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ELISA **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

Microwell Method

HBsAg Sensitive

Ref. Z12360 **Ref. Z12354** **Ref. Z12355**

For in vitro Diagnostic Use

P r o d u c t I n s e r t

Double antibody Sandwich Elisa for the **cut-off** determination of Hepatitis B Surface antigen (HBsAg) in human serum or plasma.

IVD CE 1293

Microwell Method - 96 wells

12 x 8-well Antibody coated Strips

Individual breakaway

GENERAL INFORMATION

- **Wavelength**

Measurement Filter: 450 nm

Reference Filter: 630 nm

- **Incubation Time**

120 minutes at 37°C (60/30/30)

- **Enzyme Conjugate**

HRP (Horseradish Peroxidase)

- **Substrate Solution A**

TMB (3,3',5,5'-Tetramethyl-Benzidine)

- **Substrate Solution B**

Urea Hydrogen peroxide

- **Sample**

Serum or plasma

- **Shelf life and Stability of Kit Components**

- **Sensitivity: 0.05 ng/ml (0.1 IU/ml NIBSC 00/588)**

Kit:	See expiration date on the label.
Kit Components:	See expiration date on the label.
Microwell plate:	See expiration date on the label.

KIT COMPONENTS

Z12360	Z12354 = 5x Z12360	Z12355 = 6x Z12360
Microwell plate	1 Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. 12×8-well strips per plate. Each well contains monoclonal antibodies reactive to HBsAg (anti-HBs). The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2-8°C. **Once open, stable for one month at 2-8°C.**	
Positive Control	1 ml, red-colored liquid filled in a vial with red screw cap. HBsAg diluted in protein-stabilized buffer. Ready to use as supplied. Contains 0.1% ProClin™ 300 as preservative. **Once open, stable for one month at 2-8°C.**	
Negative Control	1 ml, yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for HBsAg. Ready to use as supplied. Contains 0.1% ProClin™ 300 as preservative. **Once open, stable for one month at 2-8°C.**	
Specimen Diluent	5 ml, green-colored liquid in a vial with blue screw cap. Serum base, casein, and sucrose solution. Contains 0.1% ProClin™ 300 as preservative. **Once open, stable for one month at 2-8°C.**	
Enzyme Conjugate	6 ml, red-colored liquid filled in a vial with red screw cap. Horseradish peroxidase (POX) conjugated anti HBs antibodies. Ready to use as supplied. **Once open, stable for one month at 2-8°C.**	
Substrate Solution A	6 ml, colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. **Once open, stable for one month at 2-8°C.**	
Substrate Solution B	6 ml, colorless liquid filled in a black vial with black screw cap. TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. **Once open, stable for one month at 2-8°C.**	
Stop Solution	6 ml, colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. **Once open, stable for one month at 2-8°C.**	
Wash Buffer	30 ml, colorless liquid filled in a clear bottle with white screw cap, pH 7.4, 20 × PBS. The concentrate must be diluted **1 to 20** with distilled/ deionized water before use. Contains Tween 20 as a detergent. **Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.**	
Cardboard Plate Cover	3 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED

- ❑ **Micropipettes** capable of delivering 5 µl to 1000 µl.
- ❑ **Multichannel Micropipette, disposable pipette tips**
- ❑ **Freshly distilled or deionized water** for Wash Buffer.
- ❑ **Microplate reader** capable of reading absorbance values at 450nm. If a dual wavelength microplate reader is available, the reference filter should be set at 630nm. Refer to the instruction manual supplied with your instrument.
- ❑ **Automated microplate washer** capable of dispensing 300 µl (optional).
- ❑ **Incubator at 37°C**
- ❑ **Graph paper** or a computerized curve-fitting statistical software package.
- ❑ **Disposable gloves and timer, absorbent tissue.**
- ❑ **Waste container for potentially contaminated materials**
- ❑ **Micro shaker**

--- Microplate reader and microplate washers are available from Dialab Company. ---

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of HBsAg Sensitive ELISA achieved.

INTENDED USE

Dialab HBsAg Sensitive ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma. It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis B virus.

SUMMARY

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic test are used for screening, clinical diagnosis and management of the disease. Hepatitis B surface antigen or HBsAg, previously described as Australia antigen, is the most important protein of the envelope of Hepatitis B Virus. The surface antigen contains the determinant “a”, common to all known viral subtypes and immunologically distinguished in two distinct subgroups (ay and ad). HBV has 10 major serotypes and four HBsAg subtypes have been recognized (adw, ady, ayw, and ayr). HBsAg can be detected 2 to 4 weeks before the ALT levels become abnormal and 3 to 5 weeks before symptoms develop. The serological detection of HBsAg is a powerful method for the diagnosis and prevention of HBV infection and ELISA has become an extensively used analytical system for screening of blood donors and clinical diagnosis of HBV in infected individuals.

TEST PRINCIPLES

For detection of HBsAg, Dialab 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 microwells. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. Then the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of HBsAg is added into the wells. During the second incubation step, these HRP-conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP-conjugate is then removed by washing. After washing to remove 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” immunocomplex, 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.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this HBsAg ELISA kit, during storage protect the reagents from contamination with microorganisms or chemicals.

SPECIMEN COLLECTION, TRANSPORTING AND STORAGE

1. Sample Collection: No special patient’s preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible

particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.

2. Plasma specimens collected into EDTA, sodium citrate or heparin may be tested, but **highly lipaemic, icteric, or haemolytic specimens should not be used** as they can give false results in the assay. **Do not heat inactivate specimens**. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents..

REMARKS AND PRECAUTIONS

TO BE USED ONLY FROM PROFESSIONALS. FOR IN VITRO USE ONLY

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION – CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. The use of automatic pipettes is recommended. Never pipette solutions by mouth.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding samples, avoid touching the well's bottom with the pipette tip.
11. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
12. The enzymatic activity of the HRP-Conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the kit. These materials have been tested with test kits with accepted performance and found negative for antibodies to HIV 1&2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE-free geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
17. Chemicals should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 2 hours at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.

19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution (0.5M H_2SO_4) is an acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.
21. ProClin™300 0.1% used as a preservative can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

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 or contact technical support for further assistance.

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-Conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution (final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted 1:20 before use. For one plate, mix 30ml of the concentrate with 570ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

PREPARATIONS

1. **Reagents preparation:** Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1:20** with distilled or deionized water. Use only clean vessels to dilute the buffer.
2. Allow the test samples, controls, conjugate, diluted wash solution, substrate, aluminum bag containing the microplate and the vial containing TMB to come to ambient temperature before use.

TEST PROCEDURE

1. **Number wells:** Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) 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.
2. **Adding Diluent:** Add **20 µl** of Specimen Diluent into each well except the Blank.
3. **Adding Sample:** Add **100 µl** 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.
4. **Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37°C**.

5. **Adding HRP-Conjugate:** At the end of the incubation, remove and discard the plate cover. Add **50 µl** HRP-Conjugate into each well except the Blank, and mix by tapping the plate gently.
6. **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
7. **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.
8. **Coloring:** Add **50 µl of Chromogen A and 50 µl Chromogen B** solutions into each well including the Blank. **Incubate the plate at 37°C for 30 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBsAg positive sample wells.
9. **Stopping Reaction:** Using a multichannel pipette or manually, add **50 µl** Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-HBsAg positive sample wells.
10. **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. 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).

INTERPRETATION OF RESULTS AND QUALITY CONTROL

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. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

CALCULATION OF THE RESULTS

Calculation of the Cut-off value (C.O.) = Nc + 0.06 (Nc = the mean absorbance value for three negative controls).

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.

The A value of the **Blank well**, which contains only Chromogen 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 two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control

Blank well A value: A1 = 0.025 at 450nm

(Note: blanking is required only when reading with single filter at 450nm)

Well No.:	B1	C1	D1
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	E1	F1	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range

2. Calculation of Nc: = (0.020+0.012+0.016) : 3 = 0.016

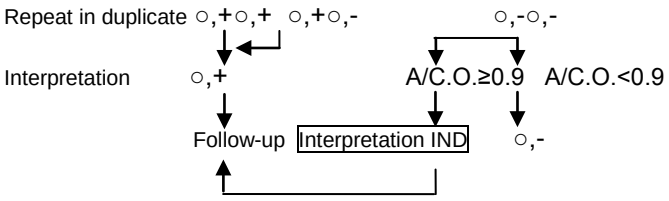
3. Calculation of the Cut-off: (C.O.) = 0.016 +0.06 = 0.076

INTERPRETATION OF 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 Hepatitis B virus surface antigen has been detected with Dialab HBsAg Sensitive ELISA, therefore the patient is probably not infected with HBV and the blood unit do not contain hepatitis B virus surface antigen and could be transfused in case that other infectious diseases markers are also absent.
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 Hepatitis B virus surface antigen has probably been detected using Dialab HBsAg Sensitive ELISA. All initially reactive specimens should be retested in duplicates using Dialab HBsAg Sensitive ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for Hepatitis B virus surface antigen with Dialab HBsAg Sensitive ELISA.
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 (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP
ALL INITIALY REACTIVE OR BORDERLINE SAMPLES



IND = non interpretable

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 Hepatitis B virus surface antigen and therefore the patient is probably infected with HBV and the blood unit must be discarded.
After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone samples, or “uninterpretable” for the time of testing.

PERFORMANCE CHARACTERISTICS

Evaluation studies demonstrated the following performance characteristics of HBsAg Sensitive ELISA.

Specificity: When evaluated on European blood donors (n=5038), the overall diagnostic specificity of the kit was 99.78%.

During multi-center evaluation Dialab HBsAg Sensitive ELISA demonstrated specificity of 99.92%.

Laboratory	Number	Dialab HBsAg ELISA		
		-	+	Specificity
Blood bank A	1958	1955	3	99.85%
Blood bank B	2518	2516	2	99.92%
Blood bank C	6344	6340	4	99.94%
Total	10820	10811	9	99.92%

Sensitivity: Dialab HBsAg Sensitive ELISA was evaluated for sensitivity on 22 HBV commercial available HBV seroconversion panels, and on total 403 HBsAg positive including 146 HBsAg HBV genotyped and HBsAg subtyped plasma samples. With respect to seroconversion sensitivity, the results for Dialab HBsAg Sensitive ELISA on the 22 HBV seroconversion panels showed a sensitivity level at least equivalent with the range of current CE marked HBsAg screening assays. 10 additional seroconversion panels were tested in-house. The seroconversion sensitivity was comparable to other CE-marked HBsAg screening tests. With respect to diagnostic sensitivity Dialab HBsAg Sensitive ELISA detected all positive samples as positive, including the HBV genotypes A-F or HBsAg subtypes examined.

In conclusion, the overall score of Dialab HBsAg Sensitive ELISA for the seroconversion sensitivity was comparable with other CE marked HBsAg test kits for which PEI holds data and all 403 HBsAg positive samples were reactive giving an overall sensitivity of 100%.

Analytical sensitivity: 0.1IU/ml (NIBSC 00/588)

Analytical specificity: No interference was observed with samples from patients with high-level of rheumatoid factor, and pregnant woman. Same day and frozen specimens have been tested to check for interferences due to collection and storage. Total of 100 samples reactive for anti-HBc, anti-HCV and anti-HIV-1 were screened for HBsAg with Dialab HBsAg Sensitive ELISA. 98 out of 100 samples were negative for HBsAg. 200 blood samples from patients were also tested with Dialab HBsAg Sensitive ELISA. 191 out of 200 samples had negative screening results for HBsAg. 8 out of 9 samples with initial reactive screening results had repeat reactive test results with Dialab HBsAg Sensitive ELISA, but Hepatitis B virus was not confirmed in all cases.

Detection of mutations: Panel of 108 samples collected and sequenced by PCR were tested to demonstrate the performance of Dialab HBsAg Sensitive ELISA in detection of HBsAg mutations. The results are given in the table below.

Background		**Number**	**Dialab HBsAg Sensitive ELISA**
adr (+)	wild type	35	33
	4 mutations	5	4
adw (+)	wild type	37	34
	16 mutations	25	24
ayw (+)	wild type	2	2
	2 mutations	2	2
ayr (+)	2 mutations	2	2
Total		108	101

Reproducibility:

Reproducibility		Within Run		Between Run	
Specimen Type	n	Mean OD	CV%	Mean OD	CV%
0.1IU/ml HBsAg	10	0.155	10.6%	0.150	11.0%
Weak positive	10	0.457	9.0%	0.432	9.5%
Moderate positive	10	1.572	7.0%	1.437	7.5%
Strong positive	10	2.327	4.2%	2.302	4.4%
Positive control	10	2.322	4.1%	2.315	4.2%

LIMITATIONS

1 Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2 Antigens may be undetectable during the early stage of the disease. Therefore, negative results obtained with Dialab HBsAg Sensitive ELISA are only indication that the sample does not contain detectable level of Hepatitis B virus surface antigen and any negative result should not be considered as conclusive evidence that the individual is not infected with HBV or the blood unit is not infected with HBV.
3 If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many

very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.

4 The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly haemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.

5 The prevalence of the marker will affect the assay's predictive values.

6 This assay cannot be utilized to test pooled (mixed) plasma. Dialab HBsAg Sensitive ELISA has been evaluated only with individual serum or plasma specimens.

7 Dialab HBsAg Sensitive ELISA is a qualitative assay and the results cannot be used to measure antigen concentration.

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CE-MARKING SYMBOLS

CE Marking – IVDD 98/76/CE

In Vitro Diagnostic Medical Device

Use by

Content Sufficient For <n> Tests

Instructions for use

+2°C~+8°C Storage conditions

Catalog number

Batch number

Manufacturer

EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	...										
C	Neg.	...										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:	
Microwell plate	One/96 wells
Negative control	One/1 ml
Positive control	One/1 ml
Specimen Diluent	One/5 ml
HRP-Conjugate	One/6 ml
Wash Buffer (20xconc.)	One/30 ml
Substrate A	One/6 ml
Substrate B	One/6 ml
Stop solution	One/6 ml
Note: the components of individual kits are not interchangeable	

SUMMARY OF THE ASSAY PROCEDURE:	
Add specimen diluent	20 µl
Add sample	100 µl
Incubate	60 minutes
Add HRP-Conjugate	50 µl
Incubate	30 minutes
Wash	5 times
Coloring	50 µl A + 50 µl B
Incubate	30 minutes
Stop the reaction	50 µl stop solution
Read the absorbance	450 nm or 450/630 nm

ELISA **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay

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