Elecsys HBsAg Confirmatory Test cobas®

REF 09127127190 09127127500

English

Intended use

Immunoassay for in vitro confirmation of the presence of hepatitis B surface antigen in human serum and plasma samples repeatedly reactive when tested with the Elecsys HBsAg II assay.

Summary

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). The disease remains an important global public health problem with significant morbidity and mortality.[1,2] The external envelope of the HBV particle is composed of a polypeptide of varying size, namely hepatitis B surface antigen (HBsAg).[3] 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.[4] After recovery from an acute HBV infection, the level of HBsAg becomes undetectable.[5] Persistence of HBsAg for more than 6 months implies chronic HBV infection, which is conventionally diagnosed by a repeat positive test for HBsAg, 6 months after the initial positive test.[6]

Therefore, HBsAg assays are used within routine diagnostic procedures to identify persons with acute or chronic HBV infection as well as to monitor the course of their disease and the efficacy of the selected therapy.[3,4,5,6,7] HBsAg assays are also used to detect HBV in blood donors in order to prevent the transmission of the virus by blood and blood products.[8] As part of prenatal care, HBsAg assays are additionally used to detect HBV in pregnant women in order to determine initiation of suitable measures for preventing as far as possible the transmission of the virus from the mother to the newborn child.[9]

The Elecsys HBsAg Confirmatory Test assay is 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 the hepatitis B surface antigen and thereby block the binding sites for the antibodies used in the Elecsys HBsAg II assay.

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 II assay. The positive control, PreciControl HBsAg II 2, should be run in parallel as a performance check.

Sample pretreatment:

- Samples found to be repeatedly reactive in the Elecsys HBsAg II assay 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 II assay this leads to a reduction in the cutoff index (COI) value (signal of sample/cutoff) in comparison to the value obtained for the sample treated with the control reagent.

Elecsys HBsAg II assay:

- 1st incubation: The 2 pretreated sample preparations react with 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)] to form a sandwich complex with accessible HBsAg.
- 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/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 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-HBsAg (sheep) ≥ 500000 IU/L in sheep serum; MES[b)] buffer 85 mmol/L, pH 6.5; preservative.

HBsAg Confirmatory Test 2
Control reagent (white cap), 2 bottles of 1.0 mL each:
Sheep serum negative for anti-HBsAg; MES buffer 80 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

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.

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: all countries: +49-621-7590

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

Reagent handling

The reagents in the kit are ready-for-use. Avoid contamination. Store at 2-8 °C after use.

Storage and stability

Store at 2-8 °C.

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 II assay.

The conditions regarding stability and specimen collection described for the Elecsys HBsAg II assay also apply here.

Materials provided

See “Reagents – working solutions” section for reagents.

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Materials required (but not provided)

- REF 08814856190, Elecsys HBsAg II reagent kit for 100 tests or
  REF 08814864190, Elecsys HBsAg II reagent kit for 200 tests or
  REF 08814848190, Elecsys HBsAg II reagent kit for 300 tests

  (the materials required for performing the Elecsys HBsAg II assay are listed in the Elecsys HBsAg II Method Sheet)
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent or
  REF 07299001190, Diluent Universal, 36 mL sample diluent
- **cobas e** analyzer

Assay

Sample pretreatment:

The cutoff index in the Elecsys HBsAg II assay is needed to select the correct sample pretreatment volume. Selection of the sample pretreatment volume should be based on the majority approach for the previously obtained Elecsys HBsAg II results. The following volumes are pipetted into Elecsys sample cups:

- For positive samples having a cutoff index < 7.0

  270 µL sample + 30 µL HBsAg Confirmatory Test 1 (confirmatory reagent)

  270 µL sample + 30 µL HBsAg Confirmatory Test 2 (control reagent)

or

- For positive samples having a cutoff index between 7.0 and < 30

  150 µL sample + 150 µL HBsAg Confirmatory Test 1 (confirmatory reagent)

  150 µL sample + 150 µL HBsAg Confirmatory Test 2 (control reagent)

or

- For positive samples having a cutoff index ≥ 30

  Predilute samples 1:20 with Diluent Universal

  150 µL diluted sample + 150 µL HBsAg Confirmatory Test 1 (confirmatory reagent)

  150 µL diluted sample + 150 µL HBsAg Confirmatory Test 2 (control reagent)

PreciControl HBsAg II 2, the positive control, should always be run in parallel as a check on performance:

270 µL PreciControl HBsAg II 2 + 30 µL HBsAg Confirmatory Test 1 (confirmatory reagent)

270 µL PreciControl HBsAg II 2 + 30 µL HBsAg Confirmatory Test 2 (control reagent)

Incubation of the reactants: 30-60 minutes at 15-25 °C or overnight at 2-8 °C.

Elecsys HBsAg II assay:

The pretreated samples are placed in the sample zone and registered by entering the sample identification data.

The 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 II assay.

Quality control

PreciControl HBsAg II 2 should always be run in parallel with the samples needing confirmation. Verification is done by the user.

For the Elecsys HBsAg II assay the conditions given in the Method Sheet apply.

Calculation

Elecsys HBsAg II assay:
The analyzer calculates the cutoff automatically on the basis of measurements on the 2 Elecsys HBsAg II calibrators contained in the kit. The result of a sample is given either as reactive, borderline or non-reactive as well as in the form of a cutoff index (COI, signal sample/cutoff).

Elecsys HBsAg Confirmatory Test calculation:
The confirmation result of the Elecsys HBsAg Confirmatory Test assay for samples and PreciControl HBsAg II 2 is manually calculated as follows:

$$\text{Confirmation result (\%)} = \frac{\text{COI (sample + confirmatory reagent)}}{\text{COI (sample + control reagent)}} \times 100$$

Evaluation and interpretation of the results

Verification of the validity of the test
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 assay, the following criteria are fulfilled:
The COI of PreciControl HBsAg II 2 with the control reagent must be found ≥ 0.81 and the confirmation result must be ≤ 60 %.
If these criteria are not fulfilled it is necessary to check the test conditions. Where appropriate, repeat the test with fresh reagent.

Evaluation and interpretation of the results

Numeric result		Interpretation	Further action
Sample with control reagent	Confirmation result		
COI ≥ 0.81	≤ 60 %	Positive	None
COI ≥ 0.81	> 60 %	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.
COI < 0.81	> 60 %	Non-valid	"Non-valid" results should be repeated using fresh reagent. In case the result remains "non-valid", a follow-up sample should be examined.

A COI < 0.81 in the reaction with the control reagent indicates that dilution is too high. Such samples should be retested undiluted or at a lower dilution. In case a high-dose hook sample is assumed please refer to the section "Limitations - interference" regarding higher predilution of such samples.

Limitations - interference

Due to the high-dose hook effect, samples having very high HBsAg concentrations (> 1 mg/mL or > 550000 IU/mL) may not be adequately neutralized by the confirmatory reagent at the stated volume, and may therefore not be 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 original Elecsys HBsAg II assay (dilution effect). The confirmatory test for these samples must be repeated at a higher predilution (at least 1:100).

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

No false confirmation of samples containing human anti-sheep antibodies up to 1000 µg/mL.

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

Limits and ranges

Detection limit

In order to determine the analytic sensitivity of the Elecsys HBsAg Confirmatory Test, 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 for HBsAg (NIBSC code number: 12/226 HBV genotype B4, HBsAg subtypes ayw1/adw2) in human HBV-negative serum was tested with 3 lots of Elecsys HBsAg Confirmatory Test. The lowest tested concentration of 0.045 IU/mL (Second WHO Standard) and 0.074 IU/mL (Third WHO Standard) was found positive with the confirmatory assay.

Specific performance data

Representative performance data for manual sample pretreatment followed by the Elecsys HBsAg II assay on the analyzers are shown below. Results obtained in individual laboratories may differ.

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Precision

The precision of the Elecsys HBsAg II assays are given in the respective Method Sheet.

Precision of the manual test steps was determined using 3 sera of differing HBsAg concentrations and PreciControl HBsAg II 2 (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 the **cobas e** 601 analyzer using Elecsys reagents, calibrators and controls.

	Control reaction			Confirmatory reaction			
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %	Mean confirmation result, %
HS*, COI < 7.0 (COI 1.51)	1.16	0.03	2.4	0.292	0.016	5.3	25.1
HS, COI 7.0 - < 30 (COI 18.6)	9.10	0.16	1.7	0.266	0.031	11.6	2.9
HS, COI ≥ 30 (COI 2987)	6844	52	0.8	0.292	0.024	8.3	0.0
PC** HBsAg II 2 (COI 4.07)	3.46	0.05	1.3	0.261	0.036	13.8	7.6

* HS = human serum

** PC = PreciControl

Analytical specificity

55 potentially cross-reacting HBsAg-negative samples were spiked with ≤ 10 % of an Elecsys HBsAg II confirmed reactive sample. The resulting HBsAg-positive samples containing the potential cross-reactants were confirmed by the Elecsys HBsAg II Confirmatory Test assay, demonstrating that the potential cross-reactants do not interfere with sample confirmation.

The following specimens were tested:

- containing antibodies against CMV, HAV, HCV, HIV, EBV, Rubella, Toxoplama gondii
- containing autoantibodies (ANA)
- after vaccination against HBV and influenza
- risk group patients suffering from non-viral induced liver diseases

Confirmation of seroconversion panels

15 commercial seroconversion panels were tested. At least the first sample (first bleeding) in each panel was negative for HBsAg. All samples positive with the Elecsys HBsAg II assay in each seroconversion were confirmed by the Elecsys HBsAg Confirmatory Test assay.

Confirmation of common HBsAg mutants and HBV genotypes

20 native samples with HBsAg mutations in the immunodominant “a” determinant region (amino acids 124-147) were tested. All samples with HBsAg mutations were positive with the Elecsys HBsAg II assay and were confirmed with the Elecsys HBsAg Confirmatory Test assay.

1st WHO International Reference Panel for Hepatitis B Virus (HBV) Genotypes for Hepatitis B Surface Antigen (HBsAg) Assays (PEI code 6100/09) representing subgenotypes A1, A2, B2, C2, D1, D2, D3, E, F2 and H was evaluated. All samples were positive with the Elecsys HBsAg II assay and confirmed with the Elecsys HBsAg Confirmatory Test assay.

Sensitivity

340 HBsAg-positive samples, including samples from acute and chronic hepatitis B infection were tested with the Elecsys HBsAg Confirmatory Test assay in-house. The presence of HBsAg was confirmed in all 340 samples, resulting in a 100 % sensitivity. The 95 % lower confidence limit was 99.12 %.

In addition 59 low titer samples 1 < COI ≤ 2 (spiked samples) were tested with the Elecsys HBsAg Confirmatory Test assay and presence of HBsAg was confirmed in all 59 samples.

Specificity

3 native false positive samples and 7 artificial interfering false positive samples were tested with the Elecsys HBsAg Confirmatory Test assay in-house. The Elecsys HBsAg Confirmatory Test assay correctly did not confirm those samples.

References

1 World Health Organization (WHO). Guidelines on hepatitis B and C testing. February 2017.

2 European Association for the Study of the Liver (EASL). EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370-398.

3 Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384(9959):2053-2063.

4 Song JE, Kim DY. Diagnosis of hepatitis B. Ann Transl Med 2016;4(18):338.doi:10.21037/atm.2016.09.11

5 Yuen MF, Chen DS, Dusheiko GM, et al. Hepatitis B virus infection. Nature Reviews Disease Primers 2018;18035. doi: 10.1038/nrdp.2018.35.

6 European Association for Study of Liver & Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. Journal of Hepatology 2015;63:237-264.

7 Seto WK, Wong DKH, Fung J, et al. Linearized hepatitis B surface antigen and hepatitis B core-related antigen in the natural history of chronic hepatitis B. Clin Microbiol Infect 2014;20:1173-1180.

8 World Health Organization. (2009). Screening donated blood for transfusion-transmissible infections: recommendations. https://apps.who.int/iris/handle/10665/44202

9 Schillie S, Vellozzi C, Reingold A., et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. Morbidity and Mortality WeeklyReport, Recomm Rep 2018;67(1):1-31. DOI: http://dx.doi.org/10.15585/mmwr.rr6701a1.

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

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

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

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

CE 0123

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