

**FOR *IN VITRO* DIAGNOSTIC USE**

<b>cobas<sup>®</sup> 4800 System Sample Preparation Kit</b>	<b>c4800 SMPL PREP</b>	960 Tests 240 Tests	P/N: 05235804190 P/N: 05235782190
<b>cobas<sup>®</sup> 4800 HPV Amplification/Detection Kit</b>	<b>c4800 HPV AMP/DET</b>	960 Tests 240 Tests	P/N: 05235898190 P/N: 05235880190
<b>cobas<sup>®</sup> 4800 HPV Controls Kit</b>	<b>c4800 HPV CTLS</b>	10 Sets	P/N: 05235855190
<b>cobas<sup>®</sup> 4800 System Liquid Cytology Preparation Kit</b>	<b>c4800 LIQ CYT</b>	960 Tests 240 Tests	P/N: 05235839190 P/N: 05235812190
<b>cobas<sup>®</sup> 4800 System Wash Buffer Kit</b>	<b>c4800 WB</b>	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. 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 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.

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, who are pregnant or who have other risk factors (e.g. HIV+, immunocompromised, history of STD).

## 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)<sup>1-3</sup>. The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide<sup>3</sup>. 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<sup>4,5</sup>, and approximately 40 different HPVs that can infect the human anogenital mucosa<sup>6,7</sup>. However, only a subset of approximately 14 of these types is considered high-risk for the development of cervical cancer and its precursor lesions<sup>3,8-13</sup>. 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<sup>14</sup>. 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<sup>15-20</sup>. An infection with any HPV type can produce cervical intraepithelial neoplasia (CIN) although this also usually resolves once the HPV infection has been cleared<sup>21</sup>.

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

Papillomavirus is extremely difficult to culture *in vitro*, and not all patients infected with HPV have a demonstrable antibody response. Nucleic acid (DNA) testing by PCR is a non-invasive method for determining the presence of a cervical HPV infection. Proper implementation of nucleic acid testing for HPV may increase the sensitivity of cervical cancer screening programs by detecting high-risk lesions earlier in women 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**<sup>®</sup> HPV Test is based on two major processes: (1) automated specimen preparation to simultaneously extract HPV and cellular DNA; (2) PCR amplification<sup>22</sup> of target DNA sequences using both HPV and  $\beta$ -globin specific complementary primer pairs and real-time detection of cleaved fluorescent-labeled HPV and  $\beta$ -globin specific oligonucleotide detection probes. The concurrent extraction, amplification and detection of  $\beta$ -globin in the **cobas**<sup>®</sup> HPV Test monitors the entire test process.

The master mix reagent for the **cobas**<sup>®</sup> HPV Test contains primer pairs and probes specific for the 14 high-risk HPV types and  $\beta$ -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  $\beta$ -globin signals are each detected with their own dedicated fluorescent dye.

### Specimen Preparation

Specimen preparation for the **cobas**<sup>®</sup> HPV Test is automated with the use of the **cobas** x 480 instrument. Cervical specimens collected in PreservCyt solution are digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids, along with the  $\beta$ -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**<sup>®</sup> 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)<sup>3,6-13,23</sup>. Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers.

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

#### Target Amplification

Eagle Z05<sup>®</sup> DNA Polymerase<sup>24</sup>, a chemically modified version of *Thermus* species Z05 DNA polymerase<sup>25</sup>, is utilized for "hot start" amplification of the HPV targets and the  $\beta$ -globin control. First, the PCR reaction mixture is heated to activate Eagle Z05<sup>®</sup> DNA Polymerase, to denature the viral DNA and genomic DNA and to expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The Eagle Z05<sup>®</sup> 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  $\beta$ -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  $\beta$ -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  $\beta$ -globin gene between the appropriate primer pair. The entire genome is not amplified.

#### Automated Real-time Detection

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

#### Selective Amplification

Selective amplification of target nucleic acid from the patient specimen is achieved in the **cobas**<sup>®</sup> 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<sup>26</sup>, 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**<sup>®</sup> HPV Test has been demonstrated to inactivate at least 10<sup>3</sup> copies of deoxyuridine-containing HPV amplicon per PCR.

## REAGENTS

### **cobas**<sup>®</sup> 4800 System Sample Preparation Kit

(P/N: 05235782190)

c4800 SMPL PREP

240 Tests

#### **MGP**

10 x 4.5 mL

(**cobas**<sup>®</sup> 4800 System Magnetic Glass Particles)

Magnetic Glass Particles

93% Isopropanol

Xi  93% (w/w) Isopropanol

Irritant

F  93% (w/w) Isopropanol

Highly Flammable

R: 11-36-67, S: 7-16-24/25-26

#### **EB**

10 x 18 mL

(**cobas**<sup>®</sup> 4800 System Elution Buffer)

Tris-HCl buffer 0.09% Sodium azide			
<b>cobas<sup>®</sup> 4800 System Sample Preparation Kit</b> (P/N: 05235804190)	<b>c4800 SMPL PREP</b>		<b>960 Tests</b>
<b>MGP</b> (cobas <sup>®</sup> 4800 System Magnetic Glass Particles)			10 x 13.5 mL
Magnetic Glass Particles 93% Isopropanol			
Xi  93% (w/w) Isopropanol Irritant			
F  93% (w/w) Isopropanol Highly Flammable			
R: 11-36-67, S: 7-16-24/25-26			
<b>EB</b> (cobas <sup>®</sup> 4800 System Elution Buffer)			10 x 18 mL
Tris-HCl buffer 0.09% Sodium azide			
<b>cobas<sup>®</sup> 4800 System Wash Buffer Kit</b> (P/N: 05235863190)	<b>c4800 WB</b>		<b>240 Tests</b>
<b>WB</b> (cobas <sup>®</sup> 4800 System Wash Buffer)			10 x 55 mL
Sodium citrate dihydrate 0.05% N-Methyl isothiazolone HCl			
<b>cobas<sup>®</sup> 4800 System Wash Buffer Kit</b> (P/N: 05235871190)	<b>c4800 WB</b>		<b>960 Tests</b>
<b>WB</b> (cobas <sup>®</sup> 4800 System Wash Buffer)			10 x 200 mL
Sodium citrate dihydrate 0.05% N-Methyl isothiazolone HCl			
<b>cobas<sup>®</sup> 4800 System Liquid Cytology Preparation Kit</b> (P/N: 05235812190)	<b>c4800 LIQ CYT</b>		<b>240 Tests</b>
<b>PK</b> (cobas <sup>®</sup> 4800 Proteinase K)			10 x 0.9 mL
Tris-HCl buffer EDTA Glycerol Calcium chloride Calcium acetate < 2% Proteinase K			
Xi  < 2% Proteinase K Irritant			
<b>SDS</b> (cobas <sup>®</sup> 4800 System SDS Reagent)			10 x 3 mL
Tris-HCl buffer 0.2% SDS 0.09% Sodium azide			

**LYS**

10 x 10 mL

**(cobas® 4800 System Lysis Buffer)**

Tris-HCl buffer

37% (w/w) Guanidine HCl

Xn,  37% (w/w) Guanidine HCl

Harmful

R: 22-36/38, S: 13-26-36-46

N  < 5 % Polydocanol

Dangerous For The Environment

R: 22-41-50, S: 26-39-61

**cobas® 4800 System Liquid Cytology Preparation Kit**

c4800 LIQ CYT

**960 Tests**

(P/N: 05235839190)

**PK**

20 x 1.2 mL

**(cobas® 4800 Proteinase K)**

Tris-HCl buffer

EDTA

Glycerol

Calcium chloride

Calcium acetate

&lt; 2% Proteinase K

Xi  < 2% Proteinase K

Irritant

**SDS**

10 x 9 mL

**(cobas® 4800 System SDS Reagent)**

Tris-HCl buffer

0.2% Sodium dodecyl sulfate

0.09% Sodium azide

**LYS**

10 x 36 mL

**(cobas® 4800 System Lysis Buffer)**

Tris-HCl buffer

37% (w/w) Guanidine HCl

Xn,  37% (w/w) Guanidine HCl

Harmful

R: 22-36/38, S: 13-26-36-46

N  < 5 % Polydocanol

Dangerous ForThe Environment

R: 22-41-50, S: 26-39-61

**cobas® 4800 HPV Amplification/Detection Kit**

c4800 HPV AMP/DET

**240 Tests**

(P/N: 05235880190)

**HPV MMX**

10 x 0.5 mL

**(cobas® 4800 HPV Master Mix)**

Tricine buffer

Potassium acetate

Potassium hydroxide

Glycerol

&lt; 0.13 % dATP, dCTP, dGTP, dUTP

&lt; 0.01 %Upstream and downstream HPV primers

&lt; 0.01 %Upstream and downstream β-globin primers

&lt; 0.01 %Fluorescent-labeled HPV probes

&lt; 0.01 %Fluorescent-labeled β-globin probes

&lt; 0.10 % Eagle Z05® DNA polymerase (microbial)

&lt; 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)

0.09% Sodium azide

<b>HPV Mg/Mn</b>		10 x 1.0 mL
(cobas <sup>®</sup> 4800 HPV Mg/Mn Solution)		
Magnesium acetate		
Manganese acetate		
< 0.02% Glacial acetic acid		
0.09% Sodium azide		
<b>cobas<sup>®</sup> 4800 HPV Amplification/Detection Kit</b>	<b>c4800 HPV AMP/DET</b>	<b>960 Tests</b>
(P/N: 05235898190)		
<b>HPV MMX</b>		20 x 1.0 mL
(cobas <sup>®</sup> 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 % Eagle Z05 <sup>®</sup> DNA polymerase (microbial)		
< 0.10 % AmpErase (uracil-N-glycosylase) enzyme (microbial)		
0.09% Sodium azide		
<b>HPV Mg/Mn</b>		10 x 1.0 mL
(cobas <sup>®</sup> 4800 HPV Mg/Mn Solution)		
Magnesium acetate		
Manganese acetate		
< 0.02% Glacial acetic acid		
0.09% Sodium azide		
<b>cobas<sup>®</sup> 4800 HPV Controls Kit</b>	<b>c4800 HPV CTLs</b>	<b>10 Sets</b>
(P/N: 05235855190)		
<b>HPV (+) C</b>		10 x 0.5 mL
(cobas <sup>®</sup> 4800 HPV Positive Control)		
Tris-HCl buffer		
EDTA		
0.05% Sodium azide		
< <b>0.00001%</b> Poly rA RNA (synthetic)		
< <b>0.00001%</b> Non-infectious plasmid DNA (microbial) containing HPV-16, 18, 39 sequences		
< <b>0.00001%</b> Non-infectious plasmid DNA (microbial) containing β-globin sequences		
<b>(-) C</b>		10 x 0.5 mL
(cobas <sup>®</sup> 4800 System Negative Control)		
Tris-HCl buffer		
EDTA		
0.05% Sodium azide		
< <b>0.00001%</b> Poly rA RNA (synthetic)		

#### WARNINGS AND PRECAUTIONS

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

- B. Do not pipette by mouth.
- C. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- D. Avoid microbial and DNA contamination of reagents.
- E. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- F. Do not use reagents after their expiration dates.
- G. Do not pool reagents.
- H. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.
- I. Gloves must be worn and must be changed between handling specimens and cobas<sup>®</sup> 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*<sup>23</sup> and in the CLSI Document M29-A3<sup>30</sup>.
- K. **LYS** contains guanidine hydrochloride. **Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.** If liquid containing guanidine

hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, **FIRST** clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.

- L. **MGP** contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.
- M. **EB, SDS, HPV MMX, HPV Mg/Mn, (-)C, and HPV (+)C** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- N. Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- O. All disposable items are for one time use. Do not reuse.
- P. Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x 480** instrument or **cobas z 480** analyzer. Clean the **cobas x 480** instrument or **cobas z 480** analyzer according to procedures described in the **cobas**<sup>®</sup> 4800 System Operator's Manual.
- Q. For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x 480** instrument or **cobas z 480** analyzer, consult the **cobas**<sup>®</sup> 4800 System Operator's Manual.

**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 2-8°C. These reagents are stable until the expiration date indicated.
- C. Store the Wash Buffer Kit (**WB**) at 15-25°C. This reagent is stable until the expiration date indicated.

**MATERIALS PROVIDED**

<p><b>A. cobas<sup>®</sup> 4800 System Sample Preparation Kit</b> (P/N: 05235782190) <b>MGP</b> (cobas<sup>®</sup> 4800 System Magnetic Glass Particles) <b>EB</b> (cobas<sup>®</sup> 4800 System Elution Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 SMPL PREP</div>	<b>240 Tests</b>
<p><b>B. cobas<sup>®</sup> 4800 System Sample Preparation Kit</b> (P/N: 05235804190) <b>MGP</b> (cobas<sup>®</sup> 4800 System Magnetic Glass Particles) <b>EB</b> (cobas<sup>®</sup> 4800 System Elution Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 SMPL PREP</div>	<b>960 Tests</b>
<p><b>C. cobas<sup>®</sup> 4800 System Wash Buffer Kit</b> (P/N: 05235863190) <b>WB</b> (cobas<sup>®</sup> 4800 System Wash Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 WB</div>	<b>240 Tests</b>
<p><b>D. cobas<sup>®</sup> 4800 System Wash Buffer Kit</b> (P/N: 05235871190) <b>WB</b> (cobas<sup>®</sup> 4800 System Wash Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 WB</div>	<b>960 Tests</b>
<p><b>E. cobas<sup>®</sup> 4800 System Liquid Cytology Preparation Kit</b> (P/N: 05235812190) <b>PK</b> (cobas<sup>®</sup> 4800 Proteinase K) <b>SDS</b> (cobas<sup>®</sup> 4800 System SDS Reagent) <b>LYS</b> (cobas<sup>®</sup> 4800 System Lysis Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 LIQ CYT</div>	<b>240 Tests</b>
<p><b>F. cobas<sup>®</sup> 4800 System Liquid Cytology Preparation Kit</b> (P/N: 05235839190) <b>PK</b> (cobas<sup>®</sup> 4800 Proteinase K) <b>SDS</b> (cobas<sup>®</sup> 4800 System SDS Reagent) <b>LYS</b> (cobas<sup>®</sup> 4800 System Lysis Buffer)</p>	<div style="border: 1px solid black; padding: 2px; display: inline-block;">c4800 LIQ CYT</div>	<b>960 Tests</b>

<p><b>G. cobas<sup>®</sup> 4800 HPV Amplification/Detection Kit</b> (P/N: 05235880190) <b>HPV MMX</b> (cobas<sup>®</sup> 4800 HPV Master Mix) <b>HPV Mg/Mn</b> (cobas<sup>®</sup> 4800 HPV Mg/Mn Solution)</p>	c4800 HPV AMP/DET	<b>240 Tests</b>
<p><b>H. cobas<sup>®</sup> 4800 HPV Amplification/Detection Kit</b> (P/N: 05235898190) <b>HPV MMX</b> (cobas<sup>®</sup> 4800 HPV Master Mix) <b>HPV Mg/Mn</b> (cobas<sup>®</sup> 4800 HPV Mg/Mn Solution)</p>	c4800 HPV AMP/DET	<b>960 Tests</b>
<p><b>I. cobas<sup>®</sup> 4800 HPV Controls Kit</b> (P/N: 05235855190) <b>HPV (+) C</b> (cobas<sup>®</sup> 4800 HPV Positive Control) <b>(-) C</b> (cobas<sup>®</sup> 4800 System Negative Control)</p>	c4800 HPV CTLs	<b>10 Sets</b>

**MATERIALS REQUIRED BUT NOT PROVIDED**

**Specimen and Reagent Handling**

- CORE Tips, 1000 µL, rack of 96 (P/N: 04639642001 or Hamilton P/N: 235905)
- 50 mL Reagent Reservoir (P/N: 05232732001)
- 200 mL Reagent Reservoir (P/N: 05232759001)
- cobas<sup>®</sup> 4800 System Extraction (deep well) Plate (P/N: 05232716001)
- cobas<sup>®</sup> 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<sup>®</sup> 4800 System control unit with system software version 1.1 or higher
- cobas<sup>®</sup> 4800 Work Order Editor version 1.1.0.1016 or higher
- Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500 (optional, for PCR Only workflow)
- Stand-alone magnetic plate (P/N: 05440777001, optional, for PCR Only workflow)

**SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

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

**A. Specimen Collection**

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

**B. Specimen Transport**

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

**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<sup>®</sup> HPV test. See PreservCyt solution labeling for storage requirements prior to cytology processing. PreservCyt specimens should not be frozen.

**INSTRUCTIONS FOR USE**

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

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

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

### Run Size

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

### Workflow

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

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

#### HPV Full Workflow

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

#### HPV PCR Only Workflow

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

### Specimens

Pre-cytology PreservCyt specimens must be aliquoted into properly barcoded 13 mL round-based secondary (Sarstedt) tubes prior to processing on the cobas x 480 instrument. For primary screening, pre-cytology primary vials should not be placed directly on the x 480 instrument because the impact on cytology has not been assessed. However PreservCyt solution specimens may 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 (Sarstedt) tube on the cobas x 480 instrument. Consult the cobas® 4800 System Operator's Manual for proper barcoding procedures and the list of acceptable barcodes for the cobas® 4800 System. 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:** *ThinPrep 20 mL primary containers should not be placed directly on the cobas® 4800 System for processing prior to performing cytology from the container.*

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

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

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

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

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

### Workflows

#### Performing a Full Workflow:

- A. The cobas® HPV Test may be used for runs of 1 to 22 specimens plus one cobas® 4800 System Negative Control and one cobas® 4800 HPV Positive Control (24-test format) and for runs of 1 to 94 specimens plus one cobas® 4800 System Negative Control and one cobas® 4800 HPV Positive Control (96-test format).
- B. Perform the system startup and maintenance procedures by following the instructions in the cobas® 4800 System Operator's Manual in the Operation section.
- C. Create a Work Order file for a full run by following the instructions in the cobas® 4800 System Operator's Manual. A Work Order file is not required if an LIS is in use.
- D. Select the test subtype and media type (PreservCyt) for each specimen.
  - Choose test subtype "HPV High Risk Panel" to report High Risk HPV test results without separate reporting of HPV16 and HPV18 results.
  - Choose test subtype "HPV High Risk Panel Plus Genotyping" to report High Risk HPV and separate HPV16 and HPV18 results.
- E. Start the new run by following the software wizard guide. Select the test type as "HPV workflow".
- F. Follow the software wizard guide to load specimens and the Work Order file.

**NOTE:** Specimens can be loaded in barcoded primary containers or secondary tubes in any order as long as their barcodes match those in the Work 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. Follow the software wizard guide to load all consumables.

H. 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.

I. 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

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

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

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

**NOTE:** The cobas® 4800 Software 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.

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

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

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

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

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

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

O. Follow the instructions in the cobas® 4800 System Operator's Manual to review and accept results.

#### Performing a PCR Only Workflow

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

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

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

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

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

- a. Refer to the result printout or the result export file for sample barcodes, media types, sub-test types and positions in the cobas® 4800 deep well plate for the run which requires a repeat of the amplification/detection.
- b. For the positive and negative controls, edit the last 4 digits to identify a reuse of the control barcodes for amplification and detection only workflow by following the instructions in the cobas® 4800 System Operator's Manual.

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

- a. For a run of up to 24 tests, add 240 µL of HPV Mg/Mn to one vial of HPV MMX (0.5 mL vial from 240 Test Kit).
- b. For a run of up to 96 tests, add 450 µL of HPV Mg/Mn to each of the two vials of HPV MMX (1.0 mL vials from 960 Test Kit).

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

- C. Thoroughly mix working master mix by carefully inverting the vial(s). Do not vortex the working master mix.
- D. Transfer 25 µL of working master mix to each of the required wells in the microwell plate.
- E. Place the deep well plate from the run to be repeated onto the stand-alone magnetic plate.
- F. Manually transfer 25 µL of eluate from the deep well plate wells to the corresponding wells in the microwell plate. Ensure that well positions are maintained (e.g. eluate in A1 well in deep well plate is transferred to A1 on the microwell plate). Ensure that no MGP is carried over to the microwell plate.
- G. Follow the instructions in the **cobas**<sup>®</sup> 4800 System Operator's Manual to seal the microwell plate.
- H. Centrifuge the microwell plate using a swinging bucket rotor for at least 5 seconds at 1500 RCF.
- I. Transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.
- J. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.
- K. Follow the instructions in the **cobas**<sup>®</sup> 4800 System Operator's Manual to review and accept results.

#### **Interpretation of Results**

**NOTE:** *All assay and run validation is performed by the cobas<sup>®</sup> 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 1**  
**Result Interpretation of the cobas® HPV Test for Presence of HPV DNA**

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

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

**Table 2**  
**Result Interpretation of the cobas<sup>®</sup> HPV Test\***

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

\*HPV testing should not be performed on women younger than 25 years of age except for ASC-US triage (ASC-US triage is acceptable for ages 21-24). In an adjunct testing population (cytology and HPV performed concurrently), women with ASC-H, AGC or HSIL cytology should proceed to colposcopy regardless of their HPV test results.

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

**Other HR HPV NEG, HPV16 POS, HPV18 Invalid**

The positive and negative results should be interpreted as shown in Table 1. In this example, HPV18 results are invalid. Original specimen should be re-tested no more than 2 times to obtain valid results. If results are still invalid, a new specimen should be obtained.

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

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

#### **QUALITY CONTROL**

One set of cobas<sup>®</sup> 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<sup>®</sup> 4800 Software to display the reportable cobas<sup>®</sup> 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<sup>®</sup> 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.

#### **PROCEDURAL LIMITATIONS**

1. The cobas<sup>®</sup> 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<sup>34</sup>.
2. The cobas<sup>®</sup> 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<sup>®</sup> HPV Test for primary screening 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<sup>®</sup> HPV Test.
4. The performance of the cobas<sup>®</sup> HPV Test has not been adequately established for HPV vaccinated individuals<sup>35</sup>.
5. Test only the indicated specimen type. The cobas<sup>®</sup> 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<sup>®</sup> Pap Test<sup>™</sup> PreservCyt<sup>®</sup> Solution. The endocervical brush/spatula utilized in the performance studies was a Pap Perfect<sup>®</sup> plastic spatula and Cytobrush<sup>®</sup> plus GT gentle touch.
6. 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.
7. 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.
8. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer.
9. A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer, but indicates a low likelihood of CIN2-3 or cancer.
10.  $\beta$ -globin amplification and detection is included in the cobas<sup>®</sup> 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  $\beta$ -globin signal within a pre-defined range

to be identified as valid negatives by the **cobas**<sup>®</sup> 4800 System. The  $\beta$ -globin control does not differentiate between targeted (cervical) and non-targeted nucleated cell types.

11. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Package Insert and the **cobas**<sup>®</sup> 4800 System Operator's Manual.
12. The addition of AmpErase enzyme into the **cobas**<sup>®</sup> 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.
13. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**<sup>®</sup> 4800 System.
14. The **cobas**<sup>®</sup> 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.
15. 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.
16. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
17. Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the **cobas**<sup>®</sup> HPV Test's primers and/or probes may result in failure to detect the presence of the viral DNA.
18. The presence of PCR inhibitors may cause false negative or invalid results.
19. 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**<sup>®</sup> 4800 System. If concentrations of whole blood exceed 1.5% (dark red or brown coloration) in PreservCyt solution, there is a likelihood of obtaining a false-negative result. The **cobas**<sup>®</sup> HPV Test performance has not been validated with PreservCyt specimens which have been treated with glacial acetic acid for removal of red blood cells. Any such processing of PreservCyt specimens prior to HPV testing would invalidate the **cobas**<sup>®</sup> HPV Test results.
20. Cross-contamination of samples can cause false positive results. The sample to sample cross-contamination rate of the **cobas**<sup>®</sup> HPV Test on the **cobas**<sup>®</sup> 4800 System has been determined in a non-clinical study to be 0.71%. Run to run cross-contamination has not been observed. In an analytical study using post-cytology 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 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.

## EXPECTED RESULTS

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  $\geq 21$  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% of women in age group 21-29 years, ~27% in age group 30-39 years, and ~48% of women in age group  $\geq 40$  years. A total of 90.0% of women had NILM cytology, and 4.1% of women had ASC-US cytology.

A total of 1,918 women (ASC-US population with age  $\geq 21$  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**<sup>®</sup> HPV Test.

A total of 32,260 women (NILM population  $\geq 30$  years) were evaluable; evaluable women were eligible women  $\geq 30$  years who had NILM cytology results and also had valid results from the IUO HR HPV Test, IUO HPV genotyping Test, and the **cobas**<sup>®</sup> HPV Test.

A total of 40,944 women (Primary Screening population  $\geq 25$  years) were evaluable; evaluable women had valid results from cytology and the **cobas**<sup>®</sup> HPV Test.

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

**Table 3**  
**Summary of HPV Prevalence by the **cobas**<sup>®</sup> HPV Test by Testing Sites and Study Population**

Testing Site	<b>cobas</b> <sup>®</sup> HPV Test – HPV Prevalence			
	<b>All Eligible Women (<math>\geq 21</math> Years)</b>	<b>ASC-US Population (<math>\geq 21</math> Years)</b>	<b>NILM Population (<math>\geq 30</math> Years)</b>	<b>Primary Screening Population (<math>\geq 25</math> Years)</b>
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**<sup>®</sup> 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<sup>®</sup> HPV Test Result by Age and Study Population**

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**<sup>®</sup> 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<sup>®</sup> HPV Test Result by Age Group for Evaluable ASC-US Women (≥ 21 Years)**

Age Group (Years)	cobas <sup>®</sup> 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<sup>®</sup> HPV Test Result by Age Group for Evaluable NILM Women (≥ 30 Years)**

Age Group (Years)	cobas <sup>®</sup> 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% (6,728/7,106)	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<sup>®</sup> HPV Test Result by Age Group for Evaluable Women (≥ 25 Years)**

Age Group (Years)	cobas <sup>®</sup> 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

### Clinical Performance

#### Baseline Phase

A multicenter, prospective study (ATHENA Study) was conducted to evaluate the performance of the **cobas<sup>®</sup>** 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<sup>®</sup>** HPV Test as well as an investigational use only (IUO) HR HPV test and an IUO HPV genotyping test. For testing with the **cobas<sup>®</sup>** 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<sup>36</sup>.

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 (CPR) consisting of three expert pathologists, and discordant results adjudicated according to a pre-defined protocol. For all analyses, the clinical performance of the **cobas<sup>®</sup>** HPV Test was measured against CPR histology results. The analyses were performed for those women with histology ≥ CIN2 and ≥ CIN3 by CPR. Women with a CPR diagnosis of ≥ CIN2 by CPR exited the study. All women who had undergone colposcopy and biopsy, without a diagnosis of ≥ CIN2 by CPR were invited to proceed to the Follow-up Phase of the study.

#### Follow-Up Phase

All women who did not have histology ≥ CIN2 by CPR 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<sup>®</sup>** 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 Central Pathology Review Panel. All women with ≥ CIN2 by CPR exited the study and those with < CIN2 by CPR 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 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<sup>®</sup>** HPV Test was measured against histology results of ≥ CIN2 and ≥ CIN3 by CPR.

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

All 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 CPR.

All women ≥ 30 years who were invited to colposcopy and did not have histology ≥ CIN2 by CPR were eligible to participate in a 3 year longitudinal study for the **cobas<sup>®</sup>** 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 CPR 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 measured according to the baseline HPV

status (as determined by the **cobas**<sup>®</sup> 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  $\geq$  CIN2 and  $\geq$  CIN3 was determined by CPR.

**STUDY DESIGN TO DEMONSTRATE CLINICAL PERFORMANCE OF THE COBAS HPV TEST 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**<sup>®</sup> HPV Test was measured against histology results of  $\geq$  CIN2 and  $\geq$  CIN3 by CPR and compared to the performance of cytology alone.

**Performance Characteristics in the ASC-US Population ( $\geq$  21 Years)**

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

**Table 8**  
**Results of the cobas<sup>®</sup> HPV Test and Central Pathology Review Panel Diagnosis in the ASC-US Population ( $\geq$  21 Years)**

cobas <sup>®</sup> HPV Test Result	Central Pathology Review Panel Diagnosis					Total
	Undetermined	Normal	CIN1	CIN2	$\geq$ 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 CPR results were due to biopsy sample(s) collected out of study visit window or biopsy sample(s) found to be inadequate for diagnosis. These were excluded from the analysis, resulting in 1578 valid biopsy results.

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

The performance of the **cobas**<sup>®</sup> HPV Test in detecting high-grade cervical disease ( $\geq$  CIN2 and  $\geq$  CIN3) is presented in Table 9. The sensitivity and the specificity of the test for detecting  $\geq$  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**<sup>®</sup> HPV Test result is 3.1 times more likely in women with  $\geq$  CIN2 than in women with  $<$  CIN2. The negative likelihood ratio (NLR) was estimated as 0.1, which implies that a negative **cobas**<sup>®</sup> HPV Test result is 10 (1/0.1) times more likely in women with  $<$  CIN2 than in women with  $\geq$  CIN2.

The sensitivity and specificity of the **cobas**<sup>®</sup> HPV Test for detecting  $\geq$  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<sup>®</sup> HPV Test in Detecting  $\geq$  CIN2 and  $\geq$  CIN3 in the ASC-US Population ( $\geq$  21 Years)**

Performance	CPR Panel Diagnosis $\geq$ CIN2		CPR Panel Diagnosis $\geq$ 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**<sup>®</sup> HPV Test in detecting high-grade cervical disease ( $\geq$  CIN2 and  $\geq$  CIN3) and the performance of the FDA approved HPV Test are presented in Table 10.

The sensitivity for detecting  $\geq$  CIN2 histology was 90.0% ((72/80) with 95% CI: 81.5% to 94.8%) for the **cobas**<sup>®</sup> 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  $\geq$  CIN2 histology was 70.5% (1,056/1,498) with 95% CI: 68.1% to 72.7%) for the **cobas**<sup>®</sup> 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  $\geq$  CIN3 histology was 93.5% ((43/46) with 95% CI: 82.5% to 97.8%) for the **cobas**<sup>®</sup> 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  $\geq$  CIN3 histology was 69.3% ((1,053/1,517) with 95% CI: 66.9% to 71.5%) for the **cobas**<sup>®</sup> 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<sup>®</sup> HPV Test and an FDA approved HPV test in Detecting**  
**≥ CIN2 and ≥ CIN3 in the ASC-US Population**

	cobas <sup>®</sup> HPV Test		FDA approved HPV Test	
	Point Estimate	95% CI	Point Estimate	95% CI
<b>≥ CIN2</b>				
Sensitivity (%)	90.0 (72/80)	(81.5, 94.8)	87.2 (68/78) <sup>1</sup>	(78.0, 92.9)
Specificity (%)	70.5 (1,056/1,498)	(68.1, 72.7)	71.1 (1,056/1,485) <sup>2</sup>	(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)
<b>≥ CIN3</b>				
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)

<sup>1</sup> 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

<sup>2</sup> 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<sup>®</sup> HPV Test for detecting ≥ CIN2 and ≥ CIN3 evaluated by age group is presented in Table 11. The sensitivity of the cobas<sup>®</sup> 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<sup>®</sup> HPV Test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population by Age Group**

Performance	21-29 Years	30-39 Years	≥ 40 Years
N	514	422	642
<b>≥ CIN2</b>			
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)
<b>≥ CIN3</b>			
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  $\geq$  CIN2 and  $\geq$  CIN3 in the ASC-US Population by Age Group**

Performance	21-29 Years	30-39 Years	$\geq$ 40 Years
N	506	417	640
<b><math>\geq</math> CIN2</b>			
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)
$\geq$ 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)
<b><math>\geq</math> CIN3</b>			
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)
$\geq$ 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 ( $\geq$  21 Years) Population – Likelihood Ratios and Risk Estimates

Likelihood ratios (LRs) and the risks of disease ( $\geq$  CIN2 and  $\geq$  CIN3) along with 95% CIs for **cobas**<sup>®</sup> HPV Test results (HR HPV16 positive/18 positive, 12 Other HR, and HR HPV negative) are presented in Table 13 for the ASC-US ( $\geq$ 21 years) population.

For  $\geq$  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 ( $\geq$  CIN2) than in a subject without disease ( $<$  CIN2). The risk of a  $\geq$  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**<sup>®</sup> 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 from a subject with disease ( $\geq$  CIN2).

The risk of disease ( $\geq$  CIN2) is the chance/probability of having the disease given an HPV test outcome. The risk of disease ( $\geq$  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  $\geq$  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 ( $\geq$  CIN3) was 2.9% in the ASC-US population (see Table 13). The risk of  $\geq$  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**<sup>®</sup> HPV Test Result in Detecting  $\geq$  CIN2 and  $\geq$  CIN3 in the ASC-US Population**

Target condition	<b>cobas</b> <sup>®</sup> HPV Test Result	Likelihood Ratio (95% CI)	Risk of Disease (%) Given the Test Result (95% CI)
$\geq$ 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%
$\geq$ 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 – Absolute and Relative Risk Estimates

The CPR diagnosis by all possible **cobas**<sup>®</sup> HPV Test result in ASCUS population is presented in table 14.

**Table 14**  
**Summary of cobas<sup>®</sup> HPV Test Result and Central Pathology Review Panel Diagnosis in the ASC-US Population (≥ 21 years)**

<b>cobas<sup>®</sup> HPV Test Result</b>	<b>Central Pathology Review Diagnosis</b>					<b>Total</b>
	<b>Undetermined</b>	<b>Negative</b>	<b>CIN1</b>	<b>CIN2</b>	<b>≥ CIN3</b>	
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
<b>Overall</b>	<b>32</b>	<b>1,342</b>	<b>158</b>	<b>34</b>	<b>46</b>	<b>1,612</b>

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 CPR diagnosis > CIN3.

The CPR diagnosis and the absolute risk of disease (≥ CIN2 and ≥ CIN3) by **cobas**<sup>®</sup> HPV Test result is 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  $\geq$  CIN2 and  $\geq$  CIN3 for Different cobas<sup>®</sup> HPV Test Results in the ASC-US Population ( $\geq$  21 Years)**

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

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

Note 2: HPV16 positive and/ or HPV18 positive include all 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 ( $\geq$  CIN2 and  $\geq$  CIN3) were calculated for women with different cobas<sup>®</sup> HPV Test results by RR and its associated 95% CIs, as presented in Table 16. The estimated RRs of  $\geq$  CIN2 and of  $\geq$  CIN3 for women with positive vs. negative cobas<sup>®</sup> 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  $\geq$  CIN2 histology and 29.7 times more likely to have  $\geq$  CIN3 histology than were women with a negative test result.

Similarly, women who have HPV16 and/or HPV18 positive results from the cobas<sup>®</sup> HPV Test were significantly more likely to have  $\geq$  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  $\geq$  CIN2 than the women with a negative result. Similar results were observed for  $\geq$  CIN3 histology.

**Table 16**  
**Relative Risks of  $\geq$  CIN2 and  $\geq$  CIN3 for Different cobas<sup>®</sup> HPV Test Results in the ASC-US Population ( $\geq$  21 Years)**

cobas <sup>®</sup> HPV Test Result	CPR Diagnosis $\geq$ CIN2		CPR Diagnosis $\geq$ 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 ( $\geq$  CIN2 and  $\geq$  CIN3) were calculated for women with different cobas<sup>®</sup> HPV Test results among different age groups and are presented in Table 17. The RRs of all comparisons were significantly greater than 1 for  $\geq$  CIN2 histology, except for HPV16 positive/18 positive vs. 12 Other HR HPV positive in  $\geq$  40 years.

**Table 17**  
**Relative Risks of  $\geq$  CIN2 and  $\geq$  CIN3 by cobas<sup>®</sup> HPV Test Result Stratified by Age in the ASC-US Population**

cobas <sup>®</sup> HPV Test Result	Age Group (Years)		
	21-29	30-39	$\geq$ 40
<b>Relative Risk for <math>\geq</math> CIN2</b>			
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%
<b>Relative Risk for <math>\geq</math> CIN3</b>			
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 ( $\geq$ 30 Years) Population

The risks of disease in the NILM ( $\geq$  30 years) population were compared in women with a positive cobas<sup>®</sup> HPV Test result to those with a negative cobas<sup>®</sup> 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 ( $\geq$  CIN2 or  $\geq$  CIN3) between subject groups with positive vs. negative cobas<sup>®</sup> 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 CPR diagnosis by all possible cobas<sup>®</sup> HPV Test result in the NILM ( $\geq$  30 years) population is presented in table 18.

**Table 18**  
**Summary of cobas<sup>®</sup> HPV Test Result and Central Pathology Review Panel Diagnosis in the NILM Population ( $\geq$  30 years)**

cobas <sup>®</sup> HPV Test Result	Central Pathology Review Diagnosis					Total
	Undetermined	Negative	CIN1	CIN2	$\geq$ CIN3	
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  $\geq$  CIN3 women, 75 are CIN3 and 5 are ACIS.

The CPR diagnosis and the crude estimate of absolute risk of disease ( $\geq$  CIN2 and  $\geq$  CIN3) by cobas<sup>®</sup> HPV Test result is presented in table 19. HPV16 positive had the highest crude absolute risk for both  $\geq$  CIN2 and  $\geq$  CIN3. In general, the crude absolute risks for both  $\geq$  CIN2 and  $\geq$  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)**

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

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

Note 2: HPV16 positive and/ or HPV18 positive include all 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**

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

**Table 21**  
**Performance of cobas® HPV Test In the NILM (≥ 30 years) Population (Unadjusted Estimates)**

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 — 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)**

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥ CIN2	HPV 16 positive /18 positive	<b>4.2</b> {(45/131)/(340/4,121)} ( 3.2, 5.4)
	12 Other HR HPV positive	<b>1.6</b> {(64/131)/(1,295/4,121)} ( 1.3, 1.9)
	HPV Negative	<b>0.3</b> {(22/131)/(2,492/4,121)} ( 0.2, 0.4)
≥ CIN3	HPV 16 positive /18 positive	<b>5.7</b> {(38/80)/347/4,178} ( 4.4, 7.3)
	12 Other HR HPV positive	<b>1.3</b> {(34/80)/1,325/4,178} ( 1.0, 1.7)
	HPV Negative	<b>0.2</b> {(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)**

Disease Endpoint	cobas® HPV Test Result	Likelihood Ratio (95% CI)
≥ CIN2	HPV16 positive/18 positive	<b>10.7</b> ( 6.5, 19.6)
	12 Other HR HPV positive	<b>4.0</b> ( 2.4, 7.2)
	HPV Negative	<b>0.7</b> ( 0.4, 0.8)
≥ CIN3	HPV16 positive / 18 positive	<b>20.2</b> ( 10.7, 39.4)
	12 Other HR HPV positive	<b>4.6</b> ( 2.4, 9.4)
	HPV Negative	<b>0.5</b> ( 0.1, 0.8)

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

Estimates of absolute risks of ≥CIN2 and ≥CIN3 for cobas® HPV Test results are presented in Table 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)**

cobas® HPV Test Result	≥ CIN2	≥ CIN3
<b>Unadjusted Estimates</b>		
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)
<b>Verification Bias Adjusted Estimates</b>		
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 /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**

Age Group	cobas® HPV Test Result	≥ CIN2	≥ CIN3
30-39 Years	<b>Unadjusted Estimates</b>		
	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%
	<b>Verification Bias Adjusted Estimates</b>		
	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	<b>Unadjusted Estimates</b>		
	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%
	<b>Verification Bias Adjusted Estimates</b>		
	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 also significantly higher in women who were HPV16 and/or 18 positive than women with (a) a positive result for 12 Other HR HPV types, or (b) a negative result.

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

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

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

\*95% CI is 2.5 and 97.5 percentile of RR distribution based on 1000 bootstrap samples.

Note 1: 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

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  $\geq$ 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  $\geq$ CIN2 and 11.9%, 3.0% and 0.31% for  $\geq$ CIN3 respectively.

**Table 28**  
**Current Risk and Current + Future Risk Based on Various cobas HPV Test Results in the NILM ( $\geq$ 30 Years) Population**

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)
$\geq$ 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)
$\geq$ 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 $\geq$ 21 Years and, NILM $\geq$ 30 Years Populations

The analytical performance of the **cobas**<sup>®</sup> 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  $\beta$ -globin and PGMY primers. The  $\beta$ -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<sup>37</sup>. PGMY-positive extracts were then amplified using HR HPV type-specific primers for subsequent sequencing reactions<sup>38</sup>.

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

The analytical accuracy of the **cobas**<sup>®</sup> 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  $\beta$ -globin amplification produced null result during sequencing. All women tested for analytical accuracy had valid **cobas**<sup>®</sup> HPV Test results.

**Table 29**  
**Percent Agreement of the cobas<sup>®</sup> HPV Test vs. the Composite Comparator**

Population	cobas <sup>®</sup> HPV Test Result	HPV Composite Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Indeterminate		
ASC-US $\geq$ 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 $\geq$ 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<sup>®</sup> HPV Test HPV16 Result vs. the HPV16 Sequencing Comparator**

Population	cobas <sup>®</sup> HPV Test: HPV16 Result	HPV 16 Sequencing Comparator			Total	Agreement Estimate & 95% CI
		Positive	Negative	Invalid		
<b>ASC-US ≥ 21 Years</b>	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%)
<b>NILM ≥ 30 Years</b>	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<sup>®</sup> HPV Test HPV18 Result vs. the HPV18 Sequencing Comparator**

Population	cobas <sup>®</sup> 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<sup>®</sup> HPV Test 12 Other HR HPV Result vs. the 12 Other HR HPV Sequencing Comparator**

Population	cobas <sup>®</sup> 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<sup>®</sup> HPV Test for Primary vs. Secondary Vials of Clinical Samples**

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 samples 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**

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**

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 with ≥CIN2 and for all women without ≥CIN2); then the changes in sensitivity and specificity were calculated for this scenario and 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**

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

### Primary Screening Population (≥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**<sup>®</sup> HPV Test result, and a valid cytology result. The percent of Invalid **cobas**<sup>®</sup> 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**<sup>®</sup> HPV Test) proceeded to colposcopy. Diagnosis of ≥CIN2 (by CPR) was observed in 431(5.5%) of 7,829 women with valid CPR 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**<sup>®</sup> HPV Test and cytology results are shown in Table 36. A correction for verification bias was applied due to the different rate of colposcopy in each category. Number of 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.

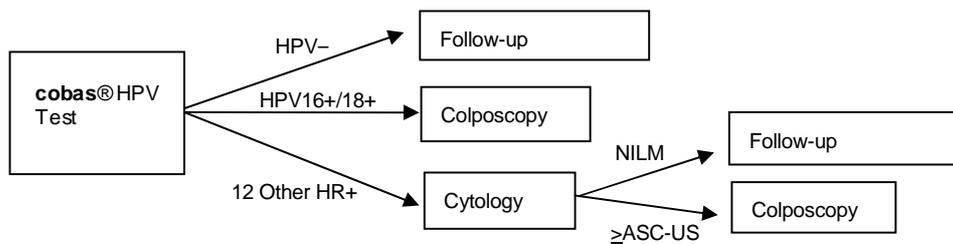
**Table 36**  
**Number of Patients with Colposcopy Results by cobas<sup>®</sup> HPV Test and cytology results**

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

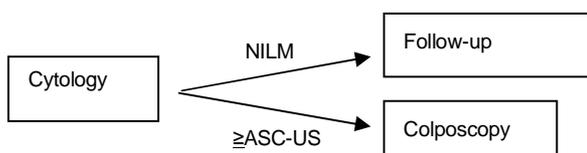
### Screening Algorithms

The use of the **cobas**<sup>®</sup> 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  $\geq$ CIN2 and  $\geq$ 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 (Table 39 and 40).

**Table 37**  
**Performance Comparison of the Primary Screening Algorithm and Cytology Algorithm ( $\geq$  CIN2)**

Algorithm	Prevalence of $\geq$ 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 ( $\geq$  CIN3)**

Algorithm	Prevalence $\geq$ 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  $\geq$  25 Years*

The performance comparisons of the HPV Primary Screening Algorithm and Cytology algorithm by age group for  $\geq$ 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  $\geq$ CIN3 (1.53%) is higher than 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  $\geq$ 30.

**Table 39**  
**Performance Comparison of the Primary Screening Algorithm and the Cytology Algorithm in age group 25-29 ( $\geq$  CIN3)**

Algorithm	Prevalence $\geq$ 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 ( $\geq$  CIN3)**

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

Algorithm	Prevalence=1.09 with 95% CI (0.89, 1.28)						
	%Pos	PPV	1-NPV	Sensitivity	1-Spec	PLR	NLR
<b>95% CI</b>	(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)
<b>Difference</b>	-1.55	6.78	-0.25	21.53	-1.82	7.95	-0.24
<b>95% CI</b>	(-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)
<b>Statistical Significant?</b>	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)**

Algorithm	Prevalence=0.83 with 95% CI (0.40, 1.53)						
	%Pos	PPV	1-NPV	Sensitivity	1-Spec	PLR	NLR
<b>Primary Screening</b>	2.78	12.58	0.50	41.98	2.45	17.14	0.59
<b>95% CI</b>	(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)
<b>Cytology</b>	6.22	5.05	0.55	37.72	5.95	6.34	0.66
<b>95% CI</b>	(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)
<b>Difference</b>	-3.44	7.53	-0.05	4.26	-3.50	10.80	-0.07
<b>95% CI</b>	(-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)
<b>Statistical Significant?</b>	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)**

Algorithm	Prevalence=0.63 with 95% CI (0.18, 1.51)						
	%Pos	PPV	1-NPV	Sensitivity	1-Spec	PLR	NLR
<b>Primary Screening</b>	1.96	8.72	0.47	27.26	1.80	15.11	0.74
<b>95% CI</b>	(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)
<b>Cytology</b>	3.77	4.50	0.48	27.04	3.63	7.46	0.76
<b>95% CI</b>	(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)
<b>Difference</b>	-1.81	4.22	-0.01	0.22	-1.83	7.65	-0.02
<b>95% CI</b>	(-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)
<b>Statistical Significant?</b>	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 (Table 43), of the primary screening population ≥25 years 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 with 12 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)**

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

Age Group	Category	Percent of patients with results (%)	Risk of ≥ CIN3 (%) (95% CI)	Risk of ≥ CIN2 (%) (95% CI)
<b>25-29 Years</b>	HPV 16/18 +	<b>6.97</b>	<b>12.7</b> ( 9.65, 16.1 )	<b>19.4</b> ( 15.7, 23.6 )
	12 Other HR HPV + and ≥ASC-US cytology	<b>3.61</b>	<b>5.83</b> ( 2.81, 9.57 )	<b>15.0</b> ( 10.1, 19.7 )

	12 Other HR HPV + and NILM cytology	<b>10.55</b>	<b>3.56</b> ( 2.09, 5.20 )	<b>5.56</b> ( 3.79, 7.52 )
	HR HPV -	<b>78.87</b>	<b>0.08</b> ( 0.00, 0.17 )	<b>0.30</b> ( 0.15, 0.49 )
<b>30-39 Years</b>	HPV 16/18 +	<b>3.18</b>	<b>20.2</b> ( 16.2, 24.5 )	<b>24.9</b> ( 20.4, 29.6 )
	12 Other HR HPV + and ≥ASC-US cytology	<b>2.09</b>	<b>7.42</b> ( 4.07, 11.5 )	<b>12.1</b> ( 8.10, 16.6 )
	12 Other HR HPV + and NILM cytology	<b>6.22</b>	<b>3.01</b> ( 1.87, 4.48 )	<b>5.77</b> ( 4.08, 7.69 )
	HR HPV -	<b>88.41</b>	<b>0.10</b> ( 0.05, 0.16 )	<b>0.18</b> ( 0.09, 0.26 )
<b>40-49 Years</b>	HPV 16/18 +	<b>1.56</b>	<b>14.3</b> ( 8.85, 19.9 )	<b>16.5</b> ( 10.6, 22.1 )
	12 Other HR HPV + and ≥ASC-US cytology	<b>1.22</b>	<b>10.5</b> ( 5.30, 16.8 )	<b>18.2</b> ( 12.2, 26.0 )
	12 Other HR HPV + and NILM cytology	<b>4.33</b>	<b>2.77</b> ( 1.42, 4.69 )	<b>4.94</b> ( 3.04, 7.34 )
	HR HPV -	<b>92.89</b>	<b>0.39</b> ( 0.01, 1.13 )	<b>0.80</b> ( 0.07, 1.84 )
<b>≥50 Years</b>	HPV 16/18 +	<b>1.18</b>	<b>8.20</b> ( 3.45, 14.2 )	<b>9.84</b> ( 4.39, 15.7 )
	12 Other HR HPV + and ≥ASC-US cytology	<b>0.78</b>	<b>8.64</b> ( 2.35, 16.4 )	<b>11.1</b> ( 3.90, 18.6 )
	12 Other HR HPV + and NILM cytology	<b>4.08</b>	<b>0.95</b> ( 0.00, 2.00 )	<b>2.13</b> ( 0.68, 3.60 )
	HR HPV -	<b>93.95</b>	<b>0.45</b> ( 0.01, 1.36 )	<b>1.67</b> ( 0.44, 3.27 )

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

For the Primary Screening Algorithm, where women who are 12 Other HR HPV positive 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. This results in approximately the same PPV, a small improvement in NPV and an 11% increase in the number of colposcopies (5.13%/4.62% =1.11).

**Table 45**

#### **Performance Comparison of Blinded and Unblinded Cytology Using the Primary Screening Algorithm (≥CIN3)**

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

#### **Analysis of Unsatisfactory (UNSAT) Cytology 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 men with satisfactory cytology. Taking this into account, for the 737 subjects 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)**

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, based on cytology slides read with/without knowledge of HPV status) and 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,  $\geq$ 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 ( $\geq$ 25 Years) (per 10,000 Women)**

Algorithm	Number of Test and Procedures			Benefit		Risk		
	Cytology	Cobas® HPV Test	Colposcopy	True Positive		False Negative		False positive
				$\geq$ CIN3	CIN2	$\geq$ 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 ( $\geq$ 25 Years) Population per 100 Colposcopy Procedures**

Benefit and risk per 100 colposcopy procedures in women  $\geq$ 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 ( $\geq$ 25 Years) (per 100 Colposcopy Procedures)**

Algorithm	Number of Test and Procedures			Benefit		Risk		
	Cytology	Cobas® HPV Test	Colposcopy	True Positive		False Negative		False positive
				$\geq$ CIN3	CIN2	$\geq$ 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 biased adjusted (VBA) estimates) of high-grade cervical disease ( $\geq$ CIN2 and  $\geq$ 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 ( $\geq$ 25 years) among women with different results from the **cobas** HPV Test and cytology results.

The risks at the Baseline for women with HPV16 positive/HPV18 positive results were 19.83% and 15.04% for the  $\geq$ CIN2 and  $\geq$ CIN3 endpoints, respectively (Table 46). 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  $\geq$ CIN2 and  $\geq$ CIN3 endpoints, respectively.

The risks at the baseline for women with 12 Other HR HPV positive and  $\geq$ ASC-US cytology results were 14.17% and 7.78% for the  $\geq$ CIN2 and  $\geq$ CIN3 endpoints, respectively (Table 49). The cumulative risks from Baseline to follow up Year 3 for women with 12 Other HR HPV positive and  $\geq$ ASC-US cytology results were 20.56% and 11.11% for the  $\geq$ CIN2 and  $\geq$ 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  $\geq$ ASC-US Cytology in the Primary Screening ( $\geq$ 25 Years) Population**

	Current Risk (%) (95% CI)	Current + Future Risk (%) at Year 3 (95% CI)

<b>≥CIN2</b>	<b>HPV16+/18+</b>	19.83 (17.39, 22.41)	28.03 (24.91, 31.07)
	<b>HPV16+</b>	23.54 (20.56, 26.71)	32.34 (28.73, 36.20)
	<b>HPV18+</b>	10.33 (6.73, 13.55)	17.02 (12.02, 21.75)
	<b>12 Other HR HPV+ and ≥ASC-US</b>	14.17 (11.36, 17.06)	20.56 (17.10, 23.94)
<b>≥CIN3</b>	<b>HPV16+/18+</b>	15.04 (12.98, 17.43)	21.11 (18.47, 23.90)
	<b>HPV16+</b>	17.72 (15.19, 20.72)	25.09 (21.89, 28.95)
	<b>HPV18+</b>	8.21 (5.10, 11.14)	10.94 (7.06, 14.49)
	<b>12 Other HR HPV+ and ≥ASC-US</b>	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 the Baseline and sum of the current risk and future risk at year 1, 2, and 3 is 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**

	<b>≥CIN2 (95% CI)</b>	<b>≥CIN3 (95% CI)</b>
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 the Baseline and sum of the 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**

	<b>≥CIN2 (95% CI)</b>	<b>≥CIN3 (95% CI)</b>
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*

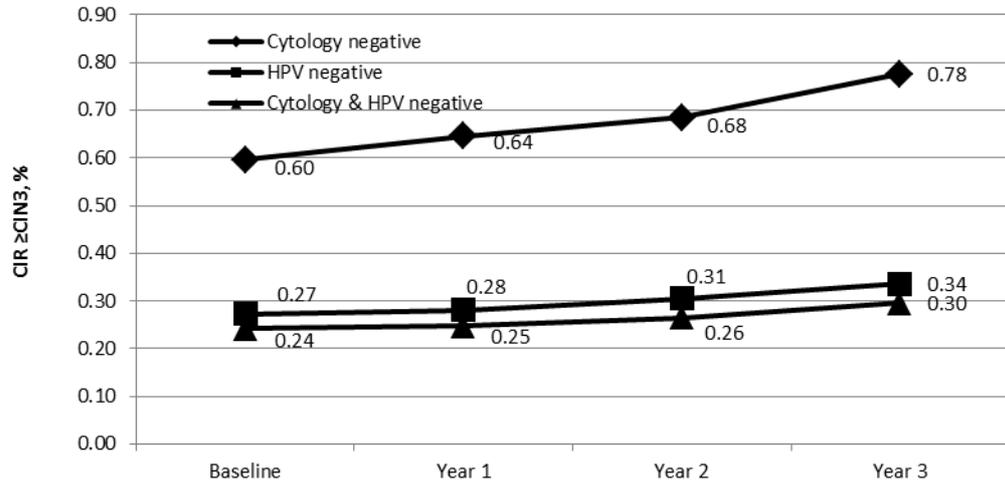
The risks of disease were compared in the primary screening population (≥25 years) between women with a NILM cytology result at the baseline versus women with a HR HPV negative results at the baseline (Table 52 and Figure 3). For those with a HR HPV negative result at baseline, the 3-year cumulative 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 result. The addition of NILM cytology result to a HR HPV negative result (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**

<b>Disease Endpoint</b>	<b>Baseline cobas HPV /Cytology Result</b>	<b>Current Risk ,% (95% CI)</b>	<b>Current + Future Risk at Year 3, %, (95% CI)</b>
<b>≥CIN2</b>	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)
<b>≥CIN3</b>	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**



## ANALYTICAL PERFORMANCE

### Clinical Cutoff Determination of the cobas® HPV Test

The clinical cutoff for detecting high-grade cervical disease ( $\geq$ 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<sup>40</sup> and was chosen to achieve a pre-defined level of sensitivity of 93% for  $\geq$  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 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 53 contains results from the reagent lot producing the most conservative (highest) LOD in the analysis.

**Table 53**  
**Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18)**

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

### Inclusivity Verification

To verify that the **cobas**® HPV Test is capable of accurately detecting all HPV high risk genotypes, the Limit of Detection (LOD) at the clinical cutoff was determined for genotypes 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Quantified plasmid stocks of each HPV genotype were diluted into a background of pooled HPV negative patient specimens collected in PreservCyt solution to concentrations below, above and at the expected LOD levels. Two lots of reagents were used to produce a minimum of 24 replicates for each positive level with each lot of reagents. For each HPV type, the reported LOD was defined as the lowest testing concentration having a > 95% positive hit rate. Table 54 contains results from the reagent lot producing the most conservative (higher) LOD in the analysis.

**Table 54**  
**Summary of High Risk Genotype Limit Of Detection For cobas® 4800 HPV Genotype Inclusivity Study**

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

68	1200	24/24	38.0	100.0%	85.7%	100.0%
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\*The LOD of the **cobas**<sup>®</sup> HPV Test for HPV genotypes 16, 18 and 31 was determined as described above in this Package Insert.

**Reproducibility**

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

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

All valid test results were included in the analyses that reported the percentage of correct results. There were no false positive results in 216 tests performed on the negative panel members (background negative cell and the pooled negative clinical sample; see Table 55 below).

For the percents of positive results for the positive panel members were presented in Table 56. 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 57) 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 55**  
**Results by Sample Type and Negative Panel Member for Lot and Site/Instrument**

Sample Type	Panel Member	Ct SD	Ct CV %	Number Negative / Total Number Valid Results					
				Lot			Site/ Instrument		
				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
				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 56**  
**Results by Sample Type and Positive Panel Member for Lot and Site/Instrument**

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

Sample Type	Panel Member	Ct SD	Ct CV %	Number Positive / Total Number Valid Results					
				Lot			Site/Instrument		
				ID	Positive/Valid	%	ID	Positive/Valid	%
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 57**  
**Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold,**  
**Estimated from Valid Samples of Positive Sample Type Panel Members**

Sample Type <sup>1</sup> / Conc. <sup>2</sup> (cells/mL)	Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]															
	n <sup>3</sup> N	Mean CT	Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site/Instrument		Total	
			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	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%

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]													
Sample Type <sup>1</sup> / Conc. <sup>2</sup> (cells/mL)			Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-Site/Instrument		Total	
	n <sup>3</sup> N	Mean CT	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
(27/mL)																
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%

<sup>1</sup> Moderate is abbreviated as mod.

<sup>2</sup> Analyte concentrations are given for the SiHa and HeLa cell lines.

<sup>3</sup> 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 50 (and % CV) may be underestimated.

#### Precision

In-house Precision was examined using a panel composed of HPV positive and negative cell lines diluted into PreservCyt solution and pooled HPV positive and negative cervical specimens collected in PreservCyt solution. The precision panel was designed to include members below (< 70% positivity rate), at (90% to 99% positivity rate) and above (> 99% positivity rate) the Limit of Detection of the cobas<sup>®</sup> 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 58. 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 59) 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 58**  
**Summary of the Precision Panel and Hit Rates For cobas® HPV Precision Study**

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

\*Results shown from detection channel 2 (HPV16)

\*\* Results shown from detection channel 3 (HPV18)

**Table 59**  
**Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold, Estimated from**  
**Valid Samples of Positive Sample Type Precision Panel Members**

#	Sample Type / Conc. <sup>1</sup> (cells/mL)	Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]													
		N <sup>2</sup> N	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%
1	SiHa HPV16 (25/mL)	<u>80</u> 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)	<u>138</u> 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)	<u>144</u> 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)	<u>142</u> 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)	<u>96</u> 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)	<u>143</u> 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)	<u>142</u> 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)	<u>144</u> 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	<u>140</u> 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	<u>143</u> 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	<u>140</u> 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	<u>144</u> 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	<u>142</u> 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	<u>144</u> 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	<u>133</u> 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	<u>144</u> 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)	<u>88</u> 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%

			Standard Deviation [SD] and Percent Coefficient of Variation [CV(%)]												
*18	SiHa HPV16 (60/mL)	<u>144</u>	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%
	HeLa HPV18 (22/mL)	144													
*19	SiHa HPV16 (80/mL)	<u>144</u>	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%
	HeLa HPV18 (27/mL)	144													
*20	SiHa HPV16 (150/mL)	<u>144</u>	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%
	HeLa HPV18 (50/mL)	144													
**17	SiHa HPV16 (25/mL)	<u>103</u>	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%
	HeLa HPV18 (8/mL)	143													
**18	SiHa HPV16 (60/mL)	<u>143</u>	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%
	HeLa HPV18 (22/mL)	144													
**19	SiHa HPV16 (80/mL)	<u>142</u>	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%
	HeLa HPV18 (27/mL)	144													
**20	SiHa HPV16 (150/mL)	<u>144</u>	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%
	HeLa HPV18 (50/mL)	144													

<sup>1</sup> Analyte concentrations are given for the SiHa and HeLa cell lines.

<sup>2</sup> 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 50 (and % CV) may be underestimated.

\*Results shown from detection channel 2 (HPV16)

\*\* Results shown from detection channel 3 (HPV18)

N/A = Not applicable

#### Analytical Specificity

A panel of bacteria, fungi and viruses, including those commonly found in the female urogenital tract, as well as several Human papillomavirus types classified as low or undetermined risk were tested with the **cobas**<sup>®</sup> HPV Test to assess analytical specificity. The organisms listed in Table 60 were spiked at high concentrations ( $\geq 1 \times 10^5$  \*units/reaction with the exception of *Treponema pallidum* and Adenovirus-5, which were both tested at  $1 \times 10^5$  \*units/reaction) into HPV negative specimen in PreservCyt solution and into HPV negative specimen in PreservCyt solution spiked with HPV31, HPV16 and HPV18 plasmid DNA at 3 times the limit of detection. Results indicated that none of these organisms interfered with detection of HPV 31, HPV16 and HPV18 or produced false positive results in the HPV negative specimen.

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

**Table 60**  
**Microorganisms Tested for Analytical Specificity**

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

Interfering Substances

HPV positive and HPV negative cervical specimens as well as contrived specimens were used to assess the effects of endogenous and exogenous interfering substances that could potentially be present in cervical specimens. Testing materials used in these studies are described in Table 61. 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 62. No interference was seen for PBMC or cervical mucus at all levels tested. Whole blood showed no interference when present in visually detectable amounts of up to 1.5%.

**Table 61**  
**Interference Testing Sample Descriptions**

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

**Table 62**  
**Interference Testing Results with Endogenous Interferents**

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

A total of 18 over-the-counter (OTC) feminine hygiene and contraceptive products were tested as potential interfering substances. Types of potential interferents tested and performance observations in 3 x LOD pools prepared from HPV negative cervical specimens in PreservCyt solution are described in Table 63.

**Table 63**  
**Interference Testing Results with Exogenous Interferents**

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

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Doc Rev. TBD TBD/2013	<b>INTENDED USE</b> statement revised to include primary screening claims <b>Clinical Performance</b> section revised to include data to support primary screening claims Please contact your local Roche Representative if you have any questions
Doc Rev. 1.0 04/2011	<b>First publishing.</b>



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