HBsAg

Hepatitis B Surface Antigen (ELISA)

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REF: DS177751 Σ 96

Intended use
The DiaSino HBsAg assay is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of HBsAg virus in human serum and plasma.

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]

Test principle
Sandwich principle.Total duration of assay: **70 minutes.**
The HBsAg ELISA uses antibody "sandwich" ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwell together with a second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and directed against a different epitope of HBsAg. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-conjugate, Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody (HRP) "sandwich" immuno complex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

Reagents
Materials provided

- **HBsAg Coated Microplate - symbol** HBsAg PLATE 8 x 12 strips, 96 wells. Pre-coated with monoclonal antibodies to HBsAg
- **HBsAg Negative Control - symbol** HBsAg NC 1 vial, 1.0 mL, ready to use. Contains 0.1% ProClin300 preservative.
- **HBsAg Positive Control - symbol** HBsAg PC 1 vial, 1.0 mL, ready to use. Contains 0.1% ProClin300 preservative.
- **HBsAg Enzyme Conjugate - symbol** HBsAg CONJ 1 vial, 6.0 mL, ready to use. Contains 0.1% ProClin300 preservative. HRP-labeled anti-HBsAg antibodies.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU:** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)

- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings

- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.
- **Indications of instability deterioration of the reagent:** Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one.

Storage and stability

- Store at 2-8℃.
- Don't freeze.
- Seal and return 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.

Specimen collection and preparation

- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but ***highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay.
- 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.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples and reagents are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.

- **Preparation:** Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample:** Add **50µL** of **Positive control, Negative control**, and **Specimen** into their respective wells except the Blank.
- Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.
- **Adding Conjugate:** Add **50µL** of **Conjugate** into each well except the Blank, and mix by tapping the plate gently.
- **Incubation:** Cover the plate with the plate cover and incubate for **60 minutes** at **37°C.**
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100µL** of **TMB Substrate** into each well including the Blank. Incubate for **10 minutes** at **room temperature** avoiding light. The enzymatic reaction between the TMB substrate and the Conjugate produces blue color in Positive control and HBsAg positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** into each well and mix gently. Intensive yellow color develops in Positive control and HBsAg positive sample wells.
- **Measuring:** Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results.
  Note: read the absorbance within 10 minutes after stopping the reaction.

Quality control and calculation
Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. The results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = 2.1×**NC** (**NC** = the mean absorbance value for two negative controls).

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Important: If the NC is lower than 0.05, take it as 0.05.
Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

Example:

1. Quality control

Blank well A value: A1= 0.025 at 450nm

Well No.:	**B1**	**C1**
Negative control A values after blanking:	0.028	0.030
Well No.:	**D1**	**E1**
Positive control A values after blanking:	2.421	2.369

All control values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.028+0.030)}{2}$ = 0.029 (Nc is lower than 0.05, so take it as 0.05)

3. Calculation of the Cut-off: (C.O.) = 2.1×0.05= 0.105

- The A value of the Blank well, which contains only TMB Substrate and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining value. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Interpretations of the results

Negative Results (A/C.O. < 1)

Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HBsAg has been detected with this ELISA kit. Therefore, the patient is probably not infected with HBsAg.

Positive Results (A/C.O. ≥ 1)

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HBsAg has probably been detected using this ELISA kit. All initially reactive specimens should be retested in duplicates using this ELISA kit before the final assay results interpretation. Repeatedly reactive specimens could be considered positive for HBsAg and therefore there are serological indications for infection with HBsAg.

Borderline (A/C.O. = 0.9-1.1)

Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results. Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for HBsAg.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance characteristics

Specificity

The clinical performances of this assay have been evaluated by a panel of samples obtained from 2280 healthy blood donors and by samples from 300 HBsAg positive samples from patients with well characterized clinical history and confirmed Western Blot and PCR positive results. The evaluation results are given below.

Sample	n	-	+	Confirmed positive	Specificity	False positive
Donors	2280	2271	9	6	99.87%	3
Patients	300	273	27	26	99.63%	1

Sensitivity

The clinical sensitivity of HBsAg ELISA was calculated by a panel of samples obtained from 1120 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. Licensed HBsAg ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

	n	+	Sensitivity
Acute	420	418	99.52%
Chronic	600	598	99.67%
Recovery	100	100	100%

Analytical Specificity

- No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
- No interference from rheumatoid factors up to 2000U/mL observed.
- No high dose hook effect up to HBsAg concentrations of 200000ng/ml observed during clinical testing.
- Frozen specimens have been tested too to check for interferences due to collection and storage.
- Analytical Endpoint Sensitivity (lower detection limit):
- The sensitivity of the assay has been calculated by means of the reference standards provided from the Reference Laboratory for Immunology Product under the Ministry of Health, China. The assay shows sensitivity at the Cut-off of 0.5ng/ml (adr) and 0.5ng/ml (adw, ay).

Limitations

- This reagent kit is to be used for un-pooled human serum or plasma only.
- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Non-repeatable false positive results may occur due to non-specific binding of the sample and conjugate to the wall of the well(s).

References

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Symbols

IVD **In vitro diagnostic medical device**	8°C / 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**		

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