# Elecsys Anti-HBc IgM

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| REF | | Σ | SYSTEM |
|---|---|---|---|
| **07026811** 119 | 07026811500 | 300 | **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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

**Note**: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 07026811190 for the Elecsys Anti-HBc IgM assay. The last 3 digits -190 have been replaced by -119 for logistic purposes.

**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 nucleocapside 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-Fdγ reagent to block specific IgG.
- 2nd incubation: Biotinylated monoclonal h-IgM-specific antibodies, HBcAg labeled with a ruthenium complex[a)] 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 ($Ru(bpy)_3^{2+}$)

**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-Fdγ-antibody (sheep) > 0.05 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R 2 Anti-h-IgM-Ab~biotin; HBcAg~$Ru(bpy)_3^{2+}$, 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:

n-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

All human material should be considered potentially infectious.

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 applied were FDA-approved 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.

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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 **cobas e** 801 analyzer | 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 **cobas e** 801 analyzer 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, $K_2$-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.

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 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** 801 analyzer

Accessories for the **cobas e** 801 analyzer:

- 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 Cups, 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). 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 with PreciControl Anti-HBc IgM 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

**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.

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**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 |
| 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 analyzer is 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** 801 analyzer | | | | | |
|---|---|---|---|---|---|
| | | **Repeatability**[c] | | **Intermediate precision**[d] | |
| Sample | Mean COI | 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.

**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**


1 World Health Organization (WHO), 2015. Hepatitis B. Fact sheet N°204. Available at: http://www.who.int/mediacentre/factsheets/fs204/en/

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4 Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 2015;386(10003):1546-1555

5 Song Y, Bian Y, Petzold M, et al. Prevalence and Trend of Major Transfusion-Transmissible Infections among Blood Donors in Western China, 2005 through 2010. PLoS One. 2014 Apr 8;9(4):e94528.

6 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.

7 World Health Organization (WHO), 2009. Screening Donated Blood for Transfusion-Transmissible Infections. Recommendations. Available at: at http://www.who.int/bloodsafety/ScreeningTTI.pdf (last access January, 2016).

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

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

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

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

12 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.

13 Hadziyannis JS, Hadziyannis AS, Dourakis S, et al. Clinical Significance of Quantitative Anti-HBc IgM assay in Acute and Chronic HBV Infection. Hepato Gastroenterol 1993;40:588-592.

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 |



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche