08498610500V2.0

Elecsys Anti-HBs II

cobas®

REF		Σ	SYSTEM
08498610190	08498610500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
A-HBS 2	10179

Intended use

Immunoassay for the in vitro quantitative determination of human antibodies to the hepatitis B surface antigen (HBsAg) in human serum and plasma.

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

Regulatory approval

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for testing of blood donations and, according to Paul-Ehrlich-Institut (PEI) recommendation,[1] for use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating).

Summary

Anti-HBs is a specific (generally IgG) antibody that is directed against the hepatitis B surface antigen (HBsAg).[2,3] Anti-HBs can be detected several weeks after the disappearance of hepatitis B surface antigen.[4,5] Anti-HBs can be formed following a hepatitis B infection or after hepatitis B vaccination.[4,5] Antibodies are formed against the HBsAg determinant a, which is common to all subtypes, and against subtype-specific determinants.[2,6,7]

Anti-HBs assays are used within the scope of hepatitis B vaccination to check the necessity and success of vaccination.[3,5,8] In addition, anti-HBs assays are used to monitor the course of disease following acute hepatitis B infection.[4]

The Elecsys Anti-HBs II assay uses a mixture of purified antigens from human serum (HBsAg subtype ad), and recombinant HBsAg subtype ay from CHO (Chinese Hamster Ovary) cells.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Anti-HBs in the sample (24 µL), biotinylated HBsAg (ad/ay), and HBsAg (ad/ay) labeled with a ruthenium complex[a)] react 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 II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

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 A-HBS 2.

M Streptavidin-coated microparticles, 1 bottle, 13.2 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HBsAg~biotin, 1 bottle, 16.7 mL:
Biotinylated HBsAg (ad/ay) human/recombinant, > 0.5 mg/L; MES[b)] buffer 85 mmol/L, pH 6.5; preservative.

R2 HBsAg~$Ru(bpy)_3^{2+}$, 1 bottle, 15.8 mL:
HBsAg (ad/ay) human/recombinant, labeled with ruthenium complex > 0.3 mg/L; MES buffer 85 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

A-HBSII Cal1 Calibrator 1, 1 bottle of 1.3 mL:
Anti-HBs (human) in human serum; preservative.

A-HBSII Cal2 Calibrator 2, 1 bottle of 1.3 mL:
Anti-HBs (human) 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.

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 dust/fume/gas/mist/vapours/spray.

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

All human material should be considered potentially infectious.

The calibrators (A-HBSII Cal1 and A-HBSII Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The HBsAg starting material used was inactivated prior to labeling with biotin or ruthenium by heating to 60 °C for 15 hours. In addition, any virus particles remaining were removed by ultracentrifugation.

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).

Reagent handling

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

Calibrators

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

Unless the entire volume is necessary for calibration on the analyzer, 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 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 analyzers	16 weeks

Stability of the calibrators:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	16 weeks
on the analyzers at 20-25 °C	use only once

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

Specimen collection and preparation

Specimen collected from living patients, blood donors, or individual organ, tissue or cell donors may be used, including donor samples obtained while the donor's heart is still beating.
Performance for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) was established according to Paul-Ehrlich-Institut recommendation[1] with samples obtained within 24 hours after death.[11] Qualitative differences of neat (non-reactive) or spiked (reactive) specimens from cadaveric compared to living donors were not observed.

Criterion: Mean value of cadaveric specimens compared to specimens from living donors within a recovery of 75-125 %.

Only the specimens listed below were tested and found acceptable.

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

K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 1.00 ± 0.15 + intercept 0 ± 2 IU/L + bias at 10 IU/L: ≤ 30 %.

Stability:
For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 5 times.
For cadaveric specimens: Stable for 3 days at 20-25 °C, 7 days at 2-8 °C. The samples may be frozen 3 times.

For plasma treated with lithium heparin, lithium heparin with gel or sodium heparin, the values found were on average up to 20 % lower than those obtained in serum. For plasma treated with sodium citrate, the values found were on average up to 30 % lower than those obtained with serum.

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.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators 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 Anti-HBs II assay has not been established with body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 11876317122, PreciControl Anti-HBs, 16 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- 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 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 11298500316, 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.

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.

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 1st WHO Reference Standard 1977.

The predefined master curve is adapted to the analyzer using A-HBSII Cal1 and A-HBSII Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using A-HBSII Cal1, A-HBSII 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 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Anti-HBs.

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

Calculation

The analyzer automatically calculates the analyte concentration of each sample in IU/L.

Interpretation of the results

Numeric result	Result message	Interpretation
< 10 IU/L	Non-reactive	Negative for anti-HBs
≥ 10 IU/L	Reactive	Positive for anti-HBs

Note: Due to the diversity of the antibodies, the measured anti-HBs value can vary depending on the testing procedure used. Results obtained from a single sample using tests from different manufacturers can therefore differ by up to a factor of 4 (or even a factor of 10 in rare cases). If there is a change in the assay procedure used during the monitoring of vaccination protection, then the anti-HBs values obtained upon changing over to the new method must be confirmed by parallel measurements by both methods. Vaccination strategies in certain risk groups are based on the measured anti-HBs concentration. Respective recommendations are given by national or regional guidelines.

Limitations - interference

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

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 513 µmol/L or ≤ 30 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7.0 g/dL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL

Criterion: Recovery for samples from Limit of Detection to 10 IU/L: ≤ ± 2 IU/L, and samples > 10 IU/L: ≤ ± 20 % of initial value.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs used in hepatitis B therapy were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
Peginterferon alfa-2a	≤ 0.18
Peginterferon alfa-2b	≤ 1.6
Lamivudine	≤ 300
Adefovir	≤ 10
Entecavir	≤ 10
Tenofovir	≤ 245
Telbivudine	≤ 600

Due to high-dose hook effect[c], results from anti-HBs concentrations of > 200000 IU/L may be found below the upper limit of the measuring range of 1000 IU/L. In rare cases, a high-dose hook effect from anti HBs concentrations of < 200000 IU/L cannot be excluded. Therefore in case of any unexpected low result the sample should be diluted 1:100 (refer to chapter "Dilution") and tested again.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. The test contains additives which minimize these effects.

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

c) High-dose hook effect: A sample with a true concentration clearly above the measuring range, but found within the measuring range.

Limits and ranges

Measuring range

2-1000 IU/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 2 IU/L. Values above the measuring range are reported as > 1000 IU/L (or up to 100000 IU/L for 100-fold diluted samples).

Dilution

Samples with anti-HBs concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:100 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 10 IU/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Manual dilution can also be made with negative human serum.

Note: Antibodies to HBsAg are heterogeneous. In some isolated cases, this may lead to non-linear dilution behavior.

Specific performance data

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

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability[d]		**Intermediate precision**[e]	
Sample	Mean IU/L	SD IU/L	CV %	SD IU/L	CV %
Human serum 1	8.15	0.377	4.6	0.566	6.9
Human serum 2	11.7	0.502	4.3	0.810	6.9
Human serum 3	102	1.84	1.8	4.02	3.9
Human serum 4	579	14.1	2.4	22.6	3.9
Human serum 5	964	15.3	1.6	44.3	4.6
PC[f] Anti-HBs 1	< 2.0	-	-	-	-
PC Anti-HBs 2	103	1.65	1.6	3.74	3.6

d) Repeatability = within-run precision

e) Intermediate precision = between-run precision

f) PC = PreciControl

Analytical specificity

No cross-reactions with HAV, HCV, HEV, CMV, EBV, HIV, Rubella, Toxoplasma gondii, Treponema pallidum, rheumatoid arthritis, autoimmune response or alcoholic liver disease were observed.

Measurements were performed on each of the pathogens listed above using ≥ 8 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens.

Relative sensitivity

Performance of the Elecsys Anti-HBs II assay has been assessed by testing a total of 669 samples at two different study sites. 296 samples from vaccinated persons and 373 samples from patients recovered from a hepatitis B infection have been measured with the Elecsys Anti-HBs II

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assay and another commercially available fully automated anti-HBs assay. Discrepant samples were tested with additional anti-HBs assays to achieve a consensus.

Characterization of samples	N	Elecsys Anti-HBs II reactive	Anti-HBs comparison test reactive	Sensitivity %
Anti-HBs positive: vaccinees	296	296	296	100
Anti-HBs positive: recovered from a hepatitis B infection	373	373	373	100
Total	669	669	669	100

Relative specificity

Performance of the Elecsys Anti-HBs II assay has been assessed by testing 2673 samples from blood donors negative for anti-HBs at two different study sites and 1623 anti-HBs negative samples from laboratory routine at three different study sites. Discrepant samples were tested with additional anti-HBs assays to achieve a consensus.

Characterization of samples	N	Elecsys Anti-HBs II false positive	Specificity %
Anti-HBs negative: blood donors	2673	6	99.78
Anti-HBs negative: routine samples	1623	9	99.45

References

1 Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, anti-HCV-Assays, HBsAg and Anti-HBc assays for Use with Cadaveric Samples; PEI 08/05/2014.

2 Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Field's Virology, Knipe DM, Howley RM (eds), 2007 5th edition, Lippincott Williams and Wilkins, Philadelphia, USA. Chapter 76, pp2977-3029.

3 WHO. Hepatitis B vaccines. Wkly Epidemiol Rec 2009;84:405-420.

4 Liaw YF, Chu CM. Hepatitis B virus infection. Lancet 2009;373:582-592.

5 Caspari G, Gerlich WH. The serologic markers of hepatitis B virus infection – proper selection and standardized interpretation. Clin Lab 2007;53:335-343.

6 Kramvis A, Kew M, François G. Hepatitis B virus genotypes. Vaccine 2005;23:2409-2423.

7 Michel ML, Tiollais P. Hepatitis B vaccines: protective efficacy and therapeutic potential. Pathol Biol 2010;58:288-295.

8 Elgouhari HM, Abu-Rajab Tamimi TI, Carey WD. Hepatitis B virus infection: understanding its epidemiology, course, and diagnosis. Cleve Clin J Med 2008;75:881-889.

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.

11 Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

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).

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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