Elecsys HSV-2 IgG

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REF		Σ	SYSTEM
08948887160	08948887501	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System information

For **cobas e** 411 analyzer: test number 920
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 270

Warning

- Federal law restricts this device to sale and distribution by or on the order of a physician, or to the clinical laboratory; and use is restricted by or on the order of a physician.
- Assay performance characteristics have not been established in patients under the age of 18 or in populations of immunocompromised or immunosuppressed patients.
- This assay has not been FDA licensed for the screening of blood, plasma and tissue donors.

Intended use

Immunoassay for the in vitro qualitative determination of IgG class antibodies to HSV-2 in human serum and lithium-heparin plasma, K_2-EDTA plasma, and K_3-EDTA plasma. The test is intended for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-2 infection. The test results may not determine the state of active lesions or associated disease manifestations, particularly for primary infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-2.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

This test is not FDA-cleared for screening blood or plasma donors.

The performance of this assay has not been established for use in a pediatric population, neonates, immunocompromised patients, or for use at point-of-care facilities.

Summary

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are 2 members of the family Herpesviridae. The prevalence of HSV-1 infections in the general population is estimated to be around 70-80 %, for HSV-2 around 17-25 %.[1,2] Transmission of HSV-1 and HSV-2 depends on intimate, personal contact between a seronegative individual and someone excreting the virus.[3] Infection with HSV-1 and HSV-2 can produce a wide spectrum of symptoms, e.g. mucous membrane and skin lesions and ocular, visceral, and central nervous system (CNS) disease. In immunosuppressed patients HSV-infection can be associated with severe and extensive lesions.[4] Although HSV-1 and HSV-2 are usually transmitted by different routes and involve different areas of the body, much overlap is seen between the epidemiology and clinical manifestations of these 2 viruses.[2,5,6,7]

Primary HSV-1 infections are typically acquired during childhood. Following oropharyngeal infection, the trigeminal ganglion becomes colonized and harbors latent virus. A major manifestation of HSV-1 infection in young children is gingivostomatitis, a serious infection of the gums, tongue, mouth, lip, facial area, and pharynx. In older people infected with HSV-1 upper respiratory tract infections and mononucleosis-like syndrome are very common.[2] Recurrent skin lesions are the hallmark of HSV pathogenesis. Nearly all people with clinically recognized HSV-1 infection develop at least 1 recurrent episode within 1 year after the primary infection. Reactivation is associated with mucosal ulcerations or lesions at the mucocutaneous junction of the lips.[8]

Genital herpes can be induced by either HSV-1 or HSV-2.[9] Approximately 85 % of the symptomatic primary genital HSV-infections are caused by HSV-2, the rest is caused by HSV-1. Genital HSV-1 results from self-inoculation or from oral sexual practices.[10]

Neonatal herpes – which can be caused by HSV-1 as well as HSV-2 – has the most severe implications and is usually acquired during the intrapartum period through exposure in the genital tract.[7,11] In most cases the mothers have no reported history of HSV infection.[12] Neonatal HSV infections may remain localized to the site of infection (skin, eye, mouth), extend to the CNS, or disseminate to multiple organs.[13] Neonates have the highest frequency of visceral and CNS involvement of all HSV-infected patients.[14,15,16]

HSV infection is frequently not recognized. Subclinical viral shedding and unrecognized infections seem to be major factors in transmission.[12] Genital HSV infection is frequently not recognized and diagnosis based on the clinical presentation alone has a low sensitivity.[8] Serologic tests have been recommended for pregnant women as well as for asymptomatic patients and patients at risk of HIV infection.[4] Type-specific serologic tests allow the identification of silent carriers of HSV-2 infection in patients with or without pre-existing antibodies to HSV-1.[17,18] Testing algorithms have been described in guidelines.[19,20,21,22,23]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, biotinylated recombinant HSV-2-specific antigens, and HSV-2-specific recombinant antigens labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as HSV-2.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HSV-2-Ag~biotin (gray cap), 1 bottle, 9 mL: Biotinylated HSV-2-specific antigen (recombinant, E. coli), > 150 µg/L, MES[b)] buffer 50 mmol/L, pH 6.5; preservative.

R2 HSV-2-Ag~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL: HSV-2-specific antigen (recombinant, E. coli) labeled with ruthenium complex > 150 µg/L; MES buffer 50 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

HSV-2 Cal1 Negative calibrator 1 (white cap), 2 bottles (lyophilized) for 1.0 mL each: Human serum, non-reactive for HSV-2 IgG; preservative.

HSV-2 Cal2 Positive calibrator 2 (black cap), 2 bottles (lyophilized) for 1.0 mL each: Human serum, reactive for HSV-2 IgG; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

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For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing dust.
P273	Avoid release to the environment.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

Use only protocols described in this Method Sheet. Incubation times or temperatures other than those specified may give erroneous results.

Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.

Do not substitute reagents from other sources or manufacturers.

Avoid cross-contamination of reagents.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[24,25]

Both calibrators (HSV-2 Cal1, HSV-2 Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The serum containing HSV-2 IgG (HSV-2 Cal1, HSV-2 Cal2) was sterilized by filtration.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Avoid any sample cross-contamination during sample preparation.

Reagent handling

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

Calibrators (lyophilized):

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

cobas e 411 analyzer: The reconstituted calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per calibrator bottle set should be performed.

cobas e 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the freshly reconstituted calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note for **cobas e** 602 analyzers: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. Please turn the vial cap 180° into the correct position so that the barcode between the yellow markers can be read by the system. Place the vial on the analyzer as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks but not beyond the stated expiration date
on the analyzers at 20-25 °C	28 days but not beyond the stated expiration date

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after reconstitution at 2-8 °C	14 days but not beyond the stated expiration date
on **cobas e** 411 analyzer at 20-25 °C	up to 5 hours
on **cobas e** 601 and **cobas e** 602 analyzers at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Assay performance for sodium citrate plasma has not been evaluated.

Test samples as soon as possible after collection. Store samples at 2-8 °C if not tested immediately. Sample stability studies were performed using serum only.

Samples are stable for 48 hours at 20-25 °C, 7 days at 2-8 °C, 12 weeks at -20 °C (± 5 °C). The samples may be frozen and thawed up to 5 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (biocides, anti-oxidants or substances that could possibly change the pH of the sample) in order to avoid erroneous results.

Pooled samples and other artificial material may have different effects on different assays and thus may lead to discrepant findings.

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Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Sample stability claims were established by experimental data by the manufacturer only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific sample stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 05572207160, PreciControl HSV, for 4 x 3.0 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** analyzer
- Distilled or deionized water

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibrators:

Place the reconstituted calibrators (in the system-compatible bottles with barcoded labels) in the sample zone.

Only keep open during calibration.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (**cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against a Roche standard. The units have been selected arbitrarily.

Calibration frequency: Calibration must be performed once per reagent lot using HSV-2 Cal1, HSV-2 Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (HSV-2 Cal1): 600-7000 (**cobas e** 411 analyzer), 400-4000 (**cobas e** 601 and **cobas e** 602 analyzers)

Positive calibrator (HSV-2 Cal2): 28000-300000 (**cobas e** 411 analyzer), 24000-260000 (**cobas e** 601 and **cobas e** 602 analyzers)

Quality control

For quality control, use PreciControl HSV.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

The recommended quality control material is serum based. The user is responsible for providing alternate control material for plasma samples when necessary.

Note: The controls are not barcode-labeled, and therefore, all values and ranges must be entered manually. The exact lot-specific target values and ranges are printed on the value sheet which is included in the control kit or reagent kit (or electronically available). Please refer to the section "QC" in the operator's manual or to the online help of the instrument software for instructions on entering these values. Always consult the value sheet located in the rackpack or PreciControl kit to make sure the correct target values are used.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of HSV-2 Cal1 and HSV-2 Cal2.

Interpretation of the results

The cutoff for the Elecsys HSV-2 IgG assay was initially established by measuring a total of 267 serum samples from 2 cohorts: sexually active adults and pregnant women. The distribution of positive and negative results was compared with the predicate assay, an FDA-cleared immunoblot. The cutoffs were set as noted below. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff). Results obtained with the Elecsys HSV-2 IgG assay can be interpreted as follows:

Non-reactive:	< 1.0 COI
Reactive:	≥ 1.0 COI

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Samples with a cutoff index < 1.0 are non-reactive in the Elecsys HSV-2 IgG assay. These samples are considered negative for HSV-2 IgG-specific antibodies and do not need further testing.

Samples with a cutoff index ≥ 1.0 are considered reactive in the Elecsys HSV-2 IgG assay.

The HSV-2 IgG results for a given specimen, as determined by assays from different manufacturers, can vary due to differences in reagents and assay methods.

If control results are out of their specified range, no patient results should be reported.

Limitations - interference

A negative test result does not completely rule out the possibility of an infection with HSV-2 as individuals may not exhibit any detectable IgG antibodies at the early stage of acute infection.

False negative results may occur when the HSV virus is glycoprotein G (gG) deficient (0.2 % HSV isolates were gG deficient).[26]

The detection of HSV-2-specific IgG antibodies in a single sample indicates a previous exposure to HSV-2 but does not give any information of the time point of an exposure.

Results from the Elecsys HSV-2 IgG immunoassay should be used in conjunction with the patient's medical history and clinical symptoms.

The results in HIV patients, in patients undergoing immunosuppressive therapy, or in patients with other disorders leading to immune suppression, should be interpreted with caution.

Specimens from neonates, cord blood, pretransplant patients or body fluids other than serum and plasma, such as urine, saliva or amniotic fluid have not been tested.

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1 g/dL

Criterion: Mean recovery of positive samples within ± 20 % of serum value. Correct assignment of negative samples and recovery of positive samples ± 20 %.

Biotin interference

For biotin, serum samples that contain biotin at a concentration of 1200 ng/mL demonstrate less than or equal to 10 % bias in COI values. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[27] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[28]

In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on Famciclovir, Acyclovir and Valacyclovir. No interference with the assay was found.

Assay performance for sodium citrate plasma has not been evaluated.

In rare cases, interference due to extremely high titers of antibodies to streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Cross-reactivity for HPV and various types of bacterial vaginosis-causing agents (e.g., Mobiluncus sp., Gardnerella vaginalis, and Bacteroides sp.) were not evaluated in the performance analysis of this assay. The influence of the serological response against any of these agents on the results of the Elecsys HSV-2 IgG assay is unknown.

Sample stability studies were performed using serum only.

The results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Expected values

The Elecsys HSV-2 IgG immunoassay was used to evaluate the prevalence of HSV-2 IgG antibodies in the intended use populations: expectant mothers and sexually active populations. With the sexually active individuals (n = 594), the observed prevalence with HSV-2 IgG was 32.2 % (151/469). In the population of pregnant women (n = 125), the observed prevalence with HSV-2 IgG was 36.8 % (46/125). The data have been summarized according to age group in decades, gender, number of reactive results, and number of non-reactive results.

Expected results for Elecsys HSV-2 IgG immunoassay in sexually active subjects

Age range	Gender	Elecsys HSV-2 IgG results				Total
		Reactive		Non-reactive		
		N	%	N	%	
18 to 19	Male	1	20.0	4	80.0	5
	Female	1	4.35	22	95.7	23
20 to 29	Male	7	14.0	43	86.0	50
	Female	38	32.5	79	67.5	117
30 to 39	Male	5	23.8	16	76.2	21
	Female	29	43.3	38	56.7	67
40 to 49	Male	8	42.1	11	57.9	19
	Female	15	36.6	26	63.4	41
50 to 59	Male	7	26.9	19	73.1	26
	Female	12	38.7	19	61.3	31
60 to 69	Male	6	30.0	14	70.0	20
	Female	9	47.4	10	52.6	19
70 to 79	Male	3	30.0	7	70.0	10
	Female	7	63.6	4	36.4	11
80 to 89	Male	0	0.00	2	100	2
	Female	2	40.0	3	60.0	5
Unknown	Male	0	0.00	1	100	1
	Female	1	100	0	0.00	1
All ages	Male	37	24.0	117	76.0	154
	Female	114	36.2	201	63.8	315
Total		151	32.2	318	67.8	469

Expected results for Elecsys HSV-2 IgG immunoassay in pregnant subjects

Age range	Elecsys HSV-2 IgG results				Total
	Reactive		Non-reactive		
	N	%	N	%	
18 to 19	0	0.00	12	100	12
20 to 29	28	36.4	49	63.6	77
30 to 39	17	50.0	17	50.0	34
40 to 49	1	50.0	1	50.0	2
All ages / total	46	36.8	79	63.2	125

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute). Results were generated from 5 human serum samples (covering a range of

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negative, near cutoff, and positive values), and 2 controls assayed in 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	**Mean COI**[c]	**SD**[d] **COI**	**CV %**	**SD COI**	**CV %**
HS[e] 1	0.098	0.001	1.3	0.002	1.6
HS 2	1.15	0.015	1.3	0.024	2.1
HS 3	12.0	0.138	1.2	0.223	1.9
HS 4	49.4	0.970	2.0	1.19	2.4
HS 5	0.861	0.011	1.3	0.020	2.3
PC[f] HSV_1	0.287	0.003	0.9	0.005	1.8
PC HSV_2	7.72	0.075	1.0	0.169	2.2

c) COI = cutoff index

d) SD = standard deviation

e) HS = human serum

f) PC = PreciControl

cobas e 601 analyzer[g]					
		Repeatability		**Intermediate precision**	
Sample	**Mean COI**	**SD COI**	**CV %**	**SD COI**	**CV %**
HS 1	0.060	0.001	1.1	0.001	1.3
HS 2	0.960	0.012	1.3	0.015	1.6
HS 3	1.14	0.013	1.2	0.019	1.7
HS 4	12.3	0.115	0.9	0.161	1.3
HS 5	50.7	0.595	1.2	0.745	1.5
PC HSV_1	0.251	0.003	1.0	0.003	1.3
PC HSV_2	7.76	0.078	1.0	0.150	1.9

g) Precision results on the **cobas e** 601 analyzer are comparable to the **cobas e** 602 analyzer.

Reproducibility was performed following CLSI EP5-A2 and CLSI EP15-A2 at 3 external sites incorporating an 8 member panel consisting of 4 serum pools (high negative, moderately positive, low positive, and high positive) and 2 controls that were assayed for 5 days, 2 runs per day, 3 replicates per run on the **cobas e** 411 analyzer. The analysis of data was based on guidance from CLSI documents EP5-A2 and EP15-A2. Data from all runs were combined to achieve SD and percent CV for repeatability (within-run), between-run (intermediate precision), between-day, between-lot, between-site, and reproducibility (total precision). The overall imprecision data is summarized in the following tables:

Reproducibility on **cobas e** 411 analyzer						
			Repeatability		**Between-run**	
Sample	**N**	**Mean COI**	**SD COI**	**CV %**	**SD COI**	**CV %**
HSP[h] 01	90	0.461	0.008	1.6	0.005	1.1
HSP 02	90	0.637	0.008	1.3	0.014	2.2
HSP 03	90	0.995	0.013	1.3	0.022	2.2
HSP 04	90	1.62	0.023	1.4	0.040	2.5
HSP 05	90	3.70	0.052	1.4	0.092	2.5
HSP 06	90	4.55	0.059	1.3	0.128	2.8
HSP 07	90	14.4	0.158	1.1	0.351	2.4
HSP 08	90	28.3	0.270	1.0	0.788	2.8
PC HSV_1	90	0.340	0.004	1.2	0.007	2.1
PC HSV_2	90	7.27	0.104	1.4	0.220	3.0

h) HSP = human serum pool

Reproducibility on **cobas e** 411 analyzer								
			Between-day		**Between-site**		**Reproducibility (total)**	
Sample	**N**	**Mean COI**	**SD COI**	**CV %**	**SD COI**	**CV %**	**SD COI**	**CV %**
HSP 01	90	0.461	0.00[i]	0.0	0.013	2.7	0.016	3.4
HSP 02	90	0.637	0.00	0.0	0.016	2.5	0.023	3.6
HSP 03	90	0.995	0.00	0.0	0.012	1.2	0.028	2.8
HSP 04	90	1.62	0.00	0.0	0.004	0.3	0.046	2.9
HSP 05	90	3.70	0.012	0.3	0.015	0.4	0.107	2.9
HSP 06	90	4.55	0.00	0.0	0.000	0.0	0.141	3.1
HSP 07	90	14.4	0.00	0.0	0.087	0.6	0.395	2.7
HSP 08	90	28.3	0.00	0.0	0.000	0.0	0.833	2.9
PC HSV_1	90	0.340	0.00	0.0	0.015	4.4	0.017	5.1
PC HSV_2	90	7.27	0.00	0.0	0.086	1.2	0.258	3.6

i) SD of zero due to variance contributed by particular component was below stated significant figure.

Clinical performance

A multi-center study was conducted in the U.S. to evaluate the ability of the Elecsys HSV-2 IgG immunoassay to detect anti-HSV-2 IgG antibodies in specimens collected from the intended use patient population from various parts of the United States, including the East Coast, West Coast, Mid-South, and Southeast. Of the 794 samples tested on the **cobas e** 411 analyzer, 125 were from pregnant women referred for HSV testing and 594, including the 125 pregnant samples, were from sexually active individuals with a request for herpes testing. Of the 794 subjects, 564 (71.0 %) were females and 230 (29.0 %) were males. The mean age of the subjects was 31 years (age range: 16 to 90 years). All female subjects were tested for pregnancy using an FDA-cleared test. Testing of specimens on the Elecsys HSV-2 IgG immunoassay was performed at 3 clinical testing sites. All samples were tested by the reference method (FDA-cleared immunoblot) at a separate testing site. Samples that repeatedly tested equivocal on the reference method (n = 14) were resolved using a validated Western Blot analysis (University of Washington, Seattle, WA) as per the instructions of the reference package insert. The results of these studies are presented in the following table.

HSV-2 IgG results on **cobas e** 411 *analyzer versus reference method*

Cohort	**N**	**Sensitivity %**	**95 % Confidence Interval**	**Specificity %**	**95 % Confidence Interval**
Sexually active[j]	469	93.6 (147/157)	88.6-96.9	98.7 (308/312)	96.8-99.7
Expectant mothers	125	97.8 (45/46)	88.5-99.9	98.7 (78/79)	93.2-100
Low prevalence	200	75.0 (3/4)	19.4-99.4	98.5 (193/196)	95.6-100

j) One sample in the sexually active population remained unresolved after testing with Western Blot analysis. This sample was scored as discrepant against the Elecsys HSV-2 IgG immunoassay.

A panel of serum samples (n = 100) was obtained from the U.S. Centers for Disease Control and Prevention (CDC) and tested to evaluate the

performance of the Elecsys HSV-2 IgG immunoassay. The panel consisted of 54 samples negative for HSV-2 IgG and 46 samples positive for HSV-2 IgG. The Elecsys HSV-2 IgG immunoassay demonstrated 100 % positive agreement (46/46) and 100 % negative agreement (54/54) with the results from the CDC. These results are included to convey further information on the test kit and do not imply endorsement of the assay by the CDC.

Cross-reactivity

A study was conducted to evaluate the Elecsys HSV-2 IgG immunoassay for potential cross-reactivity in samples from individuals with antibodies to various medical conditions. Specimens (n = 311) were tested in duplicate with both the Elecsys HSV-2 IgG immunoassay and the predicate assay. The samples demonstrated 100 % concordance against the reference assay. These included samples with the following:

Cross-reactant	No. tested	HSV-2 IgG/ Reference Negative/Negative	HSV-2 IgG/ Reference Positive/Positive
ANA	26	26	0
Candida albicans	5	5	0
Chlamydia trachomatis	17	17	0
CMV	16	16	0
E. coli	5	5	0
EBV	19	19	0
HIV	11	11	0
HSV-1	130	130	0
Neisseria Gonorrhea	8	8	0
Rubella	25	25	0
Syphilis (*Treponema pallidum*)	26	26	0
Toxoplasma gondii	10	10	0
VZV	13	13	0
Total	311	311	0

Serum and plasma comparison

Studies were conducted to evaluate the suitability of the following 4 types of blood collection tubes to be used with the Elecsys HSV-2 IgG immunoassay: serum/gel separation tubes, Li-heparin plasma, K_2-EDTA plasma, and K_3-EDTA plasma. Samples were collected into matched serum and plasma collection tubes and assayed in duplicate. The study was conducted using negative, near cutoff, and positive samples. The results support the use of serum/gel separation tubes and the following plasma types: Li-heparin plasma, K_2-EDTA plasma, and K_3-EDTA plasma.

Plasma matrix	Number of positive specimens showing recovery to serum within various ranges		
	< 10 %	10-20 %	> 20 %
Li-heparin	14	0	0
K_2-EDTA	13	1	0
K_3-EDTA	13	0	0
Serum separation tube	23	0	0

Plasma matrix	Number of negative specimens showing recovery to serum within various ranges		
	< 10 %	10-20 %	> 20 %
Li-heparin	20	1	0
K_2-EDTA	20	0	0
K_3-EDTA	22	0	0
Serum separation tube	17	0	0

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

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