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Elecsys HBsAg II Auto Confirm

cobas®

REF		Σ	IVD	Rx Only	SYSTEM
08741034162	08741034502	300; equals to maximum 150 confirmation determinations			**cobas pro** serology solution

English

For use in the USA only

System information

Short name	ACN[a)]	Assay type	To be used for
HBSAGC B	12507	**cobas e** flow	samples repeatedly reactive, independently of Elecsys HBsAg II assay titer
HBSAGACB	11507	embedded application	reagent calibration and quality control
CNHBSAGB	11508	embedded application	reaction with control pretreatment in Elecsys HBsAg II Auto Confirm **cobas e** flow
CFHBSAGB	11509	embedded application	reaction with confirmatory pretreatment in Elecsys HBsAg II Auto Confirm **cobas e** flow

a) Application code number

Intended use

Elecsys HBsAg II Auto Confirm is an in vitro immunoassay for the qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma samples repeatedly reactive when tested with the Elecsys HBsAg II assay. Elecsys HBsAg II Auto Confirm is intended to confirm HBsAg presence in individual human donors, including volunteer donors of whole blood, blood components and source plasma.

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 is 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 in order 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 in order 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]

HBsAg confirmatory testing is performed to confirm the infectious status of donors deferred on the basis of repeat reactive HBsAg first-line screening tests, allowing for appropriate donor management to be initiated.[14,15,16] According to WHO, donors who are confirmed positive should be deferred from blood donation, notified of their infection status, counselled and referred for clinical management as soon as possible.[14,16] Based on FDA guidance, the decision of whether an initially reactive donor is to be reevaluated is left to the blood establishment.[14]

The Elecsys HBsAg II Auto Confirm assay is a fully automated confirmatory assay, based on the principle of specific antibody neutralization, intended to be used for samples repeatedly reactive in the Elecsys HBsAg II assay. Polyclonal HBsAg-specific antibodies bind to the immunodominant epitopes of HBsAg and thereby block the binding sites for the antibodies used in the Elecsys HBsAg II assay. Automation of the confirmatory assay mitigates risks of manual sample pretreatment, sample handling and result calculation.

Test principle

The test principle is based on 2 parallel measurements that are implemented into a **cobas e** flow (see also section "**cobas e** flow"). All steps are automated by the analytical unit.

For the first measurement the sample is treated with the control pretreatment (PT2) prior to immunoreaction. This measurement serves as a reference.

For the second measurement the sample is treated with the confirmatory pretreatment (PT1) prior to immunoreaction. During incubation with confirmatory pretreatment, unlabeled polyclonal anti-HBsAg antibodies are bound to the sample HBsAg and thereby block the binding sites for the labeled antibodies used in the following immunoreaction. The confirmation result (%) is automatically assessed by determining the ratio of both measurements.

The Elecsys HBsAg II Auto Confirm assay is based on the Elecsys HBsAg II assay which uses the sandwich principle.

For the initial determination in the **cobas e** flow, the sample is automatically pre-diluted with Diluent Universal followed by the 1st incubation step. Depending on the obtained result, automated retesting may be performed by the **cobas e** flow with either the undiluted sample or a higher dilution of the sample, before restarting with the 1st incubation step. 24-132 µL sample volume is required for testing.

- 1st incubation: The sample is incubated with control pretreatment and confirmatory pretreatment. During confirmatory pretreatment incubation, unlabeled polyclonal anti-HBsAg antibodies form a complex with the sample HBsAg, inhibiting binding of labeled antibodies in the 2nd incubation phase.
- 2nd incubation: 2 biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex[b)] form a sandwich complex with accessible HBsAg.
- 3rd 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 Elecsys HBsAg II Auto Confirm embedded calibration. The Elecsys HBsAg II Auto Confirm result is calculated automatically based on signal to cutoff ratio (cutoff index, COI).

The confirmation result (%) is automatically calculated by the software by determining the ratio between the cutoff index (COI) obtained for the measurement with confirmatory pretreatment (result displayed as

Elecsys HBsAg II Auto Confirm

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"CFHBSAGB") and the COI obtained for the measurement with control pretreatment (result displayed as "CNHBSAGB").

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

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment **cobas e** pack (PT1, PT2) are labeled as HBSAGACB.

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.

PT1 Confirmatory pretreatment, 1 bottle, 5.0 mL:
Anti-HBsAg (sheep) ≥ 500000 IU/L in sheep serum; MES[c] buffer 85 mmol/L, pH 6.5; preservative.

PT2 Control pretreatment, 1 bottle, 5.0 mL:
Serum from sheep not immunized against HBsAg; MES buffer 80 mmol/L, pH 6.5; preservative.

c) MES = 2-morpholino-ethane sulfonic acid

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

HBSAGACB 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.

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 (HBSAGACB Cal1 and HBSAGACB Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAGACB 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 (HBSAGACB 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.[17,18]

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

Reagent handling

The reagents (M, R1, R2, PT1, PT2) 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 and the pretreatment reagents:

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 the **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.

Samples that were repeatedly reactive in the Elecsys HBsAg II assay.

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 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

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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 Auto Confirm 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 08741107162, PreciControl HBsAg Auto Confirm, 8 x 1.3 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- 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: The Elecsys HBsAg II assay has been standardized against the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).

Calibration frequency:

Calibration must be performed once per reagent lot using HBSAGACB Cal1, HBSAGACB 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 and PreciControl HBsAg Auto Confirm.

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 and PreciControl HBsAg Auto Confirm values must be within the ranges specified in the control value sheet. When the assay control values are within range, sample results are generated. 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 and contact US Customer Technical Support.

Calculation

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

The Elecsys HBsAg II Auto Confirm **cobas e** flow automatically calculates the confirmation result (%) of the sample. The confirmation result (%) is shown as the main result, together with its qualitative interpretation (confirmed reactive/confirmed non-reactive/indeterminate/confirmation not valid).

The confirmation result is determined as follows:

$$\text{Confirmation result (\%)} = \frac{\text{COI confirmatory reaction (CFHBSAGB)}}{\text{COI control reaction (CNHBSAGB)}} \times 100$$

For traceability of the main result, the result of the control reaction CNHBSAGB (COI), confirmatory reaction CFHBSAGB (COI) and the corresponding qualitative interpretation (non-reactive/reactive) is shown as sub-results. Due to dilution/pretreatment steps during the reaction, the sub-results do not concur with the COI obtained for the sample tested with the Elecsys HBsAg II assay.

For samples with a COI of 0.81 to < 0.90 in the control reaction, the sub-result is interpreted as "non-reactive". These samples can have a "confirmed reactive" main result (see also section "Interpretation of the results") if the confirmation result is ≤ 60 %.

Main result and sub-results are also uploaded to the Laboratory Information System (LIS).

Interpretation of the results

Numeric result Control reaction CNHBSAGB (sub-result)	Numeric result Confirmation result (main result)	Result message	Further action
COI ≥ 0.81	≤ 60 %	Confirmed reactive	None
COI ≥ 0.81	> 60 %	Confirmed non-reactive	None
COI < 0.81	≤ 60 %	Indeterminate	"Indeterminate" results should be repeated. In case the result remains "Indeterminate", a follow-up sample should be examined with Elecsys HBsAg II and if repeatedly reactive with Elecsys HBsAg II Auto Confirm.

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Numeric result Control reaction CNHBSAGB (sub-result)	Numeric result Confirmation result (main result)	Result message	Further action
COI < 0.81	> 60 %	Confirmation not valid	"Confirmation not valid" results should be repeated. In case the result remains "Confirmation not valid", a follow-up sample should be examined with Elecsys HBsAg II and if repeatedly reactive with Elecsys HBsAg II Auto Confirm.

Recommendations in case the following result messages are obtained:

Result message	Further action
Implausible	Recalibrate using a fresh aliquot of calibrators. Repeat quality control measurement and repeat confirmation for this sample.
Inconsistent result in **cobas e** flow	Repeat sample measurement with "HBSAGC B". In case the result remains "Inconsistent result in**cobas e** flow", a follow-up sample should be examined with Elecsys HBsAg II and if repeatedly reactive with Elecsys HBsAg II Auto Confirm.

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 following **cobas e** flow (short name HBSAGC B) is available to perform the Elecsys HBsAg II Auto Confirm assay:

cobas e flow	Time to result	Function
HBSAGC B	27-54 minutes	"HBsAg II Auto Confirm - all titer" performs a fully automated confirmation independently of sample HBsAg titer. It triggers an automated 1:50 dilution with Diluent Universal and confirmation reaction. A rerun of the sample is performed: a) if the sample COI is too low for the 1:50 dilution (COI < 2 in the control reaction). It is rerun undiluted. b) if the sample is not confirmed reactive in the 1:50 dilution. It is rerun with a 1:400 dilution to ensure confirmation of very high titer samples.

Limitations of test

Samples that are repeatedly reactive with the Elecsys HBsAg II and are not confirmed reactive with the Elecsys HBsAg II Auto Confirm may be false positive samples. False positive samples may show an atypical dilution behavior with the Diluent Universal or the Control pretreatment (PT2) resulting in "Confirmation not valid" or "Inconsistent result in **cobas e** flow" results.

Dilution

In the **cobas e** flow "HBSAGC B", the sample is automatically prediluted with Diluent Universal in the initial testing. Please ensure that Diluent Universal is on the analytical unit if running "HBSAGC B".

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 Auto Confirm assay and 1 lot of PreciControl HBsAg Auto Confirm. Panel members and controls were tested in 4 replicates, 1 run per day for 21 days. The following results were obtained.

Sample	Main confirmation result (%)	Repeatability		Intermediate precision	
		SD	% CV	SD	% CV
HSP* 01	0.443	0.038	8.6	0.049	11.1
HSP 02	1.06	0.104	9.9	0.127	12.0
HSP 03	16.8	0.963	5.7	1.06	6.3
HSP 04	0.071	0.005	6.4	0.005	7.0
HSP 05	33.0	3.24	9.8	4.48	13.6
PC** HBSAGCB	8.97	0.748	8.3	0.780	8.7

* 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 Auto Confirm reagent kit and 1 lot of PreciControl HBsAg Auto Confirm and PreciControl HBsAg II. Panel members and PreciControl HBsAg Auto Confirm were tested in 2 runs per day for 5 days with 3 sample replicates per run. Within the **cobas e** flow HSP 01 and PreciControl HBsAg Auto Confirm are tested undiluted. HSP 02 is tested 1:50 pre-diluted with Diluent Universal. The precision and reproducibility for the Elecsys HBsAg II Auto Confirm are presented respectively in the following tables:

Overall repeatability and reproducibility for control reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP* 01	1.63	0.059	3.63	0.010	0.589
HSP 02	4.86	0.129	2.66	0.058	1.20
PC**HBSAGCB	3.63	0.100	2.74	0.065	1.78

* HSP = Human specimen

** PC = PreciControl

Overall repeatability and reproducibility for control reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	1.63	0.018	1.10	0.063	3.83
HSP 02	4.86	0.055	1.14	0.152	3.13
PC HBSAGCB	3.63	0.082	2.25	0.144	3.97

Overall repeatability and reproducibility for control reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	1.63	0.020	1.25	0.088	5.38
HSP 02	4.86	0.110	2.27	0.313	6.45
PC HBSAGCB	3.63	0.000	0.000	0.343	9.44

Overall repeatability and reproducibility for control reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	1.63	0.110	6.72
HSP 02	4.86	0.365	7.52
PC HBSAGCB	3.63	0.372	10.2

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Overall repeatability and reproducibility for confirmatory reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Repeatability SD (COI)	Repeatability % CV	Between run SD (COI)	Between run % CV
HSP 01	0.301	0.028	9.43	0.014	4.68
HSP 02	0.710	0.034	4.76	0.000	0.000
PC HBSAGCB	0.414	0.036	8.77	0.010	2.45

Overall repeatability and reproducibility for confirmatory reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Between day SD (COI)	Between day % CV	Intermediate precision SD (COI)	Intermediate precision % CV
HSP 01	0.301	0.010	3.21	0.033	11.0
HSP 02	0.710	0.011	1.56	0.036	5.01
PC HBSAGCB	0.414	0.004	0.960	0.038	9.16

Overall repeatability and reproducibility for confirmatory reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Between site SD (COI)	Between site % CV	Between lot SD (COI)	Between lot % CV
HSP 01	0.301	0.002	0.780	0.026	8.52
HSP 02	0.710	0.003	0.457	0.030	4.26
PC HBSAGCB	0.414	0.006	1.55	0.022	5.23

Overall repeatability and reproducibility for confirmatory reaction Elecsys HBsAg II Auto Confirm

Sample	Mean (COI)	Reproducibility SD (COI)	Reproducibility % CV
HSP 01	0.301	0.042	13.9
HSP 02	0.710	0.047	6.59
PC HBSAGCB	0.414	0.044	10.7

Overall repeatability and reproducibility for main confirmation result (%) Elecsys HBsAg II Auto Confirm

Sample	Mean (%)	Repeatability SD (%)	Repeatability % CV	Between run SD (%)	Between run % CV
HSP 01	18.6	1.88	10.1	0.756	4.07
HSP 02	14.7	0.768	5.22	0.000	0.000
PC HBSAGCB	11.5	0.987	8.56	0.308	2.67

Overall repeatability and reproducibility for main confirmation result (%) Elecsys HBsAg II Auto Confirm

Sample	Mean (%)	Between day SD (%)	Between day % CV	Intermediate precision SD (%)	Intermediate precision % CV
HSP 01	18.6	0.477	2.57	2.08	11.2
HSP 02	14.7	0.251	1.71	0.809	5.50
PC HBSAGCB	11.5	0.090	0.782	1.04	9.00

Overall repeatability and reproducibility for main confirmation result (%) Elecsys HBsAg II Auto Confirm

Sample	Mean (%)	Between site SD (%)	Between site % CV	Between lot SD (%)	Between lot % CV
HSP 01	18.6	0.377	2.03	2.49	13.4
HSP 02	14.7	0.226	1.53	1.63	11.1
PC HBSAGCB	11.5	0.202	1.75	1.78	15.4

Overall repeatability and reproducibility for main confirmation result (%) Elecsys HBsAg II Auto Confirm

Sample	Mean (%)	Reproducibility SD (%)	Reproducibility % CV
HSP 01	18.6	3.26	17.6
HSP 02	14.7	1.83	12.4
PC HBSAGCB	11.5	2.07	18.0

Results: All determinations per sample were found "confirmed reactive" for all samples tested. The precision and reproducibility of the Elecsys HBsAg II Auto Confirm assay demonstrated minor variability from run to run, day to day and between reagent lots.

Analytical sensitivity

In order to determine the analytic sensitivity of the Elecsys HBsAg II Auto Confirm assay, a serial dilution of the WHO Second International Standard for HBsAg (NIBSC code number: 00/588; subtype adw2, genotype A) and WHO Third International Standard (NIBSC code number: 12/226; WHO Third International Standard for HBsAg, HBV genotype B4, HBsAg subtypes ayw1/adw2) in human HBV-negative serum was tested. The lowest tested concentration of 0.039 IU/mL (Second WHO Standard) and 0.037 IU/mL (Third WHO Standard) was found confirmed reactive with the confirmatory assay.

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 interference

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

For the interference study a low positive HBsAg sample was spiked with the endogenous substance and tested with the Elecsys HBsAg II Auto Confirm. Up to the listed concentration, no interference with the confirmation of a HBsAg positive sample was observed.

For further information on limitations and interferences, please refer to the Elecsys HBsAg II Method Sheet.

Seroconversion

Seroconversion panels

Seroconversion sensitivity of the Elecsys HBsAg II Auto Confirm assay was demonstrated by testing 20 commercially available seroconversion panels comparing Elecsys HBsAg II results to commercially licensed product and reactivity by Elecsys HBsAg II Auto Confirm. 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

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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 (+/-)
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

Confirmation of HBsAg reactive specimens

a. Specificity cohorts: Presumed negative specimens from the following categories were evaluated using the Elecsys HBsAg II assay. Specimens that were repeatedly reactive were evaluated using the Elecsys HBsAg II Auto Confirm assay.

Percentage of Elecsys HBsAg II Repeatedly Reactive Samples Confirmed as Positive

Specimen category	Number tested	Repeatedly reactive (RR) (% of Total)	Reactive by Elecsys HBsAg II AC (% of RR)	Number confirmed positive[d] (% of RR)
Volunteer Blood Donors - Serum	5569	1 (0.02)	0 (0.00)	0 (0.00)
Volunteer Blood Donors – Plasma	5713	3 (0.05)	1 (33.33)	1 (33.33)
Total Voluntary Blood Donors	11282	4 (0.04)	1 (25.00)	1 (25.00)
Plasmapheresis Donors	3002	2 (0.07)	0 (0.00)	0 (0.00)
Total Donors	14284	6 (0.04)	1 (16.67)	1 (16.67)

d) Number confirmed positive by supplemental testing

Increased risk specimens and specimens from individuals recovered from HBV infection were evaluated using the Elecsys HBsAg II assay. Specimens that were repeatedly reactive were evaluated using the Elecsys HBsAg II Auto Confirm assay.

Percentage of Elecsys HBsAg II Repeatedly Reactive Samples Confirmed as Positive

Specimen category	Number tested	Repeatedly reactive (RR) (% of Total)	Reactive by Elecsys HBsAg II AC (% of RR)	Number confirmed positive[d] (% of RR)[e]
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 (N/A)
Total	462	5 (1.08)	2 (40.0)	2 (40.0)

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

b. Sensitivity cohorts: Known positive HBsAg specimens from the following categories that were repeatedly reactive by the Elecsys HBsAg II assay were evaluated using the Elecsys HBsAg II Auto Confirm assay.

Percentage of Elecsys HBsAg II Repeatedly Reactive Samples Confirmed as Positive

Specimen category	Number tested	Repeatedly reactive (RR) (% of Total)	Reactive by Elecsys HBsAg II AC (% of RR)	Sensitivity (95 % CI[f])
Acute HBV	80	80 (100)	80 (100)	100 (80/80) (95.42-100.00)
Chronic HBV	186	186 (100)	186 (100)	100 (186/186) (97.98-100.00)
HBsAg (Genotype A-H)	19	19 (100)	19 (100)	100 (19/19) (83.18-100.00)
HBsAg positive	297	297 (100)	297 (100)	100 (297/297) (98.72-100.00)
Total	582	582 (100)	582 (100)	100 (582/582) (99.34-100.00)

f) CI = confidence interval

Confirmation of HBsAg mutants

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 Auto Confirm 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 Auto Confirm and found confirmed reactive.

Recombinant HBsAg proteins with mutations

Sample	Mutation	Elecsys HBsAg II reactivity	Confirmation of reactivity by Elecsys HBsAg II AC 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	+	+

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Sample	Mutation	Elecsys HBsAg II reactivity	Confirmation of reactivity by Elecsys HBsAg II AC reactivity
Mutant 12	M125T, T127P, P142A, G145R	+	+
Mutant 13	T131I	+	+
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	Confirmation of reactivity by Elecsys HBsAg II AC 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

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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.

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