ms_11820613122V15.0

Anti-HBe

cobas®

Antibody to hepatitis B e antigen (anti-HBe)

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11820613 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro qualitative determination of human antibodies to the hepatitis B e antigen (HBeAg) 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.

Summary

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

The hepatitis B e antigen (HBeAg) is a product of the pre-C/C gene which has been found in the hepatocytes during proliferation of the hepatitis B virus. Following proteolysis, the HBe protein is secreted in non-particulate form (size varying from 16 kD to 20 kD) into the serum.

HBeAg appears in serum during acute HBV infections and is detectable for a short period (days to weeks). The detection of HBeAg is generally associated with the presence of large quantities of virus. In the recovery phase following acute hepatitis B, HBeAg is the first serological marker which becomes negative and is replaced by the corresponding antibody (anti-HBe). Acute and persistent HBV infections can also occur without HBeAg being detectable. Demonstration of anti-HBe in these persons is an indication of the presence of precore stop codon mutants. These may be associated with high, low or non-detectable quantities of virus.

The anti-HBe test is therefore meaningful in association with the HBeAg test for monitoring the course of a HBV infection.

The Elecsys Anti-HBe assay uses recombinant HBe-antigen and monoclonal anti-HBe antibodies.

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: Anti-HBe in the sample (35 µL) binds to the added HBeAg.
- 2nd incubation: After addition of biotinylated antibodies and ruthenium complex[a)] -labeled antibodies specific for HBeAg, together with streptavidin-coated microparticles, the still-free binding sites on the HBe-antigens become occupied. The entire complex is then 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-HBE.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HBeAg (gray cap), 1 bottle, 12 mL:

HBeAg (E. coli, rDNA) > 7 ng/mL; HEPES[b)] buffer 36 mmol/L, pH 7.4; preservative.

R2 Anti-HBeAg-Ab~biotin; anti-HBeAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 12 mL:

Biotinylated monoclonal anti-HBe antibody (mouse) > 0.8 mg/L; monoclonal anti-HBe antibody (mouse) labeled with ruthenium complex > 0.2 mg/L; HEPES buffer 36 mmol/L, pH 7.4; preservative.

b) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

A-HBE Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.0 mL each:

Human serum, preservative.

A-HBE Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each:

Anti-HBe (human) approx. 3 PEI-U/mL[c)] in human serum; preservative.

c) 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.

The negative calibrator (A-HBE 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.

The positive calibrator (A-HBE Cal2) containing anti-HBe was tested for HIV and hepatitis C infections. The findings were negative. The serum containing anti-HBe 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.[10,11]

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.

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.

Anti-HBe

cobas®

Antibody to hepatitis B e antigen (anti-HBe)

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.

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 6 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.

Centrifuge samples containing precipitates, frozen samples, and samples for repeat measurements 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 11876384122, PreciControl Anti-HBe, for 8 x 1.3 mL each of PreciControl Anti-HBe 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 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.

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 "HBe-Reference Serum 82 (anti-HBe IgG)" of the Paul-Ehrlich-Institute, Langen (Germany). The units given - U/mL - are units used by the Paul-Ehrlich-Institute.

Calibration frequency: Calibration must be performed once per reagent lot using A-HBE Cal1, A-HBE 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-HBe 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-HBE Cal1): 300000-1500000, positive calibrator (A-HBE Cal2): 1000-6000.

Quality control

For quality control, use PreciControl Anti-HBe.

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

Anti-HBe

Antibody to hepatitis B e antigen (anti-HBe)

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-HBE Cal1 and A-HBE 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 non-reactive in the Elecsys Anti-HBe assay. These samples are considered negative for anti-HBe and do not need further testing.

Samples with a cutoff index ≤ 1.0 are reactive in the Elecsys Anti-HBe assay.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 85.5 µmol/L or < 5.0 mg/dL); higher concentrations can reduce the cutoff index by up to 30 %. In isolated cases, this may lead to false-positive results in the vicinity of the cutoff. The assay is also unaffected by 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.

No interference was observed from rheumatoid factors up to a concentration of 2400 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, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: In case the Elecsys HBsAg II/Anti-HBs and HBeAg/Anti-HBe assay combinations are processed, make sure that these assays are entered in the “Special Wash” section of the system software and “Step1” (wash execute) is checked. Please refer to the operator's manual.

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: < 0.2 PEI-U/mL

The stated sensitivity was determined by reading off the anti-HBe concentration corresponding to the signal of the cutoff value from standard curves obtained by serial dilution of the Paul-Ehrlich-Institute anti-HBe reference material in human serum free from hepatitis B.

Dilution

Use Diluent Universal or human HBV-negative serum to dilute samples.

Expected values

Anti-HBe could be detected in samples from 210 (83.7 %) out of 251 persons with chronic or past HBV infections. 14 (1.4 %) out of 1000 samples of randomly selected blood donors were reactive for anti-HBe.

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[d]			**Intermediate precision**[e]		
Sample	Mean COI[f]	SD COI	CV %	Mean COI	SD COI	CV %
HS[g], negative	1.14	0.03	2.4	1.54	0.04	2.6
HS, weakly positive	0.96	0.02	2.0	0.89	0.02	2.2
HS, positive	0.15	0.004	3.0	0.35	0.01	3.5
PC[h] A-HBE1	1.54	0.03	2.1	1.50	0.03	2.0
PC A-HBE2	0.63	0.01	2.1	0.62	0.02	2.5

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

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

f) COI = cutoff index

g) HS = human serum

h) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability[i]			**Intermediate precision**[j]		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	1.60	0.066	4.1	1.71	0.081	4.8
HS, weakly positive	0.76	0.030	4.0	0.72	0.047	6.6
HS, positive	0.003	0.0001	2.7	0.003	0.0002	5.5
PC A-HBE1	1.60	0.066	4.1	1.56	0.064	4.1
PC A-HBE2	0.72	0.027	3.8	0.70	0.039	5.7

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

j) 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*, HTLV*, CMV*, EBV, HSV, E. coli, Toxoplasma gondii, Rubella, and Treponema pallidum were observed.

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, AMA).

* 1 out of each of 20 was false-positive.

Clinical sensitivity

Samples from patients in various stages of HBV infection and from patients in a high-prevalence group (HBsAg and/or anti-HBc positive) were investigated using the Elecsys Anti-HBe assay and various comparison tests. All samples showing discrepant measurements were in the vicinity of the cutoff.

Patient samples	Number tested	Elecsys Anti-HBe assay positive / negative	Anti-HBe comparison tests positive / negative	Discrepant
Past HBV infection	192	173 / 19	154 / 38	19
Chronic HBV infection	59	37 / 22	36 / 23	1
High prevalence group	153	77 / 76	75 / 78	2

Anti-HBe

cobas®

Antibody to hepatitis B e antigen (anti-HBe)

Clinical specificity

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

Group	Number tested	Number reactive	Confirmed positive	Specificity[k)] %	Specificity[l)] %
Blood donors	1000	12	13	99.9	100

k) Confirmed positive samples (i.e. confirmed by another anti-HBe test and positive anti-HBc and anti-HBs findings) were not considered for calculation of the %-specificity.

l) Confirmed positive samples and one sample with unclear HBV-serology were not considered for calculation of the %-specificity.

204 out of 242 samples from hospitalized patients, pregnant women, and dialysis patients (without symptoms of existing HBV infection) were negative with the Elecsys Anti-HBe assay; with a comparison test the figures were 202 out of 242. 38 samples were found to be positive by both tests. Two samples were negative in the Elecsys Anti-HBe assay, positive in the comparison test and positive for anti-HBc antibodies.

References

1 Bruss V, Gerlich WH. Formation of transmembranous hepatitis B e-antigen by translational in vitro processing of the viral precore protein. Virol 1988;163:268-275.

2 Wang J, Lee AS, Ou JH. Proteolytic conversion of hepatitis B virus e antigen precursor to end product occurs in a postendoplasmatic reticulum compartment. J Virol 1991;65:5080-5083.

3 Milich DR, Jones JE, Hughes JL, et al. Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? Proceedings of the National Academy of Sciences of the USA 1990;87:6599-6603.

4 Brunetto MR, Stemler M, Bonino F, et al. A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. J Hepatol 1990;10:258-261.

5 Carman WE, Jacyna MR, Hadziyannis S, et al. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet 1989;588-591.

6 Kuhns MC, McNamara AL, Perrillo RP, et al. Quantitation of hepatitis B virus DNA by solution hybridization: comparison with DNA polymerase and hepatitis B e antigen during antiviral therapy. J Med Virol 1989;27:274-281.

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

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

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

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

11 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

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

Roche