



Hepatitis B (HBsAg)

ELISA

3rd generation enzyme-linked immunosorbent assay (ELISA) for the detection of the Hepatitis B virus surface antigen (HBsAg)

SUMMARY

Hepatitis B is a viral disease characterized for a long incubation period (45-160 days). The virus that causes this pathology (HBV) is a particle consisting of an inner area or "core", where the DNA is, and an antigenic outer coat known as the surface antigen (HBsAg). Its presence in the serum indicates an active disease. The hepatitis B surface antigen and its corresponding antibody are specific to infections by virus B. The antigen can be detected in the patient's serum and secretions during the acute phase or in HBV chronic infections.

HBsAg is usually the first evidence of HBV infection, which can precede in weeks or months any other laboratory manifestation or clinical symptoms and signs of the disease. In some cases, it can be the only indicator of asymptomatic carriers in individuals with chronic hepatitis B.

Therefore, HBsAg detection is important not only for the diagnosis of the acute disease, but also for the screening of donors in blood banks, dialysis units and hospital areas where there is a risk of transmission of the disease.

PRINCIPLE

The sample is placed in contact with an anti-HBs monoclonal antibody immobilized onto a solid support. If it contains the HBsAg antigen, it will form a complex with the antibodies and will bind to the support. The unbound fraction is eliminated by washing, after which another anti-HBs monoclonal antibody conjugated to peroxidase is added. If a reaction was produced during the first stage of the process, the conjugate will bind. After a new wash the enzymatic substrate is added. If HBsAg is present in the sample, a light blue color is developed. The reaction is stopped by adding sulfuric acid, which makes the light blue color change to yellow.

PROVIDED REAGENTS

Coated microtitration plate: microtitration plates with removable strips with wells containing immobilized anti-HBs monoclonal antibody.

Concentrated Conjugate: anti-HBs monoclonal antibody conjugated to peroxidase.

Conjugate Diluent: Tris buffer containing additives and preservatives.

Substrate A: 60 mmol/l hydrogen peroxide in 50 mmol/l citrate buffer.

Substrate B: 0.01 mol/l tetramethylbenzidine (TMB) in 0.1 N hydrochloric acid.

Stopper: 2 N sulfuric acid.

Concentrated Wash Buffer: 1.4 mol/l sodium chloride in

100 mmol/l phosphate buffer and 0.1 g/l non-ionic surfactant.

Positive Control: dilution of inactivated serum reactive to HBsAg.

Negative Control: dilution of non-reactive inactivated serum.

INSTRUCTIONS FOR USE

Wash Buffer: at low temperature, the reagent's constituents may crystallize. In that case, before diluting, place in a water bath at 37°C for a couple of minutes, then mix by inversion. Dilute 1+4 with distilled water (1 part Concentrated Wash Buffer + 4 parts distilled water) to use.

Coated microtitration plate: ready to use.

Conjugate: to improve the efficiency of the reagent, before diluting, it is convenient to gently centrifuge the Concentrated Conjugate to wash down what could have been left on the tube walls. Prepare it diluting 1 part Concentrated Conjugate + 50 parts Conjugate Diluent (e.g.: for one strip, place 20 µl Concentrated Conjugate + 1 ml Conjugate Diluent or equivalent volumes).

Substrates A: ready to use.

Substrates B: ready to use.

Stopper: ready to use.

Positive Control: ready to use.

Negative Control: ready to use.

WARNINGS

- All samples should be handled as capable of transmitting infection. Controls are inactivated, however, they should be handled as infectious material.

- The Control sera have been tested and found non-reactive to HIV.

- Discard all materials used to perform the test in order to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for 1 hour at 121°C. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.

- Do not exchange reagents from different kits and lots.

- Do not use foreign reagents.

- The Microtitration plates must be incubated in incubator. Do not use water bath. Do not open the incubator during the process.

- Avoid the contact of the microtitration plates with hypochlorite fumes from the biohazard disposal containers or other sources, as hypochlorite affects the reaction.

- Reagents are for "in vitro" diagnostic use.

- Avoid contact of the sulfuric acid (Stopper) with the skin and eyes. If this occurs, rinse the affected area with copious quantities of water. R36/38: irritates eyes and skin, R34

causes burns, S24/25: avoid contact with the eyes and skin. S26: if splashing onto skin occurs, rinse the affected area with copious quantities of water and seek medical attention, S28: after contact with skin, wash immediately with plenty of water, S37/39: wear suitable gloves and eye/face protection.

- Avoid any spilling of liquids or spraying.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze.

Wash Buffer: is stable 3 months at room temperature.

Coated microtitration plate: well strips with the immobilized antibody are provided in vacuum-sealed pouches with desiccant. Do not open the pouch until performing the test and it has reached room temperature, otherwise the contents can get moistened. Unused well strips should be kept in the pouch with the desiccant, sealed and stored at 2-10°C. Under these conditions, the strips can be used within 5 months, as long as the expiration date has not elapsed.

Conjugate: is stable 24 hours in refrigerator (2-10°C).

SAMPLE

Serum or plasma

a) Collection: obtain the sample in the usual way. Do not use heat-inactivated samples.

b) Additives: if plasma is used, any anticoagulant commonly used for transfusions can be employed.

c) Known interfering substances: hemolysis, hyperlipemia and other causes of turbidity may yield erroneous results. Clarify these samples by centrifugation.

d) Stability and storage instructions: samples can be stored for 7 days at 2-10°C. If they have to be stored for longer periods, they should be frozen at -20°C or less. Avoid repeated freezing and thawing. There is evidence that repeated freezing may cause erroneous results.

If samples must be transported, they should be packed according to local regulations for the shipment of infectious materials.

REQUIRED MATERIAL (non-provided)

- Micropipettes and pipettes for measuring the volumes indicated in the PROCEDURE.
- Stopwatch.
- Incubator at 37°C.
- Adequate volumetric material.
- Spectrophotometer for reading microtitration plates (optional).

ASSAY CONDITIONS

- Primary wavelength: 450 nm
- Secondary wavelength (bichromatic): 620-650 nm
- Instrument calibration: set the spectrophotometer to zero with the Reagent Blank, processing in the same manner as a test, but without adding the Sample and Conjugate, only the Substrate A, Substrate B and Stopper.
- Total reaction time: 2 hours 30 minutes
- Reaction temperature: 37°C and room temperature
- Sample volume: 100 ul

PROCEDURE

Note: Once the test has begun, it should be performed without interruptions.

Bring the reagents and the samples to room temperature before starting the test. Simultaneously process 2 Positive Controls (PC), 3 Negative Controls (NC) and the Unknown (U). In the microtitration plate wells to be used, place:

	U	PC	NC
Sample	100 ul	-	-
Positive Control	-	100 ul	-
Negative Control	-	-	100 ul

To avoid evaporation, cover the plate with an adhesive tape and incubate in incubator for 60 minutes at 37°C. Then, carefully aspirate the fluid from each well into a biohazardous container with 5% sodium hypochlorite. Then, wash 5 times with Wash Buffer, using approximately 350 ul/time/well. After each wash, the liquid should be discarded into the container with hypochlorite. An automated washer can be use.

After the last wash, completely discard the residual liquid, inverting and tapping the microtitration plate several times onto absorbent paper, gently pressing with the hand the long sides to prevent the well strips from falling. Then, add to each well:

Conjugate	100 ul	100 ul	100 ul
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Mix by gently tapping the sides of the microtitration plate for 10 seconds. To avoid evaporation, cover the plate with an adhesive tape and incubate 60 minutes in incubator at 37°C. Then, carefully aspirate the fluid from each well into a biohazardous container with 5% sodium hypochlorite, and wash as indicated above.

After the last wash, completely discard the residual liquid, inverting and tapping the microtitration plate several times onto absorbent paper, gently pressing with the hand the long sides to prevent the well strips from falling. Then, add:

Substrate A	1 drop	1 drop	1 drop
Substrate B	1 drop	1 drop	1 drop

If using an automated micropipette, dispense 50 ul. Mix by gently tapping the sides of the microtitration plate for 10 seconds. Incubate 30 minutes at room temperature. Then, add:

Stopper	1 drop	1 drop	1 drop
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If using an automated micropipette, dispense 50 ul. Mix by gently tapping the sides of the microtitration plate for 10 seconds. Read in spectrophotometer at 450 nm or bichromatic at 450/620-650 nm, or evaluate the result with the naked eye by comparing it with the Positive and Negative Controls.

STABILITY OF FINAL REACTION

The reaction color is stable for 30 minutes, thus the results must be read within that period of time.

RUN VALIDATION CRITERIA

The run is valid if the following two conditions are simultaneously met:

- a) Readings of at least 2 out of the 3 Negative Controls, corrected against the Reagent Blank, should be lower than or equal to 0.150 O.D.
- b) The mean of the Positive Controls corrected readings, should be higher than or equal to 0.600 O.D.

If any of these conditions is not met, rerun. Remember that the obtained readings will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

a) With optical instrument

The sample reactivity is determined relating the sample's absorbance to the cut-off value.

Cut-off = NC + 0.060 O.D.

where NC = Negative Control average readings.

Reactive samples: samples with an absorbance higher than or equal to the cut-off value.

Initially reactive samples should be tested again by duplicate using the same method, before their final interpretation. If one or both duplicates are reactive, the sample should be considered repeatedly reactive.

Non-reactive samples: samples with an absorbance below the cut-off value.

b) Visual interpretation

If this interpretation is selected, any sample that does not show a coloration more intense than the Negative Control, should be considered non-reactive. On the other hand, a yellow sample is considered reactive. Light colors higher than the Negative Control, require an instrumental interpretation.

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

The following are causes of erroneous results:

- Improper wash of the reagent wells.
- Cross contamination of non-reactive samples with an antigen from a reactive sample.
- Contamination of the chromogenic solution with oxidant agents (chlorine, etc.)
- Stopper contamination.
- Improper storage of unused well strips.
- Use of water bath instead of incubator.
- Diluted Wash Buffer contamination. Verify that the bottles where it is prepared and stored are perfectly clean. If turbidity or precipitation is observed when prepared, discard.

A negative result does not exclude the possibility of exposure or infection with HBV.

Occasionally, when performing bichromatic readings, negative absorbances may be obtained that do not invalidate the determination. This is due to the fact that some samples yield readings below the Reagent Blank.

Verify that the washing system in use (Wiener Washer or other) totally aspirates the contents of the wells, and that the washing solution level is even.

PERFORMANCE

a) Sensitivity: using the International Standard for Hepatitis B Surface Antigen (subtype ad) NIBSC (code 80/549) diluted in 5% PBS-BSA, 0.1% sodium azide, 0.5 UI/ml antigen quantities are detected. According to the report of the WHO Working Group on International Reference Preparations, 1 UI is equivalent to 0.58 PEI units (Paul Ehrlich Institute) or 1.93 French "ng" or 5.59 Abbott "ng".

b) Specificity: different studies were performed on a population of hospitalized and ambulatory individuals, from different health institutions of the city of Rosario, Argentina. The method's specificity was compared with others with a similar reaction principle, obtaining the following results:

- Research 1: 85 individuals were tested, obtaining 100% specificity.
- Research 2: 76 individuals were tested, obtaining 98.7% specificity.
- Research 3: 127 individuals were tested, obtaining 100% specificity.

c) Population-based studies: in a population that includes healthy individuals and donors, the correlation in regards to other tests available in the market was 99.8%.

WIENER LAB. PROVIDES

- Kit for 96 tests (Cat. 1483253).
- Kit for 192 tests (Cat. 1483256).

REFERENCES

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SYMBOLS EXPLANATION

Policubeta **Sensib.**

Coated microtitration plate

Diluyente **Conj.**

Conjugate Diluent

Conjugado **Conc.**

Concentrated Conjugate

Buf. Lavado **Conc.**

Concentrated Wash Buffer

Revelador **A**

Substrate A

Revelador **B**

Substrate B

Control **+**

Positive Control

Control **-**

Negative Control

Stopper

Stopper

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC **REP** Authorized representative in the European Community

IVD "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL+ Positive Control

CONTROL- Negative Control

REF Catalog number

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