HBeAg

Hepatitis B e antigen

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REF	Σ	SYSTEM
11820583 160	100	MODULAR ANALYTICS E170

English

For use in the USA only

Warning

- Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted by or on the order of a physician.
- Assay performance characteristics have not been established in pregnant women, or in populations of immunocompromised or immunosuppressed patients.
- This assay has not been FDA licensed for the screening of blood, plasma and tissue donors.

Intended use

The Elecsys HBeAg immunoassay is intended for the in vitro qualitative determination of hepatitis B e antigen (HBeAg) in human serum or plasma (K_2-EDTA, lithium or sodium heparin, and sodium citrate) in adult patients with symptoms of hepatitis or at risk for hepatitis B virus (HBV) infection. The assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B or recovery from hepatitis B infection.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the MODULAR ANALYTICS E170 analyzer.

Summary

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).[1] Following proteolysis, the HBe protein is secreted from infected cells as a 15 kD protein.[1] The detection of HBeAg is generally associated with the presence of large quantities of virus as it is a surrogate of viral replication.[1,2] HBeAg appears in serum during acute HBV infections and usually disappears when alanine aminotransferase (ALT) levels peak, followed by the presence of the corresponding antibody (anti-HBe).[1,2] However, the presence of HBeAg for more than 10 weeks is indicative of a transition to persistent infection.[2] HBeAg can also be detected during chronic infection when viral replication is high, before loss of HBeAg and seroconversion to anti-HBe as viral titers decline.[1,3,4] HBV infections can, however, 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.[1,4,5]

The Elecsys HBeAg assay uses monoclonal anti-HBe antibodies (mouse) for the detection of HBeAg.

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 M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the instrument 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) (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 derived) ≥ 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 only.
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.[6,7]

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.

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 entered in from the respective reagent barcodes.

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 MODULAR ANALYTICS E170 analyzer	8 weeks

Stability of the calibrators	
after opening at 2-8 °C	8 weeks
on MODULAR ANALYTICS E170 analyzer	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

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Specimen collection and preparation

Only the specimens listed below were tested in a sufficient number and found acceptable.

- Serum collected using standard sampling tubes or tubes containing separating gel.
- Sodium heparin, lithium heparin, K_2-EDTA, and sodium citrate plasma.

Samples are stable for 7 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen and thawed 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.

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, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 11876376160, PreciControl HBeAg, 8 x 1.3 mL each of PreciControl HBeAg 1 and 2
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 analyzer

Accessories for MODULAR ANALYTICS E170 analyzer:

- 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
- REF 11298500160, 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. Enter 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 HBEAG Cal1 and HBEAG Cal2 in the sample zone of the analyzer. Only keep open during calibration. All information necessary for calibration is encoded on the barcoded bottle labels and is entered automatically. Discard after calibration has been performed.

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 frequency: Calibration must be performed once per reagent lot using Elecsys HBEAG Cal1, HBEAG 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 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 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. Therefore always refer to the value sheet included in the rackpack 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.

Note: The recommended negative and positive PreciControls are in two different matrices. The negative control is in human serum. The positive control is in HEPES buffer with protein and sugar stabilizers containing recombinant HBeAg protein from *E.coli*. The user is responsible for providing alternate control material for serum or plasma based controls as necessary.

Results

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) with a result interpretation of:

"non-reactive" (< 0.9 COI)

"border"[d)] (0.9 ≤ COI < 1.1)

"reactive" (COI ≥1.1)

d) border = borderline

Interpretation of the results

Initial Elecsys HBeAg assay result			
COI	**Result**	**Interpretation of results**	**Retest procedure**
< 0.9	Non-reactive	No HBeAg detected	No retest required
0.9 ≤ COI < 1.1	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys HBeAg assay
≥ 1.1	Reactive	HBeAg detected	Presumptive evidence of the presence of HBeAg.

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Final Elecsys HBeAg assay result			
COI	**Result after retest (COI)**	**Final results**	**Interpretation of results**
< 0.9	No retest required	NON-REACTIVE[e)]	HBeAg not detected. Does not exclude the possibility of exposure to HBV.
0.9 ≤ COI < 1.1	At least 2/3 results < 1.0	NON-REACTIVE	HBeAg not detected. Does not exclude the possibility of exposure to HBV.
	At least 2/3 results ≥ 1.0	REACTIVE	HBeAg detected.
≥ 1.1	No retest required	REACTIVE	HBeAg detected.

e) Please note: A negative HBeAg result can indicate that the patient is either susceptible to HBV infection due to no past exposure, is in the recovery phase with HBV, or is immune to HBV due to a resolved past infection or vaccination.

Cutoff determination

The cutoff value was established with in-house studies on the Elecsys 2010 analyzer and verified on the MODULAR ANALYTICS E170 analyzer by measuring a panel of 192 samples. A Receiver Operator Curve (ROC) analysis was used to verify the cutoff. The cutoff sensitivity of the Elecsys HBeAg immunoassay was determined to be around 0.24 PEI U/mL. Validation of the cutoff was performed by external clinical studies.

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), biotin (< 164 nmol/L or < 40 ng/mL), total protein < 12 g/dL and HAMA < 805 ng/mL.

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.

Drug interference studies were performed in vitro, and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.

There is no high-dose hook effect at HBeAg concentrations up to 330 U/mL (PEI U/mL).

A reactive HBeAg result does not exclude co-infection by another hepatitis virus. False positive results due to non-specific reactivity cannot be ruled out with the Elecsys HBeAg assay.

A non-reactive test result does not exclude the possibility of exposure to HBV. Negative HBeAg results may occur during early infection due to delayed seroconversion. False negative results may occur due to antigen levels below the detection limit of this assay or if the patient's antigen does not react with the antibody used in this test.

Results obtained with the Elecsys HBeAg assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

In rare cases, interference due to extremely high titers of antibodies to immunological components, streptavidin and 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.

Specific performance data

Representative performance data on the MODULAR ANALYTICS E170 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined on one MODULAR ANALYTICS E170 analyzer at one site using one lot of Elecsys reagent to test 5 serum pools and 2 controls according to the CLSI (Clinical and Laboratory Standards Institute) guidelines EP5-A2: 2 runs per day with 2 replicates each for 12 days (n = 48). Results are presented below.

MODULAR ANALYTICS E170 analyzer					
		Within-run precision		**Within-day precision**	
Sample	Mean COI[f)]	SD COI	CV %	SD COI	CV %
HS[g)]1, high negative	0.886	0.040	4.5	0.000	0.00
HS2, high negative	0.913	0.038	4.2	0.000	0.00
HS3, low positive	1.15	0.041	3.6	0.000	0.00
HS4, low positive	1.13	0.049	4.3	0.000	0.00
HS5, positive	2.70	0.116	4.3	0.072	2.7
PC[h)] HBeAg 1	0.091	0.004	4.5	0.003	3.3
PC HBeAg 2	19.0	0.210	1.1	0.143	0.75

f) COI = cutoff index

g) HS = human serum

h) PC = PreciControl

MODULAR ANALYTICS E170 analyzer					
		Between-days		**Total precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS1, high negative	0.886	0.017	2.0	0.043	4.9
HS2, high negative	0.913	0.020	2.2	0.043	4.7
HS3, low positive	1.15	0.015	1.3	0.044	3.8
HS4, low positive	1.13	0.035	3.1	0.060	5.3
HS5, positive	2.70	0.000	0.0	0.137	5.1
PC HBeAg 1	0.091	0.002	2.7	0.006	6.2
PC HBeAg 2	19.0	0.336	1.8	0.421	2.2

Reproducibility study

Precision was further evaluated at three external sites incorporating between-run, between-day, between-lot, and between-site variation. A reproducibility study was conducted following CLSI EP5-A2 and CLSI EP15-A2 at 3 sites incorporating a 7 member panel consisting of 5 human serum pools and Elecsys PreciControl HBeAg 1 and 2 that were assayed for 5 days, 2 runs per day, 3 replicates per run. Data from all 3 reagent lots (two lots at each site: AB, BC, AC), were combined to achieve SD and percent CV for repeatability (within-run), between-run, between-day, between-lot, between-site and reproducibility (n = 180). The overall imprecision data are summarized in the following tables:

MODULAR ANALYTICS E170 analyzer							
		Repeatability		**Between-run**		**Between-day**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %	SD COI	CV %
HS1	0.853	0.017	2.0	0.017	1.9	0.019	2.2
HS2	0.939	0.018	1.9	0.003	0.3	0.022	2.3
HS3	1.08	0.022	2.0	0.011	1.1	0.025	2.3
HS4	1.24	0.025	2.0	0.012	1.0	0.033	2.6
HS5	2.42	0.043	1.8	0.039	1.6	0.046	1.9
PC[i)]1	0.099	0.007	7.4	0.007	7.0	0.000[j)]	0.0
PC2	12.1	0.171	1.4	0.182	1.5	0.169	1.4

i) PC = PreciControl HBeAg

j) SD of 0.000 because the variance was below the stated significant figures

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MODULAR ANALYTICS E170 analyzer							
		Between-lot		**Between-site**		**Total**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %	SD COI	CV %
HS1	0.853	0.095	11.2	0.000*	0.0	0.100	11.7
HS2	0.939	0.086	9.2	0.000*	0.0	0.091	9.7
HS3	1.08	0.111	10.4	0.000*	0.0	0.117	10.9
HS4	1.24	0.105	8.4	0.000*	0.0	0.113	9.1
HS5	2.42	0.240	9.9	0.000*	0.0	0.251	10.4
PC1	0.099	0.017	16.7	0.000*	0.0	0.019	19.6
PC2	12.1	0.685	5.7	0.000*	0.0	0.748	6.2

*SD of 0.000 because the variance was below the stated significant figures.

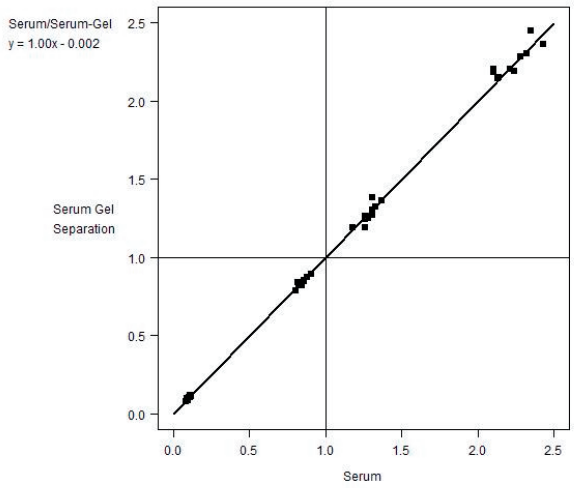
Drug interference

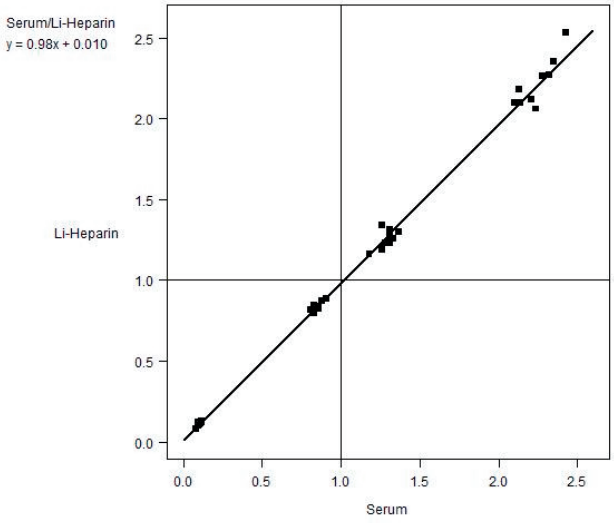
A drug interference study was performed with 21 common therapeutic drugs. Each drug was tested three-fold spiked into a negative, a low positive and a positive sample. Each drug was found to be non-interfering at the following concentrations:

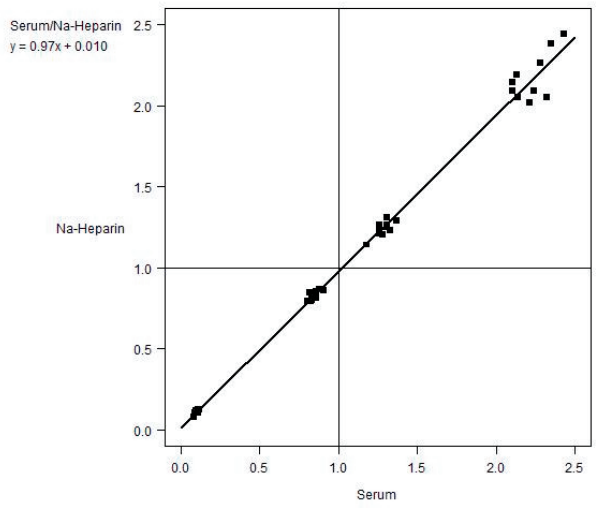
Compound	**Concentration**
Acetyl cysteine	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Tetracycline	50 mg/L
Acetylsalicylic acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L
PEG interferon alpha 2a	0.036 mg/L
Zidovudine	500 mg/L
Acyclovir	600 mg/L

Matrix effects

Studies were conducted to evaluate the suitability of the following 5 types of blood collection tubes: serum/gel separation tubes, lithium heparin plasma, sodium heparin plasma, K_2-EDTA plasma and sodium citrate plasma. Samples were collected into matched serum and plasma collection tubes from 40 donors and assayed in triplicate. The study was conducted using negative, high-negative, low-positive and positive samples for HBeAg. The results are shown below.







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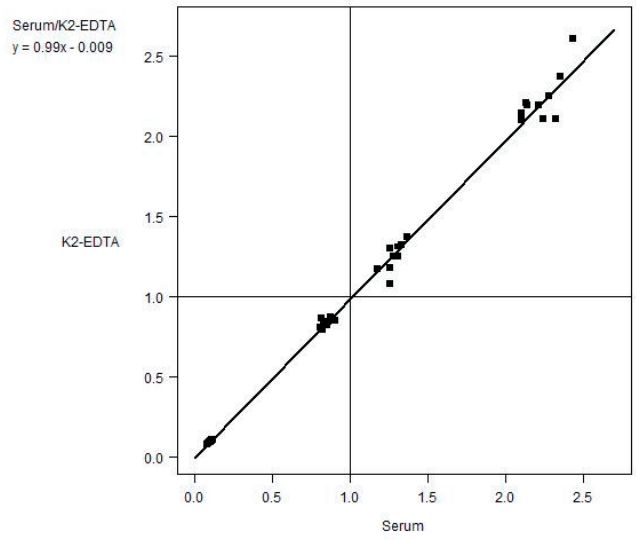

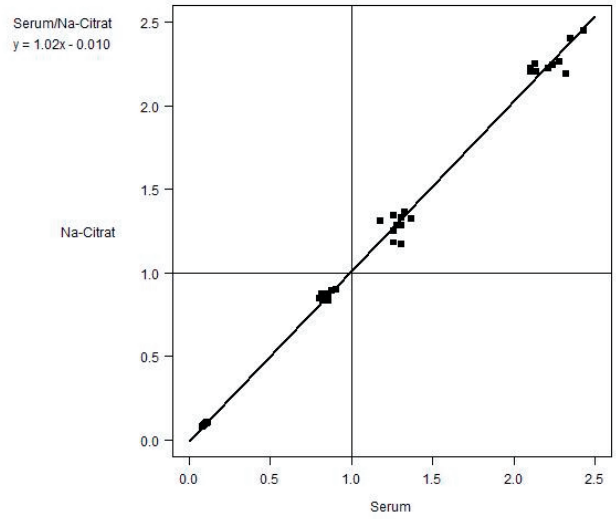

Analytical specificity

A study was conducted to evaluate the Elecsys HBeAg assay on the MODULAR ANALYTICS E170 analyzer for potential cross-reactivity in specimens from individuals with medical conditions unrelated to hepatitis B infection. All specimens in the study were evaluated with the Elecsys HBeAg assay and the reference assay. The results are summarized in the following table:

Reactivity of the Elecsys HBeAg assay in individuals with medical conditions unrelated to hepatitis B infection

Category	Reference HBeAg assay				Total
	Reactive		Non-reactive		
	Elecsys HBeAg assay				
	RX[k)]	NR[l)]	RX	NR	
Autoimmune (AMA, ANA, SLE)	0	0	0	15	15
Cytomegalovirus (anti-CMV)	0	0	0	12	12
Epstein-Barr Virus (anti-EBV)	0	0	0	12	12
E. coli infection	0	0	0	12	12
Flu Vaccination	0	0	0	10	10
Hepatitis A Virus (anti-HAV)	0	0	0	10	10
HAV Vaccination	0	0	0	10	10

Reactivity of the Elecsys HBeAg assay in individuals with medical conditions unrelated to hepatitis B infection

Category	Reference HBeAg assay				Total
	Reactive		Non-reactive		
	Elecsys HBeAg assay				
	RX[k)]	NR[l)]	RX	NR	
HBV Vaccination	0	0	0	10	10
Hepatitis C Virus (anti-HCV)	0	0	0	12	12
Hepatitis D Virus (anti-HDV)	0	0	0	4	4
Hepatitis E Virus (anti-HEV)	0	0	0	12	12
Human Immunodeficiency Virus (anti-HIV-1)	0	0	0	12	12
Herpes Simplex Virus (anti-HSV)	0	0	0	12	12
HTLV I / II (anti-HTLV)	0	0	0	12	12
Non-Viral Liver Disease	0	0	0	40	40
Parvovirus B19 infection	0	0	0	12	12
Pregnancy	0	0	0	12	12
Rheumatoid factor	0	0	0	11	11
Rubella (anti-Rubella)	0	0	0	12	12
Syphilis	0	0	0	12	12
Toxoplasmosis (anti-Toxo)	0	0	0	12	12
Varicella Zoster Virus (anti-VZV)	0	0	0	12	12
Total	0	0	0	278	278

k) RX = reactive

l) NR = non-reactive

Potential cross-reactivity due to viral- and bacterial-materials

The purpose of this study was to determine if the bacterial and viral antigens present in the culture would interfere with the Elecsys HBeAg assay, a viral hepatitis antigen assay. The results of the Elecsys HBeAg assay testing of the blank control and spiked specimens are presented below.

Aliquot culture level	Serum pool	
	HBeAg non-reactive (COI)	HBeAg reactive (COI)
Blank, no spike (Reference)	0.088	2.88
S. aureus at 900 cfu/mL	0.096	5.88
S. aureus at 9000 cfu/mL	0.091	2.68
P. aeruginosa at 900 cfu/mL	0.088	2.92
P. aeruginosa at 9000 cfu/mL	0.095	2.67
E. coli at 900 cfu/mL	0.092	2.85
E. coli at 9000 cfu/mL	0.096	2.59
EBV at 0.9 µg/mL	0.094	2.99
EBV at 0.9 ng/mL	0.088	2.78
Cytomegalovirus at 0.9 µg/mL	0.090	3.75
Cytomegalovirus at 0.9 ng/mL	0.096	2.63
Rubella at 0.9 µg/mL	0.093	3.46
Rubella at 0.9 ng/mL	0.096	2.80
Varicella Zoster Virus at 0.9 µg/mL	0.094	3.16
Varicella Zoster Virus at 0.9 ng/mL	0.094	2.53

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys HBeAg assay was shown by testing eleven commercially-sourced seroconversion panels in comparison to that of the reference assay.

The comparison of seroconversion timing in days for the Elecsys HBeAg assay on the MODULAR ANALYTICS E170 module and for the Reference HBeAg assay for each panel is shown in the table below.

	Reference HBeAg assay		Elecsys HBeAg assay		Reactivity difference in days between Elecsys and Reference	Disease Status
Panel ID	NR	RX	NR	RX		
6278	12	16	12	16	0	Acute
6281	22	33	N/A	N/A	N/A[m)]	Acute to early recovery
6282	21	26	21	26	0	Acute
6284	61	64	61	64	0	Acute
9072	108	128	128	135	-7 (1 draw)	Early acute
11015	43	70	43	70	0	Potentially acute or chronic
11024	43	49	49	54	-5 (1 draw)	Chronic
PHM933	16	144	16	144	0	Acute
PHM934	10	14	10	14	0	Acute
PHM935A	30	35	30	35	0	Acute
PHM935B	203	128[n)]	203	128[n)]	0	Early recovery

m) The entire panel was NR with the Elecsys HBeAg assay.

n) No initial negative samples were available for this panel.

Testing of eleven seroconversion panels generated data that reflected the seroconversion sensitivity of the Elecsys HBeAg assay. Overall seroconversion performance was comparable to the reference performance.

Expected values

The clinical study population for the Elecsys HBeAg immunoassay consisted of 1842 subjects. Of these 1641 were enrolled prospectively. In addition 201 retrospective samples were also tested. A demographic summary of the overall adult specimen population by age, race and gender is provided in the following tables:

Demographic Summary of Adult Specimen Population by Age

	Asymptomatic		Symptomatic		Supplemental		Overall	
Age group	n	%	n	%	n	%	n	%
21-30	172	15.4	75	14.4	56	27.9	303	16.4
31-40	244	21.8	87	16.7	29	14.4	360	19.5
41-50	435	38.8	199	38.2	30	14.9	664	36.0
51-60	239	21.3	131	25.1	22	10.9	392	21.3
61-70	25	2.23	26	4.99	14	6.96	65	3.53
71-80	5	0.00	3	0.58	13	6.47	21	1.14
> 80	-	-	-	-	1	0.50	1	0.05
Unknown	-	-	-	-	36	17.9	36	1.95
Total	1120	100	521	100	201	100	1842	100

Demographic Summary of Adult Specimen Population by Race

Ethnicity	Asympto-matic		Sympto-matic		Supple-mental		Total	
Race	n	%	n	%	n	%	n	%
American Indian/ Alaska Native	7	0.62	3	0.58	0	0.00	10	0.54
Asian	2	0.18	4	0.77	10	4.98	16	0.87
African American/ Black	645	57.5	237	45.5	7	3.48	889	48.2
Caucasian/White	438	39.1	274	52.6	8	3.98	720	39.1
Pacific Islander	2	0.18	2	0.38	0	0.00	4	0.22
Unknown	0	0.00	0	0.00	138	68.7	138	7.49
Other	26	2.32	1	0.19	38	18.9	65	3.53
Total	1120	100	521	100	201	100	1842	100

Demographic Summary of Adult Specimen Population by Gender

Gender	Asymptomatic		Symptomatic		Supplemental		Total	
	n	%	n	%	n	%	n	%
Male	801	71.5	377	72.4	121	60.2	1299	70.5
Female	319	28.5	144	27.6	43	21.4	506	27.5
Unknown	0	0.00	0	0.00	37	18.4	37	2.01
Total	1120	100	521	100	201	100	1842	100

The table below summarizes the expected (observed) values with the Elecsys HBeAg immunoassay and Elecsys PreciControl with different study populations.

Elecsys HBeAg Test Results from Different Studies

Patient population	No. tested	Gender			Age range	% Pos
		% Male	% Female	% Unknown		
Prospective: High risk	1120	71.5	28.5	-	21 to 79	3.21
Prospective: Symptomatic	521	72.4	27.6	-	21 to 73	1.34
Retrospective	201	60.2	21.4	17.9	21 to 81	31.8

Summary of clinical performance

A multicenter study was conducted to evaluate the ability of the Elecsys HBeAg assay on the MODULAR ANALYTICS E170 analyzer to detect HBe antigen in specimens from an intended use population.

Of the total 1641 adult specimens tested in the Elecsys HBeAg prospective clinical study, 1120 were in the asymptomatic at-risk for HBV group and 521 were in the symptomatic group. To supplement the study, 201 samples were obtained from subjects with increased risk for hepatitis due to living in areas endemic for hepatitis or who were potential candidates for acute disease or reactive HBeAg status.

The prospective samples for these cohorts were collected from multiple US sites including Miami, Florida; Los Angeles, CA; Newark, NJ and Atlanta, GA. Final analysis included 1641 subjects with 71.8 % male and 28.2 % female subjects. Ages for both cohorts ranged from 21 to 80 with a median of 43.0 years.

The Elecsys HBeAg assay was evaluated at three clinical laboratories to assess the performance of the assay in a testing environment which most closely resembles that of the final user. Results were compared to a reference HBeAg detection assay currently marketed in the US.

Results by specimen classification

HBV classifications were determined based on the constellation of test results from an FDA-approved HBV marker panel. Using the reference HBeAg assay, the specimens were assigned an HBV status based on the algorithm provided in the following table:

HBeAg

Hepatitis B e antigen

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Serological Classification by HBV Markers						
	HBsAg	**HBeAg**	**Anti-HBc IgM**	**Anti-HBc**	**Anti-HBe**	**Anti-HBs**
Acute	(+)	(+)	(+)	(+)	(-)	(-), eq
Acute	(+)	(+), (-)	(+)	(+)	(+), qns	(-)
Acute	(+)	(+)	(-)	(-)	(-)	(-)
Acute	(+)	(+)	(eq)	(+)	(+)	(-)
Acute	(+)	(-)	(eq)	(+)	(+)	(-)
Acute (late)	(+)	(-)	(+)	(+)	(+)	(+), eq
Chronic	(+) > 6 mo.	(-)	(-)	(+)	(+), eq, (-)	(-)
Chronic	(+) > 6 mo.	(+)	(eq)	(+)	(-)	(-)
Chronic	(+)	(+)	(-)	(+)	(-)	(-), (+), eq
Chronic	(+)	(+)	(+)	(+)	(-), (+)	(+)
Chronic	(+)	(+)	(-)	(+)	(+)	(-)
Chronic	(+)	(-)	(-)	(+)	(+), eq	(-)
Early recovery	(-)	(-)	(-)	(+)	(+), (-), eq,qns	(-)
Early recovery	(-)	(-)	(+), eq	(+)	(+)	(+)
Early recovery	(-)	(-)	eq	(+)	(+)	eq
Recovery	(-)	(-)	(-)	(+),(-)	(+)	(+)
Recovery	(-)	(-)	(-)	(+)	(+)	eq
Recovery	(-)	(-)	(-)	(+)	eq	(+)
Recovered or Immune due to natural infection	(-)	(-)	(-)	(+)	(-)	(+), eq
HBV vaccine response	(-)	(-)	(-)	(-)	(-)	(+)
HBV vaccine response (?)	(-)	(-)	(-)	(-)	(-)	eq
Not previously infected	(-), rr uncnf	(-)	(-)	(-)	(-)	(-)
Not interpretable	qns	(-)	(-), (+)	(+)	(+)	(-)
Not interpretable	qns	(-)	(-)	(-)	(-)	(-)
Not interpretable	qns	(+)	(-)	(+)	(-)	(-)
Not interpretable	(-)	(-)	(-)	qns	(-)	(+)
Not interpretable	(-)	(-)	(-)	(-)	(+)	(-)
Not interpretable	(-)	(-)	(-)	(-)	(-)	nd
Not interpretable	(-)	(-)	(-)	(+)	qns	(+)
Not interpretable	(-)	(-)	(-)	nd	(-)	(+), (-), qns
Not interpretable	(-)	(-)	(-)	nd	(+)	(-)

Serological Classification by HBV Markers						
	HBsAg	**HBeAg**	**Anti-HBc IgM**	**Anti-HBc**	**Anti-HBe**	**Anti-HBs**
Not interpretable	(-)	(+)	(-)	(-)	(-)	(+), (-)
Not interpretable	(+)	(+)	nd	(+)	(+), (-)	(-)

Key: **nd** = not done; **qns** = testing incomplete due to inadequate sample volume; **eq** = equivocal or indeterminate or borderline; **rr uncnf** = repeatedly reactive HBsAg with (-) confirmatory testing

Results by HBV classification

Adult asymptomatic at risk population

The table below summarizes the percent agreement between the Elecsys HBeAg assay and the reference HBeAg assay for samples classified by disease status in the asymptomatic at risk cohort. The table also provides the upper and lower 95 % confidence interval.

HBV classification	**Positive percent agreement % (n/N)o)**	**95 % Score confidence interval**	**Negative percent agreement % (n/N)**	**95 % Score confidence interval**
Acute	100 (12/12)	75.8-100	100 (1/1)	20.7-100
Chronic	100 (21/21)	84.5-100	95.7 (22/23)	79.0-99.2
Early recovery	N/A	N/A	100 (75/75)	95.1-100
Recovery	N/A	N/A	100 (172/172)	97.8-100
Recovered	N/A	N/A	100 (105/105)	96.5-100
HBV vaccination	N/A	N/A	100 (219/219)	98.3-100
Not previously infected	N/A	N/A	100 (483/483)	99.2-100
Not interpretable	100 (3/3)	43.9-100	100 (6/6)	61.0-100
Total	100 (36/36)	90.4-100	99.9 (1083/1084)	99.5-99.98

o) n/N = number of results over total number tested

Adult symptomatic at risk population

The table below summarizes the percent agreement between the Elecsys HBeAg assay and the reference HBeAg assay for samples classified by disease status in the symptomatic at risk cohort. The table also provides the upper and lower 95 % confidence interval.

HBV classification	**Positive percent agreement % (n/N)**	**95 % Score confidence interval**	**Negative percent agreement % (n/N)**	**95 % Score confidence interval**
Acute	100 (1/1)	20.7-100	N/A	N/A
Chronic	100 (4/4)	51.0-100	100 (7/7)	64.6-100
Early recovery	N/A	N/A	100 (37/37)	90.6-100
Recovery	N/A	N/A	100 (71/71)	94.9-100
Recovered	N/A	N/A	100 (34/34)	89.9-100
HBV vaccination	N/A	N/A	100 (136/136)	97.3-100
Not previously infected	N/A	N/A	100 (227/227)	98.3-100
Not interpretable	66.7 (2/3)	20.8-93.9	100 (1/1)	20.7-100
Total	87.5 (7/8)	52.9-97.8	100 (513/513)	99.3-100

Combined adult asymptomatic and symptomatic at risk population

The table below summarizes the percent agreement between the Elecsys HBeAg assay and the reference HBeAg assay for samples in the combined asymptomatic and symptomatic at risk cohorts.

HBeAg

Hepatitis B e antigen

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HBV classification	Positive percent agreement % (n/N)[p)]	95 % Score confidence interval	Negative percent agreement % (n/N)	95 % Score confidence interval
Acute	100 (13/13)	77.2-100	100 (1/1)	20.7-100
Chronic	100 (25/25)	86.7-100	96.7 (29/30)	83.3-99.4
Early recovery	N/A	N/A	100 (112/112)	96.7-100
Recovery	N/A	N/A	100 (243/243)	98.4-100
Recovered	N/A	N/A	100 (139/139)	97.3-100
HBV vaccination	N/A	N/A	100 (355/355)	98.9-100
Not previously infected	N/A	N/A	100 (710/710)	99.5-100
Not interpretable	83.3 (5/6)	43.9-97.0	100 (7/7)	64.6-100
Total	97.7 (43/44)	88.2-99.6	99.9 (1596/1597)	99.7-99.99

p) n/N = number of results over total number tested

Adult supplemental at risk population

The table below summarizes the percent agreement between the Elecsys HBeAg assay and the reference HBeAg assay for samples classified by disease status in the supplemental at risk cohort. The table also provides the upper and lower 95 % confidence interval.

HBV classification	Positive percent agreement % (n/N)	95 % Score confidence interval	Negative percent agreement % (n/N)	95 % Score confidence interval
Acute	91.5 (43/47)	80.1-96.6	100 (22/22)	85.1-100
Chronic	90.0 (18/20)	69.9-97.2	100 (13/13)	77.2-100
Early recovery	N/A	N/A	100 (11/11)	74.1-100
Recovery	N/A	N/A	100 (7/7)	64.6-100
Recovered	N/A	N/A	100 (4/4)	51.0-100
HBV vaccination	N/A	N/A	100 (23/23)	85.7-100
Not previously infected	N/A	N/A	100 (35/35)	90.1-100
Not interpretable	75.0 (3/4)	30.6-95.4	100 (15/15)	79.6-100
Total	90.1 (64/71)	81.0-95.1	100 (130/130)	97.1-100

References

1 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, 2977–3029.

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

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

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

7 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).

Limited License

The Elecsys HBeAg assay shall not be used by blood banks, donor centers, or other institutions which exclusively or predominantly use the test for the safety or screening of blood and blood products.

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

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US Customer Technical Support 1-800-428-2336

Roche

07049510001V1.3

PreciControl HBeAg

cobas®

REF **11876376** 160 **16 x 1.3 mL**

English

For use in the USA only

Intended use

Elecsys PreciControl HBeAg is used for quality control of the Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 analyzer.

Summary

PreciControl HBeAg is a set of ready-for-use controls supplied in buffer for the positive concentration range and in human serum for the negative concentration range. The controls are used for monitoring the performance of the Elecsys HBeAg immunoassay.

Reagents - working solutions

- PC HBEAG1: 8 bottles, each containing 1.3 mL of control human serum, negative for HBeAg; preservatives (N-Methylisothiazolone and Oxy-pyrion).
  Target range for the cutoff index: 0-0.5
- PC HBEAG2: 8 bottles, each containing 1.3 mL of control positive for HBeAg (*E. coli*, rDNA derived) approximately 2.5 U/mL (Paul-Ehrlich-Institute units) in HEPES[a)] buffer, pH 7.4 with protein and sugar stabilizers and preservatives (N-Methylisothiazolone and Oxy-pyrion).
  Target value for cutoff index: approximately 13

The exact target ranges (target value ± 44 %), given in the form of a cutoff index, are encoded in the barcodes as well as printed on the enclosed (or electronically available) value sheet.

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

Target values and ranges

The target values and ranges were determined and evaluated by Roche. They were obtained using the Elecsys HBeAg assay reagents and analyzers available at the time of testing.

Traceability information is given in the Method Sheet of the relevant Elecsys assay.

Results must be within the specified ranges. In the event that increasing or decreasing trends, or any other suddenly occurring deviations beyond the range limits are observed, all test steps must be checked.

When necessary, measurement of the patient sample tested should be repeated.

Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination, must be entered manually. Therefore always refer to the value sheet included in the rackpack 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.

Note: The negative and positive PreciControls are in two different matrices. The negative control is human serum. The positive control is in HEPES buffer with protein and sugar stabilizers containing recombinant HBeAg protein from *E. coli.*

Precautions and warnings

For in vitro diagnostic use only.
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.

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.
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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.[1,2]

The controls should not be used as calibrators.

The controls may not be used after the expiration date.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Handling

The controls are supplied ready-for-use in bottles compatible with the system. The controls should only be left on the analyzer during performance of quality control. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 7 quality control procedures per bottle should be performed.

Storage and stability

Store at 2-8 °C.

Store controls **upright** in order to prevent the control solution from adhering to the snap-cap.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	up to 6 hours

Materials provided

- PreciControl HBeAg, control barcode sheet

Materials required (but not provided)

- MODULAR ANALYTICS E170 immunoassay analyzer and assay reagents

See the assay Method Sheet and the operator's manual for additionally required material.

Assay

Treat the control serum in the system-compatible labeled bottles for analysis in the same way as patient samples.

Enter the barcode information on the control vial label into the analyzer.

Ensure the controls are at 20-25 °C prior to measurement.

Run controls daily in parallel with patient samples, once per reagent kit, and whenever a calibration is performed. The control intervals and limits should be adapted to each laboratory's individual requirements.

Follow the applicable government regulations and local guidelines for quality control.

References

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

2 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.

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→	Volume after reconstitution or mixing

PreciControl HBeAg

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