# HBeAg

cobas®

| REF | Σ | SYSTEM |
|---|---|---|
| **11820583** 119 | 100 | MODULAR ANALYTICS E170<br>**cobas e** 411<br>**cobas e** 601<br>**cobas e** 602 |

**English**

**System information**

For **cobas e** 411 analyzer: test number 440
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 073

**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 Elecsys and **cobas e** immunoassay analyzers.

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: 11820583122 for the HBeAg assay.
The last 3 digits -122 have been replaced by -119 for logistic purposes.

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

The Elecsys HBeAg test uses monoclonal anti-HBe antibodies (mouse) for the HBeAg determination.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: HBe antigen from 35 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/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 HBEAG.

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

R1 Anti-HBeAg-Ab~biotin (gray cap), 1 bottle, 12 mL:
Biotinylated monoclonal anti-HBeAg antibody (mouse) > 0.8 mg/L; TRIS buffer 50 mmol/L, pH 7.4; preservative.

R2 Anti-HBeAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 12 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 (white cap), 2 bottles of 1.0 mL each:
Human serum; preservative.

HBEAG Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each:
HBeAg (E. coli, rDNA) ≥ 3.5 PEI U/mL[b)] in HEPES[c)] buffer, pH 7.4; preservative.

b) Paul-Ehrlich-Institute units

c) 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 used assays approved by the FDA 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.[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.

**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 the analyzers | 8 weeks |

| *Stability of the reagent 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.

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 COI 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, 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 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 4 bottle labels

**Materials required (but not provided)**

- REF 11876376122, PreciControl HBeAg, 16 x 1.3 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 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).

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 Antigen 82 (HBeAg)" of the Paul-Ehrlich-Institute, Langen (Germany). The units given - U/mL - are units used by the Paul-Ehrlich-Institute.

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 reagent kit was registered on the analyzer).

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

Renewed calibration is recommended as follows:

*Calibration frequency:*

- 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 HBeAg outside the defined limits
- more frequently when this is required by pertinent regulations

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.

# 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 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 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

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

Samples with a cutoff index ≥ 1.0 are reactive in the Elecsys HBeAg assay. These samples are considered positive for HBeAg.

### Limitations - interference

The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 0.99 mmol/L or < 1.6 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 164 nmol/L or < 40 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.

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

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: ≤ 0.30 PEI U/mL

In order to determine the sensitivity, the HBeAg concentration which corresponds to the measuring signal of the cutoff value was read off the standard curves of serial dilutions of HBeAg standards and HBeAg-positive human sera in human HBV-negative serum.

| | PEI standard | | Human sera | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample | HBeAg 82 | | Serum 1 | | Serum 2 | | Serum 3 | |
| | COI | U/mL | COI | U/mL | COI | U/mL | COI | U/mL |
| 1 | 5.87 | 1.25 | 5.67 | 1.38 | 6.71 | 1.67 | 4.74 | 1.14 |
| 2 | 3.07 | 0.63 | 3.00 | 0.69 | 3.34 | 0.84 | 2.40 | 0.57 |
| 3 | 1.59 | 0.31 | 1.56 | 0.34 | 1.74 | 0.42 | 1.24 | 0.28 |
| 4 | 0.88 | 0.16 | 0.82 | 0.17 | 0.90 | 0.21 | 0.70 | 0.14 |
| Cutoff sensitivity | 0.19 U/mL | | 0.22 U/mL | | 0.22 U/mL | | 0.20 U/mL | |

### Dilution

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

### 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**[d] | | | **Intermediate precision**[e] | | |
| Sample | Mean COI | SD COI | CV % | Mean COI | SD COI | CV % |
| HS[f], negative | 0.12 | 0.005 | 4.0 | 0.14 | 0.01 | 4.0 |
| HS, negative | - | - | - | 0.50 | 0.01 | 1.7 |
| HS, positive | 33.0 | 1.32 | 4.0 | - | - | - |
| HS, positive | 235 | 6.63 | 2.8 | 64.8 | 3.17 | 4.9 |
| PreciControl HBeAg 1 | 0.14 | 0.01 | 6.6 | 0.13 | 0.01 | 4.5 |
| PreciControl HBeAg 2 | 10.6 | 0.16 | 1.6 | 10.2 | 0.17 | 1.7 |

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

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

f) HS = human serum

| MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers | | | | | | |
|---|---|---|---|---|---|---|
| | **Repeatability**[g] | | | **Intermediate precision**[h] | | |
| Sample | Mean COI | SD COI | CV % | Mean COI | SD COI | CV % |
| HS, negative | 0.11 | 0.006 | 5.1 | 0.15 | 0.017 | 11.0 |
| HS, weakly positive | 13.3 | 0.177 | 1.3 | 15.1 | 0.655 | 4.3 |
| HS, positive | 1880 | 22.25 | 1.2 | 1393 | 69.76 | 5.0 |
| PreciControl HBeAg 1 | 0.10 | 0.007 | 6.4 | 0.12 | 0.012 | 10.2 |
| PreciControl HBeAg 2 | 12.8 | 0.321 | 2.5 | 12.5 | 0.511 | 4.1 |

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

h) 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, CMV, EBV, HSV, HIV 1+2, HTLV, 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 (AMA, SLE).

### Clinical sensitivity

Of 334 samples from patients with acute and persistent HBV infection, 132 samples were consistently found to be reactive and 190 samples non-reactive using the Elecsys HBeAg assay and comparison tests. Discrepant results were only found with weakly reactive samples (7 samples reactive and 5 samples non-reactive with the Elecsys HBeAg

assay). In sample material from a commercial panel, 61 out of 61 samples were found to be consistently reactive.

**Clinical specificity**

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

| Group | Number tested | Number reactive | Specificity (%) |
|---|---|---|---|
| Blood donors | 1002 | 0 | 100 |

239 out of 242 samples from hospitalized patients, pregnant women, and dialysys patients were non-reactive using the Elecsys HBeAg assay and 222 out of 242 samples were non-reactive using a comparison test. Two of the samples found to be reactive in both tests and one sample found to be reactive in the Elecsys HBeAg test but not in the comparison test were also positive for HBsAg. 18 samples found to be non-reactive with the Elecsys HBeAg assay and weakly reactive in the comparison test were HBsAg-negative.

**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 (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 |



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