cobas® HSV 1 and 2 Test

for use on the cobas® 4800 System

For in vitro diagnostic use

IVD

cobas® 4800 System Sample Preparation Kit	240 Tests 960 Tests	P/N: 05235782190 P/N: 05235804190
cobas® 4800 System Lysis Kit 1	240 Tests 960 Tests	P/N: 06768253190 P/N: 06768270190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Internal Control Kit 1	20 Runs	P/N: 06768318190
cobas® 4800 HSV 1 and 2 Amplification/Detection Kit	80 Tests	P/N: 06768199190
cobas® 4800 HSV 1 and 2 Controls and Cofactor Kit	10 Runs	P/N: 06768296190

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Intended Use

The **cobas**® HSV 1 and 2 Test on the **cobas**® 4800 System is an automated, qualitative in vitro diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection and typing of Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) DNA in clinician-collected anogenital lesion specimens from symptomatic male and female patients. The **cobas**® HSV 1 and 2 Test is intended for use as an aid in diagnosis of anogenital HSV-1 and HSV-2 infections in symptomatic patients.

Summary and Explanation of the Test / Principle of the Procedure

Background: Screening of HSV-1 and HSV-2

Genital herpes is a sexually transmitted disease (STD) caused by HSV-1 and HSV-2, ubiquitous double-stranded neurotropic DNA viruses of the Herpesviridae family.[1] Following primary infection via secretions the virus persists lifelong. The virus may remain latent for a prolonged time or cause recurrent episodes of reactivated symptomatic disease; the number of recurrences tends to decrease over a period of years. Most genital herpes are caused by HSV-2. HSV-1 can cause genital herpes, but it more commonly causes infections of the mouth and lips. Transmission of genital herpes is commonly caused by individuals that are unaware of their infections, or those with asymptomatic infections.[2] Pre- or perinatal transmission of HSV to the neonate may result in severe disease or even death.

Genital herpes infection is common in the United States, where an HSV-2 seroprevalence of up to 30% has been observed in pregnant women and may be higher than 50% in high-risk groups including HIV-infected individuals and commercial sex workers.[2] An HSV-2 seroprevalence of up to 80% has been observed in adults[3]; seroprevalence in women is up to twice as high as in men, and increases with age.[4,5]

Signs and symptoms associated with genital herpes may vary, and in a significant number[6] of infected individuals the infection remains asymptomatic or undiagnosed. Typically, 4 to 7 days after sexual contact, local pain or tingling occurs followed by bilateral clusters of erythematous papules and vesicles on the external genitalia[2]; lesions can also arise in the perianal region. Fever, headache, malaise, and inguinal lymphadenopathy may be present at the same time. Primary infection with HSV-1 cannot be distinguished from primary HSV-2 infection based on clinical criteria. About 70% to 90% of people with symptomatic HSV-2, and about 20% to 50% with symptomatic genital HSV-1 infection will have a recurrence within the first year.[7-9] The definitive diagnosis of infection and typing of the virus is therefore of importance for the initiation of treatment and further management of patients.[10]

The diagnosis of genital herpes can be established by testing of a swab specimen taken from the anogenital lesions using culture followed by type-specific immunofluorescence, or by molecular techniques. Polymerase chain reaction (PCR)-based assays combine an increased sensitivity and a shorter time to test result compared to culture.[11,12] Tests to detect antibodies are used for the diagnosis of latent HSV infections. Rapid administration of antiviral therapy to infected individuals will help to minimize transmission of HSV and complications from HSV infection.

The **cobas**® HSV 1 and 2 Test processes anogenital lesion swab specimens collected with the COPAN MSwab Collection, Transport and Preservation kit. These primary specimens are loaded on the **cobas**® 4800 System, and nucleic acid extraction and PCR reaction set up occurs by an automated process. The subsequent real-time PCR process detects and types HSV-1 and HSV-2 specific DNA target in the sample, if present. The test can be run with the **cobas**® Cdiff and **cobas**® MRSA/SA tests in mixed batch fashion in the same run. All three tests share the same automated specimen extraction process as well as PCR profile for amplification and detection.

Explanation of the Test

The **cobas**® HSV 1 and 2 Test contains two major processes: (1) automated sample preparation to extract nucleic acids from the anogenital lesion specimens; (2) simultaneous PCR amplification of target DNA sequences using HSV-1 and HSV-2 specific primers, and real-time detection of cleaved fluorescent-labeled HSV-1 and HSV-2 specific oligonucleotide detection probes. An Internal Control, containing unrelated randomized DNA sequence, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

Principles of the Procedure

Sample Preparation

Sample preparation for the **cobas**® HSV 1 and 2 Test is automated with the use of the **cobas**® x 480 instrument. Viruses in the anogenital lesion samples are lysed with chaotropic agent, proteinase K, and SDS reagents. Released nucleic acids, along with added Internal Control DNA, are bound by magnetic glass particles. They are washed and then eluted into a small volume of buffer. The instrument then takes an aliquot of the eluted material and sets up the PCR reaction with an activated Master Mix.

PCR Amplification and TaqMan® Detection

The PCR cycling steps and detection of target signal occurs in the **cobas**® z 480 analyzer. The Master Mix reagent contains primer pairs and probes for five targets: the DNA polymerase region B and Thymidine Kinase region C of HSV-1; the Glycoprotein B 3' end region and Thymidine Kinase region C of HSV-2, and Internal Control. The dual target design for HSV-1 and HSV-2 enhances the assay robustness. If the targets nucleic acid sequences are present, amplification with the corresponding primers will occur by a thermostable DNA polymerase, generating PCR products (amplicons). These products are detected by specific TaqMan probes containing a fluorescent dye and a quencher. Normally, the quencher suppresses the fluorescence of the dye. However, if the PCR product is present, the probe hybridizes to the product and gets cleaved by the 5' to 3' nuclease activity of the polymerase. This reaction allows the fluorescence to be emitted from the dye, and the signal is recorded in real time during each PCR cycle by the **cobas**® z 480 analyzer. The signal is interpreted by the **cobas**® 4800 System Software and reported as final results.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® HSV 1 and 2 Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[13], 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 at the alkaline pH of Master Mix, 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. The **cobas**® HSV 1 and 2 Test has been demonstrated to inactivate at least 1000 copies of deoxyuridine-containing HSV 1 and 2 amplicon per PCR.

Materials, Reagents, and Specimen

Materials and Reagents Provided

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Sample Preparation Kit 240 Tests (P/N: 05235782190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol**	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 mist or vapours. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing 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

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Sample Preparation Kit 960 Tests (P/N: 05235804190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol**	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 mist or vapours. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing 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

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 240 Tests (P/N: 06768253190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer 1) Sodium citrate 5% Polydocanol** 42.6% Guanidinium thiocyanate** Dithiothreitol**	10 x 10 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 240 Tests (P/N: 06768253190)	**PK** (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K** Glycerine	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 mist or vapours. 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 Sodium dodecyl sulfate 0.09% Sodium azide	10 x 3 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 960 Tests (P/N: 06768270190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer 1) Sodium citrate 5% Polydocanol** 42.6% Guanidinium thiocyanate** Dithiothreitol**	10 x 36 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 960 Tests (P/N: 06768270190)	**PK** (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K** Glycerine	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 mist or vapours. 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 Sodium dodecyl sulfate 0.09% Sodium azide	10 x 9 mL	N/A
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl**	10 x 55 mL	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist or vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 26172-54-3 2-methyl-2H-isothiazol-3-one hydrochloride

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl**	10 x 200 mL	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist or vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 26172-54-3 2-methyl-2H-isothiazol-3-one hydrochloride
cobas® 4800 System Internal Control Kit 1 20 Runs (P/N: 06768318190)	**IC-1** (**cobas**® 4800 IC-1) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious, synthetic internal control DNA encapsulated in Lambda bacteriophage coat protein	20 x 0.5 mL	N/A
cobas® 4800 HSV 1 and 2 Amplification/Detection Kit 80 Tests (P/N: 06768199190)	**HSV MMX** (**cobas**® HSV 1 and 2 Master Mix) Tricine buffer EDTA Potassium acetate Potassium hydroxide Tween 20 Glycerol < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream HSV-1, HSV-2 and Internal Control primers < 0.01% Fluorescent-labeled HSV-1, HSV-2 and Internal Control probes < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	10 x 0.3 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 HSV 1 and 2 Controls and Cofactor Kit 10 Runs (P/N: 06768296190)	**HSV (+) C** (**cobas**® HSV 1 and 2 Positive Control) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious plasmid DNA (microbial) containing HSV 1 sequence < 0.01% Non-infectious plasmid DNA (microbial) containing HSV 2 sequence	10 x 0.5 mL	N/A
	(–) C (**cobas**® 4800 System Negative Control) Tris buffer EDTA 0.05% Sodium azide < 0.01% Poly rA RNA (synthetic)	10 x 0.5 mL	N/A
	Cofactor-2 (**cobas**® 4800 Cofactor-2) Manganese acetate Magnesium acetate 0.09% Sodium azide	10 x 1.7 mL	N/A

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent Storage and Handling

Reagent	Storage Temperature	Storage Time
cobas® 4800 System Sample Preparation Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Internal Control Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 HSV 1 and 2 Amplification/Detection Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 HSV 1 and 2 Controls and Cofactor Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiration date indicated

Note: Do not freeze reagents.

Reagent expiry date is based on the Coordinated Universal Time (UTC). Local time for reagent expiry could be offset by plus or minus 12 hours, depending on the local time zone relative to UTC.

Additional Materials Required

Materials	P/N
CORE Tips, 1000 µL, rack of 96	04639642001
50 mL Reagent Reservoir	05232732001
200 mL Reagent Reservoir	05232759001
cobas® 4800 System Extraction (deep well) Plate	05232716001
cobas® 4800 System AD (microwell) Plate 0.3 mL and Sealing Film	05232724001
Sealing foil applicator	04900383001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
MSwab Collection, Transport and Preservation System	7007248190 or COPAN 404C.R
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Optional Materials

Materials	P/N
Deep well plate cover	Hamilton 6474-01
Caps, white color (for recapping post-run primary specimens)	07033893001 or COPAN 2U008N100.R

For more information regarding the optional materials, contact your local Roche representative.

Instrumentation and Software Required but Not Provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas**® x 480 instrument **cobas**® z 480 analyzer Control Unit
cobas® 4800 system **cobas**® HSV 1 and 2 AP Software Version 1.0.0 or higher
cobas® 4800 system Application Software (Core) Version 2.2.0 or higher

For more information regarding the materials sold separately, contact your local Roche representative.

Precautions and Handling Requirements

Warning and Precautions

As with any test procedure, good laboratory practice 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, specimens and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- Avoid microbial and DNA contamination of reagents and specimens.
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- LYS-1 reagent contains guanidine thiocyanate. Do not allow direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.
- MGP contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.
- EB, HSV 1 and 2 MMX, SDS, Cofactor-2, (–)C, HSV 1 and 2 (+)C and IC-1 contain sodium azide.
- 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. If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System - User Assistance.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

Note: For specific instructions, see "Specimen Collection, Transport, and Storage".

Good Laboratory Practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens and kit reagents.
- 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.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Contamination

- Gloves must be worn and must be changed between handling specimens and **cobas**® HSV 1 and 2 Test reagents to prevent contamination. Avoid contaminating gloves when handling specimens and controls. Wear lab gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of specimens is not prevented during specimen handling.
- Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[14] and in the CLSI Document M29-A3.[15]

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.
- Do not use reagents or containers that are visibly damaged or show signs of leakage.

Disposal

- **cobas**® 4800 reagents and the **cobas**® HSV 1 and 2 Test specific reagents contain sodium azide (see "Warnings and precautions"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the cobas® 4800 System – User Assistance.

Spillage and Cleaning

- LYS-1 reagent contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the **cobas**® 4800 System - User Assistance to clean.
- 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 the **cobas**® 4800 System - User Assistance.

Specimen Collection, Transport, and Storage

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

Specimen Collection

Anogenital lesion swab specimens collected with the MSwab Collection, Transport and Preservation System have been validated for use with the **cobas**® HSV 1 and 2 Test. Specimens should be collected following the procedure detailed in the Specimen Collection Procedure section and according to your institution's standard operating procedures.

Specimen Transport Storage and Stability

Anogenital lesion swab specimens collected with the MSwab Collection, Transport and Preservation System are stable for transport and storage at 2-30°C for 4 days, or 2-8°C for 14 days, and frozen for at least 30 days before testing on the **cobas**® 4800 System (this was demonstrated by testing specimens after consecutive storage at 31±1°C for 4 days, followed by 2-8°C for 14 days, followed by -20°C for 30 days).

Transportation of HSV 1 and 2 specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Instructions for use

Running the Test

Workflow

Figure 1 cobas® HSV 1 and 2 Workflow

1	Start up the system.
2	Perform instrument maintenance.
3	Remove samples and reagents from storage.
4	Start run: • Load racks with samples.
5	With LIS: confirm work order Without LIS: create work order
6	Load consumables (deepwell plate, microwell plate, tip racks) and reagents
7	Start sample preparation run
8	Unload and seal microwell plate
9	Remove samples, used reagents, and deepwell plate.
10	Load microwell plate into analyzer
11	Review results
12	With LIS: send results to LIS
13	Unload analyzer

Test procedure

Specimen Collection Procedure

Proper specimen collection from the patient is extremely critical for optimal results. Specific guidance regarding specimen collection and the detection of viruses can be found in published reference manuals (CLSI M41-A).[17]

For optimal results, specimens for **cobas®** HSV 1 and 2 Test should be collected in the acute stage of the disease whenever possible, preferably within 3 days and less than 7 days after onset of illness (eruption of lesions).

Specimens should be collected according to your institution's standard operating procedures and/or the following:

A. Vesicles present (clear fluid-filled blister)
   1. Wash/wipe the surface of the lesion with sterile saline.
   2. Carefully uncap (disrupt) the vesicle with a FLOQSwab (preferred), needle or scalpel and collect the fluid with the FLOQSwab.
   3. With the same FLOQSwab, vigorously rub the base of the vesicle to collect cells at the base of the lesion.
   4. Transfer the swab to its MSwab transport tube. Leverage the swab shaft against the edge of the tube to break at pre-scored point.

5. Close the cap firmly while ensuring that the upper end of the swab shaft is in the center of the cap.

B. Vesicles absent (ruptured, weeping vesicle or crusted ulcer)
   1. If crust absent (ruptured and/or weeping vesicle)
      a. Using a dry FLOQSwab or one pre-moistened with two drops of sterile physiological saline, collect cells by vigorously rubbing the base of the lesion.
      b. Transfer swab to the MSwab transport tube. Leverage the swab shaft against the edge of the tube to break at pre-scored point.
      c. Close the cap firmly while ensuring that the upper end of the swab shaft is in the center of the cap.
   2. If there is crust on the lesion (crusted ulcer)
      a. Gently remove crust using a FLOQSwab pre-moistened with sterile saline
      b. Collect specimen by vigorously rubbing the base of the lesion.
      c. Alternatively, gently abrade the lesion with a sterile scalpel or needle until serous fluid emerges (avoid bleeding) and collect the sample with a pre-moistened FLOQSwab by vigorously rubbing the base of the vesicle.
      d. Transfer swab sample to MSwab transport tube. Leverage the swab shaft against the edge of the tube to break at pre-scored point.

   Label the sample and transport to testing laboratory according to your institution's standard operating procedures (refer to "Specimen Collection, Storage and Transport" section also). Refer to the "Workflow" section for notes on specimens.

All reagents except HSV 1 and 2 MMX and Co-factor 2 must be at ambient temperature prior to loading on the **cobas**® x 480 instrument. The HSV 1 and 2 MMX and Co-factor 2 reagents 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: Refer to the the cobas® 4800 System - User Assistance for detailed operating instructions.

Run Size

The **cobas**® 4800 System is designed to support mixed-batch runs between the **cobas**® HSV 1 and 2, **cobas**® Cdiff and **cobas**® MRSA/SA tests. The generic **cobas**® 4800 System Sample Preparation Kit, generic **cobas**® 4800 System Lysis Kit 1 and generic **cobas**® 4800 System Wash Buffer Kit are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and specimens for all assays to be run. The **cobas**® 4800 HSV 1 and 2 Amplification/Detection Kit is available in two sizes, each sufficient to test up to either 80 or 240 samples, which include HSV 1 and 2 controls and specimens to be run. Multiple vials of the **cobas**® 4800 HSV 1 and 2 Master Mix reagent can be used as appropriate in one run, as long as they are the same kit size. The generic **cobas**® 4800 System Internal Control Kit 1 and the **cobas**® 4800 HSV 1 and 2 Controls & Cofactor Kit are available in a single kit size , which is sufficient for 20 and 10 runs, respectively, and can support all run configurations. For each run containing HSV 1 and 2 specimens, one **cobas**® 4800 HSV 1 and 2 Positive Control and one **cobas**® 4800 System Negative Control must be run (see "Quality Control"). For a single test run, the maximum number of samples allowed is 94 specimens and 2 controls.

Note: Although not an optimal use of reagents, a 96-Test generic reagent can be used for a run containing 1-22 specimens. However, different sizes of the cobas® 4800 System Wash Buffer (WB) Kit, cobas® 4800 System Sample Preparation Kit and cobas® 4800 System Lysis Kit 1 cannot be mixed. For example, if a 96-Test WB reagent bottle is scanned at the start of the run, 96-Test size reagents from the other two kits must also be used.

Note: Although not an optimal use of reagents, a 24-Test cobas® 4800 HSV 1 and 2 MMX can be used for a run containing 1-6 HSV specimens. See the cobas® 4800 System – User Assistance for details on how to change kit size.

Workflow

The **cobas**® HSV 1 and 2 Test is run using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas**® x 480 instrument followed by amplification/detection on the **cobas**® z 480 analyzer. The run can be HSV 1 and 2 only, or mixed-batched with the **cobas**® Cdiff and/or **cobas**® MRSA/SA tests. Refer to the **cobas**® 4800 System - User Assistance for details.

Specimens

Note: The cobas® HSV 1 and 2 Test has been validated for use with the MSwab Collection, Transport and Preservation System. Do not use other swab collection devices or media types.

Note: A properly collected anogenital lesion swab specimen should have a single FLOQ swab with the shaft captured by the cap. Incoming specimens with no swabs or with more than one swab have not been collected according to the instructions, and should not be tested.

Note: Do not process anogenital lesion swab specimens that appear bloody or have a dark brown color.

Note: Specimens must be in the primary specimen containers with a proper barcode for 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: To avoid cross-contamination, it is recommended that primary tubes be processed on the cobas® 4800 System prior to other processing and testing.

Note: To avoid cross-contamination of processed specimens, additional caps for MSwab specimen container in an alternate color (white; see "Optional Materials") should be used to recap specimens after processing.

Note: Anogenital lesion swab specimens collected with the MSwab Collection, Transport and Preservation System contain sufficient volume to be tested twice on the cobas® 4800 System. Minimum volume to conduct a cobas® HSV 1 and 2 run is 700 µL in the primary MSwab specimen container.

Performing the cobas® HSV 1 and 2 Test

Note: Mixed batch runs between cobas® HSV 1 and 2 and cobas® Cdiff and/or cobas® MRSA/SA tests can be conducted. Refer to the cobas® 4800 System – User Assistance for more information.

1. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System - User Assistance.
2. Collect all reagents and consumables needed. Reagents must be at room temperature by the time the run is started with the exception of **cobas**® HSV 1 and 2 MMX and Cofactor-2 reagents.

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.

3. Check the appearance of anogenital lesion swab specimens collected in MSwab media to make sure they meet the requirements in the "Specimens" section. Ensure that all caps have been tightened. Vortex the specimen for a minimum of 5 seconds. Uncap the tube (top of the swab should be captured

by the cap) and swirl the swab around the inside wall of the tube to drain excess liquid. Discard the cap with the swab just before loading on the **cobas**® 4800 System. Make sure swab is taken out with the cap. Swab left in the sample vial will interfere with the **cobas**® HSV 1 and 2 Test.

4. Start a new run and define the work order for the run. There are three ways to create a work order:
   - By using the sample editor before sample rack is loaded into into **cobas**® x 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary.
   - By following the software wizard for the new run and loading specimens into **cobas**® x 480 instrument when prompted. The specimen barcodes will be automatically scanned, and the requested results for each specimen must be defined.
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System - User Assistance for more details. When selecting the requested results, check "HSV 1 and 2".
5. Load samples and define/select workorder or use LIS as appropriate. The "Unload sample carriers after transferring to deep well plate" option is selected by default. This allows the operator to retrieve the remaining specimens as soon as possible after they are aliquoted for processing by **cobas**® x 480 instrument. Specimen containers should be re-capped with fresh closure (see "Optional Materials") if storage is needed.
6. Follow the software wizard guide and load consumables. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
7. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - 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.

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

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial <u>immediately prior</u> to dispensing into the reagent reservoir.

8. Load amplification/detection reagents (HSV 1 and 2 MMX and Co-factor 2), Proteinase K (PK) and controls [HSV 1 and 2 (+) C, IC and (–) C] directly onto the reagent carriers. In order to prevent contamination, it is required to change gloves after handling positive controls.

Note: The software wizard will calculate the optimal number and size of cobas® HSV 1 and 2 MMX reagent to use. This will be reflected in the "Kit size" column on the MMX and Co-factor loading screen. To use a different size of cobas® HSV 1 and 2 MMX reagent, click the "Change kit size" button.

9. Start sample preparation by clicking on "Start run".
10. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results

then select "Unload" to unload the plate carrier. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System - User Assistance.

11. 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 activated master mix. Amplification and detection should be started as soon as possible but no later than 90 minutes after the end of the cobas® x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.

12. When the amplification and detection run is completed, unload the microwell plate from the **cobas**® z 480 analyzer.
13. Follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.

Results

Quality Control and Validity of Results

One set of **cobas**® HSV 1 and 2 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**® HSV 1 and 2 Test results from that run.

Positive Control

The HSV 1 and 2 (+) Control contains non-infectious DNA plasmids of both HSV-1 and HSV-2. The HSV 1 and 2 (+) Control monitors the nucleic acid extraction, amplification, and detection steps in a given run of the test. The HSV 1 and 2 (+) Control result must be 'Valid'. If the HSV 1 and 2 (+) 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.

Internal Control

The Internal Control is a lambda phage molecule that contains randomized sequences and targets for internal control-specific primers and probe. The Internal Control is added to all specimens and the Positive and Negative Controls during sample preparation on the **cobas**® x 480 instrument. The Internal Control monitors nucleic acid extraction, amplification, and detection steps for a given specimen. The Internal Control is also required for validation of the run controls.

Interpretation of Results

Note: All assay and run validation is determined 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 Table 1.

Table 1 Result Interpretation of the cobas® HSV 1 and 2 Test

cobas® HSV 1 and 2 Test	Result Report and Interpretation
POS HSV 1, POS HSV 2	**HSV-1 Positive, HSV-2 Positive** Specimen is positive for the presence of both HSV-1 and HSV-2 DNA.
NEG HSV 1, NEG HSV 2	**HSV-1 Negative*, HSV-2 Negative*** Neither HSV-1 nor HSV-2 DNA, if present, could be detected.
NEG HSV 1, POS HSV 2	**HSV-1 Negative*, HSV-2 Positive** HSV-1 DNA, if present, could not be detected. Specimen is positive for the presence of HSV-2 DNA.
POS HSV 1, NEG HSV 2	**HSV-1 Positive, HSV-2 Negative*** Specimen is positive for the presence of HSV-1 DNA. HSV-2 DNA, if present, could not be detected.
Invalid HSV 1, NEG HSV 2	**HSV-1 Invalid, HSV-2 Negative*** HSV-1 result is Invalid. Original specimen should be re-tested to obtain valid HSV-1 result. HSV-2 DNA, if present, could not be detected.
NEG HSV 1, Invalid HSV 2	**HSV-1 Negative*, HSV-2 Invalid** HSV-1 DNA, if present, could not be detected. HSV-2 result is Invalid. Original specimen should be re-tested to obtain valid HSV-2 result.
Invalid HSV 1, Invalid HSV 2	**HSV-1 Invalid, HSV-2 Invalid** Both HSV-1 and HSV-2 results is Invalid. Original specimen should be re-tested to obtain valid HSV-1 and HSV-2 results.
Invalid HSV 1, POS HSV 2	**HSV-1 Invalid, HSV-2 Positive** HSV-1 result is Invalid. Original specimen should be re-tested to obtain valid HSV-1 result. Specimen is positive for the presence of HSV-2 DNA.
POS HSV 1, Invalid HSV 2	**HSV-1 Positive, HSV-2 Invalid** Specimen is positive for the presence of HSV-1 DNA. HSV-2 result is Invalid. Original specimen should be re-tested to obtain valid HSV-2 result.
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 vortexed for a minimum of 5 seconds and re-tested to obtain valid HSV results.

*A negative result does not preclude the presence of HSV-1 and/or HSV-2 because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.

Invalid results may be obtained if the specimen contains inhibitory substances that prevent nucleic acid target extraction and/or amplification and detection. See “Procedural limitations” for known interference substances. In case of invalid results, dilute the original specimen by adding 0.2 mL of it into a new MSwab vial, vortex for a minimum of 5 seconds and re-test.

Failed results may be obtained if the specimen contains clots that interfere with the sample preparation procedure on the **cobas**® 4800 instrument.

List of result flags

The following table lists flags which are relevant for result interpretation.

Table 2 List of flags for the cobas® HSV 1 and 2 Test

cobas® HSV 1 and 2 Test	cobas® HSV 1 and 2 Test	Result Report and Interpretation
R20	The positive control is invalid.	An external control is invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R21	The negative control is invalid.	An external control is invalid. 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.

Procedural Limitations

1. The **cobas**® HSV 1 and 2 Test has only been validated for use with anogenital lesion swab specimens collected with the MSwab Collection, Transport and Preservation System.
2. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert), Package Inserts for the MSwab Collection, Transport and Preservation System and the **cobas**® 4800 System - User Assistance.
3. False negative or invalid results may occur due to interference from various substances. The Internal Control is included in the **cobas**® HSV 1 and 2 Test to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. Known interference includes, but may not be limited to the following:
   - Specimens containing blood in the amount greater than 40% of absorbed volume per swab may generate false negative results. Do not test samples that appear dark red or brown in color.
   - Specimens containing greater than 4.8 mg mucin per swab may generate false negative results.
   - Specimens containing greater than 1.6 mg feces per swab may generate false negative results.
   - Specimens containing 20 mg or more of Vagisil Crème may generate false negative results
4. A positive result is indicative of the presence of HSV DNA and not necessarily viable viruses. A negative result does not rule out the presence of HSV due to insufficient DNA in the clinical sample.
5. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown variants, resulting in a false negative result with the **cobas**® HSV 1 and 2 Assay.
6. The predictive value of an assay depends on the prevalence of the disease in any particular population.

7. The addition of AmpErase enzyme into the **cobas**® 4800 HSV 1 and 2 Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
8. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
9. 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.
10. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies.
11. Cross-contamination can cause false positive results. In a non-clinical study the sample to sample cross-contamination rate of the **cobas**® HSV 1 and 2 Test on the **cobas**® 4800 System has been determined to be 1.18% when alternating very high positive and negative samples were tested over multiple runs. Run to run cross-contamination has not been observed.

Non-clinical Performance Evaluation

Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® HSV 1 and 2 Test was determined by analyzing quantified HSV-1 and HSV-2 viral cultures diluted at multiple concentration levels into a simulated anogenital lesion swab matrix. The simulated matrix composed of mucin and human cells and mimics the effect of the clinical anogenital background for the **cobas**® HSV 1 and 2 Test. All levels were tested with at least 21 replicated using the full **cobas**® HSV 1 and 2 Test workflow across five lots of **cobas**® HSV 1 and 2 Test reagents. LOD for this test is defined as the target concentration which can be detected as positive in ≥ 95% of the replicates tested, based on results generated by the worst performing lot.

HSV-1 MacIntyre and HSV-2 G strains were tested in the analytical sensitivity study. The **cobas**® HSV 1 and 2 Test LOD on these isolates is shown in Table 3.

Table 3 cobas® HSV 1 and 2 Test LOD (Limit of Detection)

Organism	Strain	ATCC ID	Levels Tested	LOD ($TCID_{50}$ units/mL)
HSV-1	MacIntyre	VR-539	6	0.479
HSV-2	G	VR-734	6	0.112

Detection of HSV-1 and HSV-2 Strains

The limits of detection of the **cobas**® HSV 1 and 2 Test on 4 HSV-1 strains (VR-260, VR-733, VR-735 and VR-1493) and 3 HSV-2 strains (VR-1779, VR-1781 and VR-540) were verified by testing 40 replicates per level at multiple concentration levels. Dilutions and testing samples were prepared in a similar fashion as in the Limit of Detection (LOD) study. The lowest levels that had at least 95% observed hit rate are summarized in Table 4 and Table 5.

Table 4 cobas® HSV 1 and 2 Test LOD (Limit of Detection) on HSV-1 Strains

HSV-1 Strains	ATCC ID	Levels Tested	LOD ($TCID_{50}$ units/mL)
KOS	VR-1493	5	3.41
HF	VR-260	6	14.07
F	VR-733	5	5.20
MP	VR-735	4	0.04

Table 5 cobas® HSV 1 and 2 Test LOD (Limit of Detection) on HSV-2 Strains

HSV-2 Strains	ATCC ID	Levels Tested	LOD ($TCID_{50}$ units/mL)
ATCC-2011-2	VR-1779	5	0.26
ATCC-2011-4	VR-1781	5	0.24
MS	VR-540	5	2.34

Precision

In-house precision was conducted with HSV-1 and HSV-2 viruses diluted in a simulated anogenital swab matrix to concentration levels below Limit of Detection (LOD), near LOD and above LOD of the **cobas**® HSV 1 and 2 Test. A negative level composed of only the simulated anogenital swab matrix was also tested. The study used three unique lots of **cobas**® HSV 1 and 2 Test reagents and three instruments for a total of 36 runs over 12 days. A description of the precision panels and the study results are shown in Table 6. An analysis of the variance of the Ct values from valid tests was performed on positive panel members at above LOD concentrations. The analysis yielded overall CV (%) of 2.2% for HSV-1 Ct and 1.9% for HSV-2 Ct (see Table 7 and Table 8).

Table 6 In-House Precision Study Hit Rate Analysis

Panel Member	Concentration		HSV-1 (N=72)			HSV-2 (N=72)		
	HSV-1	HSV-2	Positive Results	Hit rate	95% 2-Sided Upper CL	Positive Results	Hit rate	95% 2-Sided Upper CL
P1	Negative	Negative	0	0%	5%	0	0%	5%
P2	< LOD	< LOD	36	50%	62%	40	56%	67%
P3	~ LOD	Negative	72	100%	100%	0	0%	5%
P4	Negative	~ LOD	0	0%	5%	71	99%	100%
P5	~3 x LOD	~ LOD	72	100%	100%	72	100%	100%
P6	~ LOD	~ 3 x LOD	72	100%	100%	72	100%	100%

Table 7 Variance Components Analysis For Precision Panel at 3 x LOD (Limit of Detection)

Target	HSV Level	Mean Ct	Variance Components/Percent Contribution to Total					
			Lot	Kit Size	Instrument	Run/Day	Random	Total
HSV-1	~ 3 x LOD	37.4	0	0.06	0	0.355	0.289	0.704
			0%	8.60%	0%	50.40%	41.10%	100%
HSV-2	~ 3 x LOD	38.2	0.035	0	0.049	0.102	0.345	0.53
			6.50%	0%	9.10%	19.30%	65.00%	100%

Table 8 Standard Deviations and Coefficients of Variation (%) Analysis For Precision Panel at 3 x LOD (Limit of Detection)

Target	N	Mean Ct	Standard Deviation Components/CV (%)					
			Lot	Kit Size	Instrument	Run/Day	Random	Total
HSV-1	72	37.4	0	0.245	0	0.595	0.538	0.839
			0%	0.70%	0%	1.60%	1.40%	2.20%
HSV-2	72	38.2	0.186	0	0.22	0.32	0.587	0.728
			0.50%	0%	0.60%	0.80%	1.50%	1.90%

Competitive Inhibition

Panels were constructed with HSV-1 at ~ 3 x LOD (Limit of Detection), and competing HSV-2 at ~ 300 x LOD of the **cobas**® HSV 1 and 2 Test; and vice versa. One hundred fold higher concentration of HSV-1 did not affect the detection of HSV-2 at ~ 3 x LOD concentration and one hundred fold higher concentration of HSV-2 did not affect the detection of HSV-1 at ~ 3 x LOD concentration.

Analytical Specificity

To assess the analytical specificity of the **cobas**® HSV 1 and 2 Test, the following organism panels were tested: 1) 71 bacteria, fungi and viruses that may be found in anogenital swab specimens (Table 9), 2) human cells (Table 9), and 3) high titer HSV-1 or HSV-2 (Table 9). All organisms, human cells, HSV-1 and HSV-2 viruses were spiked to 1 x 10^6 Units*/mL or higher except for *Treponema pallidum*; Human Herpes Virus 8, *Chlamydia trachomatis serovar H*, *Mycoplasma genitalium*, and *Mycoplasma hominis* which were spiked to lower concentrations due to stock concentration limitations. Testing was performed with the organisms and human cells alone to determine the analytical specificity of the **cobas**® HSV 1 and 2 Test or tested in presence of HSV-1 and HSV-2 at ~ 3 x LOD to assess the potential interference of the organisms and the human cells on detection of HSV-1 and HSV-2 by the **cobas**® HSV 1 and 2 Test. Results indicated that none of these organisms or high concentration of human cells produced false positive results when there was no HSV-1 and HSV-2 target present. None of these organisms or high concentration of human cells interfered with the detection of HSV-1 and HSV-2 targets. High concentration of HSV-1 (1e6 vp/mL) did not produce false HSV-2 positive results and high concentration of HSV-2 (1e6 vp/mL) did not produce false HSV-1 positive results.

*All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydia trachomatis serovar H* and *Chlamydia trachomatis serovar I* which were quantified as Inclusion Forming Units (IFU); *Toxoplasma gondii* and *Treponema pallidum* which were quantified as DNA copies and *Trichomonas vaginalis* which was quantified in cells. Cytomegalovirus (HHV5), Human Herpes Virus 6B Strain Z29, Human Herpes Virus 7 Strain SB, Human Herpes Virus 8, Echovirus 11, Human enterovirus 71 and Rubella Virus were quantified as $TCID_{50}$ units; HHV-6A strain GS, HSV-1 and HSV-2 were quantified in viral particles, HIV-1 Strain IIIB and HBV were quantified in International Units (IU). HIV-2 Strain NIH-Z, Epstein-Barr Virus (HHV4), Varicella-Zoster Virus (HHV3) and HPV plasmids (HPV11, HPV16, HPV18, HPV06) were quantified as DNA copies. Human Peripheral Blood Mononuclear Cells (PBMC) were quantified as number of cells.

Table 9 Microorganisms and Cells Tested for Analytical Specificity

Human Adenovirus type 7	*Staphylococcus aureus* (MRSA)	*Moraxella catarrhalis*
Cytomegalovirus (HHV5)	*Staphylococcus aureus* (MSSA)	*Moraxella lacunata*
Epstein-Barr Virus (HHV4)	*Staphylococcus epidermidis*	*Mycobacterium tuberculosis*
Varicella-Zoster Virus (HHV3)	*Propionibacterium acnes*	*Mycoplasma genitalium**
Human Herpes Virus 6A strain GS	*Escherichia coli*	*Mycoplasma hominis**
Human Herpes Virus 6B Strain Z29	*Chlamydia trachomatis* serovar H*	*Neisseria gonorrhoeae*
Human Herpes Virus 7 Strain SB	*Chlamydia trachomatis* serovar I	*Neisseria meningitidis*
Human Herpes Virus 8*	*Clostridium perfringens*	*Prevotella melaninogenica*
Echovirus 11	*Clostridium difficile*	*Proteus vulgaris*
Enterovirus 71	*Corynebacterium genitalium*	*Pseudomonas aeruginosa*
HBV	*Cryptococcus neoformans*	*Staphylococcus saprophyticus*
HIV-1 Strain IIIB	*Enterobacter cloacae*	*Streptococcus agalactiae*
HIV-2 Strain NIH-Z	*Enterococcus faecalis vanB*	*Streptococcus mitis*
HPV11	*Enterococcus faecium vanA*	*Streptococcus mutans*
HPV16	*Fusobacterium nucleatum*	*Streptococcus pneumoniae*
HPV18	*Gardnerella vaginalis*	*Streptococcus pyogenes*
HPV6	*Gemella haemolysans*	*Streptococcus salivarius*
Rubella Virus	*Haemophilus ducreyi*	*Toxoplasma gondii*
Acinetobacter calcoaceticus	*Haemophilus influenzae*	*Treponema pallidum**
Acinetobacter lwoffii	*Kingella kingae*	*Trichomonas vaginalis*
Actinomyces israelii	*Klebsiella pneumoniae subsp. ozaenae*	*Veillonella parvula*
Alcaligenes faecalis	*Lactobacillus acidophilus*	PBMC (human genomic DNA)
Bacteroides fragilis	*Listeria monocytogenes*	Herpes Simplex Virus-1
Candida albicans	*Mobiluncus curtisii spp. curtisii*	Herpes Simplex Virus-2
Candida glabrata	*Mobiluncus mulieris*	-

* Human Herpes Virus 8 was tested at 1.3 x 10^4 $TCID_{50}$ units/mL; *Chlamydia trachomatis* serovar H was tested at 1.9 x 10^4 IFU/mL; *Mycoplasma genitalium* was tested at 1.0 x 10^5 CFU/mL; *Mycoplasma hominis* was tested at 1.5 x 10^4 CFU/mL; *Treponema pallidum* was tested at 9.0 x 10^4 copies/mL.

Interference

Twenty commonly used OTC products and anti-viral medications, as well as whole blood, human serum albumin, urine, feces, and mucin, were tested for potential interference effects with the **cobas**® HSV 1 and 2 Test. All OTC products were tested at or above (20 mg or 40 mg per swab respectively for solids and100% of swab capacity for liquids) the levels what could be reasonably expected to be collected by a swab in an anogenital lesion specimen. All anti-viral medicine was tested at 3 x Cmax in the collected specimen. HSV-1 and HSV-2 were spiked to ~ 3 x LOD (Limit of Detection) of the **cobas**® HSV 1 and 2 Test and used as targets in the tests.

No interference was observed for OTC products except for Vagisil Crème (interference observed at 20 mg). For whole blood, no interference was observed up to 40% of the swab capacity; for mucin, no interference was observed up to 4.8 mg per swab: for urine, no interference was observed up to 100% of the swab capacity; for feces, no interference was observed up to 1.6 mg per swab and for human serum albumin, no interference was observed up to 16 mg per swab. These results are summarized in Table 10.

Table 10 Results from Interference Substances Testing

Substance	Interpretation
Whole blood	No interference observed up to 40% of swab capacity
Mucin	No interference observed up to 4.8 mg per swab
Urine	No interference observed
Feces	No interference observed up to 1.6 mg per swab
Human Serum Albumin	No interference observed up to 16 mg per swab
K-Y Brand Jelly (Personal Lubricant)	No interference observed
Gynol II (Contraceptive jelly)	No interference observed
YeastGard Suppositories	No interference observed
Monistat 1	No interference observed
Monistat 3	No interference observed
VagiStat 1	No interference observed
Clotrimazole vaginal cream	No interference observed
Preparation H Hemorrhoidal cream	No interference observed
Abreva cold sore treatment	No interference observed
Releev cold sore treatment	No interference observed
Acyclovir Cream	No interference observed
Vagisil Crème	Interference observed at 20 mg*
Balneol Hygienic Cleansing lotion	No interference observed up to 20 mg*
Vagicaine Anti-Itch Cream	No interference observed up to 20 mg*
VH Essentials Douche	No interference observed
Denavir	No interference observed
Famciclovir	No interference observed
Valacyclovir	No interference observed
Cidofovir	No interference observed
Acyclovir	No interference observed

*20 mg is typical average amount of non-liquid product directly absorbed by a FLOQ swab.

Clinical Performance using Clinical Specimens

The performance of the **cobas**® HSV 1 and 2 Test was compared to a FDA cleared and CE marked State-of-the-Art comparator nucleic acid test (NAAT). Two anogenital swab specimens were collected from each subject enrolled in the study. The specimen for the **cobas**® HSV 1 and 2 Test was collected with MSwab Collection, Transport and Preservation System, and the specimen for the comparator test was collected with COPAN Universal Viral Transport System.

A total of 370 anogenital lesion swabs were collected at multiple sites across the United States and tested by **cobas**® HSV-1 and 2 Test and by the comparator's test. There were 33 HSV-1 positive and 147 HSV-2 positive specimens by the **cobas**® HSV 1 and 2 Test (prevalence: HSV-1 8.9%, HSV-2 39.7%). The performance of the **cobas**® HSV 1 and 2 Test and the comparator NAAT without discrepant resolution is shown in Table 11. Discrepant results between the **cobas**® HSV 1 and 2 Test and the comparator's test were resolved by sequencing. After discrepant resolution, the sensitivity and specificity of the **cobas**® HSV 1 and 2 Test were estimated and the results are shown in Table 12.

Table 11 cobas® HSV 1 and 2 Test and Comparator Nucleic Acid Test (NAAT)

HSV-1		**Comparator NAAT**		
		Positive	**Negative**	Total
cobas® HSV 1 and 2	**Positive**	31	2	33
	Negative	1	336	337
	Total	32	338	370
		Estimate	95% 1-Sided Lower CI	
Percent Positive Agreement		96.9%	86.0%	
Percent Negative Agreement		99.4%	98.1%	

HSV-2		**Comparator NAAT**		
		Positive	**Negative**	Total
cobas® HSV 1 and 2	**Positive**	127	20	147
	Negative	0	223	223
	Total	127	243	370
		Estimate	95% 1-Sided Lower CI	
Percent Positive Agreement		100%	97.7%	
Percent Negative Agreement		91.8%	88.3%	

Table 12 cobas® HSV-1 and 2 Test and Comparator Nucleic Acid Test (NAAT) after Discrepant Resolution

HSV-1		Comparator NAAT		
		Positive	**Negative**	Total
cobas® HSV 1 and 2	**Positive**	32	1	33
	Negative	0	337	337
	Total	32	338	370
		Estimate	95% 1-Sided Lower CI	
Sensitivity*		100%	91.1%	
Specificity*		99.7%	98.6%	

HSV-2		Comparator NAAT		
		Positive	**Negative**	Total
cobas® HSV 1 and 2	**Positive**	141	5	146
	Negative	0	223	223
	Total	141	228	369**
		Estimate	95% 1-Sided Lower CI	
Sensitivity*		100%	97.9%	
Specificity*		97.8%	95.4%	

*: only discrepant specimens were sequenced to resolve the discrepancy.

**: one discrepant specimen for which the sequencing data was not available was excluded from the analysis.

Based on the sensitivity and specificity estimates after the discrepant resolution by sequencing, observed positive predictive values of the **cobas**® HSV 1 and 2 Test for study samples are 97.0% for HSV-1 and 96.8% for HSV-2; observed negative predictive values are 100% for both HSV-1 and HSV-2.

Additional Information

Key Assay Features

Sample type	Anogenital lesion specimens
Amount of sample required	1.6 mL of MSwab media in primary vial, a minimum of 700 µL is required for a **cobas**® HSV 1 and 2 Test.
Test duration	Results are available within 2.5 hours after loading the specimens on the system (1-22 specimens).
Analytical sensitivity	0.479 $TCID_{50}$ units/mL for HSV-1 (MacIntyre Strain, ATCC VR-539); 0.112 $TCID_{50}$ units/mL for HSV-2 (G strain, ATCC VR-734)
Specificity	No cross-reactivity with 71 closely related organisms or organisms typically found in anogenital lesion specimens. No false positive HSV-2 in presence of 1x 10^6 vp/mL HSV-1; no false positive HSV-1 in presence of 1x 10^6 vp/mL HSV-2.
Inclusivity	5 HSV-1 strains and 4 HSV-2 strains tested

Symbols

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

Table 13 Symbols used in labeling for Roche PCR diagnostic products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

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

Manufacturer and importer

Table 14 Manufacturer and importer

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876, USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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EC REP

Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

CE
2797

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Document Revision

Document Revision Information	
Doc. Rev. 4.0 02/2024	Updated Lysis Kits 1 hazard information. Updated **Trademarks and patents** section, including the link. Updated **cobas**® branding. Please contact your local Roche Representative if you have any questions.
Doc. Rev. 5.0 08/2024	Updated Wash Buffer Kits hazard information. Updated competent authority statement. Updated Sample Preparation kits hazard information. Removed Rx Only from front page. Updated the harmonized symbol page. 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: htts://ec.europa.eu/tools/eudamed