cobas®

Rx Only

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 240 Tests	P/N: 06768199190 P/N: 06768202190
cobas® 4800 HSV 1 and 2 Controls and Cofactor Kit	10 Runs	P/N: 06768296190

TABLE OF CONTENTS

Non-clinical performance evaluation

Clinical performance

Additional information

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 differentiation of Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) DNA in clinician-collected, external 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.

***Warning**: The **cobas**® HSV 1 and 2 Test is not FDA cleared for use with cerebrospinal fluid (CSF) and is not intended to be used for prenatal screening or for individuals under the age of 18 years*.

Summary and explanation of the test

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 infections 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.

Signs and symptoms associated with genital herpes may vary, and in a significant number[3] 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.[4-6] The definitive diagnosis of infection and typing of the virus is therefore of importance for the initiation of treatment and further management of patients.[7]

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.[8,9] 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.

Principles of the procedure

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) 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.

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 gene; the Glycoprotein B 3' end region and Thymidine Kinase region C of HSV-2 gene, 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 (amplicon). 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[10], 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 and reagents

Materials and reagents provided

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test
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
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL
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
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL
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
	PK (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K Glycerine	10 x 0.9 mL
	SDS (**cobas**® 4800 System SDS Reagent) Tris buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 3 mL

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test
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
	PK (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K Glycerine	20 x 1.2 mL
	SDS (**cobas**® 4800 System SDS Reagent) Tris buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 9 mL
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
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
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

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test
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
cobas® 4800 HSV 1 and 2 Amplification/Detection Kit 240 Tests (P/N: 06768202190)	**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 0.7 mL

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test
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
	(–) C (**cobas**® 4800 System Negative Control) Tris buffer EDTA 0.05% Sodium azide < 0.01% Poly rA RNA (synthetic)	10 x 0.5 mL
	Cofactor-2 (**cobas**® 4800 Cofactor-2) Manganese acetate Magnesium acetate 0.09% Sodium azide	10 x 1.7 mL

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
Sealing mat or deep well plate cover	Roche 04789288001 or 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.

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[11] and in the CLSI Document M29-A4.[12]

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.

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 **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 at -20°C for 90 days before testing on the **cobas**® 4800 System. It 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 90 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

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 carriers 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).[13]

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 "Specimens" 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 cobas® 4800 System – User Assistance for detailed operating instructions.

Run size

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-batch format with tests that share the same automated specimen extraction process and PCR profile for amplification and detection. The software will display tests that are compatible for mixed batching with the **cobas**® HSV 1 and 2 at the test selection step. 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: Refer to the cobas® 4800 System – User Assistance for more information on performing mixed batched runs.

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 **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 work order 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 immediately prior 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 negative (–) Control contains a buffer solution. 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 more than 5 mg 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 but may be 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 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. High positive samples in the study were prepared to generate a Ct that exceeds 95% or more of signal obtained from specimens of infected patients in the intended use population. The likelihood of encountering such specimens in the routine use of the **cobas**® HSV 1 and 2 Test is proportional to HSV prevalence in the testing population. Therefore the sample to sample cross-contamination rate in routine use of the **cobas**® HSV 1 and 2 Test will likely be less than 1.18% x 5% x HSV prevalence in the testing population. Even at a maximum prevalence of 100%, the cross-contamination rate would be 1 x 0.0118 x 0.05, which is equivalent to 0.06%.

Expected results

A. Prevalence

The prevalence of HSV-1 and HSV-2 observed during a multi-center clinical trial was calculated for the **cobas**® HSV 1 and 2 Test. The prevalence rates for HSV-1 and HSV-2 were individually established as 20.6% (84/408) and 40.9% (167/408) for anogenital samples. The gender and age distribution from anogenital specimens are shown in Table 3 and the expected prevalence values for the **cobas**® HSV 1 and 2 Test from anogenital specimens is shown by age for males in Table 4 and females in Table 5.

Table 3: Gender and age distribution for the cobas® HSV-1 and 2 Test from anogenital specimens (N = 408 evaluable results)

Age	Gender		Total (N)
	Male (n)	Female (n)	
17	0	2	2
18 to <21	15	41	56
≥21	188	162	350
Total (N)	203	205	408

Table 4: Expected prevalence values for the cobas® HSV-1 and 2 Test for anogenital specimens from males by age (n = 203 evaluable results)

Age	Total (N)	HSV-1		HSV-2	
		Positive (n)	Prevalence (%)	Positive (n)	Prevalence (%)
12 to <18	0	0	0	0	0
18 to <21	15	3	20.0	5	33.3
≥21	188	30	16.0	77	41.0
Total	203	33	16.3	82	40.4

Table 5: Expected prevalence values for the cobas® HSV-1 and 2 Test for anogenital specimens from females by age (n = 205 evaluable results)

Age	Total (N)	HSV-1		HSV-2	
		Positive (n)	Prevalence (%)	Positive (n)	Prevalence (%)
12 to <18	2	0	0	1	50.0
18 to <21	41	17	41.5	13	31.7
≥21	162	34	21.0	71	43.8
Total	205	51	24.9	85	41.5

B. Positive and Negative Predictive Value

Hypothetical positive and negative predictive values (PPV & NPV) for the **cobas**® HSV 1 and 2 Test are shown in Table 6 below. These calculations are based on hypothetical prevalence and overall sensitivity and specificity for anogenital swab specimens as determined in the clinical trial.

For HSV-1, the calculations are based upon an overall sensitivity and specificity of 92.9% and 98.8%, respectively.

For HSV-2, these calculations are based upon an overall sensitivity and specificity of 97.0% and 94.6%, respectively.

Table 6: Hypothetical Positive and Negative Predictive Values (PPV & NPV) for the cobas® HSV 1 and 2 Test from anogenital specimens

Hypothetical Prevalence (%)	HSV-1		HSV-2	
	PPV[a] (%)	NPV[b] (%)	PPV[a] (%)	NPV[b] (%)
5	79.8	99.6	48.6	99.8
10	89.3	99.2	66.6	99.6
15	93.0	98.7	76.0	99.4
20	95.0	98.2	81.8	99.2
25	96.2	97.6	85.7	99.0
30	97.0	97.0	88.5	98.7
40	98.0	95.4	92.3	97.9

[a] PPV = (Sensitivity x Prevalence) / (Sensitivity x Prevalence + [1 - Specificity] x [1 - Prevalence]).

[b] NPV = (Specificity x [1 - Prevalence]) / ([1 - Sensitivity] x Prevalence + Specificity x [1 - Prevalence]).

Non-clinical performance evaluation

Analytical sensitivity (Limit of Detection)

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 replicates 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 7.

Table 7: cobas® HSV 1 and 2 Test LOD (Limit of Detection)

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

Analytical inclusivity

Four HSV-1 strains (VR-260, VR-733, VR-735 and VR-1493) and three HSV-2 strains (VR-1779, VR-1781 and VR-540) were tested for reactivity with the cobas® HSV 1 and 2 Test. These strains were obtained from ATCC and were cultured and quantified by Virapur, LLC (California, US). Each strain was diluted in a similar fashion as in the Limit of Detection study and was tested in 40 replicates near the LoD. All strains were detected by the assay, demonstrating that the cobas® HSV 1 and 2 Test can detect a broad range of both HSV-1 and HSV-2 isolates.

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 8. 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 9 and Table 10).

Table 8: 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 CI	Positive Results	Hit rate	95% 2-Sided CI
P1	Negative	Negative	0	0%	0 - 5%	0	0%	0 - 5%
P2	< LOD	< LOD	36	50%	38 - 62%	40	56%	43 - 67%
P3	~ LOD	Negative	72	100%	95 - 100%	0	0%	0 - 5%
P4	Negative	~ LOD	0	0%	0 - 5%	71	99%	93 - 100%
P5	~3 x LOD	~ LOD	72	100%	95 - 100%	72	100%	95 - 100%
P6	~ LOD	~ 3 x LOD	72	100%	95 - 100%	72	100%	95 - 100%

Table 9: 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 10: 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/cross-reactivity and microbial panel

The analytical specificity of the **cobas**® HSV 1 and 2 Test was assessed by testing a panel of organisms that could be present in anogenital swab specimens (Table 11). The panel consists of 1) 71 bacteria, fungi and viruses that may be found in anogenital swab specimens, 2) human cells, and 3) high titer HSV-1 or HSV-2. Testing was performed with the organisms and human cells alone to determine the analytical specificity of the **cobas**® HSV 1 and 2 Test or 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.

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*; *Chlamydia trachomatis serovar H*, and *Mycoplasma genitalium* which were spiked to lower concentrations due to stock concentration limitations.

*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, 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, HPV6) were quantified as DNA copies. Two sources of Human Herpes Virus 8 were used, one was quantified as DNA copies, the other was quantified in cells and estimated as 150 DNA copies per cell. Human Peripheral Blood Mononuclear Cells (PBMC) were quantified as number of cells.

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 (1x10^6 vp/mL) did not produce false HSV-2 positive results and high concentration of HSV-2 (1x10^6 vp/mL) did not produce false HSV-1 positive results.

Table 11: Cross reactivity panel

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*	-

* Two sources of Human Herpes Virus 8 were tested at 1.0 x 10^6 copies/mL (HHV8 viral DNA and HHV8 containing human cell line BCP-1)

** *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; *Treponema pallidum* was tested at 9.0 x 10^4 copies/mL

Interference

Twenty commonly used over the counter (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 and 100% of swab capacity for liquids) the levels that could be reasonably expected to be collected by a swab in an anogenital lesion specimen. Anti-viral medicine was tested at 3 x Cmax (maximum concentration of the drug as defined in the drug's labeling) 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 the OTC products except for Vagisil Crème (interference observed at 10 mg and above). 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 12.

Table 12: Interfering substances

Substance/Product Name	Composition	Testing Level/Swab
Whole blood	Human whole blood	40%, 50%
Mucin	Mucin Type II from porcine stomach	4.8 mg, 8 mg, 12 mg, 20 mg
Urine	Human urine	70%, 100%
Feces	Human feces	1.6 mg, 4 mg
Human Serum Albumin	Human serum albumin, fatty acid and globulin free	8 mg, 16 mg
K-Y Brand Jelly (Personal Lubricant)	Glycerin, Hydroxyethylcellulose, Chlorhexidine Gluconate, Methylparaben, Sodium Hydroxide, Water	20 mg, 40 mg
Gynol II (Contraceptive jelly)	3% Nonoxynol-9, Lactic Acid, Methylparaben, Povidone, Propylene Glycol, Purified Water, Sodium Carboxymethulcellulose, Sorbic Acid, Sorbitol Solution	20 mg, 40 mg
YeastGard Suppositories	Pulsatilla, Candida Parapsilosis, Candida Albicans, Bacillus Coagulans, Polyethylene Glycols	20 mg, 40 mg
Monistat 1	2% Miconazole nitrate, Benzoic Acid, Cetyl Alcohol, Isopropyl Myristate, Polysorbate 60, Potassium Hydroxide, Propylene Glycol, Purified Water, Stearyl Alcohol	20 mg, 40 mg
Monistat 3	4% Miconazole nitrate, Benzoic Acid, Cetyl Alcohol, Isopropyl Myristate, Polysorbate 60, Potassium Hydroxide, Propylene Glycol, Purified Water, Stearyl Alcohol	20 mg, 40 mg
VagiStat 1	6.5% Tioconazole, Butylated Hydroxyanisole, Magnesium Aluminium Silicate, White Petrolatum	20 mg, 40 mg
Clotrimazole vaginal cream	1% Clotrimazole, Benzyl Alcohol, Cetostearyl Alcohol, Cetyl Esters Wax, 2-Octyldodecanol, Polysorbate 60, Purified Water, Sodium Phosphate Monobasic, Sorbitan Monostearate	20 mg, 40 mg
Preparation H Hemorrhoidal cream	14.4% Glycerin, 0.25% Phenylephrine HCl, 1% Pramoxine HCl, 15% white Petrolatum, Aloe Barbadensis Leaf Extract, Anhydrous Citric Acid, Butylated Hydroxyanisole, Carboxymethylcellulose Sodium, Cetyl Alcohol, Citric Acid Monohydrate	20 mg, 40 mg
Abreva cold sore treatment	10% Docosanol, Benzyl Alcohol, Light Mineral Oil, Propylene Glycol, Purified Water, Sucrose Distearate, Sucrose Stearate	20 mg, 40 mg
Releev cold sore treatment	Benzalkonium Chloride, Purified Water, Viracea, Methyl Cellulose, Methyl Paraben, Potassium Sorbate, Propyl Paraben	20 mg, 40 mg
Acyclovir Cream	5% Acyclovir, Polyethylene Glycol	20 mg, 40 mg

Substance/Product Name	Composition	Testing Level/Swab
Vagisil Crème	20% Benzocaine, 3% Resorcinol, Water, Mineral Oil, Cetyl Alcohol, Propylene Glycol, Glyceryl Stearate, PEG-100 Stearate, Isopropyl Palmitate, Aloe Barbadensis Leaf Juice, Tocopherl Acetate, Retinyl Plamitate, Zea Mays Oil	2.5 mg, 5 mg, 10 mg, 20 mg, 40 mg
Balneol Hygienic Cleansing lotion	Water, Mineral Oil, Propylene Glycol, Glyceryl Stearate, PEG-100 Stearate, PEG-40 Stearate, Laureth 4, PEG-4 Dilaurate, Lanolin Oil, Sodium Acetate, Carbomer 934, Triethanolamine, Methylparaben	20 mg, 40 mg
Vagicaine Anti-Itch Cream	20% Benzocaine, 3% Resorcinol, Aloe barbadensis Leaf Extract, Carbomer Homopolymer Type C, Cetyl Alcohol, Cholecalciferol, Corn Oil, Glyceryl Monostearte, Isopropyl Myristate, Isopropyl Palmitate, Lanolin Alcohol, Methylparaben	20 mg, 40 mg
VH Essentials Douche	3% Povidone-iodine, Purified Water, USP, Octylphenoxypolyethoxyethanol	100%
Denavir	1% Penciclovir, Cetomacrogol 1000BP, Cetosteryl Alcohol, Mineral Oil, Propylene Glycol, Purified Water, White Petrolatum	20 mg, 40 mg
Famciclovir	Famciclovir, Hydroxypropyl Cellulose, Hydroxypropyl Methylcellulose, Lactose, Magnesium Stearate, Polyethylene Glycols, Sodium Starch Glycolate, Titanium Dioxide	0.016 mg
Valacyclovir	Valacyclovir Hydrochloride, Carnauba Wax, Colloidal Silicon Dioxide, Crospovidone, Hypromellose, Magnesium Stearate, Microcrystalline Cellulose, Polyethylene Glycol	0.027 mg
Cidofovir	Cidofovir, Sodium hydroxide, Sterile Water	0.552 mg
Acyclovir	Acyclovir, Magnesium Stearate, Microcrystalline Cellulose, Povidone, Sodium Starch Glycolate	0.008 mg

Reproducibility

The reproducibility of the **cobas**® HSV 1 and 2 Test on the **cobas**® 4800 System was established in a multi-site, investigation using contrived clinical samples evaluated across lot, site/instrument, operator, day, and run.

HSV test panels were prepared by spiking HSV-1 (MacIntyre strain) and/or HSV-2 (G strain) virus into contrived sample matrix in transport media at one of three concentrations (Below LOD, 1 × LOD, and 3 × LOD); HSV-1 and HSV-2 negative panel members were included as panel member controls. In all, there were 6 members per test panel with 3 replicates per panel member included in each run, not including external positive and negative assay controls. Panels were tested at 3 sites by 2 operators per site with 1 valid run per operator per day, for 6 days per lot over 2 lots for a total of 1,296 valid tests (216 tests/panel member or 108 tests/panel member/lot).

Table 13 summarizes the percent agreement (two-sided 95% exact CI) for the negative panel member and HSV-1 positive panel members.

Table 13: Percent agreement by panel member – HSV-1

		HSV-1	
Panel Member	**Number of Valid Test Results**	**Agreement (n/N)**	**95% CI[a]**
Negative[b]	216	100.0% (216/216)	(98.3%, 100.0%)
Below LOD (HSV-1/HSV-2)	216	63.4% (137/216)	(56.6%, 69.9%)
1 x LOD (HSV-1)[c]	216	100.0% (216/216)	(98.3%, 100.0%)
1 x LOD (HSV-2)[c]	216	99.5% (215/216)	(97.4%, 100.0%)
3 x LOD (HSV-1)/ 1 x LOD (HSV-2)	216	100.0% (216/216)	(98.3%, 100.0%)
1 x LOD (HSV-1)/ 3 x LOD (HSV-2)[c]	216	100.0% (216/216)	(98.3%, 100.0%)

[a] 95% CI = 95% exact binomial confidence interval.

[b] Negative panel members for HSV-1: percent negative agreement was 99.8% (431/432) with 95% CI (98.7%, 100.0%).

[c] Panel members with 1 x LOD HSV-1: percent positive agreement was 100.0% (432/432) with 95% CI (99.1%, 100.0%).

Note: Results are included as agreement when a positive panel member has a valid positive result for that analyte or when the negative panel member has a valid negative result for both analytes.

CI = confidence interval; LOD = limit of detection.

Table 14 presents the percent agreement (negative or positive) by lot, site/instrument, operator, and day for HSV-1 test results for each panel member.

Table 14: Percent agreement by panel member for lot, site/instrument, operator, and day – HSV-1

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
Negative	N/A	N/A	N/A	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
Below LOD (HSV-1/HSV-2)	41.1	1.41	3.4	1	60.2 (65/108)	1	56.9 (41/72)	1	58.3 (21/36)	1	58.3 (21/36)
				2	66.7 (72/108)	2	68.1 (49/72)	2	55.6 (20/36)	2	58.3 (21/36)
						3	65.3 (47/72)	3	63.9 (23/36)	3	66.7 (24/36)
								4	72.2 (26/36)	4	72.2 (26/36)
								5	63.9 (23/36)	5	66.7 (24/36)
								6	66.7 (24/36)	6	58.3 (21/36)
1 x LOD (HSV-1)	38.8	1.18	3.0	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)
1 x LOD (HSV-2)	N/A	N/A	N/A	1	99.1 (107/108)	1	98.6 (71/72)	1	97.2 (35/36)	1	97.2 (35/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
3 x LOD (HSV-1)/ 1 x LOD (HSV-2)	37.0	1.10	3.0	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)
1 x LOD (HSV-1)/ 3 x LOD (HSV-2)	38.7	1.15	3.0	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)

* For the negative panel member, percent agreement = (number of negative results/total valid results) x 100; for the positive panel members, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; CV = coefficient of variation; ; Inst. = instrument; LOD = limit of detection; N/A = not applicable; SD = standard deviation.

Table 15 presents the SD and CV (%) of Ct values for HSV-1 positive panel members overall and attributable to lot, site/instrument, operator, day, and within-run. Across HSV-1 positive panel members, the total SD ranged from 1.10 to 1.41, and the total CV (%) ranged from 3.0% to 3.4%.

Table 15: Overall mean, standard deviations, and coefficients of variation (%) for Ct values from valid results for positive panel members – HSV-1

			Standard Deviation and Percent Coefficients of Variation											
			Site/Inst.		Lot		Operator		Day		Within-Run		Total	
Panel Member	N	Mean Ct	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
Below LOD (HSV-1/HSV-2)	137	41.1	0.00	0.0%	0.51	1.3%	0.00	0.0%	0.71	1.7%	1.10	2.7%	1.41	3.4%
1 x LOD (HSV-1)	216	38.8	0.14	0.4%	0.53	1.4%	0.00	0.0%	0.00	0.0%	1.05	2.7%	1.18	3.0%
3 x LOD (HSV-1)/ 1 x LOD (HSV-2)	216	37.0	0.00	0.0%	0.64	1.7%	0.00	0.0%	0.14	0.4%	0.89	2.4%	1.10	3.0%
1 x LOD (HSV-1)/ 3 x LOD (HSV-2)	216	38.7	0.00	0.0%	0.47	1.2%	0.15	0.4%	0.16	0.4%	1.03	2.7%	1.15	3.0%

Ct = cycle threshold; CV = coefficient of variation; Inst. = instrument; LOD = limit of detection; SD = standard deviation.

In summary, the positive percent agreement for the HSV-1 positive panel member "Below LOD (HSV-1/HSV-2)" was 63.4% (95% CI: 56.6% to 69.9%) and the positive percent agreement for all other positive panel members was 100.0% (95% CI: 98.3% to 100.0%). For the negative panel members, negative percent agreement was 99.8% (95% CI: 98.7% to 100.0%). The total SD and total %CV across all panel members were ≤ 1.41 and ≤ 3.4%, respectively.

HSV-2 reproducibility results

Table 16 summarizes the percent agreement (two-sided 95% exact CI) for the negative panel member and HSV-2 positive panel members.

Table 16: Percent agreement by panel member – HSV-2

		HSV-2	
Panel Member	Number of Valid Test Results	Agreement (n/N)	95% CI[a]
Negative[b]	216	100.0% (216/216)	(98.3%, 100.0%)
Below LOD (HSV-1/HSV-2)	216	56.5% (122/216)	(49.6%, 63.2%)
1 x LOD (HSV-1)[b]	216	100.0% (216/216)	(98.3%, 100.0%)
1 x LOD (HSV-2)[c]	216	100.0% (216/216)	(98.3%, 100.0%)
3 x LOD (HSV-1)/1 x LOD (HSV-2)[c]	216	100.0% (216/216)	(98.3%, 100.0%)
1 x LOD (HSV-1)/3 x LOD (HSV-2)	216	100.0% (216/216)	(98.3%, 100.0%)

[a] 95% CI = 95% exact binomial confidence interval.

[b] Negative panel members for HSV-2: percent negative agreement was 100.0% (432/432) with 95% CI (99.1%, 100.0%).

[c] Panel members with 1 x LOD HSV-2: percent positive agreement was 100.0% (432/432) with 95% CI of (99.1%, 100.0%).

Note: Results are included as agreement when a positive panel member has a valid result of positive for that analyte or when the negative panel member has a valid result of negative for both analytes.

CI = confidence interval; LOD = limit of detection.

Table 17 presents the percent agreement (negative and positive) by lot, site/instrument, operator, and day for HSV-2 test results for each panel member.

Table 17: Percent agreement by panel member for lot, site/instrument, operator, and day – HSV-2

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
Negative	N/A	N/A	N/A	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)
Below LOD (HSV-1/HSV-2)	40.3	0.89	2.2	1	51.9 (56/108)	1	65.3 (47/72)	1	58.3 (21/36)	1	55.6 (20/36)
				2	61.1 (66/108)	2	50.0 (36/72)	2	72.2 (26/36)	2	50.0 (18/36)
						3	54.2 (39/72)	3	58.3 (21/36)	3	47.2 (17/36)
								4	38.9 (14/36)	4	66.7 (24/36)
								5	61.1 (22/36)	5	63.9 (23/36)
								6	50.0 (18/36)	6	55.6 (20/36)

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
1 x LOD (HSV-1)	N/A	N/A	N/A	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)
1 x LOD (HSV-2)	39.0	0.92	2.3	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)
3 x LOD (HSV-1)/ 1 x LOD (HSV-2)	38.8	0.86	2.2	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)

				Percent Agreement (n/N)*							
Panel Member	**Ct**			**Lot**		**Site/Inst.**		**Operator**		**Day**	
	Mean	**SD**	**CV %**								
1 x LOD (HSV-1)/ 3 x LOD (HSV-2)	37.8	0.73	1.9	1	100.0 (108/108)	1	100.0 (72/72)	1	100.0 (36/36)	1	100.0 (36/36)
				2	100.0 (108/108)	2	100.0 (72/72)	2	100.0 (36/36)	2	100.0 (36/36)
						3	100.0 (72/72)	3	100.0 (36/36)	3	100.0 (36/36)
								4	100.0 (36/36)	4	100.0 (36/36)
								5	100.0 (36/36)	5	100.0 (36/36)
								6	100.0 (36/36)	6	100.0 (36/36)

* For the negative panel member, percent agreement = (number of negative results/total valid results) x 100; for the positive panel members, percent agreement = (number of positive results/total valid results) x 100.
Ct = cycle threshold; CV = coefficient of variation; Inst = instrument; LOD = limit of detection; N/A = not applicable; SD = standard deviation.

Table 18 presents the SD and CV (%) of Ct values for HSV-2 positive panel members overall and attributable to lot, site/instrument, operator, day, and within-run. Across HSV-2 positive panel members, the total SD ranged from 0.73 to 0.92, and the total CV (%) ranged from 1.9% to 2.3%.

Table 18: Overall mean, standard deviations, and coefficients of variation (%) for Ct values from valid results for positive panel members – HSV-2

			Standard Deviation and Percent Coefficient of Variation											
			Site/Inst.		**Lot**		**Operator**		**Day**		**Within-Run**		**Total**	
Panel Member	**N**	**Mean Ct**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Below LOD (HSV-1/HSV-2)	122	40.3	0.08	0.2%	0.36	0.9%	0.00	0.0%	0.26	0.7%	0.76	1.9%	0.89	2.2%
1 x LOD (HSV-2)	216	39.0	0.03	0.1%	0.68	1.7%	0.00	0.0%	0.31	0.8%	0.53	1.4%	0.92	2.3%
3 x LOD (HSV-1)/ 1 x LOD (HSV-2)	216	38.8	0.00	0.0%	0.64	1.7%	0.00	0.0%	0.21	0.5%	0.54	1.4%	0.86	2.2%
1 x LOD (HSV-1)/ 3 x LOD (HSV-2)	216	37.8	0.06	0.2%	0.58	1.5%	0.11	0.3%	0.23	0.6%	0.37	1.0%	0.73	1.9%

Ct = cycle threshold; CV = coefficient of variation; Inst. = instrument; LOD = limit of detection; SD = standard deviation.

In summary, the positive percent agreement for the HSV-2 positive panel member “Below LOD (HSV-1/HSV-2)” was 56.5% (95% CI: 49.6% to 63.2%), whereas the positive percent agreement for all other positive panel members was 100.0% (95% CI: 98.3% to 100.0%). For the HSV-2 negative panel member, negative percent agreement was 100.0% (95% CI: 99.1% to 100.0%). The total SD and total CV (%) across all panel members were ≤ 0.92 and ≤ 2.3%, respectively.

Clinical performance

The clinical performance of the **cobas**® HSV 1 and 2 Test was established in the prospective, multi-site, investigation comparing to the combined results of culture and Sanger sequencing as the Reference Method using clinician-collected external anogenital lesion swab specimens from patients with possible HSV infection. In addition, the performance of the **cobas**® HSV 1 and 2 Test was compared to culture alone (ELVIS® HSV ID and D^3 Typing Test).

Two external anogenital swab specimens were collected from symptomatic eligible male and female subjects 17 years of age or older attending family planning, OB/GYN and sexually transmitted disease clinics at eight geographically diverse sites (seven across the United States and one in the United Kingdom). The first swab was used for (a) culture by the ELVIS® HSV ID and D^3 Typing Test (Diagnostic Hybrids, Inc.), (b) PCR followed by bi-directional Sanger sequencing for HSV-1 and HSV-2, and (c) discordant analysis using a FDA-cleared nucleic acid amplification test. The second swab was for the **cobas**® HSV 1 and 2 Test.

The clinical performance of the **cobas**® HSV 1 and 2 Test was established compared to a composite Reference Method derived from the combined results of culture (ELVIS® HSV ID and D^3 Typing Test) and Sanger sequencing using the "any positive rule".

Comparison with composite reference method (culture and Sanger sequencing)

There were a total of 408 specimens from 205 female and 203 male subjects evaluated in the study. There were 243 HSV positive subjects by the Composite Reference method; 84 HSV-1 (51 female, 33 male) and 167 HSV-2 (85 female, 82 male) positive subjects, with 8 (2%) subjects positive for both HSV-1 and HSV-2. The clinical performance of the **cobas**® HSV 1 and 2 Test compared to the composite reference method is summarized in Table 19.

Table 19: Comparison of cobas® HSV 1 and 2 Test with the composite reference method

		Composite Reference Method					
		HSV 1			HSV 2		
		Positive	Negative	Total	Positive	Negative	Total
cobas® HSV 1 and 2 Test	Positive	78	4[a]	82	162	13[c]	175
	Negative	6[b]	320	326	5[d]	228	233
	Total	84	324	408	167	241	408
		HSV 1			HSV 2		
		Sensitivity:	92.9% (78/84) (95% CI = 85.3% - 96.7%)		Sensitivity:	97.0% (162/167) (95% CI = 93.2% - 98.7%)	
		Specificity:	98.8% (320/324) (95% CI = 96.9% - 99.5%)		Specificity:	94.6% (228/241) (95% CI = 91.0% - 96.8%)	
		PPV:	95.1% (78/82) (95% CI = 88.1% - 98.1%)		PPV:	92.6% (162/175) (95% CI = 87.7% - 95.6%)	
		NPV:	98.2% (320/326) (95% CI = 96.0% - 99.2%)		NPV:	97.9% (228/233) (95% CI = 95.1% - 99.1%)	

[a] Of the 4 specimens with HSV-1 false-positive **cobas**® HSV 1 and 2 Test results relative to the Reference Method, 2 were HSV-1 positive by a FDA-cleared nucleic acid amplification test.

[b] Of the 6 specimens with HSV-1 false-negative **cobas**® HSV 1 and 2 Test results relative to the Reference Method, all 6 were HSV-1 negative by a FDA-cleared nucleic acid amplification test.

[c] Of the 13 specimens with HSV-2 false-positive **cobas**® HSV 1 and 2 Test results relative to the Reference Method, 5 were HSV-2 positive by a FDA-cleared nucleic acid amplification test.

[d] Of the 5 specimens with HSV-2 false-negative **cobas**® HSV 1 and 2 Test results relative to the Reference Method, all 5 were HSV-2 negative by a FDA-cleared nucleic acid amplification test.

Comparison with culture

The clinical performance of the **cobas**® HSV 1 and 2 Test compared to the ELVIS® HSV ID and D^3 Typing Test system is summarized in Table 20. The ELVIS® HSV ID and D^3 Typing Test system used in this study is unable to detect patients co-infected with HSV-1 and HSV-2 when HSV-2 is detected. Only HSV-2 negative anogenital specimens can be typed for HSV-1. Therefore, the number of samples used for the calculation of HSV-1 clinical performance equals the total number of specimens (408) minus the number of samples positive for HSV-2 by culture (129).

Table 20: Comparison of cobas® HSV 1 and 2 Test with culture

		Culture Reference Method[a]					
		HSV 1			HSV 2		
		Positive	Negative	Total	Positive	Negative	Total
cobas® HSV 1 and 2 Test	Positive	67	13[b]	80	128	47[c]	175
	Negative	0	199	199	1[d]	232	233
	Total	67	212	279	129	279	408
		HSV 1			HSV 2		
		Sensitivity:	100.0% (67/67) (95% CI = 94.6% - 100.0%)		Sensitivity:	99.2% (128/129) (95% CI = 95.7% - 99.9%)	
		Specificity:	93.9% (199/212); (95% CI = 89.8% - 96.4%)		Specificity:	83.2% (232/279) (95% CI = 78.3% - 87.1%)	
		PPV:	83.8% (67/80) (95% CI = 74.2% - 90.3%)		PPV:	73.1% (128/175) (95% CI = 66.1% - 79.2%)	
		NPV:	100.0% (199/199) (95% CI = 98.1% - 100.0%)		NPV:	99.6% (232/233) (95% CI = 97.6% - 99.9%)	

[a] The reference viral culture and typing method (ELVIS® HSV ID and D^3 Typing Test system) used in this study is unable to detect co-infected patients. Only HSV-2 negative specimens can be typed for HSV-1. Therefore, the number of samples used for the calculation of HSV-1 clinical performance equals the total number of evaluable specimens (408) minus the number of samples positive for HSV-2 by culture (129) for 279 evaluable specimens.

[b] Of the 13 specimens with HSV 1 false-positive **cobas**® HSV 1 and 2 Test results relative to the culture and typing, 6 were HSV-1 positive by a FDA-cleared nucleic acid amplification test and 4 of which were also HSV-1 positive by Sanger sequencing; 5 additional samples were HSV-1 positive by Sanger sequencing alone.

[c] Of the 47 specimens with HSV 2 false-positive **cobas**® HSV 1 and 2 Test results relative to the culture and typing, 32 were HSV-2 positive by a FDA-cleared nucleic acid amplification test.

[d] The one specimen with a HSV 2 false-negative **cobas**® HSV 1 and 2 Test result relative to the culture and typing was HSV-2 negative by a FDA-cleared nucleic acid amplification test.

Note: CI = (Score) confidence interval, PPV = positive predictive value, NPV = negative predictive value

Summary of Ct values for test positive samples

Histogram plots of Ct values from **cobas**® HSV 1 and 2 Test positive samples are presented in Figure 2 and Figure 3 for HSV-1 and HSV-2, respectively.

Figure 2: Frequency distribution of cycle threshold values for HSV-1 positive test results

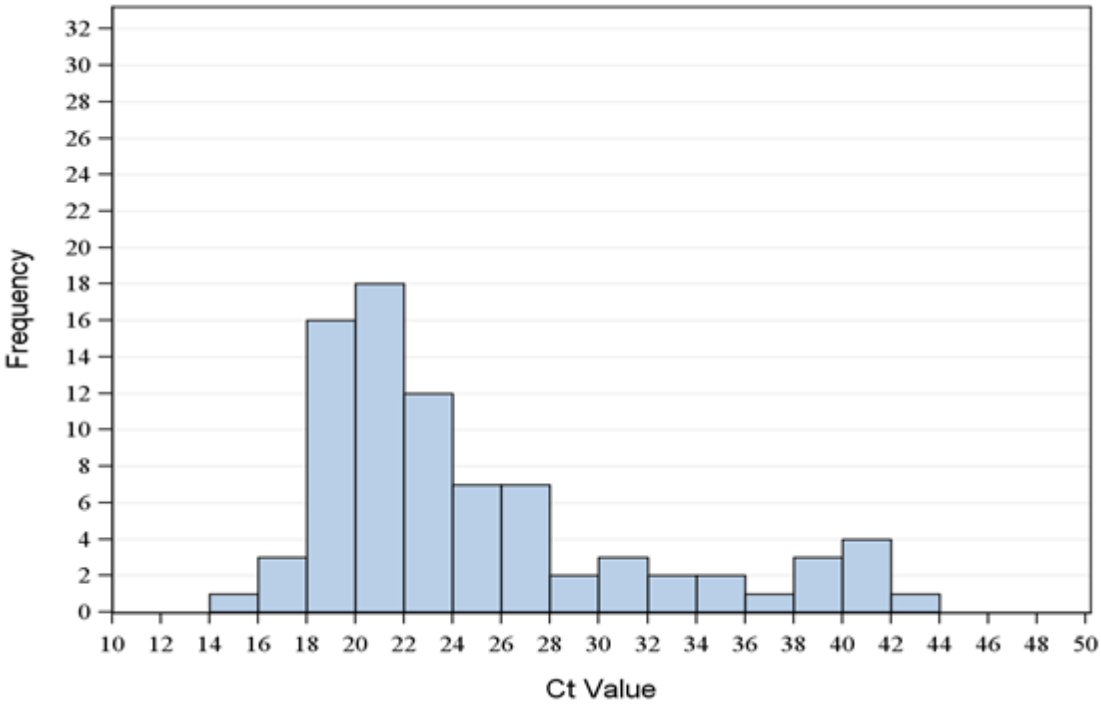
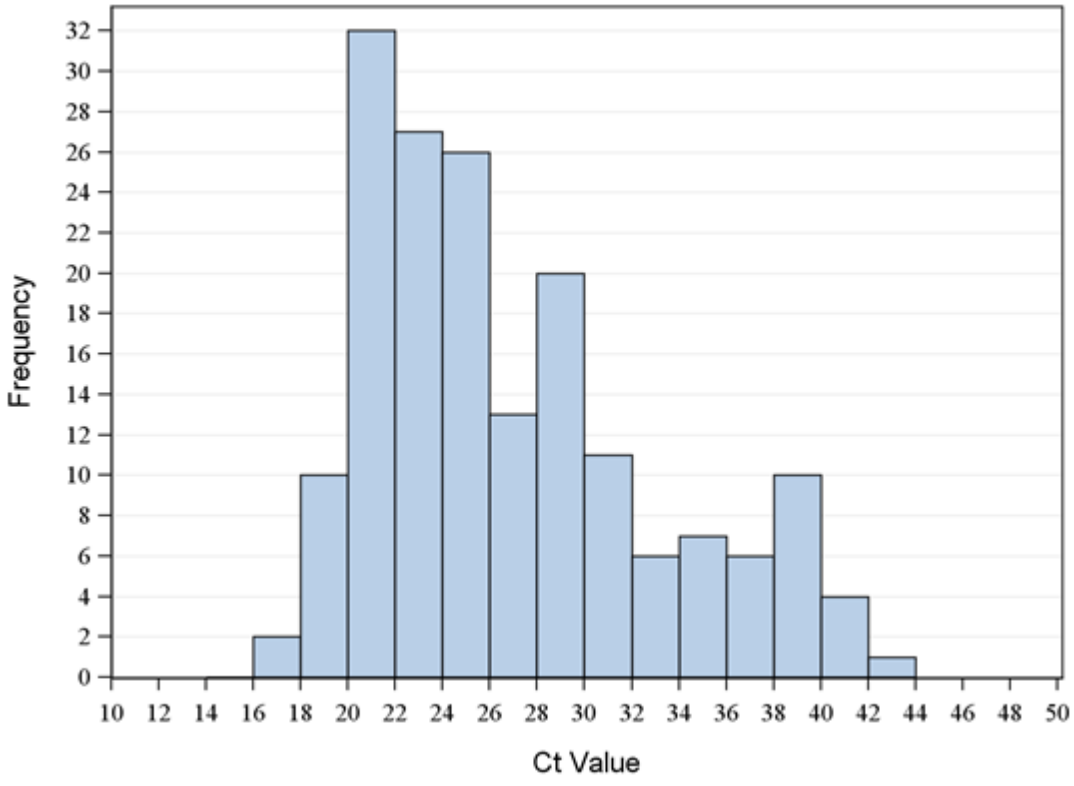


Figure 3: Frequency distribution of cycle threshold values for HSV-2 positive test results



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 21: Symbols Used in labeling for Roche PCR diagnostic products

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

This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 22: Manufacturer and distributors

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

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

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

Copyright

Bibliography

1. Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. J Am Acad Dermatol. 2007;57(5):737-763; quiz 764-766.
2. Gupta R, Warren T, Wald A. Genital herpes. Lancet. 2007;370(9605):2127-2137.
3. Langenberg AG, Corey L, Ashley RL, Leong WP, Straus SE. A prospective study of new infections with herpes simplex virus type 1 and type 2. Chiron HSV vaccine study group. N Engl J Med. 1999;341(19):1432-1438.
4. Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. N Engl J Med. 1987;316(23):1444-1449.
5. Engelberg R, Carrell D, Krantz E, Corey L, Wald A. Natural history of genital herpes simplex virus type 1 infection. Sex Transm Dis. 2003;30(2):174-177.
6. Benedetti J, Corey L, Ashley R. Recurrence rates in genital herpes after symptomatic first-episode infection. Ann Intern Med. 1994;121(11):847-854.
7. Ryan C, Kinghorn G. Clinical assessment of assays for diagnosis of herpes simplex infection. Expert Rev Mol Diagn. 2006;6(5):767-775.
8. Scoular A, Gillespie G, Carman WF. Polymerase chain reaction for diagnosis of genital herpes in a genitourinary medicine clinic. Sex Transm Infect. 2002;78(1):21-25.
9. Strick LB, Wald A. Diagnostics for herpes simplex virus: is PCR the new gold standard? Mol Diagn Ther. 2006;10(1):17-28.
10. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990;93(1):125-128.
11. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. HHS Publication No. (CDC) 21-1112. Biosafety in microbiological and biomedical laboratories. 5th edition. Revised December 2009.
12. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.
13. Clinical and Laboratory Standards Institute (CLSI). Viral culture; Approved Guideline. Volume 26 Number 35. CLSI Document M41-A:Wayne, PA;CLSI, 2006.

Document revision

Document Revision Information	
Doc. Rev. 2.0 10/2018	Update Software (Core) version to 2.2.0 or higher. Add reference to **cobas**® 4800 System - User Assistance. Remove reference to **cobas**® 4800 System - System Manual. Remove reference to **cobas**® 4800 System – Operator's Manual for **cobas**® HSV 1 and 2 Test. Changed "Tris-HCl buffer" to "Tris buffer" as a reagent component. Add a Procedural Limitation that 100% agreement between results should not be expected in correlation studies. Updated descriptions of and added Rx Only symbol and description to the harmonized symbol page at the end of the package insert. Added Roche web address www.roche.com. Please contact your local Roche Representative if you have any questions.