HBc IgM

"Capture" Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibody to Hepatitis B Virus core Antigen in human plasma and sera

- for "in vitro" diagnostic use only -

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REF BCM.CE
96 Tests

HBc IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Hepatitis B Virus core Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral agent and for the follow-up of chronic patients under therapy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV).
Particles have a size of 27nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAg. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles ; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg IgM antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen.
Anti HBcAg IgM titers, very high during the acute phase, decrease along the illness, as IgG antibodies appear, down to undetectable levels in convalescent patients.
In chronic hepatitis, however, spikes of anti HBcAg IgM synthesis are present, confirming reactivation of HBV in hepatocites and giving origin to permanent IgM low titers.
The determination of anti HBcAg IgM antibodies has become very important for the fast classification of the virus, of the phase of the illness and for the monitoring of patients under treatment with interferon.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified anti human IgM specific mouse monoclonal antibody, post-coated with bovine serum proteins 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°…
6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on the HBcIgM reference preparation supplied by Paul Erlich Institute (HBc-Referenzserum-IgM 84), ranging: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml.
It contains chemical inactivated HBcIgM positive human plasma, 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The Calibration Curve is coded with blue alimentary dye.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

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 (Immunocomplex) : CONJ
1x16.0 ml/vial. Ready-to-use solution. Contains an immunocomplex formed by a specific mouse monoclonal antibody, labelled with HRP, and a purified recombinant HBcAg.
The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The component is red colour coded.

5. Specimen Diluent : DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 2% Casein, 0.1% Kathon GC and 0.09% sodium azide as preservatives. The component is blue color coded.

6. Control Serum : CONTROL …ml
1 vial. Lyophilized. Contains fetal bovine serum, human HBcIgM positive human plasma calibrated at 20 ± 10% PEI U/ml. 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Important Notes
1. The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .
2. Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

7. 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 or TMB and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (Xi R36/38; S2/26/30)

9. Plate sealing foils: n°2

10. Package insert: n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 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.
6. Calibrated ELISA microwell reader with 450nm (reading) and if possible with 620-630nm (blanking) 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-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (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 temp erature 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.
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 external (primary container) and internal (vials) labels.
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 washing solution or in transferring components into other containers of automated workstations, in order to avoid 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. 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 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 Stop Solution 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 RECOMMANDATIONS

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. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed 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.
5. 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.
6. 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.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

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 Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve:
Ready to use. Mix well on vortex before use.

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml 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.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Specimen Diluent
Ready to use. Mix on vortex before use.

Control Serum
Dissolve the content of the vial with EIA grade water as reported in the label. Mix well on vortex before use. The dissolved control serum is ready to use.
Note: The control after dissolution is not stable. Store frozen in aliquots at –20°C.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

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.

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 (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. 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 (tole rance of +/- 0.5°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 and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 350ul/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 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 by, 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 reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes 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 the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, 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 and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended 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 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 (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium 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 and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
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 is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn 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 equipment is 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 plastic holder and carefully identify the wells for standards and samples.
2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators and the dissolved Control Serum as they are ready-to-use.
3. Leave the A1+B1 wells empty for blanking purposes.
4. Pipette 100 µl of the Calibrators in duplicate, 100 µl dissolved Control Serum in duplicate followed by 100 µl of diluted 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.
5. Incubate the microplate **for 60 min at +37°C**.

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.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. In all the wells except A1+B1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1+B1 included.

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

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction.. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and if possible a 620-630nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

M.2 Qualitative analysis

1 Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
2 Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
3 Leave the A1 well empty for blanking purposes.
4 Pipette 100 µl Calibrator 0 U/ml in duplicate, 100 µl Calibrator 10 U/ml in duplicate and 100 µl Calibrator 100 U/ml in single. Then dispense 100 µl diluted samples in proper sample wells. Check that Calibrators and samples have been correctly added.
5 Incubate the microplate **for 60 min at +37°C**.

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.*

6 When the first incubation is finished, wash the microwells as previously described (section I.3)
7 In all the wells except A1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8 When the second incubation is finished, wash the microwells as previously described (section I.3)
9 Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

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

10 Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11 Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12 Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and if possible a 620-630nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

Important 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.*

N. ASSAY SCHEME

The assay protocol can be summarized in the table below:

Calibrators & diluted samples & dissolved Control Serum	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing steps	n°4-5
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n°4-5
Chromogen/Substrate	100ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm

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	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS	S7									
H	CAL3	CS	S8									

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	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL3	S 6	S 14									
E	CAL3	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

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:

Parameter	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 PEI U/ml coefficient of variation	< 0.150 OD450nm after blanking < 30%
Calibrator 5 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 5SD and anyway > OD450nm Cal 0 U/ml + 0.100
Calibrator 10 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 0.200
Calibrator 100 PEI U/ml	> 1.000 OD450nm
Control Serum	OD450nm = OD450nm of the Calibrator 20 U/ml ± 10%

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 U/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 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 (dispensation of positive calibrators instead of Cal 0); 4. that no contamination of the Cal O, or of the wells where this 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 5 U/ml < CAL 0 + 5SD or < CAL 0 + 0.100	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 calibrator has occurred.
Calibrator 10 U/ml < CAL 0 + 0.200	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 calibrator has occurred.
Calibrator 100 U/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the 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 calibrator 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

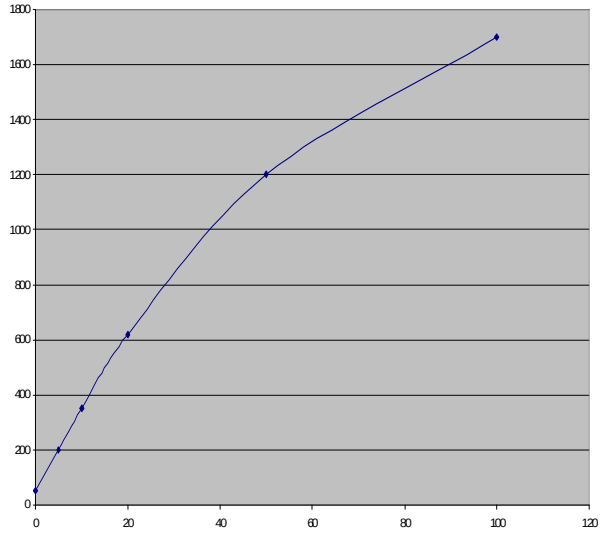
P. RESULTS

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 HBc IgM antibody in samples.

An example of Calibration curve is reported below.



Important Note: *Do not use this example to make real calculations on samples.*

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 U/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 U/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 10 U/ml: 0.350 – 0.330 OD450nm
Mean Value: 0.340 OD450nm
Higher than Cal 0 + 0.200 – Accepted
Calibrator 100 U/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Q.1 Qualitative results

For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc IgM $\geq$ 10 PEI U/ml.

Test results are therefore interpreted as a ratio of the sample OD450nm and the OD450nm of the Cal 10 PEI U/ml (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Q.2 Quantitative results
The calibration curve is used to determine the concentration of IgM antibodies to HBcAg in samples.
Samples with a concentration lower than 5 PEI U/ml are considered negative for HBcIgM.
Samples with a concentration between 5 and 10 PEI U/ml are considered in a gray-zone.
In the follow up of chronic hepatitis, however, values higher of 5 PEI U/ml may be considered positive for HBcIgM, when in presence of other clinical signs.
Samples with a concentration higher than 10 PEI U/ml are considered positive for HBcIgM.

Important general notes:
1. *When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to produce the calibration curve, calculate sample concentration and generate the correct interpretation of results.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
3. *A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.*
4. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
5. *Diagnosis of viral hepatitis infection has to be taken by 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 :

1.1 the HBcIgM reference preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on which the Standard Curve has been calibrated.
1.2 Accurun 113 (cat. N° A113-5001) supplied by Boston Biomedica Inc., USA

Results of Quality Control for three lots are given in the following tables:

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
PEI U/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
negative	0.040		0.035		0.044	

BBI Accurun # 113 lot # 48-9999-0621

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
BBI 113	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
negative	0.040		0.040		0.052	

Moreover the BBI's panel # PHE 102 was also examined in three lots of product; data are reported below with reference to a European kit (BBI's results).

BBI – Panel code PHE 102

	Lot # 0103	Lot # 0103/2	Lot # 0303	Sorin EIA
Member	S/Co	S/Co	S/Co	S/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Diagnostic Sensitivity:
It is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
The diagnostic sensitivity has been tested internally and externally in a qualified Clinical Laboratory on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different patients and from different HBV pathologies (acute and chronic hepatitis).
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
A Seroconversion panel produced by BBI, USA, code # PHM 935A, havs also been studied; results are reported below with reference to two commercial kits (BBI's results).

BBI Panel PHM 935A

	Lot # 0103	Abbott EIA	DiaSorin EIA
Member #	S/Co	S/Co	S/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**5.2**

3. Diagnostic Specificity:

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

The diagnostic specificity has been determined internally and externally in a qualified Clinical Laboratory on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.

A total number of more than 400 negative specimens were tested. A diagnostic specificity > 98% has been found.

Moreover, the diagnostic specificity was assessed by testing more than 50 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.).

No interference was observed in the study.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

4. Precision:

It has been calculated on three samples examined in 16 replicate in three different runs, carried out on three different lots. The values found were as follows:

BCM.CE: lot # 0103
Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.055	0.053	0.051	0.053
Std.Deviation	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.324	0.308	0.321	0.318
Std.Deviation	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.109	2.048	2.052	2.070
Std.Deviation	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lot # 0103/2
Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.053	0.054	0.055
Std.Deviation	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.332	0.331	0.322	0.328
Std.Deviation	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.311	2.208	2.212	2.244
Std.Deviation	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

BCM.CE: lot # 0303
Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.043	0.042	0.040	0.042
Std.Deviation	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.320	0.326	0.314	0.320
Std.Deviation	0.023	0.024	0.026	0.024
CV %	7.1	7.4	8.2	7.6

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.150	2.163	2.092	2.135
Std.Deviation	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.

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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