DRG® HBs Ab ELISA (EIA-4135)

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INTENDED USE

Enzyme ImmunoAssay (ELISA) for determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.

For Research Use only.

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.

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 (Xi R36/38; S2/26/30)

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 at about 50 +10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

9. Plate sealing foil n° 4

10. Package insert n° 1

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 possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

SPECIMEN: PREPARATION AND WARNINGS
1. Blood is drawn aseptically by venepuncture 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 freezed/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 0.2-0.8u 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 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

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 dark green, indicating a defect in manufacturing.
In this case, call DRG'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 (Xi R36/38; S2/26/30)
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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. In case Procedure B is used (see below) the instrument has to ensure shaking at 1300 rpm +150.
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 300ul/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 ideally with a second filter (620-630nm) 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. DRG'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.

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.

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.

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 possibly at 620-630nm (blanking), blanking the instrument on A1 and B1 wells.

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. The control serum may be used to verify that the whole analytical system works as expected. 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:

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 possibly at 620-630nm (blanking), 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.*

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/H2O2 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 = Calibrator 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	S1										
B	CAL1	S2										
C	CAL1	S3										
D	CAL2	S4										
E	CAL2	S5										
F	CAL5	S6										
G	CS	S7										
H	CS	S8										

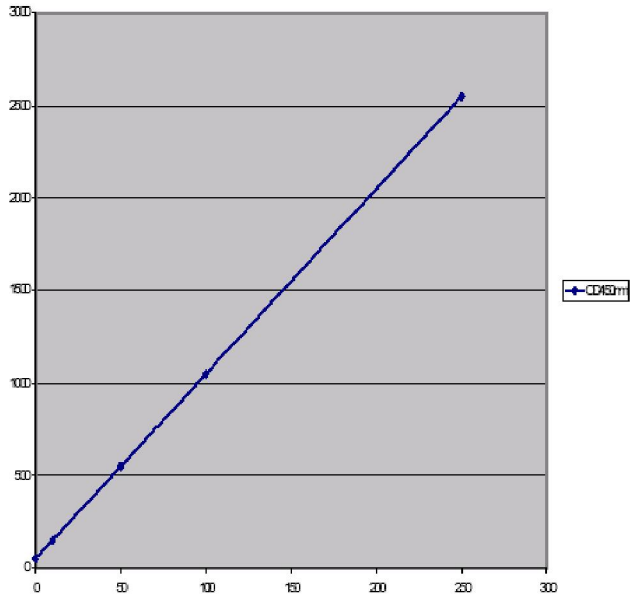
Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

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.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

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 or 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

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