Elecsys Anti-HBc II

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REF		Σ	SYSTEM
09014926190	09014926500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
AHBC 2	10142

Intended use

Immunoassay for the in vitro qualitative determination of IgG and IgM antibodies to the hepatitis B core antigen 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 screening 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

The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBcAg). The hepatitis core antigen comprises 183-185 amino acids.[2]

During an infection with HBV, antibodies to HBcAg are generally produced, which often persist for life. Anti-HBc appears shortly after the onset of HBV infection and can usually be detected in serum soon after the appearance of HBsAg.[3] Anti-HBc persists in persons who have recovered from HBV infection and inactive carriers. Therefore, they are an indicator of existing or past HBV infection.[2,3,4]

In rare cases, HBV infection can also run its course without the appearance of immunologically detectable anti-HBc (usually in immunosuppressed patients). Anti-HBc is not produced after vaccination.[5]

Due to the persistence of anti-HBc following infection with HBV, screening for anti-HBc can be used to identify previously infected individuals.[6]

Determination of anti-HBc in association with other hepatitis B tests permits the diagnosis and monitoring of HBV infections. In the absence of other hepatitis B markers (HBsAg-negative persons), anti-HBc may be the only indication of an existing HBV infection.[3,7,8]

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: Pretreatment of 24 µL of sample with reducing agent.
- 2nd incubation: After addition of HBcAg, a complex is formed with anti-HBc antibodies in the sample.
- 3rd incubation: After addition of biotinylated antibodies and ruthenium complex[a)] -labeled antibodies specific for HBcAg, together with streptavidin-coated microparticles, the still-free binding sites on the HBc-antigens become occupied. The entire 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 reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

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

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagent (R0) are labeled as AHBC 2.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R0 DTT, 1 bottle, 6.3 mL:
1,4-dithiothreitol 110 mmol/L; citrate buffer 50 mmol/L.

R1 HBcAg, 1 bottle, 15.8 mL:
HBcAg (E. coli, rDNA) > 25 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-HBcAg-Ab~biotin; anti-HBcAg-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 15.8 mL:
Biotinylated monoclonal anti-HBc antibody (mouse) 700 ng/mL; monoclonal anti-HBc antibody (mouse) labeled with ruthenium complex 200 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

AHBC 2 Cal1 Negative calibrator 1, 1 bottle of 1.0 mL:
Human serum, preservative.

AHBC 2 Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
Anti-HBc (human) > 8 WHO IU/mL[b)] in human serum; preservative.

b) WHO international units

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.

H319 Causes serious eye irritation.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

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

P337 + P313 If eye irritation persists: 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.

All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (AHBC 2 Cal1 only) and antibodies to HCV and HIV.

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

The serum containing anti-HBc (AHBC 2 Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[9,10]

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

Reagent handling

The reagents (M, R0, 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 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.

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma.

Plasma tubes containing separating gel can be used.

Criterion: Correct assignment of positive and negative samples. Samples with a COI (cutoff index) > 1.0: ± 20 % recovery; samples with a COI ≤ 1.0: ± 0.20 recovery.

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.

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.

Attention: Particularly important for the Elecsys Anti-HBc II assay: Thawed samples, samples containing precipitates, and samples for repeat measurements must be carefully centrifuged before performing the assay.

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-HBc II assay has not been established with body fluids other than serum or plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 4 bottle labels

Materials required (but not provided)

- REF 04927931190, PreciControl Anti-HBc II, 16 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- 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 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 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 anti-HBc reference material WHO Standard (NIBSC code 95/522).

Calibration frequency: Calibration must be performed once per reagent lot using AHBC 2 Cal1, AHBC 2 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.

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Renewed calibration is recommended as follows:

- after 8 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

Range for electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (AHBC 2 Cal1): 100000-700000
Positive calibrator (AHBC 2 Cal2): 100-3000

Quality control

For quality control, use PreciControl Anti-HBc II.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **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 cutoff based on the measurement of AHBC 2 Cal1 and AHBC 2 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

Numeric result	Result message	Interpretation/further steps
COI > 1.0	Non-reactive	Negative for anti-HBc, no further testing needed.
COI ≤ 1.0	Reactive	All initially reactive samples must be retested in duplicate using the Elecsys Anti-HBc II assay.

Retest result	Interpretation
One or both of the duplicate retests have a COI ≤ 1.0.	Repeatedly reactive
Both of the duplicate retests have a COI > 1.0.	Negative for anti-HBc

Retesting of samples with an initial cutoff index ≤ 1.0 can be automatically performed (see section "**cobas e** flows").

cobas e flows

cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.
A **cobas e** flow is available to perform a repetition of measurements in duplicate automatically for samples with an initial cutoff index ≤ 1.0.
Both sub-results and the overall result message will be reported.

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	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7 g/dL
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1 g/dL

Criterion: Samples with a COI > 1.0: ± 20 % recovery; samples with a COI ≤ 1.0: ± 0.20 recovery.

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
Peginterferon alfa-2a	≤ 0.036 mg/L
Peginterferon alfa-2b	≤ 0.036 mg/L
Lamivudine	≤ 300 mg/L
Adefovir	≤ 10 mg/L
Entecavir	≤ 1 mg/L
Telbivudine	≤ 600 mg/L
Tenofovir	≤ 245 mg/L

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

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: ≤ 0.8 WHO IU/mL

The stated sensitivity was determined by reading off the anti-HBc concentration corresponding to the signal of the cutoff value from standard curves obtained by serial dilution of the WHO anti-HBc reference material in human serum free from HBV.

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[c]		Intermediate precision[d]	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[e], negative	2.13	0.032	1.5	0.037	1.7
HS, weakly positive	0.920	0.017	1.9	0.029	3.1
HS, positive	0.005	0.0001	1.2	0.0001	2.2
PC[f] Anti-HBc II 1	2.41	0.029	1.2	0.044	1.8
PC Anti-HBc II 2	0.640	0.009	1.3	0.018	2.8

c) Repeatability = within-run precision

d) Intermediate precision = between-run precision

e) HS = human serum

f) PC = PreciControl

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Analytical specificity

309 samples containing potentially interfering substances were tested with the Elecsys Anti-HBc II assay comprising specimens:

- containing antibodies against HAV, HCV, HIV, HSV, Rubella, CMV, EBV, Toxoplasma gondii, Treponema pallidum
- positive for E. coli
- after vaccination against HAV and HBV
- non-viral induced liver diseases
- autoimmune diseases (ANA and SLE)

No false reactive results were found with the Elecsys Anti-HBc II assay (after repetition) resulting in a specificity of 100 %. 81 samples were identified as congruently positive with the Elecsys Anti-HBc II and a commercially available anti-HBc assay. 1 sample was found to be indeterminate and was excluded from calculation.

Clinical sensitivity

Of 793 samples from HBV infected patients in different stages of the disease, 793 were found repeatedly reactive with the Elecsys Anti-HBc II assay. The sensitivity of the Elecsys Anti-HBc II assay in this study was found to be 100 %.

Stage of disease	N	Reactive
Chronic or acute HBV infection (anti-HBc positive, HBsAg positive)	568	568
Passed HBV infection (anti-HBc positive, HBsAg negative, anti-HBs positive, anti-HBe negative)	56	56
Passed or recovered HBV infection (anti-HBc positive, HBsAg negative, anti-HBs negative, anti-HBe positive)	9	9
Passed or recovered HBV infection (anti-HBc positive, HBsAg negative, anti-HBs positive, anti-HBe positive)	160	160

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HBc II assay has been investigated by testing 10 commercially available seroconversion panels. The Elecsys Anti-HBc II assay was shown to be sensitive in early detection of infection in line with other anti-HBc assays and additional HBV serological markers.

Clinical specificity

A total of 20101 samples from blood donors, diagnostic routine, pregnant women and dialysis patients was tested in 7 European sites with the Elecsys Anti-HBc II assay.

The resulting specificity in the study on a repeatedly reactive basis (RR) was 99.93 % in blood donors (serum), the 95 % confidence interval (2-sided) was 99.84-99.97 %.

Specificity in blood donors (plasma) was found to be 99.88 %, the 95 % confidence interval (2-sided) was 99.78-99.94 %.

Specificity in diagnostic routine/hospitalized patients was found to be 100 % (95 % confidence interval (2-sided): 99.60-100 %), in dialysis patients 99.31 % (95 % confidence interval (2-sided): 98.23-99.81 %) and in pregnant women 100 % (95 % confidence interval (2-sided): 99.62-100 %).

Cohort	N	Specificity, IR*	95 % CI**, IR	Specificity, RR***	95 % CI, RR
Blood donors (serum)	8163	99.93 %	99.84-99.97 %	99.93 %	99.84-99.97 %
Blood donors (EDTA plasma)	9162	99.88 %	99.78-99.94 %	99.88 %	99.78-99.94 %
Diagnostic routine / hospitalized patients	997	100 %	99.60-100 %	100 %	99.60-100 %
Dialysis patients	779	99.13 %	97.99-99.72 %	99.31 %	98.23-99.81 %
Pregnant women	1000	100 %	99.62-100 %	100 %	99.62-100 %

* IR = initially reactive

** CI = confidence interval

*** RR = repeatedly reactive

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.

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

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

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

6 WHO. Hepatitis B. WHO/CDS/CSR/LYO/2002.2:Hepatitis B. Available at: http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf, accessed February 2012.

7 Pondé RA, Cardoso DD, Ferro MO. The underlying mechanisms for the 'anti-HBc alone' serological profile. Arch Virol 2010;155:149-158.

8 Guidelines for vaccinating kidney dialysis patients and patients with chronic kidney disease. Summarized from recommendations of the advisory committee on immunization practices (ACIP). December 2012. http://www.cdc.gov/dialysis/PDFs/Vaccinating_Dialysis_Patients_and_Patients_dec20-12.pdf

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

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CE 0123

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