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HBsAg Confirmatory Test

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HBsAg Confirmatory Test

REF **11820648** 122

English

Intended use

Immunoassay for in vitro confirmation of the presence of hepatitis B surface antigen in human serum and plasma samples repeatedly reactive when tested with the Elecsys HBsAg II assay.

Summary

References[1,2,3,4,5,6,7,8]

The Elecsys HBsAg Confirmatory Test is based on the principle of specific antibody neutralization. Polyclonal HBsAg-specific antibodies bind to the immunodominant epitopes of the hepatitis B surface antigen and thereby block the binding sites for the antibodies used in the Elecsys HBsAg II assay.

Test principle

The test principle is based on pretreatment of the samples with confirmatory reagent and control reagent followed by the assay procedure using the Elecsys HBsAg II assay. The positive control, PreciControl HBsAg II 2, should be run in parallel as a performance check.

Sample pretreatment:

- Samples found to be repeatedly reactive in the Elecsys HBsAg II assay are treated in parallel with confirmatory reagent and control reagent and then incubated. The excess anti-HBs antibodies in the confirmatory reagent neutralize any HBsAg in the sample. In the subsequent Elecsys HBsAg II assay this leads to a reduction in the cutoff index (COI) value (signal of sample/cutoff) in comparison to the value originally obtained for the sample.

Elecsys HBsAg II assay:

- 1st incubation: The two pretreated sample preparations react with biotinylated, monoclonal HBsAg-specific antibodies and mono-/polyclonal HBsAg-specific antibodies labeled with a ruthenium complex[a)] to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration. This is followed by manual verification of the validity of the assay and interpretation of the findings.

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

Reagents - working solutions

HBsAg Confirmatory Test 1
Confirmatory reagent (black cap), 2 bottles of 1.0 mL each:
Anti-HBs (human) > 200000 IU/L in human serum; preservative.

HBsAg Confirmatory Test 2
Control reagent (white cap), 2 bottles of 1.0 mL each:
Human serum, anti-HBs < 3 IU/L; 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.

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.[9,10]

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

Reagent handling

The reagents in the kit are ready for use. Avoid contamination. Store at 2-8 °C after use.

Storage and stability

Store at 2-8 °C.

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

Specimen collection and preparation

Samples that were repeatedly reactive in the Elecsys HBsAg II assay.

The conditions regarding stability and specimen collection described for the Elecsys HBsAg II assay also apply here.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 04687787, HBsAg II reagent kit for 100 tests
  (the materials required for performing the Elecsys HBsAg II assay are listed in the Elecsys HBsAg II Method Sheet)
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Assay

Sample pretreatment:

Selection of the reactant volumes is dependent on the magnitude of the respective cutoff index of the samples which were reactive in the Elecsys HBsAg II assay. The following volumes are pipetted into Elecsys sample cups:

- For positive samples having a cutoff index < 7.0
  270 µL sample + 30 µL confirmatory reagent
  270 µL sample + 30 µL control reagent

or

- For positive samples having a cutoff index between 7.0 and < 30
  150 µL sample + 150 µL confirmatory reagent
  150 µL sample + 150 µL control reagent

or

- For positive samples having a cutoff index ≥ 30
  Predilute samples 1:20 with Diluent Universal
  150 µL diluted sample + 150 µL confirmatory reagent
  150 µL diluted sample + 150 µL control reagent

PreciControl HBsAg II 2, the positive control, should always be run in parallel as a check on performance:

270 µL PreciControl HBsAg II 2 + 30 µL confirmatory reagent

270 µL PreciControl HBsAg II 2 + 30 µL control reagent

Incubation of the reactants: 30-60 minutes at 15-25 °C or overnight at 2-8 °C.

Elecsys HBsAg II assay:

The pretreated samples are placed in the sample zone and registered by entering the sample identification data.

The Elecsys HBsAg II assay is performed in accordance with the instructions given in the Method Sheet of the test reagent kit.

Calibration

For calibration, calibration frequency, and calibration verification, see data given in the Method Sheet for the Elecsys HBsAg II assay.

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Quality control

PreciControl HBsAg II 2 should always be run in parallel with the samples needing confirmation. Verification is done by the user.

For the Elecsys HBsAg II assay the conditions given in the Method Sheet apply.

Calculation

The analyzer calculates the cutoff automatically on the basis of measurements on the two Elecsys HBsAg II calibrators contained in the kit.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI, signal sample/cutoff).

The cutoff index is needed for selection of the correct sample pretreatment volumes for the confirmatory test.

Limitations - interference

Due to the high-dose hook effect, samples having very high HBsAg concentrations (> 1 mg/mL or > 550000 IU/mL) can give a cutoff index < 30 in the Elecsys HBsAg II assay. Such samples are not adequately neutralized by the confirmatory reagent at the stated volume, and are therefore not confirmed as positive. These samples can be recognized by the fact that the COI in the test with the control reagent is higher than the COI for the samples in the original HBsAg assay (dilution effect). The confirmatory test for these samples must be repeated at a higher predilution (at least 1:100).

For the Elecsys HBsAg II assay the data given in the Method Sheet of the test reagents on “Limitations - interference” apply.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Evaluation and interpretation of the results

Verification of the validity of the test

Prior to evaluation, the validity of the test must be verified. Evaluation can be made when, in addition to the conditions applying to the Elecsys HBsAg II assay, the following criteria are fulfilled:

- The cutoff index of PreciControl HBsAg II 2 in the test with confirmatory reagent must be ≤ 60 % of that for the test with control reagent:

  COI for test with control reagent ≙ 100 %

  COI for test with confirmatory reagent ≙ x %

  If x > 60 %, it is necessary to check the test conditions. Where appropriate, repeat the test with fresh reagent.

- For evaluation of the sample to be valid, the cutoff index for the sample with the control reagent must be ≥ 0.81.

  A cutoff index < 0.81 indicates that dilution is too high. Such samples should be retested undiluted or at a lower dilution.

Evaluation and interpretation of the results

- In order to confirm a positive result for a sample, the cutoff index for the sample with the confirmatory reagent must be ≤ 60 % of that with the control reagent, which must have a cutoff index of ≥ 0.81.

  Evaluation:

  COI for test with control reagent ≙ 100 %

  COI for test with confirmatory reagent ≙ x %

  Interpretation:

  x > 60 % and COI for control reagent ≥ 0.81 = non-reactive

  x > 60 % and COI for control reagent < 0.81 = non-valid

  x ≤ 60 % and COI for control reagent ≥ 0.81 = positive

  x ≤ 60 % and COI for control reagent < 0.81 = indeterminate

Non-valid results must be repeated using fresh reagent.

In case the result remains non-valid, a follow-up sample should be examined. Indeterminate results should be repeated. In case the result remains indeterminate, a follow-up sample should be examined. In case a high dose hook sample is assumed please refer to the section “Limitations - interference” regarding higher predilution of such samples.

Specific performance data

Representative performance data for manual sample pretreatment followed by the Elecsys HBsAg assay on Elecsys analyzers are shown below. Results obtained in individual laboratories may differ.

Precision

Precision of the manual test steps was determined using 3 sera of differing HBsAg concentrations (8-10 times per sample with both the control and confirmatory reagents). After a 30-minute period of incubation at 20 °C the pretreated samples were determined on Elecsys analyzers using Elecsys reagents, calibrators and controls.

Results from original HBsAg assay - without sample pretreatment (repeatability, n = 8-10):

Sample	Mean COI	SD COI	CV %
HS[b)], COI < 7.0	1.65	0.04	2.2
HS, COI 7.0 - < 30	11.3	0.11	1.0
HS, COI ≥ 30	669	16.0	2.4

b) HS = human serum

Results after manual sample pretreatment:

	Control reaction			Confirmatory reaction			
Sample	Mean COI	SD COI	CV %	Mean COI	%	SD COI	CV %
HS, COI < 7.0	1.57	0.03	2.1	0.42	27	0.04	9.1
HS, COI 7.0 - < 30	4.85	0.10	2.1	0.40	8.2	0.02	3.8
HS, COI ≥ 30	1321	11.6	0.9	1.31	0.1	0.10	7.9

Detection limit

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

Dilutions of ≤ 0.1 U/mL and ≤ 0.1 IU/mL were definitely confirmed for the Paul-Ehrlich-Institute standard (subtype ad, 1987) and the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A) using the Elecsys HBsAg Confirmatory Test.

Method comparison

A comparison of Elecsys HBsAg Confirmatory Test with a commercially available HBsAg confirmatory test (MEIA) in 90 repeatedly reactive patient samples gave the following result:

HBsAg confirmatory test	**Confirmed positive**	**Negative**	**Agreement**
Elecsys test	90	0	100 %
MEIA comparison test	90	0	100 %

Clinical sensitivity

20 out of 20 samples within the range 1-2 of the cutoff index could be confirmed with the Elecsys HBsAg Confirmatory Test.

Clinical specificity

The specificity of the Elecsys HBsAg Confirmatory Test was tested using confirmed positive and false-positive samples. 318 positive samples were confirmed as positive. One false positive blood donor sample and 19 artificially produced false-positive interference samples were found negative. The specificity of the Elecsys HBsAg Confirmatory Test is therefore 100 % for both groups.

Extended specific performance data

Comparison of the Elecsys HBsAg and HBsAg II assay

A panel with n = 88 HBsAg positive samples from Korea served to show comparability of the Elecsys HBsAg and Elecsys HBsAg II assay (1 sample was excluded due to wrong sample pre-selection; 1 sample was negative in both HBsAg assays (COI 0.81-0.88) but initially positive (COI 1.12) when measured in Korea; 2 samples were not determined with the Elecsys

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HBsAg Confirmatory Test due to insufficient sample volume). Samples were first measured with the Elecsys HBsAg and Elecsys HBsAg II assay, treated with Elecsys HBsAg Confirmatory Test reagents and measured again with both assays. For the remaining 84 HBsAg positive samples both Elecsys HBsAg and Elecsys HBsAg II assays showed identical neutralization behaviour (correlation factor 0.995).

Neutralization of samples with HBsAg mutations

A panel with n = 19 native mutant samples (undiluted and diluted) was used to test applicability of the Elecsys HBsAg Confirmatory Test for neutralization of mutant samples. Samples were measured with the Elecsys HBsAg II assay and additionally with 3 registered HBsAg assays. 2 samples (mutation M133L/M143T/G145R and mutation T45S/I49R/113T114/I186P, respectively) were negative with all assays, 1st mutation tested with 3 assays (COI 0.03-0.76), 2nd mutation tested with 4 assays (COI 0.03-0.78). 5 samples were not determined with the Elecsys HBsAg Confirmatory Test due to insufficient sample volume (including both samples negative with all assays tested). The remaining 14 native mutant samples were efficiently neutralized with the Elecsys HBsAg Confirmatory Test (55.6-99.7 % neutralization).

References

1 Gerlich W. Viral Hepatitis. Section 2, Churchil Livingstone, Ed. Zuckermann AJ, Thomas HC, 1993:83-113.

2 Hollinger FB. Hepatitis B virus. In: Fields BN, Knipe DM (eds). Virology ed. 2 New York Raven Press 1990;2:2171-2236.

3 Couroucé-Pauty AM, Plancon A, Soulier JP. Distribution of HBsAg Subtypes in the World. Vox Sang 1983;44:197-211.

4 Frösner G. Moderne Hepatitisdiagnostik. Frösner G Kilian Verlag, Marburg 1996.

5 Hoofnagle JH, Di Bisceglie AM. Serologic Diagnosis of Acute and Chronic Viral Hepatitis. Seminars in Liver Disease 1991;11/2:73-83.

6 Frösner G, Schomerus H, Wiedmann KH, et al. Diagnostic significance of quantitative HBsAg determination in acute and chronic hepatitis B infection. Eur J Clin Microbiol 1982;1:52-58.

7 CDC. Prevention of Perinatal Transmission of Hepatitis B Virus: Prenatal Screening of all Pregnant Women for Hepatitis-B-Surface Antigen: Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR 1988;37:341-355.

8 STIKO Recommendations, Bundesgesundheitsblatt 1996;1/96:32-42.

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

10 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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

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

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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

Roche