Elecsys HBeAg

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REF		Σ	SYSTEM
07027427119	07027427500	300	**cobas e** 801

English

System information

Short name	ACN (application code number)
HBEAG	10036

Intended use

Immunoassay for the in vitro qualitative determination of 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 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: 07027427190 for the Elecsys HBeAg 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The last 3 digits -190 have been replaced by -119 for logistic purposes.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 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] In addition, some of these HBV markers are 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.[4,8,9] HBeAg can be detected in serum shortly after HBsAg during acute HBV infections 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, both in self-limited infections and in chronic hepatitis B; its presence for more than 10 weeks is indicative of a transition to 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), marking a transition from the immune-active phase of the disease to the inactive carrier state.[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 HBeAg test is, therefore, meaningful in association with the anti-HBe test for monitoring the course of HBV infection and the effect of treatment for chronic hepatitis B.[6,8,9,11] The Elecsys HBeAg assay uses monoclonal anti-HBe antibodies (mouse) for detection of HBeAg.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: HBe antigen from 21 µL sample, a biotinylated monoclonal HBeAg-specific antibody, and a monoclonal HBeAg-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex 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 HBEAG.

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

R1 Anti-HBeAg-Ab~biotin, 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-HBeAg antibody (mouse) > 0.8 mg/L; TRIS buffer 50 mmol/L, pH 7.4; preservative.

R2 Anti-HBeAg-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 14.8 mL:
Monoclonal anti-HBeAg antibody (mouse) labeled with ruthenium complex > 0.3 mg/L; TRIS buffer 50 mmol/L, pH 7.4; preservative.

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

HBEAG Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
HBeAg (E. coli, rDNA) ≥ 3.5 IU/mL in HEPES[b)] buffer, pH 7.4; preservative.

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

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. All products derived from human blood are 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.

However, as no 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.

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.

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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: 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 (plasma), 11 days at 2-8 °C (serum), 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 manufacturer.

Centrifuge samples containing precipitates, thawed samples, and samples for repeat measurements before performing the assay.

Do not use heat-inactivated samples.

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 HBeAg 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 11876376122, PreciControl HBeAg, 16 x 1.3 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 WHO 1st International Standard Hepatitis B virus e antigen (HBeAg), code 129097/12 of the Paul-Ehrlich-Institut, Langen (Germany).

Calibration frequency: Calibration must be performed once per reagent lot using HBEAG Cal1, HBEAG 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:

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 HBeAg outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (HBEAG Cal1): 400-2000
Positive calibrator (HBEAG Cal2): 20000-100000

Quality control

For quality control, use PreciControl HBeAg.

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 HBEAG Cal1 and HBEAG 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
COI < 1.0	Non-reactive	Negative for HBeAg
COI ≥ 1.0	Reactive	Positive for HBeAg

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.

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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	≤ 164 nmol/L or ≤ 40 ng/mL
Rheumatoid factors	≤ 1200 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.

The high-dose hook effect does not lead to false-negative results in the Elecsys HBeAg 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	≤ 0.18
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

Cutoff sensitivity: ≤ 0.3 IU/mL

The stated sensitivity was determined by reading off the HBeAg concentration corresponding to the signal of the cutoff value from standard curves obtained by serial dilution of the WHO HBeAg reference material in human serum free from HBV.

Dilution

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

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	0.103	0.004	4.1	0.004	4.1
HS, weakly positive	1.08	0.017	1.6	0.034	3.1
HS, positive	2.34	0.036	1.5	0.056	2.4
PreciControl HBeAg 1	0.095	0.004	3.8	0.004	4.2
PreciControl HBeAg 2	15.9	0.138	0.9	0.450	2.8

c) Repeatability = within-run precision

d) Intermediate precision = between-run precision

e) HS = human serum

Analytical specificity

No cross-reactions with HAV, HCV, HEV, CMV, EBV, HSV, HIV, E. coli, Toxoplasma gondii, Rubella and Treponema pallidum were observed.

Measurements were performed on each of the pathogens listed above using ≥ 5 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (ANA).

No false positive results were found with specimens from patients after vaccination against HBV or Influenza or from patients with non-viral induced liver disease.
No false positive results were found with specimens from hemophiliacs, homosexuals and intravenous drug abusers.

Clinical sensitivity

245 samples from patients with acute or persistent HBV infection were tested and were consistently found to be reactive using the Elecsys HBeAg 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	3[f]	100

f) 3 out of 1000 samples from routine or from hospitalized patients were found reactive using the Elecsys HBeAg assay and the comparison test and could be confirmed HBV positive. They were excluded from the specificity calculation.

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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8 Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Field's Virology, Knipe DM, Howley RM (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.

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12 Negro F. Management of chronic hepatitis B: an update. Swiss Med Wkly 2011;141:w13264.

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:

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

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