPV Test Result	Age Group (Years)		
	21-29	30-39	≥40
Relative Risk for ≥ CIN2			
Positive vs. Negative	11.9 (3.7, 37.9)	87.9 (5.4, 1443.3)*	10.3 (3.6, 29.6)
HPV16 positive /18 positive vs. Negative	20.4 (6.3, 65.4)	163.6 (9.8, 2729.1)*	12.9 (3.3, 51.0)
HPV16 positive /18 positive vs. Other 12 HR HPV positive	3.3 (1.8, 6.1)	2.9 (1.3, 6.5)	1.4 (0.4, 4.8)
12 Other HR HPV positive vs. Negative	6.2 (1.8, 21.3)	56.1 (3.3, 959.0)*	9.5 (3.1, 29.3)
Prevalence	8.8%	4.7%	2.3%
Relative Risk for ≥ CIN3			
Positive vs. Negative	40.7 (2.5, 666.9)*	48.3 (2.9, 816.3)*	13.8 (3.7, 51.1)
HPV16 positive /18 positive vs. Negative	80.1 (4.9, 1315.5)*	89.2 (5.1, 1566.9)*	21.5 (4.6, 101.3)
HPV16 positive /18 positive vs. Other 12 HR HPV positive	5.6 (2.2, 14.6)	2.9 (0.9, 8.8)	1.9 (0.5, 7.4)
12 Other HR HPV positive vs. Negative	14.2 (0.8, 258.5)*	31.2 (1.7, 565.4)*	11.4 (2.8, 46.6)
Prevalence	4.7	2.6	1.7

* 0.5 was added to a cell with zero frequency in age group 21-29 years and 30-39 years and also for the HPV negative result.
Note 1: HPV16 positive and/ or HPV18 positive include all subjects with either or both of these genotypes occurring with or without 12 Other HR HPV positive results
Note 2: 12 Other HR HPV positive include all subjects with positive results for 12 other HR genotypes with negative results for HPV16 and HPV18 .

NILM (≥30 Years) Population

The risks of disease in the NILM (≥ 30 years) population were compared in subjects with a positive result to those with a negative result from the cobas® HPV Test. In this population, all subjects with a positive result from the IUO HPV HR test or IUO HPV genotyping test were selected to proceed to Study Visit 2, whereas a random subset of subjects (1 of 35) with a negative result from both IUO HPV tests were randomized to Study Visit 2. To compare the risks of high-grade cervical disease (≥ CIN2 or ≥ CIN3) between subject groups with positive vs. negative cobas® HPV Test results, an adjustment for verification bias was applied to account for the different rate of selection in these groups. This was accomplished by calculating the likely number of diseased cases that would have been found if all the subjects in a given subgroup had undergone colposcopy.

Table 17 presents the CPR diagnosis by all possible **cobas®** HPV Test results in the NILM (≥ 30 years) population.

Table 17
Summary of cobas® HPV Test Result and Central Pathology Review Panel Diagnosis in the NILM Population (≥ 30 years)

	Central Pathology Review Diagnosis					
cobas® HPV Test Result	**Undetermined**	**Negative**	**CIN1**	**CIN2**	**≥ CIN3**	**Total**
Other HR HPV NEG, HPV16 NEG, HPV18 NEG	63	2,391	101	14	8	2,577
Other HR HPV NEG, HPV16 NEG, HPV18 POS	2	78	7	2	6	95
Other HR HPV NEG, HPV16 POS, HPV18 NEG	6	147	13	3	24	193
Other HR HPV NEG, HPV16 POS, HPV18 POS	0	1	0	0	1	2
Other HR HPV POS, HPV16 NEG, HPV18 NEG	41	1,199	96	30	34	1,400
Other HR HPV POS, HPV16 NEG, HPV18 POS	0	27	4	0	1	32
Other HR HPV POS, HPV16 POS, HPV18 NEG	1	51	8	2	6	68
Other HR HPV POS, HPV16 POS, HPV18 POS	0	4	0	0	0	4
Overall	113	3,898	229	51	80	4,371

Note 1: Undetermined results include inadequate biopsy sample for diagnosis and sample collected outside the Study Visit window.

Note2: Of the 80 ≥ CIN3 subjects, 75 are CIN3 and 5 are ACIS.

Table 18 presents the CPR diagnosis and the crude estimate of absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas®** HPV Test result. HPV16 positive had the highest crude absolute risk for both ≥ CIN2 and ≥ CIN3. In general, the crude absolute risks for both ≥ CIN2 and ≥ CIN3 were higher in subjects with any results of HPV positive than in subjects with an HPV negative result.

Table 18
Central Pathology Review Diagnosis and Different cobas® HPV Test Results in the NILM Population (≥30 Years)

cobas® HPV Test Result	**Total**	**Central Pathology Review Diagnosis**					**Crude Absolute Risk for ≥ CIN2 (%)**	**Crude Absolute Risk for ≥ CIN3 (%)**
		Undetermined	**Normal**	**CIN1**	**CIN2**	**≥CIN3**		
HPV positive	1794	50	1507	128	37	72	6.3 (109/1,744)	4.1 (72/1,744)
HPV16 positive and/or HPV18 positive	394	9	308	32	7	38	11.7 (45/385)	9.9 (38/385)
HPV16 positive	267	7	203	21	5	31	13.8 (36/260)	11.9 (31/260)
HPV18 positive	127	2	105	11	2	7	7.2 (9/125)	5.6 (7/125)
12 Other HR HPV positive	1400	41	1199	96	30	34	4.7 (64/1,359)	2.5 (34/1,359)
HPV negative	2577	63	2391	101	14	8	0.9 (22/2,514)	0.3 (8/2,514)

Note1: Undetermined results include inadequate biopsy sample for diagnosis and sample collected outside the Study Visit window.
Note 2: HPV16 positive and/ or HPV18 positive include all subjects with either or both of these genotypes occurring with or without 12 other HR positive results
Note 3: 12 Other HR HPV positive include all subjects with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18 .

The subjects in various subgroups are classified as shown in Table 19. The combined results of the two IUO HPV Test were considered positive if either of the two test results was positive. The combined results were considered negative if both tests results were negative.

Table 19
Classification of Evaluable NILM Subjects (≥ 30 Years) by cobas® HPV Test Result, Disease Status (≥ CIN2 and ≥ CIN3), and Disease Verification Status

cobas® HPV Test Result	Combined Results From Two IUO HPV Test	Total No. Subjects	Verified Disease Status: ≥ CIN2		Verified Disease Status: ≥ CIN3		No. Subjects with Unknown Disease Status (Unverified)
			No. Diseased Subjects (≥ CIN2)	No. Non-Diseased Subjects (< CIN2)	No. Diseased Subjects (≥ CIN3)	No. Non-Diseased Subjects (< CIN3)	
HPV16 positive/18 positive	Positive	470	45	339	38	346	86
	Negative	11	0	1	0	1	10
12 Other HR HPV positive	Positive	1,634	64	1,292	34	1,322	278
	Negative	55	0	3	0	3	52
Negative	Positive	2,187	16	1,774	6	1,784	397
	Negative	27,903	6	718	2	722	27,179
Total		32,260	131	4,127	80	4,178	28,002

NILM (≥ 30 Years) Population – Performance Evaluation

For the NILM (≥30 years) population, estimates of sensitivity and specificity along with 95% CIs for HR HPV positive vs. HR HPV negative are presented in Table 20 for unadjusted results and Table 21 for verification bias adjusted results, respectively.

The unadjusted sensitivity and the specificity of the test for ≥ CIN2 histology were 83.2% ((109/131) with 95% CI:75.9% to 88.6%) and 60.4% ((2492/4127) with 95% CI:58.9% to 61.9%), respectively. The unadjusted sensitivity and specificity of the **cobas®** HPV Test for detecting ≥ CIN3 histology were 90.0% ((72/80) with 95% CI: 81.5% to 94.8%) and 60.0% ((2506/4178) with 95% CI: 58.5% to 61.5%), respectively.

The verification bias adjusted sensitivity for ≥ CIN2 and ≥ CIN3 histology were 34.5% (with 95% CI: 22.1% to 61.4%) and 51.2% (with 95% CI: 29.3% to 94.4%), respectively, and the verification bias adjusted specificity for ≥ CIN2 and ≥ CIN3 histology were 93.6% (with 95% CI: 93.3%, to 93.9%) and 93.5% (with 95% CI: 93.2%, to 93.8%), respectively.

Table 20
Performance of cobas® HPV Test In the NILM (≥30 years) Population (Unadjusted Estimates)

CPR Diagnosis	Performance	Estimate	95% CI
≥CIN2	Sensitivity (%)	83.2 (109/131)	(75.9, 88.6)
	Specificity (%)	60.4 (2492/4127)	(58.9, 61.9)
	PPV(%)	6.3 (109/1744)	(5.8, 6.8)
	NPV(%)	99.1 (2492/2514)	(98.7, 99.4)
	Prevalence (%)	3.1(131/4258)	(2.6, 3.6)
≥CIN3	Sensitivity (%)	90.0 (72/80)	(81.5, 94.8)
	Specificity (%)	60.0 (2506/4178)	(58.5, 61.5)
	PPV(%)	4.1 (72/1744)	(3.8, 4.5)
	NPV(%)	99.7 (2506/2514)	(99.4, 99.8)
	Prevalence (%)	1.9(80/4258)	(1.5, 2.3)

Table 21
Performance of cobas® HPV Test In the NILM (≥30 years) Population
(Verification Bias Adjusted Estimates)

CPR Diagnosis	Performance	Estimate and 95% CI
≥CIN2	Sensitivity (%)	34.5 (22.1, 61.4)
	Specificity (%)	93.6 (93.3, 93.9)
	PPV(%)	6.1 (4.9, 7.2)
	NPV(%)	99.2 (98.5, 99.7)
	Prevalence(%)	1.2 (0.6, 1.8)
≥CIN3	Sensitivity (%)	51.2 (29.3, 94.4)
	Specificity (%)	93.5 (93.2, 93.8)
	PPV(%)	4.1 (3.1, 5.0)
	NPV(%)	99.7 (99.3, 100.0)
	Prevalence(%)	0.5 (0.3, 0.9)

NILM (≥ 30 Years) Population – Likelihood Ratios and Risk Estimates

Unadjusted estimates of likelihood ratios along with 95% CIs for HR HPV 16 positive /18 positive, 12 Other HR, and HR HPV negative for the NILM (≥30 years) population are presented in Table 22. The risks of ≥CIN2 and ≥CIN3 are 11.7% (45/385) and 9.9% (38/385), respectively for a NILM subject with HPV 16 positive /18 positive. The risks of ≥CIN2 and ≥CIN3 are 0.9% (22/2,514) and 0.3% (8/2,514) for a NILM subject with HPV negative, respectively.

Table 22
Likelihood Ratios by cobas® HPV Test Result in Detecting ≥CIN2 and ≥CIN3 in the NILM Population
(Unadjusted Estimates)

CPR Diagnosis	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥CIN2	HPV 16 positive /18 positive	**4.2** (3.2, 5.4)
	12 Other HR HPV positive	**1.6** (1.3, 1.9)
	HPV Negative	**0.3** (0.2, 0.4)
≥CIN3	HPV 16 positive /18 positive	**5.7**(4.4, 7.3)
	12 Other HR HPV positive	**1.3** (1.0, 1.7)
	HPV Negative	**0.2** (0.1, 0.4)

Verification bias adjusted estimates of likelihood ratios along with 95% CIs for HR HPV 16 positive /18 positive, 12 Other HR, and HR HPV negative for the NILM (≥30 years) population are presented in Table 23

Table 23
Likelihood Ratios by cobas® HPV Test Result in Detecting ≥CIN2 and ≥CIN3 in the NILM Population
(Verification-Bias Adjusted Estimates)

CPR Diagnosis	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥CIN2	HPV 16 positive/18 positive	**10.7** (6.5, 19.6)
	12 Other HR HPV positive	**4.0** (2.4, 7.2)
	HPV Negative	**0.7** (0.4, 0.8)
≥CIN3	HPV 16 positive / 18 positive	**20.2** (10.7, 39.4)
	12 Other HR HPV positive	**4.6** (2.4, 9.4)
	HPV Negative	**0.5** (0.1, 0.8)

NILM (≥30 Years) Population – Absolute and Relative Risk Estimates

Estimates of absolute risks of ≥CIN2 and ≥CIN3 for **cobas®** HPV Test results are presented in Table 24. The estimates are calculated with and without adjusting for verification bias. The risks of ≥CIN2 and ≥CIN3 are 11.4% (with 95% CI: 8.3% to 14.7%) and 9.8% (with 95% CI:

6.9% to 12.6%) for a NILM subject with HPV 16 positive /18 positive. The risks of ≥CIN2 and ≥CIN3 are 0.8% (with 95% CI: 0.3% to 1.5%) and 0.3% (with 95% CI: 0.0% to 0.7%), respectively for a NILM subject with HPV negative.

Table 24
Absolute Risk of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the NILM Population (≥ 30 Years)

cobas® HPV Test Result	≥CIN2	≥CIN3
Unadjusted Estimates		
HPV positive	6.3% (5.2, 7.5)	4.1% (3.3, 5.2)
HPV 16 positive/18 positive	11.7% (8.9, 15.3)	9.9% (7.3, 13.3)
Other 12 HR positive	4.7% (3.7, 6.0)	2.5% (1.8, 3.5)
HPV Negative	0.9% (0.6, 1.3)	0.3% (0.2, 0.6)
Verification Bias Adjusted Estimates		
HPV positive	6.1% (4.9, 7.2)	4.1% (3.1, 5)
HPV 16 positive/18 positive	11.4% (8.3, 14.7)	9.7% (6.9, 12.6)
Other 12 HR positive	4.6% (3.5, 5.7)	2.4% (1.6, 3.3)
HPV Negative	0.8% (0.3, 1.5)	0.3% (0, 0.7)

Note 1: HPV 16 positive /18 positive include all subjects with either or both of these genotypes occurring with or without 12 Other HR HPV positive results
Note 2: 12 Other HR HPV positive include all subjects with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18 .

Estimates of absolute risk of ≥CIN2 and ≥CIN3 for **cobas®** HPV Test results stratified by age group are presented in Table 25. The risk of disease decreases with age for **cobas®** HPV Test results of HPV 16 positive/18 positive and for 12 Other HR HPV positive results. The risk of disease with a **cobas®** HPV Test negative result remains similar for the 30-39 years age group as well as for ≥40 years.

Table 25
Absolute Risk Estimates in the NILM(≥30 Years) Population by cobas® HPV Test Result and Age

Age Group	cobas® HPV Test Result	≥CIN2	≥CIN3
30-39 Years	**Unadjusted Estimates**		
	HPV 16 positive/18 positive	16.1 (11.9, 21.5)	13.5 (9.6, 18.6)
	Other 12 HR positive	5.8 (4.2, 8.0)	3.1 (2.0, 4.8)
	HPV Negative	0.8 (0.4, 1.6)	0.3 (0.1, 0.9)
	Prevalence	4.4%	2.8%
	Verification Bias Adjusted Estimates		
	HPV 16 positive/18 positive	16.1(11.4, 20.8)	13.5 (9.1, 18.1)
	Other 12 HR positive	5.6 (3.8, 7.7)	3.0 (1.7, 4.5)
	HPV Negative	0.1 (0, 0.2)	0 .0(0, 0.1)
	Prevalence	0.8%	0.6%
≥40 Years	**Unadjusted Estimates**		
	HPV 16 positive/18 positive	5.6 (3.0, 10.2)	4.9 (2.5, 9.4)
	Other 12 HR positive	3.8 (2.6, 5.4)	2.0 (1.2, 3.3)
	HPV Negative	0.9 (0.6, 1.5)	0.3 (0.1, 0.8)
	Prevalence	2.1%	1.1%
	Verification Bias Adjusted Estimates		
	HPV 16 positive/18 positive	5.6 (2, 8.9)	4.7 (1.8, 8.1)
	Other 12 HR positive	3.7 (2.3, 5)	1.9 (1, 3.1)
	HPV Negative	1.2 (0.4, 2.2)	0.4 (0, 1)
	Prevalence	1.4%	0.5%

The relative risks of disease (≥ CIN2 and ≥ CIN3) were calculated between subjects with different **cobas®** HPV Test results and are presented in Table 26. Subjects with positive **cobas®** HPV Test results are 7.3 (95% CI = 3.99 to 22.11) times more likely to have ≥ CIN2 and 14.5 (95% CI = 5.81 to 230.4) times more likely to have ≥ CIN3, respectively, compared with subjects with a negative **cobas®** HPV

Test result. The risks of disease (both ≥ CIN2 and ≥ CIN3) were significantly higher in subjects with a positive compared with subjects with a negative HPV test result.

Also the risks of disease (≥ CIN2 and ≥ CIN3) were significantly higher in subjects who were HPV 16 and/or 18 positive than subjects with (a) a positive result for 12 Other HR HPV types, or (b) a negative result.

Similar results were also observed for risk of ≥ CIN3 by different **cobas®** HPV Test results. The RRs of the ≥ CIN3 were higher than the RRs of the ≥ CIN2 for each comparison.

Table 26
Relative Risks of ≥ CIN2 and ≥ CIN3 for Different the cobas® HPV Test Results in the NILM Population (≥ 30 Years)

cobas® HPV Test Result	CPR Diagnosis ≥ CIN2		CPR Diagnosis ≥ CIN3	
	Relative Risk	95% CI*	Relative Risk	95% CI*
HPV Positive vs. Negative	7.29	(3.99, 22.11)	14.53	(5.81, 230.4)
HPV16 positive /18 positive vs. Negative	13.71	(7.31, 41.92)	35.02	(12.96, 559.4)
HPV16 positive /18 positive vs. 12 Other HR HPV positive	2.51	(1.73, 3.61)	4.03	(2.57, 6.59)

*95% CI is 2.5 and 97.5 percentile of RR distribution based on 1000 bootstrap samples.
Note 1: HPV 16 positive and/ or HPV 18 positive include all subjects with either or both of these genotypes occurring with or without 12 Other HR HPV positive results
Note 2: 12 other HR HPV positive include all subjects with positive results for 12 Other HR HPV genotypes with negative results for HPV 16 and HPV 18 .

Agreement with a Composite Comparator for the ASC-US ≥ 21 and, NILM ≥ 30

The analytical performance of the **cobas®** HPV Test was evaluated by comparing results from the test with a composite comparator composed of HPV DNA sequencing and an FDA-approved HR HPV DNA test or directly with DNA sequencing. Sequencing was performed at a commercial lab. DNA was extracted from cervical specimens followed by a PCR amplification utilizing both ß-globin and PGMY primers. The ß-globin amplification serves as a process control. The PGMY primers are a pool of consensus primers designed to amplify a portion of the polymorphic L1 region of the HPV genome[37]. PGMY-positive extracts were then amplified using HR HPV type-specific primers for subsequent sequencing reactions[38].

Representative cervical samples were selected from 2 subsets of subjects from the ATHENA Study: women ≥ 21 years who had ASC-US cytology results (n = 999) and women ≥ 30 years with NILM cytology results (n = 747).

The analytical accuracy of the **cobas®** HPV Test was evaluated by estimating the positive percent agreement (PPA), negative percent agreement (NPA), overall percent agreement (OPA) and 95% confidence intervals (CIs) compared with the composite comparator (Table 27) or genotype-specific HPV DNA sequencing results (Tables 28, 29 and 30). The indeterminate and invalid results are presented in the tables but not included in the calculation of percent agreement. The composite comparator result was indeterminate if results were discordant between HPV DNA sequencing result and the FDA-approved HR HPV DNA test result, or if the result from the FDA-approved test was indeterminate, or if HPV DNA sequencing result was invalid. The sequencing comparator result was invalid if ß-globin amplification produced null result during sequencing. All subjects tested for analytical accuracy had valid **cobas®** HPV Test results.

Table 27
Percent Agreement of the cobas® HPV Test vs. the Composite Comparator

Population	cobas® HPV Test Result	HPV Composite Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Indeterminate		
ASC-US ≥21 Years	Positive	268	28	29	325	PPA: 97.8% (268/274) 95% CI: (95.3%, 99.0%)
	Negative	6	618	50	674	NPA: 95.7% (618/646) 95% CI: (93.8%, 97.0%)
	Total	274	646	79	999	OPA: 96.3% (886/920) 95% CI: (94.9%, 97.3%)
NILM ≥30 Years	Positive	156	82	86	324	PPA: 96.3% (156/162) 95% CI: (92.2%, 98.3%)
	Negative	6	388	29	423	NPA: 82.6% (388/470) 95% CI: (78.9%, 85.7%)
	Total	162	470	115	747	OPA: 86.1% (544/632) 95% CI: (83.2%, 88.6%)

Note: subjects with indeterminate results were excluded from percent agreement calculation

Table 28
Percent Agreement of the cobas® HPV Test HPV16 Result vs. the HPV16 Sequencing Comparator

Population	cobas® HPV Test: HPV16 Result	HPV 16 Sequencing Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Invalid		
ASC-US ≥21 Years	Positive	69	8	0	77	PPA: 97.2% (69/71) 95% CI: (90.3%, 99.2%)
	Negative	2	918	2	922	NPA: 99.1% (918/926) 95% CI: (98.3%, 99.6%)
	Total	71	926	2	999	OPA: 99.0% (987/997) 95% CI: (98.2%, 99.5%)
NILM ≥30 Years	Positive	39	17	0	56	PPA: 100.0% (39/39) 95% CI: (91.0%, 100.0%)
	Negative	0	689	2	691	NPA: 97.6% (689/706) 95% CI: (96.2%, 98.5%)
	Total	39	706	2	747	OPA: 97.7% (728/745) 95% CI: (96.4%, 98.6%)

Note: subjects with invalid results were excluded from percent agreement calculation

Table 29
Percent Agreement of the cobas® HPV Test HPV18 Result vs. the HPV18 Sequencing Comparator

Population	cobas® HPV Test: HPV18 Result	HPV 18 Sequencing Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Invalid		
ASC-US ≥21 Years	Positive	38	0	0	38	PPA: 95.0% (38/40) 95% CI: (83.5%, 98.6%)
	Negative	2	957	2	961	NPA: 100.0% (957/957) 95% CI: (99.6%, 100.0%)
	Total	40	957	2	999	OPA: 99.8% (995/997) 95% CI: (99.3%, 99.9%)
NILM ≥30 Years	Positive	17	6	0	23	PPA: 94.4% (17/18) 95% CI: (74.2%, 99.0%)
	Negative	1	721	2	724	NPA: 99.2% (721/727) 95% CI: (98.2%, 99.6%)
	Total	18	727	2	747	OPA: 99.1% (738/745) 95% CI: (98.1%, 99.5%)

Note: subjects with invalid results were excluded from percent agreement calculation

Table 30
Percent Agreement of cobas® HPV Test 12 Other HR HPV Result vs. the 12 Other HR HPV Sequencing Comparator

Population	cobas® HPV Test: 12 Other HR HPV Result	12 Other HR HPV Sequencing Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Invalid		
ASC-US ≥21 Years	Positive	226	32	1	259	PPA: 94.6% (226/239) 95% CI: (90.9%, 96.8%)
	Negative	13	726	1	740	NPA: 95.8% (726/758) 95% CI: (94.1%, 97.0%)
	Total	239	758	2	999	OPA: 95.5% (952/997) 95% CI: (94.0%, 96.6%)
NILM ≥30 Years	Positive	168	96	1	265	PPA: 88.4% (168/190) 95% CI: (83.1%, 92.2%)
	Negative	22	459	1	482	NPA: 82.7% (459/555) 95% CI: (79.3%, 85.6%)
	Total	190	555	2	747	OPA: 84.2% (627/745) 95% CI: (81.4%, 86.6%)

Note: subjects with invalid results were excluded from percent agreement calculation

ANALYTICAL PERFORMANCE

Clinical Cutoff Determination of the cobas® HPV Test

The clinical cutoff for detecting high-grade cervical disease (≥CIN2) for the **cobas®** HPV test was selected based on approximately 29,000 subjects enrolled in Phase 1 of the ATHENA study. The method for selection of cutoff was based on Kondratovich[39] and was chosen to achieve a pre-defined level of sensitivity of 93% for ≥ CIN2 in the ASC-US population. Based on these criteria, the cutoff values of (40.0, 40.5, 40.0) in the 3 channels (12 Other HR HPV, HPV 16 and HPV 18, respectively) were selected for the **cobas®** HPV test.

Limit of Detection at the Clinical Cutoff

The Limit of Detection (LOD) at the clinical cutoff of high risk HPV genotypes HPV16, HPV18 and HPV31 was determined for the **cobas**® HPV Test. The LODs were assessed using 1) plasmids of HPV31, HPV16 and HPV18 in the background of pooled HPV negative patient specimens collected in PreservCyt solution, and 2) HPV positive cell lines SiHa (HPV16) and HeLa (HPV18) in PreservCyt solution containing an HPV negative cell line (HCT-15) background. Plasmid and cell lines were diluted to concentrations below, above and at the expected LOD levels. A minimum of 60 replicates were tested for each plasmid or cell line level for each of 3 reagent lots. A total of 30 runs were performed in a period of 5 days using 4 instrument systems. The LOD at the clinical cutoff is the level of HPV DNA in the sample that has positive test results (above the clinical cutoff) at least 95% of the time. Table 31 contains results from the reagent lot producing the most conservative (highest) LOD in the analysis.

Table 31
Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18)

HPV Type	Concentration (copies or cells/mL)	Number of Positive/Tested	Mean CT	% Positives	95% Confidence Interval	
					Lower	Upper
31	600	60/60	36.6	100.0%	94.0%	100.0%
	300	**59/61**	**37.9**	**96.7%**	**88.7%**	**99.6%**
	150	49/60	38.7	81.7%	69.6%	90.5%
16	1500	60/60	36.5	100.0%	94.0%	100.0%
	600	**60/60**	**37.7**	**100.0%**	**94.0%**	**100.0%**
	300	55/61	39.1	90.2%	79.8%	96.3%
18	1,500	60/60	36.9	100.0%	94.0%	100.0%
	600	**60/60**	**38.0**	**100.0%**	**94.0%**	**100.0%**
	300	42/61	39.6	68.9%	55.7%	80.1%
SiHa (HPV16)	200	60/60	36.9	100.0%	94.6%	100.0%
	100	**60/60**	**38.0**	**100.0%**	**94.6%**	**100.0%**
	50	53/60	39.3	88.3%	77.4%	95.2%
HeLa (HPV18)	80	60/60	35.7	100.0%	94.0%	100.0%
	40	**60/60**	**36.8**	**100.0%**	**94.0%**	**100.0%**
	20	56/60	38.2	93.3%	83.8%	98.1%

Inclusivity Verification

To verify that the **cobas**® HPV Test is capable of accurately detecting *all HPV high risk genotypes, the Limit of Detection (LOD) at the clinical cutoff was determined for genotypes 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Quantified plasmid stocks of each HPV genotype were diluted into a background of pooled HPV negative patient specimens collected in PreservCyt solution to concentrations below, above and at the expected LOD levels. Two lots of reagents were used to produce a minimum of 24 replicates for each positive level with each lot of reagents. For each HPV type, the reported LOD was defined as the lowest testing concentration having a > 95% positive hit rate. Table 32 contains results from the reagent lot producing the most conservative (higher) LOD in the analysis.

Table 32
Summary of High Risk Genotype Limit Of Detection For cobas® HPV Genotype Inclusivity Study

HPV DNA *Type	LOD (copies/mL)	Number of Positive/Tested	Mean CT	Hit Rate	95% Confidence Interval	
					Lower	Upper
33	300	24/24	38.2	100.0%	85.7%	100.0%
35	600	23/24	38.4	95.8%	78.8%	99.8%
39	300	24/24	37.9	100.0%	85.7%	100.0%
45	150	23/24	38.0	95.8%	78.8%	99.8%
51	300	24/24	38.4	100.0%	85.7%	100.0%
52	2400	24/24	39.1	100.0%	85.7%	100.0%
56	1200	23/24	38.4	95.8%	78.8%	99.8%
58	600	24/24	38.6	100.0%	85.7%	100.0%
59	300	23/24	39.0	95.8%	78.8%	99.8%
66	1200	24/24	37.7	100.0%	85.7%	100.0%
68	1200	24/24	38.0	100.0%	85.7%	100.0%

*The LOD of the **cobas**® HPV Test for HPV genotypes 16, 18 and 31 was determined as described above in this Package Insert.

Reproducibility

An 18-member panel composed of pools made from clinical samples collected into PreservCyt solution, and from samples derived from SiHa and HeLa cell lines was tested for Reproducibility Each panel member was tested for 18 days (6 days per kit lot), 2 replicates per run, at 3 testing sites. Two operators at each of 3 sites performed 2 runs per day for 3 days each on each of 3 reagent lots. A run was defined as 36 panel-member aliquots and 1 positive and 1 negative control.

Overall, 111 runs were performed to obtain 108 valid runs. The 3 invalid runs were due to instrument errors (percent of invalid runs was 2.7% (3/111) with 95% CI: 0.6%, 7.7%). A total of 3,888 tests were performed on the 18 panel members in the valid runs; 5 of those tests were invalid due to instrument errors.

All valid test results were included in the analyses that reported the percentage of correct results. There were no false positive results in 216 tests performed on the negative panel members (background negative cell and the pooled negative clinical sample; see Table 33 below).

For the percents of positive results for the positive panel members were presented in Table 34. With respect to sites, site 1 tended to have a lower percent positive for some weak-positive and moderate-positive panel members. This trend can be attributed to operator 1, who tended to have lower percent positive values in the weak positive and moderate positive panel members.

Analysis of variance of the Ct values from valid tests performed on positive panel members (see Table 35) yielded overall CV (%) ranges of 1.1% to 2.5% for the SiHa cell lines, 1.5% to 2.5% for the HeLa cell lines, and 3.5% to 10.3% for the pooled clinical samples.

1046
1047

Table 33
Results by Sample Type and Negative Panel Member for Lot and Site/Instrument

Sample Type	Panel Member	Ct SD	Ct CV %	Number Negative / Total Number Valid Results					
				Lot			Site/ Instrument		
				Lot ID	Negative/ Valid	%	Site ID	Negative/ Valid	%
Background cell line	Negative cell line	n/a	n/a	1	72/72	100.0	1	72/72	100.0
				2	72/72	100.0	2	72/72	100.0
				3	72/72	100.0	3	72/72	100.0
Pooled negative clinical sample	Negative	n/a	n/a	1	72/72	100.0	1	72/72	100.0
				2	72/72	100.0	2	72/72	100.0
				3	72/72	100.0	3	72/72	100.0

1048

1049
1050

Table 34
Results by Sample Type and Positive Panel Member for Lot and Site/Instrument

Sample Type	Panel Member	Ct SD	Ct CV %	Number Positive / Total Number Valid Results					
				Lot			Site/Instrument		
				Lot ID	Positive/ Valid	%	Site ID	Positive/ Valid	%
SiHa cell line	HPV16 - weak positive A (25 cells/mL)	0.45	1.1	1	41/72	56.9	1	22/72	30.6
				2	25/72	34.7	2	38/72	52.8
				3	23/72	31.9	3	29/72	40.3
SiHa cell line	HPV16 - weak positive B (60 cells/mL)	0.68	1.7	1	66/72	91.7	1	56/72	77.8
				2	64/72	88.9	2	71/72	98.6
				3	63/72	87.5	3	66/72	91.7
SiHa cell line	HPV16 - weak positive C (80 cells/mL)	0.68	1.8	1	68/72	94.4	1	61/72	84.7
				2	67/72	93.1	2	72/72	100.0
				3	69/72	95.8	3	71/72	98.6
SiHa cell line	HPV16 – positive (150 cells/mL)	0.94	2.5	1	71/72	98.6	1	71/72	98.6
				2	71/72	98.6	2	72/72	100.0
				3	72/72	100.0	3	71/72	98.6
HeLa cell line	HPV18 - weak positive A (8 cells/mL)	0.60	1.5	1	43/72	59.7	1	34/72	47.2
				2	35/72	48.6	2	46/72	63.9
				3	42/72	58.3	3	40/72	55.6
HeLa cell line	HPV18 - weak positive B (22 cells/mL)	0.90	2.4	1	67/72	93.1	1	59/72	81.9
				2	63/72	87.5	2	72/72	100.0
				3	67/72	93.1	3	66/72	91.7
HeLa cell line	HPV18 - weak positive C (27 cells/mL)	0.90	2.4	1	69/72	95.8	1	65/72	90.3
				2	67/72	93.1	2	71/72	98.6
				3	72/72	100.0	3	72/72	100.0
HeLa cell line	HPV18 – positive (50 cells/mL)	0.91	2.5	1	70/72	97.2	1	69/72	95.8
				2	71/72	98.6	2	72/72	100.0
				3	72/72	100.0	3	72/72	100.0
Pooled HPV 16 clinical sample	HPV16 - moderate positive	1.59	4.3	1	66/71	93.0	1	64/72	88.9
				2	66/71	93.0	2	68/70	97.1
				3	69/72	95.8	3	69/72	95.8
Pooled HPV 16 clinical sample	HPV16 - positive	1.21	3.5	1	72/72	100.0	1	72/72	100.0
				2	71/71	100.0	2	72/72	100.0
				3	72/72	100.0	3	71/71	100.0
Pooled HPV 18 clinical sample	HPV18 - moderate positive	2.30	6.1	1	62/71	87.3	1	56/71	78.9
				2	63/72	87.5	2	71/72	98.6
				3	67/72	93.1	3	65/72	90.3
Pooled HPV 18 clinical sample	HPV18 - positive	3.51	10.3	1	72/72	100.0	1	71/71	100.0
				2	72/72	100.0	2	72/72	100.0
				3	71/71	100.0	3	72/72	100.0

Sample Type	Panel Member	Ct SD	Ct CV %	Number Positive / Total Number Valid Results					
				Lot			Site/Instrument		
				Lot ID	Positive/ Valid	%	Site ID	Positive/ Valid	%
Pooled HPV 31 clinical sample	HPV31 - moderate positive	2.95	8.0	1	67/72	93.1	1	61/72	84.7
				2	62/72	86.1	2	68/72	94.4
				3	63/72	87.5	3	63/72	87.5
Pooled HPV 31 clinical sample	HPV31 - positive	3.01	8.3	1	72/72	100.0	1	70/72	97.2
				2	68/72	94.4	2	72/72	100.0
				3	72/72	100.0	3	70/72	97.2
Pooled HPV 45 clinical sample	HPV45 - moderate positive	1.88	5.0	1	70/72	97.2	1	66/72	91.7
				2	66/72	91.7	2	70/72	97.2
				3	64/72	88.9	3	64/72	88.9
Pooled HPV 45 clinical sample	HPV45 - positive	1.80	5.0	1	72/72	100.0	1	72/72	100.0
				2	72/72	100.0	2	72/72	100.0
				3	72/72	100.0	3	72/72	100.0

Table 35
Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated from Valid Samples of Positive Sample Type Panel Members

Sample Type[1] / Conc.[2] (cells/mL)			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]													
			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site/ Instrument		Total	
	n[3] / N	Mean CT	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
SiHa GT 16 weak positive A (25/mL)	89/216	39.80	0.38	0.96%	0.20	0.50%	0.08	0.21%	0.00	0.00%	0.09	0.23%	0.00	0.00%	0.45	1.13%
SiHa GT 16 weak positive B (60/mL)	193/216	39.14	0.53	1.36%	0.17	0.43%	0.19	0.48%	0.03	0.08%	0.25	0.64%	0.23	0.59%	0.68	1.74%
SiHa GT 16 weak positive C (80/mL)	204/216	38.73	0.58	1.50%	0.00	0.00%	0.18	0.47%	0.08	0.21%	0.21	0.55%	0.21	0.54%	0.68	1.76%
SiHa GT 16 positive (150/mL)	214/216	37.89	0.45	1.19%	0.22	0.57%	0.35	0.91%	0.35	0.91%	0.21	0.57%	0.58	1.53%	0.94	2.47%
HeLa GT 18 weak positive A (8/mL)	120/216	39.02	0.57	1.45%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.12	0.32%	0.16	0.41%	0.60	1.54%
HeLa GT 18 weak positive B (22/mL)	197/216	38.10	0.72	1.89%	0.38	1.00%	0.11	0.29%	0.13	0.33%	0.17	0.44%	0.30	0.78%	0.90	2.36%
HeLa GT 18 weak positive C (27/mL)	208/216	37.77	0.73	1.93%	0.13	0.35%	0.17	0.44%	0.31	0.83%	0.25	0.67%	0.26	0.69%	0.90	2.38%

HeLa GT 18 positive (50/mL)	213/216	36.76	0.64	1.74%	0.07	0.20%	0.29	0.79%	0.38	1.05%	0.32	0.87%	0.29	0.80%	0.91	2.48%
Clinical GT 16 weak positive	201/214	37.33	1.46	3.92%	0.44	1.18%	0.44	1.17%	0.00	0.00%	0.00	0.00%	0.00	0.00%	1.59	4.26%
Clinical GT 16 positive	215/215	34.95	1.05	3.02%	0.50	1.44%	0.00	0.00%	0.00	0.00%	0.18	0.51%	0.27	0.76%	1.21	3.46%
Clinical GT 18 weak positive	192/215	37.63	2.27	6.02%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.39	1.05%	2.30	6.11%
Clinical GT 18 positive	215/215	34.17	3.16	9.25%	1.26	3.68%	0.00	0.00%	0.42	1.23%	0.00	0.00%	0.73	2.13%	3.51	10.26 %
Clinical GT 31 weak positive	192/216	36.91	2.95	7.98	0.00	0.00%	0.00	0.00%	0.22	0.60%	0.00	0.00%	0.00	0.00%	2.95	8.00%
Clinical GT 31 positive	212/216	36.49	2.81	7.69%	0.00	0.00%	0.67	1.84%	0.00	0.00%	0.00	0.00%	0.86	2.35%	3.01	8.25%
Clinical GT 45 weak positive	200/216	37.37	1.88	5.03%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%	1.88	5.03%
Clinical GT 45 positive	216/216	35.66	1.74	4.87%	0.21	0.58%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.41	1.14%	1.80	5.04%

[1] Moderate is abbreviated as mod.
[2] Analyte concentrations are given for the SiHa and HeLa cell lines.
[3] n is the number of positive tests, which contribute CT values to the analysis. N is the total number of valid tests for the panel member. Because only positive test results were included, estimates of SD (and %CV) may be underestimated.

Precision

In-house Precision was examined using a panel composed of HPV positive and negative cell lines diluted into PreservCyt solution and pooled HPV positive and negative cervical specimens collected in PreservCyt solution. The precision panel was designed to include members below (< 70% positivity rate), at (90% to 99% positivity rate) and above (> 99% positivity rate) the Limit of Detection of the **cobas**® HPV Test. Panel members 1-9 and 19-22 were prepared with HPV positive and negative cell lines (SiHa, HPV16; HeLa, HPV18; HCT-15, HPV negative) diluted at different levels into PreservCyt solution (panel level 1 was prepared with HPV negative cell line only). Panel members 10-18 were prepared with high risk HPV positive specimen in PreservCyt solution pools (HPV16, HPV18, HPV31 and HPV45) diluted at different levels into pooled HPV negative specimens in PreservCyt solution (panel level 10 was prepared with HPV negative specimen pool only).

A description of the precision panel, anticipated performance in % positivity rate and the actual study performance in % positivity rate are shown in Table 36. All panel levels at and above the limit of detection yielded the anticipated positivity rates. Analysis of variance of the Ct values from valid tests performed on positive panel members (see Table 37) yielded overall CV (%) ranges of 1.1% to 1.7% for the SiHa cell lines, 1.5% to 2.2% for the HeLa cell lines, and 3.7% to 8.5% for the pooled clinical samples.

1069 **Table 36**

1070 **Summary of the Precision Panel and Hit Rates For cobas® HPV Precision Study**

Panel Number	HPV Target	Description	Anticipated Positivity Rate	N Tested	N Pos	Positivity Rate	95% CI	
							Lower	Upper
1	N/A	HCT15 cell line (HPV negative)	0%	144	0	0.0%	0%	3%
2	HPV16	SiHa cell line	< 70%	143	80	55.9%	47%	64%
3	HPV16	SiHa cell line	90% — 95%	144	138	95.8%	91%	98%
4	HPV16	SiHa cell line	95% — 99%	144	144	100.0%	97%	100%
5	HPV16	SiHa cell line	> 99%	143	142	99.3%	96%	100%
6	HPV18	HeLa cell line	< 70%	144	96	66.7%	58%	74%
7	HPV18	HeLa cell line	90% — 95%	144	143	93.3%	96%	100%
8	HPV18	HeLa cell line	95% — 99%	144	142	98.6%	95%	100%
9	HPV18	HeLa cell line	> 99%	144	144	100.0%	97%	100%
10	N/A	Pooled HPV neg specimen	0%	141	1	0.7%	0%	4%
11	HPV16	High Risk HPV positive specimen	90% — 99%	144	140	97.2%	93%	99%
12	HPV16	High Risk HPV positive specimen	> 99%	143	143	100.0%	97%	100%
13	HPV18	High Risk HPV positive specimen	90% — 99%	144	140	97.2%	93%	99%
14	HPV18	High Risk HPV positive specimen	> 99%	144	144	100.0%	97%	100%
15	HPV31	High Risk HPV positive specimen	90% — 99%	143	142	99.3%	96%	100%
16	HPV31	High Risk HPV positive specimen	> 99%	144	144	100.0%	97%	100%
17	HPV45	High Risk HPV positive specimen	90% — 99%	144	133	92.4%	87%	96%
18	HPV45	High Risk HPV positive specimen	> 99%	144	144	100.0%	97%	100%
*19	HPV16 & HPV18	SiHa & HeLa cell lines	< 70%	143	88	61.5%	53%	70%
*20	HPV16 & HPV18	SiHa & HeLa cell lines	90% — 95%	144	144	100.0%	97%	100%
*21	HPV16 & HPV18	SiHa & HeLa cell lines	95% — 99%	144	144	100.0%	97%	100%
*22	HPV16 & HPV18	SiHa & HeLa cell lines	> 99%	144	144	100.0%	97%	100%
**19	HPV16 & HPV18	SiHa & HeLa cell lines	< 70%	143	103	72.0%	64%	79%
**20	HPV16 & HPV18	SiHa & HeLa cell lines	90% — 95%	144	143	93.3%	96%	100%
**21	HPV16 & HPV18	SiHa & HeLa cell lines	95% — 99%	144	142	98.6%	95%	100%
**22	HPV16 & HPV18	SiHa & HeLa cell lines	> 99%	144	144	100.0%	97%	100%

1071

1072 **Table 37**
1073 **Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold,**
1074 **Estimated from Valid Samples of Positive Sample Type Precision Panel Members**

				Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
#	Sample Type /Conc.[1] (cells/mL)			Between-Lot		Between-Run/System		Between-Operator		Between-Day		Within-Run		Total	
		N[2] / N	Mean CT	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	SiHa HPV16 (25/mL)	80/143	39.8	0.000	0.000%	0.000	0.000%	0.065	0.20%	0.168	0.40%	0.410	1.00%	0.448	1.10%
2	SiHa HPV16 (60/mL)	138/144	38.8	0.172	0.40%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.640	1.70%	0.663	1.70%
3	SiHa HPV16 (80/mL)	144/144	38.4	0.055	0.10%	0.000	0.00%	0.116	0.30%	0.142	0.40%	0.569	1.50%	0.601	1.60%
4	SiHa HPV16 (150/mL)	142/143	37.3	0.067	0.20%	0.092	0.20%	0.000	0.00%	0.284	0.80%	0.405	1.10%	0.508	1.40%
5	HeLa HPV18 (8/mL)	96/144	38.9	0.116	0.30%	0.073	0.20%	0.000	0.00%	0.000	0.00%	0.665	1.70%	0.680	1.70%
6	HeLa HPV18 (22/mL)	143/144	37.7	0.000	0.00%	0.000	0.00%	0.076	0.20%	0.074	0.20%	0.811	2.20%	0.818	2.20%
7	HeLa HPV18 (27/mL)	142/144	37.5	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.229	0.60%	0.675	1.80%	0.712	1.90%
8	HeLa HPV18 (50/mL)	144/144	36.5	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.157	0.40%	0.578	1.60%	0.599	1.60%
9	Clinical HPV16	140/144	37.2	0.000	0.00%	0.258	0.70%	0.000	0.00%	0.000	0.00%	1.650	4.40%	1.670	4.50%
10	Clinical HPV16	143/143	34.5	0.220	0.60%	0.135	0.40%	0.000	0.00%	0.441	1.30%	1.183	3.40%	1.288	3.70%
11	Clinical HPV18	140/144	36.7	0.378	1.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	3.081	8.40%	3.104	8.50%
12	Clinical HPV18	144/144	34.9	0.000	0.00%	0.692	2.00%	0.000	0.00%	1.291	3.70%	2.180	6.20%	2.626	7.50%
13	Clinical HPV31	142/143	37.1	0.000	0.00%	0.255	0.70%	0.323	0.90%	0.000	0.00%	2.351	6.30%	2.387	6.40%
14	Clinical HPV31	144/144	35.8	0.190	0.50%	0.000	0.00%	0.000	0.00%	0.746	2.10%	2.825	7.90%	2.928	8.20%
15	Clinical HPV45	133/144	37.3	0.000	0.00%	0.186	0.50%	0.101	0.30%	0.000	0.00%	1.915	5.10%	1.926	5.20%
16	Clinical HPV45	144/144	35.0	0.393	1.10%	0.246	0.70%	0.000	0.00%	0.000	0.00%	1.780	5.10%	1.839	5.30%
*17	SiHa HPV16 (25/mL) HeLa HPV18 (8/mL)	88/143	39.8	0.000	0.00%	0.000	0.00%	0.014	0.00%	0.000	0.00%	0.461	1.20%	0.461	1.20%
*18	SiHa HPV16 (60/mL) HeLa HPV18 (22/mL)	144/144	38.4	0.106	0.30%	0.000	0.00%	0.034	0.10%	0.000	0.00%	0.591	1.50%	0.601	1.60%

#	Sample Type /Conc.[1] (cells/mL)			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
				Between-Lot		Between-Run/System		Between-Operator		Between-Day		Within-Run		Total	
		$\frac{N^2}{N}$	Mean CT	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
*19	SiHa HPV16 (80/mL) HeLa HPV18 (27/mL)	144/144	38.3	0.134	0.30%	0.060	0.20%	0.000	0.00%	0.238	0.60%	0.405	1.10%	0.479	1.30%
*20	SiHa HPV16 (150/mL) HeLa HPV18 (50/mL)	144/144	37.2	0.088	0.20%	0.039	0.10%	0.000	0.00%	0.238	0.60%	0.405	1.10%	0.479	1.30%
**17	SiHa HPV16 (25/mL) HeLa HPV18 (8/mL)	103/143	38.8	0.000	0.00%	0.127	0.30%	0.065	0.20%	0.274	0.70%	0.579	1.50%	0.656	1.70%
**18	SiHa HPV16 (60/mL) HeLa HPV18 (22/mL)	143/144	37.6	0.182	0.50%	0.000	0.00%	0.000	0.00%	0.145	0.40%	0.710	1.90%	0.747	2.00%
**19	SiHa HPV16 (80/mL) HeLa HPV18 (27/mL)	142/144	37.3	0.000	0.00%	0.062	0.20%	0.000	0.00%	0.131	0.40%	0.626	1.70%	0.643	1.70%
**20	SiHa HPV16 (150/mL) HeLa HPV18 (50/mL)	144/144	36.4	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.244	0.70%	0.481	1.30%	0.540	1.50%

[1] Analyte concentrations are given for the SiHa and HeLa cell lines.

[2] n is the number of positive tests, which contribute CT values to the analysis. N is the total number of valid tests for the panel member. Because only positive test results were included, estimates of SD (and %CV) may be underestimated.

*Results shown from detection channel 2 (HPV16)

** Results shown from detection channel 3 (HPV18)

N/A = Not applicable

Analytical Specificity

A panel of bacteria, fungi and viruses, including those commonly found in the female urogenital tract, as well as several Human papillomavirus types classified as low or undetermined risk were tested with the **cobas**® HPV Test to assess analytical specificity. The organisms listed in Table 38 were spiked at high concentrations ($\geq 1 \times 10^6$ *units/reaction with the exception of *Treponema pallidum* and Adenovirus-5, which were both tested at $\geq 1 \times 10^5$ *units/reaction) into HPV negative specimen in PreservCyt solution and into HPV negative specimen in PreservCyt solution spiked with HPV31, HPV16 and HPV18 plasmid DNA at 3 times the limit of detection. Results indicated that none of these organisms interfered with detection of HPV 31, HPV16 and HPV18 or produced false positive results in the HPV negative specimen.

*All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydia trachomatis* as Elementary Bodies (EBs). *Treponema pallidum* and all HPV genotypes were quantified as DNA copies. Adenovirus was quantified as Plaque Forming Units (PFU). CMV, EBV, HSV-1 and HSV-2 were quantified as Viral Particles (VP). HBV and HIV-1 were quantified in International Units (IU) and SV40 was quantified in Infection Units (IU).

Table 38
Microorganisms Tested for Analytical Specificity

Achromobacter xerosis	*Erysipelothrix rhusiopathiae*	*Mycoplasma hominis*	*Weissella paramesenteroides*
Acinetobacter calcaceticus	*Escherichia coli*	*Neisseria gonorrhea*	*Yersinia enterocolitica*
Acinetobacter lwoffi	*Ewingella americana*	*Neisseria meningitidis Serogroup A*	HPV 6
Acinetobacter sp. Genospecies 3	*Fusobacterium nucleatum*	*Pasteurella maltocida*	HPV 11
Actinomyces isrealii	*Gemella morbillorum*	*Pediococcus acidilactica*	HPV 26
Adenovirus 5	*Gardnerella vaginalis*	*Peptostreptococcus anaerobius*	HPV 30
Aerococcus viridans	*Haemophilus ducreyi*	*Propionibacterium acnes*	HPV 34
Alcaligenes faecalis	Hepatitis B virus (HBV)	*Proteus mirabilis*	HPV 40
Bacillus thuringiensis	Herpes simplex virus 1 (HSV-1)	*Proteus vularis*	HPV 42
Bacteroides fragilis	Herpes simplex virus 2 (HSV-2)	*Providencia stuartii*	HPV 53
Bacteroides ureolyticus	Human immunodeficiency virus (HIV-1)	*Pseudomonas aeruginosa*	HPV 54
Bifidobacterium longum	*Kingella kingae*	*Ruminococcus productus*	HPV 55B
Bifidobacterium adolescentis	*Klebsiella pneumoniae ss ozaenae*	*Salmonella minnesota*	HPV 61
Bifidobacterium brevi	*Lactobacillus acidophilus*	*Serratia marcescens*	HPV 62
Campylobacter jejuni	*Lactobacillus crisptus*	*Staphylococcus aureus*	HPV 64
Candida albicans	*Lactobacillus delbrueckii s. lactis*	*Staphylococcus epidermidis*	HPV 67
Chlamydia trachomatis	*Lactobacillus jensenii*	*Staphylococcus saprophyticus*	HPV 69
Chromobacter violaceum	*Lactobacillus vaginalis*	*Streptococcus agalactiae*	HPV 70
Citrobacter braakii	*Lactococcus lactis cremoris*	*Streptococcus anginosus*	HPV 71
Clostridium perfringens	*Legionella pneumophila*	*Streptococcus pyogenes*	HPV 72
Corynebacterium genitalium	*Micrococcus luteus*	*Streptococcus sanguis*	HPV 73
Cytomegalovirus (CMV)	*Mobiluncus curtsil s. curtsii*	Simian Virus 40 (SV40)	HPV 81
Eikenella corrodens	*Moraxella osloensis*	*Treponema Pallidum*	HPV 82
Enterobacter cloacae	*Morganella morganii*	*Trichomonas vaginalis*	HPV 83
Enterococcus faecalis	*Mycobacterium avium*	*Ureaplasma urealyticum*	HPV 84
Enterococcus faecium	*Mycobacterium smegmatis*	*Veillonela parvula*	HPV 85
Epstein Barr Virus (EBV)	*Mycoplasma genitalium*	*Vibrio parahaemolyticus*	HPV 89 (CP6108)

Interfering Substances

HPV positive and HPV negative cervical specimens as well as contrived specimens were used to assess the effects of endogenous and exogenous interfering substances that could potentially be present in cervical specimens. Testing materials used in these studies are described in Table 39. The concentrations of endogenous and exogenous substances tested represent conditions that could occur during specimen collection.

Whole blood, Peripheral Blood Mononuclear Cells (PBMC) and cervical mucus were tested as potential endogenous interfering substances found in cervical specimens. Levels of each potential interfering substance tested and performance observations are described in Table 40. No interference was seen for PBMC or cervical mucus at all levels tested. Whole blood showed no interference when present in visually detectable amounts of up to 1.5%.

Table 39
Interference Testing Sample Descriptions

Sample type	Description	Study
HPV Positive Cervical Specimens	10 individual HPV positive cervical specimens in PreservCyt solution were aliquoted for testing with and without endogenous interfering substances	Endogenous Interference
HPV Negative Cervical Specimens	10 individual HPV negative cervical specimens in PreservCyt solution were aliquoted for testing with and without endogenous interfering substances	Endogenous Interference
Contrived HPV Positive Cervical Specimen	Cervical specimens in PreservCyt solution positive for one of the high risk HPV types other than HPV16 and/or HPV18 were diluted with HPV negative specimen to generate signal consistent with approximately 3 fold LOD. HPV types 16 and 18 plasmids were then added at concentrations of approximately 3 fold LOD.	Endogenous Interference
3 x LOD PreservCyt Specimen Pools	HPV types 31, 16, 18 plasmids were each diluted to 3 fold LOD into pools of negative cervical specimen in PreservCyt solution.	Exogenous Interference

Table 40
Interference Testing Results with Endogenous Interferents

Interferent Tested	Concentrations Tested	Interference Observed
Whole Blood	1%, 1.5%, 2%, 3% v/v	Above 1.5%
PBMC	10^4, 10^5, 10^6 cells/mL	None
Cervical Mucus	Mucus obtained from standard cervical cleaning procedure	None

A total of 18 over-the-counter (OTC) feminine hygiene and contraceptive products were tested as potential interfering substances. Types of potential interferents tested and performance observations in 3 x LOD pools prepared from HPV negative cervical specimens in PreservCyt solution are described in Table 41.

Table 41
Interference Testing Results with Exogenous Interferents

Product Name	Active Ingredients	Interference Observed
Prodium	Phenazopyridine Hydrochloride	None
Vaginal Contraceptive Foam	Nonoxynol-9	None
Clotrimazole 7	Clotrimazole	None
Gyne-Lotrimin 7	Clotrimazole	None
Gynecort	Hydrocortisone	None
Vagisil Satin	Hydrocortisone	None
Vagi-Gard (Douche)	Povidone-iodine	None
Miconazole	Miconazole nitrate	None
Monistat 3 Cream	Miconazole nitrate	None
Equate tioconazole 1	Tiocanazole	None
Vagi-Gard Medicated Cream	Benzocaine	None
Vagicaine Anti-Itch Cream	Benzocaine	None
Yeast Gard	Pulsatilla, Candida Parapsilosis, Candida Albicans	None
Norforms	PEG-32,PEG-18, Peg-20 stearate	None
KY Jelly	Hydroxyethylcellulose, Chlorhexidine Gluconate	None
Vagisil Moisturizer	DMDM Hydantoin, Diazolidinyl urea	None
Replens	Polycarbophil,	None
Vagi-Gard (Lube Gel)	Glucano Delta Lactone, Chlorhexidine Gluconate	None

REFERENCES

1. Burd, Eileen M. 2003. Human Papillomavirus and Cervical Cancer. Clinical Microbiology Reviews. **16**:1-17.
2. zur Hausen, H. 2002. Papillomaviruses and Cancer: From Basic Studies to Clinical Application. Nat Rev Cancer. **2(5)**:342-50.
3. Walboomers, Jan M.M., Jacobs, Marcel V., Manos, M.M., et al. 1999. Human Papillomavirus is a Necessary Cause of Invasive Cervical Cancer Worldwide. Journal of Pathology. **189**:12-19.
4. Bernard HU. Review: The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. J Clin Virol. 2005; **32S**, S1-6.
5. Molijn A, Kleter B, Quint W, van Doorn, L. Review: Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol. 2005; **32S**:S43-51.
6. zur Hausen H. Roots and perspectives of contemporary papillomavirus research. J Cancer Res Clin Oncol. 1996; **122**: 3-13.
7. de Villiers EM, Fauguet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004; **324**:17-27.
8. Franco EL, Rohan TE, Villa LL. Epidemiologic evidence and human papillomavirus infection as a necessary cause of cervical cancer. J Natl Cancer Inst. 1999; **91**:506-511.
9. Lorincz AT, Reid R, Jenson AB, et al. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol. 1992; **79**:328-37.
10. Bosch, F.X., Manos, M.M., Munoz, N., et al. 1995. International Biological Study on Cervical Cancer (IBSCC) Study Group. Prevalence of Human Papillomavirus in Cervical Cancer: a Worldwide Perspective. Journal of the National Cancer Institute, Vol. 87, No. **11**:796-802.
11. Bosch, F.X., A. Lorincz, N. Muñoz, C.J.L.M. Meijer, K.V. Shah (2002) "The causal relation between human papillomavirus and cervical cancer" J Clin Path **55**: 244-265.
12. Muñoz N, F.X. Bosch, S. de Sanjosé, R. Herrero, X. Castellsagué, K.V. Shah, P.J.F. Snijders, and Chris J.L.M. Meijer, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. (2003) "Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer" N Engl J Med **348(6)**: 518-527.

13. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer. 2003; **88**:63-73.

14. Koutsky, L. 1997. Epidemiology of genital human papillomavirus infection. American Journal of Medicine. **102(5A)**:3-8.

15. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis. 2005;**191**:731-738.

16. Moscicki, A, Schiffman M, Kjaer S, Villa L. Updating the natural history of HPV and anogenital cancer. Vaccine 2006; **24(S3)**; 42-51.

17. Moscicki AB, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. J Infect Dis. 2004 Jul 1;**190(1)**:37-45.

18. Palmer Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Wacholder S, Tarone R, Burk RD. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis. 2005 Jun 1;**191(11)**:1808-16.

19. Zielinski GD, Snijders PJF, Rozendaal I, et al. High-risk HPV testing in women with borderline and mild dyskaryosis; long term follow-up data and clinical relevance. J Pathol 2001;**195**:300-306.

20. Holowaty P, Miller AB, Rohan T, To T. Natural history of dysplasia of the uterine cervix, J Natl Cancer Inst 1999; **91**:252-58.

21. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Bezemer PD, Verheijen RH, Meijer CJ. Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. Lancet. 2001;**358(9295)**:1782-1783.

22. Myers, T.W. and Gelfand, D.H. 1991. Reverse transcription and DNA amplification by a *Thermus thermophilus* DNA polymerase. Biochemistry. **30**:7661-7666.

23. Davies, P., Kornegay, J., Iftner, T. 2001. Current methods of testing for human papillomavirus. Best Practice and Research Clinical Obstetrics and Gynecology. Vol. 15, No. **5**:677-700.

24. Birch, D.E. 1996. Nature. Vol 381, No 6581:445-446...or US patent no.5,677,152.

25. Meng et al. 2001. J Clin Microbiol. Vol 39, No 8 :2937-2945.

26. 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.

27. Higuchi, R., Dollinger, G., Walsh, P.S., and Griffith, R. 1992. Simultaneous amplification and detection of specific DNA sequences. Bio/Technology **10**:413-417.

28. Heid, C.A., Stevens, J., Livak, J.K., and Williams, P.M. 1996. Real time quantitative PCR. Genome Research **6**:986-994.

29. Richmond, J.Y. and McKinney, R.W. eds. 1999. *Biosafety in Microbiological and Biomedical Laboratories*. HHS Publication Number (CDC) 93-8395.

30. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections. Approved Guideline-Third Edition. CLSI Document M29-A3 Villanova, PA:CLSI, 2005.

31. International Air Transport Association. Dangerous Goods Regulations, 48th Edition. 2007.

32. Wheeler, C.M., Hunt, W.C., Joste, N.E., Key, C.R., Quint, W.G.V. and Castle, P.E. 2009. Human Papillomavirus Genotype Distributions: Implications for Vaccination and Cancer Screening in the United States. J Natl Cancer Inst. 101: 475-487

33. Khan, M.J., Castle, P.E., Lorincz, A.T., Wacholder, S., Sherman, M., Scott, D.R., Rush, B.B., Glass, A.G. and Schiffman, M. 2005. The Elevated 10-Year Risk of Cervical Precancer and Cancer in Women With Human Papillomavirus (HPV) Type 16 and 18 and the Possible Utility of Type-Specific HPV Testing in Clinical Practice. J Natl Cancer Inst. 97: 1072-1079.

34. Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, and Solomon D. 2007. 2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests. Am J Obstet Gynecol 197 (4); 346-355.

35. Saslow D, Runowicz CD, Solomon D, Moscicki A-B, Smith RA, Eyre HJ, Cohen C. 2002, American Cancer Society Guideline for the Early Detection of Cervical Neoplasia and Cancer. CA Can Jour Clin 53: 342-362.

36. hc2 High-Risk HPV DNA Test® [package insert]. Gaithersburg, MD: Digene Corporation, 2007.

37. Bauer, H.M., Greer, C.E., and Manos, M.M. 1992. Determination of Genital Human Papillomavirus Infection Using Consensus PCR, p. 132-152. *In* Herrington, C.S., and McGee, J.O.D.(ed.), Diagnostic Molecular Pathology: A Practical Approach. Oxford University Press, Oxford, United Kingdom

38. Gravitt, P., C. L. Peyton, T. Q. Alessi, C. Wheeler, F. Coutlée, A. Hildesheim, M. Schiffman, D. R. Scott, and R. J. Apple. 2000. *Improved amplification of genital human papillomaviruses.* J. Clin. Microbiol. **38**:357-361

39. Kondratovich MV, Yousef WA. Evaluation of Accuracy and 'Optimal' cutoff of Diagnostic Devices in the Same Study. *Joint Statistical Meeting Proceedings.* 2005:2547-2551

The following symbols are now used in labeling for Roche PCR diagnostic products.

 Ancillary Software	 Distributed by
 Authorized representative	 For *in vitro* diagnostic use
 Batch code	 Manufactured by
 Biological Risk (Potentially biohazardous material)	 Store in the dark
 Catalogue Number	 Temperature limitation (Store At)
 Consult instructions for use	 Test Definition File
 Contains sufficient for < n > tests	 Use by (last day of month)
 Contents of kit	

US Customer Technical Support 1-800-428-2336

[SP6ML-01]
05641268001-01
(Variable IFU M/N Rev)

Document Revision Information

Doc Rev. 1.0, ##/2010	First publishing

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

D

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the Roche
Response Center toll-free: 1-800- 526-1247)

XX/2010 05641268001-01

Doc Rev. 1.0