Atlas Medical

Hepatitis B Surface Antigen (ELISA)

IVD *For in-vitro diagnostic use only*

Store at 2 to 8°C

96 Tests

INTRODUCTION

Hepatitis B is a disease caused by viral infection. The route of infection can be improper needle puncture, blood transfusion or even by taking contaminated food or water.

Hepatitis B has become a significant problem for public health management. Almost one in every ten adults, who have been infected by Hepatitis B Virus (HBV), develops some form of chronic liver disease and becomes a long-term carrier of HBV. Screening for Hepatitis B is therefore urgently needed.

Hepatitis B is an immune disease. Invasion of the human body by HB virus induces autoimmune reactions, which damage the liver. The components of the virus (antigens) and the host responses (antibodies), the so-called immunologic markers have often been used as diagnostic tools.

There are six immunologic markers of HBV: HBsAg, HBcAg, HBeAg and their respective antibodies. The HBsAg however is the first marker to appear in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier.

PRINCIPLE

This assay is based upon the one-step sandwich method. Sample, An- ti-HBs coated microplate and enzyme-labeled Anti-HBs are combined. During the incubation, HBsAg present in the sample is allowed to react simultaneously with the two antibodies, resulting in the HBsAg being sandwiched between the solid phase and enzyme-linked antibodies. After washing, a complex is generated between the solid phase, the HBsAg within the sample and antibody in enzyme conjugate by immunological reactions. Substrate A and substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromo- genic reaction is measured as absorbance. The color intensity is propor- tional to the amount of HBsAg in the sample.

MATERIALS

MATERIALS PROVIDED

- **Coated microplates:**
  One plate of 96 wells pre-coated with mouse monoclonal Anti-HBs. Store at 2-8° C.
- **Enzyme Conjugate:**
  One red cap vial containing 7.5 ml of HRP (horseradish peroxidase) labeled sheep polyclonal Anti-HBs in a buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin 300® preservative.
- **HBsAg Negative Control:**
  One green cap vial (1 ml) containing of phosphate buffered solution containing proteins of bovine origin. Contains 0.1% ProClin 300® preservative. Store at 2-8° C.
- **HBsAg Positive Control:**
  One red cap vial (1 ml) of phosphate buffered solution containing heat-inactivated human plasma positive for HBsAg and proteins of bovine origin. Contains 0.1% ProClin 300® preservative. Store at 2-8° C.
- **Substrate Solution (A)**
  One blue cap vial (7.5 ml) containing Hydrogen Peroxide. Store at 2-8° C.
- **Substrate Solution (B)**
  One brown cap vial (7.5 ml) containing stabilized 3,3'-5,5' Tetramethylbenzidine (TMB) in buffer solution. Store at 2-8° C.
- **Stop Solution**
  One yellow cap vial (7.5 ml) containing 0.62 mol/l H_2SO_4. Ready to use.
- **Wash Buffer (20X Conc.)**
  One transparent cap vial (30 ml) of 20 times working strength PBS-Tween wash buffer.
- **Package insert.**
- **1 piece of plate lid.**
- **1 zip-lock bag.**

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

1. Absorbent paper or paper towel.
2. Automated microplate strip washer.
3. Distilled water.
4. Disposable reagent troughs.
5. Incubator
6. Magnetic stirrer.
7. Micropipettes and multichannel micropipettes of appropriate volumes.
8. Microplate reader.
9. Plate shaker.

PRECAUTION

- The Positive Control is made of HBsAg positive sera and has been heated at 60° C for 10 hours. This is a generally accepted method to inactivate the Hepatitis agent. However, for safety precautions, it must be treated as potentially infectious material. Both positive and negative controls have been tested and found negative for HIV 1-2 and Hepatitis C virus. However, they must be treated as potentially infectious agents.
- Do not smoke or eat where specimens or reagents of the kit are handled.
- Do not pipette by mouth. Wear PVC gloves when handling reagent kits or specimens and wash hands thoroughly afterwards.
- Non acid containing spills should be wiped up thoroughly with 5% sodium hypochlorite solution.
- All waste material should be properly disinfected before disposal. Both liquid and solid waste material can be autoclaved for one hour at 121° C. Solid waste material can also be incinerated. Non-acid waste material can be treated with sodium hypochlorite (bleach solution) diluted to a final concentration of 1.0%. Acid liquid waste material requires neutralization before similar treatment and should stand for 30 minutes to obtain effective disinfections.

STORAGE

1. Store all components at 2-8 ℃. Do not freeze. Avoid strong light.
2. Place unused wells in the Zip-lock bag with desiccant provided, then seal the Zip-lock bag in the aluminum foiled pouch with a plate lid and return to 2-8 ℃, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
3. Seal and return all the other unused reagents to 2-8 ℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

REAGENT PREPARATION

1. Bring all reagents to room temperature (18-25 ℃) prior to use for at least 30 minutes. Mix all reagents through gently inverting prior to use. Do not induce foaming.
2. Adjust the incubator to 37 ℃.
3. Add 1 volume of wash solution concentrate to 19 volumes of distilled water to give the required volume, and mix well with a magnetic stirrer. The wash solution is stable at room temperature for 2 months.

SPECIMEN COLLECTIONS AND STORAGE

1. Either serum or plasma can be used with this diagnostic kit.
2. Collect samples in accordance with correct medical practices.
3. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.

PROCEDURE

1. Bring all reagents and specimens to room temperature (15°C-25°C) before beginning the assay. Swirl gently before use. Adjust the incubator at +37°C, if necessary.
2. Write down the relative numbers of specimens and wells on the data sheet. One well for the blank, five additional wells for the controls and one well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the zip lock bag (included in the kit) which contains desiccant.
3. Reserve one well for blank, add **50ul** of the Negative Control to B1 ,C1 and D1 and **50ul** of the Positive Control to E1 and F1 and add **50ul** of specimen to respective wells, then Pipette **50ul** of Anti-HBsAg peroxidase-conjugate working solution into each well, except the blank (A1) according to the following scheme:

   A1 : Blank
   B1, C1, D1 : Negative Control Serum
   E1, F1 : Positive Control Serum

Notes:

- Use an individual tip for each pipetting to avoid cross contaminations.
- If more than 2 or 3 runs per plate are required and reliable apparatus (i.e. pipettes, washers, etc.) are used, one can pipette the Negative Control in duplicate and the Positive control in singleton.

4. Incubate the plate in a +37°C incubator for 30 minutes.
5. At the end of the incubation wash the strips 6 times with the working wash solution either manually or with an automatic washer. After the final washing, make sure that whole solution is perfectly removed from each well.

Note: Proper washing procedure is essential for good assay performance.

6. Dispense **50ul** of Substrate solution A and **50ul** of Substrate solution B, including 1A. Mix horizontally for 15 seconds and incubate for 10 minutes at +37°C (avoid exposure to direct sun light).
7. Stop the reaction by adding **50ul** of stop solution to each well (including the blank) in the same order adopted for the addition of the substrate solution.
8. Read the absorbance within 20 minutes at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a mi-croplate reader. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the ab-sorbance of the blank well at 450 nm.

CONTROL PROCEDURE

The result is valid if the following criteria for the controls are both met:

Negative Control:

1. Mean negative control is lower than 0.10 O.D.

Positive Control:

2. Mean positive control is equal to or higher than 0.6 O.D.

MEASUREMENT RESULTS

Each plate must be considered separately when calculating and interpreting results of the assay.

1. Negative Control

Calculate the mean absorbance of the replicates of the negative control.

2. Cut-off Value

The cut-off value is 2.1 times the mean of the negative control replicates (in case the mean absorbance of negative control replicates < 0.05, use 0.05 instead of the actual mean)

3. Example

Negative Control absorbance: well 1 = 0.011, well 2 = 0.009, well 3= 0.013.
Mean Negative Control = (0.011 + 0.009+ 0.013)/3 = 0.011
Cut-off Value =2.1 x 0.05= 0.105.

INTERPRETATION OF RESULTS

1. Nonreactive

Samples giving an absorbance less than the cut-off value are considered nonreactive.

2. Reactive

Samples giving an absorbance equal to or greater than the cut-off value are considered reactive. Such samples should be retested in duplicate using the original sample source. Samples that are reactive in at least one of the re-tests are presumed to contain HBsAg and should be confirmed by using a confirmatory kit and tests for other HBV markers. Samples that are nonreactive in both wells on retest should be considered nonreactive.

PERFORMANCE CHARACTERISTICS

PRECISION

This assay is designed to have a within-run precision of <10%. 1 bovine serum based panel member was assayed, using 1 batch of reagents, in replicates of 96. Data from this study are summarized in the following table:

Panel Member	Batch	n	Mean	Within-run precision SD	%CV
1	1	96	1.177	0.068	5.58

This assay is designed to have a between-run precision of < 15%. 1 human plasma based panel member was assayed, using 1 batch of reagents, in replicates of 8, across 12 separate runs. Data from this study are summarized in the following table:

Panel Member	Batch	n	Mean	Between-run precision SD	%CV
1	1	96	1.150	0.084	7.35

SENSITIVITY

Sensitivity: 100 %

SPECIFICITY

Specificity: > 99,5%

LIMITATION OF THE PROCEDURE

- This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
- If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Heterophilic antibodies and rheumatoid factors in samples may in-terfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immu-noassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
- 4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibo-dies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis

REFERENCE

1. Dienstag JL. Hepatitis B virus infection. N. Engl. J. Med. 2008;359(14):1486-1500.
2. Knowles B, Howe C, Aden D. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science. 1980;209(4455):497-499.
3. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T–lymphocyte response. Nat Med. 1996;2(10):1104-1108.
4. Gust ID. Epidemiology of hepatitis B infection in the Western Pacific and South East Asia. Gut. 1996;38 Suppl 2:S18-23.
5. Toukan A. Strategy for the control of hepatitis B virus infection in the Middel East and North Africa. Vaccine. 1990; 8:S100-S106.

ATLAS MEDICAL
Ludwig-Erhard Ring 3
15827 Blankenfelde-Mahlow
Germany
Tel: +49 - 33708 – 3550 30
Email: Info@atlas-medical.com

PPI1423A01,Rev A (02.09.2019)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry