

HBsAb

**Enzyme Immunoassay for
qualitative/quantitative determination of
antibodies to Hepatitis B surface Antigen
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child-to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or

cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon* or *lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV). This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.

Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.

Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.

The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.

After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.

The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.

The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.

Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.

Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: **CAL N° ...**

5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml. Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: **WASHBUF 20X**

1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : **CONJ**

1x16.0 ml/vial. Ready-to-use solution and red color coded. It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

5. Chromogen/Substrate: **SUBS TMB**

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H₂O₂). **Note: To be stored protected from light as sensitive to strong illumination.**

6. Sulphuric Acid: **H₂SO₄ 0.3 M**

1x15ml/vial. Contains 0.3 M H₂SO₄ solution. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: **DILSPE**

1x8ml. 10 mM Tris Buffered solution pH 7.4 +/-0.1, suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: **CONTROL ...ml**

1 vial. Lyophilized. Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2.8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.

10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.

11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..

14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water

16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.

3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.

4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be freeze/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 µl of each specimen with 450 µl of Cal 0 (1:10). Then 50 µl of the 1:10 dilution are diluted with 450 µl of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.

In this case, call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve

Ready to use. Mix well on vortex before use.

3. Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

Note: The control after dissolution is not stable. Store frozen in aliquots at -20°C.

4. Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

5. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:

Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.

If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning H statements:

H315 – Causes skin irritation.

H319 – Causes serious eye irritation.

Precautionary P statements:

P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.

P332 + P313 – If skin irritation occurs: Get medical advice/attention.

P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337 + P313 – If eye irritation persists: Get medical advice/attention.

P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

2. The ELISA incubator has to be set at +37°C (tolerance of ±1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of ±5%.

5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the

values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed. Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: This additive is added before distributing samples and controls into specific wells and is particularly

intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.

3. Wash the microplate as reported in section I.3.
4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes**.

Important note:

- 1) Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.
- 2) Mix thoroughly the Enzyme Conjugate on vortex before use.

5. Wash the microplate as described.
6. Pipette 100µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes**.

Important note:

- 3) Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.
- 4) Mix thoroughly the Enzyme Conjugate on vortex before use.

5. Wash the microplate as described.
6. Pipette 100µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

Important general notes:

1. If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.
3. The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME (standard procedure)

Specimen Diluent	50 ul
Calibrators	100 ul
Control Serum	100 ul
Samples	100 ul
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme Conjugate	100 ul
2nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂ mix	100 ul
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3	S11									
B	CAL1	S4	S12									
C	CAL1	S5	S13									
D	CAL2	S6	S14									
E	CAL2	S7	S15									
F	CAL5	S8	S16									
G	S1	S9	S17									
H	S2	S10	S18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

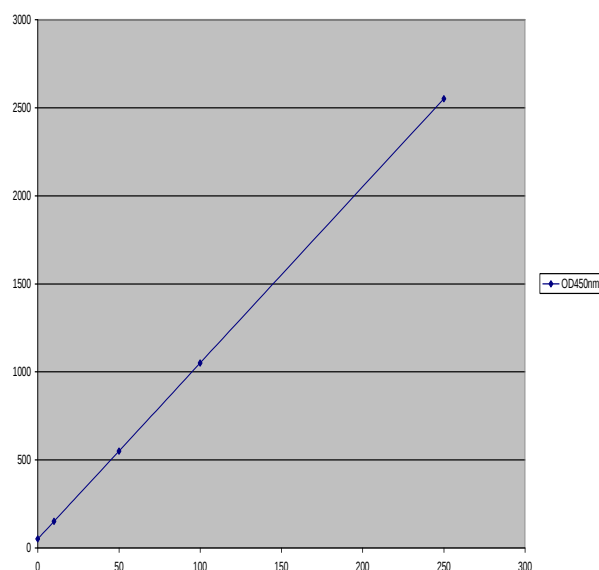
If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200	1. that the washing procedure and the washer settings are as validated in the pre qualification study;
coefficient of variation > 30%	2. that the proper washing solution has been used and the washer has been primed with it before use;
	3. that no mistake has been done in the assay procedure when the dispensation of standards is carried out;
	4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate;
	5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate
	6. that the washer needles are not blocked or partially obstructed.

Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: a) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. b) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Example of Calibration Curve :



Important Note:

Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.

An example of Calibration curve is reported in the next page.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.

Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.

In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:

1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier. Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.

More than 500 negative specimens were tested, internally and externally, against a European company.

A diagnostic specificity of 98.8% was assessed. .

Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.

Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.

More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

5. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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