Elecsys Anti-HBe

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REF		Σ	SYSTEM
07026838190	07026838500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
AHBE	10033

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 **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 the cases 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] Some of these HBV markers are also routinely used in patient and donor screening.[7]

The hepatitis B e antigen (HBeAg) is a product of the pre-C/C gene that has been found in hepatocytes during proliferation of the hepatitis B virus (HBV) and is an important diagnostic tool to determine the status of ongoing HBV infections. The detection of HBeAg is generally associated with the presence of large quantities of virus as it is a surrogate of viral replication.[8,9] During acute HBV infection HBeAg can be detected in serum shortly after hepatitis B surface antigen (HBsAg) and usually disappears before HBsAg, when alanine aminotransferase (ALT) levels peak, followed by the presence of the corresponding antibody (anti-HBe).[8,9,10] HBeAg can usually be detected when viral replication is high; its presence for more than 10 weeks is indicative of a persistent infection. HBeAg seroconversion to anti-HBe suggests the end of active viral replication and is therefore associated with clinical resolution (self-limited) or remission (chronic disease).[6,8,9,11] HBV infections can occur without detectable HBeAg due to infection with HBV variants containing precore stop codon mutants; while the virus can no longer produce HBeAg, disease activity is ongoing and anti-HBe may be present.[8,12,13]

The anti-HBe test, therefore, is meaningful in association with the HBeAg test for monitoring the course of a HBV infection and the effect of treatment for chronic hepatitis B.[6,8,9,11] The Elecsys Anti-HBe assay uses recombinant HBeAg and monoclonal anti-HBe antibodies to detect anti-HBe.

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: Anti-HBe in the sample (21 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 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 AHBE.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HBeAg, 1 bottle, 21.9 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~$Ru(bpy)_3^{2+}$, 1 bottle, 13.9 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

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

AHBE Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
Anti-HBe (human) approximately 3 IU/mL in human serum; preservative.

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:

2-methyl-2H-isothiazol-3-one hydrochloride

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 (AHBE 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.

The positive calibrator (AHBE 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.[14,15]

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.

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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, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma.

Criterion: 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 6 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, thawed 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 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-HBe 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 4 bottle labels

Materials required (but not provided)

- REF 11876384122, PreciControl Anti-HBe, for 16 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- 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 WHO 1st International Standard Anti-hepatitis B virus e antigen (anti-HBe), code 129095/12 of the Paul-Ehrlich-Institut, Langen (Germany).

Calibration frequency: Calibration must be performed once per reagent lot using AHBE Cal1, AHBE 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 12 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-HBe outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (AHBE Cal1): 300000-1500000
Positive calibrator (AHBE 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 **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 AHBE Cal1 and AHBE 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).

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Interpretation of the results

Numeric result	Result message	Interpretation/ further steps
COI > 1.0	Non-reactive	Negative for anti-HBe, no further testing needed.
COI ≤ 1.0	Reactive	Positive for anti-HBe.

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	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 1.24 mmol/L or ≤ 2000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 410 nmol/L or ≤ 100 ng/mL
Rheumatoid factors	≤ 2400 IU/mL
Albumin	≤ 7.0 g/dL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL

Criterion: 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.

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	≤ 0.18
Peginterferon alfa-2b	≤ 0.08
Lamivudine	≤ 300
Adefovir	≤ 10
Entecavir	≤ 1
Telbivudine	≤ 600
Tenofovir	≤ 245

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.

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 IU/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 WHO anti-HBe reference material in human serum free from hepatitis B.

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.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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					
		Repeatability[c]		**Intermediate precision**[d]	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[e], negative	1.12	0.015	1.3	0.017	1.5
HS, weakly positive	0.891	0.010	1.1	0.014	1.5
HS, positive	0.313	0.005	1.7	0.007	2.3
PC[f] Anti-HBe 1	1.48	0.020	1.3	0.021	1.4
PC Anti-HBe 2	0.640	0.008	1.2	0.010	1.5

c) Repeatability = within-run precision

d) Intermediate precision = within-laboratory precision

e) HS = human serum

f) PC = PreciControl

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 16 samples was indeterminate.

** 1 out of each 20 samples was indeterminate.

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

Clinical specificity

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

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Group	Number tested	Confirmed positive	Elecsys Anti-HBe reactive	Specificity* %	Specificity** %
Blood donors	1000	12	13	99.9	100

* 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.

** 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

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13 Marcellin P. Hepatitis B and hepatitis C in 2009. Liver Int 2009;29(S1):1-8.

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

15 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 (for USA: see dialog.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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