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Elecsys HBsAg II

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REF		Σ	IVD	Rx Only	SYSTEM
08814848162	08814848502	20 x 300			**cobas pro** serology solution

English

For use in the USA only

System information

Short name	ACN (application code number)
HBSAG2B	10502
HBSAG2BE (embedded application)	11502
HBSAG2BR (for use with **cobas e** flow)	12502

Intended use

Elecsys HBsAg II is an in vitro immunoassay intended for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma. Elecsys HBsAg II is intended to screen individual human donors, including volunteer donors of whole blood, blood components and source plasma. The assay is also intended to be used to screen organ, tissue and cell donors, when donor samples are obtained while the donor's heart is still beating. It is not intended for use on cord blood specimens.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use with **cobas pro** serology solution equipped with the **cobas e** 801 analytical unit.

Summary

Hepatitis B virus (HBV) is transmitted by percutaneous or mucosal exposure to infected blood and various body fluids including saliva, menstrual, vaginal, and seminal fluids.[1] The majority of adult patients recover completely from their HBV infection, but up to 10 % of them become asymptomatic carriers or develop chronic hepatitis which may lead to cirrhosis and/or liver cancer.[2,3] Despite immunization, HBV is still prevalent worldwide with approximately 300 million chronically infected patients and a serious threat to blood transfusion safety, especially in highly endemic countries.[1,4,5] Serological diagnosis of HBV infection involves the detection of HBV specific antigens and/or antibodies to identify different phases of the HBV infection to determine whether a patient has acute or chronic HBV infection, is susceptible to infection, or is immune to HBV as a result of prior infection or vaccination.[6,7] In addition, some of these HBV markers are routinely used in patient and donor screening.[7]

The external envelope of the hepatitis B virus (HBV) particle is composed of a polypeptide of varying size, namely hepatitis B surface antigen (HBsAg).[8] Detection of HBsAg in human serum or plasma is the standard serological test to confirm an acute or chronic HBV infection. Particularly, after an acute exposure to HBV, HBsAg appears in serum within 1 to 10 weeks.[9] After recovery from an acute HBV infection, the level of HBsAg becomes undetectable.[10] Persistence of HBsAg for more than 6 months implies chronic HBV infection, which is conventionally diagnosed by a repeat reactive test for HBsAg, 6 months after the initial reactive test.[11]

HBsAg assays are used to detect HBV in blood donors to prevent the transmission of the virus by blood and blood products.[7]

HBsAg assays are also used to screen organ and tissue donors.[12,13] The objective of blood screening is to detect markers of infection to prevent the release of infected blood and blood components for clinical use. Blood screening strategies are designed to assure the safety of blood units, but should not be used for notifying blood donors of reactive test results.[14]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, 2 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 II 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 sample with the cutoff value obtained by HBsAg embedded calibration. The Elecsys HBsAg II result is calculated automatically based on signal to cutoff ratio (cutoff index, COI).

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

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as HBSAG2B.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HBsAg-Ab~biotin, 1 bottle, 15.8 mL:
2 biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.

R2 Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 13.9 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.

HBSAG2B Cal1 Non-reactive calibrator 1, 2 vials of 1.3 mL each:
Human serum, non-reactive for HBsAg; preservative.

HBSAG2B Cal2 Reactive calibrator 2, 2 vials of 1.3 mL each:
Human serum, reactive for HBsAg; preservative.

Precautions and warnings

For in vitro diagnostic use.

This test is not intended for use as an aid in diagnosis of hepatitis B infection.

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.

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

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

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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: 1-866-744-6397

All human material should be considered potentially infectious.

The calibrators (HBSAG2B Cal1 and HBSAG2B Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAG2B Cal1 only) and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A).

The serum containing HBsAg (HBSAG2B 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 donor specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[15,16]

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

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators

The calibrators are supplied ready-for-use in vials compatible with the system.

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

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack:

unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analytical unit	16 weeks

Stability of the calibrators:

unopened at 2-8 °C	up to the stated expiration date
on **cobas e** 801 analytical unit at 20-25 °C	use only once, stable onboard for up to 5 hours

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the lid of the vial.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum and Li-heparin, K_2-EDTA, K_3-EDTA, CPD, and Na-citrate plasma collected using standard sampling tubes.

Serum and Li-heparin and K_2-EDTA plasma collected in tubes containing separating gel.

Samples on-the-clot are stable for 7 days at 15-30 °C and 14 days at 2-8 °C. Do not freeze samples on-the-clot.
Samples off-the-clot are stable for 7 days at 20-25 °C, 14 days at 2-8 °C and 1 month at -20 °C (± 5 °C). Samples off-the-clot may be frozen up to 4 times.

Specimens collected by plasmapheresis, which have not been frozen, do not require centrifugation. All other whole-blood samples and samples containing precipitates need to be centrifuged before performing the assay for 10 to 15 minutes at 2000 to 4000 RCF (relative centrifugal force = x g).

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/collection system manufacturer.

Do not use pools of samples.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

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

Sample stability claims were established by experimental data by the manufacturer only for the temperatures/time frames as stated in the method sheet.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04687876162, PreciControl HBsAg II, 16 x 1.3 mL
- REF 09366717190, PreciControl Release HBsAg II, 16 x 1.3 mL
- General laboratory equipment
- The **cobas pro** serology solution is a combination of the **cobas pro** serology controller, **cobas pro** integrated solutions (**cobas e** 801 analytical units only) and applicable licensed or cleared donor screening assays.

Additional materials for **cobas e** 801 analytical unit:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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 analytical unit concerned. Refer to the appropriate user guide for analytical unit-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Note: Anti-CMV and any HBsAg assay shall not be assigned to the same measuring cell on a **cobas pro** analytical unit, avoiding a potential signal carryover from samples with high CMV antibodies on a measuring cell. When assigned to the same measuring cell as Anti-CMV or HBsAg II, confirmation of HBsAg-reactive samples using HBsAg II Auto Confirm must only be run with all results reported and released (all brackets closed). All samples must be finalized in processing on the instrument (**cobas pro** integrated solution). This is to further reduce a potential signal carryover with high HBsAg II samples on a measuring cell.

Calibrators:

Place the calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

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).

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Calibration frequency:

Calibration must be performed once per reagent lot using HBSAG2B Cal1, HBSAG2B Cal2 and fresh reagent (i.e., not more than 24 hours since the **cobas e** pack was registered on the analytical unit).

Recalibration is required as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analytical unit
- as required: e.g., quality control findings outside the defined limits

Quality control

For quality control, use PreciControl HBsAg II.

Controls for the various concentration ranges must be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

PreciControl HBsAg II values must be within the ranges specified in the control value sheet. When the assay control values are within range, sample results are generated, and a valid release control result is required to release test results. If an assay control value is not within range, sample results are not generated for in-process or scheduled samples. For troubleshooting information, refer to User Assistance **cobas pro** serology solution or contact US Customer Technical Support.

Release control

For release control, use PreciControl Release HBsAg II.

Result validation is based on test result batches that are concluded by release control measurements. A release control result within defined limits is required to validate a batch of previously measured test results utilizing the **cobas pro** serology controller software. Initial reactive results will not be invalidated by a failed release control and must be retested in duplicate. Repeatedly reactive results will not be invalidated by a failed release control and stay reactive. Other results rendered invalid due to a failed release control result must be retested after resolving the cause for the failed control measurement.

For a valid batch of sample results, the release control is tested at user-defined intervals with a maximum span of every 300 samples or 350 determinations within 24 hours from the PreciControl and must be tested in order to release the test results. Reactive results will not be invalidated. The release control must meet specifications defined in the PreciControl Release HBsAg II value sheet in order to validate the system functionality and release test results. For troubleshooting information, refer to User Assistance **cobas pro** serology solution or contact US Customer Technical Support.

Calculation

The analytical unit automatically calculates the cutoff based on the measurement of HBSAG2B Cal1 and HBSAG2B Cal2.

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).

Interpretation of the results

Initial result

Numeric result	Result	Interpretation / further steps
COI < 0.90	Non-reactive	Non-reactive for HBsAg. No further testing needed.
COI ≥ 0.90	Reactive	Reactive in the Elecsys HBsAg II assay. All initially reactive samples should be retested in duplicate using Elecsys HBsAg II assay. Redetermination of samples with an initial COI ≥ 0.90 can be performed automatically (see section **cobas e** flow).

Final result

Numeric result	Final result	Interpretation / further steps
One or both of the duplicate retests have a COI ≥ 0.90	Repeatedly reactive	Presumptive evidence of HBV. Repeatedly reactive samples must be confirmed using a neutralization test (Elecsys HBsAg II Auto Confirm).
Both of the duplicate retests have a COI < 0.90	Non-Reactive	Non-reactive for HBsAg. No further testing needed.

cobas e flow

A **cobas e** flow is a procedure programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.
The **cobas e** flow (short name HBSAG2BR) is available to perform a repetition of measurements in duplicate automatically for samples with an initial cutoff index ≥ 0.90.

Limitations of the test

A non-reactive test result does not completely rule out the possibility of an infection with HBV. Serum or plasma samples from the very early (pre-seroconversion) phase can occasionally yield non-reactive findings. HBV variants may also lead to non-reactive HBsAg results.

The detection of HBsAg is not a diagnosis of HBV. It is recommended that repeatedly reactive specimens be confirmed by supplemental testing. Vaccination with a recombinant HBsAg Hepatitis B vaccine may cause transient positive results caused by a passive transfer of antigen by vaccination.[17] Individuals who are repeatedly reactive should be referred for medical evaluation which may include additional testing.

Specific performance data

Representative performance data is given below. Results obtained in individual laboratories may differ.

Precision

A study was performed based on guidance from CLSI EP05 A3 (n = 84). Testing was conducted at 1 site using 1 lot of the Elecsys HBsAg II assay and 1 lot of PreciControl HBsAg II. Panel members and controls were tested in 4 replicates, 1 run per day for 21 days. The following results were obtained.

Sample	Mean (COI)	Repeatability		Intermediate precision	
		SD	% CV	SD	% CV
HSP* 01	0.254	0.018	7.1	0.030	11.7
HSP 02	0.784	0.030	3.8	0.038	4.8
HSP 03	0.962	0.038	4.0	0.045	4.7
HSP 04	1.12	0.036	3.3	0.043	3.9
HSP 05	1.22	0.036	2.9	0.046	3.8
HSP 06	10.8	0.295	2.7	0.365	3.4
PC** HBSAG1B	0.363	0.036	9.9	0.040	11.0
PC HBSAG2B	4.17	0.091	2.2	0.125	3.0

* HSP = Human specimen

** PC = PreciControl

Reproducibility

A study was performed based on guidance from CLSI EP05-A3 (n = 270). Testing was conducted at 3 external sites using 3 lots of the Elecsys HBsAg II reagent kit and 1 lot each of the PreciControl HBsAg II and PreciControl Release HBsAg II. Panel members and PreciControl HBsAg II were tested in 2 runs per day for 5 days with 3 sample replicates per run. The precision and reproducibility for the Elecsys HBsAg II assay are presented in the following tables:

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Overall repeatability and reproducibility for Elecsys HBsAg II

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP* 01	1.74	0.067	3.85	0.009	0.492
HSP 02	7.04	0.182	2.59	0.092	1.31
PC** HBSAG1B	0.411	0.030	7.28	0.000	0.000
PC HBSAG2B	3.81	0.098	2.56	0.025	0.642

* HSP = Human specimen

** PC = PreciControl

Overall repeatability and reproducibility for Elecsys HBsAg II

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	1.74	0.024	1.38	0.072	4.12
HSP 02	7.04	0.145	2.06	0.250	3.56
PC HBSAG1B	0.411	0.014	3.45	0.033	8.06
PC HBSAG2B	3.81	0.059	1.56	0.117	3.07

Overall repeatability and reproducibility for Elecsys HBsAg II

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	1.74	0.030	1.75	0.123	7.07
HSP 02	7.04	0.149	2.12	0.601	8.54
PC HBSAG1B	0.411	0.012	2.89	0.018	4.46
PC HBSAG2B	3.81	0.067	1.76	0.382	10.0

Overall repeatability and reproducibility for Elecsys HBsAg II

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	1.74	0.146	8.37
HSP 02	7.04	0.668	9.49
PC HBSAG1B	0.411	0.040	9.65
PC HBSAG2B	3.81	0.405	10.6

Results: The precision and reproducibility of the Elecsys HBsAg II assay demonstrated minor variability from run to run, day to day and between reagent lots.

Analytical sensitivity

For determination of the cutoff sensitivity, a dilution series of 2 HBsAg reference standards (WHO standard 00/588, 2nd International Standard for HBsAg. Subtype adw2. Genotype A, and WHO standard 12/226, 3rd International Standard for HBsAg, HBV genotype B4, HBsAg subtype ayw1/adw2) were prepared. The measurements were performed in duplicate with 3 reagent lots on 1 **cobas e** 801 analytical unit at 1 site. The results from 3 kit lots at the COI of 0.90 were 0.023, 0.027, and 0.028 IU/mL (average 0.026 IU/mL) for NIBSC code 00/588 and 0.025, 0.026, and 0.028 IU/mL (average 0.026 IU/mL) for NIBSC code 12/226.

Analytical specificity

The effect of the following endogenous substances 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	≤ 753 µmol/L or ≤ 44 mg/dL
Hemoglobin	≤ 0.311 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Albumin	≤ 7.0 g/dL

Additionally, naturally elevated samples for bilirubin, rheumatoid factor, triglycerides (lipemic), hemoglobin and albumin were tested; no false reactive results were found.

No false non-reactive 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.

In rare cases, interference due to extremely high titers of antibodies to immunological components, streptavidin or ruthenium can occur and these effects are minimized by assay formulation and design.

Seroconversion

Seroconversion panels

Seroconversion sensitivity of the Elecsys HBsAg II assay was demonstrated by testing 20 commercially available seroconversion panels comparing Elecsys HBsAg II results to commercially licensed product. Results are summarized in the table below.

Panel ID	Elecsys HBsAg II first reactive result (bleed number)	Reactivity status by Elecsys HBsAg II AC	Reference method first reactive result (bleed number)	Difference in number of bleeds (+/-)
HBV6271	3	Reactive	3	0
HBV6272	20	Reactive	20	0
HBV6274	1	Reactive	1	0
HBV6276	7	Reactive	7	0
HBV6277	6	Reactive	6	0
HBV6279	6	Reactive	6	0
HBV6286	5	Reactive	5	0
HBV6292	7	Reactive	7	0
HBV9072	12	Reactive	12	0
HBV9073	14	Reactive	14	0
HBV9074	17	Reactive	17	0
HBV11002	3	Reactive	3	0
HBV11011	9	Reactive	9	0
HBV11012	4	Reactive	4	0
HBV11016	6	Reactive	6	0
HBV11024	11	Reactive	11	0
HBV11029	9	Reactive	9	0
HBV11058	5	Reactive	5	0
HBV11059	5	Reactive	6	+1
HBV11069	9	Reactive	9	0

Clinical specificity

A total of 5569 fresh serum specimens and 5713 fresh plasma specimens from volunteer blood donors and 3002 plasmapheresis samples were collected at 4 donor centers. The initial and repeat reactive rates were 0.02 % (1/5569) for the serum specimens, 0.07 % (4/5713) and 0.05 % (3/5713), respectively, for the plasma specimens, and 0.07 % (2/3002) for the plasmapheresis samples. Repeatedly reactive specimens were further tested using the Elecsys HBsAg II Auto Confirm; 1 specimen was confirmed positive and 5 specimens were not confirmed. The 1 confirmed positive specimen was positive by a FDA-licensed HBV Qualitative DNA assay.

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Specificity of Elecsys HBsAg II

Specimen category	Number tested	Initially reactive (% of total)	Repeatedly reactive (RR) (% of total)	Reactive by Elecsys HBsAg II AC (% of RR)	Number confirmed positive* (% of RR)	Specificity (%) (95 % CI**)
Volunteer blood donors - serum	5569	1 (0.02)	1 (0.02)	0 (0.00)	0 (0.00)	99.98 (5568/5569) (99.90-100.00)
Volunteer blood donors - plasma	5713	4 (0.07)	3 (0.05)	1 (33.33)	1 (33.33)	99.96 (5710/5712) (99.87-99.99)
Total volunteer blood donors	11282	5 (0.04)	4 (0.04)	1 (25.00)	1 (25.00)	99.97 (11278/11281) (99.91-100.00)
Plasmapheresis donors - plasma	3002	2 (0.07)	2 (0.07)	0 (0.00)	0 (0.00)	99.93 (3000/3002) (99.76-99.98)
Total donors	14284	7 (0.05)	6 (0.04)	1 (16.67)	1 (16.67)	99.96 (14278/14283) (99.92-99.99)

* Number confirmed positive by supplemental testing

**CI = confidence

Specificity based on assumed zero prevalence of HBsAg in whole blood and plasmapheresis donors was estimated in this study to be 99.96 % (14278/14283) with a 95 % confidence interval of 99.92 % to 99.99 %.

Clinical sensitivity

A total of 582 specimens from the categories shown in the table below were tested using the Elecsys HBsAg II assay. All repeatedly reactive specimens were tested using the Elecsys HBsAg II Auto Confirm assay. Sensitivity was estimated to be 100 % (582/582) with a 95 % confidence interval of 99.34 % to 100 % for preselected positive specimens.

Sensitivity of Elecsys HBsAg II

Specimen category	Number tested	Repeatedly reactive (RR) (% of total)	Reactive by Elecsys HBsAg II AC (% of RR)	Sensitivity (95 % CI*)
Acute HBV	80	80 (100)	80 (100)	100 (80/80) (95.42-100)
Chronic HBV	186	186 (100)	186 (100)	100 (186/186) (97.98-100)
HBsAg (Genotypes A-H)	19	19 (100)	19 (100)	100 (19/19) (83.18-100)
HBsAg positive	297	297 (100)	297 (100)	100 (297/297) (98.72-100)
Total	582	582 (100)	582 (100)	100 (582/582) (99.34-100)

*CI = confidence interval

A total of 462 specimens from individuals at increased risk for hepatitis and from individuals recovered from HBV infection were tested to demonstrate assay performance in an untested population of specimens which could potentially provide more positive outcomes than in a donor population.

Specimen category	Number tested	Repeatedly reactive (RR) (% of total)	Reactive by Elecsys HBsAg II AC (% of total)	Number confirmed positive* (% of RR)**
Increased risk for hepatitis infection	409	4 (0.98)	2 (50.0)	2 (50.0)
HBV recovered	53	1 (1.89)	0 (N/A)	0 (NA)
Total	462	5 (1.08)	2 (40.0)	2 (40.0)

*Number confirmed positive by supplemental testing

**The sensitivity and 95% confidence intervals are not estimated due to the small sample size

Other specimen conditions or disease states

A total of 311 samples containing potentially interfering substances were tested with the Elecsys HBsAg II assay comprising specimens:

- containing antibodies against acute HIV, HCV, HTLV-I/II, HDV, VZV, HAV, HSV, EBV, HEV
- from pregnant women with both single and multiparous pregnancy
- containing heterophilic autoantibodies (ANA), EBV, or human anti-mouse antibodies (HAMA)
- containing antibodies against *Candida* sp., *Escherichia coli*, *Treponema pallidum* (syphilis), *Toxoplasma gondii*, *Chlamydia trachomatis*, parvovirus, and rubella virus
- after vaccination against hepatitis A (HAV) and influenza
- for hyper-IgG/ IgM interference
- containing autoimmune antibodies for Morbus Crohn and Colitis Ulcerosa
- from patients with alcohol-induced hepatitis /cirrhosis
- from patients with systemic lupus erythematosus (SLE)

Only one false reactive sample (antibody against infectious agent of chlamydia) was found in samples with potentially cross-reacting factors.

Mutant detection

A total of 20 recombinant HBsAg proteins with mutations and 21 native samples with HBsAg mutations (including different HBV genotypes) 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). All mutations were recognized with Elecsys HBsAg II.

Recombinant HBsAg proteins with mutations

Sample	Mutation	Elecsys HBsAg II reactivity
Mutant 1	F8L, R24K, N40S, G43R, L94S, M103I, 113A114, M133T, P142L, D144G	+
Mutant 2	T/A45S, C107R, M195I	+
Mutant 3	S132Y, P142S, G145R	+
Mutant 4	T123N	+
Mutant 5	G145K	+
Mutant 6	D144G	+
Mutant 7	D144A	+
Mutant 8	G145R	+
Mutant 9	122RA123	+
Mutant 10	Q129P, F134R, P142L, D144E, G145K, S171F, L175S	+
Mutant 11	R122I	+
Mutant 12	M125T, T127P, P142A, G145R	+
Mutant 13	T131I	+

Elecsys HBsAg II

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Sample	Mutation	Elecsys HBsAg II reactivity
Mutant 14	C147S	+
Mutant 15	K141E	+
Mutant 16	S143L	+
Mutant 17	P142L	+
Mutant 18	Y134S	+
Mutant 19	E164D	+
Mutant 20	I126S	+

Native samples with HBsAg mutation

Sample	Mutation	Elecsys HBsAg II reactivity
Mutant 1	A128V	+
Mutant 2	G145R	+
Mutant 3	S143T	+
Mutant 4	M133L	+
Mutant 5	G130R	+
Mutant 6	T125M, S143M	+
Mutant 7	G130N	+
Mutant 8	T125M	+
Mutant 9	S143L	+
Mutant 10	G145A	+
Mutant 11	M133I	+
Mutant 12	Q129H	+
Mutant 13	T140I	+
Mutant 14	G145V	+
Mutant 15	P127T	+
Mutant 16	S132F, G145R	+
Mutant 17	T126A	+
Mutant 18	S132Y	+
Mutant 19	M133T	+
Mutant 20	F134L	+
Mutant 21	T126I	+

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For further information, please refer to the appropriate user guide for the analytical unit 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 navifyportal.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
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Elecsys HBsAg II

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