

Elecsys Anti-HBc IgM

REF			SYSTEM
07026811190	07026811500	300	cobas e 402 cobas e 801

English

System information

Short name	ACN (application code number)
AHBCIGM	10040

Intended use

Immunoassay for the in vitro qualitative determination of IgM antibodies to the hepatitis B core antigen in human serum and plasma.

The electrochemiluminescence immunoassay "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.

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 250 million chronically infected patients and a serious threat to blood transfusion safety, especially in highly endemic countries.^{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

HBV consists of an external envelope (HBsAg) and an inner core. The hepatitis B core antigen (HBcAg) is a highly immunogenic nucleocapsid protein.⁸ During an infection with HBV, antibodies to HBcAg appear shortly after the onset of HBV infection and can usually be detected in serum soon after the appearance of HBsAg. Free HBcAg or core particles are not detectable in serum.⁶

Anti-HBc IgM antibodies are one of the first serologic markers of HBV infection and usually persist for up to 6 months, being then replaced by anti-HBc IgG antibodies.^{1,8,9} High titers of anti-HBc IgM are detected during acute hepatitis B infection while low titers can be detected during chronic hepatitis B infection (CHB), and moderately high titers can occur in cases of CHB associated with viral replication and inflammatory activity.^{6,10} Tests to detect anti-HBc IgM antibodies are used, in conjunction with HBsAg determinations, to identify acute HBV infections.⁸ However, what occasionally appears to be an acute hepatitis B can occur in undiagnosed CHB carriers and additional tests are required to differentiate between chronic and acute infection.⁹

Test principle

μ-Capture test principle. Total duration of assay: 18 minutes.

- 1st incubation: Pretreatment of 6 μL of sample (automatically prediluted 1:400 with Diluent Universal) with anti-Fdy reagent to block specific IgG.
- 2nd incubation: Biotinylated monoclonal h-IgM-specific antibodies, HBcAg labeled with a ruthenium complex⁹ and streptavidin-coated microparticles are added to the pretreated sample. Anti-HBc IgM antibodies present in the sample react with the ruthenium-labeled HBcAg and the biotinylated anti-h-IgM to form a sandwich complex which 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)₃²⁺)

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as AHBCIGM.

- M Streptavidin-coated microparticles, 1 bottle, 16 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Pretreatment anti-HBc IgM, 1 bottle, 18.8 mL:
Sample pretreatment reagent: Anti-human-Fdy-antibody (sheep) > 0.05 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.
- R2 Anti-h-IgM-Ab~biotin; HBcAg~Ru(bpy)₃²⁺, 1 bottle, 18.8 mL:
Biotinylated monoclonal anti-h-IgM antibody (mouse) > 600 ng/mL; HBcAg (E. coli, rDNA), labeled with ruthenium complex > 200 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

AHBCIGM Cal1 Negative calibrator 1, 1 bottle of 1.0 mL:
Human serum; preservative.

AHBCIGM Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
Anti-HBc IgM (human) > 100 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.

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.

Elecsys Anti-HBc IgM

The negative calibrator (AHBCIGM Cal1) has 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.

Positive calibrator (AHBCIGM Cal2): Materials of human origin were tested for HIV and hepatitis C. The findings were negative. The serum containing anti-HBc IgM 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.^{11,12}

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

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

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, K₂-EDTA, K₃-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 : $\pm 20\%$ recovery; samples with a COI < 1.0 : ± 0.20 recovery.

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.

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 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 IgM assay has not been established with cadaveric samples or body fluids other than serum or plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- [REF] 11876333122, PreciControl Anti-HBc IgM, 16 x 1.0 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] 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 "HBc Reference Serum 84 (anti-HBc IgM)" of the Paul-Ehrlich-Institut, Langen (Germany). For the Elecsys Anti-HBc IgM assay, the cutoff (cutoff index 1.0) was set to approximately 100 PEI-U/mL.¹³

Calibration frequency: Calibration must be performed once per reagent lot using AHBCIGM Cal1, AHBCIGM Cal2 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack 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 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 the electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (AHBCIGM Cal1): 400-3500

Positive calibrator (AHBCIGM Cal2): 18000-130000

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Quality control

For quality control, use PreciControl Anti-HBc IgM.

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 AHBCIGM Cal1 and AHBCIGM 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 IgM
COI ≥ 1.0	Reactive	Positive for anti-HBc IgM

Note: According to the recommendations of the Paul-Ehrlich-Institut, Langen (Germany), an equivocal range should be allowed for the assessment of results from anti-HBc IgM tests. For the Elecsys Anti-HBc IgM assay the equivocal cutoff index range is 0.9-1.1.

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	≤ 428 μmol/L or ≤ 25 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 410 nmol/L or ≤ 100 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7 g/dL
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL

Samples with a COI ≥ 1.0: ± 20 % recovery; samples with a COI < 1.0: ± 0.20 recovery.

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.

As with many μ-capture assays an interference with unspecific human IgM is observed. Increasing amounts of unspecific human IgM may lead to a decrease in the recovery of positive samples with the Elecsys Anti-HBc IgM assay.

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	≤ 0.036

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

In rare cases, interference due to extremely high titers of antibodies to immunological components, streptavidin and ruthenium can occur.

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

Dilution

Use Diluent Universal for automatic sample predilution.

Expected values

For the Elecsys Anti-HBc IgM assay, the cutoff (cutoff index 1.0) was set to approximately 100 PEI-U/mL. In acute HBV infections the anti-HBc IgM level is generally far above this limit. After recovery from hepatitis B the anti-HBc IgM levels are below this. Chronic hepatitis B can produce values in the vicinity of the cutoff.

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					
Sample	Mean COI	Repeatability ^{c)}		Intermediate precision ^{d)}	
		SD COI	CV %	SD COI	CV %
HS ^{e)} , negative	0.069	0.001	1.6	0.001	1.9
HS, weakly positive	1.03	0.031	3.0	0.034	3.3
HS, positive	2.06	0.063	3.1	0.070	3.4
PC ^{f)} Anti-HBc IgM 1	0.071	0.001	1.7	0.001	2.0
PC Anti-HBc IgM 2	1.39	0.037	2.6	0.045	3.2

c) Repeatability = within-run precision

d) Intermediate precision = between-run precision

e) HS = human serum

f) PC = PreciControl

Analytical specificity

131 samples containing potentially interfering substances were tested with the Elecsys Anti-HBc IgM 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 IgM assay resulting in a specificity of 100 %.

Cutoff sensitivity

Approximately 100 PEI-U/mL for the Elecsys Anti-HBc IgM assay. Assays of other manufacturers may be set differently.

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Clinical sensitivity

245 samples from patients with different stages of HBV infection (acute, late acute/early recovery) were tested and were consistently found to be reactive using the Elecsys Anti-HBc IgM assay and a comparison test.

Clinical specificity

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

Group	Number tested	Number reactive	Specificity %
Blood donors	1000	0	100
Samples from routine and from hospitalized patients	1000	1 ^{g)}	100

g) 1 out of 1000 samples from routine or from hospitalized patients was found discrepantly reactive with the comparison assay. It could be confirmed as true negative.

References

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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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume for reconstitution
	Global Trade Item Number

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