Elecsys HBsAg II quant II

cobas®

REF		Σ	SYSTEM
			cobas e 411
08814899119	08814899500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 2110
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 131

Intended use

Immunoassay for the in vitro quantitative determination of hepatitis B surface antigen (HBsAg) in confirmed HBsAg positive human serum and plasma. Assay results in conjunction with HBV DNA quantification and clinical information, may be used as an aid to monitor treatment of individuals with chronic hepatitis B.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **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: 08814899190 for the HBsAg II quant II. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary

The hepatitis B surface antigen (HBsAg), a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus (HBV) particle.[1,2] In addition to the intact infectious viral particles, the blood of persons infected with HBV contains large amounts of non-infectious particles which consist only of an outer coat containing HBsAg.[3] After infection, HBsAg is the first immunological marker detectable in serum and is usually present weeks to months before the onset of clinical symptoms and the appearance of other biochemical markers.[4] In the case of acute HBV infection with recovery, HBsAg is detectable in serum for up to 6 months after its appearance.[4] If HBsAg persists for more than 6 months after acute hepatitis, the presence of chronic hepatitis B (CHB) infection must be assumed.

Classifying the stage of CHB infection is essential for identifying patients who require treatment and monitoring, as well as assessing the likelihood of responding to treatment and risk of progression to more severe liver disease.[5,6,7] A CHB patient with elevated aminotransferase levels, high HBV DNA viral load, and histological abnormalities will be considered for therapy and two different treatment strategies are applicable: treatment of finite duration with pegylated interferon alpha or long-term treatment with nucleoside/nucleotide analogs (NUCs).[5] Monitoring HBsAg levels, in addition to HBV DNA, before[8,9] and during pegylated interferon-based therapy can help physicians to predict the likely response and implement the response-guided therapy algorithms, as recommended in the guidelines, to achieve the optimal outcome, which is sustained HBsAg loss with or without seroconversion to anti-HBs.[5,6,8,9,10,11] There is also some evidence suggesting that HBsAg quantification may have value for monitoring response to NUC therapy and identifying patients able to achieve a sustained response after terminating treatment.[3,12,13,14,15] This is based on the suggestion that HBsAg levels decline during antiviral therapy with NUCs reflecting an improvement in the degree of host immune control of the virus, with lower HBsAg levels at end of treatment being associated with continued remission.[11,16,17] However, further studies in larger cohorts are required.

For patients in the immune clearance phase of CHB, HBV DNA levels have traditionally been used to determine the disease progression risk. However, HBsAg monitoring can provide additional information and distinguish true inactive carriers (HBV DNA < 2000 IU/mL and HBsAg < 1000 IU/mL), who are at the lowest risk of progression from those at a higher risk of developing cirrhosis or hepatocellular carcinoma (HCC). An HBsAg level ≥ 1000 IU/mL in hepatitis B 'e' antigen negative patients with HBV DNA < 2000 IU/mL has been identified as an independent risk factor for progression to HCC.[5,6,11,18,19,20]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies 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 via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as HBSAGQN2.

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

R1 Anti-HBsAg-Ab~biotin (gray cap), 1 bottle, 8 mL: Two biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.

R2 Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 7 mL: Monoclonal anti-HBsAg antibody (mouse), polyclonal anti-HBsAg antibodies (sheep) labeled with ruthenium complex > 1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative.

HBSAGQN2 Cal1	Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each: Human serum, buffered, pH 6.5; preservative.
HBSAGQN2 Cal2	Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each: HBsAg approximately 50 IU/mL in human serum, buffered, pH 6.5; preservative.
HBSAGQN2 Dil HepB	2 bottles of 36 mL each (white cap): Human serum negative for HBsAg and anti-HBs, buffered, pH 6.5; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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

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

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

All human material should be considered potentially infectious.

The calibrators and HBSAGQN2 Dil HepB have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAGQN2 Cal1 and HBSAGQN2 Dil HepB only) and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing HBsAg (HBSAGQN2 Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[21,22]

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

Pre-dilution of samples is mandatory according to the test algorithm (see "Dilution" section).

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.

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 and HBSAGQN2 Dil HepB	
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 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 **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 and sodium citrate plasma.

Criterion: Slope 1.00 ± 0.1 + intercept ≤ ± 0.5 IU/mL + coefficient of correlation ≥ 0.95.

Stable for 7 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 5 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 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.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 07143745190, PreciControl HBsAg II quant II, for 15 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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

Additional materials for **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

Additional materials 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. **Pre-dilution of samples is mandatory according to the test algorithm (see "Dilution" section).** 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.

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 (**cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).

Every Elecsys HBsAg II quant II reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using HBSAGQN2 Cal1 and HBSAGQN2 Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using HBSAGQN2 Cal1, HBSAGQN2 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:

- 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

Quality control

For quality control, use PreciControl HBsAg II quant II.

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.

Controls 1, 2 and 3 are not barcode-labeled. All values and ranges must be entered manually. Please refer to the "QC" section in the operator`s manual or to the online help for the instrument software.
PC HBSAGQN1 and PC HBSAGQN2 must be run like external controls.
PC HBSAGQN3 (dilution control) must be run as a patient sample in order to request an automatic dilution.
PC HBSAGQN2 must be run before PC HBSAGQN1 and PC HBSAGQN3 (dilution control).
PC HBSAGQN3 will be measured on only one measuring cell (**cobas e** 601, **cobas e** 602) and printed in the same way as a sample result. Results for PC HBSAGQN3 will not be printed on the QC chart.

Non-barcode labeled controls: Only one target value and range for each control level can be entered in the analyzer. The reagent lot-specific target values have to be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run.

The exact lot-specific target values and ranges are printed on the enclosed (or electronically available) value sheet in the reagent kit or PreciControl kit.

Please make sure that the correct values are used.

Calculation

The analyzer automatically calculates the analyte concentration (IU/mL) based on the measurement of HBSAGQN2 Cal1 and HBSAGQN2 Cal2. In case of a manual pre-dilution, the dilution factor needs to be accounted for manual calculation of the final result.

Limitations - interference

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

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 684 µmol/L or ≤ 40 mg/dL
Hemoglobin	≤ 0.311 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery within ± 20 % of initial value.

No high-dose hook effect was found with the Elecsys HBsAg II quant II assay up to a concentration of 8.7 x 10^5 IU/mL when samples were analyzed according to instructions for use.

There is no indication for a significant loss in sensitivity or specificity with samples having elevated levels of albumin up to 14 g/dL.

No significant interfering effects of 22 commonly used therapeutic drugs could be detected (including lamivudine, peginterferon alpha-2a, entecavir, telbivudine and adefovir).

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.

cobas e 601 and **cobas e** 602 analyzers:

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys HBsAg II quant II assay is combined with **all assays** performed on the analyzer - including the Elecsys HBsAg II quant II assay itself:

From test	Step	To test	Step 0	Step 1	Step 2
Elecsys HBsAg II quant II	1	Elecsys HBsAg II quant II	x	x	x
Elecsys HBsAg II quant II	1	each other assay	x	x	x

If new tests are installed make sure that the Special Wash List is updated accordingly.

For the Elecsys Anti-HBs II assay make sure that "Step 1" and "Step 2" are activated:

From test	Step	To test	Step 0	Step 1	Step 2
Elecsys Anti-HBs II	1	Elecsys HBsAg II quant II	-	x	x

The described additions to the Special Wash List have to be entered manually. 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

Measuring range

Measuring range for pre-diluted samples:

5-13000 IU/mL for 100-fold diluted samples (**cobas e** 411 analyzer).
Values below the measuring range are reported as < 5 IU/mL.
Values above the measuring range are reported as > 13000 IU/mL.

20-52000 IU/mL for 400-fold diluted samples (**cobas e** 601 and **cobas e** 602 analyzers).
Values below the measuring range are reported as < 20 IU/mL.
Values above the measuring range are reported as > 52000 IU/mL.

Measuring range for undiluted samples:

0.05-130 IU/mL (defined by the Limit of Detection and the maximum of the master curve).
Values below the Limit of Detection are reported as < 0.05 IU/mL.
Values above the measuring range are reported as > 130 IU/mL.

Lower limits of measurement

Limit of Blank and Limit of Detection

Limit of Blank = 0.03 IU/mL

Limit of Detection = 0.05 IU/mL

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Dilution

Every sample has to be initially diluted with HBSAGQN2 Dil HepB (mandatory dilution to be ordered on the respective platform).

Samples with concentrations above the extended measuring range must be further diluted manually with HBSAGQN2 Dil HepB.

Elecsys HBsAg II quant II

The dilution factor for dilution by the **cobas e** 411 analyzer is 1:100.

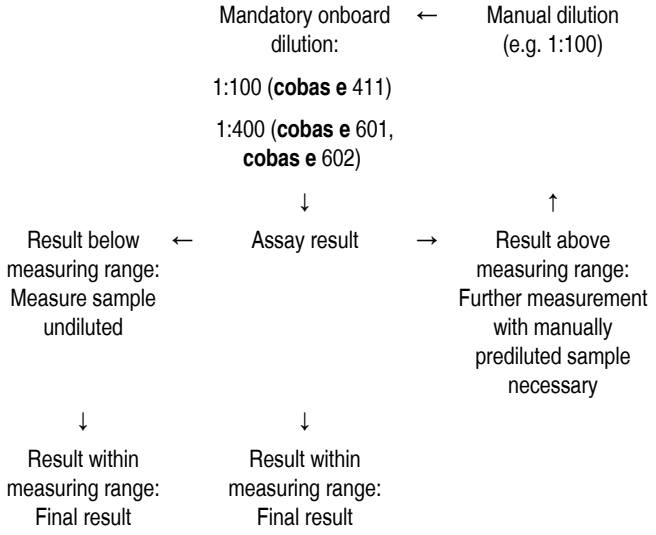
The dilution factor for dilution by the **cobas e** 601 and **cobas e** 602 analyzers is 1:400.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Due to different dilutions performed on the different instrument platforms, minor deviations between the measurements on the **cobas e** 411 analyzer and on the **cobas e** 601/**cobas e** 602 analyzers might occur.

In highly concentrated patient samples, further manual dilution steps might be necessary to achieve results within the measuring range for pre-diluted samples. After manual dilution, multiply the result by the dilution factor chosen for the respective dilution step.

Test algorithm for samples:



Initial onboard dilution is mandatory for every sample. Therefore every sample has to be run first with a dilution step ordered by the user and performed by the analyzer (1:100 on the **cobas e** 411 analyzer and 1:400 on the **cobas e** 601 and **cobas e** 602 analyzers).

If a result is found within 5-13000 IU/mL for 100-fold diluted samples or 20-52000 IU/mL for 400-fold diluted samples **no further dilution is necessary** and endpoint result is achieved.

If a result is found **below** the above mentioned **lower ranges**, the sample has to be run **undiluted** and should be found within 0.05-130 IU/mL.

If a result is found > 13000 IU/mL for 100-fold diluted samples or > 52000 IU/mL for 400-fold diluted samples **further manual dilution steps (e.g. additional 1:100 sample predilution prior to 1:100/1:400 instrument dilution to achieve a final 1:10000/1:40000 dilution)** are recommended until result is found within the measuring range.

Expected values

Note: Where indicated, data have been generated using the Elecsys HBsAg II quant assay. Since the reagents (M, R1, R2) of the Elecsys HBsAg II quant assay are the same as those of the Elecsys HBsAg II quant II assay (only the controls and calibrators have been modified) the data generated with the Elecsys HBsAg II quant assay are transferable to the Elecsys HBsAg II quant II assay and no new data needed to be generated.

From 611 samples obtained from a multicenter evaluation the following values have been reported with the Elecsys HBsAg II quant assay.

IU/mL	MCE (n = 611)	% of total
< 1	17	2.78
1-< 10	20	3.27
10-< 100	35	5.73
100-< 1000	127	20.8
1000-< 10000	239	39.1
10000-< 100000	147	24.1
100000-< 1000000	26	4.26

The final result was determined from the first measurement in 70.0 % of the samples on the **cobas e** 411 analyzer (1:100 dilution) and 85.6 % of the samples on the **cobas e** 601 and **cobas e** 602 analyzers (1:400 dilution).

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability[b]		**Intermediate precision**[c]	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	0.153	0.003	1.7	0.005	3.4
Human serum 2	2.84	0.038	1.3	0.088	3.1
Human serum 3	58.5	0.635	1.1	1.48	2.5
Human serum 4	110	1.33	1.2	3.05	2.8
Human serum 5	342	7.09	2.1	13.2	3.8
Human serum 6	5019	121	2.4	243	4.8
PC[d] HBsAg II quant II 1	3.87	0.055	1.4	0.146	3.8
PC HBsAg II quant II 2	89.9	1.61	1.8	3.39	3.8
PC HBsAg II quant II 3	91.8	2.19	2.4	3.82	4.2

b) Repeatability = within-run precision

c) Intermediate precision = between-run precision

d) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	0.151	0.004	2.8	0.005	3.2
Human serum 2	2.74	0.040	1.4	0.049	1.8
Human serum 3	58.0	0.629	1.1	0.886	1.5
Human serum 4	112	1.55	1.4	1.91	1.7
Human serum 5	317	11.0	3.5	14.5	4.6
Human serum 6	4606	113	2.4	203	4.4
PC HBsAg II quant II 1	3.68	0.054	1.5	0.085	2.3
PC HBsAg II quant II 2	87.7	1.11	1.3	1.72	2.0
PC HBsAg II quant II 3	83.3	2.37	2.8	4.08	4.9

Method comparison

A comparison of the Elecsys HBsAg II quant II assay, REF 08814899190 (**cobas e** 601 analyzer; y), with the Elecsys HBsAg II quant II assay, REF 07143737190 (**cobas e** 601 analyzer; x), gave the following correlation (IU/mL):

Number of samples measured: 220

Passing/Bablok[23]	y = 0.897x + 0.000
Pearson	r = 0.998

The sample concentrations were between 0 and 35811 IU/mL.

Quantitation of potentially cross reactive samples

1285 samples containing potentially interfering substances were tested with the Elecsys HBsAg II quant assay comprising specimens:

- containing antibodies against HAV, HCV, HIV, HTLV, CMV, EBV, HSV, Rubella, Parvo virus, VZV, Toxoplasma gondii, Treponema pallidum
- containing autoantibodies (ANA, SLE), elevated titers of rheumatoid factor or HAMA antibodies
- positive for Mumps, Measles, Malaria
- after vaccination against HBV and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma, patients undergoing dialysis or patients suffering from alcoholic liver disease
- from pregnant women

No results were found ≥ 0.05 IU/mL.

Quantitation of HBV mutants

A total of 50 samples comprising different HBsAg mutations were tested with the Elecsys HBsAg II quant assay. Results of observed concentrations are displayed.

Mutant panel	Elecsys HBsAg II quant (IU/mL)[e)]
Native mutant panel (strains displaying amino acid substitutions either linked to vaccine resistance, resistance to therapy with human HB immunoglobulin or impaired HBsAg reactivity)	< 0.05 (n = 2) 0.05-324 (n = 17)
Recombinant mutant panel	> 0.05-6.9 (n = 31)

e) Observed concentrations with HBV mutants might differ compared to competitor assays and are a characteristic of the individual assays.

Seroconversion panels

18 seroconversion panels were analyzed with the Elecsys HBsAg II quant assay. In all panels the Elecsys HBsAg II quant assay showed a significant increase in concentration upon seroconversion correlated to the shift as it is detectable in qualitative screening assays. Observed concentrations ranged from < Limit of Detection for negative samples draws, and 0.058-92300 IU/mL for conversion samples (confirmed positives).

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, pp2977–3029.

2 Lee JM, Ahn SH. Quantification of HBsAg: basic virology for clinical practice. World J Gastroenterol 2011;17:283-289.

3 Liaw YF. Clinical utility of hepatitis B surface antigen quantification in patients with chronic hepatitis B: a review. Hepatology 2011;54:E1-E9.

4 Liaw YF, Chu CM. Hepatitis B infection. Lancet 2009;373:582-592.

5 European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-185.

6 Liaw YF, Kao JH, Piratvisuth T, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531-561.

7 Lok ASF, McMahon B. Chronic hepatitis B: update 2009. AASLD Practice Guidelines. Available at: http://www.aasld.org/practiceguidelines/Documents/Bookmarked%20Practice%20Guidelines/Chronic_Hep_B_Update_2009%208_24_2009.pdf (accessed January 2013).

8 Piratvisuth T, Marcellin P, Popescu M, et al. Hepatitis B surface antigen: association with sustained response to peginterferon alfa-2a in hepatitis B e antigen positive patients. Hepatol Int 2013;7:429-436.

9 Marcellin P, Bonino F, Yurdaydin C, et al. Hepatitis B surface antigen levels: association with 5 year response to peginterferon alfa-2a in hepatitis B e antigen-negative patients. Hepatol Int 2013;7:88-97.

10 Sonneveld MJ, Hansen BE, Piratvisuth T, et al. Response-guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen levels. Hepatology 2013;58:872-880.

11 Martinot-Peignoux M, Lapalus M, Asselah T, et al. The role of HBsAg quantification for monitoring natural history and treatment outcomes. Liver Int 2013;33 Suppl1:125-132.

12 Janssen HLA, Sonneveld MJ, Brunetto MR. Quantification of serum hepatitis B surface antigen: is it useful for the management of chronic hepatitis B? Gut 2012;61:641-645.

13 Chan HL, Thompson A, Martinot-Peignoux M, et al. Hepatitis B surface antigen quantification: why and how to use it in 2011 – a core report. J Hepatol 2011;55:1121-1131.

14 Wang CC, Tseng TC, Wang PC, et al. Baseline hepatitis B surface antigen quantitation can predict virologic response in entecavir-treated chronic hepatitis B patients. J Formos Med Assoc Epub ahead of print Aug 1 2013.

15 Liang Y, Jiang J, Su M, et al. Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. Aliment Pharmacol Ther 2011;34:344-352.

16 Tseng T-C, Kao J-H. Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: new trick of old dog. J Gastroenterol 2013;48:13-21.

17 Pérez-Cameo C, Pons M, Esteban R. New therapeutic perspectives in HBV: when to stop NAs. Liver Int 2014;34 Suppl 1:146-153.

18 Tseng TC, Liu CJ, Yang HC, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140-1149.

19 Chen CJ, Lee MH, Liu J, et al. Quantitative serum levels of hepatitis B virus DNA and surface antigen are independent risk predictors of hepatocellular carcinoma. Hepatology 2011;54:881A (abstract 1095).

20 Tseng TC, Liu CJ, Yang HC, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology 2013;57:441-450.

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

22 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.

23 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator

Elecsys HBsAg II quant II

→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com