Elecsys HBeAg

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REF		Σ	SYSTEM
07027427190	07027427501	300	**cobas e** 402 **cobas e** 801

English

For use in the USA only

System information

Short name	ACN (application code number)
HBEAG	10036

Warning

- Federal law restricts this device to sale 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

Immunoassay 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 **cobas e** immunoassay analyzers.

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 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 WHO[b)] in HEPES[c)] buffer, pH 7.4; preservative.

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

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

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

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Contact phone: 1-800-428-2336

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.[6,7]

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.

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 in a sufficient number and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA, and Na-citrate plasma.

Samples are stable for 4 days at 20-25 °C, 7 days at 2-8 °C, 3 months at -20 °C (± 5 °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 and thawed samples 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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 11876376160, PreciControl HBeAg, 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 11298500160, 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:

- 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.

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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.

Note: The recommended negative and positive PreciControls are in 2 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.

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) 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[e)]	**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.

e) COI = cutoff index

Final Elecsys HBeAg assay result			
COI	**Result after retest (COI)**	**Final results**	**Interpretation of results**
< 0.9	No retest required	NON-REACTIVE[f)]	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.

f) 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

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.

Endogenous interferences

The effect of the following endogenous substances and pharmaceutical compounds on the Elecsys HBeAg assay performance was tested on the MODULAR ANALYTICS E170 analyzer. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Interferent tested	No interference up to
Bilirubin	428 µmol/L or 25 mg/dL
Hemoglobin	0.99 mmol/L or 1.6 g/dL
Intralipid	1500 mg/dL
Biotin	164 nmol/L or 40 ng/mL
Total protein	12 g/dL
HAMA (human anti-mouse antibody)	805 ng/mL

Drug interference

A drug interference study was performed with 21 common therapeutic drugs. This study was performed on the MODULAR ANALYTICS E170 analyzer. Each drug was tested 3-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/L
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L

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Compound	Concentration
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

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

Specific performance data

Representative performance data are given below. The precision data was generated on the **cobas e** 402 and **cobas e** 801 analyzers. However, since the **cobas e** 402 and **cobas e** 801 analyzers are members of the Elecsys instrument family of analyzers, some of the data below may have been generated on other members of the Elecsys instrument family. Results obtained in individual laboratories may differ.

Precision

Precision was determined on the **cobas e** 402 and **cobas e** 801 analyzers using 1 lot of Elecsys reagent, spanning 2 calibration cycles, to test 6 (**cobas e** 801) and 8 (**cobas e** 402) human serum pools and 2 controls and calculated according to CLSI (Clinical and Laboratory Standards Institute) guideline EP05-A3. Each sample was separated in 2 aliquots measured in duplicate on 2 runs per day for 12 days (n = 96). The following results were obtained:

cobas e 801 analyzer					
		Repeatability[g]		**Intermediate precision**[h]	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
Human serum 1	0.109	0.004	3.4	0.004	3.5
Human serum 2	0.780	0.012	1.5	0.016	2.1
Human serum 3	0.858	0.011	1.2	0.019	2.2
Human serum 4	1.13	0.018	1.6	0.033	2.9
Human serum 5	1.17	0.019	1.7	0.028	2.4
Human serum 6	2.47	0.032	1.3	0.044	1.8
PreciControl HBeAg 1	0.101	0.003	2.8	0.003	3.2
PreciControl HBeAg 2	16.7	0.115	0.7	0.258	1.5

g) Repeatability = within-run precision

h) Intermediate precision = between-run precision

cobas e 402 analyzer					
		Repeatability[g]		**Intermediate precision**[h]	
Sample Material	Mean COI	SD COI	CV %	SD COI	CV %
Human serum 1	25.3	0.337	1.3	1.03	4.1
Human serum 2	1.25	0.016	1.3	0.046	3.7
Human serum 3	0.875	0.011	1.2	0.031	3.5
Human serum 4	1.16	0.012	1.0	0.043	3.7
Human serum 5	1.01	0.011	1.1	0.039	3.9
Human serum 6	0.068	0.003	4.3	0.004	5.7
Human serum 7	0.915	0.009	1.0	0.030	3.3
Human serum 8	1.14	0.016	1.4	0.042	3.7
PreciControl HBeAg 1	0.082	0.003	4.3	0.004	4.7
PreciControl HBeAg 2	12.3	0.116	0.9	0.541	4.4

Reproducibility

Precision was evaluated incorporating between-run, between-day, and between-site variation with 3 **cobas e** 402 and 3 **cobas e** 801 analyzers (3 different sites: 2 external and 1 internal). A reproducibility panel was conducted following CLSI EP05-A3 consisting of 6 human sera and 2 levels of PreciControl (1 positive and 1 negative) that were assayed for 5 days, 2 runs per day, 3 replicates per run. Calibration was performed on day 1. Sample distribution was as follows:

- 1 negative sample (target ≤ 0.5 COI)
- 2 high negative samples (at approximately C_5 concentration) (range 0.6 - < 0.9 COI)
- 2 positive samples (at approximately C_{95} concentration) (range 1.1 - 1.4 COI)
- 1 moderate positive sample (approximately 2-3 times the concentration of the clinical cut-off) (range 1.6 - 3.0 COI)

The overall reproducibility (imprecision) data on the **cobas e** 402 and **cobas e** 801 analyzers are summarized in the following tables:

cobas e 801 analyzer							
		Repeatability		**Between-run**		**Between-day**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %	SD COI	CV %
HS[i]1	0.105	0.005	4.6	0.0002	0.1	0.002	2.0
HS2	0.770	0.019	2.4	0.010	1.3	0.008	1.0
HS3	0.854	0.014	1.6	0.000	0.0	0.010	1.2
HS4	1.15	0.020	1.7	0.006	0.5	0.015	1.3
HS5	1.16	0.020	1.7	0.010	0.9	0.009	0.7
HS6	2.42	0.039	1.6	0.022	0.9	0.029	1.2
PC[j]1	0.104	0.004	3.6	0.001	1.4	0.000	0.0
PC2	17.3	0.231	1.3	0.181	1.0	0.261	1.5

i) HS = human serum

j) PC = PreciControl HBeAg

cobas e 801 analyzer					
		Between-site		**Reproducibility**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS1	0.105	0.012	11.2	0.013	12.3
HS2	0.770	0.035	4.5	0.042	5.4
HS3	0.854	0.026	3.0	0.031	3.6
HS4	1.15	0.032	2.8	0.041	3.6
HS5	1.16	0.039	3.3	0.046	3.9
HS6	2.42	0.087	3.6	0.102	4.2
PC[j]1	0.104	0.010	9.8	0.011	10.5
PC2	17.3	0.239	1.4	0.459	2.7

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cobas e 402 analyzer							
		Repeatability		**Between-run**		**Between-day**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %	SD COI	CV %
HS[i])1	2.98	0.053	1.8	0.062	2.1	0.097	3.2
HS2	1.22	0.028	2.3	0.017	1.4	0.036	3.0
HS3	1.22	0.013	1.1	0.016	1.3	0.036	3.0
HS4	0.823	0.009	1.1	0.010	1.2	0.023	2.7
HS5	0.910	0.009	1.0	0.014	1.5	0.027	3.0
HS6	0.083	0.003	3.8	0.000	0.1	0.002	2.2
PC[i])1	0.099	0.003	3.3	0.002	2.4	0.001	1.0
PC2	13.1	0.187	1.4	0.231	1.8	0.512	3.9

cobas e 402 analyzer					
		Between-site		**Reproducibility**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS1	2.98	0.095	3.2	0.158	5.3
HS2	1.22	0.031	2.6	0.058	4.8
HS3	1.22	0.029	2.3	0.050	4.1
HS4	0.823	0.021	2.6	0.034	4.1
HS5	0.910	0.026	2.8	0.041	4.5
HS6	0.083	0.004	5.1	0.006	6.8
PC[i])1	0.099	0.004	4.1	0.006	5.9
PC2	13.1	0.268	2.0	0.65	5.0

Method comparison

A method comparison study was performed to compare the Elecsys HBeAg immunoassay on the **cobas e** 801 analyzer with the MODULAR ANALYTICS E170 analyzer. 1 set of 220 samples was tested on 3 different MODULAR ANALYTICS E170 analyzers and 3 different **cobas e** 801 analyzers.

The median of the values measured on the 3 MODULAR ANALYTICS E170 analyzers was taken as the reference value used for comparison to the values generated on each of the 3 different **cobas e** 801 analyzers (220 samples were assayed, therefore 660 results were obtained on the 3 **cobas e** 801 analyzers).

The negative and positive percent agreement (NPA and PPA) rates are presented in the following tables:

	Median value MODULAR ANALYTICS E170 analyzer			
cobas e 801 analyzer	Non-reactive < 0.9	Border 0.9 ≤ x < 1.1	Reactive ≥ 1.1	Total
Non-reactive < 0.9	305	17	0	322
Border 0.9 ≤ x < 1.1	4	16	5	25
Reactive ≥ 1.1	0	0	313	313
Total	309	33	318	660

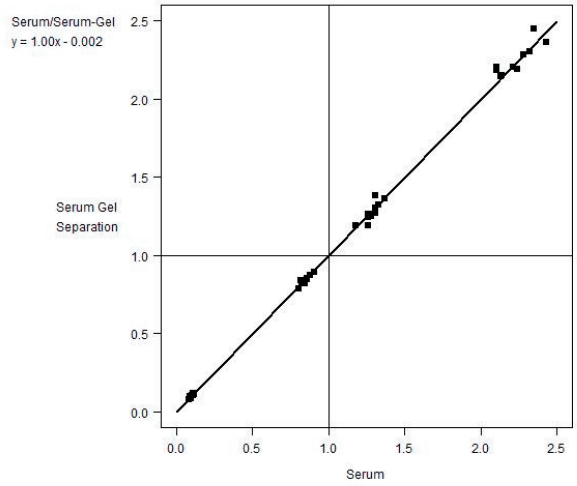
	Absolute	Relative	Two-sided 95 % CI
NPA	305/309	98.71 %	96.72; 99.50
PPA	313/318	98.43 %	96.37; 99.33

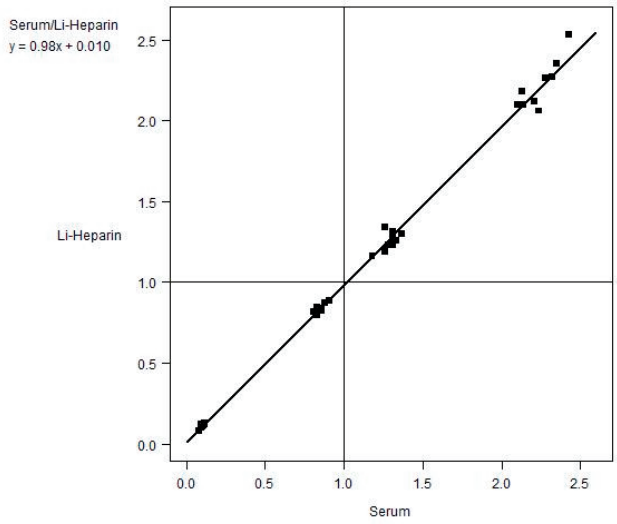
Of the 660 determinations, 26 determinations close to the cut-offs (0.9 COI and 1.1 COI) showed a discrepant result between the median value of the MODULAR ANALYTICS E170 analyzers and the result on at least 1 of 3 **cobas e** 801 analyzers in the 3 sites. Samples affected are borderline or close to the C_{95} concentration on MODULAR ANALYTICS E170 analyzer.

The Method Comparison study was verified on the **cobas e** 402 analyzer showing equivalent performance to **cobas e** 801 analyzer.

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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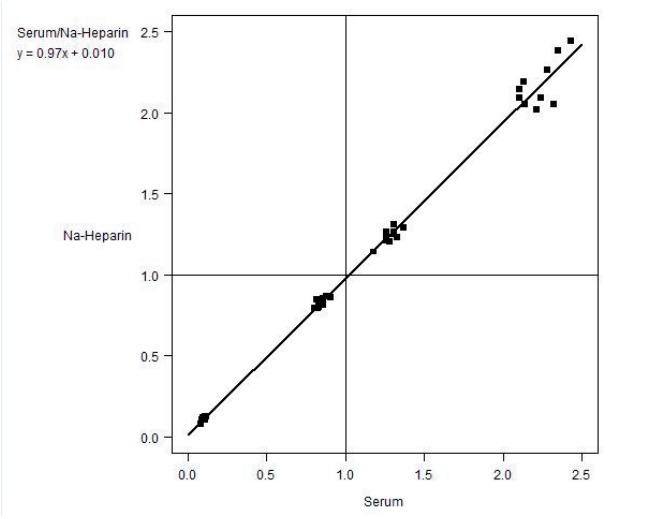

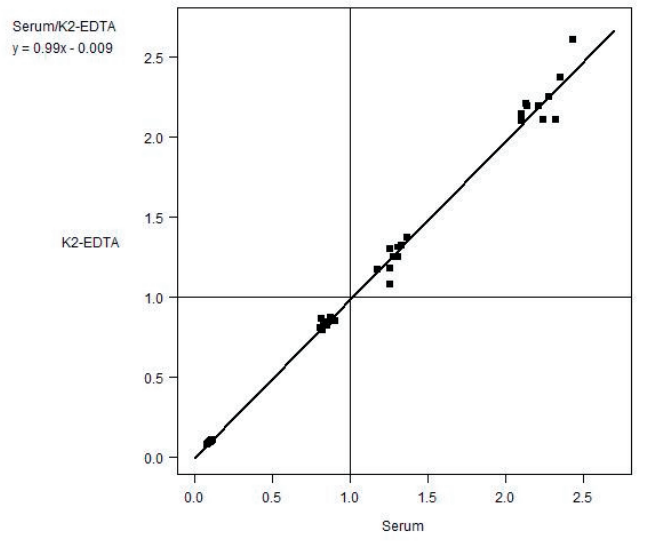

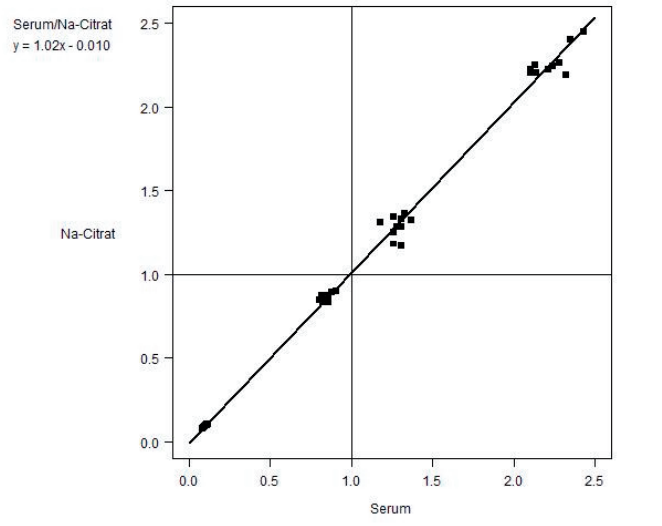

Analytical specificity

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
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

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Aliquot culture level	Serum pool	
	HBeAg non-reactive (COI)	HBeAg reactive (COI)
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

Eleven (11) well-characterized seroconversion panels were tested with the Elecsys HBeAg assay on the MODULAR ANALYTICS E170 analyzer and on the **cobas e** 801 analyzer in parallel to show comparable performance.

Seroconversion sensitivity of Elecsys HBeAg assay on the MODULAR ANALYTICS E170 analyzer compared to the **cobas e** 801 analyzer

Panel ID	Results	Number of bleeds		Discrepant results
		MODULAR ANALYTICS E170	cobas e 801	
Panel 1	Non-reactive	4	4	0
	Reactive	1	1	
Panel 2	Non-reactive	16	16	0
	Reactive	3	3	
Panel 3	Non-reactive	13	13	0
	Reactive	3	3	
Panel 4	Non-reactive	9	10	1 m)
	Reactive	2	1	
Panel 5	Non-reactive	9	9	0
	Reactive	3	3	
Panel 6	Non-reactive	6	6	0
	Reactive	1	1	
Panel 7	Non-reactive	4	4	0
	Reactive	2	2	
Panel 8	Non-reactive	21	21	0
	Reactive	2	2	
Panel 9	Non-reactive	23	23	0
	Reactive	13	13	
Panel 10	Non-reactive	4	4	0
	Reactive	2	2	
Panel 11	Non-reactive	8	8	0
	Reactive	9	9	

m) The discrepant result is caused by 1 panel sample with testing results in the gray zone.

Testing of 11 seroconversion panels generated data that demonstrated the seroconversion sensitivity of the Elecsys HBeAg assay in the **cobas e** 801 analyzer compared with the MODULAR ANALYTICS E170 analyzer. In 10 out of 11 panels tested, the Elecsys HBeAg assay in the **cobas e** 801 analyzer had reactive value results in the same bleeds as in the MODULAR ANALYTICS E170 analyzer (in Panel 4, in a sample close to the cutoff, seroconversion was detected 1 bleed later in the **cobas e** 801 analyzer compared with the MODULAR ANALYTICS E170 analyzer).

The seroconversion study was verified on the **cobas e** 801 analyzer and on the **cobas e** 402 analyzer showing equivalent performance to the MODULAR ANALYTICS E170 analyzer.

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	-	21-79	3.21

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Patient population	No. tested	Gender			Age range	% Pos
		% Male	% Female	% Unknown		
Prospective: Symptomatic	521	72.4	27.6	-	21-73	1.34
Retrospective	201	60.2	21.4	17.9	21-81	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, FL; 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 3 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:

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	(+)	(-)
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)[n)]	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

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HBV classification	Positive percent agreement % (n/N)[n)]	95 % Score confidence interval	Negative percent agreement % (n/N)	95 % Score confidence interval
Total	100 (36/36)	90.4-100	99.9 (1083/1084)	99.5-99.98

n) 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.

HBV classification	Positive percent agreement % (n/N)[o)]	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

o) 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

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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 and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 for reconstitution
GTIN	Global Trade Item Number

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