cobas® HPV Test

FOR *IN VITRO* DIAGNOSTIC USE.

Rx Only

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.

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INTENDED USE

The **cobas**® HPV Test is a qualitative *in vitro* test for the detection of Human Papillomavirus in cervical specimens collected by a clinician using an endocervical brush/spatula and placed in the ThinPrep® Pap Test™ PreservCyt® Solution or using a cervical broom and placed in SurePath™ Preservative Fluid. 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 HPV16 and HPV18 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 detect high-risk HPV genotypes 16 and 18. This information, together with the physician's assessment of screening 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 detect high risk HPV types. This information, together with the physician's assessment of screening 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 detect HPV genotypes 16 and 18. This information, together with the physician's assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management.

(e) In women 25 years and older, the cobas® HPV Test can be used for specimens **collected only in ThinPrep® Pap TestTM PreservCyt® Solution** as a first-line primary cervical cancer screening test to detect high risk HPV, including genotyping for 16 and 18. Women who test negative for high risk HPV types by the cobas® HPV Test should be followed up in accordance with the physician's assessment of screening and medical history, other risk factors, and professional guidelines. Women who test positive for HPV genotypes 16 and/or 18 by the cobas® HPV Test should be referred to colposcopy. Women who test high risk HPV positive and 16/18 negative by the cobas® HPV Test (12 other HR HPV positive) should be evaluated by cervical cytology to determine the need for referral to colposcopy.

WARNING

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 HPV16/18 positive should be monitored carefully for the development of high-grade cervical dysplasia according to current practice guidelines.
- for women who have undergone hysterectomy.
- for use with samples other than those collected by a clinician using an endocervical brush/spatula and placed in the ThinPrep® Pap Test™ PreservCyt® Solution or collected by a clinician using a cervical broom and placed in the Surepath™ Preservative Fluid.

Cervical specimens collected in SurePath™ Preservative Fluid have not been evaluated for use with the HPV test in primary screening.

HPV-negative cancers of the cervix do occur in rare circumstances. Also, no cancer screening test is 100% sensitive. Use of this device for primary cervical cancer screening should be undertaken after carefully considering the performance characteristics put forth in this label, as well as recommendations of professional 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).

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[5,7]. 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 or ablated 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 25 years and older 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 HPV16, HPV18 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. Specimens collected in SurePath Preservative Fluid must first undergo the preanalytic procedure (addition of the **cobas**® Sample Prep Buffer with heating). On the cobas x 480 instrument, pre-treated SurePath specimens and PreservCyt specimens 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

EagleZ05® 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 EagleZ05® 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 EagleZ05® 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 amplicon (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 (c4800 SMPL PREP) **240 Tests** (P/N: 05235782190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
MGP **(cobas® 4800 System Magnetic Glass Particles)**	Magnetic glass particles 93% Isopropanol[b]	10 x 4.5 mL	 **DANGER** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 +P378: In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
EB **(cobas® 4800 System Elution Buffer)**	Tris-HCl buffer 0.09% Sodium azide	10 x 18 mL	N/A

cobas® 4800 System Sample Preparation Kit (c4800 SMPL PREP) **960 Tests** (P/N: 05235804190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
MGP **(cobas® 4800 System Magnetic Glass Particles)**	Magnetic glass particles 93% Isopropanol[b]	10 x 13.5 mL	 **DANGER** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves/ eye protection/ face protection.

cobas® 4800 System Sample Preparation Kit (c4800 SMPL PREP) **960 Tests** (P/N: 05235804190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
			P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 +P378: In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
EB **(cobas® 4800 System Elution Buffer)**	Tris-HCl buffer 0.09% Sodium azide	10 x 18 mL	N/A

cobas® 4800 System Wash Buffer Kit (c4800 WB) **240 Tests** (P/N: 05235863190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
WB **(cobas® 4800 System Wash Buffer)**	Sodium citrate dihydrate 0.05% N-Methyl isothiazolone HCl	10 x 55 mL	N/A

cobas® 4800 System Wash Buffer Kit (c4800 WB) **960 Tests** (P/N: 05235871190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
WB **(cobas® 4800 System Wash Buffer)**	Sodium citrate dihydrate 0.05% N-Methyl isothiazolone HCl	10 x 200 mL	N/A

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT) **240 Tests** (P/N: 05235812190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
PK **(cobas® 4800 Proteinase K)**	Tris-HCl buffer[b] EDTA Glycerol Calcium chloride Calcium acetate < 2% Proteinase K[b]	10 x 0.9 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves. P284: Wear respiratory protection.

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT) **240 Tests** (P/N: 05235812190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
			P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
SDS **(cobas® 4800 System SDS Reagent)**	Tris-HCl buffer 0.2% SDS 0.09% Sodium azide	10 x 3 mL	N/A
LYS **(cobas® 4800 System Lysis Buffer)**	Tris-HCl buffer 37% (w/w) Guanidine HCl[b] < 5% Polydocanol[b]	10 x 10 mL	 **DANGER** H302: Harmful if swallowed. H315: Causes skin irritation. H318: Causes serious eye damage. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ eye protection/ face protection. P301 + P312 + P330: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P501: Dispose of contents/ container to an approved waste disposal plant.

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT) **960 Tests** (P/N: 05235839190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
PK **(cobas® 4800 Proteinase K)**	Tris-HCl buffer[b] EDTA Glycerol Calcium chloride Calcium acetate < 2% Proteinase K[b]	20 x 1.2 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves. P284: Wear respiratory protection.

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT) **960 Tests** (P/N: 05235839190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
			P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
SDS **(cobas® 4800 System SDS Reagent)**	Tris-HCl buffer 0.2% Sodium dodecyl sulfate 0.09% Sodium azide	10 x 9 mL	N/A
LYS **(cobas® 4800 System Lysis Buffer)**	Tris-HCl buffer 37% (w/w) Guanidine HCl[b] < 5% Polydocanol[b]	10 x 36 mL	 **DANGER** H302: Harmful if swallowed. H315: Causes skin irritation. H318: Causes serious eye damage. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ eye protection/ face protection. P301 + P312 + P330: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P501: Dispose of contents/ container to an approved waste disposal plant.

cobas® 4800 HPV Amplification/Detection Kit (c4800 HPV AMP/DET) **240 Tests** (P/N: 05235880190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HPV MMX **(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 β-globin primers < 0.01% Fluorescent-labeled HPV probes < 0.01% Fluorescent-labeled β-globin	10 x 0.5 mL	N/A

cobas® 4800 HPV Amplification/Detection Kit (c4800 HPV AMP/DET) **240 Tests** (P/N: 05235880190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
	probes < 0.10% EagleZ05® DNA polymerase (microbial) < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide		
HPV Mg/Mn (cobas® 4800 HPV Mg/Mn Solution)	Magnesium acetate Manganese acetate < 0.02% Glacial acetic acid 0.09% Sodium azide	10 x 1.0 mL	N/A

cobas® 4800 HPV Amplification/Detection Kit (c4800 HPV AMP/DET) **960 Tests** (P/N: 05235898190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HPV MMX (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 β-globin primers < 0.01% Fluorescent-labeled HPV probes < 0.01% Fluorescent-labeled β-globin probes < 0.10% EagleZ05® DNA polymerase (microbial) < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	20 x 1.0 mL	N/A
HPV Mg/Mn (cobas® 4800 HPV Mg/Mn Solution)	Magnesium acetate Manganese acetate < 0.02% Glacial acetic acid 0.09% Sodium azide	10 x 1.0 mL	N/A

cobas® 4800 HPV Controls Kit (c4800 HPV CTLs) **10 Sets** (P/N: 05235855190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
HPV (+) C (cobas® 4800 HPV Positive Control)	Tris-HCl buffer EDTA 0.05% Sodium azide < 0.00001% Poly rA RNA (synthetic)	10 x 0.5 mL	N/A

cobas® 4800 HPV Controls Kit (c4800 HPV CTLs) **10 Sets** (P/N: 05235855190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning[a]**
	< 0.00001% Non-infectious plasmid DNA (microbial) containing HPV-16, 18, 39 sequences < 0.00001% Non-infectious plasmid DNA (microbial) containing β-globin sequences		
(–) C **(cobas® 4800 System Negative Control)**	Tris-HCl buffer EDTA 0.05% Sodium azide < 0.00001% Poly rA RNA (synthetic)	10 x 0.5 mL	N/A

NOTE: ***Consult the correct Operator's Manual when required. Software version 1.1.2 users should consult the cobas® 4800 System Operator's Manual. Software version 2.1 or higher users should consult the cobas® 4800 System Manual and the cobas® HPV Test Operator's Manual.***

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. Safety Data Sheets (SDS) 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*[23] 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 appropriate **cobas®** 4800 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 appropriate **cobas®** 4800 Operator's Manual.

R. Cervical specimens collected in SurePath™ Preservative Fluid must undergo the preanalytic procedure prior to running the HPV test or risk obtaining false negative test results.

S. SurePath specimens taken from patients who are using the over-the-counter product Replens® vaginal moisturizer may give invalid or false negative results.

STORAGE AND HANDLING REQUIREMENTS

A. Do not freeze reagents.

B. Store the Sample Preparation Kit (**MGP, EB)**, Liquid Cytology Preparation Kit **(PK, SDS, LYS)**, HPV Amplification/Detection Kit **(HPV MMX, HPV Mg/Mn)** and HPV Controls Kit **[HPV (+) C** and **(–) C]** at 2-8ºC. These reagents are stable until the expiration date indicated.

C. Store the Wash Buffer Kit **(WB)** and CSPB Kit at 15-25°C. These reagents are 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** (P/N: 05235898190) **HPV MMX** (**cobas®** 4800 HPV Master Mix) **HPV Mg/Mn** (**cobas®** 4800 HPV Mg/Mn Solution)	**c4800 HPV AMP/DET**	**960 Tests**
I.	**cobas® 4800 HPV Controls Kit** (P/N: 05235855190) **HPV (+) C** (**cobas®** 4800 HPV Positive Control) **(–) C** (**cobas®** 4800 System Negative Control)	**c4800 HPV CTLS**	**10 Sets**

MATERIALS REQUIRED BUT NOT PROVIDED

Specimen and Reagent Handling

- **cobas**® Sample Prep Buffer (Roche P/N 06526985190; Buffer containing a detergent)
- 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)
- For HPV ASAP v2.0.1 use **cobas®** 4800 System Extraction (deep well) Plate 1.6 mL (P/N: 05232716001)
- **cobas®** 4800 System AD (microwell) Plate and Sealing Film (P/N: 05232724001)
- Rack Sample Carrier, SMP-CAR-12-D35, PreservCyt (P/N: 05329973001)
- 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: 60.540.500) 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 2.1 or higher
- **cobas**® 4800 System **cobas**® HPV AP software version 2.0.1 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)
- Heat-resistant barcode labels (RACO Industries; Cat # RAC-225075-9501, or equivalent)
- Thermometer -20/150ºC (VWR Cat# 89095-600) or equivalent
- Digital Heater Block 120V (VWR Cat# 12621-096) or equivalent
- 12-Hole Heat Block Module 16mm (VWR Cat# 13259-162) or equivalent

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 or collected in SurePath™ Preservative Fluid using a cervical broom 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 or SurePath™ Preservative Fluid can be transported at 2-30ºC. 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. Cervical specimens collected in SurePath™ Preservative Fluid may be stored at 2-8°C for up to 6 months or at 15-30°C for up to 4 weeks after the date of collection provided that SurePath™ Preservative Fluid matrix-induced crosslinks are reversed through treatment with cobas® Sample Prep Buffer prior to HPV testing. PreservCyt and SurePath specimens should not be frozen.

SurePath specimens that have undergone the preanalytic procedure may be stored at 2-30°C for up to 4 weeks prior to HPV testing on the cobas® 4800 System.

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 and SurePath™ Preservative Fluid must be at ambient temperature before loading on the cobas x 480 instrument.***

NOTE: Refer to the appropriate cobas® 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 94 specimens plus controls (up to 96 tests per run). Each **cobas**® 4800 System Sample Preparation Kit, **cobas**® 4800 System Liquid Cytology Preparation Kit, and **cobas**® 4800 System Wash Buffer 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 Amplification/Detection Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit); multiple vials of cobas HPV MMX can be used to optimize reagent usage for 48 or 72 tests. The **cobas**® 4800 HPV Controls Kit contains reagents sufficient for a total of 10 runs (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 System Sample Preparation 960 Test Kit can be used for a 24 sample run and a HPV Amplification/Detection 960 Test Kit can be used for a 24, 48, or 72 sample run.***

The **cobas**® HPV Test can be run using either of two workflows, referred to as "Full workflow" or "Recovery" 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. Refer to the "Performing a Full Workflow" section below and the appropriate **cobas**® Operator's Manual for details.

HPV Recovery Workflow

The "HPV Recovery workflow" consists of manual PCR plate setup using eluate from the processed deep well plate followed by amplification/detection on the **cobas z** 480 analyzer. Refer to the "Performing a Recovery Workflow" section below and the appropriate **cobas**® Operator's Manual for details.

Specimens Collected into PreservCyt® Solution

NOTE: ***ThinPrep 20 mL primary containers should not be placed directly on the cobas® 4800 System for processing prior to performing cytology from the container***.

Pre-cytology PreservCyt specimens aliquoted into properly barcoded 13 mL round-based secondary tubes may be processed on the cobas x 480 instrument. PreservCyt® Solution specimens may also be tested after cytology processing on the ThinPrep T2000 or T3000 processor directly out of the 20 mL primary container with a proper barcode or out of a properly barcoded 13 mL round-based secondary tube on the **cobas x** 480 instrument. Consult the appropriate **cobas**® Operator's Manual for proper barcoding procedures and the list of acceptable barcodes for the **cobas**® 4800 System. PreservCyt primary containers in PreservCyt racks and PreservCyt specimens aliquoted into barcoded secondary tubes can be processed together in the same run. Residual PreservCyt® Solution specimens from ThinPrep processors other than the T2000 or T3000 have not been evaluated and should not be used.

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 (other than SurePath™ Preservative Fluid) . 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: ***ThinPrep 20 mL primary containers can be placed directly on the cobas 4800 system after cytology processing; the performance of the cobas® HPV Test for containers placed directly on the system prior to cytology processing has not been validated***.

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: ***The minimum volume required in the PreservCyt® Solution primary container is 3.0 mL. When using 13 mL round-based secondary tubes for PreservCyt® specimens, fill to a minimum volume of 1.0 mL and a maximum volume of 4.0 mL.***

Specimens Collected into SurePath™ Preservative Fluid

SurePath™ Preservative Fluid specimens must be transferred into a properly barcoded 13 mL round-based secondary tube and treated to reverse matrix-induced crosslinks before processing 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.

NOTE: ***It will be necessary to aliquot SurePath 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. See SurePath labeling for detailed instructions on aliquot removal.***

NOTE: ***Use only SurePath™ Preservative Fluid and a cervical broom to collect cervical specimens for the cobas® HPV Test. The cobas® HPV Test has not been validated with other collection devices or media types (other than PreservCyt® Solution). 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: ***When testing SurePath™ specimens treated to reverse matrix-induced cross-links, the secondary tube input volume is 1.0 mL (0.5 mL of*** cobas® Sample Prep Buffer and 0.5 mL of SurePath specimen***).***

NOTE: ***It is recommended to perform all specimen handling steps in a biological hood to reduce the potential for cross-contamination***

NOTE: ***Heat-resistant barcodes are required for tubes used to reverse matrix-induced cross-links (see the Instrumentation and Software section)***

Treatment of SurePath Specimens with cobas® Sample Prep Buffer to reverse matrix-induced crosslinks

A. Prepare a barcoded 13 mL round-based tube with 0.5 mL of cobas® Sample Prep Buffer for each SurePath specimen to be tested.

B. Vortex SurePath specimens for 10 seconds prior to transfer. Transfer 0.5 mL of each SurePath specimen into a 13 mL round-based tube prepared in step A. Re-cap each tube before moving to the next. Always change pipet tips for each specimen.

C. Vortex each tube for 1 second.

D. Transfer tubes to the heating unit (see Optional Equipment and Materials section). Up to 48 tubes can be processed per batch.

E. Heat to 120°C for 20 minutes.

F. After Heating, remove tubes to a collection rack and cool at ambient temperature for 10 minutes.

G. Vortex each tube for 5 seconds.

H. Transfer tubes to 24 position cobas® 4800 specimen racks, discard caps and process on the cobas® 4800 System for HPV testing.

SurePath specimens treated with cobas® Sample Prep Buffer can be stored for future HPV testing if, for example, cytology evaluation is required first. The following procedure should be followed:

A. Follow the treatment procedure above to step G.

B. Store tubes with SurePath specimens treated with cobas® Sample Prep Buffer at 2-30°C for up to 4 weeks prior to HPV testing on the cobas® 4800 System.

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

A single run can have any combination of specimens (PreservCyt® Solution and/or SurePath™ Preservative Fluid) and each specimen can be tested with either the HPV High Risk or HPV High Risk Plus Genotyping sub-tests.

Workflows

Performing a Full Workflow:

A. The **cobas®** HPV Test may be used for runs of 1 to 94 specimens plus one **cobas®** 4800 System Negative Control and one **cobas®** 4800 HPV Positive Control.

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

C. Start a new run by clicking on the "New run" button.

D. In the Select test window, select Workflow type "Full" and then select the Test "HPV".

E. Enter a run name or leave as the default run name, then click "OK" to proceed.

F. Follow the software wizard guide to load specimens.

NOTE: ***Specimens can be loaded in barcoded primary containers (PreservCyt® specimens only) or secondary tubes in any order.***

NOTE: ***If primary containers for PreservCyt® Solution specimens are used for processing, vortex each specimen thoroughly to resuspend cells immediately prior to loading.***

G. Select a Specimen type for each specimen.
- Choose "PC" for ordering a PreservCyt specimen.
- Choose "SP" for ordering a pretreated SurePath specimen

H. Select the Requested result 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.

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 sufficient reagents are loaded on the instrument.***

NOTE: ***The cobas® 4800 Software tracks 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.

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

O. Follow the instructions in the appropriate **cobas®** 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 appropriate **cobas®** Operator's Manual to review and accept results.

Performing a Recovery Workflow

NOTE: ***The Recovery 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 Recovery run. System surveillance for reagents and consumables is limited during the Recovery run. No sample position tracking is provided when using the Recovery Workflow – the end user must ensure that the actual position of a sample on the microwell plate corresponds to the one designated in the Recovery Plate Layout Report. 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 Recovery run within 24 hours if stored at 2°C to 30°C.***

A. Start a Recovery run by clicking the "New run" button.

B. In the Selection test window, select the Workflow type "Recovery" then select the Test "HPV".

C. Enter a run name or leave as the default run name, then click "OK" to proceed.

D. Select a run to recover.

E. Enter the new MWP ID.

F. Enter the Master Mix and Metal Ions IDs for all Amplification/Detection reagent vials in the kit.

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

1. For a 240 Test Kit, add 240 µL of **HPV Mg/Mn** to one vial of **HPV MMX** (0.5 mL vial from 240 Test Kit).
2. For a 960 Test Kit, 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 Recovery 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 Recovery workflow. The end user must ensure that amplification and detection is started within the allotted time.***

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

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

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

K. 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.

L. Follow the instructions in the appropriate **cobas®** Operator's Manual to seal the microwell plate.

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

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

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

P. Follow the instructions in the appropriate **cobas®** 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:

Table 1A
Result Interpretation of the cobas® HPV Test for Presence of HPV DNA

cobas® HPV Test	**Result Report and Interpretation**
Requested Result "HPV High Risk Panel":	
POS HR HPV	**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.
NEG HR HPV	**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. For PreservCyt specimens, the original specimen should be retested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath specimens, the original specimen should be retested if there is sufficient post-quot volume. If the results are still invalid a new specimen should be obtained.
Failed	**No Result for Specimen** Consult the appropriate **cobas®** Operator's Manual for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid result.
Requested Result "HPV High Risk Panel Plus Genotyping"	
POS Other HR HPV	**Other High Risk HPV Positive** 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.
NEG Other HR HPV	**Other High Risk HPV Negative*** HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid Other HR HPV	**Invalid Other High Risk HPV** The result for Other HR HPV is Invalid. For PreservCyt specimens, the original specimen should be retested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath specimens, the original specimen should be retested if there is sufficient post-quot volume. If the results are still invalid a new specimen should be obtained.
POS HPV16	**HPV16 Positive** Specimen is positive for HPV type 16 DNA.
NEG HPV16	**HPV16 Negative*** HPV type 16 DNA was undetectable or below the pre-set threshold.
Invalid HPV16	**Invalid HPV16** The result for HPV16 is Invalid. For PreservCyt specimens, the 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. For SurePath specimens the original specimen should be retested if there is sufficient post-quot volume. If the results are still invalid a new specimen should be obtained
POS HPV18	**HPV18 Positive** Specimen is positive for HPV type 18 DNA.
NEG HPV18	**HPV18 Negative*** HPV type 18 DNA was undetectable or below the pre-set threshold.
Invalid HPV18	**Invalid HPV18** The result for HPV18 is Invalid. For PreservCyt specimens, the 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. For SurePath specimens the original specimen should be retested if there is sufficient post-quot volume. If the results are still invalid a new specimen should be obtained
Failed	**No Result for Specimen** Consult the appropriate **cobas®** 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 2A
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**
NEG Other HR HPV**, NEG HPV16, NEG HPV18	Very low likelihood of underlying ≥ CIN2;	Lowest likelihood of underlying ≥ CIN2.
POS Other HR HPV**, NEG HPV16, NEG HPV18	Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy.	Low likelihood of underlying ≥ CIN2.
POS HPV16 and/or POS HPV18	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: ***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 may be 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.***

NOTE: ***In addition to the results tabulated above, invalid results for one or more combinations are also possible and are reported out specifically for each channel.***

The impact of an invalid HPV result on patient management will depend on the screening algorithm that is being used. For the ASC-US triage intended use, a positive result in any channel will refer women ≥25 years to colposcopy independent of an invalid result in any other channel; for women 21-24 years with ASC-US cytology and any HR HPV positive result, the preferred management according to the 2012 guidelines would be repeat cytology in 12 months. For women ≥30 years with a NILM cytology result, an invalid result for either HPV16 or HPV18 and a negative result for the remaining genotype would be considered invalid for management decisions based on the genotyping algorithm that sends women positive for HPV16 or HPV18 to immediate colposcopy. However, under these same circumstances, positive results for Other HR HPV would be valid for the alternate management strategy of deferral of patients with NILM cytology and any HR HPV positive result to follow up in 12 months. Invalid results when the cobas® HPV Test is used in primary screening should be interpreted for management decisions as follows:

Table 2B

cobas® HPV Test Result			**Result for management decision**
POS, NEG or Invalid Other HR HPV	POS HPV16	NEG or Invalid HPV18	Valid
POS, NEG or Invalid Other HR HPV	POS HPV18	NEG or Invalid HPV16	Valid
POS or NEG Other HR HPV	Invalid HPV16	NEG HPV18	Invalid
POS or NEG Other HR HPV	Invalid HPV18	NEG HPV16	Invalid

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 20 mL primary containers should not be placed directly on the **cobas**® 4800 System for processing prior to performing cytology from the container.
2. As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
3. Handle all specimens as if they are capable of transmitting infectious agents.
4. To reduce the risk of obtaining false negative results, all specimens collected in SurePath™ Preservative Fluid must undergo pretreatment prior to performing the **cobas**® HPV.
5. The **cobas**® HPV Test should not be used for the HPV primary screening indication for specimens collected in SurePath™ Preservative Fluid

PROCEDURAL LIMITATIONS

1. The **cobas**® 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**® 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**® HPV Test for primary screening (PreservCyt specimens only) has only been established where women who are 12 other HR HPV positive have cytology results read from the same cytology vial that was used to perform the **cobas**® HPV Test.
4. The performance of the cobas® HPV Test with ThinPrep® Pap TestTM PreservCyt® Solution has not been adequately established for HPV vaccinated individuals[35]. The performance of the cobas HPV Test with the SurePath™ Preservative has been established only for a limited population of vaccinated individuals.
5. Test only the indicated specimen type. The **cobas**® HPV Test has only been validated for use with cervical specimens collected by a clinician using an endocervical brush/spatula and placed in the ThinPrep® Pap Test™ PreservCyt® Solution or using a cervical broom and placed into the SurePath™ Preservative Fluid. The endocervical brush/spatula utilized in the performance studies was a Pap Perfect® plastic spatula and Cytobrush® plus GT gentle touch. The cervical broom utilized in the clinical performance studies was a Rovers® Cervex-Brush (Rovers Medical Devices BV).
6. Cell pellets obtained after processing on the BD PrepStain™ Slide Processor cannot be used for the **cobas**® HPV Test.
7. 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.
8.. 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.
9.. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2, CIN3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2, CIN3 or cancer.
10. A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2, CIN3 or cancer, but indicates a low likelihood of CIN2, CIN3 or cancer.
11. β-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 β-globin signal within a pre-defined range to be identified as valid negatives by the **cobas**® 4800 System. The β-globin control does not differentiate between targeted (cervical) and non-targeted nucleated cell types.
12. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Package Insert and the appropriate **cobas**® 4800 Operator's Manual.
13. 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.
14. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
15. 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.
16. 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.
17. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
18. 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.
19. The presence of PCR inhibitors may cause false negative or invalid results.
20. 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 exceed 1.5% (dark red or brown coloration) in PreservCyt® Solution or 2% in SurePath™ Preservative Fluid prior to treatment with cobas Sample Prep Buffer there is a likelihood of obtaining a false-negative result. The **cobas**® HPV Test performance has not been validated with specimens which have been treated with glacial acetic acid for removal of red blood cells. Any such processing of specimens prior to HPV testing would invalidate the **cobas**® HPV Test results.
21. 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% when alternating very high positive and negative samples were tested over multiple runs using both PreservCyt® Solution and SurePath™ Preservative Fluid. The cross-contamination study using SurePath™ Preservative Fluid also produced 11 negative samples with signal above the clinical cutoff (7.7%; 95% CI: 3.9% to 13.4%); and all of these results remained negative. In an analytical study using post-cytology PreservCyt primary vials, the percent of negative clinical specimens with Ct values increased by 16.7% (95% CI: 4.7% to 29.8%) when processed subsequent to moderate to high positive PreservCyt clinical specimens on the ThinPrep T3000 processor. All of these Ct values remained above the cutoff of the assay and the results remained negative.

    No cross-contamination was seen on the T2000 processor with PreservCyt® specimens. A 1.4% cross-contamination rate was noted with SurePath™ specimens on the BD PrepMate.

EXPECTED RESULTS FOR SPECIMENS COLLECTED IN PRESERVCYT SOLUTION

A total of 47,208 women 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%) women were eligible to participate in the study. Eligible women 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 women was 39 years, with ~25% women in age group 21-29 years, ~27% in age group 30-39 years, and ~48% women in age group ≥ 40 years. A total of 90.0% of women had NILM cytology, and 4.1% women had ASC-US cytology.

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

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

A total of 40, 944 women (Primary Screening population ≥ 25 years) were evaluable; evaluable women had valid results from cytology and the **cobas**® HPV Test.

Table 3 shows HPV prevalence by the **cobas**® HPV Test by testing site and study population. The overall HPV prevalence was 12.6% in all eligible women (≥ 21 years), 31.9% in the ASC-US (≥ 21 years) population, 6.7% in the NILM (≥ 30 years) population and 10.5% in the Primary Screening (≥ 25 years) population.

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

Testing Site	cobas® HPV Test – HPV Prevalence			
	All Eligible Women (≥ 21 Years)	**ASC-US Population (≥ 21 Years)**	**NILM Population (≥ 30 Years)**	**Primary Screening Population (≥ 25 Years)**
1	12.2% (1,578/12,966)	32.8% (165/503)	6.4% (572/8,925)	10.3% (1,163/11,332)
2	12.0% (1,020/8,500)	35.5% (99/279)	6.5% (395/6,041)	9.9% (753/7,570)
3	12.9% (834/6,456)	36.5% (74/203)	7.1% (309/4,370)	10.8% (600/5,560)
4	13.4% (1,084/8,115)	34.6% (106/306)	7.0% (387/5,539)	11.1% (783/7,082)
5	12.6% (1,336/10,564)	26.8% (168/627)	6.9% (507/7,385)	10.5% (984/9,400)
Overall	12.6% (5,854/46,601)	31.9% (612/1,918)	6.7% (2,170/32,260)	10.5% (4,283/40,944)

Table 4 shows HPV prevalence by **cobas**® HPV Test result by age and study population. HPV prevalence decreased with age in each study population. In the ASC-US population, HPV prevalence dropped from 58.2% in 21-24 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. In the primary screening population ≥ 25 years, HPV prevalence decreased from 21.1% in the 25-29 year range to 11.6% in the 30-39 year range and remained relatively constant at 5%-7% after 40 years old.

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

Age Group (Years)	ASC-US Population (≥ 21 Years)	NILM Population (≥ 30 Years)	Primary Screening Population (≥ 25 Years)
	Positive	**Positive**	**Positive**
21-24	58.2% (167/ 287)	N/A	N/A
25-29	49.6% (168/339)	N/A	21.1% (1,406/6,654)
30-39	29.7% (151/508)	9.0% (1,029/11,398)	11.6% (1,421/12,260)
40-49	15.0% (76/508)	5.7% (627/10,944)	7.1% (831/11,695)
50-59	19.3% (40/207)	5.3% (378/7,106)	6.3% (472/7,435)
60-69	17.3% (9/52)	4.9% (111/2,287)	5.3% (125/2,354)
≥ 70	5.9% (1/17)	4.8% (25/525)	5.1% (28/ 546)

The **cobas**® HPV Test results, stratified into four groups by age is presented in Table 5 for the ASC-US population (≥ 21 years), in Table 6 for the NILM population (≥ 30 years) and in Table 7 for the primary screening population (≥ 25 years). In all 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 for each category decreases with age in all three populations.

Table 5
Summary of Four-Category cobas® HPV Test Result by Age Group for Evaluable ASC-US Women (≥ 21 Years) - PreservCyt

Age Group (Years)	cobas® HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	Negative	
21–24	18.1% (52/287)	4.9% (14/287)	35.2% (101/287)	41.8% (120/287)	287
25–29	13.3% (45/339)	6.2% (21/339)	30.1% (102/339)	50.4% (171/339)	339
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 Women (≥ 30 Years) - PreservCyt

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, 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 positive.

Table 7
Summary of Four-Category cobas® HPV Test Result by Age Group for Evaluable Women (≥ 25 Years) - PreservCyt

Age Group (Years)	cobas® HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	Negative	
25–29	5.3% (355/6,654)	1.6% (109/6,654)	14.2% (942/6,654)	78.9% (5,248/6,654)	6654
30–39	2.3% (282/12,260)	1% (120/12,260)	8.3% (1019/12,260)	88.4% (10839/12,260)	12,260
40–49	1.1% (126/11,695)	0.5% (56/11,695)	5.5% (649/11,695)	92.9% (10,864/11,695)	11,695
50–59	0.8% (56/7,435)	0.5% (37/7,435)	5.1% (379/7,435)	93.7% (6,963/7,435)	7,435
60–69	0.8% (18/2,354)	0.2% (5/2,354)	4.3% (102/2,354)	94.7% (2,229/2,354)	2,354
≥ 70	0.7% (4/ 546)	0.4% (2/ 546)	4% (22/ 546)	94.9% (518/ 546)	546

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 positive.

PERFORMANCE CHARACTERISTICS WHEN TESTING SAMPLES COLLECTED IN PRESERVCYT SOLUTION

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, as an adjunctive test to cervical cytology to guide management decisions and also as a first-line primary screen for cervical cancer screening. The study consisted of a Baseline Phase, as well as a 3 year Follow-up Phase. In the Baseline Phase, women ≥ 21 years old undergoing routine cervical cancer screening were invited to participate in the study. In total, 47,208 women 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 on pre-aliquoted samples in secondary vials prior to cytology processing at five different laboratories. LBC testing was conducted at four of these five laboratories. Cytology samples were classified according to the criteria of the 2001 Bethesda System. A cervical sample 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 women with ASC-US cytology test results was tested with an FDA-approved test according to the manufacturer's instructions[36].

Those women ≥ 21 years old with ≥ ASC-US cytology were invited to undergo colposcopy. In addition, all women ≥ 25 years old with 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 women (approximately 1:35) with NILM (negative for intraepithelial lesions or malignancy) 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 (CPRP) consisting of three expert pathologists, and discordant results adjudicated according to a pre-defined protocol. For all analyses, the clinical performance of the **cobas**® HPV Test was measured against CPRP histology results. The analyses were performed for those women with histology ≥ CIN2 and ≥ CIN3 by CPR. Women

with a CPRP diagnosis of ≥ CIN2 by CPRP exited the study. All women who had undergone colposcopy and biopsy, without a diagnosis of ≥ CIN2 by CPRP were invited to proceed to the Follow-up Phase of the study.

Follow-Up Phase

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

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

Those women ≥ 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 the **cobas**® HPV Test was measured against histology results of ≥ CIN2 and ≥ CIN3 by CPRP.

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

All women ≥ 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 women (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 CPRP.

All women ≥ 30 years who were invited to colposcopy and did not have histology ≥ CIN2 by CPRP were eligible to participate in a 3 year longitudinal study for the **cobas**® HPV Test. All women with follow-up cytology ≥ ASC-US were invited to proceed to colposcopy; colposcopy and biopsies were performed in a standardized manner as describe above. All cervical biopsies were examined by the CPRP and all women with ≥ CIN2 exited the study. Exit colposcopy and ECC were offered to all women. The objectives of the follow-up phase of the study were to determine the 3-year risk (cumulative incidence rates, CIRs) of developing ≥ CIN2 and ≥ CIN3 in women ≥ 30 years with NILM cytology. Risk was 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 was determined by CPRP.

STUDY DESIGN TO DEMONSTRATE CLINICAL PERFORMANCE OF THE COBAS® HPV TEST COLL ECTED IN PRESERVCYT AS A FIRST-LINE PRIMARY TEST FOR CERVICAL CANCER SCREENING

Baseline and Follow-Up data from the ATHENA study were evaluated for all evaluable women 25 years and older as described above. The clinical performance of the primary screening indication for the **cobas**® HPV Test was measured against histology results of ≥ CIN2 and ≥ CIN3 by CPRP and compared to the performance of cytology alone.

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

Of 1,918 evaluable women in the ASC-US population, 1,610 completed colposcopy procedures. The results of the **cobas**® HPV Test reported as (HR HPV) Positive or (HR HPV) Negative together with the CPRP diagnosis are presented in Table 8. In a total of 1,578 ASC-US women with valid CPR panel diagnoses, 80 women had a ≥ CIN2 result (prevalence of ~5.1%), and 46 women had a ≥ CIN3 result (prevalence of ~2.9%).

Table 8
Results of the cobas® HPV Test and Central Pathology Review Panel Diagnosis in the ASC-US Population (≥ 21 Years) - PreservCyt

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	1,342	158	34	46	1,612

Note: The 32 Undetermined CPRP 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 result was 0.12% (2/1612) with 95% CI: 0.03% to 0.45%.

The 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 women with ≥ CIN2 than in women 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 women with < CIN2 than in women 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 9
Performance of the cobas® HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population (≥ 21 Years) - PreservCyt

Performance	**CPRP Panel Diagnosis ≥ CIN2**		**CPRP 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 10.

The sensitivity for detecting the ≥ 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 10
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 – PreservCyt

Performance	cobas® HPV Test in PreservCyt		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 women 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 women with a < CIN2 diagnosis could not be determined by the FDA approved HPV Test due to insufficient volume resulting from repeated testing.

The performance of the **cobas**® HPV Test for detecting ≥ CIN2 and ≥ CIN3 evaluated by age group is presented in Table 11. 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 for 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%).

Performance of the FDA approved HPV test in detecting ≥ CIN2 and ≥ CIN3 by age group is presented in Table 12.

Table 11
Performance of the cobas® HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population by Age Group – PreservCyt

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)

Table 12
Performance of an FDA Approved HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population by Age Group – PreservCyt

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 Within Samples Collected in PreservCyt – 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 HPV16 positive/18 positive, 12 Other HR, and HR HPV negative are presented in Table 13 for the ASC-US (≥21 years) population.

For ≥ 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 occur in a subject with disease (≥ CIN2) than in 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 occur in a subject without disease (< CIN2) than in 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 13). 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 13
Likelihood Ratios and Risk of Disease by cobas® HPV Test Result in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population – PreservCyt

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
≥ CIN2	HPV16 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	HPV16 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 in Samples Collected in PreservCyt – Absolute and Relative Risk Estimates

The CPRP diagnosis by all possible **cobas**® HPV Test result in ASCUS population is presented in Table 14.

Table 14
Summary of cobas® HPV Test Result and Central Pathology Review Panel Diagnosis in the ASC-US Population (≥ 21 years) – PreservCyt

	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

Note 1: Undetermined results include inadequate biopsy sample for diagnosis and sample collected outside the study visit window.
Note 2: None of the women in the ASC-US population had a CPRP diagnosis > CIN3.

The CPRP diagnosis and the absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas®** HPV Test result are presented in Table 15. HPV16 positive/18 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3. In general, the absolute risks for both ≥ CIN2 and ≥ CIN3 were higher in women with results of HPV positive, HPV16 positive/18 positive, or 12 Other HR positive than in women with an HPV negative result.

Table 15
Central Pathology Review Diagnosis and Absolute Risk of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the ASC-US Population (≥ 21 Years) – PreservCyt

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)

Note 1: 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 women 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 women with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18.

The relative risks (RRs) of disease (≥ CIN2 and ≥ CIN3) were calculated for women with different **cobas®** HPV Test results by RR and its associated 95% CIs as presented in Table 16. The estimated RRs of ≥ CIN2 and of ≥ CIN3 for women with positive vs. negative **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 women with a positive result were 18.6 times more likely to have ≥ CIN2 histology and 29.7 times more likely to have ≥ CIN3 histology than were women with a negative test result.

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

Table 16
Relative Risks of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the ASC-US Population (≥ 21 Years) – PreservCyt

cobas® HPV Test Result	**CPRP Diagnosis ≥ CIN2**		**CPRP 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%	

Note 1: HPV16 positive and/ or HPV18 positive include all women 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 women 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 women with different **cobas®** HPV Test results among different age groups and are presented in Table 17. The RRs of all comparisons were significantly greater than 1 for ≥ CIN2 histology, except for HPV16 positive /18 positive vs. 12 Other HR HPV positive in ≥ 40 years.

Table 17
Relative Risks of ≥ CIN2 and ≥ CIN3 by cobas® HPV Test Result Stratified by Age in the ASC-US Population – PreservCyt

cobas® HPV Test Result	Age Group (Years)		
	21-29	30-39	≥ 40
Relative Risk for ≥ CIN2 (95% CI)			
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 (95% CI)			
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 women 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 women with positive results for 12 other HR genotypes with negative results for HPV16 and HPV18.

NILM (≥ 30 Years) Population Within Samples Collected in PreservCyt

The risks of disease in the NILM (≥ 30 years) population were compared in women with a positive **cobas**® HPV Test result to those with a negative **cobas**® HPV Test result. In this population, all women with a positive result from the IUO HPV HR test or IUO HPV genotyping test were selected to proceed to colposcopy, as well as a random subset of women (1 of 35) with a negative result from both IUO HPV tests. 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 women in a given subgroup had undergone colposcopy.

The CPRP diagnosis by all possible **cobas**® HPV Test result in the NILM (≥ 30 years) population is presented in Table 18.

Table 18
Summary of cobas® HPV Test Result and Central Pathology Review Panel Diagnosis in the NILM Population (≥ 30 years) – PreservCyt

	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.
Note 2: Of the 80 ≥ CIN3 women, 75 are CIN3 and 5 are ACIS.

The CPRP diagnosis and the crude estimate of absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas**® HPV Test result are presented in Table 19. 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 women with any results of HPV positive than in women with an HPV negative result.

Table 19
Central Pathology Review Diagnosis and Different cobas® HPV Test Results in the NILM Population (≥ 30 Years) - PreservCyt

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)

Note 1: 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 women 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 women with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18.

The women in various subgroups are classified as shown in Table 20. The combined results of the two IUO HPV Tests 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 20
Classification of Evaluable NILM Women (≥ 30 Years) by cobas® HPV Test Result, Disease Status (≥ CIN2 and ≥ CIN3), and Disease Verification Status - PreservCyt

cobas® HPV Test Result	Combined Results From Two IUO HPV Test	Total No. Women	Verified Disease Status: ≥ CIN2		Verified Disease Status: ≥ CIN3		No. Women with Unknown Disease Status (Unverified)
			No. Diseased Women (≥ CIN2)	No. Non-Diseased Women (< CIN2)	No. Diseased Women (≥ CIN3)	No. Non-Diseased Women (< 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 in Samples Collected in PreservCyt – 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 21 for unadjusted results and Table 22 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 sensitivities 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 specificities 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 21
Performance of cobas® HPV Test In the NILM (≥ 30 years) Population (Unadjusted Estimates) - PreservCyt

Disease Endpoint	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 22
Performance of cobas® HPV Test In the NILM (≥ 30 years) Population (Verification Bias Adjusted Estimates)

Disease Endpoint	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 in Samples Collected in PreservCyt – Likelihood Ratios and Risk Estimates

Unadjusted estimates of likelihood ratios along with 95% CIs for HR HPV16 positive /18 positive, 12 Other HR, and HR HPV negative for the NILM (≥ 30 years) population are presented in Table 23. The risks of ≥ CIN2 and ≥ CIN3 were 11.7% (45/385) and 9.9% (38/385), respectively for a NILM subject with HPV16 positive /18 positive. The risks of ≥ CIN2 and ≥ CIN3 were 0.9% (22/2,514) and 0.3% (8/2,514) for a NILM subject with HPV negative, respectively.

Table 23
Likelihood Ratios by cobas® HPV Test Result in Detecting ≥ CIN2 and ≥ CIN3 in the NILM Population (Unadjusted Estimates) – PreservCyt

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥ CIN2	HPV16 positive /18 positive	**4.2** {(45/131)/(340/4,121)} (3.2, 5.4)
	12 Other HR HPV positive	**1.6** {(64/131)/(1,295/4,121)} (1.3, 1.9)
	HPV Negative	**0.3** {(22/131)/(2,492/4,121)} (0.2, 0.4)
≥ CIN3	HPV16 positive /18 positive	**5.7** {(38/80)/347/4,178} (4.4, 7.3)
	12 Other HR HPV positive	**1.3** {(34/80)/1,325/4,178} (1.0, 1.7)
	HPV Negative	**0.2** {(8/80)/2,506/4,178} (0.1, 0.4)

Verification bias adjusted estimates of likelihood ratios along with 95% CIs for HR HPV16 positive /18 positive, 12 Other HR, and HR HPV negative for the NILM (≥30 years) population are presented in Table 24.

Table 24
Likelihood Ratios by cobas® HPV Test Result in Detecting ≥ CIN2 and ≥ CIN3 in the NILM Population (Verification-Bias Adjusted Estimates) – PreservCyt

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥ CIN2	HPV16 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	HPV16 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 in Samples Collected in PreservCyt – Absolute and Relative Risk Estimates

Estimates of absolute risks of ≥CIN2 and ≥CIN3 for **cobas®** HPV Test results are presented in Table 25. The estimates were calculated with and without adjusting for verification bias. The risks of ≥ CIN2 and ≥ CIN3 were 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 HPV16 positive /18 positive. The risks of ≥ CIN2 and ≥ CIN3 were 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 25
Absolute Risk of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the NILM Population (≥ 30 Years) – PreservCyt

cobas® HPV Test Result	≥ CIN2	≥ CIN3
Unadjusted Estimates		
HPV positive	6.3% (5.2, 7.5)	4.1% (3.3, 5.2)
HPV16 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)
HPV16 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: HPV16 positive and/or 18 positive include all women 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 women 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 26. The risk of disease decreased with age for **cobas®** HPV Test results of HPV16 positive/18 positive and for 12 Other HR HPV positive results. The risk of disease with a **cobas®** HPV Test negative result remained similar for the 30-39 and ≥ 40 years age groups.

Table 26
Absolute Risk Estimates in the NILM (≥ 30 Years) Population by cobas® HPV Test Result and Age – PreservCyt

Age Group	cobas® HPV Test Result	≥ CIN2	≥ CIN3
30-39 Years	**Unadjusted Estimates**		
	HPV16 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		
	HPV16 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**		
	HPV16 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		
	HPV16 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 women with different **cobas**® HPV Test results and are presented in Table 27. Women with positive **cobas**® HPV Test results were 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 women with a negative **cobas**® HPV Test result. The risks of disease (both ≥ CIN2 and ≥ CIN3) were significantly higher in women with a positive compared with women with a negative HPV test result.

The risks of disease (≥ CIN2 and ≥ CIN3) were significantly higher in women who were HPV16 and/or 18 positive than women with (a) a negative result , or (b) a positive result for 12 Other HR HPV types.

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 27
Relative Risks of ≥ CIN2 and ≥ CIN3 for Different the cobas® HPV Test Results in the NILM Population (≥ 30 Years) – PreservCyt

cobas® HPV Test Result	CPRP Diagnosis ≥ CIN2		CPRP 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: HPV16 positive and/ or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18.

Current and Future Risk of Disease in the NILM (≥ 30 Years) Population in Samples Collected in PreservCyt

Among the 4,291 NILM women who were eligible for the Follow-Up phase, a total of 3,542 women completed Year 1 Pap visits, 3,086 completed Year 2 Pap visits, and 2,810 completed Year 3 Pap visits.

Risks and 3-Year Cumulative Incidence Risks of High-Grade Cervical Disease

The current risk at Baseline and current + cumulative risks (both crude and VBA estimates) at follow-up Year 3 for high-grade disease (≥ CIN2 and ≥ CIN3) were calculated in the NILM population (≥ 30 years) among women with different baseline **cobas**® HPV Test results (Table 28). The data show that 4.0% of women were found to have ≥ CIN3 at Baseline and a total of 5.0% women would be diagnosed with ≥ CIN3 in a 3-year period if the Baseline **cobas**® HPV Test results were positive. By comparison, if the **cobas**® HPV Test results were negative, only 0.28% of women would have ≥ CIN3 at baseline, and a total of 0.31% women would have ≥ CIN3 detected in a 3-year period. The current risks at the baseline for HPV16 positive/HPV18 positive, 12 Other HR HPV positive and HR HPV negative women were 11.2%, 4.6% and 0.83% for ≥ CIN2 and 9.6%, 2.4% and 0.28% for ≥ CIN3 respectively. The sum of the current and cumulative risks at follow-up year 3 for HPV16 positive/HPV18 positive , 12 Other HR HPV positive and HR HPV negative women were 16.0%, 7.0% and 0.89% for ≥ CIN2 and 11.9%, 3.0% and 0.31% for ≥ CIN3 respectively.

Table 28
Current Risk and Current + Future Risk Based on Various cobas® HPV Test Results in the NILM (≥ 30 Years) Population – PreservCyt

Disease Endpoint	Baseline cobas® HPV Test Result	Crude		VBA	
		Current Risk, (%) (95% CI)	Current+Future Risk (%) at Year 3 (95% CI)	Current Risk, (%) (95% CI)	Current+Future Risk (%) at Year 3 (95% CI)
≥ CIN2	HPV+	6.25 (5.21, 7.49)	9.31 (7.96, 10.86)	6.04 (4.90, 7.14)	8.99 (7.69, 10.49)
	HPV16+/18+	11.69 (8.85, 15.28)	16.77 (13.19, 21.09)	11.23 (8.24, 14.52)	16.01 (12.38, 20.17)
	HPV16+	13.85 (10.17, 18.57)	20.29 (15.61, 25.95)	13.27 (9.42, 17.84)	19.44 (14.58, 25.08)
	HPV18+	7.20 (3.83, 13.12)	9.15 (5.13, 15.82)	7.01 (2.86, 11.61)	8.92 (4.23, 14.75)
	12 Other HR HPV+	4.71 (3.71, 5.97)	7.20 (5.86, 8.80)	4.56 (3.48, 5.72)	6.99 (5.63, 8.47)
	HPV-	0.88 (0.58, 1.32)	1.46 (1.04, 2.06)	0.83 (0.30, 1.48)	0.89 (0.35, 1.54)
≥ CIN3	HPV+	4.13 (3.29, 5.17)	5.14 (4.17, 6.33)	4.01 (3.07, 4.94)	4.98 (3.96, 6.04)
	HPV16+/18+	9.87 (7.28, 13.26)	12.43 (9.37, 16.32)	9.56 (6.77, 12.50)	11.85 (8.74, 15.47)
	HPV16+	11.92 (8.53, 16.43)	15.62 (11.52, 20.85)	11.42 (7.80, 15.48)	14.82 (10.64, 20.22)
	HPV18+	5.60 (2.74, 11.11)	5.60 (2.69, 11.28)	5.73 (1.47, 9.84)	5.73 (1.47, 9.84)
	12 Other HR HPV+	2.50 (1.80, 3.48)	3.09 (2.27, 4.20)	2.43 (1.58, 3.34)	3.02 (2.08, 4.00)
	HPV-	0.32 (0.16, 0.63)	0.53 (0.30, 0.95)	0.28 (0.02, 0.68)	0.31 (0.03, 0.71)

Current Risk = Absolute Risk at baseline; Current + Future Risk at Year 3 = Cumulative Risk from baseline to follow up year 3; VBA = Verification Bias Adjusted.

Agreement with a Composite Comparator for the ASC-US ≥ 21 Years and, NILM ≥ 30 Years Populations Within Samples Collected in PreservCyt

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 β-globin and PGMY primers. The β-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 women 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 29) or genotype-specific HPV DNA sequencing results (Tables 30, 31 and 32). 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 β-globin amplification produced null result during sequencing. All women tested for analytical accuracy had valid **cobas**® HPV Test results.

Table 29
Percent Agreement of the cobas® HPV Test vs. the Composite Comparator – PreservCyt

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: women with indeterminate results were excluded from percent agreement calculation

Table 30
Percent Agreement of the cobas® HPV Test HPV16 Result vs. the HPV16 Sequencing Comparator – PreservCyt

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: women with invalid results were excluded from percent agreement calculation

Table 31
Percent Agreement of the cobas® HPV Test HPV18 Result vs. the HPV18 Sequencing Comparator – PreservCyt

Population	cobas® HPV Test: HPV18 Result	HPV18 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: women with invalid results were excluded from percent agreement calculation

Table 32
Percent Agreement of cobas® HPV Test 12 Other HR HPV Result vs. the 12 Other HR HPV Sequencing Comparator – PreservCyt

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: women with invalid results were excluded from percent agreement calculation

Comparison of Results From the **cobas®** HPV Test for Primary vs. Secondary Vials of Clinical Samples Collected in PreservCyt

Results of the **cobas®** HPV Test were compared using samples from primary vials vs. pre-cytology aliquots from secondary vials. Testing was done after processing primary vials on the ThinPrep 2000 processor (T2000) and the ThinPrep 3000 processor (T3000), For the T2000 study, a total of 1,256 archived samples from a subset of women enrolled in the baseline phase of the ATHENA study whose cytology had been tested with the T2000 System were randomly selected to be tested in the primary vial. The selection of 1,100 samples reflected the screening population of all women ≥ 25 years of age in the baseline phase of the ATHENA study. An extra 156 women from women with an ASC-US cytology result were added to obtain a larger sample size for the ASC-US ≥ 21 year sub-population. The samples were tested on 3 separate **cobas®** 4800 instruments. Comparisons of results between the primary vial and secondary vial along with the estimates of agreement are shown in Table 33.

Table 33
Comparison of cobas® HPV Test Results from the Primary Vial and Secondary Vial: T2000 Study – PreservCyt

Population	Positive Percent Agreement (%) 95% CI	Negative Percent Agreement (%) 95% CI
ASC-US Population (≥ 21 Years)	95.5 (63/66) (87.5, 98.4)	96.3 (129/132) (91.6, 98.4)
NILM Population (≥ 30 Years)	86.2 (50/58) (75.1, 92.8)	99.8 (805/807) (99.1, 99.9)

For the T3000 study, a total of 352 archived samples from women enrolled in the baseline phase of the ATHENA study whose cytology was processed on the T3000 System were selected to be tested in the primary vial. Additionally, a random sample of 748 samples selected from 1,500 freshly collected samples was tested in the primary vial. The combined archived and freshly collected samples had distributions of subject age, cytology, and secondary vial HPV results similar to those in the baseline phase of the ATHENA study. All available extra samples with an ASC-US cytology result (n=52) that had either been archived (n=21) or were fresh (n=31) were added to obtain a larger sample size for the ASC-US ≥ 21 year sub-population. The samples were tested on 3 separate **cobas®** 4800 instruments. Comparisons of results between the primary vial and secondary vial along with the estimates of agreement are shown in Table 34.

Table 34
Comparison of cobas® HPV Test Results from the Primary Vial and Secondary Vial: T3000 Study – PreservCyt

Population	Positive Percent Agreement (%) 95% CI	Negative Percent Agreement (%) 95% CI
ASC-US Population (≥ 21 Years)	95.6 (43/45) (85.2, 98.8)	91.5 (43/47) (80.1, 96.6)
NILM Population (≥ 30 Years)	84.2 (48/57) (72.6, 91.5)	99.1 (770/777) (98.2, 99.6)

A systematic, but small decrease of signal was observed between results from the primary vials vs results from secondary vials for samples that had been processed on the ThinPrep processors. The clinical significance of this systematic difference was investigated for the NILM ≥ 30 population given that the positive percent agreement in this population was less than 95%. For this, the clinical data of the secondary vials from the NILM ≥ 30 study for the **cobas**® HPV Test were evaluated considering the same change in signal (for all women ≥ CIN2 and for all women without ≥ CIN2); then the changes in sensitivity and specificity were calculated for this scenario and the effects on positive and negative predictive values were evaluated. It was determined that the NPV decreased by 0.1%.

For the NILM population (≥ 30 years), a combined comparator of the secondary vial results and Sanger sequencing was used for comparisons of results to the primary vial; the estimates of agreement are shown in Table 35.

Table 35
Agreement of Comparator vs. cobas® HPV Test with Primary Vial in the NILM (≥30) Population – PreservCyt

Pap Processor	Primary Vial	Comparator (HPV Positive/Negative)				Primary Vial	Comparator (HPV16/18 Positive/Negative)			
		Positive	Negative	Indeterminate	Total		16/18 Positive	16/18 Negative	Indeterminate	Total
T2000	Positive	34	2	16	52	16/18 Positive	7	0	4	11
	Negative	2	805	6	813	16/18 Negative	0	854	0	854
	Total	36	807	22	865	Total	7	854	4	865
	PPA(%)	94.4 (81.9, 98.5)				PPA(%)	100 (64.6, 100)			
	NPA(%)	99.8 (99.1, 99.9)				NPA(%)	100 (99.6, 100)			
T3000	Positive	38	7	10	55	16/18 Positive	10	2	2	14
	Negative	1	770	8	779	16/18 Negative	0	818	2	820
	Total	39	777	18	834	Total	10	820	4	834
	PPA(%)	97.4 (86.8, 99.5)				PPA(%)	100 (72.2, 100)			
	NPA(%)	99.1 (98.2, 99.6)				NPA(%)	99.8 (99.1, 99.9)			

Primary Screening Population in Samples Collected in PreservCyt (≥ 25 years)

Description of the Primary Screening (≥ 25 Years) Population

Among the 47,208 women enrolled in the study, a total of 40,944 were evaluable for the analysis of the primary screening population. To be evaluable, the women must have been eligible for study enrollment at Baseline, have been 25 years or older with a valid **cobas**® HPV Test result, and a valid cytology result. The percent of Invalid **cobas**® HPV Test results was 0.43% (181/41,864) with 95% CI: 0.37% to 0.50%.

The median age of evaluable women in the primary screening population was 41 years with ~16% women in the age group 25-29 years and ~30% in the age group 30-39 years; the remaining ~54% women were ≥ 40 years. Approximately 83% of women were White and most (98%) had a high school or above education. Approximately 91% of women had cytology performed in the previous 5 years, and ~93% did not have a colposcopy in the previous 5 years. About 20% of women had an HPV test in the previous 5 years and among them ~18% were HPV positive.

A total of 8,073 women (3,612 positive and 4,461 negative by the **cobas**® HPV Test) proceeded to colposcopy. Diagnosis of ≥ CIN2 (by CPRP) was observed in 431(5.5%) of 7,829 women with valid CPRP results at colposcopy. A total of 7,642 women were eligible for the Follow-Up phase. A total of 6,168 women completed the Follow-Up Year 1 visit, 5,203 women completed the Follow-Up Year 2 visit, and 4,666 completed the Follow-Up Year 3 visit.

The number of patients with colposcopy results for each combination of **cobas**® HPV Test and cytology results is shown in Table 36. A correction for verification bias was applied due to the different rate of colposcopy in each category. Cases of disease were imputed for the women who did not have colposcopy data from the women who did go to colposcopy in each category based on their IUO HPV Test results, cytology results and their age.

Table 36
Number of Patients with Colposcopy Results by cobas® HPV Test and Cytology Results – PreservCyt

cobas® HPV Test	Cytology			Total
	> ASC-US	ASC-US	NILM	
HPV 16/18 Pos	250 Colpo: 216	139 Colpo: 121	781 Colpo: 630	1,170
12 Other HR HPV Pos	414 Colpo: 348	306 Colpo: 255	2,393 Colpo: 1,934	3113
HR HPV Neg	322 Colpo: 279	1,187 Colpo: 968	35,152 Colpo: 3,078	36,661
Total	986	1,632	38,326	**40,944**

Screening Algorithms

The use of the **cobas**® HPV Test as a first line screening method was evaluated by comparing the Primary Screening algorithm with the Cytology algorithm, shown in Figures 1 and 2, respectively.

Figure 1
Primary Screening Algorithm

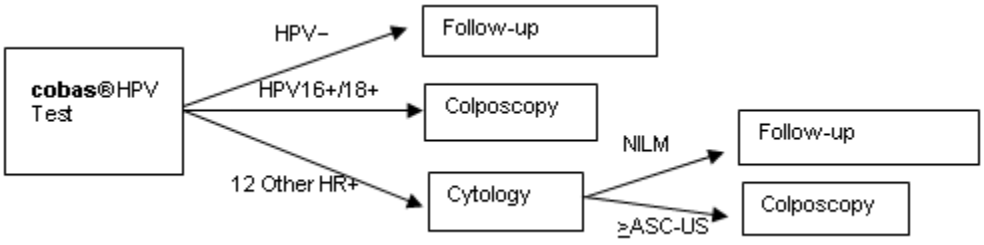
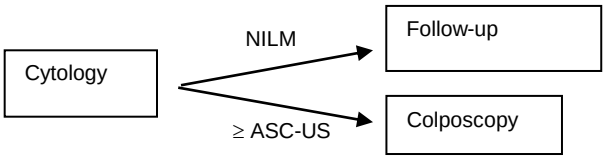


Figure 2
Cytology Algorithm



Performance Evaluation of the Primary Screening Algorithm in the Primary Screening (≥ 25 Years) Population

Performance of the Primary Screening algorithm (HPV 16/18 Genotyping with reflex to Cytology) and the Cytology algorithm (Cytology alone) was evaluated and compared in the primary screening population by estimating the sensitivity, specificity, PLR, NLR, prevalence, PPV, and NPV in the identification of high-grade cervical disease; results are presented in Table 37 for ≥ CIN2 and Table 38 for ≥ CIN3.

The performance of the Primary Screening algorithm was significantly better than the Cytology algorithm for both ≥ CIN2 and ≥ CIN3 endpoints in that the Primary Screening algorithm had significantly higher sensitivity, PPV and PLR, and also significantly lower (1-specificity), (1-NPV) and NLR compared with the Cytology algorithm. Also, the Primary Screening algorithm required 1.77% fewer colposcopies (Pos %) compared to the Cytology algorithm (Tables 37 and 38).

Table 37
Performance Comparison of the Primary Screening Algorithm and Cytology Algorithm (≥ CIN2) - PreservCyt

Algorithm	Prevalence of ≥ CIN2 =1.79 with 95% CI (1.37, 2.25)						
	Pos (%)	PPV (%)	1-NPV (%)	Sensitivity (%)	1-Spec (%)	PLR	NLR
Primary Screening	4.62	17.62	1.03	45.41	3.87	11.73	0.57
95% CI	(4.42, 4.82)	(15.80, 19.54)	(0.60, 1.49)	(35.81, 59.65)	(3.68, 4.06)	(9.15, 15.43)	(0.42, 0.67)
Cytology	6.39	9.89	1.24	35.31	5.87	6.02	0.69
95% CI	(6.16, 6.62)	(8.68, 11.20)	(0.81, 1.72)	(27.60, 46.74)	(5.64, 6.09)	(4.66, 8.01)	(0.57, 0.77)
Difference	-1.77	7.73	-0.21	10.1	-2	5.71	-0.12
95% CI	(-2.01, -1.55)	(6.51, 8.93)	(-0.27,-0.15)	(6.57, 14.45)	(-2.22,-1.77)	(4.31, 7.66)	(-0.16,-0.08)
Statistically Significant.	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 38
Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm (≥ CIN3) - PreservCyt

Algorithm	Prevalence ≥ CIN3 =0.97 with 95% CI (0.74, 1.28)						
	Pos (%)	PPV (%)	1-NPV (%)	Sensitivity (%)	1-Spec (%)	PLR	NLR
Primary Screening	4.62	12.25	0.42	58.26	4.09	14.24	0.44
95% CI	(4.42, 4.82)	(10.69, 13.91)	(0.20, 0.74)	(44.02, 74.37)	(3.89, 4.28)	(10.77, 18.29)	(0.27, 0.58)
Cytology	6.39	6.47	0.59	42.63	6.04	7.06	0.61
95% CI	(6.16, 6.62)	(5.54, 7.50)	(0.36, 0.92)	(31.75, 55.41)	(5.81, 6.27)	(5.24, 9.26)	(0.47, 0.73)
Difference	-1.77	5.78	-0.17	15.63	-1.95	7.18	-0.17
95% CI	(-2.01, -1.55)	(4.72, 6.94)	(-0.23, -0.12)	(10.28, 22.16)	(-2.18, -1.71)	(5.34, 9.40)	(-0.24, -0.12)
Stat Sign.	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Performance Evaluation by Age Group for the Primary Screening Algorithm in Women ≥ 25 Years

The performance comparisons of the HPV Primary Screening Algorithm and Cytology algorithm by age group for ≥ CIN3 endpoint are shown in Tables 39 to 42. The percent of women referred to colposcopy is significantly higher in the 25-29 age group for the HPV Primary Screening Algorithm but significantly lower in all other age groups. Also of note, the prevalence of ≥ CIN3 (1.53%) is higher in the 25-29 age group than in any other age group. Both the PPV and PLR of the HPV Primary Screening Algorithm are significantly higher than the Cytology algorithm for all age groups. The point estimate of sensitivity, (1-NPV) and NLR all indicate superior performance of the HPV Primary Screening Algorithm over the Cytology algorithm for all 4 age groups, but the difference is not statistically significant for the age groups 40-49 and 50 and older. The estimate of (1-specificity) is significantly lower for all age groups ≥ 30.

Table 39
Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm in age group 25-29 (≥ CIN3) - PreservCyt

Algorithm	Prevalence ≥ CIN3 =1.53 with 95% CI (1.22, 1.84)						
	%Pos	PPV	1-NPV	Sensitivity	1-Spec	PLR	NLR
Primary Screening	10.58	10.42	0.48	71.88	9.63	7.47	0.31
95% CI	(9.84, 11.31)	(8.02, 13.06)	(0.30, 0.67)	(62.04, 81.44)	(8.92, 10.34)	(6.37, 8.66)	(0.20, 0.42)
Cytology	9.80	6.77	0.96	43.29	9.28	4.67	0.63
95% CI	(9.11, 10.51)	(4.81, 8.93)	(0.69, 1.23)	(33.50, 54.31)	(8.55, 10.03)	(3.57, 5.93)	(0.50, 0.73)
Difference	0.78	3.65	-0.48	28.59	0.35	2.80	-0.32
95% CI	(0.03, 1.47)	(1.87, 5.45)	(-0.69, -0.28)	(17.41, 38.77)	(-0.39, 1.01)	(1.55, 4.10)	(-0.43,-0.19)
Statistical Significant?	Yes	Yes	Yes	Yes	No	Yes	Yes

Table 40

Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm in age group 30–39 (≥ CIN3) – PreservCyt

Algorithm	Prevalence=1.09 with 95% CI (0.89, 1.28)						
	%Pos	PPV	1-NPV	Sensitivity	1-Spec	PLR	NLR
Primary Screening	5.37	15.14	0.29	74.86	4.60	16.26	0.26
95% CI	(4.98, 5.77)	(12.26, 17.98)	(0.20, 0.40)	(66.54, 81.75)	(4.23, 5.00)	(14.06, 18.52)	(0.19, 0.35)
Cytology	6.92	8.36	0.54	53.33	6.42	8.31	0.50
95% CI	(6.48, 7.37)	(6.43, 10.39)	(0.41, 0.70)	(43.98, 62.11)	(5.99, 6.85)	(6.82, 9.91)	(0.40, 0.60)
Difference	-1.55	6.78	-0.25	21.53	-1.82	7.95	-0.24
95% CI	(-1.98, -1.10)	(4.68, 8.74)	(-0.37, -0.14)	(11.99, 31.14)	(-2.23, -1.36)	(5.77, 10.13)	(-0.34, -0.13)
Statistical Significant?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 41

Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm in age group 40–49 (≥ CIN3) – PreservCyt

	Prevalence=0.83 with 95% CI (0.40, 1.53)						
Algorithm	**%Pos**	**PPV**	**1-NPV**	**Sensitivity**	**1-Spec**	**PLR**	**NLR**
Primary Screening	2.78	12.58	0.50	41.98	2.45	17.14	0.59
95% CI	(2.50, 3.09)	(8.54, 16.62)	(0.11, 1.22)	(20.51, 77.96)	(2.19, 2.75)	(8.41, 32.49)	(0.23, 0.81)
Cytology	6.22	5.05	0.55	37.72	5.95	6.34	0.66
95% CI	(5.80, 6.67)	(3.36, 6.83)	(0.14, 1.29)	(18.61, 71.57)	(5.52, 6.41)	(3.09, 12.11)	(0.30, 0.87)
Difference	-3.44	7.53	-0.05	4.26	-3.50	10.80	-0.07
95% CI	(-3.87, -3.01)	(4.73, 10.43)	(-0.13, 0.01)	(-3.52, 15.69)	(-3.94, -3.08)	(5.10, 21.88)	(-0.18, 0.02)
Statistical Significant?	Yes	Yes	No	No	Yes	Yes	No

Table 42

Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm in age group ≥ 50 years (≥ CIN3) – PreservCyt

	Prevalence=0.63 with 95% CI (0.18, 1.51)						
Algorithm	**%Pos**	**PPV**	**1-NPV**	**Sensitivity**	**1-Spec**	**PLR**	**NLR**
Primary Screening	1.96	8.72	0.47	27.26	1.80	15.11	0.74
95% CI	(1.71, 2.23)	(4.68, 13.08)	(0.04, 1.34)	(9.39, 83.22)	(1.56, 2.07)	(5.15, 47.43)	(0.17, 0.92)
Cytology	3.77	4.50	0.48	27.04	3.63	7.46	0.76
95% CI	(3.42, 4.16)	(2.40, 6.85)	(0.05, 1.37)	(9.29, 80.44)	(3.28, 4.01)	(2.54, 22.81)	(0.20, 0.94)
Difference	-1.81	4.22	-0.01	0.22	-1.83	7.65	-0.02
95% CI	(-2.18, -1.45)	(1.66, 7.17)	(-0.07, 0.04)	(-13.95, 15.21)	(-2.19, -1.47)	(2.05, 27.67)	(-0.17, 0.14)
Statistical Significant?	Yes	Yes	No	No	Yes	Yes	No

Baseline Risks of High-Grade Cervical Disease for the Primary Screening Algorithm

Women with HPV16/18+ and 12 Other HR HPV+ with ≥ ASC-US cytology account for 2.86% and 1.76%, respectively, of the primary screening population ≥ 25 years (Table 43) and will be referred for immediate colposcopy by the Primary Screening Algorithm. The risks of ≥ CIN2 were 19.8% (95%CI, 17.4-22.4) for HPV16/18+ and 14.2% (95% CI, 11.4-17.1) for 12 Other HR HPV+ with ≥ ASC-US cytology. These high risk estimates justify referral of these women for colposcopy. Women with12 Other HR HPV+ and NILM cytology account for 5.84% and had a risk of ≥ CIN2 of 4.9%. The majority of women (89.6%) were HPV- and had a risk of 0.77% for ≥ CIN2.

Table 43

The Risk of Disease in Each Category Related to the Primary Screening Algorithm (≥ 25 Years) – PreservCyt

Category	Proportion of women with results (%)	Risk of ≥ CIN3 (%) (95% CI)	Risk of ≥ CIN2 (%) (95% CI)
HPV 16/18 +	**2.86**	**15.0** (13.0, 17.4)	**19.8** (17.4, 22.4)
12 Other HR HPV + and ≥ ASC-US cytology	**1.76**	**7.8** (5.6, 10.2)	**14.2** (11.4, 17.1)
12 Other HR HPV + and NILM cytology	**5.84**	**2.8** (2.1, 3.5)	**4.9** (3.9, 5.9)
HR HPV -	**89.54**	**0.27** (0.05, 0.60)	**0.77** (0.33, 1.29)

Baseline Risks of High-Grade Cervical Disease by Age Group for the Primary Screening Algorithm

The risks of high-grade cervical disease by age group for the Primary Screening Algorithm are presented in Table 44. The risks of ≥ CIN2 are all above 10% in each age group for women with HPV16/18+ and women with 12 Other HR HPV + and ≥ ASC-US cytology. The risk of ≥ CIN3 is below 0.45% in each age group for women with a negative HPV test result.

Table 44
The Risk of Disease in Each Category Related to the Primary Screening Algorithm by Age Groups – PreservCyt

Age Group	Category	Percent of patients with results (%)	Risk of ≥ CIN3 (%) (95% CI)	Risk of ≥ CIN2 (%) (95% CI)
25–29 Years	HPV 16/18 +	**6.97**	**12.7** (9.65, 16.1)	**19.4** (15.7, 23.6)
	12 Other HR HPV + and ≥ ASC-US cytology	**3.61**	**5.83** (2.81, 9.57)	**15.0** (10.1, 19.7)
	12 Other HR HPV + and NILM cytology	**10.55**	**3.56** (2.09, 5.20)	**5.56** (3.79, 7.52)
	HR HPV -	**78.87**	**0.08** (0.00, 0.17)	**0.30** (0.15, 0.49)
30–39 Years	HPV 16/18 +	**3.18**	**20.2** (16.2, 24.5)	**24.9** (20.4, 29.6)
	12 Other HR HPV + and ≥ ASC-US cytology	**2.09**	**7.42** (4.07, 11.5)	**12.1** (8.10, 16.6)
	12 Other HR HPV + and NILM cytology	**6.22**	**3.01** (1.87, 4.48)	**5.77** (4.08, 7.69)
	HR HPV -	**88.41**	**0.10** (0.05, 0.16)	**0.18** (0.09, 0.26)
40–49 Years	HPV 16/18 +	**1.56**	**14.3** (8.85, 19.9)	**16.5** (10.6, 22.1)
	12 Other HR HPV + and ≥ ASC-US cytology	**1.22**	**10.5** (5.30, 16.8)	**18.2** (12.2, 26.0)
	12 Other HR HPV + and NILM cytology	**4.33**	**2.77** (1.42, 4.69)	**4.94** (3.04, 7.34)
	HR HPV -	**92.89**	**0.39** (0.01, 1.13)	**0.80** (0.07, 1.84)
≥ 50 Years	HPV 16/18 +	**1.18**	**8.20** (3.45, 14.2)	**9.84** (4.39, 15.7)
	12 Other HR HPV + and ≥ ASC-US cytology	**0.78**	**8.64** (2.35, 16.4)	**11.1** (3.90, 18.6)
	12 Other HR HPV + and NILM cytology	**4.08**	**0.95** (0.00, 2.00)	**2.13** (0.68, 3.60)
	HR HPV -	**93.95**	**0.45** (0.01, 1.36)	**1.67** (0.44, 3.27)

Effect of Knowledge of HPV status on Cytology (Unblinded Results) for the Primary Screening Algorithm

For the Primary Screening Algorithm, in which women with 12 Other HR HPV positive results are reflexed to cytology, the sensitivity of the Primary Screening Algorithm for ≥ CIN3 increases by approximately 5% (Table 45) and specificity decreases by approximately 0.5% if the cytologists are unblinded to HPV results. Unblinded leads to approximately the same PPV, a small improvement in NPV and an 11% increase in the number of colposcopies (Table 45).

Table 45
Performance Comparison of Blinded and Unblinded Cytology Using the Primary Screening Algorithm (≥ CIN3) – PreservCyt

Algorithm	Prevalence(%)=0.97 with 95% CI (0.74, 1.28)						
	Pos (%)	PPV (%)	1-NPV (%)	Sensitivity (%)	1-Spec (%)	PLR	NLR
HPV Primary Screening Algorithm (Blinded to HPV status)	4.62	12.25	0.42	58.26	4.09	14.24	0.44
HPV Primary Screening Algorithm (Unblinded to HPV status)	5.13	11.91	0.38	63.14	4.58	13.80	0.39
Difference	-0.51	0.34	0.04	-4.88	-0.49	0.44	0.04

Analysis of Unsatisfactory (UNSAT) Cytology from ThinPrep on the Performance of the Primary Screening Algorithm

In this clinical study 1.77% (737 out of 41,681) of women ≥ 25 years had UNSAT cytology results. The proportions of women with **cobas**® HPV Test negative, HPV 16/18 positive and 12 Other HR HPV positive results were similar for both women with satisfactory and UNSAT cytology results. These results do not contradict an assumption that the risk of ≥ CIN3 for the women with UNSAT cytology is similar to the risk of ≥ CIN3 for women with satisfactory cytology. Taking this into account, for the 737 women with UNSAT cytology, the risk of having ≥ CIN3 was estimated by their **cobas**® HPV Test status and age group. The performances of the Primary Screening Algorithm in women with UNSAT cytology and without UNSAT cytology showed no differences (Table 46).

Table 46
Performance of the Primary Screening Algorithm with and Without UNSAT Cytology (≥ CIN3) – PreservCyt

Primary Screening Algorithm	Pos (%)	PPV (%)	1-NPV (%)	Sensitivity (%)	1-Spec (%)	PLR	NLR
Without UNSAT Cytology	4.62	12.25	0.42	58.26	4.09	14.24	0.44
With UNSAT Cytology	4.70	12.05	0.42	58.48	4.18	14.00	0.43

Benefit and Risk for Primary Screening (≥ 25 Years) Population per 10,000 Women

Benefit and risk per 10,000 screened women ≥ 25 years for the Primary Screening algorithm (Blinded to HPV status and Unblinded to HPV status, ie based on cytology slides read with/without knowledge of HPV status) and for the Cytology algorithm were evaluated for detection of high-grade cervical disease (CIN2, ≥ CIN3) (Table 47). The Primary Screening algorithm (Unblinded to HPV status) detected more disease cases when compared with the Cytology algorithm (88 vs. 63, respectively), with fewer colposcopies (514 vs. 639, respectively) and approximately the same number of screening tests (10,760 vs. 10,000). Additionally, fewer cases of high-grade cervical disease (CIN2, ≥ CIN3) were missed by the Primary Screening algorithm (Unblinded to HPV status) when compared to the Cytology algorithm (91 vs. 116). In addition, fewer false positive cases were identified with the Primary Screening algorithm vs. the Cytology algorithm (426 vs. 576).

Table 47

Benefit and Risk of the Primary Screening Algorithm and the Cytology Algorithm for the Primary Screening Population (≥ 25 Years) (per 10,000 Women)

(PreservCyt)

Algorithm	Number of Test and Procedures			Benefit		Risk		
	Cytology	cobas® HPV Test	Colposcopy	True Positive		False Negative		False positive
				≥ CIN3	CIN2	≥ CIN3	CIN2	
Primary Screening (Blinded to HPV status)	760	10000	461	57	24	40	58	380
Primary Screening (Unblinded to HPV status)	760	10000	514	61	27	36	55	426
Cytology	10000	0	639	41	22	56	60	576

Benefits and Risk for the Primary Screening (≥ 25 Years) Population per 100 Colposcopy Procedures

Benefit and risk per 100 colposcopy procedures in women ≥ 25 years for the Primary Screening algorithm and Cytology algorithm are presented in Table 48. The Primary Screening Algorithm (Unblinded to HPV status) detected more cases of disease (17 = 12+5) per 100 colposcopies performed than the Cytology algorithm and also had the lower false positive rate (83 vs. 90). Although the Primary Screening algorithm had the same number of false negatives (18= 7+11) as the Cytology algorithm (18=9+9) per 100 colposcopies performed, a larger number of women were screened by the Primary Screening Algorithm than by the Cytology algorithm in order to identify women for 100 colposcopy procedures (24% more women, (1947/1,564)). In addition, the probability of disease among women not referred to colposcopy was 1.0% (18/1847) by the Primary Screening algorithm, which was lower compared with the Cytology algorithm, 1.2% (18/1464).

Table 48

Benefit and Risk of the Primary Screening Algorithm and the Cytology Algorithm for the Primary Screening Population (≥ 25 Years) (per 100 Colposcopy Procedures)

(PreservCyt)

Algorithm	Number of Test and Procedures			Benefit		Risk		
	Cytology	cobas® HPV Test	Colposcopy	True Positive		False Negative		False positive
				≥ CIN3	CIN2	≥ CIN3	CIN2	
Primary Screening (Blinded to HPV status)	165	2169	100	12	5	9	13	83
Primary Screening (Unblinded to HPV status)	148	1947	100	12	5	7	11	83
Cytology	1564	0	100	7	3	9	9	90

Baseline and 3-Year Cumulative Risks of High-Grade Cervical Disease for the Primary Screening Algorithm

The risks (verification bias adjusted (VBA) estimates) of high-grade cervical disease (≥ CIN2 and ≥ CIN3) at Baseline (Current Risk) and the sum of Current Risk and Future risk at Year 3 (cumulative risk at Year 3 Follow-Up) were calculated in the primary screening population (≥ 25 years) among women with different results from the **cobas**® HPV Test and cytology results.

The risks at Baseline for women with HPV16 positive/HPV18 positive results were 19.83% and 15.04% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively (Table 49). The cumulative risks from Baseline to follow up Year 3 for women with HPV16 positive/HPV18 positive results were 28.03% and 21.11% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively.

The risks at the baseline for women with 12 Other HR HPV positive and ≥ ASC-US cytology results were 14.17% and 7.78% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively (Table 49). The cumulative risks from Baseline to follow up Year 3 for women with 12 Other HR HPV positive and ≥ ASC-US cytology results were 20.56% and 11.11% for the≥ CIN2 and ≥ CIN3 endpoints, respectively.

These high risk estimates justify referral of these women for colposcopy.

Table 49
Risk of Disease in Women with HPV16 Positive/HPV18 Positive or with 12 Other HR HPV Positive and ≥ ASC-US Cytology in the Primary Screening (≥ 25 Years) Population (PreservCyt)

Disease Endpoint	Category	Current Risk (%) (95% CI)	Current + Future Risk (%) at Year 3 (95% CI)
≥ **CIN2**	**HPV16+/18+**	19.83 (17.39, 22.41)	28.03 (24.91, 31.07)
	HPV16+	23.54 (20.56, 26.71)	32.34 (28.73, 36.20)
	HPV18+	10.33 (6.73, 13.55)	17.02 (12.02, 21.75)
	12 Other HR HPV+ and ≥ ASC-US	14.17 (11.36, 17.06)	20.56 (17.10, 23.94)
≥ **CIN3**	**HPV16+/18+**	15.04 (12.98, 17.43)	21.11 (18.47, 23.90)
	HPV16+	17.72 (15.19, 20.72)	25.09 (21.89, 28.95)
	HPV18+	8.21 (5.10, 11.14)	10.94 (7.06, 14.49)
	12 Other HR HPV+ and ≥ ASC-US	7.78 (5.57, 10.15)	11.11 (8.37, 13.92)
Current Risk = Absolute Risk at baseline; Current + Future Risk at Year 3 = Cumulative Risk from baseline to follow up year 3; VBA = Verification Bias Adjusted.			

The risks for women with positive results for 12 Other HR HPV genotypes and NILM cytology at Baseline and the sum of current risk and future risk at year 1, 2, and 3 are presented in Table 50. The risks at the Baseline were 4.89% and 2.76% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively. The cumulative risks from Baseline to follow up Year 3 for women with 12 Other HR HPV positive and NILM cytology results were 7.90% and 3.64% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively.

Table 50
Risk of Disease in Women with 12 Other HR HPV Positive and NILM Cytology in the Primary Screening (≥ 25 Years) Population – PreservCyt

	≥ CIN2 (95% CI)	≥ CIN3 (95% CI)
Current Risk (%)	4.89 (3.94, 5.87)	2.76 (2.06, 3.45)
Current + Future Risk at Year 1 (%)	6.14 (5.00, 7.24)	3.13 (2.39, 3.88)
Current + Future Risk at Year 2 (%)	6.60 (5.38, 7.69)	3.34 (2.59, 4.15)
Current + Future Risk at Year 3 (%)	7.90 (6.59, 9.25)	3.64 (2.80, 4.52)

The risks for women with HR HPV negative results at Baseline and the sum of current risk and future risk at year 1, 2, and 3 are presented in Table 51. The risks at the Baseline were 0.77% and 0.27% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively. The cumulative risks from Baseline to follow up Year 3 for women with HR HPV negative results were 0.94% and 0.34% for the ≥ CIN2 and ≥ CIN3 endpoints, respectively.

Table 51
Risk of Disease in Women with HR HPV Negative Results in the Primary Screening (≥ 25 Years) Population – PreservCyt

	≥ CIN2 (95% CI)	≥ CIN3 (95% CI)
Current Risk (%)	0.77 (0.33, 1.29)	0.27 (0.05, 0.60)
Current + Future Risk at Year 1 (%)	0.81 (0.36, 1.31)	0.28 (0.06, 0.61)
Current + Future Risk at Year 2 (%)	0.87 (0.42, 1.38)	0.31 (0.08, 0.64)
Current + Future Risk at Year 3 (%)	0.94 (0.47, 1.45)	0.34 (0.11, 0.66)

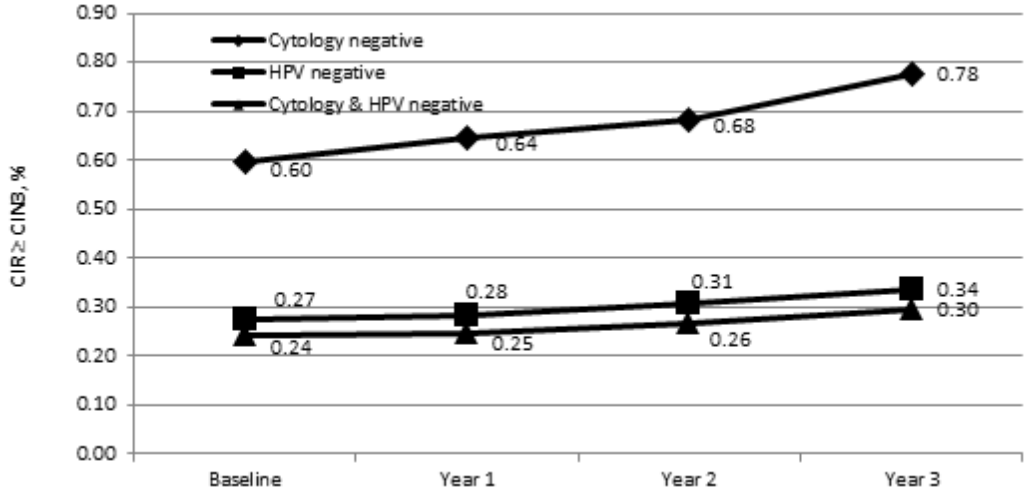
Comparing Risks of Disease for Women with NILM Cytology and Negative cobas® HPV Test Results

The risks of disease were compared in the primary screening population (≥ 25 years) between women with a NILM cytology result at baseline versus women with a HR HPV negative results at baseline (Table 52 and Figure 3). For those with HR HPV negative result at baseline, the 3-year cumulative risk of ≥ CIN3 was 0.34% compared with 0.78% for those with NILM cytology, indicating that women with a HR HPV negative result have one half the risk of being diagnosed with ≥ CIN3 over 3 years than women with NILM cytology results. The addition of NILM cytology results to HR HPV negative results (co-testing) decreased this risk of ≥ CIN3 marginally (0.34 vs. 0.30).

Table 52
Comparison of the Risk of Disease Between Women With a HR HPV Negative Result vs. a NILM Cytology Result at Baseline in the Primary Screening (≥ 25 Years) Population –PreservCyt

Disease Endpoint	Baseline cobas® HPV /Cytology Result	Current Risk ,% (95% CI)	Current + Future Risk at Year 3, %, (95% CI)
≥ CIN2	NILM	1.24 (0.81, 1.72)	1.67 (1.23, 2.15)
	HR HPV Neg	0.77 (0.33, 1.29)	0.94 (0.47, 1.45)
	NILM &HR HPV Neg	0.73 (0.28, 1.26)	0.85 (0.38, 1.37)
≥ CIN3	NILM	0.60 (0.36, 0.92)	0.78 (0.53, 1.11)
	HR HPV Neg	0.27 (0.05, 0.60)	0.34 (0.11, 0.66)
	NILM &HR HPV Neg	0.24 (0.02, 0.58)	0.30 (0.06, 0.64)
Current Risk = Absolute Risk at baseline; Current + Future Risk at Year 3 = Cumulative Risk from baseline to follow up year 3; All numbers are verification bias adjusted.			

Figure 3
3-Year Cumulative Risk of NILM Cytology and HR HPV Negative Results Alone and in Combination at Baseline



EXPECTED RESULTS FOR SAMPLES COLLECTED IN SUREPATH ™ PRESERVATIVE FLIUD

For the ASC-US triage claim, a total of 952 women ≥21 years of age were eligible for the study and cervical specimens were tested at 3 US sites. Eligible women were ≥21 years of age who had signed informed consent, satisfied study inclusion/exclusion criteria, had not enrolled in the study previously and had not withdrawn consent before undergoing all study procedures. Of 952 eligible women, 87 pre-quot samples were missing; 865 had valid positive or negative cobas HPV Test result.

The median age of evaluable subjects was 35.4 years (range: 21 to 75) with ~41% subjects 21-29 years, ~28% 30-39 years, ~17% 40-49 years, and ~15% ≥50 years. Approximately 72% of the evaluable subjects were White, 5% were African American, and 23% were from other races. Approximately 42% were Hispanic or Latino. Of note, 31.8% of subjects were vaccinated for HPV.
Table 53 displays HPV prevalence as determined by the cobas® HPV Test by testing site for all eligible subjects. The overall HPV prevalence in ASC-US women ⩾21 years was approximately 46% and ranged from 44% to 47% across the 3 sites.

Table 53
Summary of HPV prevalence by the cobas® HPV Test by Testing Site – SurePath

	cobas® HPV Test Result		
cobas® HPV Testing Site	**ASC-US population (≥21 Years) No. of Eligible Subjects**	**Positive n (%)**	**Negative n (%)**
1	349	155 (44.4)	194 (55.6)
2	284	132 (46.5)	152 (53.5)
3	232	109 (47.0)	123 (53.0)
Overall	865	396 (45.8)	469 (54.2)

Table 54 shows HPV prevalence by cobas® HPV test results by age. In the ASC-US population, HPV prevalence decreased with age from 60.6% in the 21-24 year population to 47.2% at 30-39 years and remained between 28 to 38% after 40 years of age.

Table 54
Summary of HPV Prevalence by the cobas® HPV Test Result Stratified by Age group - SurePath

	cobas® HPV Test Result	
Age Group (Years)	Positive	Negative
Overall Eligible Subjects	45.8% (396/865)	54.2% (469/865)
21-24	60.6% (77/127)	39.4% (50/127)
25-29	47.2% (108/229)	52.8% (121/229)
30-39	51.9% (122/235)	48.1% (113/235)
40-49	33.6% (48/143)	66.4% (95/143)
50-59	28.7% (27/94)	71.3% (67/94)
60-69	38.2% (13/34)	61.8% (21/34)
≥70	33.3% (1/3)	66.7% (2/3)

Table 55 displays the four-category **cobas**® HPV Test results stratified by age. Overall, the highest prevalence was observed for 12 Other HR HPV (~37%) followed by HPV16 (~6%). The prevalence of 12 Other HR HPV was highest (~55%) in the age group 21-24 years and decreased with age. Of note, the prevalence of HPV16 was 4.8% (17/356) in the 21-29 year group, but increased to 10.6% by 30-39 years and decreased with age afterward. The prevalence of HPV18 exhibited a pattern similar to HPV16.

Table 55
Summary of Four-Category cobas® HPV Test Results by Age Group – SurePath

	cobas® HPV Test Result, (%) n				
Age Group (Years)	**HPV16 Positive**	**HPV18 Positive**	**12 Other HR HPV Positive**	**Negative**	**Total**
Overall Eligible Subjects	6.5% (56/865)	2.4% (21/865)	36.9% (319/865)	54.2% (469/865)	865
21-24	5.5% (7/127)	0.0% (0/127)	55.1% (70/127)	39.4% (50/127)	127
25-29	4.4% (10/229)	3.9% (9/229)	38.9% (89/229)	52.8% (121/229)	229
30-39	10.6% (25/235)	4.3% (10/235)	37.0% (87/235)	48.1% (113/235)	235
40-49	6.3% (9/143)	0.0% (0/143)	27.3% (39/143)	66.4% (95/143)	143
50-59	4.3% (4/94)	2.1% (2/ 94)	22.3% (21/ 94)	71.3% (67/94)	94
60-69	2.9% (1/34)	0.0% (0/34)	35.3% (12/ 34)	61.8% (21/34)	34
≥70	0.0% (0/3)	0.0% (0/3)	33.3% (1/3)	66.7% (2/3)	3

STUDY DESIGN TO DEMONSTRATE CLINICAL SENSITIVITY AND SPECIFICITY OF THE **COBAS**® HPV TEST FOR SCREENING PATIENTS WITH ASC-US CYTOLOGY RESULTS TO DETERMINE THE NEED FOR REFERRAL TO COLPOSCOPY

A multi-center prospective study was conducted to evaluate the performance of the **cobas**® HPV test using specimens collected in SurePath Preservative Fluid™ as a triage test to stratify women with ASC-US cytology for colposcopy. Women ≥21 years of age presenting for routine cervical cancer screening had 2 cervical samples taken, the first in SurePath Preservative Fluid™ and the second in specimen transport medium (STM) as part of the standard of care. Women were enrolled at 2 sites (comprised of 21 clinics) and tested at 3 sites. Cytology samples were classified according to the criteria of the 2001 Bethesda System. Those women who had ASC-US cytology results on specimens collected in SurePath were invited to enroll in the study. A total of 952 subjects met the inclusion/exclusion criteria and were enrolled in the study. After informed consent was obtained, demographic and gynecologic history information was recorded. **cobas**® HPV testing was performed using pre-aliquots and post-aliquots of SurePath Preservative Fluid™. All women with ASC-US cytology results who agreed to participate in the study were referred independent of their HPV status to colposcopy within 12 weeks of the enrollment visit. To avoid bias, health care providers, study participants and colposcopists remained blinded to all HPV results until after colposcopy. Colposcopy was conducted per protocol that required all visible cervical lesions to be biopsied; in those cases where no lesions were visible, a single random biopsy was taken at the squamocolumnar junction. An endocervical curettage (ECC) was performed in all patients in whom the squamocolumnar junction was not visualized. All biopsies were examined by a Central Pathology Review Panel (CPRP) consisting of three expert pathologists, and discordant results were adjudicated according to a pre-defined protocol. For all analyses, the clinical performance of the **cobas**® HPV Test was evaluate against CPRP histology results, with ≥CIN2 and ≥CIN3 as the primary and secondary study endpoints, respectively. The clinical performance below is presented for the pre-aliquot specimens; the clinical performance for the post-aliqouot was similar. Of 952 eligible women, 87 pre-quot samples were missing; 865 had valid positive or negative **cobas** HPV Test result. The percent of invalid **cobas**® HPV Test results when reported as positive and negative results was 0.0% (0/865) with 95% CI: 0.0% to 0.4%.

Of the 865, colposcopy were performed for 857 women; histology review for 1 woman did not follow the protocol, resulting in her being excluded from the study analysis.

Performance Characteristic in the ASC-US Population in Samples Collected in SurePath (≥ 21 Years)

Table 56 displays a summary of the CPRP diagnosis stratified by the **cobas**® HPV Test results (positive and negative) for 856 women.

Table 56
Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis – SurePath

	Central Pathology Review Panel Diagnosis					
cobas® HPV Test Result	**Undetermined**	**Normal**	**CIN1**	**CIN2**	**≥CIN3**	**Total**
Positive	3	264	64	34	28	393
Negative	7	410	33	9	4	463
Total	10	674	97	43	32	856

Ten women had undeterminate histology results which lead to 846 women being included in the clinical performance estimates analysis.

Performance of the **cobas**® HPV in Detecting ≥CIN2 and ≥CIN3 in the Non- vaccinated population – SurePath

Table 57 displays a summary of the CPRP diagnosis stratified by the cobas® HPV Test results (positive and negative) in the non-vaccinated population. The risk of ≥CIN2 in subjects with HPV positive results and with HPV negative results was ~14% (34/250) and ~2% (6/326), respectively. The risk of ≥CIN3 in subjects with HPV positive and with HPV negative results was ~7% (17/250) and ~1% (2/326), respectively.

Table 57
Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis the Non-Vaccinated ASC-US (≥21) Population- SurePath

		CPRP Diagnosis				
cobas® HPV Test Result	**Normal**	**CIN1**	**CIN2**	**CIN3**	**Cancer**	**Total**
Positive	179	37	17	15	2	250
Negative	296	24	4[a]	2[b]	0	326
Total	475	61	21	17	2	576

[a] 3 CIN2 cases were FDA approved test-negative and sequence negative; 1 CIN2 case was FDA approved test-positive and sequence negative.
[b] 1 CIN3 case was FDA approved test-positive and sequence negative; and 1 CIN3 case was FDA approved test-negative and sequence negative.

Performance of the **cobas**® HPV Test and the performance of the FDA approved HPV test in detecting ≥CIN2 and ≥CIN3 was evaluated by estimating sensitivity and specificity, PPV and NPV and PLR and NLR, as shown in Table 58 for the non-vaccinated population. The sensitivity of the **cobas**® HPV Test for detecting ≥CIN2 and ≥CIN3 was 85.0% (34/40) with 95% CI=70.9% to 92.9 and 89.5% (17/19) with 95% CI = 68.6% to 97.1%, respectively. The specificity of the **cobas**® HPV Test for detecting ≥CIN2 and ≥CIN3 was 59.7% (320/536) with 95% CI=55.5% to 63.8% and 58.2% (324/557) with 95% CI=54.0% to 62.2%, respectively.

The sensitivity of the FDA approved HPV test for detecting ≥CIN2 and ≥CIN3 was 81.8% (36/44) with 95% CI=68.0% to 90.5 and 90.5% (19/21) with 95% CI = 71.1% to 97.4%, respectively. The specificity of the FDA approved HPV test for detecting ≥CIN2 and ≥CIN3 was 58.9% (355/603) with 95% CI=54.9% to 62.7% and 57.7% (361/626) with 95% CI=53.8% to 61.5%, respectively.

Table 58

Performance of the cobas® HPV Test and an FDA Approved HPV Test in Detecting ≥CIN2 and ≥CIN3 in the Non-vaccinated ASC-US Population (≥ 21 Years) – SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	**Point Estimate**	**95% CI**	**Point Estimate**	**95% CI**
≥CIN2	Sensitivity (%)	85.0% (34/40)	(70.9, 92.9)	81.8 (36/44)	(68.0, 90.5)
	Specificity (%)	59.7% (320/536)	(55.5, 63.8)	58.9% (355/603)	(54.9, 62.7)
	PPV (%)	13.6% (34/250)	(11.4, 15.5)	12.7% (36/284)	(10.6, 14.4)
	NPV (%)	98.2% (320/326)	(96.5, 99.1)	97.8% (355/363)	(96.2, 98.9)
	PLR	2.1	(1.7, 2.5)	2.0	(1.6, 2.3)
	NLR	0.25	(0.12, 0.49)	0.31	(0.15, 0.55)
	Prevalence (%)	6.9% (40/576)		6.8% (44/647)	
≥CIN3	Sensitivity (%)	89.5% (17/19)	(68.6, 97.1)	90.5% (19/21)	(71.1, 97.4)
	Specificity (%)	58.2% (324/557)	(54.0, 62.2)	57.7% (361/626)	(53.8, 61.5)
	PPV (%)	6.8% (17/250)	(5.2, 7.8)	6.7% (19/284)	(5.3, 7.5)
	NPV (%)	99.4% (324/326)	(98.1, 99.9)	99.4% (361/363)	(98.3, 99.9)
	PLR	2.1	(1.6, 2.5)	2.1	(1.7, 2.4)
	NLR	0.18	(0.04, 0.56)	0.17	(0.03, 0.52)
	Prevalence (%)	3.3% (19/576)		3.2% (21/647)	

Table 59 displays a summary of the CPRP diagnosis by redefining disease status based on the lesion directed biopsies; therefore, subjects without lesions would had have random biopsies and would be defined as non-diseased.

Table 59

Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis for the Direct Biopsy in the Non-vaccinated ASC-US Population (≥ 21 Years) – SurePath

cobas® HPV Test Result	No Lesion	CPRP Diagnosis from lesion					
		Normal	**CIN1**	**CIN2**	**CIN3**	**Cancer**	**Total**
Positive	122	81	23	12	11	1	250
Negative	250	65	8	2[a]	1[b]	0	326
Total	372	146	31	14	12	1	576

[a] 2 CIN2 cases were FDA approved test-negative and sequence negative.
[b] 1 CIN3 case was FDA approved test-positive and sequence negative.

Table 60 presents the performance of the **cobas**® HPV Test and the performance of the FDA approved HPV Test in detecting ≥CIN2 and ≥CIN3 by redefining disease status in subjects who had random biopsies as non-diseased. Under this modified definition of disease the sensitivity of detecting ≥CIN2 was 88.9% (24/27) with 95% CI = 71.9% to 96.1% for the **cobas**® HPV Test and 90.3% (28/31) with 95% CI = 75.1% to 96.7% for the FDA approved HPV Test. The specificity for detecting ≥CIN2 was 58.8% (323/549) with 95% CI = 54.7% to 62.9% for the **cobas**® HPV Test and 58.4% (360/616) with 95% CI=54.5% to 62.3% for the FDA approved HPV Test.

Under this modified definition of disease the sensitivity of detecting ≥CIN3 was 92.3% (12/13) with 95% CI = 66.7% to 98.6% for the **cobas**® HPV Test for and 93.3% (14/15) with 95% CI = 70.2% to 98.8% for the FDA approved HPV Test. The specificity for detecting ≥CIN3 was 57.7% (325/563) with 95% CI = 53.6% to 61.7% for the **cobas**® HPV Test and 57.3%(362/632) (95% CI=53.4% to 61.1%) for the FDA approved HPV Test.

Table 60

Performance of the cobas® HPV Test and an FDA Approved HPV test Considering Subjects With Random Biopsy as Non-diseased in the Non-vaccinated ASC-US Population (≥ 21 Years) - SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	**Point Estimate**	**95% CI**	**Point Estimate**	**95% CI**
≥CIN2*	Sensitivity (%)	88.9% (24/27)	(71.9, 96.1)	90.3% (28/31)	(75.1, 96.7)
	Specificity (%)	58.8% (323/549)	(54.7, 62.9)	58.4% (360/616)	(54.5, 62.3)
	PPV (%)	9.6% (24/250)	(7.8, 10.9)	9.9% (28/284)	(8.2, 11.0)
	NPV (%)	99.1% (323/326)	(97.7, 99.7)	99.2% (316/363)	(97.9, 99.8)
	PLR	2.2	(1.7, 2.5)	2.2	(1.8, 2.5)
	NLR	0.19	(0.06, 0.48)	0.17	(0.05, 0.443)
	Prevalence (%)	4.7% (27/576)		4.8% (31/647)	
≥CIN3*	Sensitivity (%)	92.3% (12/13)	(66.7, 98.6)	93.3% (14/15)	(70.2, 98.8)
	Specificity (%)	57.7% (325/563)	(53.6, 61.7)	57.3% (362/632)	(53.4, 61.1)
	PPV (%)	4.8% (12/250)	(3.5, 5.4)	4.9% (14/284)	(3.7, 5.5)
	NPV (%)	99.7% (325/326)	(98.6, 99.9)	99.7% (362/363)	(98.7, 99.9)
	PLR	2.2	(1.6, 2.5)	2.2	(1.6, 2.5)
	NLR	0.13	(0.01, 0.60)	0.12	(0.01, 0.54)
	Prevalence (%)	2.3% (13/576)		2.3% (15/647)	

* Subjects with random biopsy (no direct biopsy) were redefined as having a normal colposcopy and are included in the analysis as non-diseased. For subjects with direct biopsies, CPRP diagnosis remained unchanged.

Table 61 presents the performance of the **cobas**® HPV Test and the performance of the FDA approved HPV test in STM in detecting ≥CIN2 and ≥CIN3 by biopsy status for the non-vaccinated subject. When confined to subjects with direct biopsies, the sensitivity of detecting ≥CIN2 was 88.9% (24/27) (95% CI = 71.9% to 96.1%) for the **cobas**® HPV Test and 90.3% (28/31) (95% CI = 75.1% to 96.7%) for the FDA approved HPV test. The specificity of detecting ≥CIN2 was 41.2% (73/177) (95% CI = 34.3% to 48.7%) for the **cobas**® HPV Test and 39.4% (82/208) (95% CI = 33.0% to 46.2%) for the FDA approved HPV test. With direct biopsies, the sensitivity of detecting ≥CIN3 was 92.3% (12/13) (95% CI = 66.7% to 98.6%) for the **cobas**® HPV Test and 93.3% (14/15) (95% CI = 70.2% to 98.8%) for the FDA approved HPV test. The specificity of detecting ≥CIN3 was 38.3% (75/191) (95% CI = 32.6% to 46.3%) for the **cobas**® HPV Test and 37.5% (84/224) (95% CI = 31.4% to 44.0%) for the FDA approved HPV test.

When confined to subjects with random biopsies, the sensitivity of detecting ≥CIN2 was 76.9% (10/13) (95% CI = 49.7% to 91.8%) for the **cobas**® HPV Test and 61.5% (8/13) (95% CI = 35.5% to 82.3%) for the FDA approved HPV test. The specificity of detecting ≥CIN2 was 68.8% (247/359) (95% CI = 63.8% to 73.4%) for the **cobas**® HPV Test and 69.1% (273/395) (95% CI = 64.4% to 73.5%) for the FDA approved HPV test. With random biopsies, the sensitivity of detecting ≥CIN3 was 83.3% (5/6) (95% CI = 43.7% to 97.0%) for the **cobas**® HPV Test and 83.3% (5/6) (95% CI = 43.7% to 97.0%) for the FDA approved HPV test. The specificity of detecting ≥CIN3 was 68.0% (249/366) (95% CI = 63.1% to 72.6%) for the **cobas**® HPV Test and 68.9% (277/407) (95% CI = 64.2% to 73.2%) for the FDA approved HPV test.

Table 61
Performance of the cobas® HPV Test and an FDA Approved HPV test by Subject Biopsy Status in the Non-vaccinated ASC-US Population (≥21 Years) – SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	Lesion-Directed Biopsy Point Estimate	No Lesion-Random Biopsy Point Estimate	Lesion-Directed Biopsy Point Estimate	No Lesion-Random Biopsy Point Estimate
≥CIN2	Sensitivity (%) 95% CI	88.9% (24/27) (71.9, 96.1)	76.9% (10/13) (49.7, 91.8)	90.3% (28/31) (75.1, 96.7)	61.5% (8/13) (35.5, 82.3)
	Specificity (%) 95% CI	41.2% (73/177) (34.3, 48.7)	68.8% (247/359) (63.8, 73.4)	39.4% (82/208) (33.0, 46.2)	69.1% (273/395) (64.4, 73.5)
	Prevalence	13.2% (27/204)	3.5% (13/372)	13.0% (31/239)	3.2% (13/408)
≥CIN3	Sensitivity (%) 95% CI	92.3%(12/13) (66.7, 98.6)	83.3% (5/6) (43.7, 97.0)	93.3% (14/15) (70.2, 98.8)	83.3% (5/6) (43.7, 97.0)
	Specificity (%) 95% CI	38.3%(75/191) (32.6, 46.3)	68.0% (249/366) (63.1, 72.6)	37.5% (84/224) (31.4, 44.0)	68.9% (277/402) (64.2, 73.2)
	Prevalence	6.4% (13/204)	1.6% (6/372)	6.3% (15/239)	1.5% (6/408)
HPV positivity		62.7% (128/204)	32.8% (122/372)	64.4% (154/239)	31.9% (130/408)

Performance of the **cobas**® HPV test in detecting ≥ CIN2 and ≥ CIN3 by age group in the non-vaccinated population is presented in Table 62. The sensitivity of **cobas**® HPV Test for detecting ≥CIN2 was 100.0% (7/7) (95% CI = 64.6% to 100%) in the 21 – 29 year age group, 85.0% (17/20) (95% CI = 64.0% to 94.8%) in the 30 – 39 year age group and 76.9% (10/13) (95% CI = 49.7% to 91.8%) in the ≥40 age group. The specificity of the test was highest in ≥40 year, with an estimate of 69.8% (173/248) (95% CI = 63.8% to 75.1%).

The sensitivity of **cobas**® HPV Test for detecting ≥CIN3 was 100.0% (4/4) (95% CI = 51.0% to 100%) in the 21 - 29 year age group, 81.8% (9/11) (95% CI = 52.3% to 94.9%) in the 30 - 39 year age group and 100% (4/4) (95% CI = 51.0% to 100.0%) in the ≥40 age group. The specificity of the test was highest in ≥40 year, with an estimate of 68.5% (176/257) (95% CI = 62.6% to 73.9%).

Table 62
Performance of the cobas® HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the Non-vaccinated ASC-US Population by Age Group - SurePath

Performance	21-29 Years	30-39 Years	≥ 40 Years
N	107	208	261
≥ CIN2			
Sensitivity (%)	100% (7/7)	85.0% (17/20)	76.9% (10/13)
95% CI (%)	(64.6, 100)	(64.0, 94.8)	(49.7, 91.8)
Specificity (%)	51.0% (51/100)	51.1% (96/188)	69.8% (173/248)
95% CI (%)	(41.3, 60.6)	(44.0, 58.1)	(63.8, 75.1)
PPV (%)	12.5% (7/56)	15.6% (17/109)	11.8% (10/85)
95% CI (%)	(8.2, 15.1)	(11.9, 18.5)	(7.6, 15.1)
NPV (%)	100% (51/51)	97.0% (96/99)	98.3% (173/176)
95% CI (%)	(95.0, 100)	(92.9, 99.0)	(96.3, 99.5)
≥ CIN2 prevalence	6.5% (7/107)	9.6% (20/208)	5.0% (13/261)
≥ CIN3			
Sensitivity (%)	100% (4/4)	81.8% (9/11)	100% (4/4)
95% CI (%)	(51.0, 100)	(52.3, 94.9)	(51.0, 100)
Specificity (%)	49.5% (51/103)	49.2% (97/197)	68.5% (176/257)
95% CI (%)	(40.1, 59.0)	(42.3, 56.2)	(62.6, 73.9)
PPV (%)	7.1% (4/56)	8.3% (9/109)	4.7% (4/85)
95% CI (%)	(3.6, 8.6)	(5.3, 10.2)	(2.3, 5.6)
NPV (%)	100% (51/51)	98.0% (97/99)	100% (176/176)
95% CI (%)	(96.0, 100)	(94.7, 99.6)	(98.9, 100)
≥ CIN3 prevalence	3.7% (4/107)	5.3% (11/208)	1.5% (4/261)

Performance of the **cobas**® HPV in Detecting ≥CIN2 and ≥CIN3 in the Vaccinated population – SurePath

Table 63 displays a summary of the CPRP diagnosis stratified by the **cobas**® HPV Test results (positive and negative) in the vaccinated population. The risk of ≥CIN2 in subjects with HPV positive results and with HPV negative results was 20% (28/140) and ~5% (7/130), respectively. The risk of ≥CIN3 in subjects with HPV positive and with HPV negative results was ~8% (11/140) and ~2% (2/130), respectively.

Table 63
Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis in the Vaccinated ASC-US(≥21 years) Population – SurePath

		Central Pathology Review Panel Diagnosis				
cobas® HPV Test Result	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	85	27	17	10	1	140
Negative	144	9	5[a]	2[b]	0	130
Total	199	36	22	12	1	270

[a] 2 CIN2 cases were FDA approved test-negative and sequence negative; 2 CIN2 cases were FDA approved test- negative and sequence positive. 1 CIN2 case was FDA approved test-positive and sequence positive.

[b] 1 CIN3 case was FDA approved test-negative and sequence negative; 1 CIN3 case was FDA approved test negative and sequence positive.

Performance of the **cobas**® HPV Test and the performance of the FDA approved HPV test in STM in detecting ≥CIN2 and ≥CIN3 was evaluated by estimating sensitivity and specificity, PPV and NPV and PLR and NLR as shown in Table 64. In the vaccinated population, the sensitivity of the **cobas**® HPV Test for detecting ≥CIN2 and ≥CIN3 was 80.0% (28/35) with 95% CI=64.1% to 90.0% and 89.5% (17/19) with 95% CI = 68.6% to 97.1%, respectively. The specificity of the **cobas**® HPV Test for detecting ≥CIN2 and ≥CIN3 was 59.7% (320/536) with 95% CI=55.5% to 63.8% and 58.2% (324/557) with 95% CI=54.0% to 62.2%, respectively.

In the vaccinated population, the sensitivity of the FDA approved HPV test for detecting ≥CIN2 and ≥CIN3 was 74.3% (26/35) with 95% CI=57.9% to 85.8% and 76.9% (10/13) with 95% CI = 49.7% to 91.8%, respectively. The specificity of the FDA approved HPV test for detecting ≥CIN2 and ≥CIN3 was 49.8% (124/249) with 95% CI=43.6% to 56.0% and 48.0% (130/271) with 95% CI=42.1% to 53.9%, respectively.

Table 64

Performance of the cobas® HPV Test and an FDA Approved HPV test in Detecting ≥CIN2 and ≥CIN3 in the Vaccinated ASC-US Population (≥21 years) – SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	Point Estimate	95% CI	Point Estimate	95% CI
≥CIN2	Sensitivity (%)	80.0% (28/35)	(64.1, 90.0)	74.3% (26/35)	(57.9, 85.8)
	Specificity (%)	52.3% (123/235)	(46.0, 58.6)	49.8% (124/249)	(43.6, 56.0)
	PPV (%)	20.0% (28/140)	(16.2, 23.3)	17.2% (26/151)	(13.7, 20.4)
	NPV (%)	94.6% (123/130)	(90.6, 97.3)	93.2% (124/133)	(89.2, 96.2)
	PLR	1.7	(1.3, 2.0)	1.5	(1.1, 1.8)
	NLR	0.38	(0.19, 0.70)	0.52	(0.28, 0.86)
	Prevalence (%)	13.0% (35/270)		12.3% (35/284)	
≥CIN3	Sensitivity (%)	84.6% (11/13)	(57.8, 95.7)	76.9% (10/13)	(49.7, 91.8)
	Specificity (%)	49.8% (128/257)	(43.7, 55.9)	48.0% (130/271)	(42.1, 53.9)
	PPV (%)	7.9% (11/140)	(5.4, 9.4)	6.6% (10/151)	(4.3, 8.2)
	NPV (%)	98.5% (128/130)	(95.8, 99.7)	97.7% (130/133)	(95.1, 99.3)
	PLR	1.7	(1.1, 2.0)	1.5	(0.9, 1.9)
	NLR	0.31	(0.06, 0.86)	0.48	(0.15, 1.07)
	Prevalence (%)	4.8% (13/270)		4.6% (13/284)	

Table 65 displays a summary of the CPRP diagnosis by redefining disease status based on the lesion directed biopsies; therefore, subjects without lesions would had have random biopsies and would be defined as non-diseased.

Table 65

Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis for the Direct Biopsy in the Vaccinated ASC-US Population (≥21 Years) - SurePath

cobas HPV Test Result	No Lesion	Central Pathology Review Panel Diagnosis from Lesion					
		Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	63	41	17	10	9	0	140
Negative	97	27	4	2[a]	0	0	130
Total	160	68	21	12	9	0	270

[a] 1 CIN2 case was FDA approved test negative and sequence negative; and 1 CIN2 case was FDA approved test negative and sequence positive.

Table 66 presents the performance of the **cobas**® HPV Test and the performance of the FDA approved HPV Test in STM in detecting ≥CIN2 and ≥CIN3 by redefining disease status in subjects who had random biopsies as non-diseased. Under this modified definition of disease the sensitivity of detecting ≥CIN2 was 90.5% (19/21) (95% CI = 71.1% to 97.4%) for the **cobas**® HPV Test and for the FDA approved HPV Test. The specificity for detecting ≥CIN2 was 51.4% (128/249) (95% CI = 45.2% to 57.6%) for the **cobas**® HPV Test and 49.8% (131/263) (95% CI=43.8% to 55.8%) for the FDA approved HPV Test.

Under this modified definition of disease the sensitivity of detecting ≥CIN3 was 100% (9/9) (95% CI = 70.1% to 100%) for the **cobas**® HPV Test and for the FDA approved HPV Test. The specificity for detecting ≥CIN3 was 49.8% (130/261) (95% CI = 43.8% to 55.8%) for the **cobas**® HPV Test and 48.4% (133/275) (95% CI=42.5% to 54.3%) for the FDA approved HPV Test.

Table 66

Performance of the cobas® HPV Test and an FDA Approved HPV test Considering Subjects With Random Biopsy as Non-diseased in the Vaccinated ASC-US Population (≥ 21 Years)- SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	Point Estimate	95% CI	Point Estimate	95% CI
≥CIN2	Sensitivity (%)	90.5% (19/21)	(71.1, 97.4)	90.5% (19/21)	(71.1, 97.4)
	Specificity (%)	51.4% (128/249)	(45.2, 57.6)	49.8% (131/263)	(43.8, 55.8)
	PPV (%)	13.6% (19/140)	(10.7, 15.6)	12.6% (19/151)	(10.0, 14.4)
	NPV (%)	98.5% (128/130)	(95.4, 99.7)	98.5% (131/133)	(95.5, 99.7)
	PLR	1.9	(1.4, 2.2)	1.8	(1.4, 2.1)
	NLR	0.19	(0.04, 0.57)	0.19	(0.04, 0.59)
	Prevalence (%)	7.8% (21/270)		7.4% (21/284)	
≥CIN3	Sensitivity (%)	100% (9/9)	(70.1, 100)	100% (9/9)	(70.1, 100)
	Specificity (%)	49.8% (130/261)	(43.8, 55.8)	48.4% (133/275)	(42.5, 54.3)
	PPV (%)	6.4% (9/140)	(4.5, 7.2)	6.0% (9/151)	(4.2, 6.6)
	NPV (%)	100% (130/130)	(97.7, 100)	100% (133/133)	(97.8, 100)
	PLR	2.0	(1.4, 2.3)	1.9	(1.3, 2.2)
	NLR	0.00	(0.00, 0.67)	0.00	(0.00, 0.69)
	Prevalence (%)	3.3% (9/270)		3.2% (9/284)	

* Subjects with random biopsy (no direct biopsy) were redefined as having a normal colposcopy and are included in the analysis as non-diseased. For subjects with direct biopsies, CPRP diagnosis remained unchanged.

Table 67 presents the performance of the **cobas**® HPV Test and the performance of the FDA approved HPV test in STM in detecting ≥CIN2 and ≥CIN3 by biopsy status in the vaccinated population. When confined to subjects with direct biopsies, the sensitivity of detecting ≥CIN2 was 90.5% (19/21) (95% CI = 71.1% to 97.4%) for the **cobas**® HPV Test and for the FDA approved HPV test. The specificity of detecting ≥CIN2 was 34.8% (31/89) (95% CI = 25.8% to 45.2%) for the **cobas**® HPV Test and 30.3% (30/99) (95% CI = 22.1% to 40.0%) for the FDA approved HPV test. With direct biopsies, the sensitivity of detecting ≥CIN3 was 100% (9/9) (95% CI = 70.1% to 100%) for the **cobas**® HPV Test and for the FDA approved HPV test. The specificity of detecting ≥CIN3 was 32.7% (33/101) (95% CI = 24.3% to 42.3%) for the **cobas**® HPV Test and 28.8% (32/111) (95% CI = 21.1% to 37.9%) for the FDA approved HPV test.

When confined to subjects with random biopsies, the sensitivity of detecting ≥CIN2 was 64.3% (9/14) (95% CI = 38.8% to 83.7%) for the **cobas**® HPV Test and 50.0% (7/14) (95% CI = 26.8% to 73.2%) for the FDA approved HPV test. The specificity of detecting ≥CIN2 was 63.0% (92/146) (95% CI = 54.9% to 70.4%) for the **cobas**® HPV Test and 62.7% (94/150) (95% CI = 54.7% to 70.0%) for the FDA approved HPV test. With random biopsies, the sensitivity of detecting ≥CIN3 was 50.0% (2/4) (95% CI = 15.0% to 85.0%) for the **cobas**® HPV Test and 25.0% (1/4) (95%

CI = 4.6% to 69.9%) for the FDA approved HPV test. The specificity of detecting ≥CIN3 was 60.9% (95/156) (95% CI = 54.9% to 70.4%) for the **cobas**® HPV Test and 61.3% (98/160) (95% CI = 53.5% to 68.5%) for the FDA approved HPV test.

Table 67

Performance of the cobas® HPV Test and an FDA Approved HPV test by Subject Biopsy Status in the Vaccinated ASC-US Population (≥21 Years) - SurePath

Disease Endpoint		cobas® HPV test		FDA approved test using STM	
	Performance	Lesion-Directed Biopsy Point Estimate	No Lesion-Random Biopsy Point Estimate	Lesion-Directed Biopsy Point Estimate	No Lesion-Random Biopsy Point Estimate
≥CIN2	Sensitivity (%) 95% CI	90.5% (19/21) (71.1, 97.4)	64.3% (9/14) (38.8, 83.7)	90.5% (19/21) (71.1, 97.4)	50.0% (7/14) (26.8, 73.2)
	Specificity (%) 95% CI	34.8% (31/89) (25.8, 45.2)	63.0% (92/146) (54.9, 70.4)	30.3% (30/99) (22.1, 40.0)	62.7%(94/150) (54.7, 70.0)
	Prevalence (%)	19.1% (21/110)	8.8% (14/160)	17.5% (21/120)	8.5% (14/164)
≥CIN3	Sensitivity (%) 95% CI	100% (9/9) (70.1, 100)	50.0% (2/4) (15.0, 85.0)	100% (9/9) (70.1, 100)	25.0% (1/4) (4.6, 69.9)
	Specificity (%) 95% CI	32.7% (33/101) (24.3, 42.3)	60.9% (95/156) (54.9, 70.4)	28.8% (32/111) (21.1, 37.9)	61.3% (98/160) (53.5, 68.5)
	Prevalence (%)	8.2% (9/110)	2.5% (4/160)	7.5% (9/120)	2.4% (4/164)
HPV positivity		70.0% (77/110)	39.4% (63/160)	73.3% (88/120)	38.4% (63/164)

Performance of the **cobas**® HPV test in detecting ≥ CIN2 and ≥ CIN3 by age group in the vaccinated population is presented in Table 68. The sensitivity of **cobas**® HPV Test for detecting ≥CIN2 was 78.1% (25/32) (95% CI = 61.2% to 89.0%) in the 21 – 29 year age group, 100% (3/3) (95% CI = 43.9% to 100%) in the 30 – 39 year age group. The specificity of the test was higher in 30 – 39 year age group, with an estimate of 56.5% (13/23) (95% CI = 36.8% to 74.4%).

The sensitivity of **cobas**® HPV Test for detecting ≥CIN3 was 81.8% (9/11) (95% CI = 52.3% to 94.9%) in the 21 – 29 year age group, 100% (2/2) (95% CI = 34.2% to 100%) in the 30 – 39 year age group. The specificity of the test was higher in 30 – 39 year, with an estimate of 54.2% (13/24) (95% CI = 35.1% to 72.1%).

Table 68

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

Performance	21-29 Years	30-39 Years	≥ 40 Years
N	244	26	NA
≥ CIN2			
Sensitivity (%)	78.1% (25/32)	100% (3/3)	NA
95% CI (%)	(61.2, 89.0)	(43.9, 100)	
Specificity (%)	51.9% (110/212)	56.5% (13/23)	NA
95% CI (%)	(45.2, 58.5)	(36.8, 74.4)	
PPV (%)	19.7% (25/127)	23.1% (3/13)	NA
95% CI (%)	(15.7, 23.3)	(10.5, 34.3)	
NPV (%)	94.0% (110/117)	100% (13/13)	NA
95% CI (%)	(89.7, 96.9)	(86.8, 100)	
Prevalence (%)	13.1% (32/244)	11.5% (3/26)	NA
≥ CIN3			
Sensitivity (%)	81.8% (9/11)	100% (2/2)	NA
95% CI (%)	(52.3, 94.9)	(34.2, 100)	
Specificity (%)	49.4% (115/233)	54.2% (13/24)	NA
95% CI (%)	(43.0, 55.7)	(35.1, 72.1)	
PPV (%)	7.1% (9/127)	15.4% (2/13)	NA
95% CI (%)	(4.6, 8.6)	(3.5, 23.3)	
NPV (%)	98.3% (115/117)	100% (13/13)	NA
95% CI (%)	(95.5, 99.6)	(89.4, 100)	
Prevalence (%)	4.5% (11/244)	7.7% (2/26)	NA

ASC-US (≥ 21 Years) Non-Vaccinated Population in Samples Collected in SurePath – Likelihood ratios and Risk Estimates

Likelihood ratios (LRs) and the risks of disease (≥ CIN2 and ≥ CIN3) with 95% CIs for **cobas**® HPV Test results (HR HPV16 positive/18 positive, 12 Other HR, and HR HPV negative) are presented in Table 69 for the non-vaccinated ASC-US (≥21 years) population.
For ≥ CIN2 histology, the estimate of the LR of HPV16 positive/18 positive was 5.2, indicating that an HPV16 positive/18 positive result is 5.2 times more likely to occur in a subject with disease (≥ CIN2) than in a subject without disease (< CIN2). The risk of a ≥ CIN2 outcome for an ASC-US subject with an HPV16 positive/18 positive result was 28.1%. The LR of 12 Other HR HPV positive was 1.4. Both LRs were significantly greater than 1.
The estimate of the LR of a negative **cobas**® HPV Test result was 0.3, indicating that a negative result was 0.3 times more likely to occur in a subject without disease (< CIN2) than in a subject with disease (≥ CIN2).

The risk of disease (≥ CIN2) is the chance (probability) of having the disease given an HPV test result. The risk of disease (≥ CIN2) was 6.9% in the ASC-US population regardless of the HPV test result (prevalence = 6.9%). 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 a HR HPV negative result.

For ≥ CIN3 histology, LR of HPV16 positive/18 positive was 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 3.3% in the ASC-US population (see Table 69). The risk of ≥ CIN3 was significantly increased for the HPV16 positive/18 positive and 12 Other HR HPV positive.

Table 69

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

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
≥CIN2	HPV 16 Positive /18 Positive	5.2 (3.2, 8.5)	28.1 (16/57) (19.4, 38.7)
	12 Other HR HPV Positive	1.4 (1.0, 2.0)	9.3 (18/193) (6.7, 12.9)
	Negative	0.3 (0.1, 0.5)	1.8 (6/326) (0.9, 3.8)
	Prevalence (%)	6.9% (5.6, 8.5)	
≥CIN3	HPV 16 Positive /18 Positive	7.0 (4.4, 11.2)	19.3 (11/57) (13.0, 27.7)
	12 Other HR HPV Positive	0.9 (0.5, 1.8)	3.1 (6/193) (1.6, 5.9)
	Negative	0.2 (0.0, 0.7)	0.6 (2/326) (0.2, 2.2)
	Prevalence (%)	3.3% (2.4, 4.5)	

ASC-US (≥ 21 Years) the Non-Vaccinated Population in Samples Collected in SurePath - Absolute and Relative Risk

The CPRP diagnosis by all possible **cobas** HPV Test results in the non-vaccinated ASC-US population is presented in Table 70.

Table 70

Summary of the cobas® HPV Test Results and Central Pathology Review Panel Diagnosis in the Non-vaccinated ASC-US Population (≥21 years) – SurePath

	Central Pathology Review Diagnosis					
cobas® HPV Test Result	**Normal**	**CIN1**	**CIN2**	**CIN3**	**Cancer**	**Total**
NEG Other HR HPV, NEG HPV16, NEG HPV18	296	24	4	2	0	326
NEG Other HR HPV, NEG HPV16, POS HPV18	4	2	0	1	0	7
NEG Other HR HPV, POS HPV16, NEG HPV18	11	2	3	6	2	24
POS Other HR HPV, NEG HPV16, NEG HPV18	146	29	12	6	0	193
POS Other HR HPV, NEG HPV16, POS HPV18	7	0	0	1	0	8
POS Other HR HPV, POS HPV16, NEG HPV18	10	4	2	1	0	17
POS Other HR HPV, POS HPV16, POS HPV18	1	0	0	0	0	1
Total	475	61	21	17	2	576

The CPRP diagnosis and the absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas**® HPV Test result are presented in Table 71 for the non-vaccinated ASC-US population . HPV16 positive/18 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3. In general, the absolute risks for both ≥ CIN2 and ≥ CIN3 were higher in women with results of HPV positive, HPV16 positive/18 positive, or 12 Other HR positive than in women with an HPV negative result.

Table 71

Central Pathology Review Diagnosis and Absolute Risk of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the Non-vaccinated ASC-US Population (≥ 21 Years) – SurePath

cobas® HPV Test Result	Total	Central Pathology Review Diagnosis					Absolute Risk for ≥ CIN2 (%)	Absolute Risk for ≥ CIN3 (%)
		Normal	CIN1	CIN2	CIN3	Cancer		
HPV16 positive	42	22	6	5	7	2	33.3% (14/42)	21.4% (9/42)
HPV18 positive	15	11	2	0	2	0	13.3% (2/15)	13.3% (2/15)
12 Other HR HPV positive	193	146	29	12	6	0	9.3% (18/193)	3.1% (6/193)
HPV negative	326	296	24	4	2	0	1.8% (6/326)	0.6% (2/326)
HPV16 positive and/or HPV18 positive	57	33	8	5	9	2	28.1% (16/57)	19.3% (11/57)
HPV positive	250	179	37	17	15	2	13.6% (34/250)	6.8% (17/250)
Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18								

The relative risks (RRs) of disease (≥ CIN2 and ≥ CIN3) were calculated for women with different **cobas**® HPV Test results by RR and its associated 95% CIs as presented in Table 72 for the non-vaccinated ASC-US population. The estimated RRs of ≥ CIN2 and of ≥ CIN3 for women with positive vs. negative **cobas**® HPV Test results were 7.4 (95% CI: 3.2 to 17.3) and 11.1 (95% CI: 2.6 to 47.5), respectively, indicating that women with a positive result were 7.4 times more likely to have ≥ CIN2 histology and 11.1 times more likely to have ≥ CIN3 histology than were women with a negative test result.

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

Table 72
Relative Risks of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the Non-vaccinated ASC-US Population (≥ 21 Years) - SurePath

cobas® HPV Test Result	CPRP Diagnosis ≥ CIN2		CPRP Diagnosis ≥ CIN3	
	Relative Risk	95% CI	Relative Risk	95% CI
HPV Positive vs. Negative	7.4	(3.2, 17.3)	11.1	(2.6, 47.5)
HPV16 positive/18 positive vs. Negative	15.3	(6.2, 37.3)	31.5	(7.2, 138.2)
HPV16 positive /18 positive vs. 12 Other HR HPV positive	3.0	(1.6, 5.5)	6.2	(2.4, 16.0)
12 Other HR HPV positive vs. Negative	5.1	(2.0, 12.5)	5.1	(1.0, 24.9)
Prevalence (%)	6.9%		3.3%	
Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18				

The absolute risks of disease (≥ CIN2 and ≥ CIN3) by **cobas**® HPV Test result stratified by age group in the non-vaccinated population are presented in Table 73. HPV16 positive/18 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3 in each age group, followed by Other 12 HR positive.

Table 73
Absolute Risk of ≥ CIN2 and ≥ CIN3 by cobas HPV Test Result Stratified by Age in the Non-vaccinated ASC-US Population (≥ 21 Years) – SurePath

	Age Group (Years)		
cobas HPV Test Result	**21-29**	**30-39**	**≥ 40**
Absolute Risk for ≥ CIN2 (%) (95% CI)			
HPV16 positive /18 positive	30.0% (3/10) (10.8, 60.3)	25.8% (8/31) (13.7, 43.2)	31.3% (5/16) (14.2, 55.6)
Other 12 HR HPV positive	8.7% (4/46) (3.4, 20.3)	11.5% (9/78) (6.2, 20.5)	7.2% (5/69) (3.1, 15.9)
Negative	0.0% (0/51) (0.0, 7.0)	3.0% (3/99) (1.0, 8.5)	1.7% (3/176) (0.6, 4.9)
Prevalence (%)	6.5% (7/107)	9.6% (20/208)	5.0% (13/261)
Absolute Risk for ≥ CIN3 (%) (95% CI)			
HPV16 positive /18 positive	20.0% (2/10) (5.7, 51.0)	19.4% (6/31) (9.2, 36.3)	18.8% (3/16) (6.6, 43.0)
Other 12 HR HPV positive	4.3% (2/46) (1.2, 14.5)	3.8% (3/78) (1.3, 10.7)	1.4% (1/69) (0.3, 7.8)
Negative	0.0% (0/51) (0.0, 7.0)	2.0% (2/99) (0.6, 7.1)	0.0% (0/176) (0.0, 2.1)
Prevalence (%)	3.7% (4/107)	5.3% (11/208)	1.5% (4/261)
Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes and with negative results for HPV16 and HPV18.			

ASC-US (≥ 21 Years) Vaccinated Population in Samples Collected in SurePath – Likelihood ratios and Risk Estimates

Likelihood ratios (LRs) and the risks of disease (≥ CIN2 and ≥ CIN3) with 95% CIs for **cobas**® HPV Test results (HR HPV16 positive/18 positive, 12 Other HR, and HR HPV negative) are presented in Table 74 for the vaccinated ASC-US (≥21 years) population.

For ≥ CIN2 histology, the estimate of the LR of HPV16 positive/18 positive was 4.9, indicating that an HPV16 positive/18 positive result is 4.9 times more likely to occur in a subject with disease (≥ CIN2) than in a subject without disease (< CIN2). The risk of a ≥ CIN2 outcome for an ASC-US subject with an HPV16 positive/18 positive result was 42.1% (significantly different from 1). The LR of 12 Other HR HPV positive was 1.3.
The estimate of the LR of a negative **cobas**® HPV Test result was 0.4, indicating that a negative result was 0.4 times more likely to occur in a subject without disease (< CIN2) than in a subject with disease (≥ CIN2).

The risk of disease (≥ CIN2) is the chance (probability) of having the disease given an HPV test result. The risk of disease (≥ CIN2) was 13.0% in the ASC-US population regardless of the HPV test result (prevalence = 13.0%).

For ≥ CIN3 histology, LR of HPV16 positive/18 positive was 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 4.8% in the ASC-US population (see Table 74). The risk of ≥ CIN3 was significantly increased for the HPV16 positive/18 positive and 12 Other HR HPV positive.

Table 74
Likelihood Ratios and Risk of Disease by cobas® HPV Test Result in Detecting ≥ CIN2 and ≥ CIN3 in the Vaccinated ASC-US Population (≥ 21 Years) - SurePath

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
≥ CIN2	HPV16 positive/18 positive	4.9 (2.1, 10.9)	42.1% (8/19) (23.9, 62.0)
	12 Other HR HPV positive	1.3 (0.9, 1.8)	16.5% (20/121) (12.1, 20.9)
	HPV Negative	0.4 (0.2, 0.7)	5.4% (7/130) (2.7, 9.4)
	Prevalence (%)	13.0% (35/270)	
≥ CIN3	HPV16 positive/18 positive	11.5 (5.1, 23.5)	36.8% (7/19) (20.6, 54.3)
	12 Other HR HPV positive	0.7 (0.3, 1.3)	3.3% (4/121) (1.4, 6.2)

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
	HPV Negative	0.31 (0.06, 0.86)	1.54% (2/130) (0.32, 4.19)
	Prevalence (%)	4.8% (13/270)	

ASC-US (≥ 21 Years) Vaccinated Population in Samples Collected in SurePath - Absolute and Relative Risk

The CPRP diagnosis by all possible **cobas** HPV Test results in vaccinated ASC-US population is presented in Table 75.

Table 75
Summary of the cobas® HPV Test Results (PreQuot) and Central Pathology Review Panel Diagnosis in the Vaccinated ASC-US Population (≥ 21 Years) - SurePath

	Central Pathology Review Diagnosis					
cobas® HPV Test Result	**Normal**	**CIN1**	**CIN2**	**CIN3**	**Cancer**	**Total**
NEG Other HR HPV, NEG HPV16, NEG HPV18	114	9	5	2	0	130
NEG Other HR HPV, NEG HPV16, POS HPV18	2	0	0	0	1	3
NEG Other HR HPV, POS HPV16, NEG HPV18	2	0	0	2	0	4
POS Other HR HPV, Invalid HPV16, NEG HPV18	0	0	1	0	0	1
POS Other HR HPV, NEG HPV16, NEG HPV18	74	27	15	4	0	120
POS Other HR HPV, NEG HPV16, POS HPV18	3	0	0	0	0	3
POS Other HR HPV, POS HPV16, NEG HPV18	4	0	1	4	0	9
Total	199	36	22	12	1	270

The CPRP diagnosis and the absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas**® HPV Test result are presented in Table 76 for the vaccinated ASC-US population. HPV16 positive/18 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3. In general, the absolute risks for both ≥ CIN2 and ≥ CIN3 were higher in women with results of HPV positive, HPV16 positive/18 positive, or 12 Other HR positive than in women with an HPV negative result.

Table 76
Central Pathology Review Diagnosis and Absolute Risk of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the Vaccinated ASC-US Population (≥ 21 Years) - SurePath

cobas® HPV Test Result	Total	Central Pathology Review Diagnosis					Absolute Risk for ≥ CIN2 (%)	Absolute Risk for ≥ CIN3 (%)
		Normal	CIN1	CIN2	CIN3	Cancer		
HPV16 positive	13	6	0	1	6	0	53.8% (7/13)	46.2% (6/13)
HPV18 positive	6	5	0	0	0	1	16.7% (1/6)	16.7% (1/6)
12 Other HR HPV positive	121	74	27	16	4	0	16.5% (20/121)	3.3% (4/121)
HPV negative	130	114	9	5	2	0	5.4% (7/130)	1.5% (2/130)
HPV16 positive and/or HPV18 positive	19	11	0	1	6	1	42.1% (8/19)	36.8% (7/19)
HPV positive	140	85	27	17	10	1	20.0% (28/140)	7.9% (11/140)
Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes and with negative results for HPV16 and HPV18								

The relative risks (RRs) of disease (≥ CIN2 and ≥ CIN3) were calculated for vaccinated women with different **cobas**® HPV Test results by RR and its associated 95% CIs as presented in Table 77. The estimated RRs of ≥ CIN2 and of ≥ CIN3 for women with positive vs. negative **cobas**® HPV Test results were 3.7 (95% CI: 1.7 to 8.2) and 5.1 (95% CI: 1.2 to 22.6), respectively, indicating that women with a positive result were 3.7 times more likely to have ≥ CIN2 histology and 5.1 times more likely to have ≥ CIN3 histology than were women with a negative test result.

Similarly, women who have HPV16 and/or HPV18 positive results from the **cobas**® HPV Test were significantly more likely to have ≥ CIN2 than the women with (a) a negative result, or (b) a positive result for 12 Other HR HPV types. Women with a positive result for 12 Other HR HPV types were significantly more likely to have ≥ CIN2 than the women with a negative result. Similar results were observed for ≥ CIN3 histology. ***Table 77***

Relative Risks of ≥ CIN2 and ≥ CIN3 for Different cobas® HPV Test Results in the Vaccinated ASC-US Population (≥ 21 Years) - SurePath

cobas® HPV Test Result	CPRP Diagnosis ≥ CIN2		CPRP Diagnosis ≥ CIN3	
	Relative Risk	95% CI	Relative Risk	95% CI
HPV Positive vs. Negative	3.7	(1.7, 8.2)	5.1	(1.2, 22.6)
HPV16 positive/18 positive vs. Negative	7.8	(3.2, 19.1)	23.9	(5.4, 106.9)
HPV16 positive /18 positive vs. 12 Other HR HPV positive	2.5	(1.3, 4.9)	11.1	(3.6, 34.5)
12 Other HR HPV positive vs. Negative	3.1	(1.3, 7.0)	2.1	(0.4, 11.5)
Prevalence	13.0% (35/270)		4.8% (13/270)	
Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes and with negative results for HPV16 and HPV18				

The absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas**® HPV Test result stratified by age group in the vaccinated population are presented in Table 78 HPV16 positive/18 positive had the highest absolute risk for both ≥ CIN2 and ≥ CIN3 in each age group followed by Other 12 HR positive.

Table 78
Absolute Risk of ≥ CIN2 and ≥ CIN3 by cobas HPV Test Result Stratified by Age in the Vaccinated ASC-US Population (≥ 21 Years) – SurePath

	Age Group (Years)		
cobas HPV Test Result	**21-29**	**30-39**	**≥ 40**
Absolute Risk for ≥CIN2 (%) (95% CI)			
HPV16 positive /18 positive	40.0% (6/15) (19.8, 64.3)	50.0% (2/4) (15.0, 85.0)	NA
Other 12 HR HPV positive	17.0% (19/112) (11.1,25.0)	11.1% (1/9) (2.0,43.5)	NA
Negative	6.0% (7/117) (2.9,11.8)	0.0% (0/13) (0.0,22.8)	NA
Prevalence	13.1% (32/244) (9.4, 17.9)	11.5% (3/26) (4.0, 29.0)	NA
Absolute Risk for ≥CIN3 (%) (95% CI)			
HPV16 positive /18 positive	33.3% (5/15) (15.2, 58.3)	50.0% (2/4) (15.0, 85.0)	NA
Other 12 HR HPV positive	3.6% (4/112) (1.4, 8.8)	0.0% (0/9) (0.0, 29.9)	NA
Negative	1.7% (2/117) (0.5, 6.0)	0.0% (0/13) (0.0, 22.8)	NA
Prevalence	4.5% (11/244) (2.5, 7.9)	7.7% (2/26) (2.1, 24.1)	NA

Note 1: HPV16 positive and/or HPV18 positive include all women 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 women with positive results for 12 Other HR HPV genotypes and with negative results for HPV16 and HPV18.

STUDY DESIGN TO DEMONSTRATE ANALYTICAL PERFORMANCE OF THE **COBAS**® HPV TEST IN ASC-US WOMEN ≥ 21 YEARS

To demonstrate analytical performance of **cobas**® HPV Test approximately 700 samples with ASC-US cytology results were compared to a composite comparator comprising HPV DNA sequencing and, an FDA approved HR HPV DNA test from samples collected in STM. Cervical samples were obtained from all women ≥21 years with ASC-US cytology results who participated in the ASC-US triage study and who had valid **cobas** HPV Test results in SurePath and STM samples available for evaluation.

Agreement with a Composite Comparator in Samples Collected in STM Compared to Samples Collected in SurePath for the ASC-US ≥21 Years Population

The analytical performance of the **cobas**® HPV Test was evaluated by comparing results from the test to a composite comparator comprising of HPV DNA sequencing and an FDA-approved HR HPV DNA test. Additionally, the test was also compared directly with DNA sequencing from samples collected in STM. Sequencing was performed at a commercial lab. DNA was extracted from cervical specimens followed by a PCR amplification utilizing both β-globin and PGMY primers. The β-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, 38]. PGMY-positive extracts were then amplified using HR HPV type-specific primers for subsequent sequencing reactions.

All cervical samples were selected from the ASC-US triage study: women ≥21 years who had ASC-US cytology results, valid **cobas**® HPV Test results in SurePath and adequate STM sample volume (n = 678). Of these, 677 were from eligible subjects and had valid **cobas**® HPV Test results in the pre-quot; of these, 640 had valid sequencing results and 37 were invalid. Invalid samples were negative for both β-globin and PGMY primers. Table 79 displays the agreement between the **cobas**® HPV Test and the composite comparator (FDA-approved HPV test and HPV DNA Sequencing in STM). The PPA, NPA and OPA between the **cobas**® HPV test and the composite comparator were 95.4% (206/216) with 95% CI = 91.7% to 97.5%, 93.2% (288/309) with 95% CI = 89.8% to 95.5 % and 94.1% (495/515) with 95% CI = 91.8% to 95.8 %, respectively. Seventeen percent (17%) of the composite comparator results were indeterminate (discordant between sequencing and the FDA-approved HPV test) and 5.5% (37/678) were invalid with sequencing.

Table 79

Agreement Between the cobas® HPV Test and the Composite Comparator (Sequencing/FDA-approved HPV test in STM) in the ASC-US Population (≥21 Years)- SurePath

	Composite Comparator				
cobas® HPV Test in prequot	**Positive**	**Negative**	**Indeterminate**	**Invalid**	**Total**
Positive	206	21	57	16	301
Negative	10	288	58	21	377
Total	216 (31.9%)	309 (45.6 %)	115 (17.0 %)	37 (5.5 %)	677
PPA: 95.4% (206/216), 95% CI: (91.7 %, 97.5 %)					
NPA: 93.2% (288/309), 95% CI: (89.8 %, 95.5 %)					
OPA: 94.1% (495/526), 95% CI: (91.8 %, 95.8 %)					

Tables 80 - 82 present the HPV genotype-specific percent agreement between the **cobas**® HPV Test and HPV DNA sequencing stratified by CPRP diagnosis (≥CIN2 and <CIN2) for the detection of HPV16, HPV18 and 12 Other HR HPV. The PPA, NPA and OPA between **cobas**® HPV Test and HPV DNA sequencing was ~ 94% for the detection of HPV16 and 100% for the detection of HPV18 among subjects with CPRP diagnosis ≥CIN2.

The PPA, NPA and OPA between **cobas**® HPV Test and HPV DNA sequencing for the detection of 12 Other HR HPV were ~ 83.0%, 79.2% and 81.5% respectively among subjects with CPRP diagnosis ≥CIN2. Of the 3 ≥CIN2 cases where **cobas** HPV Test results were 12 Other HR HPV negative, 2 were HPV16 positive by the **cobas**® HPV Test whereas both HPV16 and 39 were positive by sequencing; one was HPV16 positive by **cobas**® HPV Test and both HPV16 and 59 positive by sequencing. Of the 4 ≥CIN2 cases with HPV negative by the cobas, 3 were ⩾CIN2 and positive for HPV genotypes 33, 45, 56 and 66 by sequencing; 1 case was CIN3 and HPV 52 positive by sequencing.

Table 80

Percent Agreement between the cobas® HPV Test HPV16 Results vs. HPV16 Sequencing in STM Comparator Stratified by CPRP Diagnosis of ≥ CIN2 or <CIN2 in the ASC-US Population (≥21 Years)- SurePath

	Sequencing Results						
	⩾ CIN2				< CIN2		
cobas HPV Test Result in prequot	**HPV 16 Positive**	**HPV 16 Negative**	**Total**		**HPV 16 Positive**	**HPV 16 Negative**	**Total**
HPV 16 Detected	15	3	18		12	12	24
HPV 16 Not Detected, but either HPV 18 or 12 other HR HPV Detected	1*	46	47		4	534	540
HR HPV Not Detected	0				2		
Total	16	49	65		18	546	564
PPA: 93.8% (15/16), 95% CI: (71.7 %, 98.9 %)					PPA: 66.7% (12/18), 95% CI: (43.7 %, 83.7 %)		
NPA: 93.9% (46/49), 95% CI: (83.5 %, 97.9 %)					NPA: 97.8% (534/546), 95% CI: (96.2 %, 98.7 %)		
OPA: 93.8% (61/65), 95% CI: (85.2 %, 97.6 %)					OPA: 96.8% (546/564), 95% CI: (95.0 %, 98.0 %)		

*12 Other HR HPV positive by the **cobas**® HPV Test and HPV genotypes, 51, 56, and 68 were positive by sequencing.

Table 81
Percent Agreement between the cobas® HPV Test HPV18 Results vs. HPV18 Sequencing in STM Comparator Stratified by CPRP Diagnosis of ≥ CIN2 or <CIN2 in the ASC-US Population (≥21 Years)- SurePath

	Sequencing Results						
	≥ CIN2				< CIN2		
cobas HPV Test Result in prequot	**HPV 18 Positive**	**HPV 18 Negative**	**Total**		**HPV 18 Positive**	**HPV 18 Negative**	**Total**
HPV 18 Detected	3	0	3		12	3	15
HPV 18 Not Detected, but either HPV 16 or 12 other HR HPV Detected	0	62	62		0	549	549
HR HPV Not Detected	0				0		
Total	3	62	65		12	552	564
PPA: 100.0% (3/3), 95% CI: (43.9 %, 100.0 %)					PPA: 100.0% (12/12), 95% CI: (75.8 %, 100.0 %)		
NPA: 100.0% (62/62), 95% CI: (94.2 %, 100.0 %)					NPA: 99.5% (549/552), 95% CI: (98.4 %, 99.8 %)		
OPA: 100.0% (65/65), 95% CI: (94.4 %, 100.0 %)					OPA: 99.5% (561/564), 95% CI: (98.4 %, 99.8 %)		

Table 82
Percent Agreement between the cobas® HPV Test 12 Other HR HPV Results vs. 12 Other HR HPV Sequencing in STM Comparator Stratified by CPRP Diagnosis of ≥ CIN2 or <CIN2 in the ASC-US Population (≥21 Years)- SurePath

	Sequencing Results						
	≥ CIN2				< CIN2		
cobas HPV Test Result in prequot	**12 other HR HPV Positive**	**12 other HR HPV Negative**	**Total**		**12 other HR HPV Positive**	**12 other HR HPV Negative**	**Total**
12 other HPV Detected	34	5	39		156	57	213
12 other HPV Not Detected, but either HPV 16 or HPV 18 Detected	3[a]	19	26		0	314	351
HR HPV Not Detected	4[b]				37		
Total	41	24	65		193	371	564
PPA: 82.9% (34/41), 95% CI: (68.7 %, 91.5 %)					PPA: 80.8% (156/193), 95% CI: (74.7 %, 85.8 %)		
NPA: 79.2% (19/24), 95% CI: (59.5 %, 90.8 %)					NPA: 84.6% (314/371), 95% CI: (80.6 %, 87.9 %)		
OPA: 81.5% (53/65), 95% CI: (70.4 %, 89.1 %)					OPA: 83.3% (470/564), 95% CI: (80.0 %, 86.2 %)		

[a] 2 cases were HPV16 positive by the **cobas**® HPV Test and HPV16 and 39 positive by sequencing; 1 case was HPV16 positive by the cobas® HPV Test and HPV16 and 59 positive by sequencing.

[b] 3 cases were ⩾CIN2 and HPV genotypes 33, 45, 56 and 66 by sequencing; 1 case was CIN3 and HPV 52 positive by sequencing.

Agreement Between Pre and Post-Cytology for the ASC-US Population in Samples Collected in SurePath ≥21 Years

Performance for the cytology specimen type was also assessed during the clinical study. The postquot demonstrated similar clinical performance (sensitivity and specificity) to the prequot specimen. For example, for lesion-directed biopsies, the sensitivity for detecting ≥CIN2 and ≥CIN3 in the non-vaccinated population was 88.9% (24/27) and 92.3% (12/13) respectively. The specificity for detecting ≥CIN2 and ≥CIN3 was 39.4% (69/175) and 37.6% (71/189) respectively (Table 83).

Table 83
Performance of the cobas HPV Test in the Postquot and Prequot in Detecting ≥CIN2 and ≥CIN3 for the Non-Vaccinated Population with Lesion Directed Biopsy – SurePath

Disease Endpoint		**Point Estimate**	
	Performance	**Postquot**	**Prequot**
≥CIN2	Sensitivity (%)	88.9 (24/27)	88.9% (24/27)
	95%CI	(71.9, 96.1)	(71.9, 96.1)
	Specificity (%)	39.4 (69/175)	41.2% (73/177)
	95%CI	(32.5, 46.8)	(34.3, 48.7)
	Prevalence (%)	13.4 (27/202)	13.2% (27/204)
	95%CI	(9.4, 18.7)	(9.3, 18.6)
≥CIN3	Sensitivity (%)	92.3 (12/13)	92.3%(12/13)
	95%CI	(66.7, 98.6)	(66.7, 98.6)

Disease Endpoint		Point Estimate	
	Performance	Postquot	Prequot
	Specificity (%)	37.6 (71/189)	38.3%(75/191)
	95%CI	(31.0, 44.7)	(32.6, 46.3)
	Prevalence (%)	6.4 (13/202)	6.4% (13/204)
	95%CI	(3.8, 10.7)	(3.8, 10.6)

Table 84 displays the agreement between test results by the **cobas**® HPV Test in SurePath using the prequot (pre-cytology) and postquot (post-cytology) samples. The PPA, NPA and OPA between the prequot and postquot test results from the **cobas**® HPV Test were 97.7% (374/383) (95% CI = 95.6% to 98.8%), 97.0% (453/467) (95% CI = 95.0% to 98.2 %) and 97.3% (827/850) (95% CI = 96.0% to 98.2 %), respectively.

Table 85 displays the agreement between four-category test results by the **cobas**® HPV Test in SurePath using the prequot and postquot samples by genotype.

Table 84
Agreement between the cobas® HPV Test Results in Prequot and Postquot in the ASC-US Population ≥21 Years – SurePath

	cobas® HPV Test Result in Postquot		
cobas® HPV Test Result in Prequot	**Positive**	**Negative**	**Total**
Positive	374	14	388
Negative	9	453	462
Total	383 (45.1 %)	467 (54.9 %)	850
PPA: 97.7% (374/383), 95% CI: (95.6 %, 98.8 %)			
NPA: 97.0% (453/467), 95% CI: (95.0 %, 98.2 %)			
OPA: 97.3% (827/850), 95% CI: (96.0 %, 98.2 %)			

Table 85
Summary of Four-Category cobas HPV Test Results by Pre- and Postquot in the ASC-US Population ≥21 Years - SurePath

	cobas HPV Test Result Postquot				
cobas HPV Test Result Prequot	**HPV 16 Positive**	**HPV 18 Positive**	**12 Other HR HPV Positive**	**Negative**	**Total**
HPV16	48	0	2	3	53
HPV18	2	17	0	1	20
12 Other HR HPV	0	0	305	10	315
Negative	2	0	7	453	462
Total	52	17	314	467	850

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

During Year 3 of the Follow-up phase of the ATHENA study a co-collection of samples in PreservCyt and Sure Path collection media was performed if women consented to collection of an additional (second) specimen in SurePath. All study participants either underwent colposcopy if an abnormal cytology result was obtained or were offered an exit colposcopy and ECC to maximize disease ascertainment. 88% of those remaining in Year 3 agreed to undergo the colposcopy/ECC.A total of 4882 women completed the Year 3 follow-up. Of these, 4023 women had valid test results for PreservCyt and SurePath cytology and **cobas**® HPV Test. Similarity between the performance estimates for specimens collected in PreservCyt and SurePath media was demonstrated.

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 women enrolled in Phase 1 of the ATHENA study. The method for selection of cutoff was based on Kondratovich[49] 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, HPV16 and HPV18, respectively) were selected for the **cobas**® HPV Test.

Limit of Detection in PreservCyt® Solution 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 86 contains results from the reagent lot producing the most conservative (highest) LoD in the analysis.

Table 86
Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18) in PreservCyt® Solution

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%

Limit of Detection in SurePath™ Preservative Fluid 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 SurePath™ Preservative Fluid, and 2) HPV positive cell lines SiHa (HPV16) and HeLa (HPV18) in SurePath™ Preservative Fluid 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 49 runs were performed in a period of 12 days using 3 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 87 contains results from the reagent lot producing the most conservative (highest) LoD in the analysis.

Table 87
Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18) in SurePath™ Preservative Fluid

HPV Type	Concentration (copies or cells/mL)	Number of Positive/Tested	Mean CT	% Positives	95% Confidence Interval	
					Lower	Upper
31	600	60/60	37.0	100.0%	94.0%	100.0%
	300	**60/60**	**38.0**	**100.0%**	**94.0%**	**100.0%**
	150	54/60	39.1	90.0%	79.5%	96.2%
16	600	60/60	37.9	100.0%	94.0%	100.0%
	300	**60/60**	**39.0**	**100.0%**	**94.0%**	**100.0%**
	150	51/60	40.1	85.0%	73.4%	92.9%
18	1,500	60/60	36.5	100.0%	94.0%	100.0%
	600	**60/60**	**37.9**	**100.0%**	**94.0%**	**100.0%**
	300	55/59*	38.9	93.2%	83.5%	98.1%
SiHa (HPV16)	400	60/60	36.7	100.0%	94.6%	100.0%
	200	**60/60**	**37.8**	**100.0%**	**94.6%**	**100.0%**
	100	55/60	39.3	91.7%	81.6%	97.2%
HeLa (HPV18)	80	60/60	37.0	100.0%	94.0%	100.0%
	40	**59/60**	**38.3**	**98.30%**	**91.1%**	**100.0%**
	20	43/60	39.6	71.7%	58.6%	82.5%

*One sample not processed due to sample pipetting error

Inclusivity Verification in PreservCyt® Solution

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 88 contains results from the reagent lot producing the most conservative (higher) LoD in the analysis.

Table 88
Summary of High Risk Genotype Limit Of Detection For cobas® 4800 HPV Genotype Inclusivity Study in PreservCyt® Solution

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.

Inclusivity Verification in SurePath™ Preservative Fluid

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 SurePath™ Preservative Fluid 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 89 contains results from the reagent lot producing the most conservative (higher) LoD in the analysis.

Table 89
Summary of High Risk Genotype Limit Of Detection For cobas® 4800 HPV Genotype Inclusivity Study in SurePath™ Preservative Fluid

HPV DNA *Type	LoD (copies/mL)	Number of Positive/Tested	Mean CT	Hit Rate	95% Confidence Interval	
					Lower	Upper
33	600	24/24	37.5	100.0%	85.7%	100.0%
	300	24/24	38.6	100.0%	85.7%	100.0%
	150	22/24	39.4	91.7%	73.0%	99.0%
35	1200	22/24	37.9	100.0%	85.7%	100.0%
	600	23/24	39.2	95.8%	78.8%	99.8%
	300	13/24	40.1	54.2%	32.8%	74.4%
39	300	48/48**	37.2	100.0%	92.6%	100.0%
	150	24/24	38.1	100.0%	85.7%	100.0%
	80	23/24	39.1	95.8%	78.8%	99.8%
45	600	48/48**	36.7	100.0%	92.6%	100.0%
	300	24/24	37.3	100.0%	85.7%	100.0%
	150	22/24	37.9	91.7%	73.0%	99.0%
51	1200	24/24	37.5	100.0%	85.7%	100.0%
	600	23/24	38.9	95.8%	78.8%	99.9%
	300	19/24	39.5	79.2%	57.8%	92.9%
52	7200	48/48**	38.5	100.0%	92.6%	100.0%
	4800	24/24	38.9	100.0%	85.7%	100.0%
	2400	11/24	40.0	45.8%	25.6%	67.2%
56	2400	24/24	38.2	100.0%	85.7%	100.0%
	1200	23/24	39.3	95.8%	78.8%	99.8%
	600	5/24	40.5	20.8%	7.1%	42.2%
58	1200	48/48**	37.0	100.0%	92.6%	100.0%
	600	24/24	38.3	100.0%	85.7%	100.0%
	300	20/24	39.6	83.3%	62.6%	95.3%
59	1200	24/24	37.4	100.0%	85.7%	100.0%
	600	24/24	38.5	100.0%	85.7%	100.0%
	300	22/24	39.6	91.7%	73.0%	99.0%
66	2400	24/24	37.0	100.0%	85.7%	100.0%
	1200	24/24	38.6	100.0%	85.7%	100.0%
	600	16/24	39.8	66.7%	44.7%	84.4%
68	600	48/48**	37.2	100.0%	92.6%	100.0%
	300	24/24	38.4	100.0%	85.7%	100.0%
	150	19/24	39.5	79.2%	57.8%	92.9%

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

**Genotype level tested with one reagent lot

Reproducibility in PreservCyt® Solution

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 90 below).

Percent of positive results for the positive panel members are presented in Table 91. 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 92) 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.

Table 90
Results by Sample Type and Negative Panel Member for Lot and Site/Instrument

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

				Number Negative / Total Number Valid Results					
Sample Type	Panel Member	Ct SD	Ct CV %	Lot			Site/ Instrument		
				ID	Negative/ Valid	%	ID	Negative/ Valid	%
				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

Table 91
Results by Sample Type and Positive Panel Member for Lot and Site/Instrument

				Number Positive / Total Number Valid Results					
Sample Type	**Panel Member**	**Ct SD**	**Ct CV %**	**Lot**			**Site/Instrument**		
				ID	**Positive/ Valid**	**%**	**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 HPV16 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 HPV16 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 HPV18 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 HPV18 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
Pooled HPV31 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 HPV31 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 HPV45 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 HPV45 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

*****concentration in cells/mL included for SiHa and HeLa cell line levels.

Table 92
Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated from Valid Samples of Positive Sample Type Panel Members

Sample Type / Conc.[1] (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[2] / 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] 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.

Reproducibility in SurePath™ Preservative Fluid

A 13-member panel made from both HPV 16/18 cell lines and pools consisting of clinical samples collected into SurePath™ Preservative Fluid was tested for Reproducibility. Each panel member was tested for 15 days (5 days per kit lot), 3 replicates per run, at 3 testing sites. Two operators at each of 3 sites performed 1 run per day for 5 days each on each of 3 reagent lots. A run was defined as 39 panel-member aliquots and 1 positive and 1 negative control.

Overall, 92 runs were performed to obtain 90 valid runs. One invalid run was due to an invalid positive control and one run was aborted by an operator (percent of invalid runs was 2.2% (2/92) with 95% CI: 0.3%, 7.6%). A total of 3,510 tests were performed on the 13 panel members in the valid runs; 4 replicates were not processed due to pipetting error i.e. "failed" results.

All valid test results were included in the analyses that reported the percentage of correct results. There was one false positive result in 270 tests performed on the negative panel members (pooled negative clinical sample; see Table 93 below).

Percents of positive results for the positive panel members are presented in Table 94. With respect to sites, site 3 tended to have a lower percent of agreement for HPV 16/18 cell lines and this trend can be attributed to operator 5, who tended to have lower percent positive values in HPV 16/18 cell line panel members.

Analysis of variance of the Ct values from valid tests performed on positive panel members (see Table 95) yielded total CV (%) ranges of 1.7% to 5.8% across all panel members. The CV(%) ranged from 0.0% to 2.5% for the cell line samples and 0.0% to 5.6% for the pooled clinical samples.

Table 93

Results by Sample Type and Negative Panel Member for Lot and Site/Instrument – SurePath

			Number Positive / Total Number Valid Results					
Panel Member	**Ct SD**	**Ct CV %**		**Lot**		**Site/Instrument**		
			ID	**Negative /Valid**	**%**	**ID**	**Negative /Valid**	**%**
Negative Background Cell Line	n/a	n/a	1	90/90	100.0	1	90/90	100.0
			2	89/89	100.0	2	89/89	100.0
			3	90/90	100.0	3	90/90	100.0
Negative Pooled Clinical Samples	n/a	n/a	1	90/90	100.0	1	89/90	98.9
			2	89/90	98.9	2	90/90	100.0
			3	90/90	100.0	3	90/90	100.0

Table 94
Results by Sample Type and Positive Panel Member for Lot and Site/Instrument - SurePath

				Number Positive / Total Number Valid Results					
Panel Member		**Ct SD**	**Ct CV %**	**Lot**			**Site/Instrument**		
				ID	**Positive /Valid**	**%**	**ID**	**Positive /Valid**	**%**
HPV 16/18 High Negative (~0.5xLoD)	HPV 16 High Negative	1.02	2.5	1	62/90	68.9	1	61/90	67.8
				2	62/90	68.9	2	82/90	91.1
				3	67/90	74.4	3	48/90	53.3
	HPV 18 High Negative	1.17	3.0	1	60/90	66.7	1	59/90	65.6
				2	68/90	75.6	2	80/90	88.9
				3	57/90	63.3	3	46/90	51.1
HPV 16/18 Weak Positive (~1xLoD)	HPV 16 Weak Positive	0.89	2.3	1	86/90	95.6	1	88/90	97.8
				2	86/88	97.7	2	89/89	100.0
				3	87/90	96.7	3	82/89	92.1
	HPV 18 Weak Positive	1.22	3.2	1	75/90	83.3	1	85/90	94.4
				2	75/88	85.2	2	88/89	98.9
				3	87/90	96.7	3	64/89	71.9
HPV 16/18 Positive (~3xLoD)	HPV 16 Positive	0.64	1.7	1	88/90	97.8	1	89/89	100.0
				2	89/89	100.0	2	90/90	100.0
				3	90/90	100.0	3	88/90	97.8
	HPV 18 Positive	0.61	1.7	1	89/90	98.9	1	89/89	100.0
				2	89/89	100.0	2	90/90	100.0
				3	90/90	100.0	3	89/90	98.9
	Pooled HPV 16 Moderate Positive (~1xLoD)	1.69	4.6	1	87/90	96.7	1	90/90	100.0
				2	90/90	100.0	2	90/90	100.0
				3	90/90	100.0	3	87/90	96.7
	Pooled HPV 16 Positive (~3xLoD)	2.02	5.8	1	90/90	100.0	1	90/90	100.0
				2	90/90	100.0	2	90/90	100.0
				3	90/90	100.0	3	90/90	100.0
	Pooled HPV 18 Moderate Positive (~1xLoD)	1.72	4.7	1	87/90	96.7	1	89/90	98.9
				2	90/90	100.0	2	90/90	100.0
				3	89/90	98.9	3	87/90	96.7
	Pooled HPV 18 Positive (~3xLoD)	1.48	4.3	1	90/90	100.0	1	90/90	100.0
				2	90/90	100.0	2	90/90	100.0
				3	90/90	100.0	3	90/90	100.0
	Pooled HPV A Moderate Positive (~1xLoD)	1.09	2.9	1	87/90	96.7	1	90/90	100.0
				2	90/90	100.0	2	89/90	98.9
				3	89/90	98.9	3	87/90	96.7
	Pooled HPV A Positive (~3xLoD)	1.77	4.9	1	88/90	97.8	1	90/90	100.0
				2	90/90	100.0	2	90/90	100.0
				3	90/90	100.0	3	88/90	97.8
	Pooled HPV B Moderate Positive (~1xLoD)	1.72	4.6	1	85/90	94.4	1	87/90	96.7
				2	89/90	98.9	2	88/90	97.8
				3	88/90	97.8	3	87/90	96.7
	Pooled HPV B Positive (~3xLoD)	1.86	5.2	1	89/90	98.9	1	90/90	100.0
				2	90/90	100.0	2	90/90	100.0
				3	90/90	100.0	3	89/90	98.9

Table 95

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

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]					
Sample Type	**N**	**Mean CT**	**Between-Lot**	**Between-Site/ Instrument**	**Between-Operator**	**Between-Day**	**Within-Run**	**Total**
HPV 16/18 High Negative (~0.5xLoD)								
HPV 16 High Negative	270	40.2	0.00, (0.00%)	0.38, (0.95%)	0.13, (0.32%)	0.49, (1.21%)	0.80, (1.99%)	2.5
HPV 18 High Negative	270	39.5	0.11, (0.28%)	0.50, (1.28%)	0.09, (0.23%)	0.38, (0.97%)	0.98, (2.48%)	3.0
HPV 16/18 Weak Positive (~1xLoD)								
HPV 16 Weak Positive	268	39.0	0.08, (0.21%)	0.41, (1.06%)	0.08, (0.19%)	0.43, (1.09%)	0.65, (1.67%)	2.3
HPV 18 Weak Positive	268	38.6	0.00, (0.00%)	0.59, (1.53%)	0.16, (0.41%)	0.59, (1.53%)	0.88, (2.27%)	3.2
HPV 16/18 Positive (~3xLoD)								
HPV 16 Positive	269	37.1	0.00, (0.00%)	0.27, (0.72%)	0.07, (0.19%)	0.46, (1.24%)	0.36, (0.96%)	1.7
HPV 18 Positive	269	36.2	0.03, (0.09%)	0.28, (0.76%)	0.15, (0.42%)	0.38, (1.06%)	0.36, (0.99%)	1.7
Pooled HPV 16 Moderate Positive (~1xLoD)	270	37.0	0.00, (0.00%)	0.00, (0.00%)	0.30, (0.80%)	0.68, (1.84%)	1.52, (4.11%)	4.6
Pooled HPV 16 Positive (~3xLoD)	270	34.9	0.35, (1.01%)	0.33, (0.93%)	0.12, (0.34%)	0.28, (0.81%)	1.94, (5.56%)	5.8
Pooled HPV 18 Moderate Positive (~1xLoD)	270	36.9	0.28, (0.76%)	0.00, (0.00%)	0.25, (0.68%)	0.73, (1.98%)	1.51, (4.10%)	4.7
Pooled HPV 18 Positive (~3xLoD)	270	34.7	0.14, (0.40%)	0.00, (0.00%)	0.00, (0.00%)	0.38, (1.09%)	1.43, (4.11%)	4.3
Pooled HPV A Moderate Positive (~1xLoD)	270	37.6	0.09, (0.23%)	0.23, (0.62%)	0.28, (0.74%)	0.24, (0.65%)	0.99, (2.64%)	2.9
Pooled HPV A Positive (~3xLoD)	270	36.6	0.00, (0.00%)	0.14, (0.38%)	0.00, (0.00%)	0.36, (0.98%)	1.73, (4.74%)	4.9
Pooled HPV B Moderate Positive (~1xLoD)	270	37.3	0.00, (0.00%)	0.00, (0.00%)	0.36, (0.95%)	0.00, (0.00%)	1.68, (4.51%)	4.6
Pooled HPV B Positive (~3xLoD)	270	35.6	0.00, (0.00%)	0.12, (0.33%)	0.00, (0.00%)	0.23, (0.64%)	1.85, (5.18%)	5.2

Precision in PreservCyt® Solution

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 96. 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 97) 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.

Table 96
Summary of the Precision Panel and Hit Rates For cobas® HPV Precision Study in PreservCyt® Solution

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%

*Results shown from detection channel 2 (HPV16)

** Results shown from detection channel 3 (HPV18)

N/A = Not applicable

Table 97
Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated from Valid Samples of Positive Sample Type Precision Panel Members in PreservCyt® Solution

				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%
*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)

Precision in SurePath™ Preservative Fluid

In-house Precision was examined using a panel composed of HPV positive clinical specimens collected in SurePath™ Preservative Fluid and HPV positive cell lines (SiHa and HeLa) diluted into pooled negative cervical specimens collected in SurePath™ Preservative Fluid. 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 2-9 were prepared with HPV positive cell lines (SiHa, HPV16; HeLa, HPV18) diluted at different levels into SurePath™ Preservative Fluid. Panel members 10-12 were prepared with high risk HPV positive specimen in SurePath™ Preservative Fluid pools (HPV16, HPV18, and HR HPV positive) diluted into pooled HPV negative specimens in SurePath™ Preservative Fluid. Panel level 1 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 98. 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 99) 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.

Table 98
Summary of the Precision Panel and Hit Rates For cobas® HPV Precision Study in SurePath™ Preservative Fluid

Panel Number	HPV Target	Description	Anticipated Positivity Rate	N Positive	N Tested	% Hit Rate	95% CI	
							Lower	Upper
1	N/A	Pooled HPV negative specimen	0%	0	216	0	0.0	1.7
2	HPV16	SiHa cell line	90% — 95%	216	216	100	98.3	100.0
3	HPV18	HeLa cell line	90% — 95%	216	216	100	98.3	100.0
4	HPV16	SiHa cell line	95% — 99%	216	216	100	98.3	100.0
5	HPV18	HeLa cell line	95% — 99%	216	216	100	98.3	100.0
6*	HPV16 & HPV18	SiHa & HeLa cell lines	90% — 95%	216	216	100	98.3	100.0
6**	HPV16 & HPV18	SiHa & HeLa cell lines	90% — 95%	216	216	100	98.3	100.0
7	HPV16	SiHa cell line	< 70%	53	216	25	19.0	30.8
8	HPV18	HeLa cell line	< 70%	135	216	63	55.7	69.0
9 *	HPV16 & HPV18	SiHa & HeLa cell lines	< 70%	78	216	36	29.7	42.9
9 **	HPV16 & HPV18	SiHa & HeLa cell lines	< 70%	122	216	57	49.6	63.2
10	High Risk Channel 1	High Risk HPV positive specimen	90% — 95%	216	216	100	98.3	100.0
11	HPV16	High Risk HPV positive specimen	90% — 95%	216	216	100	98.3	100.0
12	HPV18	High Risk HPV positive specimen	90% — 95%	208	216	96	92.8	98.4

*Results shown from detection channel 2 (HPV16)

** Results shown from detection channel 3 (HPV18)

N/A = Not applicable

Table 99
Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated from Valid Samples of Positive Sample Type Precision Panel Members in SurePath™ Preservative Fluid

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]											
	Sample Type / Conc.[1] (cells/mL)	Mean CT	Between-Lot		Between-Run/System		Between-Operator		Between-Day		Within-Run		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
2	SiHa HPV16 (200/mL)	37.9	0.188	0.50%	0.000	0.00%	0.072	0.20%	0.000	0.00%	0.000	0.00%	0.513	1.40%
3	HeLa HPV18 (40/mL)	37.6	0.161	0.40%	0.071	0.20%	0.000	0.00%	0.000	0.00%	0.015	0.00%	0.59	1.60%
4	SiHa HPV16 (600/mL)	36.4	0.132	0.40%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.09	0.20%	0.343	0.90%
5	HeLa HPV18 (120/mL)	36.0	0.091	0.30%	0.056	0.20%	0.044	0.10%	0.000	0.00%	0.071	0.20%	0.392	1.10%
6*	SiHa HPV16 (200/mL)	37.9	0.023	0.10%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.073	0.20%	0.436	1.10%
6**	HeLa HPV18 (40/mL)	37.7	0.081	0.20%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.134	0.40%	0.604	1.60%
7	SiHa HPV16 (20/mL)	41.2	0.000	0.00%	0.092	0.20%	0.116	0.30%	0.000	0.00%	0.000	0.00%	0.979	2.40%
8	HeLa HPV18 (8/mL)	39.8	0.125	0.30%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	1.042	2.60%
9*	SiHa HPV16 (20/mL)	40.9	0.146	0.40%	0.000	0.00%	0.155	0.40%	0.000	0.00%	0.084	0.20%	0.987	2.40%
9**	HeLa HPV18 (8/mL)	39.9	0.195	0.50%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	1.095	2.70%
10	Clinical High Risk channel 1 (N/A)	37.2	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.000	0.00%	1.135	3.10%
11	Clinical HPV16 (N/A)	36.7	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.119	0.30%	0.000	0.00%	1.772	4.80%
12	Clinical HPV18 (N/A)	36.9	0.151	0.40%	0.000	0.00%	0.000	0.00%	0.000	0.00%	0.128	0.30%	1.848	5.00%

*Results shown from detection channel 2 (HPV16)

** Results shown from detection channel 3 (HPV18)

Analytical Specificity in PreservCyt® Solution

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 100 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 1×10^5 *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 100
Microorganisms Tested for Analytical Specificity in PreservCyt® Solution

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 israelii	*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 55
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)

Analytical Specificity in SurePath™ Preservative Fluid

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 101 were spiked at high concentrations ($\geq 1 \times 10^6$ *units/reaction with the exception of *Chlamydia trachomatis and all viruses*, which were all tested at 1×10^5 *units/reaction) into HPV negative specimen in SurePath™ Preservative Fluid and into HPV negative specimen in SurePath™ Preservative Fluid 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). *A*ll 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).

Table 101
Microorganisms Tested for Analytical Specificity in SurePath™ Preservative Fluid

Adenovirus 5	Epstein Barr Virus (EBV)	*Pseudomonas fluorescens*	HPV 30
Bacteroides caccae	*Escherichia coli*	*Staphylococcus aureus*	HPV 34
Bifidobacterium adolescentis	*Fusobacterium varium*	*Staphylococcus epidermidis*	HPV 53
Candida albicans	Herpes simplex virus 1 (HSV-1)	*Streptococcus agalactiae*	HPV 67
Chlamydia trachomatis	Herpes simplex virus 2 (HSV-2)	*Streptococcus faecalis*	HPV 69
Clostridium beijerinckii	*Klebsiella pneumoniae ss ozaenae*	*Streptococcus pyogenea*	HPV 70
Corynebacterium glutamicum	*Lactobacillus acidophilus*	*Trichomonas vaginalis*	HPV 73
Cytomegalovirus (CMV)	*Neisseria gonorrhea*	HPV 6	HPV 82
Enterobacter aerogenes	Peptostreptococcus anaerobius	HPV 11	HPV 85
Enterococcus faecium	*Proteus mirabilis*	HPV 26	

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 102. 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 103. 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% in PreservCyt® specimens and up to 2% % in SurePath specimens.

Table 102: 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 Specimen Pools	HPV types 31, 16, 18 plasmids were each diluted to 3 fold LoD into pools of negative cervical specimen in PreservCyt® Solution and SurePath™ Preservative Fluid.	Exogenous Interference

Table 103
Interference Testing Results with Endogenous Interferents

Interferent Tested	Collection Medium	Concentrations Tested	Interference Observed
Whole Blood	PreservCyt®	1%, 1.5%, 2%, 3% v/v	Above 1.5%
Whole Blood	SurePath™	2%, 4%, 6%, 8% v/v	Above 2%
PBMC	PreservCyt® and SurePath™	10^4, 10^5, 10^6 cells/mL	None
Cervical Mucus	PreservCyt® and SurePath™	Mucus obtained from standard cervical cleaning procedure	None

A total of 21 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 and SurePath™ Preservative Fluid are described in Table 104.

Table 104
Interference Testing Results with Exogenous Interferents

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

*Addition of 15 mg to the test sample produced false negative results

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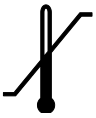

Temperature Limit

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Test Definition File

Contains Sufficient for <*n*> tests

Use-by date

Contents of kit

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