Rx Only

cobas® HPV

Qualitative nucleic acid test
for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

cobas® HPV	P/N: 07460155190
cobas® HPV Positive Control Kit	P/N: 07460171190
cobas® Buffer Negative Control Kit	P/N: 07002238190

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Intended use

cobas® HPV for use on the **cobas**® 6800/8800 Systems (**cobas**® HPV) is an automated qualitative *in vitro* test for the detection of human papillomavirus (HPV) DNA in patient specimens. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies HPV16 and HPV18 while concurrently detecting the other high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) at clinically relevant infection levels. Specimens are limited to cervical cells collected in Roche Cell Collection Medium (Roche Molecular Systems, Inc.), **cobas**® PCR Cell Collection Media (Roche Molecular Solutions, Inc.), PreservCyt® Solution (Hologic Corp.) and SurePath™ Preservative Fluid (BD Diagnostics-TriPath).

Indications for use of **cobas**® HPV are:

A. **cobas**® HPV is indicated for use in screening patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy.

B. **cobas**® HPV is indicated for use in screening patients with ASC-US cervical cytology results to assess the presence or absence of HR HPV genotypes 16 and 18.

C. **cobas**® HPV is indicated for use adjunctively with cervical cytology to assess the presence or absence of HR HPV types.

D. **cobas**® HPV is indicated for use adjunctively with cervical cytology to assess the presence or absence of HPV genotypes 16 and 18.

E. **cobas**® HPV is indicated for use as a first-line primary screening test to identify women at increased risk for the development of cervical cancer or presence of high-grade disease.

F. **cobas**® HPV is indicated for use as a first-line primary screening test to assess the presence or absence of HPV genotypes 16 and 18.

The results from **cobas**® HPV, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of **cobas**® HPV are not intended to prevent women from proceeding to colposcopy.

Summary and explanation of the test

Background

Persistent infection with human papillomavirus (HPV) is the principal cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN).[1-3] The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide.[3] HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 140 different genotypes of HPV[4,5] and approximately 40 different types that can infect the human anogenital mucosa.[6,7] However, only a subset of 14 HPV genotypes has been found to be the cause of most cervical cancer cases and precancerous cervical lesions.[3,8-13] In this document "HPV" indicates "high risk HPV", except where otherwise noted.

In developed countries with cervical cancer screening programs, cytology (Pap smear) has been used since the 1960s as the primary tool to detect early precursors to cervical cancer. Although it has dramatically decreased the incidence and death

from cervical cancer in those countries, cytology is costly, requires multiple testing at short intervals, and interpretation by highly trained cytopathologists has limited reproducibility and relatively low sensitivity for the detection of precancer. The discovery of persistent HPV as the single causative agent of cervical cancer generated interest in the use of HPV tests as a screening tool for cervical cancer and subsequent studies demonstrated that HPV-based testing was more sensitive than cytology for detection of precancer.[14] In 2001 professional guidelines first recommended the use of HPV testing as an adjunct to cytology in women ≥ 21 years, and by 2012 co-testing (cytology plus HPV testing) was designated as the preferred method of cervical cancer screening in women ≥ 30 years.[15] More recently, the **cobas®** 4800 HPV Test received FDA approval in 2014 as the first-line primary screen and interim guidance supporting HPV primary screening as an option for screening women ≥ 25 years was issued by 2 professional societies in 2015.[16]

Rationale for HPV testing

Most cervical cancer cases and deaths can be prevented through early detection of pre-cancerous changes in the cervix. Pap cytology testing has been central to cervical cancer screening programs for over 50 years and has contributed to the 70% decline in rates of cervical cancer in the developed world.[15] HPV is now recognized as a single, necessary cause of cancer of the cervix and is present in 99.7% of cases of cervical cancer.[3] Thirteen HPV genotypes are classified as carcinogenic or high-risk (HR) because of their association with cervical cancer: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and an additional genotype (66) is classified as probably carcinogenic.[17] Therefore, tests that detect infection with these HR HPV genotypes are now being used increasingly in cervical cancer screening programs. The 2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests[18] describes the utility of using a combination of cervical cytology, tests for HR HPV infection, and type-specific HPV Testing for women undergoing screening for cervical cancer. In these guidelines, the timing of additional investigations (e.g. colposcopy) and the interval for re-screening depends on the result of these tests. These guidelines have recently been revised and now recommend the combination of cytology and HR HPV testing (co-testing) as the preferred method of screening, with HPV 16/18 genotype-specific testing an added option.[15] HPV Testing provides a more sensitive method for cervical cancer screening than cytology[19] and its medical value has been clearly demonstrated as an adjunct to cytology, triage of ASC-US cytology, and as a first-line test.

Explanation of the test

cobas® HPV is a qualitative real-time[20,21] PCR test that detects 14 high-risk HPV genotypes. **cobas®** HPV uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the Master Mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).[3,9-13,22] This test utilizes β-globin DNA as an internal control to monitor the entire sample preparation and PCR amplification process so an additional primer pair targets the human β-globin gene (330 base pair amplicon). Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers. In addition, the test utilizes a low titer positive and a negative control.

cobas® HPV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification[23] and detection. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 6800/8800 software which assigns test results for all tests as positive, negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid (DNA) from patient samples and external controls is simultaneously extracted. In summary, nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and

potential PCR inhibitors are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

A thermostable DNA polymerase enzyme is used for PCR amplification. The HPV and β-globin sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step.[24] However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® HPV master mix contains detection probes specific for twelve High Risk HPV target sequences, one detection probe specific for the HPV16 target sequence, one detection probe specific for the HPV18 target sequence and one for β-globin. The amplified signal from twelve high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) is detected using the same fluorescent dye while HPV16, HPV18 and β-globin signals are each detected with their own dedicated fluorescent dye. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the HPV targets and β-globin, respectively.

Reagents and materials

cobas® HPV reagents and controls

Table 1 cobas® HPV

cobas® HPV
Store at 2-8°C
480 test cassette (P/N 07460155190)

Kit components	Reagent ingredients	Quantity per kit 480 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% Proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	38 mL
Empty Vessel (EV)	N/A	1
Elution Buffer (EB)	Tris buffer, 0.2% Methyl-4 hydroxibenzoate	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, Potassium hydroxide, < 0.1% Sodium azide	14.5 mL
HPV Master Mix Reagent 2 (HPV MMX-R2)	Tricine buffer, Potassium acetate, EDTA, Glycerol, < 18% Dimethyl sulfoxide, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.1% Tween 20, < 0.1% Sodium azide, < 0.1% Z05 DNA polymerase, < 0.10% AmpErase (uracil N-glycosylase) enzyme (microbial), < 0.1% Upstream and downstream HPV primers, < 0.01% Upstream and downstream β-globin primers, < 0.01% Fluorescent-labeled oligonucleotide probes specific for HPV and β-globin, < 0.01% Oligonucleotide aptamer	17.5 mL

Table 2 cobas® HPV Positive Control Kit

cobas® HPV Positive Control Kit
Store at 2–8°C
(P/N 07460171190)

Kit components	Reagent ingredients	Quantity per kit
HPV Positive Control (HPV (+) C)	Tris buffer, < 0.05% EDTA, < 0.1% Sodium azide, < 0.01% Non-infectious plasmid DNA (microbial) containing HPV16, HPV18 and HPV 39 sequences, < 0.01% Non-infectious plasmid DNA (microbial) containing β-globin sequences, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit
Store at 2-8°C
(P/N 07002238190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1mL)

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® HPV kit. See listing of additional materials required (Table 7).

**Product safety labeling primarily follows EU GHS guidance

*** Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® HPV	2–8°C
cobas® HPV Positive Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 describes the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the cobas® 6800/8800 Systems

Reagent	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® HPV	90 days from first usage	Max 20 runs	Max 20 hours
cobas® HPV Positive Control Kit	Not applicable	Max 16 runs	Max 10 hours
cobas® Buffer Negative Control Kit	Not applicable	Max 16 runs	Max 10 hours
cobas omni Lysis Reagent	30 days from loading	Not applicable	Not applicable
cobas omni MGP Reagent	30 days from loading	Not applicable	Not applicable
cobas omni Specimen Diluent	30 days from loading	Not applicable	Not applicable
cobas omni Wash Reagent	30 days from loading	Not applicable	Not applicable

Additional equipment and materials required

Table 7 Equipment, materials and consumables required for use with cobas® HPV

Material	P/N
cobas® Sample Prep Buffer (CSPB)*	06526985190
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container	07094361001
Tubes, 13 mL Round Base, for use as secondary sample tubes	07958048190
Caps, neutral color	07958056190
Heat-resistant barcode labels	RACO Industries, RAC-225075-9501
Vortex Mixer (single tube)	Any vendor
Thermometer -20/150ºC	VWR 89095-600 or equivalent
Digital Heater Block 120V	VWR 75838-294 or equivalent
12-Hole Heat Block Module 16mm	VWR 13259-162 or equivalent
MPA RACK 16 MM LIGHT GREEN 7001-7050 [a,b]	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR [a,b]	11902997001

*An open bottle of **cobas**® Sample Prep Buffer (CSPB) may be stored at ambient temperature (15-30°C) for up to 21 days and up to 4 separate uses for the pre-analytic treatment of SurePath™ samples.

[a] MPA 16mm and RD5 racks are required to use **cobas**® HPV. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

[b] MPA 16mm rack is the preferred rack.

Table 8 Specimen collection kits for use with cobas® HPV

Collection Kit	P/N
Roche Cell Collection Medium (250 Vials)	07994745190
cobas® PCR Cell Collection Media (250 Vials)	05619637190
ThinPrep Pap Test Physician's Kit (500 vials & Broom-like collection devices) ThinPrep Pap Test Physician's Kit (500 vials & Cytobrush/spatula collection devices)	Hologic 70136-001 Hologic 70136-002
SurePath™ GYN Preservative Vial Kit BD SurePath™ Collection Vial Kit (500 vials) BD SurePath™ Collection Vial Kit (25 vials)	Becton, Dickinson 490522, 490527 Becton, Dickinson 491253 Becton, Dickinson 491324
Cervical Collection Brush – 20 Bags, 25 Brushes each (500/Box) Cervical Collection Brush – sterilized & single packed (100/box)	08399832190 08779040190
Rovers® Cervex-Brush® Combi (500/Box)	VWR 89171-022
Cytobrush Plus GT - 25 Bags, 100 Brushes each (2,500/Box) Cytobrush Plus GT – 2 Bags, 500 Brushes each (1,000/Box) Cytobrush Plus GT – 10 Bags, 10 Brushes each (100/Box) Cytobrush Plus GT Sterile – 1 Brush per Pouch (40/Box) Cytobrush Plus GT Scored – 25 Bags, 100 Brushes each (2,500/Box) Pap-Perfect Plastic Spatulas (500/Box)	Medscand C0105 Medscand C0121 Medscand C0104 Medscand C0112 Medscand C0305 Medscand 11080
42mm Manual Replacement Caps for Vials (loose, 250/bag)	08037230190 (optional)
42mm Replacement Caps for Vials (8 trays of 48/box)	07682247001 (optional)

Instrumentation and software required

The **cobas®** 6800/8800 software and **cobas®** HPV analysis packages shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system(s).

Table 9 Instrumentation

Equipment	P/N
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories[25] and in the CLSI Document M29-A4.[26] Only personnel proficient in handling infectious materials and the use of **cobas**® HPV and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- Do not freeze any samples stored in primary or secondary tubes.
- Use only supplied or specified required consumables to ensure established test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect established test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples, reagents, or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Expended control kits contain pierced vials with residual reagent; special care should be taken during disposal to avoid spills and contact.
- **cobas**® HPV, **cobas**® HPV Positive Control Kit, **cobas**® Negative Control Kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Avoid contaminating gloves when handling samples and controls. Gloves must be changed between handling samples and **cobas**® HPV, **cobas**® HPV Positive Control Kit, **cobas**® Buffer Negative Control Kit and **cobas omni** reagents to prevent contamination.
- Wash hands thoroughly after handling samples and reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the instrument, follow the instructions in the **cobas**® 6800/8800 Systems User Guide to properly clean and decontaminate the surface of instrument(s).

Specimen collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Specimen collection

Cervical specimens collected in Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media, PreservCyt® Solution and SurePath™ Preservative Fluid have been validated for use with **cobas**® HPV. Follow the manufacturer's instructions for collecting cervical specimens.

Specimen transport

Cervical specimens collected in Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media, PreservCyt® Solution or SurePath™ Preservative Fluid 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.[27]

Specimen storage

Cervical specimens collected in Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media and PreservCyt® Solution may be stored at 2-30°C for up to 3 months after the date of collection prior to performing **cobas**® HPV. See Roche Cell Collection Medium labeling for medium storage requirements. See **cobas**® PCR Cell Collection Media Solution labeling for medium storage requirements. See PreservCyt® Solution labeling for medium storage requirements. Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media and PreservCyt® specimens should not be frozen.

SurePath™ Preservative Fluid matrix-induced cross-links are reversed through treatment with **cobas**® Sample Prep Buffer (CSPB) prior to HPV testing. The pre-analytic treatment is a mandatory step for all cervical specimens collected in SurePath™ prior to testing with **cobas**® HPV. SurePath™ specimens should not be frozen.

Primary vials of cervical specimens collected in SurePath™ Preservative Fluid may be stored at 2-8 °C for up to 3 months or

for up to 6 weeks at 15-30°C after the date of collection. If desired, SurePath™ specimens may be mixed with **cobas**® Sample Prep Buffer in a secondary tube and stored at 2-30°C for up to 6 weeks before completing the heat step as described in the "Running **cobas**® HPV" section. Alternatively, SurePath™ specimens may be stored at 2-30°C for up to 6 weeks after samples are pre-treated [as described in the "Running **cobas**® HPV" section] and prior to HPV testing.

Table 10 summarizes the acceptable specimen storage conditions prior to testing with **cobas**® HPV.

Table 10 Summary of acceptable specimen storage conditions prior to testing with cobas® HPV

Specimen Type		2-8°C	15-30°C
Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media and PreservCyt®		3 months	3 months
SurePath™*	Storage of primary vial sample prior to pre-analytic treatment	3 months	6 weeks
	or		
	Storage of sample mixed with CSPB prior to heat step	6 weeks	6 weeks
	or		
	Storage of treated sample	6 weeks	6 weeks

*An open bottle of **cobas**® Sample Prep Buffer (CSPB) may be stored at ambient temperature (15-30°C) for up to 21 days and up to 4 separate uses for the pre-analytic treatment of SurePath™ samples.

Instructions for use

Procedural notes

- Do not use **cobas**® HPV, **cobas**® HPV Positive Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of the sample racks. Refer to the **cobas**® 6800/8800 Systems User Guide for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 6800/8800 Systems User Guide for proper maintenance of instruments.

Running cobas® HPV

cobas® HPV can be run with a minimum required sample volume of 1.0 mL for Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media and PreservCyt® specimens as well as for SurePath™ specimens that have undergone the pre-analytic procedure. The pre-analytic procedure is described on the following page. The operation of the instrument is described in detail in the **cobas**® 6800/8800 Systems User's Guide. Figure 1 summarizes the procedure.

- It is necessary to aliquot specimens into barcoded 13 mL round-bottom secondary tubes for processing on the **cobas**® 6800/8800 Systems. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
- A single run can have any combination of specimens (Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media, PreservCyt® Solution and/or SurePath™ Preservative Fluid) and each specimen can be tested with either the HPV High Risk (HPV-HR) or HPV High Risk Plus Genotyping (HPV-GT) ASAPs.
- Specimens should be processed using the sample type selection in the user interface (UI) of **cobas**® HPV as described in Table 11.
- Heat-resistant barcode labels are required for tubes used for specimens collected in SurePath™ Preservative Fluid.

Table 11 Sample type selection in the user interface of cobas® HPV

Specimen	Collection kit type	Process as Sample Type
Cervical specimen	Roche Cell Collection Medium	RCCM
Cervical specimen	**cobas**® PCR Cell Collection Media	RCCM
Cervical specimen	PreservCyt® Solution (ThinPrep)	PreservCyt®
Cervical specimen	SurePath™ Preservative Fluid	Surepath™

Roche Cell Collection Medium, cobas® PCR Cell Collection Media or PreservCyt® Solution

1. Prepare a barcoded 13 mL round-bottom secondary tube for each Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® or specimen to be tested.
2. With clean gloved hands, **vortex** each Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® primary specimen vial for **10 seconds** immediately prior to transfer.
3. Uncap a primary vial and transfer at least **1.0 mL** but no more than **4.0 mL** into the prepared barcoded secondary tube from step 1. *Always use caution when transferring specimens from primary containers to secondary tube. Always use a new pipette tip for each specimen*. Transfer tube to a rack (or cap the secondary tube if testing will be performed at a future time).
4. Re-cap the primary vial with a replacement cap before moving to the next specimen. Store the primary vial upright.
5. Load the racks of uncapped secondary tubes into the Sample Supply Module and process on the **cobas®** 6800/8800 Systems for HPV testing.

SurePath™ Preservative Fluid

1. Prepare a barcoded* 13 mL round-bottom secondary tube for each SurePath™ specimen to be tested and aliquot **0.5 mL** of **cobas®** Sample Prep Buffer (CSPB) into each secondary tube.
2. With clean gloved hands, vortex each SurePath™ primary specimen vial for **10 seconds** immediately prior to transfer.
3. Uncap a primary vial and transfer **0.5 mL** of SurePath™ specimen into the prepared barcoded secondary tube from step 1. *Always use caution when transferring specimens from primary containers to secondary tube. Always use a new pipette tip for each specimen.*
4. Cap the secondary tube and re-cap the primary vial with a replacement cap before moving to the next specimen. Store the primary vial upright.
5. Vortex each secondary tube for **1 second**.
6. Transfer tubes to the heating unit set to **95°C** and incubate for **20 minutes**.
7. Remove tubes to a collection rack and cool at ambient temperature for **10 minutes**. *Use caution as the secondary tubes may be hot.*
8. Vortex each secondary tube for **5 seconds**.
9. Uncap tubes, discard caps, transfer to racks and process on the **cobas®** 6800/8800 Systems for HPV testing.
10. SurePath™ specimens treated with **cobas®** Sample Prep Buffer can be stored for future HPV testing if needed. After following the above procedure up to step 7, store the tubes with SurePath™ specimens treated with **cobas®** Sample Prep Buffer at 2-30°C for up to 6 weeks prior to HPV testing on the **cobas®** 6800/8800 Systems.

**Heat-resistant barcode labels are required for tubes used with the heat step to reverse matrix-induced cross-links. See "Additional equipment and materials required" section for recommended product numbers.*

Figure 1 cobas® HPV procedure

1

Log onto the system
Press Start to Prepare the system
Order Tests

- Choose “RCCM” for ordering Roche Cell Collection Medium or **cobas®** PCR Cell Collection Media specimens
- Choose “PreservCyt” for ordering PreservCyt® Solution specimen
- Choose “SurePath” for ordering SurePath™ Preservative Fluid specimens that have undergone the defined pre-analytic procedure

2

Refill reagents and consumables as prompted by the system

- Load test specific reagent cassette
- Load control cassettes
- Load pipette tips
- Load processing plates
- Load MGP Reagent
- Load amplification plates
- Refill Specimen Diluent
- Refill Lysis Reagent
- Refill Wash Reagent

3

Loading specimens onto the system

- For each primary Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® specimen vial:
  - Vortex for 10 seconds
  - Aliquot a minimum of 1 mL of Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® specimen into a 13 mL round-bottom secondary tube
  - Transfer tube to rack
- For each primary SurePath™ specimen vial:
  - Aliquot 0.5 mL of CSPB into a 13 mL round-bottom secondary tube
  - Vortex primary SurePath™ specimen vial for 10 seconds
  - Aliquot 0.5 mL of SurePath™ specimen into the prepared secondary tube containing 0.5 mL of CSPB and cap tightly
  - Vortex each tube for 1 second
  - Transfer tubes to a heating unit set to 95°C and incubate for 20 minutes
  - Remove tubes to a collection rack and cool at ambient temperature for 10 minutes
  - Vortex each tube for 5 seconds
  - Un-cap tube and transfer to rack
- Load sample rack and clotted tip racks into the sample supply module
- Confirm samples have been accepted into the transfer module

4

Start run

5

Review and export results

6

Remove sample tubes. If needed, cap any sample tubes meeting the minimum volume requirements for future use. Clean up instrument

- Unload empty control cassettes
- Empty amplification plate drawer
- Empty liquid waste
- Empty solid waste

Results

cobas® HPV automatically detects and discriminates 14 high risk HPV genotypes (HPV-HR) and/or 12 high risk genotypes with individual typing of HPV 16 and HPV 18 simultaneously (HPV-GT).

Quality control and validity of results

- One **cobas**® Buffer Negative Control [(–) Ctrl] and one HPV Positive Control [HPV (+) C] are processed with each batch of a requested result type (HPV-HR or HPV-GT).
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control performance.

Interpretation of results

cobas® HPV for cobas® System Software v1.2

Display examples for **cobas**® HPV for **cobas**® System Software v1.2 are shown in Figure 2 and Figure 3.

Figure 2 Example of cobas® HPV result display for the HPV-HR results request for cobas® System Software v1.2

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2	Target 3
HPV-HR	C161420284084194727902	Yes		HPV (+) C	**Valid**	Valid		
HPV-HR	C161420284090428825772	Yes		(-) Ctrl	**Valid**	Valid		
HPV-HR 400 ul	PC_HPVHRinv_01	No	Y40T	PreservCyt®	**Invalid**	Invalid		
HPV-HR 400 ul	PC_HPVHRneg_01	Yes		PreservCyt®	**Negative**	HR HPV Negative		
HPV-HR 400 ul	PC_HPVHRneg_02	Yes		PreservCyt®	**Negative**	HR HPV Negative		
HPV-HR 400 ul	PC_HPVHRneg_03	Yes		PreservCyt®	**Negative**	HR HPV Negative		
HPV-HR 400 ul	SP_HPVHRinv_01	No	Y40T	Surepath™	**Invalid**	Invalid		
HPV-HR 400 ul	SP_HPVHRneg_01	Yes		Surepath™	**Negative**	HR HPV Negative		

Figure 3 Example of cobas® HPV result display for the HPV-GT results request for cobas® System Software v1.2

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2	Target 3
HPV-GT 400 ul	SP_HPVGTpos_03	Yes		Surepath™	**Reactive**	Other HR HPV Positive	HPV 16 Negative	HPV 18 Negative
HPV-GT 400 ul	SP_HPVGTpos_05	Yes		Surepath™	**Reactive**	Other HR HPV Positive	HPV 16 Positive	HPV 18 Negative
HPV-GT 400 ul	SP_HPVGTpos_06	Yes		Surepath™	**Positive**	Other HR HPV Positive	HPV 16 Positive	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTpos_02	Yes		PreservCyt®	**Reactive**	Other HR HPV Negative	HPV 16 Negative	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTneg_01	Yes		PreservCyt®	**Negative**	Other HR HPV Negative	HPV 16 Negative	HPV 18 Negative
HPV-GT 400 ul	PC_HPVGTpos_06	No	C02H1	PreservCyt®	**Invalid**	Invalid	HPV 16 Positive	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTpos_03	No	C02H1	PreservCyt®	**Invalid**	Invalid	HPV 16 Positive	Invalid
HPV-GT 400 ul	C161420284090390657451	Yes		HPV (+) C	**Valid**	Valid	Valid	Valid
HPV-GT 400 ul	C161420284090419645071	Yes		(-) Ctrl	**Valid**	Valid	Valid	Valid

For a valid batch, check each individual sample for flags in the **cobas®** 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Samples are marked with “Yes” in the column ‘Valid’ if all requested target results reported valid results. Samples marked with “No” in the column ‘Valid’ may require additional interpretation and action.
- The values in “Overall Result” column for individual samples should be interpreted as follows:
    - Positive - All requested results are positive
    - Reactive – At least one of the requested results is positive and the other(s) negative
    - Negative – All requested results are negative
    - Invalid – At least one requested result is invalid
- Values reported in the “Overall Result” column **do not** impact the validity of results reported within individual target result columns.
- Reported target results for individual samples are valid unless indicated as “Invalid” within the individual target result column.
- Invalid results for one or more target combinations are possible with the HPV-GT result request and are reported out specifically for each channel. Refer to retesting instructions for the respective specimen type below.
- For invalid target results from Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For invalid target results from SurePath™ specimens the original specimen should be retested if there is sufficient volume. If the results are still invalid a new specimen should be obtained.

cobas® HPV for cobas® System Software v1.3

Display examples for **cobas®** HPV for **cobas®** System Software v1.3 are shown in Figure 4 and Figure 5.

Figure 4 Example of cobas® HPV result display for the HPV-HR results request for cobas® System Software v1.3

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2	Target 3
HPV-HR	C161420284084194727902	Yes		HPV (+) C	**Valid**	Valid		
HPV-HR	C161420284090428825772	Yes		(-) Ctrl	**Valid**	Valid		
HPV-HR 400 ul	PC_HPVHRinv_01	NA	Y40T	PreservCyt®	**NA**	Invalid		
HPV-HR 400 ul	PC_HPVHRneg_01	NA		PreservCyt®	**NA**	HR HPV Negative		
HPV-HR 400 ul	PC_HPVHRneg_02	NA		PreservCyt®	**NA**	HR HPV Negative		
HPV-HR 400 ul	PC_HPVHRneg_03	NA		PreservCyt®	**NA**	HR HPV Negative		
HPV-HR 400 ul	RCCM_HPVHRpos_01	NA		RCCM	**NA**	HR HPV Positive		
HPV-HR 400 ul	RCCM_HPVHRpos_02	NA		RCCM	**NA**	HR HPV Positive		
HPV-HR 400 ul	RCCM_HPVHRpos_03	NA		RCCM	**NA**	HR HPV Positive		
HPV-HR 400 ul	SP_HPVHRinv_01	NA	Y40T	Surepath™	**NA**	Invalid		
HPV-HR 400 ul	SP_HPVHRneg_01	NA		Surepath™	**NA**	HR HPV Negative		

Note: The Target 2 and Target 3 columns are reserved for HPV16 and HPV 18 results with HPV-GT request, respectively.

Figure 5 Example of cobas® HPV result display for the HPV-GT results request for cobas® System Software v1.3

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2	Target 3
HPV-GT 400 ul	RCCM_HPVGTpos_02	NA		RCCM	**NA**	Other HR HPV Negative	HPV 16 Negative	HPV 18 Positive
HPV-GT 400 ul	RCCM_HPVGTpos_01	NA		RCCM	**NA**	Other HR HPV Negative	HPV 16 Positive	HPV 18 Positive
HPV-GT 400 ul	RCCM_HPVGTpos_04	NA		RCCM	**NA**	Other HR HPV Positive	HPV 16 Negative	HPV 18 Positive
HPV-GT 400 ul	SP_HPVGTpos_03	NA		Surepath™	**NA**	Other HR HPV Positive	HPV 16 Negative	HPV 18 Negative
HPV-GT 400 ul	SP_HPVGTpos_05	NA		Surepath™	**NA**	Other HR HPV Positive	HPV 16 Positive	HPV 18 Negative
HPV-GT 400 ul	SP_HPVGTpos_06	NA		Surepath™	**NA**	Other HR HPV Positive	HPV 16 Positive	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTpos_02	NA		PreservCyt®	**NA**	Other HR HPV Negative	HPV 16 Negative	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTneg_01	NA		PreservCyt®	**NA**	Other HR HPV Negative	HPV 16 Negative	HPV 18 Negative
HPV-GT 400 ul	PC_HPVGTpos_06	NA	C02H1	PreservCyt®	**NA**	Invalid	HPV 16 Positive	HPV 18 Positive
HPV-GT 400 ul	PC_HPVGTpos_03	NA	C02H1	PreservCyt®	**NA**	Invalid	HPV 16 Positive	Invalid
HPV-GT 400 ul	C161420284090390657451	Yes		HPV (+) C	**Valid**	Valid	Valid	Valid
HPV-GT 400 ul	C161420284090419645071	Yes		(-) Ctrl	**Valid**	Valid	Valid	Valid

For a valid batch, check each individual sample for flags in the **cobas®** 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- The "Valid" and "Overall Result" columns are not applicable (NA) to sample results for **cobas®** HPV and are marked with "NA". Values reported in these columns **do not** impact the validity of results reported within individual target result columns.
- Reported target results for individual samples are valid unless indicated as "Invalid" within the individual target result column.
- Invalid results for one or more target combinations are possible with the HPV-GT result request and are reported out specifically for each channel. Refer to retesting instructions for the respective specimen type below.
- For invalid target results from Roche Cell Collection Medium, **cobas®** PCR Cell Collection Media or PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For invalid target results from SurePath™ specimens the original specimen should be retested if there is sufficient volume. If the results are still invalid a new specimen should be obtained.

Results and their corresponding interpretation for detecting HR HPV only (Table 12) and Other HR HPV, HPV 16 and HPV 18 (Table 13) are shown below. This interpretation is equally applicable to results reported with either **cobas®** System Software version.

Table 12 cobas® HPV results and interpretation for the HPV-HR result request

Target 1	Target 2	Target 3	Interpretation
HR HPV Positive	<Blank>	<Blank>	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 and 68.
HR HPV Negative			HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid			The result for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 is invalid.

Table 13 cobas® HPV results and interpretation for the HPV-GT result request

Target 1	Target 2	Target 3	Interpretation
Other HR HPV Positive	HPV 16 Positive, HPV 16 Negative, or Invalid	HPV 18 Positive, HPV 18 Negative, or Invalid	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 and 68.
Other HR HPV Negative			HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were undetectable or below the pre-set threshold.
Invalid			The result for HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is invalid.
Other HR HPV Positive, Other HR HPV Negative, or Invalid	HPV 16 Positive	HPV 18 Positive, HPV 18 Negative, or Invalid	Specimen is positive for HPV type 16 DNA.
	HPV 16 Negative		HPV type 16 DNA was undetectable or below the pre-set threshold.
	Invalid		The result for HPV type 16 is invalid.
Other HR HPV Positive, Other HR HPV Negative, or Invalid	HPV 16 Positive, HPV 16 Negative, or Invalid	HPV 18 Positive	Specimen is positive for HPV type 18 DNA.
		HPV 18 Negative	HPV type 18 DNA was undetectable or below the pre-set threshold.
		Invalid	The result for HPV type 18 is invalid.

Procedural limitations

- **cobas**® HPV has been evaluated only for use in combination with the **cobas**® HPV Positive Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- **cobas**® HPV has only been validated for use with cervical specimens collected in Roche Cell Collection Medium, **cobas**® PCR Cell Collection Media, PreservCyt® Solution and SurePath™ Preservative Fluid. Assay performance has not been validated for use with other collection media and/or specimen types. Use of other collection media and/or specimen types may lead to false positive, false negative or invalid results.
- **cobas**® HPV has been validated for testing cervical specimens collected in SurePath™ Preservative Fluid treated with **cobas**® Sample Prep Buffer to reverse SurePath™ Preservative Fluid matrix-induced cross-links. Processing SurePath™ specimens without following the pre-treatment protocol with **cobas**® Sample Prep Buffer or pre-treatment with alternate reagents may produce false negative or invalid results.
- **cobas**® HPV 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.[18]
- **cobas**® HPV 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 and for other medico-legal indications.
- 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.
- 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.
- 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.
- A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.
- β-globin amplification and detection is included in **cobas**® HPV to differentiate HPV negative specimens from those that do not exhibit HPV signal due to insufficient cell mass in the specimen. All HPV negative specimens must have a valid β-globin signal within a pre-defined range to be identified as valid negatives.
- Reliable results depend on proper sample collection, storage and handling procedures.
- The addition of AmpErase enzyme into the **cobas**® HPV Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use are necessary to avoid contamination of reagents.
- Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 6800/8800 Systems.
- 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. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies and normal variability of the tests.

- The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
- Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by **cobas**® HPV's primers and/or probes may result in failure to detect the presence of the viral DNA.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Use of over-the-counter products Replens™, RepHresh™ Vaginal Gel and RepHresh™ Clean Balance™ Kit has been associated with false-negative results.
- Use of Metronidazole Vaginal Gel has been associated with false-negative results.
- HPV negative results are not intended to prevent women from proceeding to colposcopy.
- Positive test results indicates the presence of any one or more of the high risk types, but since patients may be co-infected with low-risk types it does not rule out the presence of low-risk types in patients with mixed infections.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Clinical performance characteristics

Specimens from a multi-center, prospective, population based cohort of participants in a study designed to evaluate the performance of **cobas®** HPV for identifying high-grade cervical disease (CIN2, CIN3, cervical cancer or adenocarcinoma in situ [ACIS]) were tested. Study participants represented a general screening population with histology assessment based on central pathology review panel (CPRP). Eligible women were 25-65 years of age undergoing routine cervical cancer screening that had signed informed consent and satisfied study inclusion/exclusion criteria. Two cervical samples were collected, first in SurePath™ Preservative Fluid and a second collected in PreservCyt® Solution. Three tests were performed for all subjects for each sample type: Pap cytology test, **cobas®** HPV for use on the **cobas®** 6800/8800 Systems and **cobas®** 4800 HPV Test.

Women with ≥ ASC-US cytology in SurePath™ were invited to undergo colposcopy. In addition, all women with a positive test result for HR HPV DNA (positive by the **cobas®** 4800 HPV Test), as well as a randomly selected subset of women with NILM (negative for intraepithelial lesions or malignancy) cytology and negative HR HPV DNA (by the **cobas®** 4800 HPV Test), were selected 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 CPRP consisting of three expert pathologists, and discordant results adjudicated according to a pre-defined protocol. For each sample type, the clinical performance (sensitivity and specificity) of the **cobas®** 4800 HPV Test and **cobas®** HPV for use on the **cobas®** 6800/8800 Systems was measured against CPRP histology results. The analyses were performed for those women with histology ≥ CIN2 by CPRP. A total of 995 PreservCyt® samples (Table 14) and 841 SurePath™ samples (Table 15) collected in the clinical trial with completed histology assessment were tested. There were 65 women with histologic diagnosis of ≥ CIN2.

Table 14 Performance of cobas® HPV and cobas® 4800 HPV Test for the Detection of ≥CIN2 in PreservCyt®

	cobas® HPV		**cobas®** 4800 HPV Test	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Sensitivity	93.8% (61/65)	(85.2%, 97.6%)	93.8% (61/65)	(85.2%, 97.6%)
Specificity	41.7% (387/929)	(38.5%, 44.9%)	43.3% (403/930)	(40.2%, 46.5%)

CI = Confidence interval

Table 15 Performance of cobas® HPV and cobas® 4800 HPV Test for the Detection of ≥CIN2 in SurePath™

	cobas® HPV		**cobas®** 4800 HPV Test	
	Estimate	**95% CI**	**Estimate**	**95% CI**
Sensitivity	93.1% (54/58)	(83.6%, 97.3%)	94.8% (55/58)	(85.9%, 98.2%)
Specificity	43.4% (340/783)	(40.0%, 46.9%)	33.6% (263/783)	(30.4%, 37.0%)

CI = Confidence interval

Agreement between the cobas® HPV in SurePath™ and PreservCyt® with composite comparator

Agreement between **cobas®** HPV results and a composite comparator consisting of HPV DNA results from Qiagen Hybrid Capture 2 (hc2; high risk genotypes probe) and the **cobas®** 4800 HPV Test was assessed. A positive result from both the **cobas®** 4800 HPV Test and hc2 assays was defined as composite comparator positive, a negative result from both the **cobas®** 4800 HPV Test and hc2 was defined as composite comparator negative; samples with discordant results between the two methods were considered indeterminate and not used in calculating positive, negative and overall agreement. Positive, negative, and overall percent agreement was calculated for each media type versus the composite comparator. Final data analysis included **cobas®** HPV and composite comparator results from 2318 PreservCyt® specimens (Table 16) and 1651 SurePath™ specimens (Table 17).

Table 16 Agreement between cobas® HPV and composite comparator (hc2 in PreservCyt® and cobas® 4800 HPV in PreservCyt®) for samples collected in PreservCyt®

	Composite Comparator Result						
cobas® HPV Result	**Positive**	**Negative**	**Indeterminate***	**Total**	**PPA (95% CI)**	**NPA (95% CI)**	**OPA (95% CI)**
Positive	195	33	67	295	98.0% (195/199) (94.9%, 99.2%)	98.3% (1966/1999) (97.7%, 98.8%)	98.3% (2161/2198) (97.7%, 98.8%)
Negative	4	1966	53	2023			
Total	199	1999	120	2318			

CI = Confidence interval, NPA = Negative percent agreement, OPA = Overall percent agreement, PPA = Positive percent agreement

*hc2 and **cobas®** 4800 HPV Test did not agree

There were three invalid results by **cobas®** HPV

Table 17 Agreement between cobas® HPV and composite comparator (hc2 in PreservCyt® and cobas® 4800 HPV in SurePath™) for samples collected in SurePath™

	Composite Comparator Result						
cobas® HPV Result	**Positive**	**Negative**	**Indeterminate***	**Total**	**PPA (95% CI)**	**NPA (95% CI)**	**OPA (95% CI)**
Positive	141	13	50	204	94.0% (141/150) (89.0%, 96.8%)	99.1% (1376/1389) (98.4%, 99.5%)	98.6% (1517/1539) (97.8%, 99.1%)
Negative	9	1376	62	1447			
Total	150	1389	112	1651			

CI = Confidence interval, NPA = Negative percent agreement, OPA = Overall percent agreement, PPA = Positive percent agreement

*hc2 and **cobas®** 4800 HPV did not agree

Non-clinical performance evaluation

Performance with cervical specimens collected in Roche Cell Collection Medium has shown to be comparable to cervical specimens collected in PreservCyt® Solution. Performance testing with cervical specimens collected in SurePath™ Preservative Fluid was completed using treatment with **cobas®** Sample Prep Buffer. All concentrations listed in the following studies are reflective of the treated SurePath™ sample.

Key performance characteristics

Limit of Detection (LoD)

The LoD for HPV16 and HPV18 were assessed using SiHa and HeLa cell lines in the background of pooled HPV negative patient specimens collected in PreservCyt® Solution and SurePath™ Preservative Fluid. Cell lines were diluted to concentrations below, above and at the expected LoD levels. A minimum of 24 replicates were tested for each cell line level in both PreservCyt® Solution and SurePath™ Preservative Fluid using 3 reagent lots with an equal number of runs performed on the **cobas®** 6800 and the **cobas®** 8800 Systems. The LoD was defined as the level of HPV cells in the sample that has positive test results at least 95% of the time with all concentration levels above exhibiting positive results more than 95% of the time.

The LoD for SiHa was 16 cells/mL for both PreservCyt® and SurePath™; the LoD for HeLa was 16 cells/mL in PreservCyt® and 8 cells/mL in SurePath™. Table 18 through Table 21 contain results from the reagent lot producing the most conservative (highest) LoD in the analysis for HPV16 and HPV18 in PreservCyt® Solution and in SurePath™ Preservative Fluid, respectively.

Dilution panels of HPV16 and HPV18 cell lines in the background of pooled HPV negative patient specimens collected in Roche Cell Collection Medium and PreservCyt® Solution were tested side-by-side. The limit of detection for **cobas®** HPV was comparable.

Table 18 Limit of Detection levels for HPV16 (SiHa Cell Line) in PreservCyt® Solution

SiHa Concentration (cells/mL)	Number of Positive/Tested	% Positive	95% Confidence Interval
32	24 / 24	100%	85.8% - 100%
16	**24 / 24**	**100%**	**85.8% - 100%**
8	22 / 24	91.7%	73.0% - 100%

Table 19 Limit of Detection levels for HPV16 (SiHa Cell Line) in SurePath™ Preservative Fluid

SiHa Concentration (cells/mL)	Number of Positive/Tested	% Positive	95% Confidence Interval
32	24 / 24	100%	85.8% - 100%
16	**23 / 24**	**95.8%**	**78.9% - 100%**
8	21 / 24	87.5%	67.6% - 97.3%

Table 20 Limit of Detection levels for HPV18 (HeLa Cell Line) in PreservCyt® Solution

HeLa Concentration (cells/mL)	Number of Positive/Tested	% Positive	95% Confidence Interval
32	24 / 24	100%	85.8% - 100%
16	**24 / 24**	**100%**	**85.8% - 100%**
8	22 / 24	91.7%	73.0% - 100%

Table 21 Limit of Detection levels for HPV18 (HeLa Cell Line in SurePath™ Preservative Fluid

HeLa Concentration (cells/mL)	Number of Positive/Tested	% Positive	95% Confidence Interval
16	24 / 24	100%	85.8% - 100%
8	**24 / 24**	**100%**	**85.8% - 100%**
4	20 / 24	83.3%	62.6% - 95.3%

Inclusivity

Plasmids for high risk genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 were tested in the background of pooled HPV negative patient specimens collected in PreservCyt® Solution and SurePath™ Preservative Fluid. All 12 of the high risk genotypes tested were detected by the assay.

Precision

Within-laboratory precision was examined using a panel with each member composed from either HPV cell lines or HPV positive clinical samples diluted into a pool of negative cervical specimen matrix collected in PreservCyt® Solution and a pool of negative cervical specimen matrix collected in SurePath™ Preservative Fluid.

The precision panel was designed to include members with very low, low and medium concentrations of HPV (High Negative, < LoD, ~ LoD and > LoD) as well as HPV negative samples for each sample type. Testing was performed with three lots of **cobas®** HPV reagents on two instruments. There was an equal number of runs performed on the **cobas®** 6800 and the **cobas®** 8800 Systems over 12 days for a total of 24 runs for each panel member. A description of the precision panels and the observed hit rates are shown in Table 22 and Table 23.

All panel members tested exhibited the expected hit rates. Analysis of standard deviation and percent coefficient of variation of the Ct values from valid tests performed on positive panel members yielded overall CV (%) ranges from 4.32% to 6.34% for Other High Risk HPV (Table 24), 1.09% to 4.61% for HPV16 (Table 25) and 1.21% to 3.76% for HPV18 (Table 26).

Within-laboratory precision was also examined using panels prepared by adding SiHa and HeLa cell lines into a background of pooled HPV negative patient specimens collected in Roche Cell Collection Medium at and above the LoD. Testing of the panels prepared in Roche Cell Collection Medium demonstrated precision comparable to the precision with panels prepared in PreservCyt® Solution.

Table 22 Summary of within laboratory precision in PreservCyt® Solution

Panel Level	Expected Hit Rate	Target Source	HPV Concentration	Target Channel	N Tested	N Positive	Hit Rate	95% CI	
								LL	UL
Negative	0%	N/A	N/A	Other HR HPV	72	0	**0%**	0%	5%
Negative	0%	N/A		HPV16	72	0	**0%**	0%	5%
Negative	0%	N/A		HPV18	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample	N/A	Other HR HPV	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample		HPV16	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample		HPV18	72	5	**7%**	2%	15%
< 1x LoD	< 95%	Clinical sample	N/A	Other HR HPV	72	30	**42%**	30%	54%
< 1x LoD	< 95%	Clinical sample	N/A	HPV16	71	33	**47%**	35%	59%
< 1x LoD	< 95%	Clinical sample	N/A	HPV18	72	49	**68%**	56%	79%
< 1x LoD	20-80%	SiHa cell line	4.8 cells/mL	HPV16	72	44	**61%**	49%	72%
< 1x LoD	20-80%	HeLa cell line	4.8 cells/mL	HPV18	72	49	**68%**	56%	79%
~ 1x LoD	≥ 95%	Clinical sample	N/A	Other HR HPV	72	72	**100%**	95%	100%
~ 1x LoD	≥ 95%	SiHa cell line	16 cells/mL	HPV16	72	72	**100%**	95%	100%
~ 1x LoD	≥ 95%	HeLa cell line	16 cells/mL	HPV18	72	72	**100%**	95%	100%
> 1x LoD	≥ 99%	Clinical sample	N/A	Other HR HPV	72	72	**100%**	95%	100%
> 1x LoD	≥ 99%	SiHa cell line	48 cells/mL	HPV16	72	72	**100%**	95%	100%
> 1x LoD	≥ 99%	HeLa cell line	48 cells/mL	HPV18	72	72	**100%**	95%	100%

CI = Confidence interval, LL = Lower limit, UL = Upper limit

Table 23 Summary of within laboratory precision in SurePath™ Preservative Fluid

Panel Level	Expected Hit Rate	Target Source	HPV Concentration	Target Channel	N Tested	N Positive	Hit Rate	95% CI	
								LL	UL
Negative	0%	N/A	N/A	Other HR HPV	72	0	**0%**	0%	5%
Negative	0%	N/A		HPV16	72	0	**0%**	0%	5%
Negative	0%	N/A		HPV18	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample	N/A	Other HR HPV	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample		HPV16	72	0	**0%**	0%	5%
High Negative	≤ 5%	Clinical sample		HPV18	72	0	**0%**	0%	5%
< 1x LoD	< 95%	Clinical sample	N/A	Other HR HPV	72	64	**89%**	79%	95%
< 1x LoD	< 95%	Clinical sample	N/A	HPV16	72	11	**15%**	8%	26%
< 1x LoD	< 95%	Clinical sample	N/A	HPV18	72	36	**50%**	38%	62%
< 1x LoD	20-80%	SiHa cell line	4.8 cells/mL	HPV16	72	55	**76%**	65%	86%
< 1x LoD	20-80%	HeLa cell line	2.4 cells/mL	HPV18	72	47	**65%**	53%	76%
~ 1x LoD	≥ 95%	Clinical sample	N/A	Other HR HPV	72	72	**100%**	95%	100%
~ 1x LoD	≥ 95%	SiHa cell line	16 cells/mL	HPV16	72	72	**100%**	95%	100%
~ 1x LoD	≥ 95%	HeLa cell line	8 cells/mL	HPV18	72	70	**97%**	90%	100%
> 1x LoD	≥ 99%	Clinical sample	N/A	Other HR HPV	72	72	**100%**	95%	100%
> 1x LoD	≥ 99%	SiHa cell line	48 cells/mL	HPV16	72	72	**100%**	95%	100%
> 1x LoD	≥ 99%	HeLa cell line	24 cells/mL	HPV18	72	72	**100%**	95%	100%

CI = Confidence interval, LL = Lower limit, UL = Upper limit

Table 24 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold – Other High Risk HPV

			Random Effect															
			Day		Instrument		Operator		Lot		Between Run		Within Run		Residual		Total	
Level	Hit Rate	Mean Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Cervical samples collected in PreservCyt® Solution																		
< LoD	41.7%	33.2	0	0	0	0	0	0	0	0	0.47	1.43	0	0	1.72	5.18	1.78	5.37
~ LoD	100%	32.4	0	0	0	0	0.49	1.50	0.16	0.51	0	0	0	0	1.94	5.98	2.01	6.19
> LoD	100%	30.7	0	0	0	0	0	0	0.27	0.88	0	0	0	0	1.30	4.23	1.33	4.32
Cervical samples collected in SurePath™ Preservative Fluid																		
< LoD	88.9%	32.7	0	0	0.16	0.50	0	0	0	0	0	0	0	0	2.07	6.32	2.07	6.34
~ LoD	100%	32.1	0.45	1.41	0	0	0.32	1.01	0.75	2.32	0	0	0	0	1.65	5.13	1.89	5.89
> LoD	100%	29.9	0	0	0	0	0	0	0	0	0.31	1.04	0	0	1.82	6.09	1.85	6.18

Table 25 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold – HPV16

			Random Effect															
			Day		Instrument		Operator		Lot		Between Run		Within Run		Residual		Total	
Level	Hit Rate	Mean Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Cervical samples collected in PreservCyt® Solution																		
< LoD	46.5%	35.7	0.84	2.34	0.29	0.80	0.85	2.39	0	0	0	0	0	0	1.10	3.07	1.65	4.61
< LoD	61.1%	36.1	0.44	0.67	0	0	0.16	0.45	0.21	0.57	0	0	0	0	0.49	1.36	0.61	1.68
~ LoD	100%	35.0	0	0	0.02	0.06	0.02	0.07	0.38	1.09	0	0	0.16	0.46	0.42	1.20	0.59	1.69
> LoD	100%	34.0	0.03	0.09	0.04	0.12	0	0	0.27	0.78	0	0	0	0	0.25	0.74	0.37	1.09
Cervical samples collected in SurePath™ Preservative Fluid																		
< LoD	15.3%	36.9	0	0	0	0	0.93	2.52	0	0	0	0	0	0	0.96	2.61	1.34	3.63
< LoD	76.4%	37.1	0.27	0.72	0.10	0.28	0	0	0.25	0.67	0	0	0.32	0.87	0.58	1.58	0.77	2.07
~ LoD	100%	36.3	0	0	0.15	0.40	0	0	0.35	0.95	0.12	0.32	0.11	0.29	0.47	1.29	0.62	1.71
> LoD	100%	35.2	0	0	0.07	0.20	0	0	0.35	0.98	0.01	0.04	0	0	0.33	0.94	0.49	1.38

Table 26 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold – HPV18

			Random Effect															
			Day		Instrument		Operator		Lot		Between Run		Within Run		Residual		Total	
Level	**Hit Rate**	**Mean Ct**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Cervical samples collected in PreservCyt® Solution																		
< LoD	68.1%	35.9	0	0	0.55	1.52	0	0	0.18	0.51	0.17	0.49	0	0	1.21	3.37	1.35	3.76
< LoD	68.1%	35.3	0.19	0.54	0	0	0.02	0.06	0	0	0	0	0	0	0.97	2.75	0.99	2.80
~ LoD	100%	33.8	0	0	0	0	0	0	0.37	1.11	0	0	0	0	0.73	2.17	0.82	2.44
> LoD	100%	32.2	0	0	0	0	0	0	0.22	0.68	0.03	0.10	0	0	0.33	1.02	0.39	1.23
Cervical samples collected in SurePath™ Preservative Fluid																		
< LoD	50.0%	37.3	0.14	0.36	0	0	0.10	0.27	0.25	0.66	0	0	0	0	0.45	1.21	0.54	1.45
< LoD	65.3%	36.3	0.23	0.65	0	0	0	0	0.27	0.74	0.15	0.42	0.20	0.55	0.54	1.49	0.70	1.92
~ LoD	97.2%	35.7	0	0	0	0	0	0	0.33	0.94	0.07	0.20	0	0	0.56	1.57	0.65	1.84
> LoD	100%	34.4	0	0	0.06	0.19	0.02	0.06	0.20	0.57	0.04	0.13	0	0	0.36	1.04	0.42	1.21

Analytical specificity/cross-reactivity

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 **cobas**® HPV to assess analytical specificity. The organisms listed in Table 27 were spiked at concentrations of approximately 1 x 10^6 units*/mL for bacteria and approximately 1 x 10^5 units*/mL for viruses into pools of HPV negative cervical specimens in PreservCyt® Solution and in SurePath™ Preservative Fluid. Testing was performed with each potential interfering organism alone as well as with each organism mixed with SiHa (HPV16) and HeLa (HPV18) cell lines at approximately 3x LoD. Results indicated that none of these organisms interfered with detection of HPV16 and HPV18 DNA or produced a false positive result in the HPV negative specimen.

* All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydophila psittaci* which was quantified as Elementary Bodies (EB). All viruses were quantified as units/mL as determined by $TCID_{50}$ Endpoint Dilution Assay except Epstein Barr virus which was in copies/mL. *Trichomonas vaginalis* was quantified as cells/mL.

Table 27 Microorganisms tested for analytical specificity/cross-reactivity

Adenovirus Type 40	Herpes Simplex Virus 1	*HPV82*
Bacteroides caccae	Herpes Simplex Virus 2	*HPV83*
Bacteroides ureolyticus	HPV6	*HPV84*
Bifidobacterium adolescentis	HPV11	*HPV85*
Bifidobacterium breve	HPV26	*HPV89*
Bifidobacterium longum	HPV30	*Klebsiella oxytoca*
Candida albicans	HPV34	*Lactobacillus acidophillus*
Chlamydia trachomatis	HPV40	*Neisseria gonorrhoeae*
Chlamydophila psittaci	HPV42	*Peptostreptococcus anaerobius*
Clostridium difficile (Serogroup B)	HPV53	*Peptostreptococcus asaccharolyticus*
Clostridium perfringens	HPV54	*Peptostreptococcus magnus*
Corynebacterium genitalium	HPV55	*Proteus mirabilis*
Cytomegalovirus	HPV61	*Proteus penneri*
Enterobacter aerogenes	HPV62	*Proteus vulgaris*
Enterobacter cloacae	HPV64	*Pseudomonas aeruginosa*
Enterococcus avium	HPV67	*Pseudomonas fluorescens*
Enterococcus casseliflavus	HPV69	*Pseudomonas putida*
Enterococcus faecalis	HPV70	*Staphylococcus aureus*
Enterococcus faecium	HPV71	*Staphylococcus epidermidis*
Epstein Barr Virus	HPV72	*Streptococcus agalactiae*
Escherichia coli	HPV73	*Streptococcus pyogenes*
Fusobacterium nucleatum	HPV81	*Trichomonas vaginalis*

Interference

The effects of endogenous and exogenous substances that may be present in cervical specimens were tested for potential interference. All testing for interference was performed with each potential interfering substance alone as well as with the substance mixed with SiHa (HPV16) and HeLa (HPV18) cell lines at approximately 3x LoD in pools of HPV negative cervical specimens in Roche Cell Collection Medium, PreservCyt® Solution and in SurePath™ Preservative Fluid.

Endogenous substances tested were cervical mucus, peripheral blood mononuclear cells and whole blood. Levels of endogenous substances tolerated by the assay for specimen types are shown in Table 28. Exogenous substance testing included 17 over-the-counter (OTC) feminine hygiene and prescription products that are listed in Table 29. Of OTC feminine hygiene and prescription products tested, Metronidazole Gel, Replens™, RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance™ Feminine Freshness Kit produced false negative results.

Potential interference from the presence of glacial acetic acid was also tested in pools of HPV negative and HPV positive cervical specimens in Roche Cell Collection Medium and PreservCyt® Solution. Concentrations up to and including 5% (v/v) of glacial acetic acid were tolerated by the assay.

Table 28 Summary of endogenous substance concentrations that did not interfere with performance

Endogenous Substance	Roche Cell Collection Medium	PreservCyt®	SurePath™
Mucus	Presence*	Presence*	Presence*
Peripheral Blood Mononuclear Cells (PBMCs as cells/mL)	1.00E+06	1.00E+06	1.00E+05
Whole Blood (% v/v)	10%	10%	10%

*Presence refers to the amount of cervical mucus normally removed from the cervix prior to sampling

Table 29 List of substances tested for interference in cervical specimens

Product Name	
Clindamycin Phosphate Vaginal Cream	Norforms® Suppositories
CVS Tioconazole 1 (Equate™ tioconazole 1)	Premarin® Vaginal Cream
Equate™ Vagicaine Anti-Itch Cream	Replens™ Long-Lasting Vaginal Moisturizer*
Estrace® Cream	RepHresh™ Odor Eliminating Vaginal Gel*
K-Y® Ultra Gel	RepHresh™ Clean Balance™ Feminine Freshness Kit*
Metronidazole Vaginal Gel*	Summer's Eve® Feminine Deodorant Spray
Monistat® 3 Vaginal Antifungal Combination Pack	VCF® - Vaginal Contraceptive Foam
Monistat® Complete Care Itch Relief Cream	Yeast Gard Advanced®
Gyne-Lotrimin® 7	Glacial acetic acid**

* Metronidazole Vaginal Gel, Replens™, RepHresh™ Odor Eliminating Vaginal Gel and RepHresh™ Clean Balance™ Feminine Freshness Kit showed interference at levels that may potentially be present in clinical specimens.

**Concentrations of ≤ 5%(v/v) glacial acetic acid did not show interference. GAA testing was done in cervical specimens collected in PreservCyt® Solution only.

Cross contamination

Studies were performed to evaluate potential cross contamination on the **cobas**® 6800/8800 Systems using **cobas**® HPV. In this performance study the sample to sample cross-contamination rate of **cobas**® HPV has been determined to be 0.139% (1/719) when alternating very high positive sample representing more than 95% of the positives in the intended use population with negative samples over multiple runs. Run to run cross-contamination has been determined to be 0% (0/470). Testing was done using samples prepared in Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid.

Whole system failure

The samples tested in the whole system failure study were pooled HPV negative clinical cervical specimens collected in Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid. Each pool of clinical specimens was spiked with SiHa (HPV16) cells and HeLa (HPV18) cells to a concentration at around 3x LoD for each sample type. The results of this study determined that the hit rate was in excess of 99% in Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid.

Method correlation

The performance of **cobas**® HPV was compared to the **cobas**® 4800 HPV Test using cervical specimens collected in PreservCyt® Solution and cervical specimens collected in SurePath™ Preservative Fluid. All SurePath™ specimens were treated with **cobas**® Sample Prep Buffer according to the pre-analytic method defined for each test.

A total of 6961 cervical specimens collected in PreservCyt® Solution and 5755 cervical specimens collected in SurePath™ Preservative Fluid were tested for this correlation analysis.

The correlation results and calculated positive, negative and overall percent agreements along with 95% confidence intervals are shown in Table 30 for PreservCyt® specimens and Table 31 for SurePath™ specimens. There were 397 discordant specimens for High Risk HPV for the two sample types, combined; of which 212 were positive by **cobas**® HPV and 185 were positive by **cobas**® 4800 HPV Test.

Table 30 Correlation between cobas® HPV and the cobas® 4800 HPV Test for cervical specimens collected in PreservCyt® Solution

		cobas® 4800 HPV Test – 14 HR Result		
		Positive	Negative	**Total**
cobas® HPV - 14 HR Result	Positive	834	146	980
	Negative	57	5924	5981
	Total	891	6070	6961

Result (%)		**95% Confidence Interval**
Positive Percent Agreement	93.6%	91.8% - 95.0%
Negative Percent Agreement	97.6%	97.2% - 98.0%
Overall Percent Agreement	97.1%	96.7% - 97.5%

Agreements for HPV16/HPV18 detection between **cobas**® HPV and the **cobas**® 4800 HPV Test are (estimate and 95% confidence interval): PPA: 99.5% (97.2%-99.9%); NPA: 98.6% (98.2%-98.8%); and OPA: 98.6% (98.3%-98.8%)

Table 31 Correlation between cobas® HPV and the cobas® 4800 HPV Test for cervical specimens collected in SurePath™ Preservative Fluid

		cobas® 4800 HPV Test – 14 HR Result		
		Positive	Negative	**Total**
cobas® HPV - 14 HR Result	Positive	701	66	767
	Negative	128	4860	4988
	Total	829	4926	5755

Result (%)		**95% Confidence Interval**
Positive Percent Agreement	84.6%	81.9% - 86.9%
Negative Percent Agreement	98.7%	98.3% - 98.9%
Overall Percent Agreement	96.6%	96.1% - 97.1%

Agreements for HPV16/HPV18 detection between **cobas**® HPV and the **cobas**® 4800 HPV Test are (estimate and 95% confidence interval): PPA: 97.7% (94.3%-99.1%); NPA: 99.0% (98.7%-99.3%); and OPA: 99.0% (98.7%-99.2%)

Comparison of test performance with Roche Cell Collection Medium and PreservCyt® Solution

A comparison of **cobas**® HPV results for cervical specimens in Roche Cell Collection Medium and cervical specimens in PreservCyt® Solution was performed. Cervical samples were co-collected from the same subjects, placed into Roche Cell Collection Medium or PreservCyt® Solution in randomized order and tested. Specimens positive for any of the 14 high risk HPV genotypes detected by the test (HPV-HR) were considered positive; specimens with negative results for all of the 14 high risk HPV genotypes detected by the test were considered negative.

Performance of **cobas**® HPV with Roche Cell Collection Medium and PreservCyt® Solution was compared using the two sample test for proportions. The 95% confidence intervals for the difference in proportions (Roche Cell Collection Medium - PreservCyt® Solution) for both HPV positives and HPV negatives were inclusive of "0" which confirmed that the results for cervical specimens collected in Roche Cell Collection Medium were not statistically dissimilar from results for cervical specimens collected in PreservCyt® Solution (Table 32).

Table 32 Two sample test for proportions for cervical specimens collected in Roche Cell Collection Medium and cervical specimens collected in PreservCyt® Solution

Count Total % Column % Row %	**14 HPV-HR Positive**	**14 HPV-HR Negative**	**Total**
Roche Cell Collection Medium (RCCM)	**490** 16.57 49.80 33.04	**993** 33.58 50.33 66.96	**1483** 50.15
PreservCyt® Solution (PCYT)	**494** 16.71 50.20 33.51	**980** 33.14 49.67 66.49	**1474** 49.85
Total	**984** 33.28	**1973** 66.72	**2957**

Two Sample Test for Proportions	**Proportion Difference**	**Lower 95% Confidence Limit**	**Upper 95% Confidence Limit**
P(14 HR HPV Positive|RCCM)-P(14 HR HPV Positive|PCYT)	-0.00473	-0.03868	0.029224
P(14 HR HPV Negative|RCCM)-P(14 HR HPV Negative|PCYT)	0.004731	-0.02922	0.038676

Additional information

Key assay features

Sample types	• Cervical specimen collected in Roche Cell Collection Medium • Cervical specimen collected in **cobas®** PCR Cell Collection Media • Cervical specimen collected in PreservCyt® Solution • Cervical specimen collected in SurePath™ Preservative Fluid
Amount of sample processed	• ≥1000 µL required in sample tube for Roche Cell Collection Medium samples, instrument processes 400 µL • ≥1000 µL required in sample tube for **cobas®** PCR Cell Collection Media samples, instrument processes 400 µL • ≥1000 µL required in sample tube for PreservCyt® samples, instrument processes 400 µL • 1000 µL required in sample tube for SurePath™ samples treated with **cobas®** Sample Prep Buffer, instrument processes 400 µL
Test duration	• < 3.5 hours to first result

Symbols

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

Table 33 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In vitro* diagnostic medical device
	Authorized representative in the European Community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD performance evaluation only		Global Trade Item Number
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture

CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an *in vitro* diagnostic medical device

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 34 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
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Roche Diagnostics GmbH
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68305 Mannheim, Germany

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
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This product is covered by one or more of US Patent Nos. 8097717, 8192958, 8129118, and 6727067, and foreign equivalent patents of each.

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Document revision

Document Revision Information	
Doc Rev. 5.0 11/2020	Updated hazard warnings. Updated **trademarks and patents** section and distributors addresses. Inserted Rx Only symbol on first page. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.