Anti-HBc IgM

cobas®

REF	Σ	SYSTEM
11820567 122	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 460
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 086

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 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 testing of blood donations.

Summary

References[1,2,3,4,5,6,7,8,9,10]

Hepatitis B core antigen (HBcAg) is a non-glycosylated protein (p22) which forms the nucleocapsid (virus core) of the hepatitis B virus. The virus core encloses the HBV-DNA (virus genome) and the DNA-polymerase. In the cytosol of virus-producing hepatocytes the nucleocapsid is enveloped by the hepatitis B surface antigen (HBsAg) to form virions. Free HBcAg or non-enveloped virus cores are not detectable in serum.

IgM antibodies to HBcAg occur in serum during proliferation of active hepatitis B virus and can still be detected weeks to months after viral proliferation has ceased. High anti-HBc IgM concentrations can be found in acute hepatitis B and in attacks during chronic hepatitis B.

Tests for detecting anti-HBc IgM antibodies are used, in conjunction with HBsAg determinations, to identify acute hepatitis B viral infections. An acute attack of hitherto non-diagnosed chronic hepatitis B clinically resembles an acute hepatitis B infection and cannot be distinguished from this with certainty by determining the anti-HBc IgM. Follow-up studies, imaging procedures and liver biopsies are useful in differentiating between these two clinical pictures.

Test principle

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

- 1st incubation: Pretreatment of 10 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 HBc antigen 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/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, R1, R2) is labeled as A-HBCIGM.

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

R1 Pretreatment anti-HBc IgM (gray cap), 1 bottle, 10 mL:
Sample pretreatment reagent: Anti-human-Fdγ-antibody (sheep) > 0.05 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-h-IgM-Ab~biotin; HBcAg~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 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.

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

A-HBCIGM Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each:
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 follows EU GHS guidance.

All human material should be considered potentially infectious.

The negative calibrator (A-HBCIGM 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 (A-HBCIGM 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 in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer 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.

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 **cobas e** 411	4 weeks
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	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 **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 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. Do not use plasma treated with sodium fluoride and potassium oxalate.

Criterion: Correct assignment of negative and positive samples.

Stable for 6 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 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. 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.

The performance of the Elecsys Anti-HBc IgM assay has not been established with cadaveric samples or 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 11876333122, PreciControl Anti-HBc IgM, 16 x 1.0 mL
- 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
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

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, 48 magazines x 84 reaction cups 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 (except for the **cobas e** 602 analyzer).

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 Serum 84 (anti-HBc IgM)" of the Paul-Ehrlich-Institute, 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 A-HBCIGM Cal1, A-HBCIGM Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

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Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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 IgM 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-HBCIGM Cal1): 600-3500 (**cobas e** 411 analyzer), 400-3500 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Positive calibrator (A-HBCIGM Cal2): 24000-150000 (**cobas e** 411 analyzer), 18000-130000 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

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 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-HBCIGM Cal1 and A-HBCIGM 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

Samples with a cutoff index ≥ 1.0 are reactive in the Elecsys Anti-HBc IgM assay. These samples are considered positive for anti-HBc IgM.

Samples with a cutoff index < 1.0 are non-reactive in the Elecsys Anti-HBc IgM assay. These samples are considered negative.

Note: According to the recommendations of the Paul-Ehrlich-Institute, 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 assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 2.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 409 nmol/L or < 100 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.

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.

No interference was observed from rheumatoid factors up to a concentration of 4200 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 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.

Limits and ranges

Detection limit: ≤ 3.0 PEI-U/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the negative calibrator (negative calibrator + 2 SD, repeatability study, n = 21).

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 disease the anti-HBc IgM levels are below this. Chronic hepatitis 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, human sera and controls.

cobas e 411 analyzer						
	Repeatability[c]			**Intermediate precision**[d]		
Sample	Mean COI[e]	SD COI	CV %	Mean COI	SD COI	CV %
HS[f], negative	0.123	0.003	2.2	0.069	0.002	2.8
HS, weakly positive	1.14	0.040	3.5	1.15	0.021	1.8
HS, positive	3.58	0.131	3.7	3.98	0.135	3.4
PC[g] A-HBCIGM1	0.053	0.001	1.7	0.063	0.002	3.3
PC A-HBCIGM2	1.39	0.063	4.5	1.55	0.056	3.6

c) Repeatability = within-run precision (n = 20/21)

d) Intermediate precision = between-run precision (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	0.037	0.001	3.4	0.037	0.002	3.9
HS, weakly positive	1.32	0.032	2.4	1.33	0.054	4.1
HS, positive	4.91	0.080	1.6	5.11	0.171	3.3
PC A-HBCIGM1	0.032	0.001	1.5	0.042	0.001	1.9
PC A-HBCIGM2	1.67	0.043	2.6	1.80	0.069	3.8

h) Repeatability = within-run precision (n = 20/21)

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, HIV 1+2, CMV, EBV, HSV, E. coli, Toxoplasma gondii, Rubella, and Treponema pallidum were observed.

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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 or contained autoantibodies (SLE, ANA).

Cutoff sensitivity

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

Clinical sensitivity

	Elecsys Anti-HBc IgM assay			Anti-HBc IgM comparison test		
	pos[j]	neg[k]	discrepant	pos	neg	discrepant
Acute HBV infection clinically and serologically manifested	48	-	-	48	-	-
Acute HBV infection clinically manifested	31	4	3 (neg)	31	4	3 (pos)[l]
Acute HBV infection serologically manifested	57	6	16 (neg) 1 (pos)	57	6	16 (pos) 1 (neg)
Serologically manifested no clinical information	145	292	44 (neg)	145	292	44 (pos)

j) positive

k) negative

l) In the comparison test, the discrepant samples were weakly positive.

Clinical specificity

To investigate the specificity, samples from randomly selected blood donors were tested with the Elecsys Anti-HBc IgM assay in comparison to licensed enzyme immunoassays.

1003/1003 samples from blood donors were negative with the Elecsys Anti-HBc IgM assay (100 % specificity for this cohort).

990/1003 were negative with a comparison test (98.7 % specificity).

242/242 samples from hospitalized patients, pregnant women, and dialysis patients with no indication of a HBV infection were negative with both the Elecsys Anti-HBc IgM assay and the comparison test (100 % specificity for this cohort).

References

1 Gudat F, Bianchi L, Sonnabend W, et al. Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Laboratory investigations 1975;32(1):1-9.

2 Gerlich WH, Robinson WS. Hepatitis B virus contains protein attached to the 5' terminus of its complete DNA strand. Cell 1980;21:801-809.

3 Gerlich WH, Goldmann U, Müller R, et al. Specificity and localization of the hepatitis B virus-associated protein kinase. Journal of Virology 1982;42(3):761-766.

4 Roossinck MJ, Siddiqui A. In vivo phosphorylation and protein analysis of hepatitis B virus core antigen. J Virol 1987;61:955-961.

5 Gallina A, Bonelli F, Zentilin L, et al. A recombinant hepatitis B core antigen polypeptide with the protamine-like domain deleted self-assembles into capsid particles but fails to bind nucleic acids. J Virol 1989;63(11):4645-4652.

6 Zhou S, Stranding DN. Hepatitis B virus capsid particles are assembled from core-protein dimer precursors. Proc Natl Acad Sci USA 1992;89:10046-10050.

7 Birnbaum BS, Nassal M. Hepatitis B virus nucleocapsid assembly: primary structure requirements in the core protein. J Virol 1990;64(7):3025-3330.

8 Hollinger FB. Hepatitis B virus. In Fields BN, Knipe DM (eds) Virology 2nd ed., New York, Raven Press 1990:2171-2236.

9 Frösner G. Moderne Hepatitisdiagnostik. Kilian Verlag, Marburg 1996.

10 Hoofnagle Bisceleglie MD. Serologic diagnosis of acute and chronic viral hepatitis. Seminars in Liver Disease 1991;11(2):73-83.

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 (for USA: see https://usdiagnostics.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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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