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Anti-HBc

cobas®

Antibodies to hepatitis B core antigen (anti-HBc)

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11820559 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

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 Elecsys and **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.

Summary

The hepatitis B virus consists of an external envelope (HBsAg) and an inner core (HBcAg). The hepatitis core antigen comprises 183-185 amino acids.[1]

During an infection with the hepatitis B virus, antibodies to HBcAg are generally formed, which often persist for life. Anti-HBc appears shortly after the onset of infection with hepatitis B virus and can usually be detected in serum soon after the appearance of HBsAg. Anti-HBc antibodies persist both, in persons who have recovered from a hepatitis B infection and in those who develop HBsAg-carrier status. Accordingly, they are an indicator of existing or past hepatitis B infection.[2]

In rare cases, an HBV infection can also run its course without the appearance of immunologically detectable anti-HBc (usually in immunosuppressed patients).[3]

Due to the long persistence of anti-HBc following a hepatitis B viral infection, screening for anti-HBc provides the best information on the prevalence of hepatitis B in a particular group of persons.[4]

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 hepatitis B viral infection.[5]

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: Pretreatment of 40 µL of sample with reducing agent (Patent No. US 6150113 for USA or EP 0 341 439 B1 for Europe).[6]
- 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/ProCell 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 reagent rackpack (M, R0, R1, R2) is labeled as A-HBC.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R0 DTT (white cap), 1 bottle, 5 mL:
1,4-dithiothreitol 110 mmol/L; citrate buffer 50 mmol/L.

R1 HBcAg (gray cap), 1 bottle, 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~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Biotinylated monoclonal anti-HBc antibody (mouse) > 800 ng/mL; monoclonal anti-HBc antibody (mouse) labeled with ruthenium complex > 130 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

A-HBC Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.0 mL each:
Human serum, preservative.

A-HBC Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each:
Anti-HBc (human) > 8 PEI U/mL[b)] in human serum; preservative.

b) Paul-Ehrlich-Institute 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.

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 (A-HBC Cal1 only) and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HBc (A-HBC 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.[7,8]

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

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.

Elecsys 2010 and **cobas e** 411 analyzers: The calibrators should only be left on the analyzers during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, 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 read in from the respective reagent barcodes.

Anti-HBc

Antibodies to hepatitis B core antigen (anti-HBc)

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	8 weeks

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on Elecsys 2010 and **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 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

Only the specimens listed below were tested in a sufficient number and found acceptable.

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

Sodium heparin, K_3-EDTA and sodium citrate plasma.

Criterion: Correct assignment of negative and positive samples.

Stable for 5 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes 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 manufacturer.

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

Heat-inactivated samples may be used.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls 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.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 4 bottle labels

Materials required (but not provided)

- REF 11876325122, PreciControl Anti-HBc, for 8 x 1.3 mL each of PreciControl Anti-HBc 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- 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. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against the "HBc-Reference Material 82 (anti-HBc IgG)" of the Paul-Ehrlich-Institute, Langen (Germany).

Calibration frequency: Calibration must be performed once per reagent lot using A-HBC Cal1, A-HBC Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings with PreciControl Anti-HBc outside the defined limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (A-HBC Cal1):

Anti-HBc

Antibodies to hepatitis B core antigen (anti-HBc)

42000-250000 (Elecsys 2010 and **cobas e** 411 analyzers) or

100000-650000 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Positive calibrator (A-HBC Cal2):

100-3000 (Elecsys 2010, MODULAR ANALYTICS E170 and **cobas e** analyzers).

Quality control

For quality control, use PreciControl Anti-HBc.

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

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination, must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.

When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of A-HBC Cal1 and A-HBC 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).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 1.0 mmol/L or < 1.6 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Correct assignment of negative and positive samples.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 676 IU/mL.

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

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

Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

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 PEI U/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 Paul-Ehrlich-Institute anti-HBc reference material in human serum free from hepatitis B.

Dilution

Diluent Universal or human serum, plasma and fetal calf serum can be used to dilute the samples.

Expected values

Interpretation of the results

Samples with a cutoff index > 1.0 are non-reactive in the Elecsys Anti-HBc assay. These samples are considered negative for anti-HBc and do not need further testing.

Samples with a cutoff index ≤ 1.0 are reactive in the Elecsys Anti-HBc test. All samples found to be reactive in the initial test must be retested in duplicate with the Elecsys Anti-HBc assay. If the results in the follow-up test are "non-reactive" in both cases, then the sample is deemed negative for anti-HBc.

If at least one of the repeat measurements is reactive, then the sample is deemed repeatedly reactive.

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, human sera and controls.

Elecsys 2010 and **cobas e** 411 analyzers						
		Repeatability[c)]		**Intermediate precision[d)]**		
Sample	Mean COI[e)]	SD COI	CV %	SD COI	Mean COI	CV %
HS[f)], negative	1.73	0.020	1.2	1.78	0.024	1.4
HS, weakly positive	0.70	0.027	3.9	0.90	0.010	1.1
HS, positive	0.31	0.010	3.4	0.01	0.0001	1.3
PC[g)] A-HBC1	1.46	0.028	1.9	1.42	0.014	1.0
PC A-HBC2	0.32	0.007	2.3	0.30	0.004	1.6

c) Repeatability = within-run precision (n = 21)

d) Intermediate precision = between-run (n = 10)

e) COI = cutoff index

f) HS = human serum

g) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
		Repeatability[h)]		**Intermediate precision[i)]**		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	2.26	0.033	1.5	2.20	0.120	5.5
HS, weakly positive	0.74	0.019	2.6	0.73	0.059	8.1
HS, positive	0.004	0.00006	1.7	0.004	0.0001	1.8
PC A-HBC1	2.26	0.026	1.2	2.21	0.103	4.7
PC A-HBC2	0.43	0.008	1.9	0.49	0.033	6.8

h) Repeatability = within-run precision (n = 21)

i) Intermediate precision = within-laboratory (modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60))

Analytical specificity

No cross-reactions with HAV, HCV, CMV, EBV, HSV, Toxoplasma gondii, Rubella, and E. coli were observed.

Measurements were performed on each of the pathogens listed above using ≥ 6 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (AMA, ANA, SLE).

Clinical sensitivity

Characterization of samples	Elecsys Anti-HBc assay No. repeat. reactive	Anti-HBc comp. test No. repeat. reactive	Sensitivity %
HBsAg positive	457	457	100
Anti-HBs positive, HBsAg negative	67	67	100

Anti-HBc

cobas®

Antibodies to hepatitis B core antigen (anti-HBc)

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Samples from blood donors which had not been selected and hospitalized patients were used to determine the specificity.

Group	Number tested	Number reactive	Specificity %	Number repeat. reactive	Specificity %	Number confirmed positive
Blood donors	5111	112	99.6	106	99.7	91
Hospitalized patients from high-prevalence areas	245	95	-	89	-	87

For calculation of the specificity (%) the confirmed positive samples were not considered.

References

1 Sällberg M, Ruden U, Magnius LO, et al. Characterisation of a Linear Binding Site for a Monoclonal Antibody to Hepatitis B Core Antigen. J Med Virol 1991;33:248-252.

2 Hoofnagle JH. Type B Hepatitis: Virology, Serology and Clinical Course. Seminars in Liver Disease: I 1981;1:7-14.

3 Kumar S, Pound DC. Serologic diagnosis of viral hepatitis. Postgraduate Medicine 1992;92(4):55-65.

4 Decker RH. Section 9, Viral Hepatitis. In: Zuckerman A, Thomas HC eds. Diagnosis. Churchill Livingstone, 1993:165-184.

5 Gerlich WH, Caspari G, Uy A, et al. A critical appraisal of anti-HBc, HBV DNA and anti-HCV in the diagnosis of viral hepatitis. Biotest Bulletin 1991;4:283-293.

6 Patent owned by Abbott Laboratories, USA.

7 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

8 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.

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.

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

CE 0123

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