Elecsys HBsAg II

cobas®

REF	Σ	SYSTEM
04687787 160	100	**cobas e** 601

For use in the USA only

System information

For **cobas e** 601 analyzer: Application Code Number 663

Warning

- Federal law restricts this device to sale by or on the order of a physician.
- Assay performance characteristics have not been established when the Elecsys HBsAg II assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Assay performance characteristics have not been established for testing of newborns.
- This assay has not been FDA licensed for the screening of blood, plasma and tissue donors.

Intended use

Immunoassay for the in vitro qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric (2 to 21 years of age) serum and plasma (sodium heparin, lithium heparin, K_2-EDTA, sodium citrate). Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals at risk for infection with HBV or with signs and symptoms of hepatitis. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates at high risk of acquiring HBV during the perinatal period.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 601 immunoassay analyzer.

Summary

The hepatitis B surface antigen, a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus particle (HBV).[1,2] The blood of persons infected with HBV contains, in addition to intact infectious HBV particles, smaller non-infectious "empty" envelope particles, which are formed in great excess and also contain the hepatitis B surface antigen.[3] The HBsAg determinant **a**, against which the immune response is mainly directed, is common to all HBsAg particles. Within this **a** determinant, several HBsAg subtype determinants could be defined as **d, y, w1-w4, r** and **q**.[4] Under selective pressure (caused by antiviral therapy or by the action of the immune system itself), the virus can express many different viable HBsAg mutants (so-called *escape mutants*). Some mutants might lead to a loss of detection in commercially available HBsAg assays.[2,5] The Elecsys HBsAg II assay was especially developed in order to detect wild types and a multitude of these mutants.
The detection of HBsAg in human serum or plasma indicates an infection by the hepatitis B virus. HBsAg is the first immunological marker to appear in acute hepatitis B infection and is generally present some days or weeks before clinical symptoms begin to appear. HBsAg is observed in persons with acute and chronic hepatitis B infections.[6]
HBsAg tests are part of the diagnostic algorithm used to identify persons infected with HBV and to prevent the transmission of the hepatitis B virus by blood and blood products.[3,7]
In addition, HBsAg tests are recommended as part of prenatal care, in order to identify neonates at high risk of acquiring HBV during perinatal period.[8]

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The Elecsys HBsAg II assay uses monoclonal and polyclonal anti-HBs antibodies (mouse and sheep) for HBsAg detection.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies 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 M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

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

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as HBSAG II.

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-HBsAg-Ab~biotin (gray cap), 1 bottle, 8 mL: Two biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.
R2	Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 7 mL: Monoclonal anti-HBsAg antibody (mouse), polyclonal anti-HBsAg antibodies (sheep) labeled with ruthenium complex > 1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative.

HBSAG II Cal1	Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each: Human serum; preservative.
HBSAG II Cal2	Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each: HBsAg approximately 0.5 IU/mL in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

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

All human material should be considered potentially infectious.

The negative calibrator (HBSAG II Cal1) has been 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 used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

Positive calibrator (HBSAG II Cal2): Materials of human origin were tested for HIV and HCV. The findings were negative.

The serum containing HBsAg (HBSAG II Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or 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.[9,10]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
The reagents may not be used after the stated expiration date.

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.
Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the ready-for-use 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.

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	6 weeks
on the analyzer	4 weeks

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzer 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 in a sufficient number and found acceptable.
Serum collected using standard sampling tubes or tubes containing separating gel.
Li-heparin, Na-heparin, K_2-EDTA and Na-citrate plasma. Plasma tubes containing separating gel can be used.
Stable for 6 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 6 times.
The sample types listed were tested with a selection of sample collection tubes or systems 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.
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 and calibrators on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys HBsAg II assay has not been established with cadaveric samples or body fluids other than serum and plasma.

The claims, including those pertaining to sample stability made in the labeling of the cleared/approved reagents of Roche Diagnostics are part of the clearance of the overall IVD test system (assay). Sample stability was tested only for the temperatures/time frame as claimed by the manufacturer under the conditions claimed 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 stability criteria for its laboratory.

Materials provided

See “Reagents – working solutions” section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 04687876160, PreciControl HBsAg II, for 8 x 1.3 mL
- REF 11820648122, Elecsys HBsAg Confirmatory Test, 2 x 1 mL each of confirmatory and control reagent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** 601 analyzer

Accessories for **cobas e** 601 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 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.

PreClean M solution is necessary to be onboard the analyzer.

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.

Place the calibrators in the sample zone.

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

Ensure the calibrators are at 20-25 °C prior to measurement.

After calibration has been performed, discard the calibrators.

Special Wash Programming for the **cobas e** 601 analyzer:

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys HBsAg II assay is combined with all assays performed on the analyzer - including the Elecsys HBsAg II assay itself:

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From test	Step	To test	Step 0	Step 1	Step 2
HBsAg II	1	HBsAg II	x	x	x
HBsAg II	1	each other assay	x	x	x

If new tests are installed make sure that the Special Wash List is updated accordingly.
For the Elecsys Anti-HBs assay make sure that “Step 1” and “Step 2” are activated:

From test	Step	To test	Step 0	Step 1	Step 2
Anti-HBs	1	HBsAg II	-	x	x

The described additions to the Special Wash List have to be entered manually. Please refer to the operator's manual.

Calibration

Traceability: This method has been standardized against the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).
The following reference materials from the Paul-Ehrlich-Institute, Langen (Germany), were also measured (U/mL) and compared with the WHO standard:
PEI Standard AD (information sheet 1985, subtype AD; 1000 U/mL; inactivated)
PEI Standard AY (information sheet 1985, subtype AY; 1000 U/mL; inactivated)
(1 IU/mL WHO Standard corresponds to 0.34 U/mL PEI Standard AY and 1 IU/mL WHO Standard corresponds to 0.44 U/mL PEI Standard AD)
Calibration frequency:
Calibration must be performed once per reagent lot using HBSAG II Cal1, HBSAG II Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Calibration interval may be extended based on acceptable verification of calibration by the laboratory.
Renewed calibration is recommended as follows:

- after 1 month (28 days) 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 (HBSAG II Cal1): 300-1500, positive calibrator (HBSAG II Cal2): 2500-11000.

Quality control

For quality control, use PreciControl HBsAg II.
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.
Note: For technical reasons re-assigned target values valid only for a specific reagent and control lot combination, must be entered manually. Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

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Cutoff determination

The analyzer automatically calculates the cutoff based on the measurement of HBSAG II Cal1 and HBSAG II Cal2.

The cutoff for the Elecsys HBsAg II immunoassay was established and verified by testing 279 well characterized samples and commerical sources.

The cutoff is calculated from signals of the negative calibrator (HBSAG II Cal1) and the positive calibrator (HBSAG II Cal2) according to the following formula:

Cutoff formula

Cutoff = 0.175*counts Cal1 + 0.0396*counts Cal2

The test result is calculated in the form of a cutoff index (COI) equal to test signal/cutoff.

Interpretation of the results

Samples with a cutoff index of < 0.90 are non-reactive in the Elecsys HBsAg II assay. These samples are considered negative for HBsAg and do not require further testing.

Samples with an initial cutoff index of ≥ 1.0 are considered initially reactive. Samples with a cutoff index of ≥ 0.90 to < 1.0 are considered borderline. All initially reactive or borderline samples should be reassayed in duplicate using the Elecsys HBsAg II immunoassay. If the cutoff index values of < 1.0 are found in both cases, the sample is considered negative for HBsAg.

Initially reactive or borderline samples giving cutoff index values of ≥ 1.0 in two out of the three determinations are deemed repeatedly reactive. Repeatedly reactive samples must be confirmed using an independent neutralization test (Elecsys HBsAg Confirmatory Test). Samples confirmed by neutralization with human anti-HBs are regarded as positive for HBsAg.

Initial Elecsys HBsAg II			
COI	Initial result	Interpretation of initial results	Retest procedure
< 0.90	Non-reactive	No HBsAg detected	No retest required
≥ 0.90 to < 1.0	Border	Borderline zone (undetermined)	All initially reactive or borderline samples should be retested in duplicate using the Elecsys HBsAg II
≥ 1.0	Reactive	HBsAg detected	

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Final Elecsys HBsAg II			
Initial result	Result after retest (COI)	Final results	Interpretation of results
Non-reactive	No retest required	NON-REACTIVE	HBsAg not detected; does not exclude the possibility of exposure to HBV
Border	If 2 of the 3 results have a COI < 1.0	NON-REACTIVE	HBsAg not detected; does not exclude the possibility of exposure to HBV
	If 2 of the 3 results have a COI ≥ 1.0	REPEATEDLY REACTIVE	Presumptive evidence of HBV. Repeatedly reactive samples must be confirmed using a neutralization test (Elecsys HBsAg Confirmatory Test)
Reactive	If 2 of the 3 results have a COI ≥ 1.0	REPEATEDLY REACTIVE	
	If 2 of the 3 results have a COI < 1.0	NON-REACTIVE	HBsAg not detected; does not exclude the possibility of exposure to HBV

Limitations

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No false negative result due to high-dose hook effect was found with the Elecsys HBsAg II assay up to a concentration of 1.5 million IU/mL.

Current methods for the detection of HBsAg may not detect all infected individuals. A negative test result does not exclude with certainty a possible exposure to or an infection with the hepatitis B virus. Negative test results obtained for persons with a past exposure may be caused by an antigen concentration below the detection limit of this assay or the lack of reactivity of the antigens to the antibodies used in this assay.

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

Assay performance characteristics have not been established when Elecsys HBsAg II assay is used in conjunction with other manufacturers' assays for specific HBV serological markers.

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

Analytical sensitivity

In order to determine assay sensitivity, the HBsAg concentration which corresponds to the measuring signal of the cutoff value was read off the standard curves of serial dilutions of HBsAg standards (ad and ay) in human HBV-negative serum.

Sample	Paul-Ehrlich-Institute standards				WHO standard 00/588	
	Subtype ad, 1985		Subtype ay, 1985		Subtype adw2, genotype A	
	COI	U/mL	COI	U/mL	COI	IU/mL
1	0.394	0.000	0.389	0.000	0.287	0.000
2	3.57	0.050	2.03	0.050	1.47	0.050

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Sample	Paul-Ehrlich-Institute standards				WHO standard 00/588	
	Subtype ad, 1985		Subtype ay, 1985		Subtype adw2, genotype A	
3	68.1	1.00	32.0	1.00	23.6	1.00
4	136	2.00	62.3	2.00	48.2	2.00
5	333	5.00	138	5.00	119	5.00
Cutoff sensitivity (cutoff = 1.0)	0.010 U/mL		0.019 U/mL		0.033 IU/mL	

Specific performance data

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

Precision

Within-laboratory precision
Within-laboratory precision was determined on the **cobas e** 601 analyzer, using Elecsys reagents and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute). A precision panel consisting of four human sera and the two controls was measured - each sample was separated in two aliquots measured in duplicate on two runs per day by two operators for 12 days (n = 96). The following results were obtained:

cobas e 601 analyzer					
		Repeatability[b)]		**Intermediate precision**[c)]	
Sample n = 96	Mean COI	SD COI	CV %	SD COI	CV %
HS[d)], negative	0.264	0.021	7.94	0.024	8.89
HS, high negative	0.899	0.029	3.18	0.036	3.95
HS, low positive	1.14	0.044	3.82	0.046	4.04
HS, positive	2.72	0.055	2.04	0.091	3.34
PreciControl HBSAG II 1	0.340	0.020	5.86	0.027	8.01
PreciControl HBSAG II 2	4.20	0.088	2.10	0.127	3.02

b) Repeatability = within-run precision

c) Intermediate precision = within-laboratory precision

d) HS = human serum

All results for repeatability and intermediate precision studies met all performance specifications.

Reproducibility

The same precision panel as above was evaluated in the reproducibility study. The reproducibility results were collected on three **cobas e** 601 analyzers at three sites with three lots of reagents.
PreciControl HBSAG II 1, PreciControl HBSAG II 2 and the following sample concentrations were tested in

3 replicates per run, 2 runs per day, for 5 days in a protocol EP05-A3 of the CLSI (Clinical and Laboratory Standards Institute). The overall reproducibility (imprecision) data are summarized in the following tables:

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		Between-run		**Between-day**		**Between-lot**	
Sample n = 180	Mean COI	SD COI	CV %	SD COI	CV %	SD COI	CV %
HS1, negative	0.72	0.02	2.4	0.01	0.8	0.02	2.7
HS2, negative	0.83	0.02	1.8	0.01	1.3	0.02	2.4
HS3, negative	0.94	0.01	1.4	0.01	1.2	0.02	2.6
HS4, low positive	1.18	0.01	0.9	0.02	1.8	0.03	2.2
HS5, low positive	1.22	0.00	0.0	0.02	1.7	0.02	1.4
HS6, positive	1.68	0.03	1.5	0.03	1.5	0.05	3.0
PreciControl HBSAG II 1	0.32	0.01	2.8	0.01	4.3	0.01	4.5
PreciControl HBSAG II 2	4.22	0.07	1.6	0.04	0.9	0.06	1.3

cobas e 601 analyzer					
		Between-site		**Reproducibility**	
Sample n = 180	Mean COI	SD COI	CV %	SD COI	CV %
HS1, negative	0.72	0.00	0.0	0.04	5.9
HS2, negative	0.83	0.00	0.0	0.05	5.5
HS3, negative	0.94	0.00	0.0	0.05	5.0
HS4, low positive	1.18	0.00	0.0	0.05	4.5
HS5, low positive	1.22	0.01	1.2	0.05	4.1
HS6, positive	1.68	0.06	3.8	0.10	6.0
PreciControl HBSAG II 1	0.32	0.01	3.9	0.04	11.1
PreciControl HBSAG II 2	4.22	0.05	1.2	0.14	3.4

All results for reproducibility precision studies met all performance specifications.

Endogenous interference

To evaluate the effect of elevated levels of hemoglobin, bilirubin, intralipid, biotin, and total protein on the Elecsys HBsAg II assay, one negative, one high negative, one low positive, and one positive HBsAg serum samples were spiked with potential interferents. Each interferent was evaluated at 10 concentrations. All samples were tested in duplicate.

The results of the interferences are presented in the following table:

Compound	Concentration tested
Bilirubin	≤ 684 µmol/L or ≤ 40 mg/dL
Hemoglobin	≤ 1.37 mmol/L or ≤ 2200 mg/dL
Intralipid	≤ 2200 mg/dL
Biotin	≤ 180 nmol/L or ≤ 44 ng/mL
Albumin	≤ 22.0 g/dL

Drug interference

A drug interference study was performed with 18 common therapeutic drugs. Each drug was tested three-fold spiked into a negative, a high negative, a low positive, and a moderate positive sample. Each drug was found to be non-interfering at the following claimed concentrations:

Compound	Concentration (mg/L)
Acetyl cysteine	150
Ampicillin-Na	1000
Ascorbic acid	300
Ca-Dobesilate	200
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U/L
Intralipid	10000
Levodopa	20
Methyldopa+ 1.5	20
Metronidazole	200
Phenylbutazone	400
Tetracycline	50
Acetylsalicylic acid	1000
Rifampicin	60
Acetaminophen	200

Compound	Concentration (mg/L)
Ibuprofen	500
Theophylline	100

In addition, the following special therapeutic drugs were tested. No interference with the assay was found.
Special therapeutic drugs

Drug	Concentration tested
Lamivudin	300 mg/L
PEG interferon-alpha	180 µg/L
Entecavir	0.5 mg/L
Telbivudine	600 mg/L
Adefovir	10 mg/L

Drug interference studies were performed in vitro, and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.

Serum / plasma comparison

Studies were conducted to evaluate the suitability of the following five sample types: serum/gel separation tubes, sodium heparin plasma, sodium citrate plasma, lithium heparin plasma and K_2-EDTA plasma to be used with the Elecsys HBsAg II assay.
Samples were collected into matched serum and plasma collection tubes and assayed in duplicate. The study was conducted using negative, high negative, low positive, and moderate positive samples for HBsAg. The studies support the use of serum/gel separation tubes, and the following plasma types: Lithium heparin plasma, K_2-EDTA plasma, sodium heparin plasma, and sodium citrate plasma.

Analytical specificity

A study was conducted to evaluate the Elecsys HBsAg II assay for potential cross-reactivity in specimens from individuals with various medical conditions. The specificity of 269 samples with 29 categories of potentially interfering diseases or medical conditions was evaluated with the Elecsys HBsAg II assay and the reference assay. The results are summarized in the following table.

Category	Reference HBsAg assay		Total
	Non-reactive		
	Elecsys HBsAg II assay		
	RX[e)]	NR[f)]	
Immune disorders (n = 40)			
Serum lupus erythematosus	0	10	10
ANA anti-nuclear antibody	0	15	15

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Category	Reference HBsAg assay		Total
	Non-reactive		
	Elecsys HBsAg II assay		
	RX[e)]	NR[f)]	
Rheumatoid factor	0	15	15
Non-viral infections (n = 30)			
Syphilis	0	15	15
Toxoplasmosis	0	15	15
Viral infection (n = 149)			
Cytomegalovirus	0	15	15
Epstein-Barr Virus	0	15	15
Hepatitis A Virus	0	10	10
Hepatitis C Virus	0	11	11
Hepatitis E Virus	1	10	11
Human Immunodeficiency Virus	0	13	13
Herpes Simplex Virus	0	15	15
HTLV	0	14	14
Parvovirus B19 Infection	0	15	15
Rubella	0	15	15
Varicella Zoster Virus	0	15	15
Non-viral liver disease (n = 40)			
Various Cirrhosis	0	8	8
Chronic non-alcoholic liver disease	0	6	6
Steatohepatitis	0	6	6
Acute liver failure	0	7	7
Fatty infiltrate of liver	0	2	2
Autoimmune disorder	0	2	2
Chronic passive congestion of liver	0	2	2

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Category	Reference HBsAg assay		Total
	Non-reactive		
	Elecsys HBsAg II assay		
	RX[e)]	NR[f)]	
Jaundice	0	2	2
Liver abscess	0	1	1
Liver lesion	0	1	1
Malignant neoplasm of liver and intrahepatic bile ducts	0	1	1
Abdominal pain / pelvic mass	0	2	2
Vaccination			
Flu vaccination	0	10	10
Total	1	268	269

e) RX = reactive

f) NR = nonreactive

Conclusion: 268 samples were found to be non-reactive (negative) with both the Elecsys HBsAg II assay and the FDA-approved HBsAg reference assay while 1 sample was found to be non-reactive (0.18 COI) with the reference assay and reactive (234 COI, 229 COI, 227 COI with confirmation by neutralization) with the Elecsys HBsAg II assay. No potential interference is demonstrated by the medical conditions presented in this comparison study.

Potential interference from bacterial and viral proteins was evaluated by testing a HBsAg-reactive panel and a HBsAg-non-reactive panel with the Elecsys HBsAg II assay. Each panel contained individual samples spiked with specific bacterial or viral culture materials at two concentrations (1000 and 10000 cfu/mL for bacteria and 1 and 1000 ng/mL for viruses) and an unspiked control. The organisms tested included:

- Staphyloccus aureus
- Pseudomonas aeruginosa
- Escherichia coli
- Epstein Barr virus
- Cytomegalovirus
- Rubella
- Varicella-zoster virus

These materials were only tested in the Elecsys HBsAg II assay. The unspiked control results were compared to the results obtained with the spiked materials.

Elecsys HBsAg II results of bacterial spikes and viral antigen spikes:

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Sample ID	Description	Elecsys HBsAg II			Interpretation	
		Initial	Rpt[g)] 1	Rpt 2	Before spike	After spike
Unspiked / HBsAg non-reactive panel						
50121_A-00	control	0.41	N/A	N/A	NR	NR
Spiked / HBsAg non-reactive panels						
50121_A-01	S. aureus 1000 cfu/mL	0.46	N/A	N/A	NR	NR
50121_A-02	S. aureus 10000 cfu/mL	0.41	N/A	N/A	NR	NR
50121_A-03	P. aeruginosa 1000 cfu/mL	0.36	N/A	N/A	NR	NR
50121_A-04	P. aeruginosa 10000 cfu/mL	0.38	N/A	N/A	NR	NR
50121_A-05	E. coli 1000 cfu/mL	0.44	N/A	N/A	NR	NR
50121_A-06	E. coli 10000 cfu/mL	0.42	N/A	N/A	NR	NR
50121_A-07	Epstein Barr virus 1 ng/mL	0.41	N/A	N/A	NR	NR
50121_A-08	Epstein Barr virus 1000 ng/mL	0.93	0.44	0.38	NR	NR
50121_A-09	Cytomegalovirus 1 ng/mL	0.41	N/A	N/A	NR	NR
50121_A-10	Cytomegalovirus 1000 ng/mL	0.46	N/A	N/A	NR	NR
50121_A-11	Rubella 1 ng/mL	0.43	N/A	N/A	NR	NR
50121_A-12	Rubella 1000 ng/mL	0.44	N/A	N/A	NR	NR
50121_A-13	Varicella-zoster virus 1 ng/mL	0.45	N/A	N/A	NR	NR
50121_A-14	Varicella-zoster virus 1000 ng/mL	0.40	N/A	N/A	NR	NR
Spiked / HBsAg reactive panels						
50122_B-00	Reactive neat control	1.81	1.91	1.83	RX	RX
50122_B-01	S. aureus 1000 cfu/mL	1.75	1.91	1.83	RX	RX

Elecsys HBsAg II

Sample ID	Description	Elecsys HBsAg II			Interpretation	
		Initial	Rpt[g)] 1	Rpt 2	Before spike	After spike
50122_B-02	S. aureus 10000 cfu/mL	3.14	1.89	1.83	RX	RX
50122_B-03	P. aeruginosa 1000 cfu/mL	1.85	3.26	3.24	RX	RX
50122_B-04	P. aeruginosa 10000 cfu/mL	1.80	1.71	1.74	RX	RX
50122_B-05	E. coli 1000 cfu/mL	1.70	1.87	1.77	RX	RX
50122_B-06	E. coli 10000 cfu/mL	1.88	1.84	1.84	RX	RX
50122_B-07	Epstein Barr virus 1 ng/mL	1.71	1.72	1.76	RX	RX
50122_B-08	Epstein Barr virus 1000 ng/mL	1.65	1.80	1.69	RX	RX
50122_B-09	Cytomegalovirus 1 ng/mL	1.85	1.69	1.73	RX	RX
50122_B-10	Cytomegalovirus 1000 ng/mL	1.74	1.91	1.85	RX	RX
50122_B-11	Rubella 1 ng/mL	1.93	1.84	1.76	RX	RX
50122_B-12	Rubella 1000 ng/mL	1.77	1.8	1.71	RX	RX
50122_B-13	Varicella-zoster virus 1 ng/mL	1.77	1.86	1.81	RX	RX
50122_B-14	Varicella-zoster virus 1000 ng/mL	1.84	1.93	1.88	RX	RX

g) rpt = repeat

All samples, which were repeatedly reactive with the Elecsys HBsAg II assay, were confirmed by neutralization as reactive except for sample ID 50122_B-05, which had inadequate volume for confirmation.

Conclusion: The bacterial/viral spike performance demonstrated that the Elecsys HBsAg II assay conducted on the **cobas e** 601 analyzer was not affected by the two levels of bacterial and viral proteins introduced through culture material. The bacterial and viral spiked samples in the HBsAg non-reactive and reactive panels were all concordant with the respective unspiked control. The organisms tested included Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Epstein Barr virus, Cytomegalovirus, Rubella and Varicella-zoster virus.

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys HBsAg II assay has been shown by testing 14 commercial seroconversion panels in comparison to a commerically available FDA-approved HBsAg reference assay. In

all panels the Elecsys HBsAg II assay shows detection of seroconversion equal to the reference HBsAg assay except with one panel. The Elecsys HBsAg II assay detected seroconversion to a reactive status one draw later than the reference assay in panel PHM912.

Days to evidence of seroconversion for Elecsys HBsAg II compared to the reference assay					
	Elecsys HBsAg II assay		Reference HBsAg assay		Difference in days to Elecsys HBsAg II reactivity
Panel ID	NR[h)]	RX[i)]	NR	RX	Reference test
6272	74	94	74	94	0
6281	7	13	7	13	0
9092	37	42	37	42	0
11000	19	21	19	21	0
PHM 906	0	137	0	137	0
PHM 912	24	42	20	24	+ 18 (1 draw)
PHM 918	0	7	0	7	0
PHM 924	0	23	0	23	0
PHM 926	2	9	2	9	0
PHM 927	0	4	0	4	0
PHM 929	11	14	11	14	0
PHM 930	0	3	0	3	0
PHM 935B[j)]	-	128	-	128	-
PHM 936[k)]	-	0	-	0	-

h) NR = non-reactive

i) RX = reactive

j) Initial time point was positive (128 days). No panel member earlier than 128 days was included.

k) Positive at day 0.

Genotype panel

One commercially available HBsAg performance panel, containing 20 unique specimens with the most common hepatitis B surface antigen genotypes (A through H) was tested to validate the performance of the assay. The following tables show the Elecsys HBsAg II results against the FDA-approved reference assay and the results provided by the vendor of the performance panels.

Elecsys HBsAg II

Summary of genotype performance panel					
		Elecsys HBsAg II results			
Panel ID	**Genotype**	**Initial COI**	**Repeat 1**	**Repeat 2**	**Result**
WWHD301-01	A:A	39.5	35.3	36.8	cRX[l)]
WWHD301-02	A:A	4003	4637	4587	cRX
WWHD301-03	A:A	2634	2711	2723	cRX
WWHD301-04	A/E:na	3050	2971	2977	cRX
WWHD301-05	A/F:A	3636	3782	3781	cRX
WWHD301-06	B:B	2387	2497	2507	cRX
WWHD301-07	C:C	2771	2909	2979	cRX
WWHD301-08	C:C	2760	3180	2999	cRX
WWHD301-09	D/E:D	4750	4927	4827	cRX
WWHD301-10	D:D	443	423	430	cRX
WWHD301-11	D/F:D	1846	1814	1790	cRX
WWHD301-12	D/G:D	3600	3422	3509	cRX
WWHD301-13	E:E	1906	1803	1838	cRX
WWHD301-14	E:E	3996	3916	3922	cRX
WWHD301-15	E:E	3490	3486	3499	cRX
WWHD301-16	F:F	1686	1746	1726	cRX
WWHD301-17	F:F	1589	1661	1615	cRX
WWHD301-18	G:D	286	274	271	cRX
WWHD301-19	G:na	7.44	6.12	5.99	cRX
WWHD301-20	H:H	1626	1633	1651	cRX

l) cRX = confirmed reactive

Elecsys HBsAg II

Summary of genotype performance panel							
		FDA-approved reference assay				Commercially available samples	
Panel ID	**Genotype**	**Initial COI**	**Rpt[m)] 1**	**Rpt 2**	**Result**	**COI**	**Result**
WWHD301-01	A:A	18.3	18.7	19.1	rRX[n)]	19.1	RX
WWHD301-02	A:A	5002	5109	5195	rRX	5381	RX
WWHD301-03	A:A	4063	4060	4053	rRX	5370	RX
WWHD301-04	A/E:na	4274	4235	4143	rRX	5048	RX
WWHD301-05	A/F:A	6350	6343	6325	rRX	2919	RX
WWHD301-06	B:B	2836	2856	2843	rRX	6082	RX
WWHD301-07	C:C	6466	6539	6636	rRX	5128	RX
WWHD301-08	C:C	6437	6316	6450	rRX	4749	RX
WWHD301-09	D/E:D	6101	6136	6073	rRX	5321	RX
WWHD301-10	D:D	917	892	878	rRX	5472	RX
WWHD301-11	D/F:D	2296	2329	2216	rRX	1427	RX
WWHD301-12	D/G:D	5527	5628	5725	rRX	3741	RX
WWHD301-13	E:E	2457	2368	2338	rRX	5432	RX
WWHD301-14	E:E	4358	4292	4347	rRX	5147	RX
WWHD301-15	E:E	4418	4325	4230	rRX	5222	RX
WWHD301-16	F:F	2290	2155	2175	rRX	6044	RX
WWHD301-17	F:F	2870	2794	2786	rRX	5412	RX
WWHD301-18	G:D	415	433	458	rRX	215	RX
WWHD301-19	G:na	0.320	0.51	0.90	NR	22.1	RX
WWHD301-20	H:H	2129	1973	1967	rRX	6168	RX

m) Rpt = repeat

n) rRX = repeatedly reactive

HBsAg mutant detection

A total of 20 recombinant mutants were tested with the Elecsys HBsAg II assay to determine correct antigenic recognition of the HBsAg structure. The mutants contained important epitope clusters within amino acids

100-160, including the „a determinant“ region (amino acid 124-147) as the most important target for serological diagnosis. The recombinant mutants were diluted in individual HBsAg negative human serum to

yield a low positive sample close to the cutoff. The measurements were done in singlicate determinations. All 20 recombinant mutants were recognized with Elecsys HBsAg II.

Sample	Mutation	Sample (COI)
Mutant 1	F8L, R24K, N40R, G43R, L94S, M103I, 133A114, M133T, P142L, D144G	5.81
Mutant 2	T/A45S, C107R, M195I	4.66
Mutant 3	S132Y, P142S, G145R	7.12
Mutant 4	T123N	9.38
Mutant 5	G145K	2.28
Mutant 6	D144G	8.37
Mutant 7	D144A	6.58
Mutant 8	G145R	5.15
Mutant 9	122RA123	4.02
Mutant 10	Q129P, F134R, P142L, D144E, G145K, S171F, L175S	1.28
Mutant 11	R122I	1.59
Mutant 12	M125T, T127P, P142A, G145R	2.77
Mutant 13	T131I	5.51
Mutant 14	C147S	2.48
Mutant 15	K141E	5.10
Mutant 16	S143L	1.43
Mutant 17	P142L	1.37
Mutant 18	Y134S	3.65
Mutant 19	E164D	6.60
Mutant 20	I126S	2.55

Summary of clinical performance

Study description

A prospective multicenter study was conducted on the **cobas e** 601 analyzer to evaluate the ability of the Elecsys HBsAg II assay to detect HBsAg in specimens from an intended use diagnostic population. A total of 2059 adult, 202 pregnant and 128 pediatric (ages 2-21) specimens were obtained from individuals at increased risk of HBV infection due to lifestyle, behavior, occupation, disease state or known exposure event, or from individuals with signs and symptoms of a hepatitis infection (asymptomatic, symptomatic, pregnant and pediatric subjects at increased risk for hepatitis). Previously collected and commercially

available specimens were used in this evaluation to increase the low number of reactive HBsAg (586 pregnant from the US, 16 pregnant from outside the US, and 397 supplemental adult non-pregnant). The number of prospective and retrospective specimens is summarized in the following table:

Clinical study cohort	Adult np[o] (IR[p]) + Supplemental) (n = 2456)		Pregnant (n = 804)			Pediatric IR np[q]	Total
	IR	Supplemental	IR	IR Ex US[r]	Low or Unk Risk[s]		
	Pro[t]	Retro[u]	Pro	Retro	Retro	Pro	
Number of specimens	2059	397	202	16	586	128	3388

o) np = non-pregnant

p) IR = increased risk

q) np = non-pregnant

r) Low or unknown risk supplemental

s) Low or unknown risk supplemental

t) pro = Prospective

u) Retro = retrospective

Demographics of clinical populations by sex

A total of 3388 samples were tested at 3 clinical testing sites with the Elecsys HBsAg II assay to evaluate clinical performance of Elecsys HBsAg II assay. The following table shows the demographics for each study cohort (non-pregnant adult, pregnant, pediatric at increased risk, and supplemental and pregnant at low or unknown risk) by gender.

Increased at risk populations								
Sex	**Non-pregnant adult IR[v]**		**Pregnant IR**		**Pediatric IR**		**Total**	
	n	%	n	%	n	%	n	%
Female	940	45.7	202	100	68	53.1	1226	51.0
Male	1119	54.4	N/A	N/A	60	46.9	1179	49.0
Total	2059	100	202[w]	100	128	100	2405	100

v) IR = increased risk

w) An additional 16 specimens from pregnant women were sourced from outside the US.

Supplemental and low or unknown risk populations						
Sex	**Supplemental**		**Pregnant adult low/unknown risk**		**Total**	
	n	%	n	%	n	%
Female	65	16.4	586	100	651	66.2
Male	331	83.4	N/A	N/A	331	33.7

Supplemental and low or unknown risk populations						
Sex	**Supplemental**		**Pregnant adult low/unknown risk**		**Total**	
	n	%	n	%	n	%
Unknown	1	0.25	N/A	N/A	1	0.1
Total	397	100	586	100	983	100

Demographics of clinical populations by ethnicity

Increased risk population								
Ethnicity	**Non-pregnant adult IR**		**Pregnant IR**		**Pediatric IR**		**Total**	
	n	%	n	%	n	%	n	%
Hispanic/Latino	535	26.0	171	84.65	70	54.7	776	32.3
Not Hispanic / Latino	1517	73.7	31	15.35	57	44.5	1605	66.7
Unknown	7	0.34	0	0	1	0.78	24	1.0
Total	2059	100	202[x)]	100	128	100	2405	100

x) An additonal 16 specimens from pregnant women were sourced from outside the US.

Supplemental and low or unknown risk population						
Ethnicity	**Supplemental**		**Pregnant adult at low risk**		**Total**	
	n	%	n	%	n	%
Hispanic / Latino	7	1.76	0	0.00	7	0.7
Not Hispanic / Latino	29	7.30	0	0.00	29	3.0
Unknown	361	90.9	586	100	947	96.3
Total	397	100	586	100	983	100

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Demographics of clinical populations by race

Race	Adult non-pregnant (n = 2456)			
	Adult IR (non-pregnant)		Supplemental	
	n	%	n	%
AIAN	22	0.34	0	0
Asian	15	0.73	84	21.13
African Am/ Black	1020	49.54	182	45.84
Caucasian/ White	946	45.94	55	13.85
NHOPI	4	0.19	0	0
Unknown	8	0.39	70	17.63
Other	44	2.14	6	1.51
Total	2059	100	397	100

AIAN: American Indian/Alaska Native NHOPI: Native/Pacific Islander

	Pregnant (n = 804)				Pediatric IR (n = 128)	
Race	Pregnant IR		Pregnant Risk Low or unknown			
	n	%	n	%	n	%
AIAN	1	0.49	2	0.34	0	0
Asian	3	1.49	10	1.71	4	3.13
African Am/ Black	15	7.43	316	53.92	32	25
Caucasian/ White	176	87.13	149	25.43	86	67.19
NHOPI	1	0.49	0	0	1	0.78
Unknown	2	0.99	4	0.68	1	0.78
Other	4	1.98	105	17.92	4	3.12
Total	202[y)]	100	586	100	128	100

y) An additional 16 specimens from pregnant women were sourced from outside the US and were of black race.

*Includes 16 non US subjects

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Demographics of adult, pregnant and pediatric populations by age

Age	Non-pregnant adult IR		Pregnant IR		Pediatric IR	
	n	%	n	%	n	%
2 to 11	0	0.00	0	0.00	24	18.8
12 to 21	0	0.00	59	29.2	104	81.3
22 to 29	256	12.4	90	44.6	0	0.00
30 to 39	369	17.9	43	21.29	0	0.00
40 to 49	589	28.6	10	4.95	0	0.00
50 to 59	639	31.0	0	0.00	0	0.00
60 to 69	185	8.98	0	0.00	0	0.00
70 to 79	18	0.87	0	0.00	0	0.00
≥ 80	3	0.15	0	0.00	0	0.00
Total	2059	100	202	100	128	100
Age range (years)	22 to 84		17 to 44		2 to 21	
Median age (years)	47		25		19	

Age	Supplemental		Pregnant at low risk		Total	
	n	%	n	%	n	%
2 to 11	0	0.00	0	0.00	24	0.7
12 to 21	3	0.76	174	29.7	346	10.2
22 to 29	123	31.0	287	49.0	761	22.5
30 to 39	119	30.0	118	20.1	654	19.3
40 to 49	85	21.4	7	1.19	691	20.4
50 to 59	49	12.3	0	0.00	688	20.3
60 to 69	14	3.53	0	0.00	199	5.9
70 to 79	4	1.01	0	0.00	22	0.7
≥ 80	0	0.00	0	0.00	3	0.1
Unknown	0	0.00	0	0.00	0	0

Age	Supplemental		Pregnant at low risk		Total	
	n	%	n	%	n	%
Total	397	100	586	100	3388	100
Age range (years)	21 to 78		15 to 41		2 to 89	
Median age (years)	45		24.5		38	

Clinical performance on the cobas e 601 immunoassay analyzer

A total of 3388 samples were tested on the **cobas e** 601 immunoassay analyzer from subjects from the following cohorts:

- adult subjects at increased risk for hepatitis (symptomatic and asymptomatic)
- supplemental specimens for low prevalence disease states (adults)
- pediatric subjects at increased risk for hepatitis
- pregnant subjects at increased, low and unknown risk for hepatitis

Serological characterization using a complete hepatitis B panel of FDA-approved assays was performed on the main clinical cohort samples from the prospective increased risk collection (asymptomatic and symptomatic adults) and the adult supplemental sample cohort. A panel of hepatitis B serological markers is used to assist in the determination of the disease state at the time of the blood draw. For serological characterization the following HBV markers were evaluated: HBsAg (and HBsAg confirmatory test), Anti-HBc IgM, Anti-HBc, Anti-HBs, Anti-HBe and HBeAg.

Results by specimen classification

The following table compares the Elecsys HBsAg II results by serological classification.

Serological classification by FDA-approved HBV panel						
	HBsAg	**HBeAg**	**A-HBc IgM**	**A-HBc**	**A-HBe**	**A-HBs**
Acute	(+)	(+)	(+)	(+)	(-),(+)	(-)
Acute	(+)	(+)	(-),(+)	(-)	(-)	(-)
Acute	(+)	(-)	(-)	(-)	(-)	(-)
Acute	(+)	(+)	eq	(+)	(-),(+)	(-)
Acute	(+)	(-)	(+)	(+)	(-)	(-)
Acute	(+)	(-)	eq	(+)	(+)	(-)
Acute (late)	(+)	(-)	(+)	(+)	(+)	(-),(+)
Chronic	(+)	(+)	(+)	(+)	(+)	(+)
Chronic	(+)	(-)	(-)	(+)	(+)	(-),(+)
Chronic	(+)	(-)	(-)	(+)	eq	(-)

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Serological classification by FDA-approved HBV panel						
	HBsAg	**HBeAg**	**A-HBc IgM**	**A-HBc**	**A-HBe**	**A-HBs**
Chronic	(+)	(-)	(-)	(+)	(-)	(-),(+)
Chronic	(+)	(+)	(+)	(+)	(-)	(+)
Chronic	(+)	(+)	(-)	(+)	(-)	(-),(+)
Chronic	(+)	(+)	(-)	(+)	(+)	(-)
Early recovery	(-)	(-)	(-)	(+)	(-),(+)	(-)
Early recovery	(-)	(-)	(+)	(+)	(-)	(-),(+)
Early recovery	(-)	(-)	(+)	(+)	(+)	(-),(+)
Recovery	(-)	(-)	(-)	(-), (+)	(+)	(+)
Recovery	(-)	(-)	(-)	(+)	(+)	eq
Recovered of immune due to natural infection	(-)	(-)	(-)	(+)	(-)	(+),eq
HBV vaccine response	(-)	(-)	(-)	(-)	(-)	(+)
HBV vaccination response unknown	(-)	(-)	(-)	(-)	(-)	eq
Not previously infected	(-)	(-)	(-)	(-)	(-)	(-)
Not interpretable	(-)	(+)	(-)	(+)	(-)	(+)
Not interpretable	(-)	(-)	(-)	(-)	(+)	(-)
Not interpretable	(-)	(+)	(-)	(+)	(+)	(-)
Not interpretable	(-)	(+)	(-)	(-)	(-)	(-),eq, (+)

Pediatric, pregnant, potential interference and seroconversion and performance panel specimens were not serologically characterized. Participating testing sites included three sites with **cobas e** 601 analyzers and four FDA-approved reference methods in strict accordance wtih the manufacturer's method sheet instructions. All sites were located in the United States.
The following table shows the distribution of hepatitis B disease states across serologically characterized cohorts.

HBV classification	**Adult IR**	**Supplemental**
Acute	7	74
Chronic	32	317

Elecsys HBsAg II

HBV classification	Adult IR	Supplemental
Early recovery	198	2
Not interpretable	9	0
Not previously infected	942	1
Recovered	245	2
Recovery	131	1
Vaccination	495	0
Total	2059	397

The Elecsys HBsAg II assay was tested on the **cobas e** 601 immunoassay analyzer and an FDA-approved reference assay to establish the clinical performance characteristics of the Elecsys HBsAg II assay. The study was evaluated at three clinical laboratories. Supplemental samples used to increase the low number of reactive HBsAg samples were tested with the other increased risk subjects for test and reference results; serological characterization was based on HBV marker panel testing. The following table compares the Elecsys HBsAg II results with the results obtained on an FDA-approved HBsAg reference assay by HBV disease classification for the adult at increased risk cohort.

HBV classification	Reference HBsAg results				Total
	+		-		
	Elecsys HBsAg II results (**cobas e** 601 analyzer)				
	+	-	+	-	
Acute	7	0	0	0	7
Chronic	32	0	0	0	32
Early recovery	0	0	1	197	198
Recovery	0	0	0	131	131
Recovered	0	0	1	244	248
HBV vaccination	0	0	1	494	495
Not previously infected	0	0	2	940	942
Not interpretable	0	0	0	9	9
Total	39	0	5	2015	2059

This table presents the concordance for the adult increased risk specimens classified by each serological diagnostic status.

The following table reflects the percent agreement between the Elecsys HBsAg II assay and the reference assay for each disease classification for the adult at increased risk cohort.

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HBV classification	PPA[z)] % (n/N)	95 % confidence interval	NPA[)] % (n/N)	95 % confidence interval
Acute	100 (7/7)	64.6 - 100	N/A	N/A
Chronic	100 (32/32)	89.3 - 100	N/A	N/A
Early recovery	N/A	N/A	99.5 (197/198)	97.2 - 99.99
Recovery	N/A	N/A	100 (131/131)	97.2 - 100
Recovered	N/A	N/A	99.6 (244/245)	97.8 - 99.99
HBV vaccination	N/A	N/A	99.8 (494/495)	98.9 - 99.99
Not previously infected	N/A	N/A	99.8 (940/942)	99.2 - 99.97
Not interpretable	N/A	N/A	100 (9/9)	70.1 - 100
Total	100 (39/39)	91.0 - 100	99.8 (2015/2020)	99.4 - 99.9

z) PPA = positive percent agreement

) NPA = negative percent agreement

This table presents the percent agreement between the results for the Elecsys HBsAg II and the FDA-approved reference assay for each disease classification. The positive HBsAg cohort represents the low prevalence of acute and chronic subjects captured in an all-comers prospective at increased risk for hepatitis collection.

Clinical performance in non-pregnant adult subjects (at increased risk and supplemental)

The following table presents the Elecsys HBsAg II results by age range and sex among the 2059 at increased risk, non-pregnant adult subjects. Interpretation was based on the complete immunoassay and confirmatory testing.

Age	Sex	Elecsys HBsAg II						Total
		Pos		Indeterminate		Neg		
22 to 29	Female	1	(0.68)	0	(0.00)	147	(99.3)	148
	Male	2	(1.85)	0	(0.00)	106	(98.2)	108
30 to 39	Female	2	(1.05)	0	(0.00)	189	(99.0)	191
	Male	5	(2.81)	0	(0.00)	173	(97.2)	178
40 to 49	Female	4	(1.59)	1	(0.40)	246	(98.0)	251
	Male	14	(4.14)	0	(0.00)	324	(95.9)	338
50 to 59	Female	5	(1.93)	0	(0.00)	254	(98.1)	259
	Male	8	(2.11)	0	(0.00)	372	(97.9)	380

Elecsys HBsAg II

Age	Sex	Elecsys HBsAg II						Total
		Pos		Indeterminate		Neg		
60 to 69	Female	2	(2.56)	0	(0.00)	76	(97.4)	78
	Male	0	(0.00)	0	(0.00)	107	(100)	107
70 to 79	Female	0	(0.00)	0	(0.00)	10	(100)	10
	Male	0	(0.00)	0	(0.00)	8	(100)	8
>= 80	Female	0	(0.00)	0	(0.00)	3	(100)	3
	Male	0	(0.00)	0	(0.00)	0	(0.00)	0
Totals	Female	14	(1.49)	1	(0.11)	925	(98.4)	940
	Male	29	(2.59)	0	(0.00)	1090	(97.4)	1119
All		43	(2.09)	1	(0.05)	2015	(97.9)	2059

The following tables present the concordance and percent agreements for adult subjects at increased risk for hepatitis B across all 3 testing sites.

	Reference HBsAg assay							
	Site 1		Site 2		Site 3		All sites	
Elecsys HBsAg II	RX	NR	RX	NR	RX	NR	RX	NR
Reactive	14	0	14	4	11	0	39	4
Indeterminate	0	0	0	1	0	0	0	1
Negative	0	675	0	663	0	677	0	2015
Total	14	675	14	668	11	677	39	2020

Percent agreement for adult subjects at increased risk populations (Adult AR)				
	Site 1	Site 2	Site 3	All sites
PPA[aa)]	100 (14/14)	100 (14/14)	100 (11/11)	100 (39/39)
95 % CI	76.84 to 100	76.84 to 100	71.51 to 100	90.97 to 100
NPA[ab)]	100 (675/675)	99.26 (663/668)	100 (677/677)	99.75 (2015/2020)
95 % CI	99.45 to 100	98.26 to 99.76	99.46 to 100	99.42 to 99.92

aa) PPA = positive percent agreement

ab) NPA = negative percent agreement

The positive percent agreement for the combined sites is 100 % based on the ratio of 39/39 with confidence limits of 90.97 to 100 %. The negative percent agreement is 99.75 % based on a ratio of 2015/2020 with confidence limits of 99.42 to 99.92 %.

The following tables present the concordance and percent agreements for supplemental subjects which were selected to increase the low number of reactive HBsAg samples and disease classification subgroups.

	Reference HBsAg							
	Site 1		Site 2		Site 3		All sites	
Elecsys HBsAg II	RX	NR	RX	NR	RX	NR	RX	NR
Reactive	131	0	127	1	130	0	388	1
Negative	0	4	3	1	0	0	3	5
Total	131	4	130	2	130	0	391	6

Percent agreement for adult supplemental subjects populations				
	Site 1	Site 2	Site 3	All sites
PPA	100 (131/131)	97.69 (127/130)	100 (130/130)	99.23 (388/391)
95 % CI	97.22 to 100	93.40 to 99.52	97.20 to 100	97.77 to 99.74
NPA	100 (4/4)	50.0 (1/2)	na (0/0)	83.33 (5/6)
95 % CI	39.76 to 100	1.26 to 98.74	na	43.65 to 97.00

The positive percent agreement was 99.23 % based on the ratio of 388/391 with confidence limits of 97.77 to 99.74. The negative percent agreement is based on a small number of specimens obtained for the purpose of enhancing low prevalence markers and disease states, but which were misrepresented or mis-selected for that purpose. The negative percent agreement was 83.33 % based on the ratio of 5/6 with confidence limits of 43.65 to 97.00 due to the low number of only 6 subjects.

Clinical performance in pregnant subjects

The performance of the Elecsys HBsAg II assay on the **cobas e** 601 immunoassay analyzer and an FDA-approved HBsAg reference assay was performed on 202 US subjects and 16 Cameroon subjects at increased risk for hepatitis and 586 US subjects at low/unknown risk of hepatitis.

	Reference HBsAg assay final interpretation					
	Low/unknown risk US		At increased risk (AIR) US		At increased risk (AIR) non-US	
Elecsys HBsAg II	RX	NR	RX	NR	RX	NR
Reactive	5	1	0	0	13	0
Non-reactive	1	579	0	202	0	3
Total	6	580	0	202	13	3

Percent agreement in pregnant subject population			
	Low/unknown risk US	**At increased risk (AIR) US**	**At increased risk (AIR) non-US**
PPA	83.3 (5/6)	N/A (0/0)	100 (13/13)
95 % CI	43.65 to 96.99	N/A	77.19 to 100
NPA	99.83 (579/580)	100 (202/202)	100 (3/3)
95 % CI	99.03 to 99.97	98.13 to 100	43.85 to 100

The percent agreements for the complete pregnancy population (n = 804) were as follows:

- Positive percent agreement of 94.7 % (18/19) with 95th percentile confidence limits of 75.36 to 99.07 %.
- Negative percent agreement was 99.9 % (784/785) with 95th percentile confidence limits of 99.3 to 99.98 %.

Only two discrepant samples were observed in the pregnancy population. As expected, the reactive side of the population is low and the resultant significance is lower than that of the non-reactive side. Comparison of percent agreements among the cohorts is very similar.

Agreement for pregnant subjects by trimester

Increased risk pregnant females

Specimens from pregnant subjects can also be analyzed by trimester. Trimester information was not available for the 16 non-US subjects. Comparison of Elecsys HBsAg II assay results to the reference results for the increased risk for hepatitis specimens is shown in the following table.

	First trimester		**Second trimester**		**Third trimester**		Total
	Reference HBsAg assay						
Elecsy HBsAg II	RX	NR	RX	NR	RX	NR	
Reactive	0	0	0	0	0	0	0
Non-reactive	0	62	0	66	0	74	202
Total	0	62	0	66	0	74	202

Low risk pregnant females

Comparison of Elecsys HBsAg II assay results to the reference results for the low risk for hepatitis specimens is shown in the following table.

	First trimester		**Second trimester**		**Third trimester**		Total
	Reference HBsAg assay						
Elecsy HBsAg II	RX	NR	RX	NR	RX	NR	
Reactive	2	0	2	1	1	0	6
Non-reactive	0	196	0	192	1	191	580

	First trimester		Second trimester		Third trimester		Total
	Reference HBsAg assay						
Elecsy HBsAg II	RX	NR	RX	NR	RX	NR	
Total	2	196	2	193	2	191	586

Performance in terms of percent agreement appeared to be consistent across trimester and risk status in the pregnancy population. The overall population positive percent agreement was 94.7 % (18/19) with 95th percentile confidence limits of 74.0 to 99.9 % as a result of a single discrepant sample within a group of 19 subjects. The negative percent agreement was based on a very substantial cohort: 99.9 % (784/785) with 95th percentile confidence limits of 99.3 to 100 %.

Due to the low prevalence of HBV in the pregnant population and to ensure HBsAg detection did not differ between pregnant and non-pregnant samples, 32 samples of pregnant women and 32 samples of non-pregnant women were spiked with analyte from a high positive HBsAg sample. The level of spiking of the samples included 8 high negative (near the cutoff), 4 borderline and 20 low positive (near the cutoff). All specimens were tested with the Elecsys HBsAg II assay before and after spiking in two-fold determinations. All samples presented recoveries between 81 % and 126 % with the following distribution of the differences:

Distribution of % differences		
X < 10 %	10 % ≤ X ≤ 20 %	X > 20 %
81.2 % (26/32)	12.5 % (4/32)	6.3 % (2/32)

The results verify the detection of HBsAg does not differ between pregnant women and non-pregnant women.

Clinical performance in pediatric at increased risk subjects

The performance of the Elecsys HBsAg II assay on the **cobas e** 601 immunoassay analyzer and an FDA-approved HBsAg reference assay was performed on 128 pediatric subjects at increased risk for hepatitis from US subjects.

	Reference HBsAg assay final interpretation	
Elecsys HBsAg II	Reactive	Non-reactive
Reactive	0	0
Non-reactive	0	128
Total	0	128

Percent agreement in pediatric (IR) subject population	
PPA	N/A (0/0)
95 % CI	N/A
NPA	100 (128/128)
95 % CI	97.16 to 100

Elecsys HBsAg II

Due to the low prevalence of HBV in the pediatric population, a comparative study comparing 33 spiked pediatric samples against one adult sample was performed with Elecsys HBsAg II. 33 pediatric samples that were HBV antibody negative and one adult HBV-negative sample were spiked with analyte from a high positive pediatric or adult sample to the level of 4 times the cutoff to assess performance.
All 33 samples presented recoveries between 95 % and 109 %.
The results of all 33 pediatric samples recovered within 10 % to support the assay is suitable for the pediatric population.

References

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9 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
10 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.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 after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO

Elecsys HBsAg II

EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

HBsAg Confirmatory Test

REF	REAGENT	SYSTEM
11820648 122	Elecsys HBsAg immunoassay	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602
	Elecsys HBsAg II	**cobas e** 601

For USA: Elecsys HBsAg Confirmatory Test

For use in the USA only

Warning

- This assay has not been FDA cleared or approved for the screening of blood or plasma donors.
- Assay performance characteristics have not been established when the Elecsys HBsAg or Elecsys HBsAg II assays are used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Assay performance characteristics have not been established for testing of newborns.

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

Intended use

For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg immunoassay

Immunoassay for in vitro qualitative confirmation of the presence of hepatitis B surface antigen in human serum and plasma (sodium heparin, K_3-EDTA, sodium citrate) samples repeatedly reactive when tested with the Elecsys HBsAg immunoassay. This assay is intended for use on the Elecsys and **cobas e** immunoassay analyzers.

For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg II

Immunoassay for in vitro qualitative confirmation of the presence of hepatitis B surface antigen in human serum and plasma (sodium heparin, lithium heparin, K_2-EDTA, sodium citrate) samples repeatedly reactive when tested with Elecsys HBsAg II. This assay is intended for use on the **cobas e** 601 immunoassay analyzer.

HBsAg Confirmatory Test

cobas®

Summary

The Elecsys HBsAg Confirmatory Test is based on the principle of specific antibody neutralization.[1] Polyclonal HBsAg-specific antibodies bind to the immunodominant epitopes of the hepatitis B surface antigen and thereby block the binding sites for the antibodies used in the Elecsys HBsAg or Elecsys HBsAg II assays.

Test principle

The test principle is based on pretreatment of the samples with confirmatory reagent and control reagent followed by the assay procedure using the Elecsys HBsAg or Elecsys HBsAg II assays. The positive control, PC HBSAG2 or PC HBSAGII2, should be run in parallel as a performance check.

Pretreatment of the samples:

- Samples found to be repeatedly reactive in the Elecsys HBsAg or Elecsys HBsAg II assays are treated in parallel with confirmatory reagent and control reagent and then incubated. The excess anti-HBs antibodies in the confirmatory reagent neutralize any HBsAg in the sample. In the subsequent Elecsys HBsAg or Elecsys HBsAg II assays this leads to a reduction in the cutoff index (COI) value (signal of sample/cutoff) in comparison to the value originally obtained for the sample.

Elecsys HBsAg assay or Elecsys HBsAg II:

- 1st incubation: The two pretreated sample preparations react with a biotinylated, monoclonal HBsAg-specific antibody and a monoclonal HBsAg-specific antibody labeled with a ruthenium complex[a)] to 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. This is followed by manual verification of the validity of the assay and interpretation of the findings.

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

Reagents - working solutions

HBsAg Confirmatory Test 1 Confirmatory reagent (black cap), 2 bottles of 1.0 mL each: Anti-HBs (human) > 200000 IU/L in human serum; non-reactive for HBsAg, anti-HCV, and anti-HIV 1 + 2; preservative.

HBsAg Confirmatory Test 2 Control reagent (white cap), 2 bottles of 1.0 mL each: Human serum, anti-HBs < 3 IU/L; negative for HBsAg, anti-HCV and anti-HIV 1 + 2; preservative.

Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

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

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 applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

HBsAg Confirmatory Test

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.[2,3]
Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

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:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks

Specimen collection and preparation

Samples that were repeatedly reactive in the Elecsys HBsAg or Elecsys HBsAg II assay.
Elecsys HBsAg Immunoassay:
Stable for 5 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen and thawed 6 times.
Elecsys HBsAg II:
Stable for 14 days at 2-8 °C, 6 days at 20-25 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen and thawed 6 times.
The conditions regarding stability and specimen collection described for the Elecsys HBsAg or Elecsys HBsAg II assays also apply here.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 11820532160, HBsAg reagent kit for 100 tests (the materials required for performing the Elecsys HBsAg assay are listed in the Elecsys HBsAg Method Sheet)
- REF 04687787160, HBsAg II reagent kit for 100 tests (the materials required for performing the Elecsys HBsAg II assay are listed in the Elecsys HBsAg II Method Sheet)
- REF 07914482160, HBsAg II reagent kit for 200 tests (the materials required for performing the Elecsys HBsAg II assay are listed in the Elecsys HBsAg II Method Sheet)
- REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Assay

Pretreatment of the samples:
Selection of the reactant volumes is dependent on the magnitude of the respective cutoff index of the samples which were reactive in the Elecsys HBsAg or Elecsys HBsAg II assay. The following volumes are pipetted into Elecsys sample cups:

HBsAg Confirmatory Test

- For positive samples having a cutoff index < 7.00
  270 µL sample + 30 µL confirmatory reagent
  270 µL sample + 30 µL control reagent

or

- For positive samples having a cutoff index between 7.00 and < 30.0
  150 µL sample + 150 µL confirmatory reagent
  150 µL sample + 150 µL control reagent

or

- For positive samples having a cutoff index ≥ 30.0
  Predilute samples 1:20 with Diluent Universal
  150 µL diluted sample + 150 µL confirmatory reagent
  150 µL diluted sample + 150 µL control reagent

PC HBSAG2 or PC HBSAGII2, the positive control, should always be run in parallel as a check on performance:
270 µL PC HBSAG2 or PC HBSAGII2, + 30 µL confirmatory reagent
270 µL PC HBSAG2 or PC HBSAGII2, + 30 µL control reagent
Incubation of the reactants: 30-60 minutes at 15-25 °C or overnight at 2-8 °C.
The pretreated samples are placed in the sample zone and registered by entering the sample identification data.
The Elecsys HBsAg or Elecsys HBsAg II assay is performed in accordance with the instructions given in the Method Sheet of the test reagent kit.

Calibration

For calibration, calibration frequency, and calibration verification, see data given in the Method Sheet for the Elecsys HBsAg or Elecsys HBsAg II assay.

Quality control

PC HBSAG2 or PC HBSAGII2 should always be run in parallel with the samples needing confirmation. Verification is done by the user.
For the Elecsys HBsAg or the Elecsys HBsAg II assay the conditions given in the Method Sheet apply.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer calculates the cutoff automatically on the basis of measurements on the two Elecsys HBsAg or Elecsys HBsAg II calibrators contained in the kit.
The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI).
The cutoff index is needed for selection of the correct sample pretreatment volumes for the confirmatory test.

Elecsys HBsAg
(COI) = (signal of sample - background*/cutoff).
*background = 0.7 x $signal_{cal1}$

Elecsys HBsAg II
(COI) = (signal of sample - 0.796*$signal_{cal1}$) / (0.175*$signal_{cal1}$ + 0.0.396*$signal_{cal2}$)

HBsAg Confirmatory Test

Limitations - interference

Due to the high-dose hook effect, samples having very high HBsAg concentrations (> 1 mg/mL or > 550000 IU/mL) can give a cutoff index < 30.0 in the Elecsys HBsAg or Elecsys HBsAg II assay. Such samples are not adequately neutralized by the confirmatory reagent at the stated volume, and are therefore not confirmed as positive. These samples can be recognized by the fact that the COI in the test with the control reagent is higher than the COI for the samples in the originally performed HBsAg assay (dilution effect). The confirmatory test for these samples must be repeated at a higher predilution (1:100).

For the Elecsys HBsAg or Elecsys HBsAg II assay the data given in the Method Sheet of the test reagents on “Limitations - interference” apply.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Evaluation and interpretation of the results

For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg immunoassay

In order to confirm a reactive result for a sample, the cutoff index for the sample with the confirmatory reagent must be < 50 % of that with the control reagent, which must have a cutoff index of ≥ 1.00. This indicates ≥ 50 % neutralization of the HBsAg in the sample.

Assay evaluation

Neutralization of PreciControl HBsAg 2 (PC2) should be ≥ 50 % using the following formula:

% Neutralization of PreciControl HBsAg 2 (PC2) = [(COI of PC2 + control reagent) - (COI of PC2 + confirmatory reagent)] / (COI of PC2 + control reagent) * 100

The COI of the patient sample diluted with the control reagent must be ≥ 1.00.

% Neutralization of sample = [(COI of sample + control reagent) - (COI of sample + confirmatory reagent)] / (COI of sample + control reagent) * 100

Interpretation

% Neutralization ≥ 50 % = Confirmed positive sample or confirmed positive control

% Neutralization < 50 % = Negative (false positive) result

Once the validity of the run is established, neutralization of the patient sample is calculated (using the formula above). Samples with neutralization ≥ 50 % using the Elecsys HBsAg Confirmatory Test are regarded as confirmed positive for HBsAg.

Non-valid results must be repeated using fresh reagent. Indeterminate results should be repeated. In case the result remains indeterminate, a follow-up sample should be examined.

For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg II

Prior to evaluation, the validity of the test must be verified. Evaluation can be made when, in addition to the conditions applying to the Elecsys HBsAg II, the following criteria are fulfilled:

- The cutoff index of PC HBSAGII2 in the test with confirmatory reagent must be ≤ 60 % of that for the test with control reagent:
  - COI for test with control reagent 100 %
  - COI for test with confirmatory reagent x %

HBsAg Confirmatory Test

- If x > 60 %, it is necessary to check the test conditions. Where appropriate, repeat the test with fresh reagent.

Evaluation and interpretation of the results

In order to confirm a positive result for a sample, the cutoff index for the sample with the confirmatory reagent must be ≤ 60 % of that with the control reagent, which must have a cutoff index of ≥ 0.81.

Evaluation:

COI for test with control reagent 100 %

COI for test with confirmatory reagent x %

Interpretation:

x > 60 % and COI for control reagent ≥ 0.81 = non-reactive

x > 60 % and COI for control reagent < 0.81 = non-valid

x ≤ 60 % and COI for control reagent ≥ 0.81 = positive

x ≤ 60 % and COI for control reagent < 0.81 = indeterminate

Non-valid results must be repeated using fresh reagent.

In case the result remains non-valid, a follow-up sample should be examined. Indeterminate results should be repeated. In case the result remains indeterminate, a follow-up sample should be examined. In case a high dose hook sample is assumed please refer to the section "Limitations - interference" regarding higher predilution of such samples.

Specific performance data

The Elecsys HBsAg Confirmatory Test was evaluated with all specimens throughout the Elecsys HBsAg II clinical study. The table below contains the number of specimens repeatedly reactive with HBsAg II immunoassay and the number of specimens confirmed with the Elecsys HBsAg Confirmatory Test on the **cobas e** 601 analyzer.

Patient Population	N	HBsAg II Test Repeatedly reactive (RR)	HBsAg Confirmatory Test Reactive
Adults IR	2059	48	43[b)]
Adults at risk Supplemental	397	389	389
Pregnant with low or unknown risk (US)	586	6	6
Pregnant IR (US)	202	0	0
Pregnant IR (ex US)	16	13	13
Pediatric IR	128	0	0
Total	3388	456	451 out of 456 RR (98.90 %)

b) 43 reactive, 2 undetermined and 3 nonreactive results were obtained by Elecsys HBsAg confirmatory test in the Adults IR populaton.

Method comparison

The Elecsys HBsAg Confirmatory Test was evaluated with all specimens throughout the Elecsys HBsAg clinical study that had been confirmed positive by the FDA-approved reference HBsAg confirmatory assay. The table below compares the Elecsys HBsAg Confirmatory Test results with the reference HBsAg confirmed positive result on the Elecsys 2010 immunoassay analyzer.

HBsAg Confirmatory Test

Sample sources	N	Reference HBsAg reactive and confirmed positive by neutralization	Elecsys HBsAg Confirmatory Test confirmed positive by neutralization
First time blood donors	0	NA	NA
Subject at risk for HBV infection due to lifestyle or behavior	6	6	6
Serologically classified acute HBV infection (archived)	151	151	151
Serologically classified chronic HBV infection (archived)	111	111	111
Chronic HBV infection determined by persistent HBsAg for > 6 months (archived)	74	74	74
Pregnant women	3	3	3
Total	345	345	345 (100 %)

Detection limit

In order to determine the sensitivity, the HBsAg concentration which corresponds to the measuring signal of the cutoff value was read off the standard curves of serial dilutions of HBsAg standards in human HBV-negative serum.
Dilutions of ≤ 0.1 U/mL and ≤ 0.1 IU/mL were definitely confirmed for the Paul-Ehrlich-Institute standard (subtype ad, 1987) and the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A) using the Elecsys HBsAg Confirmatory Test.

Precision

Precision of the manual test steps was determined using 3 sera of differing HBsAg concentrations (8-10 times per sample with both the control and confirmatory reagents). After a 30-minute period of incubation at 20 °C, the pretreated samples were determined on Elecsys 2010 analyzers. Representative performance data for manual sample pretreatment followed by assay on Elecsys 2010 analyzers are shown below. Results obtained in individual laboratories may differ.
Results from original HBsAg assay - without sample pretreatment (repeatability, n = 8-10):

Sample	Mean COI[c)]	SD COI	CV[d)] %
HS[e)], COI < 7.00	1.65	0.04	2.4
HS, COI 7.00 - < 30.0	11.3	0.11	1.0
HS, COI ≥ 30.0	669	17.7	2.6

c) COI = Cutoff index

d) CV = Coefficient of variation

e) HS = human serum

Results after manual sample pretreatment:

HBsAg Confirmatory Test

	Control reaction			Confirmatory reaction		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, COI < 7.00	1.57	0.04	2.6	0.42	0.04	9.5
HS, COI 7.00 - < 30.0	4.85	0.10	2.1	0.40	0.02	5.0
HS, COI ≥ 30.0	1321	12.8	1.0	1.31	0.10	7.6

References

1 Gerlich W. Viral Hepatitis. Section 2, Churchil Livingstone, Ed. Zuckermann AJ, Thomas HC, 1993:83-113.
2 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
3 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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

Symbols

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→	Volume after reconstitution or mixing
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FOR US CUSTOMERS ONLY: LIMITED WARRANTY Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

HBsAg Confirmatory Test

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

PreciControl HBsAg II

REF **04687876** 160 **16 x 1.3 mL**

For use in the USA only

Intended use

PreciControl HBsAg II is used for quality control of the Elecsys HBsAg II immunoassay on the **cobas e** 601 immunoassay analyzer. The performance of PreciControl HBsAg II has not been established with any other HBsAg assay.

Summary

PreciControl HBsAg II is a ready-for-use control serum based on human serum both in the negative and positive concentration range. The controls are used for monitoring the accuracy of the Elecsys HBsAg II immunoassay.

Reagents - working solutions

- PC HBSAGII1: 8 bottles, each containing 1.3 mL of control serum Human serum, negative for HBsAg; preservative. Target range for the cutoff index: 0.0-0.80
- PC HBSAGII2: 8 bottles, each containing 1.3 mL of control serum HBsAg (human) approximately 0.2 IU/mL in human serum; preservative. Target range for the cutoff index: 2.6-5.0

The exact ranges, given in the form of a cutoff index (COI), are encoded in the barcodes as well as printed on the enclosed (or electronically available) value sheet.

Target values and ranges

The target values and ranges were determined and evaluated by Roche. They were obtained using the Elecsys HBsAg II assay reagents and analyzers available at the time of testing.

Traceability information is given in the Method Sheet of the relevant Elecsys assay.

Results must be within the specified ranges. In the event that increasing or decreasing trends, or any other suddenly occurring deviations beyond the range limits are observed, all test steps must be checked.

When necessary, measurement of the patient sample tested should be repeated.

Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Note: For technical reasons re-assigned target values valid only for a specific reagent and control lot combination, must be entered manually. Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.

When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

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

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 (PC HBSAGII1 only) and antibodies to HCV and HIV.

PreciControl HBsAg II

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A. The serum containing HBsAg used for the positive control (PC HBSAGII2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or 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.[1,2]

The controls may not be used after the expiration date.

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

Handling

The controls are supplied ready-for-use in bottles compatible with the system. The controls should only be left on the analyzer during performance of quality control. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 7 quality control procedures per bottle should be performed.

Storage and stability

Store at 2-8 °C.

Store controls **upright** in order to prevent the control solution from adhering to the snap-cap.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers at 20-25 °C	up to 5 hours

Materials provided

- PreciControl HBsAg II

Materials required (but not provided)

- **cobas e** 601 immunoassay analyzers and assay reagents

See the assay Method Sheet and the operator's manual for additionally required materials.

Assay

Treat the control serum in the system-compatible labeled bottles for analysis in the same way as patient samples.

Read the data into the analyzer.

Ensure the controls are at 20-25 °C prior to measurement.

Run controls daily in parallel with patient samples, once per reagent kit, and whenever a calibration is performed. The control intervals and limits should be adapted to each laboratory's individual requirements.

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

References

1 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

2 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

PreciControl HBsAg II

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

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.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 after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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