cobas® 4800 HPV Test

FOR *IN VITRO* DIAGNOSTIC USE.

cobas® 4800 System Sample Preparation Kit	c4800 SMPL PREP	960 Tests 240 Tests	P/N: 05235804190 P/N: 05235782190
cobas® 4800 HPV Amplification/Detection Kit	c4800 HPV CTLS	960 Tests 240 Tests	P/N: 05235910190 P/N: 05235901190
cobas® 4800 HPV Controls Kit	c4800 LIQ CYT	10 Sets	P/N: 05235855190
cobas® 4800 System Liquid Cytology Preparation Kit	c4800 WB	960 Tests 240 Tests	P/N: 05235839190 P/N: 05235812190
cobas® 4800 System Wash Buffer Kit	c4800 HPV AMP/DET	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.

INTENDED USE

The **cobas®** 4800 Human Papillomavirus (HPV) Test is a qualitative *in vitro* test for the detection of Human Papillomavirus in patient specimens. The test utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies (types) 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) at clinically relevant infection levels. Specimens are limited to cervical cells collected in Roche Cell Collection Medium (Roche Molecular Systems, Inc.), , PreservCyt® Solution (Cytyc Corp.) and SurePath™ Preservative Fluid (BD Diagnostics-TriPath).

Indications for use of the **cobas®** 4800 HPV Test are:

(a) The **cobas®** 4800 HPV Test is indicated for use in screening patients 21 years and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology results to determine the need for referral to colposcopy.

(b) The **cobas®** 4800 HPV Test is indicated for use in screening patients 21 years and older with ASC-US cervical cytology results, to assess the presence or absence of high-risk HPV genotypes 16 and 18.

(c) The **cobas®** 4800 HPV Test is indicated for use in patients 30 years and older adjunctively with cervical cytology to assess the presence or absence of high risk HPV types.

(d) The **cobas®** 4800 HPV Test is indicated for use in patients 30 years and older adjunctively with cervical cytology to assess the presence or absence of HPV genotypes 16 and 18.

(e) The **cobas®** 4800 HPV Test is indicated for use as a first-line primary screening test in patients 25 years and older to identify women at increased risk for the development of cervical cancer or presence of high-grade disease.

(f) The **cobas®** 4800 HPV Test is indicated for use as a first-line primary screening test in patients 25 years and older to assess the presence or absence of HPV genotypes 16 and 18.

The **cobas®** 4800 HPV Test can also be used with healthcare worker–instructed self-collected vaginal specimens collected in Roche Cell Collection Medium or PreservCyt® Solution.

The results from the **cobas®** 4800 HPV Test, 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 the **cobas®** HPV Test are not intended to prevent women from proceeding to colposcopy.

SUMMARY AND EXPLANATION OF THE TEST

Persistent infection with human papillomavirus (HPV) is the principal cause of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN)[1-3]. The presence of HPV has been implicated in greater than 99% of cervical cancers, worldwide[3]. HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 118 different types of HPV[4,5], and approximately 40 different HPVs that can infect the human anogenital mucosa[6,7]. However, only a subset of 13 to 18 of these types is considered high-risk for the development of cervical cancer and its precursor lesions[3,8-13]. In an analysis of data from the International Agency of Research on Cancer (IARC) multi-center case-control study, the pooled OR (Odds Ratio) for squamous-cell cervical cancer with HPV infection was 158.2 when the analysis was restricted to studies using well validated HPV detection techniques[12]. In this study, the odds ratios for cervical cancer ranged from 109 to 276 in studies from different parts of the world[12].

Although persistent infection with high-risk (HR) HPV is a necessary cause of cervical cancer and its precursor lesions, a very small percentage of infections progress to these disease states. Sexually transmitted infection with HPV is extremely common, with estimates of up to 75% of all women

experiencing exposure to HPV at some point[14]. However, > 90% of infected women will mount an effective immune response and clear the infection in 6 to 24 months without any long term health consequences[15-20]. An infection with any HPV type can produce cervical intraepithelial neoplasia (CIN) although this also usually resolves once the HPV infection has been cleared[21].

In developed countries with cervical cancer screening programs, the Pap smear has been used since the mid-1950s as the primary tool to detect early precursors to cervical cancer. Although it has decreased the death rates due to cervical cancer dramatically in those countries, the Pap smear requires interpretation by highly trained cytopathologists and is a relatively inaccurate test with a high rate of false negatives. Cytological abnormalities observed in the Pap smear are primarily due to infection with HPV; however, various inflammatory or sampling variations can result in false positive Pap results. Triage of an abnormal Pap smear 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. The implementation of HPV DNA testing has increased the efficiency of cervical cancer screening programs by detecting high-risk lesions earlier in women 30 years and older with NILM cytology and by reducing the need for unnecessary colposcopy and treatment in patients 21 and older with ASC-US cytology. Furthermore, the superior sensitivity of HPV testing over Pap smears for the detection of high grade disease in a screening population has been well documented[22,23]. With superior sensitivity established, HPV DNA testing as a first-line primary screening test has been proposed and adopted in some screening programs.

PRINCIPLES OF THE PROCEDURE

The **cobas**® 4800 HPV Test is based on two major processes: (1) automated specimen preparation to simultaneously extract HPV and cellular DNA; (2) PCR amplification[24] of target DNA sequences using both HPV and β-globin specific complementary primer pairs and real-time detection of cleaved fluorescent-labeled HPV and β-globin specific oligonucleotide detection probes. The concurrent extraction, amplification and detection of β-globin in the **cobas**® 4800 HPV Test monitors the entire test process.

The Master Mix reagent for the **cobas**® 4800 HPV Test contains primer pairs and probes specific for the 14 high-risk HPV types and β-globin DNA. The detection of amplified DNA (amplicon) is performed during thermal cycling using oligonucleotide probes labeled with four different fluorescent dyes. The amplified signal from 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.

Specimen Preparation

Specimen preparation for the **cobas**® 4800 HPV Test is automated with the use of the **cobas x** 480 instrument. Cervical specimens collected in Roche Cell Collection Medium, , PreservCyt® Solution or SurePath™ Preservative Fluid are digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids, along with the β-globin DNA serving as process control, are purified through absorption 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**® 4800 HPV Test uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the Master Mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)[3,8-13,25]. Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers.

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

Target Amplification

EagleZ05 DNA Polymerase[26], a chemically modified version of *Thermus species* Z05 DNA polymerase[27], is utilized for "hot start" amplification of the HPV targets and the β-globin control. First, the PCR reaction mixture is heated to activate EagleZ05 DNA Polymerase, to denature the viral DNA and genomic DNA and to expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The EagleZ05 DNA Polymerase, in the presence of divalent metal ion and excess dNTPs, extends the primer(s), and a second DNA strand is synthesized. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the target region of the HPV genome and β-globin gene. The DNA Polymerase extends the annealed primers along the target templates to produce an approximately 200-base pair double-stranded HPV target DNA molecule or a 330 base pair β-globin DNA molecule termed an amplicon. This process is repeated for a number of cycles, each cycle effectively doubling the amount of amplicon DNA. Amplification occurs only in the region of the HPV genome and/or β-globin gene between the appropriate primer pair. The entire genome is not amplified.

Automated Real-time Detection

The **cobas**® 4800 HPV Test utilizes real-time[29,30] PCR technology. Each oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher that quenches fluorescent emissions from the dye in an intact probe. As amplification progresses, probes that are complementary to the amplicon bind to specific single-stranded DNA sequences and are cleaved by the 5' to 3' nuclease activity of the EagleZ05 DNA Polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, it emits fluorescence of a characteristic wavelength when excited by the proper spectrum of light. This characteristic wavelength for each dye allows HPV16 amplicon, HPV18 amplicon, other HR amplicon (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and the β-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 clinical specimen is achieved in the **cobas**® 4800 HPV Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[28], 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 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. AmpErase enzyme in the **cobas**® 4800 HPV Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing HPV amplicon per PCR.

REAGENTS

cobas® 4800 System Sample Preparation Kit (c4800 SMPL PREP)
240 Tests (P/N: 05235782190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
MGP **(cobas® 4800 System Magnetic Glass Particles)**	Magnetic glass particles 93% Isopropanol[b]	10 x 4.5 mL	 **DANGER** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 +P378: In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish. 67-63-0 Propan-2-ol
EB **(cobas® 4800 System Elution Buffer)**	Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

[a] Product safety labeling primarily follows EU GHS guidance
[b] Hazardous substance

cobas® 4800 System Sample Preparation Kit (c4800 SMPL PREP)
960 Tests (P/N: 05235804190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
MGP **(cobas® 4800 System Magnetic Glass Particles)**	Magnetic glass particles 93% Isopropanol[b]	10 x 13.5 mL	 **DANGER** H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233: Keep container tightly closed. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P370 +P378: In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish. 67-63-0 Propan-2-ol
EB **(cobas® 4800 System Elution Buffer)**	Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

[a] Product safety labeling primarily follows EU GHS guidance
[b] Hazardous substance

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

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

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

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

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT)
240 Tests (P/N: 05235812190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
PK **(cobas® 4800 Proteinase K)**	Tris buffer < 0.05% EDTA Calcium chloride Calcium acetate Glycerol < 2% Proteinase K[b]	10 x 0.9 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves. P284: Wear respiratory protection. P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor. 39450-01-6 Proteinase, Tritirachium album serine
SDS **(cobas® 4800 System SDS Reagent)**	Tris buffer 0.2% SDS 0.09% Sodium azide	10 x 3 mL	N/A
LYS **(cobas® 4800 System Lysis Buffer)**	Tris buffer 37% (w/w) Guanidine HCl[b] < 5% Polydocanol[b]	10 x 10 mL	 **DANGER** H302: Harmful if swallowed. H315: Causes skin irritation. H318: Causes serious eye damage. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ eye protection/ face protection. P301 + P312 + P330: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P501: Dispose of contents/ container to an approved waste disposal plant. 50-01-1 Guanidinium chloride 9002-92-0 Polidocanol

[a] Product safety labeling primarily follows EU GHS guidance
[b] Hazardous substance

cobas® 4800 System Liquid Cytology Preparation Kit (c4800 LIQ CYT)
960 Tests (P/N: 05235839190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
PK **(cobas® 4800 Proteinase K)**	Tris buffer < 0.05% EDTA Calcium chloride Calcium acetate Glycerol < 2% Proteinase K[b]	20 x 1.2 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280: Wear protective gloves. P284: Wear respiratory protection. P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor. 39450-01-6 Proteinase, Tritirachium album serine
SDS **(cobas® 4800 System SDS Reagent)**	Tris-HCl buffer 0.2% SDS 0.09% Sodium azide	10 x 9 mL	N/A
LYS **(cobas® 4800 System Lysis Buffer)**	Tris-HCl buffer 37% (w/w) Guanidine HCl[b] < 5% Polydocanol[b]	10 x 36 mL	 **DANGER** **DANGER** H302: Harmful if swallowed. H315: Causes skin irritation. H318: Causes serious eye damage. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ eye protection/ face protection. P301 + P312 + P330: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P501: Dispose of contents/ container to an approved waste disposal plant. 50-01-1 Guanidinium chloride 9002-92-0 Polidocanol

[a] Product safety labeling primarily follows EU GHS guidance
[b] Hazardous substance

cobas® 4800 HPV Amplification/Detection Kit (c4800 HPV AMP/DET) **240 Tests** (P/N: 05235901190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning**
HPV MMX **(cobas® 4800 HPV Master Mix)**	Tricine buffer Potassium acetate Potassium hydroxide Glycerol < 0.13% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream HPV primers < 0.01% Upstream and downstream β-globin primers < 0.01% Fluorescent-labeled HPV probes < 0.01% Fluorescent-labeled β-globin probes < 0.10% EagleZ05 DNA polymerase (microbial) < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	10 x 0.5 mL	N/A
HPV Mg/Mn **(cobas® 4800 HPV Mg/Mn Solution)**	Magnesium acetate Manganese acetate < 0.02% Glacial acetic acid 0.09% Sodium azide	10 x 1.0 mL	N/A

cobas® 4800 HPV Amplification/Detection Kit (c4800 HPV AMP/DET) **960 Tests** (P/N: 05235910190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning**
HPV MMX **(cobas® 4800 HPV Master Mix)**	Tricine buffer Potassium acetate Potassium hydroxide Glycerol < 0.13% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream HPV primers < 0.01% Upstream and downstream β-globin primers < 0.01% Fluorescent-labeled HPV probes < 0.01% Fluorescent-labeled β-globin probes < 0.10% EagleZ05 DNA polymerase (microbial) < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	20 x 1.0 mL	N/A
HPV Mg/Mn **(cobas® 4800 HPV Mg/Mn Solution)**	Magnesium acetate Manganese acetate < 0.02% Glacial acetic acid 0.09% Sodium azide	10 x 1.0 mL	N/A

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

WARNINGS AND PRECAUTIONS

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

B. This test is for use with cervical specimens collected using Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid.

C. Self-collected vaginal specimens must be suspended in Roche Cell Collection Medium or PreservCyt® Solution after sample is collected.

D. False negative or invalid results may occur with self-collected samples if samples are not suspended in medium after collection.

E. Do not pipette by mouth.

F. 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.

G. Avoid microbial and DNA contamination of reagents.

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

I. Do not use reagents after their expiration dates.

J. Do not pool reagents.

K. Safety Data Sheets (SDS) are available on request from your local Roche office.

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

M. Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[31] and in the CLSI Document M29-A3[32].

N. **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 a spill occurs with potentially infectious agents, **FIRST** clean the affected area first with laboratory detergent and water, and then with 0.5% sodium hypochlorite.

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

P. **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.

Q. 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.

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

S. Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or **cobas z** 480 analyzer according to procedures described in the **cobas**® 4800 System - User Assistance.

T. For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance.

U. Do not use reagents or containers that are visibly damaged or show signs of leakage.

V. Inform your local competent authority about any serious incidents which may occur when using this assay.

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents.***

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

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

MATERIALS PROVIDED

A. cobas® 4800 System Sample Preparation Kit — **c4800 SMPL PREP** — **240 Tests**
(P/N: 05235782190)

MGP
(**cobas®** 4800 System Magnetic Glass Particles)

EB
(**cobas®** 4800 System Elution Buffer)

B. cobas® 4800 System Sample Preparation Kit — **c4800 SMPL PREP** — **960 Tests**
(P/N: 05235804190)

MGP
(**cobas®** 4800 System Magnetic Glass Particles)

EB
(**cobas®** 4800 System Elution Buffer)

C. cobas® 4800 System Wash Buffer Kit — **c4800 WB** — **240 Tests**
(P/N: 05235863190)

WB
(**cobas®** 4800 System Wash Buffer)

D. cobas® 4800 System Wash Buffer Kit — **c4800 WB** — **960 Tests**
(P/N: 05235871190)

WB
(**cobas®** 4800 System Wash Buffer)

E. cobas® 4800 HPV Amplification/Detection Kit — **c4800 HPV AMP/DET** — **240 Tests**
(P/N: 05235901190)

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

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

F. cobas® 4800 HPV Amplification/Detection Kit — **c4800 HPV AMP/DET** — **960 Tests**
(P/N: 05235910190)

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

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

G. cobas® 4800 System Liquid Cytology Preparation Kit — **c4800 LIQ CYT** — **240 Tests**
(P/N: 05235812190)

PK
(**cobas®** 4800 Proteinase K)

SDS
(**cobas®** 4800 System SDS Reagent)

LYS
(**cobas®** 4800 System Lysis Buffer)

H. cobas® 4800 System Liquid Cytology Preparation Kit — **c4800 LIQ CYT** — **960 Tests**
(P/N: 05235839190)

PK
(**cobas®** 4800 Proteinase K)

SDS
(**cobas**® 4800 System SDS Reagent)

LYS
(**cobas**® 4800 System Lysis Buffer)

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

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

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

MATERIALS REQUIRED BUT NOT PROVIDED

Specimen and Reagent Handling

- Roche Cell Collection Medium (Roche P/N 07994745190, optional)
- Roche Cell Collection Medium Replacement Caps (Roche P/N 08037230190, optional)
- Cervical Collection Brush (Roche P/N 08399832190, optional)
- Cervical Collection Brush, sterile (Roche P/N 08779040190, optional)
- Copan FLOQSwabs® for vaginal self-collection, 552C.80 (Roche P/N 09032932190)
- Sample suspension instructions for Copan FLOQSwabs® for vaginal self-collection, 552C.80 (Roche P/N 09652671001)
- CO-RE Tips, 1000 µL, rack of 96 (Roche P/N 04639642001)
- 50 mL Reagent Reservoir (Roche P/N 05232732001)
- 200 mL Reagent Reservoir (Roche P/N 05232759001)
- For HPV ASAP v2.0.1 use **cobas**® 4800 System Extraction (deep well) Plate 1.6 mL (Roche P/N 05232716001)
- For HPV ASAP v2.1 use **cobas**® 4800 System Extraction (deep well) Plate 2.0 mL (Roche P/N 06884008001)
- **cobas**® 4800 System AD (microwell) Plate 0.3 mL and Sealing Film (Roche P/N 05232724001)
- Solid waste bag [Roche P/N 05530873001 (small) or 04691989001 (large)]
- Hamilton STAR Plastic Chute (Roche P/N 04639669001)
- Tubes 13 mL Round Base (Roche P/N 07958048190) for use as secondary sample tubes
- Caps, neutral color (Roche P/N 07958056190; for recapping post-run specimens in 13 mL Round Base tubes)
- Disposable gloves, powderless

Instrumentation and Software

- **cobas x** 480 instrument
- **cobas z** 480 analyzer
- **cobas**® 4800 System Control Unit with System Software version 2.2 or higher
- **cobas**® 4800 System **cobas**® HPV AP software version 2.0 or higher

Optional Equipment and Materials

- **cobas**® Sample Prep Buffer (Roche P/N 06526985190; Tris buffered detergent)*
- Pipettes: capable of delivering 1000 µL
- Aerosol barrier DNase-free tips: capable of delivering 1000 µL
- Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500
- Stand-alone magnetic plate (Roche P/N 05440777001)
- Vortex Mixer (single tube)
- Multi-tube vortexer [e.g. VWR P/N 58816-116]
- Heat-resistant barcode labels (RACO Industries; Cat # RAC-225075-9501)
- Thermometer -20/150ºC (VWR Cat# 89095-600) or equivalent
- Digital Heater Block 120V (VWR Cat# 75838-294) or equivalent
- 12-Hole Heat Block Module 16mm (VWR Cat# 13259-162) or equivalent

* 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.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

A. Specimen Collection

Cervical specimens collected in Roche Cell Collection Medium, , PreservCyt® Solution and SurePath™ Preservative Fluid have been validated for use with the **cobas**® 4800 HPV Test.

Vaginal specimens collected with *FLOQSwabs® for vaginal self-collection* and suspended in Roche Cell Collection Medium and PreservCyt® Solution have been validated for use with the **cobas**® 4800 HPV Test.

Follow the manufacturer's instructions for collecting specimens.

B. Specimen Transport

Specimens collected in Roche Cell Collection Medium, , PreservCyt® Solution and SurePath™ Preservative Fluid can be transported at 2-30ºC. Transportation of HPV specimens must comply with country, federal, state and local regulations for the transport of etiologic agents[33].

C. Specimen Storage

Specimens collected in Roche Cell Collection Medium and PreservCyt® Solution may be stored at 2-30°C for up to 6 months after the date of collection. Cervical specimens collected in SurePath™ Preservative Fluid may be stored at 2-8°C for up to 6 months or may be stored at 15-30°C for up to 6 weeks after the date of collection provided that SurePath™ Preservative Fluid matrix-induced crosslinks are reversed through treatment with **cobas**® Sample Prep Buffer prior to HPV testing.

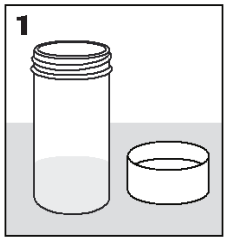
Sample suspension instructions for *Copan FLOQSwabs® for vaginal self-collection (552C.80)*

Sample handling instructions for self-collected sample using Copan FLOQSwabs® for vaginal self-collection (552C.80) for testing with **cobas**® 4800 HPV Test or **cobas**® HPV.

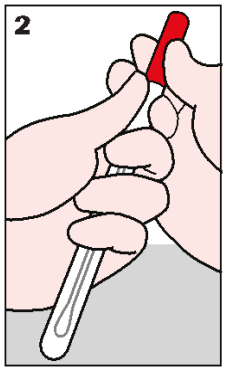
Self-collected sample must be placed into medium after sample has been collected.

- **Read all instructions before starting sample suspension.**
- For sample collection, follow the manufacturer's Instructions For Use.
- Once the sample has been collected, continue with the following instructions to preserve the sample:

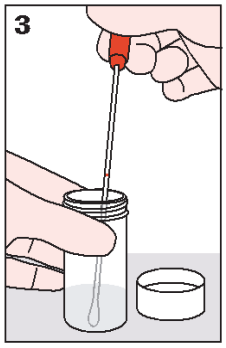
Handle the collected sample with care.



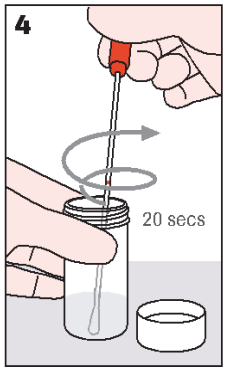
1. Carefully uncap the vial containing medium and place it on a stable, flat surface.



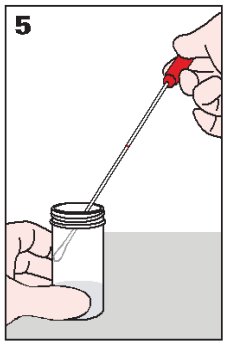
2. Slowly pull the FLOQSwab cap off to remove the swab from the tube. **Minimize touching the inner walls of the tube as you remove the FLOQSwab.**



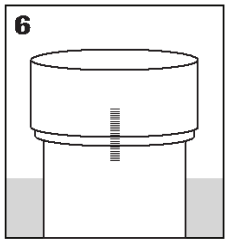
3. Hold the vial with one hand then with the other hand place the FLOQSwab tip into the vial until the FLOQSwab **tip is fully immersed in the medium** and **touching the bottom** of the vial.



4. Holding onto the vial, **swirl the FLOQSwab along the inner vial wall** for **20 seconds** while **ensuring the swab remains immersed** in the medium. Be careful not to splash.



5. Carefully draw the FLOQSwab up along the inner vial wall until the tip is no longer immersed in the medium. **Hold the tip against the inner vial wall to drain fluid** off of the swab. Place the FLOQSwab into the tube and discard.



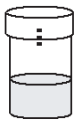
6. Re-cap the vial and tighten until **the lines on the cap and vial meet or slightly overlap** to prevent leakage. Store upright.

7. The sample can now be processed with the **cobas**® 4800 HPV Test or **cobas**® HPV.

Glossary

FLOQSwab/Swab: The self-collection device used to collect sample.

Tube: A protective container that the self-collected device will come in and can be used to temporarily store the collection device after the sample has been collected.



Vial: A container which contains 20 ml of clear solution. The specimen you collect will need to be transferred into this container and then this container will be sent to the lab for processing.

Medium: What the liquid that comes in the vial is called.

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 Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid must be at ambient temperature before loading on the cobas x 480 instrument.

NOTE: Refer to the cobas® 4800 System - User Assistance for detailed operating instructions.**Run Size:**

The **cobas**® 4800 System is designed to support the **cobas**® 4800 HPV Test with run sizes from 1 to 94 specimens plus controls (up to 94 assays per run). Each **cobas**® 4800 System Sample Preparation Kit, **cobas**® 4800 System Liquid Cytology Preparation Kit and **cobas**® 4800 System Wash Buffer Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit). Each **cobas**® 4800 HPV Amplification/Detection Kit contains reagents sufficient for 10 runs of either 24 tests (240 tests per kit) or 96 tests (960 tests per kit); multiple vials of **cobas**® HPV MMX can be used to optimize reagent usage for 48 or 72 tests. The **cobas**® 4800 HPV Controls Kit contains reagents sufficient for a total of 10 runs (10 sets per kit). The minimum run size on the **cobas**® 4800 System is 1 specimen plus controls. One replicate of the **cobas**® 4800 System Negative Control [**(–) C**] and one replicate of the **cobas**® 4800 HPV Positive Control [**HPV (+) C**] are required to perform each test run (see *"Quality Control"* section).

Workflow:

NOTE: Although not an optimal use of reagents, a System Sample Preparation 960 Test Kit can be used for a 24 sample run and an HPV Amplification/Detection 960 Test Kit can be used for a 24, 48, or 72 sample run.

The **cobas**® 4800 HPV Test can be run using either of two workflows, referred to as "Full Workflow" or "Recovery 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 - User Assistance for details.

HPV Recovery Workflow:

The "HPV Recovery Workflow" consists of manual PCR plate setup using eluate from the processed deep well plate followed by amplification/detection on the **cobas z** 480 analyzer. Refer to the "Performing a Recovery Workflow" section below and the **cobas**® 4800 System - User Assistance for details.

Specimens:

There are three cervical specimen types that can be assayed using the **cobas**® 4800 HPV Test: a) cervical specimens in Roche Cell Collection Medium, b) cervical specimens in PreservCyt® Solution, c) cervical specimens in SurePath™ Preservative Fluid. Roche Cell Collection Medium and PreservCyt® Solution may be processed directly out of their primary container with a proper barcode or out of a properly barcoded 13 mL round-based tube on the **cobas x** 480 instrument. SurePath™ Preservative Fluid specimens must be transferred into a properly barcoded 13 mL round-based tube for specimen treatment (See Treatment of SurePath™ primary specimens section) processing on the **cobas x** 480 instrument. Consult the **cobas**® 4800 System - User Assistance for proper barcoding procedures and the list of acceptable barcodes for the **cobas**® 4800 System.

NOTE: SurePath™ specimens must be treated with cobas® Sample Prep Buffer to reverse matrix-induced cross-links prior to HPV testing on the cobas® 4800 System.

Treatment of SurePath™ primary specimens with **cobas**® Sample Prep Buffer to reverse matrix-induced crosslinks

NOTE: Heat-resistant barcodes are required for tubes used to reverse matrix-induced cross-links (see the Optional Equipment and Materials section).

NOTE: It is recommended that steps B, C, G and H below are done in a biological hood to minimize possible cross-contamination.

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

   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.

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

C. Vortex each tube for 1 second.

D. Transfer tubes to the heating unit set at 120°C (see Optional Equipment and Materials section). Up to 48 tubes can be processed per batch.

E. Heat for 20 minutes.

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

G. Vortex each tube for 5 seconds.

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

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

A. Follow the treatment procedure above to step G.

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

NOTE: The minimum volume required in the Roche Cell Collection Medium and PreservCyt® Solution primary containers 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 10 mL.

NOTE: Use only Roche Cell Collection Medium, PreservCyt® Solution and SurePath™ Preservative Fluid to collect cervical specimens for the cobas® 4800 HPV Test. The cobas® 4800 HPV Test has not been validated with other media types. Using the cobas® 4800 HPV Test with other media types could lead to false negative, false positive and/or invalid results.

NOTE: It may be necessary to aliquot specimens into barcoded 13 mL round-based 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 of processed specimens, additional caps for these tubes in an alternate color (neutral; see Materials Required But Not Provided section) 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 pipet tips after each specimen.

NOTE: To avoid cross-contamination of processed specimens, additional caps for vials (see Materials Required But Not Provided section) should be used to recap specimens after processing. Re-cap tightly. Store and ship the vials in upright orientation.

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

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

Workflows

Performing a Full Workflow:

A. The **cobas**® 4800 HPV Test may be used for 1 to 94 specimens plus one **cobas**® 4800 System negative control and one **cobas**® 4800 HPV positive control.

B. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System - User Assistance.

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

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

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

F. Follow the software wizard guide to load specimens.

NOTE: Specimens can be loaded in barcoded primary or secondary tubes in any order.

NOTE: If primary containers for Roche Cell Collection Medium and PreservCyt® Solution specimens are used for processing, vortex prior to loading.

G. Select a Specimen type for each specimen.

- Choose "PC" for ordering PreservCyt® Solution or Roche Cell Collection Medium. .
- Choose "SP" for ordering SurePath™ Preservative Fluid specimens.

NOTE: Test orders may be annotated to identify self-collected samples using the instrument software and users are encouraged to do so. Refer to the cobas® 4800 System – User Assistance for annotation instructions.

H. Select the Request result for each specimen.

- Choose Requested result "HPV High Risk Panel" to report any one of, or combination of high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 test results.
- Choose Requested result "HPV High Risk Panel Plus Genotyping" to report any one of, or combination of high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and to separately report high risk HPV Type 16 and high risk HPV Type 18 test results.

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

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

NOTE: Controls [HPV (+) C and (–) C] are not loaded together with specimens. They are loaded onto the reagent carrier during reagent loading. Two positions (A1 and B1) on each of the Extraction 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 time has expired.

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

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

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

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

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

NOTE: ***All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs. The software also verifies that sufficient reagents are loaded on the instrument.***

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

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

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

NOTE: ***The status of sample preparation can be reviewed at this point, prior to clicking "Unload". See the cobas® 4800 System - User Assistance for details.***

O. Follow the instructions in the **cobas®** 4800 System - User Assistance 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.***

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

Q. Follow the instructions in the **cobas®** 4800 System - User Assistance to review and accept results.

Performing a Recovery Workflow:

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

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

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

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

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

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

D. Select a run to recover.

E. If using HPV ASAP v2.1, scan the original DWP ID from the full workflow.

F. Enter the new MWP ID.

G. Enter the Master Mix and Mg/Mn IDs for all Amplification/Detection reagent vials in the kit.

H. Prepare the **cobas**® 4800 HPV working master mix:
   1. For a 240 Test Kit, add 240 µL of **HPV Mg/Mn** to one vial of **HPV MMX** (0.5 mL vial from 240 Test Kit).
   2. For a 960 Test Kit, add 450 µL of **HPV Mg/Mn** to each of the two vials of **HPV MMX** (1.0 mL vials from 960 Test Kit).

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

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

J. Transfer 25 µL of working Master Mix to the required wells in the microwell plate.

K. Place the Extraction Plate from the run to be repeated onto the stand-alone magnetic plate.

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

M. Follow the instructions in the **cobas**® 4800 System - User Assistance to seal the microwell plate.

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

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

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

Q. Follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.

Interpretation of Results

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

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

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

Table 1
Result Interpretation of the cobas® 4800 HPV Test for Presence of HPV DNA

cobas® 4800 HPV Test	**Result Report and Interpretation**
Requested Result "HPV High Risk Panel":	
POS HR HPV	**High Risk HPV Positive** Specimen is positive for the DNA of any one of, or combination of, the following high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
NEG HR HPV	**High Risk HPV Negative*** HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid HR HPV	**High Risk HPV Invalid** The results for HR HPV are invalid. For PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath™ specimens the original specimen should be retested if sufficient volume remains. If the results are still invalid a new specimen should be obtained.
Failed	**No Result for Specimen** Consult the **cobas®** 4800 System - User Assistance for instructions to review run flags and recommended actions. Original specimen should be re-tested to obtain valid results.
Requested Result "HPV High Risk Panel Plus Genotyping":	
POS Other HR HPV	**Other High Risk HPV Positive** Specimen is positive for the DNA of any one of, or combination of, the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
NEG Other HR HPV	**Other High Risk HPV Negative*** HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid Other HR HPV	**Invalid Other High Risk HPV** The results for HR HPV are invalid. For PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath™ specimens the original specimen should be retested if sufficient volume remains. If the results are still invalid a new specimen should be obtained.
POS HPV16	**HPV16 Positive** Specimen is positive for HPV type 16 DNA.
NEG HPV16	**HPV16 Negative*** HPV type 16 DNA was undetectable or below the pre-set threshold.
Invalid HPV16	**Invalid HPV16** The result for HPV16 is Invalid. For PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath™ specimens the original specimen should be retested if sufficient volume remains. If the results are still invalid a new specimen should be obtained.
POS HPV18	**HPV18 Positive** Specimen is positive for HPV type 18 DNA.
NEG HPV18	**HPV18 Negative*** HPV type 18 DNA was undetectable or below the pre-set threshold.
Invalid HPV18	**Invalid HPV18** The result for HPV18 is invalid. For PreservCyt® specimens, the original specimen should be re-tested no more than two times to obtain valid results. If the results are still invalid a new specimen should be obtained. For SurePath™ specimens the original specimen should be retested if sufficient volume remains. If the results are still invalid a new specimen should be obtained.
Failed	**No Result for Specimen** Consult the **cobas®** 4800 System - User Assistance 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® 4800 HPV Test for Patients with Cytological Abnormalities

Results	Interpretation
NEG Other HR HPV*, NEG HPV16, NEG HPV18	Very low likelihood of underlying ≥ CIN2.
POS Other HR HPV*, NEG HPV16, NEG HPV18	Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy.
POS HPV16 and/or POS HPV18	Highest likelihood that underlying ≥ CIN2 will be detected at colposcopy[34, 35].

*Other HR HPV DNA includes the following types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.

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

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

Other HR HPV NEG, HPV16 POS, HPV18 Invalid

The positive and negative results should be interpreted as shown in Table 1. In this example, HPV 18 results are invalid. The 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.

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

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

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

LIST OF RESULT FLAGS

The following table lists common flags for the **cobas**® 4800 HPV Test which are relevant for result interpretation. Refer to the **cobas**® 4800 System - User Assistance for a full list of flags.

Table 3
List of flags for cobas® 4800 HPV Test

Flag code	Description	Recommended action
R20	Positive control is invalid.	Positive control values were invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R21	Negative control is invalid.	Negative control values were invalid. To avoid carryover, use Good Laboratory Practice. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
X3	Error: Clot was detected. Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

QUALITY CONTROL

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

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.

PROCEDURAL LIMITATIONS

1. The **cobas**® 4800 HPV Test detects DNA of the high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types[36].
2. The **cobas**® 4800 HPV Test for detection of human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is not recommended for evaluation of suspected sexual abuse.
3. The performance of the **cobas**® 4800 HPV Test has not been adequately established for HPV vaccinated individuals[37].
4. 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.
5. 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.
6. A negative high-risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.7. Test only the indicated specimen type. The **cobas**® 4800 HPV Test has only been validated for use with:
   - cervical specimens collected in Roche Cell Collection Medium,
   - cervical specimens collected in PreservCyt® Solution
   - cervical specimens collected in SurePathTM Preservative Fluid
   - vaginal specimens collected with FLOQSwabs® 552C.80 and suspended in Roche Cell Collection Medium
   - vaginal specimens collected with FLOQSwabs® 552C.80 and suspended in PreservCyt® Solution

   Assay performance has not been validated for use with other collection media and/or collection devices. Use of other collection media and/or collection devices may lead to false positive, false negative or invalid results.
8. 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.
9. Beta-globin amplification and detection is included in the **cobas**® 4800 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**® 4800 System.
10. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Package Insert and the **cobas**® 4800 System - User Assistance.
11. The addition of AmpErase enzyme into the **cobas**® 4800 HPV Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.
12. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
13. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR system can be used with this product.
14. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.
15. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
16. Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the **cobas**® 4800 HPV Test's primers and/or probes may result in failure to detect the presence of the viral DNA.
17. The presence of PCR inhibitors may cause false negative or invalid results.
18. Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the **cobas**® 4800 System. If concentrations of whole blood exceeds 2% (dark red or brown coloration) in Roche Cell Collection Medium, or PreservCyt® solution, or above 4% in SurePath™ Preservative Fluid treated with **cobas**® Sample Prep Buffer, there is a likelihood of obtaining a false-negative result.
19. Use of the vaginal moisturizer Replens® has been associated with false-negative results in SurePath™ Preservative Fluid.
20. Use of the RepHresh® vaginal hygiene products has been associated with false-negative results in Roche Cell Collection Medium and PreservCyt® Solution.
21. Removal of red blood cells from Roche Cell Collection Medium, PreservCyt®, or SurePath™ specimens through treatment with glacial acetic acid (GAA) has not been validated with the **cobas**® 4800 HPV Test. Any use of GAA treatments with the **cobas**® 4800 HPV Test must be validated by the testing laboratory.

CLINICAL PERFORMANCE USING CLINICAL SPECIMENS

Performance Comparison to a CE Mark Comparator HPV Test

Clinical sensitivity and specificity to disease status (≥ CIN2) was determined for the **cobas**® 4800 HPV Test and a CE Mark comparator HPV test[38] in a population of women at least 21 years old with ASC-US cytology results, determined through routine cervical cancer screening. All testing was carried out using PreservCyt® Solution cervical specimens. A total of 1578 subjects with an initial ASC-US cytology result underwent colposcopy and had valid HPV tests and cervical biopsy results. Disease status of the subjects was ascertained by a central pathology review panel from the biopsy specimens obtained at colposcopy. The results for an ASC-US population are summarized in Table 4 and indicate that the **cobas**® 4800 HPV Test performance was comparable to the comparator test.

Table 4
Comparison of the Performance of the cobas® 4800 HPV Test and a CE Mark Comparator HPV test in Detecting ≥ CIN2 and ≥ CIN3 in the ASC-US Population

	cobas® 4800 HPV Test		**CE Marked HPV Test**	
	Point Estimate	**95% CI**	**Point Estimate**	**95% CI**
≥ CIN2				
Sensitivity (%)	90.0 (72/80)	(81.5, 94.8)	87.2 (68/78)[1]	(78.0, 92.9)
Specificity (%)	70.5 (1,056/1,498)	(68.1, 72.7)	71.1 (1,056/1,485)[2]	(68.8, 73.4)
PPV (%)	14.0 (72/514)	(12.8, 15.3)	13.7 (68/497)	(12.4, 15.1)
NPV (%)	99.2 (1,056/1,064)	(98.6, 99.6)	99.1 (1,056/1,066)	(98.3, 99.5)
Prevalence (%)	5.1 (80/1578)	(4.1, 6.3)	5.0 (78/1563)	(4.0, 6.2)
≥ CIN3				
Sensitivity (%)	93.5 (43/46)	(82.5, 97.8)	91.3 (42/46)	(79.7, 96.6)
Specificity (%)	69.3 (1,053/1,517)	(66.9, 71.5)	70.0 (1,062/1,517)	(67.7, 72.3)
PPV (%)	8.4 (43/514)	(7.6, 9.2)	8.5 (42/497)	(7.6, 9.4)
NPV (%)	99.7 (1,061/1,064)	(99.2, 99.9)	99.6 (1,062/1,066)	(99.0, 99.9)
Prevalence (%)	2.9 (43/1578)	(2.2, 3.9)	3.0 (46/1563)	(2.2, 3.9)

[1] Results for two subjects with a ≥ CIN2 diagnosis could not be determined by the CE Mark Comparator HPV Test due to insufficient volume resulting from repeated testing.

[2] Results for thirteen subjects with a < CIN2 diagnosis could not be determined by the CE Mark Comparator HPV Test due to insufficient volume resulting from repeated testing.

In women ≥ 30 years with normal cytology, the risk of cervical disease (≥ CIN2) is 7.29 fold higher with a High Risk positive **cobas**® 4800 HPV Test result than with a negative **cobas**® 4800 HPV Test result. Relative risk estimates and their 95% confidence intervals are presented in Table 5.

In women 30 years or older, the **cobas**® 4800 HPV Test can be used to assess the presence or absence of HPV genotypes 16 and 18. The risk of cervical disease (≥ CIN2) is 13.71 fold higher with an HPV16 and/or HPV18 positive **cobas**® 4800 HPV Test result than with a negative result and the risk is 2.51 fold higher with an HPV16 and/or HPV18 positive **cobas**® 4800 HPV Test result compared to a positive **cobas**® 4800 HPV Test result for the 12 other high risk types. In all cases, the lower bound of the 95% confidence interval exceeds 1, suggesting a statistically higher risk of developing cervical disease with a positive HPV test result.

Table 5
Relative Risk for Cervical Disease (≥ CIN2 by Central Pathology Review) in Women ≥ 30 Years with Normal Cytology*

HPV Result	**Relative Risk Estimate**	**95% CI***
Pos vs. Neg	7.29	(3.99, 22.11)
16+/18+ vs. Neg	13.71	(7.31, 41.92)
16+/18+ vs. 12 other HR+	2.51	(1.73, 3.61)

Note: 0.5 was added to a zero cell of the estimated number of diseased subjects in any of the 1000 bootstrap samples.

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

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 6.

The 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 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.

Table 6
Performance of cobas® 4800 HPV Test In the NILM (≥ 30 years) Population

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

Overall (≥ 25 Years) Population – Comparison of Performance of HPV testing vs. Cytology

Clinical performance of the **cobas**® HPV Test and liquid based cytology (PreservCyt®) was determined in a population of 40,901 women 25 years and older, independent of cytology status (Overall Population). For the Overall (≥ 25 Years) population, estimates of sensitivity and specificity for the **cobas**® HPV Test vs. cytology for the detection of ≥ CIN2 and ≥ CIN3 are presented[38] in Table 7. The sensitivities of the **cobas**® HPV Test and cytology for detection of ≥ CIN2 were 88.2% (380/431) with 95% CI 84.8-90.9% and 51.5% (222/431) with CI 46.8-56.2%, respectively. The sensitivities of the **cobas**® HPV Test and cytology for detection of ≥ CIN3 were 92.0% (252/274) with 95% CI 88.1-94.6 and 53.3% (146/274) with CI 47.4-59.1%, respectively.

Table 7
Comparison of performance of the cobas® 4800
HPV Test and Cytology for the detection of ≥ CIN2 and ≥ CIN3 in the Overall (≥ 25 years) Population

	Cytology		cobas® HPV Test	
	% (n)	95% CI	% (n)	95% CI
≥ CIN2				
Sensitivity	51.5 (222/431)	(46.8–56.2)	88.2 (380/431)	(84.8–90.9)
Specificity	73.4 (5428/7392)	(72.4–74.4)	57.8 (4270/7392)	(56.6–58.9)
PPV	10.2 (222/2186)	(9.3–11.1)	10.9 (380/3502)	(10.4–11.3)
NPV	96.3 (5428/5637)	(95.9–96.6)	98.8 (4270/4321)	(98.5–99.1)
≥ CIN3				
Sensitivity	53.3 (146/274)	(47.4–59.1)	92.0 (252/274)	(88.1–94.6)
Specificity	73.0 (5509/7549)	(72.0–74.0)	56.9 (4299/7549)	(55.8–58.1)
PPV	6.7 (146/2186)	(6.0–7.4)	7.2 (252/3502)	(6.9–7.5)
NPV	97.7 (5509/5637)	(97.4–98.0)	99.5 (4299/4321)	(99.2–99.7)

Limit of Detection: PreservCyt® Solution and SurePath™ Preservative Fluid

The limit of detection (LOD) of high risk HPV genotypes HPV16, HPV18 and HPV31 was determined for the **cobas®** 4800 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 SurePath™ Preservative Fluid, and 2) HPV positive cell lines SiHa (HPV16) and HeLa (HPV18) in PreservCyt® Solution and SurePath™ Preservative Fluid containing an HPV negative cell line (HCT-15) background. Plasmid and cell lines were diluted to concentrations below, above and at the expected LOD levels. A minimum of 60 replicates were tested for each plasmid or cell line level in PreservCyt® Solution and SurePath™ Preservative Fluid for each of 3 reagent lots. All testing in SurePath™ specimen background was done using treatment with **cobas®** Sample Prep Buffer. The LOD is the level of HPV DNA in the sample that has positive test results at least 95% of the time. Tables 8 and 9 contain results from the reagent lot producing the most conservative (highest) LOD in the analysis for PreservCyt® Solution and SurePath™ Preservative Fluid, respectively.

Table 8
Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18) in PreservCyt® Solution

HPV Type	Titer (copies or cells/mL)	Number of Positive/Tested	% Positives	95% Confidence Interval	
				Lower	Upper
31	600	60/60	100%	94%	100%
31	**300**	**59/61**	**97%**	**89%**	**100%**
31	150	49/60	82%	70%	90%
16	1500	60/60	100%	94%	100%
16	**600**	**60/60**	**100%**	**94%**	**100%**
16	300	55/61	90%	80%	96%
18	1,500	60/60	100%	94%	100%
18	**600**	**60/60**	**100%**	**94%**	**100%**
18	300	42/61	69%	56%	80%
SiHa (HPV 16)	200	66/66	100%	95%	100%
SiHa (HPV 16)	**100**	**64/65**	**98%**	**92%**	**100%**
SiHa (HPV 16)	50	57/60	95%	86%	99%
HeLa (HPV 18)	80	60/60	100%	94%	100%
HeLa (HPV 18)	**40**	**60/60**	**100%**	**94%**	**100%**
HeLa (HPV 18)	20	56/60	93%	84%	98%

Table 9
Limit of Detection Levels for HPV Types 31, 16, 18 and Cell Lines SiHa (HPV16) and HeLa (HPV18) in SurePath™ Preservative Fluid

HPV Type	Titer (copies or cells/mL)	Number of Positive/Tested	% Positives	95% Confidence Interval	
				Lower	Upper
31	600	60/60	100%	94%	100%
31	**300**	**59/59**	**100%**	**94%**	**100%**
31	150	54/60	90%	80%	96%
16	600	60/60	100%	94%	100%
16	**300**	**59/60**	**98%**	**91%**	**100%**
16	150	40/60	67%	53%	78%
18	1,500	60/60	100%	94%	100%
18	**600**	**60/60**	**100%**	**94%**	**100%**
18	300	55/59	93%	84%	98%
SiHa (HPV 16)	400	60/60	100%	94%	100%
SiHa (HPV 16)	**200**	**60/60**	**100%**	**94%**	**100%**
SiHa (HPV 16)	100	55/60	92%	82%	97%
HeLa (HPV 18)	80	60/60	100%	94%	100%
HeLa (HPV 18)	**40**	**59/60**	**98%**	**91%**	**100%**
HeLa (HPV 18)	20	43/60	72%	59%	83%

Limit of Detection: Roche Cell Collection Medium

Dilution panels of HPV31 plasmid, 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 the **cobas**® 4800 HPV Test was comparable.

Inclusivity Verification

To verify that the **cobas**® 4800 HPV Test is capable of accurately detecting all HPV high risk genotypes, the limit of detection (LOD) was determined (Tables 10 and 11) for genotypes 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The sensitivity of the **cobas**® 4800 HPV Test for HPV genotypes 16, 18 and 31 was determined in the Limit of Detection Study described above in this Package Insert. Quantified plasmid stocks of each HPV genotype were diluted into either PreservCyt® Solution or SurePath™ Preservative Fluid containing HPV-negative HCT-15 cells to concentrations below, above and at the expected LOD levels. One lot of reagents was used to produce a minimum of 48 replicates for each positive level in each media. For testing in SurePath™ Preservative Fluid using treatment with **cobas**® Sample Prep Buffer (Table 11), background material was prepared from cervical specimens collected in SurePath™ Preservative Fluid and tested as 24 replicates each with two lots of reagents. For each HPV type, the reported LOD was defined as the lowest testing concentration having a ≥ 95% positive hit rate with all higher concentrations having at least a 95% hit rate.

Table 10
Summary of High Risk Genotype Limit Of Detection for cobas® 4800 HPV Genotype Inclusivity Study (PreservCyt® Solution)

HPV DNA Type	LOD (copies/mL)	Number of Positive/Tested	Hit Rate	95% Confidence Interval	
				Lower	Upper
33	190	46/48	96%	86%	99%
35	480	48/48	100%	93%	100%
39	80	48/48	100%	93%	100%
45	190	46/48	96%	86%	99%
51	100	46/48	96%	86%	99%
52	2400	48/48	100%	93%	100%
56	1400	48/48	100%	93%	100%
58	480	47/48	98%	89%	100%
59	190	46/48	96%	86%	99%
66	640	48/48	100%	93%	100%
68	450	48/48	100%	93%	100%

Table 11
Summary of High Risk Genotype Limit Of Detection for cobas® 4800 HPV Genotype Inclusivity Study (SurePath™ Preservative Fluid)

HPV DNA Type	LOD (copies/mL)	Number of Positive/Tested	Hit Rate	95% Confidence Interval	
				Lower	Upper
33	300	48/48	100%	93%	100%
35	600	47/48	100%	89%	100%
39	150	48/48	100%	93%	100%
45	300	48/48	100%	93%	100%
51	600	46/48	96%	86%	99%
52	4800	48/48	100%	93%	100%
56	1200	46/48	96%	86%	99%
58	600	48/48	100%	93%	100%
59	600	48/48	100%	93%	100%
66	1200	48/48	100%	93%	100%
68	300	48/48	100%	93%	100%

Precision: PreservCyt® Solution and SurePath™ Preservative Fluid

In-house Precision was examined using panel members prepared for the Limit of Detection Study described in this Package Insert. Levels at and above the limit of detection were used for the precision analysis. Panels were prepared by spiking plasmids of HPV31, HPV16 and HPV18 into the background of pooled HPV negative patient specimens collected in PreservCyt® Solution and SurePath™ Preservative Fluid. All testing in SurePath™ specimen background was done using treatment with **cobas®** Sample Prep Buffer.

The positive hit rates for panel members (PreservCyt® Solution and SurePath™ Preservative Fluid) at and above the LOD are shown in Tables 12 and 13, respectively. Hit rates were above 95% for all plasmid panel levels. The variance in Ct value for the test was analyzed, and contribution from reagent lot, systems, run-to-run, and within-run random factors were calculated and summarized in Table 14 for PreservCyt® Solution and Table 15 for SurePath™ Preservative Fluid. Table 16 shows the Ct value SD and %CV of components of variation in PreservCyt® Solution. Table 17 shows the Ct value SD and %CV of components of variation in SurePath™ Preservative Fluid.

Table 12
Summary of Hit Rates for cobas® 4800 HPV Precision Study At or Above LOD (in PreservCyt® Solution)

Target	Panel Level	Concentration (copies or cells/mL)	N Tests	N Pos	Hit Rate	95% CI for Hit Rate	
						Lower	Upper
HPV31	> LOD	600	186	186	100%	98%	100%
	= LOD	300	187	184	98%	95%	100%
HPV16	> LOD	1,500	186	186	100%	98%	100%
	= LOD	600	186	186	100%	98%	100%
HPV18	> LOD	1,500	186	186	100%	98%	100%
	= LOD	600	186	186	100%	98%	100%

Table 13
Summary of Hit Rates for cobas® 4800 HPV Precision Study At or Above LOD (in SurePath™ Preservative Fluid)

Target	Panel Level	Concentration (copies or cells/mL)	N Tests	N Pos	Hit Rate	95% CI for Hit Rate	
						Lower	Upper
HPV31	> LOD	300	180	180	100%	98%	100%
	= LOD	150	180	175	97%	94%	99%
HPV16	> LOD	600	180	180	100%	98%	100%
	= LOD	300	180	180	100%	98%	100%
HPV18	> LOD	1,500	180	180	100%	98%	100%
	= LOD	600	180	180	100%	98%	100%

Table 14
Analysis of Ct Value Variance Components for cobas® 4800 HPV Precision Study Panel Levels Prepared in PreservCyt® Solution

Target	Panel Level	N	Mean Elbow	Variance Components/Percent Contribution				
				Rgt Lot	System	Run	Random	Total
HPV16	> LOD	186	36.3	0.038	0	0.111	0.079	0.228
				17%	0%	49%	35%	100%
	= LOD	186	37.5	0.025	0	0.042	0.161	0.228
				11%	0%	18%	71%	100%
HPV18	> LOD	186	36.6	0.043	0	0.149	0.067	0.259
				16%	0%	58%	26%	100%
	= LOD	186	37.8	0.027	0	0.050	0.184	0.261
				10%	0%	19%	71%	100%
HPV31	> LOD	186	36.5	0.003	0.002	0.105	0.187	0.297
				1%	1%	35%	63%	100%
	= LOD	187	37.6	0.020	0	0.157	0.489	0.666
				3%	0%	24%	73%	100%

Table 15
Analysis of Ct Value Variance Components for cobas® 4800 HPV Precision Study Panel Levels Prepared in SurePath™ Preservative Fluid

Target	Panel Level	N	Mean Elbow	Variance Components/Percent Contribution				
				Rgt Lot	System	Run	Random	Total
HPV16	> LOD	180	37.2	0.014	0	0.039	0.157	0.209
				7%	0%	18%	75%	100%
	= LOD	180	38.2	0	0	0.090	0.316	0.405
				0%	0%	22%	78%	100%
HPV18	> LOD	180	36.3	0.011	0	0.119	0.073	0.204
				5%	0%	58%	36%	100%
	= LOD	180	37.7	0	0	0.148	0.219	0.366
				0%	0%	40%	60%	100%
HPV31	> LOD	180	37.2	0	0	0.099	0.393	0.493
				0%	0%	20%	80%	100%
	= LOD	180	38.1	0.026	0.015	0.038	0.684	0.764
				3%	2%	5%	90%	100%

Table 16
Analysis of Ct Value SD and %CV for cobas® 4800 HPV Precision Study Panel Levels Prepared in PreservCyt® Solution

Target	Panel Level	N	Mean Elbow	SD Components/%CV				
				Rgt Lot	System	Run	Random	Total
HPV16	> LOD	186	36.3	0.19	0	0.33	0.28	0.48
				0.50%	0.00%	0.90%	0.80%	1.30%
	= LOD	186	37.5	0.16	0	0.20	0.40	0.48
				0.40%	0.00%	0.50%	1.10%	1.30%
HPV18	> LOD	186	36.6	0.21	0	0.39	0.26	0.51
				0.60%	0.00%	1.10%	0.70%	1.40%
	= LOD	186	37.8	0.16	0	0.22	0.43	0.51
				0.40%	0.00%	0.60%	1.10%	1.30%
HPV31	> LOD	186	36.5	0.05	0.05	0.32	0.43	0.54
				0.10%	0.10%	0.90%	1.20%	1.50%
	= LOD	187	37.6	0.14	0	0.40	0.70	0.82
				0.40%	0.00%	1.10%	1.90%	2.20%

Table 17
Analysis of Ct Value SD and %CV for cobas® 4800 HPV Precision Study Panel Levels Prepared in SurePath™ Preservative Fluid

Target	Panel Level	N	Mean Elbow	SD Components/%CV				
				Rgt Lot	System	Run	Random	Total
HPV16	> LOD	180	37.2	0.12	0	0.20	0.40	0.46
				0.30%	0.00%	0.50%	1.10%	1.20%
	= LOD	180	38.2	0	0	0.30	0.56	0.64
				0.00%	0.00%	0.80%	1.50%	1.70%
HPV18	> LOD	180	36.3	0.11	0	0.34	0.27	0.45
				0.30%	0.00%	1.00%	0.70%	1.20%
	= LOD	180	37.7	0	0	0.38	0.47	0.61
				0.00%	0.00%	1.00%	1.20%	1.60%
HPV31	> LOD	180	37.2	0	0.02	0.32	0.63	0.70
				0.00%	0.10%	0.80%	1.70%	1.90%
	= LOD	180	38.1	0.16	0.12	0.20	0.83	0.87
				0.40%	0.30%	0.50%	2.20%	2.30%

Precision: Roche Cell Collection Medium

Panels were prepared by spiking HPV16 cell line and HPV18 cell line 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.

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**® 4800 HPV Test to assess analytical specificity. The organisms listed in Table 18 were spiked at high concentrations (≥ 1 x 10^3 units/reaction) into HPV negative PreservCyt® Solution specimen and into HPV negative PreservCyt® Solution specimen spiked with HPV 31, HPV16 and HPV18 plasmid DNA at 3 times the LOD. Organisms with an asterisk were also tested in SurePath™ Preservative Fluid specimen background under the same conditions. Organisms with a double asterisk were tested only in SurePath™ specimen background. All testing in SurePath™ specimen background was done using treatment with **cobas**® Sample Prep Buffer. Results indicated that none of these organisms interfered with detection of HPV31, HPV16 and HPV18 plasmid DNA or produced a false positive result in the HPV negative specimen.

Table 18
Microorganisms Tested for Analytical Specificity

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

*Tested in both PreservCyt® and SurePath™ specimen background

**Tested only in SurePath™ specimen background

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 19. 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. All testing in SurePath™ specimen background was done using treatment with **cobas®** Sample Prep Buffer. Levels of each potential interfering substance tested and performance observations are described in Table 20. 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 2% in Roche Cell Collection Medium, PreservCyt® Solution . Whole blood showed no interference when present in visually detectable amounts of up to 4% in SurePath™ Preservative Fluid.

Table 19
Interference Testing Sample Descriptions

Sample type	Description
HPV Positive Cervical Specimens	10 individual HPV positive PreservCyt® Solution specimens were aliquoted for testing with and without endogenous interfering substances
HPV Negative Cervical Specimens	10 individual HPV negative PreservCyt® Solution specimens were aliquoted for testing with and without endogenous interfering substances
Contrived HPV Positive Cervical Specimen	HPV positive (channel1) PreservCyt® Solution specimens were diluted with HPV negative specimen to an approximate level of 3 x LOD. HPV types 16 (channel 2) and 18 (channel 3) plasmids were then added at ~ 3 x LOD. HPV31 plasmid, HPV16 cell line DNA, and HPV18 cell line DNA were added at ~ 3 x LOD to HPV negative specimens collected in Roche Cell Collection Medium.
3x LOD PreservCyt® Solution and SurePath™ Preservative Fluid pools	HPV types 31, 16, 18 plasmids were each diluted to 3 x LOD in pools of PreservCyt® Solution and SurePath™ Preservative Fluid negative specimen.

Table 20
Interference Testing Results with Endogenous Interferents

Interferent Tested	Concentrations Tested	Interference Observed	
		PreservCyt®	SurePath™
Whole Blood	1%, 1.5%, 2%, 3%, 4%, 6%, 8% v/v	Above 2%	Above 4%
PBMC	10^4, 10^5, 10^6 cells/mL	None	None
Cervical Mucus	Mucus obtained from standard cervical cleaning procedure	None	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 Roche Cell Collection Medium, PreservCyt® Solution, and SurePath™ Preservative Fluid 3 x LOD pools are described in Table 21.

Table 21
Interference Testing Results with Exogenous Interferents

Interferent Description	Interference Observed
Contraceptive Gels, Foams	None
Vaginal Lubricants	*Yes
Vaginal Douche	None
Anti-fungal creams containing 1% clotrimazole, Phenazopyridine Hydrochloride, 1% Hydrocortisone, 2% Miconazole nitrate, 6.5% Tioconazole Ointment, 20% Benzocaine	None

*Replens® (topical anti-dryness gel) produced negative results in replicates of the SurePath™ Preservative Fluid 3 x LOD pool. RepHresh® vaginal hygiene products produced negative results in replicates of the 3 x LOD pools of Roche Cell Collection Medium and PreservCyt® Solution.

SurePath™ Specimen Stability for 6 Weeks at 2-30°C using Treatment with **cobas**® Sample Prep Buffer

Three SurePath™ HPV negative specimen pools were spiked with HPV Type 51 positive SurePath™ specimen material to produce positive, high positive and low positive specimen pools. The low positive pool was at ~ Limit of Detection (LOD) for the test at Day 0 prior to treatment with **cobas**® Sample Prep Buffer. These pools were stored at 32°C and tested at intervals up to 6 weeks. Pooled materials were treated to reverse matrix-induced crosslinks followed by analysis with the **cobas**® 4800 HPV Test at each timepoint. All three pools maintained Ct averages below the clinical cutoff for HPV type 51 (40.0 for channel 1) through the 6 week storage period (see Table 22).

Table 22
SurePath™ Specimen Stability Results for 6 Weeks with Treatment using* cobas® *Sample Prep Buffer

SurePath™ Pools	Average Ct Values*				
	Day 0	Week 1	Week 3	Week 4	Week 6
High Positive	28.7	30.1	30.3	30.6	31.1
Positive	32.9	33.5	34.1	33.9	34.6
Low Positive (~LOD)	36.9	37.9	38.0	38.8	38.7

*Low Positive timepoints tested as 40 replicates; Positive timepoints as 30 replicates; High Positive timepoints as 20 replicates

Correlation of Results from Self-Collected and Clinician-Collected Specimens

A comparison of results from self-collected vaginal specimens and clinician-collected cervical specimens was performed using paired samples from 744 screening-eligible women.

Each woman first collected her sample using a FLOQSwab #552C.80 (Copan, Italy) which was suspended into Roche Cell Collection Medium or PreservCyt® Solution after collection. A second sample was collected by a clinician during the same visit using the standard of care protocol; the clinician-collected sample was suspended in the same medium type as that of the self-collected sample.

The rate of invalid results for the self-collected and clinician-collected results were 4.6% and 0.3%, respectively. A total of 708 valid paired results were used for correlation analysis. 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.

The correlation results and calculated positive, negative and overall percent agreements along with 95% confidence intervals are shown in Table 23.

Table 23
Correlation of Results for Self-Collected Vaginal Specimens using FLOQSwabs #552C.80 and Clinician-Collected Cervical Specimens

		Clinician-collected cervical sample 14 HR Result		
		Positive	**Negative**	**Total**
Self-collected vaginal sample using FLOQSwabs #552C.80 14 HR Result	**Positive**	161	48	209
	Negative	22	477	509
	Total	183	525	708

	Result (%)	**95% Confidence Interval**
Positive Percent Agreement	88.0%	82.5% - 91.9%
Negative Percent Agreement	90.8%	88.1% - 93.0%
Overall Percent Agreement	90.1%	87.7% - 92.1%

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Document Revision Information	
Doc Rev. 13.0 08/2023	Updated **Warnings and Precautions** section addressing leaking vials and to advise user to reach out to local competent authority. Added weblink to the summary of safety and performance report. Removed **cobas**® PCR Cell Collection Media. Renamed "performance characteristics" to "clinical performance using clinical specimens". Updated to comply with IVDR requirements. Inserted Rx Only symbol on first page. Updated the harmonized symbol page. Added **Technical support** section and Made in statement. Removed distributors' addresses. Added importer information. Updated **Trademarks and Patents** section. Updated **Intended Use** section to include self-collected specimens. Updated **Warnings and Precautions** section to state self-collected specimens must be suspended in medium after collection. Added collection device information to **Materials Required But Not Provided** section Removed Hamilton P/N 235905 from **Materials Required But Not Provided** section Updated **Specimen Collection, Transport and Storage** section with self-collected sample information. Added suspension instructions for self-collected specimens in the **Instructions For Use** section. Updated the **Procedural Limitations** section with validated specimen types for the test. Updated the **Clinical Performance Using Clinical Specimens** section with performance data for self-collected specimen data. Please contact your local Roche Representative if you have any questions.

The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed

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Technical support

For technical support (assistance) please reach out to your local affiliate: https://www.roche.com/about/business/roche_worldwide.htm

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Carryover prevention technology in the AmpErase® enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

See http://www.roche-diagnostics.us/patents

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

Age/DOB Age or Date of Birth

SW Ancillary Software

Assigned Range [copies/mL] Assigned Range (copies/mL)

Assigned Range [IU/mL] Assigned Range (IU/mL)

EC REP Authorized representative in the European Community

BARCODE Barcode Data Sheet

LOT Batch code

Biological risks

REF Catalogue number

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

Collect Date Collect date

Consult instructions for use

Contains sufficient for <*n*> tests

CONTENT Content of kit

CONTROL Control

Date of manufacture

Device for near-patient testing

Device for self-testing

Device not for near-patient testing

Device not for self-testing

Distributor
(Note: The applicable country/region may be designated beneath the symbol)

Do not re-use

Female

For IVD performance evaluation only

GTIN Global Trade Item Number

Importer

IVD In vitro diagnostic medical device

LLR Lower Limit of Assigned Range

Male

Manufacturer

CONTROL − Negative control

NON STERILE Non-sterile

Patient Name

Patient number

Peel here

CONTROL + Positive control

QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.

QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.

SN Serial number

Site Site

Procedure Standard Standard Procedure

STERILE EO Sterilized using ethylene oxide

Store in dark

Temperature limit

TDF Test Definition File

This way up

Procedure UltraSensitive Ultrasensitive Procedure

UDI Unique Device Identifier

ULR Upper Limit of Assigned Range

Urine Fill Line Urine Fill Line

Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.

Use-by date