**HBc IgM**
*Enzyme Immunoassay Test*
**Product No. 4225**

## SUMMARY OF PROCEDURE

1. STEP 1 Place 100 µl of Calibrators, Control Serum and diluted serum or plasma samples, leaving one well for the blank.
2. Incubate for 60 min. at 37°C
3. Wash 4-5 times (350 µl)
4. STEP 2 Place 100 µl of Enzyme Conjugate in each well
5. Incubate for 60 min. at 37°C
6. Wash 4-5 times (350 µl)
7. STEP 3 Place 100 µl of Chromogen/Substrate in each well
8. Incubate for 20 min. at room temperature (18-30°C)
9. STEP 4 Add 100 µl of Stop Solution
10. Read absorbance at 450 nm & 620 nm

## INTENDED USE

Capture Enzyme Immunoassay for the qualitative and quantitative detection of IgM antibodies to Hepatitis B Virus Core Antigen (HBcAb IgM) in human serum or plasma. 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.

## SUMMARY AND EXPLANATION

The Hepatitis B Virus (HBV) is a human pathogen DNA virus with a word-wide distribution among geographic areas and population groups. He is one of the five strictly epatotropic viruses, together with the A, C, Delta and E viruses. All of these viruses can cause acute disease, with symphtoms that ranged between mild asymphtomatic infection to severe fulminant hepatitis[1-3]. The thpical symptoms are yellowing of the skin and eyes (jaundice), dark urine and extreme fatigue, nausea, vomiting and abdominal pain[4]. HBV can cause chronic infection when the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer[4], particularly if the infection was acquired in childhood than as an adult[5,6]. In this case the infection persists for more than six months. More than 90% of the infected adults will have an acute self-limiting infection[1]. Young children are the most likely to develop chronic hepatitis infection. 90% of the infants affected by HBV infection in the first year and 30-50% of the infants affected into the first 4 years can develop chronic hepatitis, and 25% of these patients will death for liver cancer or cirrhosis[4]. HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Since 1991, WHO suggested to consider the hepatitis B vaccine into their national immunization programs. The therapy is mainly based on alfa-interferon to relieve symptoms[5,6]. The response to this treatment is between 40 and 50% of patients with chronic active HBV infection[5,6]. In the chronic HBV carriers there is no evidence of hepatic damage[1,2], the infection persists and the patient maintains the ability to transmit the virus.
The transmission of the disease is mainly due to a parenteral contac (blood or blood products exchange, sexual contacts, perinatal spread from mother to newborn at the birth, unsafe injections and transfusions)[4]. The virus is not spread by contaminated food or water[4]. High prevalence of HBV infections are found in Southern areas of Eastern and Central Europe. Lower prevalence (5%) is detected in Middle and Far East, and less that 1% in Western Europe and North America[4].
In the HBV infection three phases can be identify: incubation, acute and covalescent, on the basis of several serological markers results. The first marker to appear in the serum is the hepatitis B surface antigen (HBsAg),m detectable after 4-12 weeks after the infection and before the onset of the symptoms[1,2,5].
Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of 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.

## PRINCIPLE OF THE PROCEDURE

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 human IgM 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 Horse Radish 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.

## REAGENTS

Reagents are sufficient for 96 determinations
Bring to room temperature before use

| | |
|---|---|
| Antibody Coated Wells (Microplate) | 12 x 8 breakable wells coated with purified anti-human IgM specific mouse monoclonal antibody, post-coated with bovine serum proteins and sealed into a bag with desiccant. |
| Calibration Curve | 6 vials containing 1.8 ml of ready to use chemically inactivated anti-HBc IgM positive human plasma, calibrated on the anti-HBc IgM 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; CAL5 = 50 U/ml; CAL6 = 100 U/ml. Contain 100 mM Tris buffer pH 7.4+/-0.1, 0.1% Kathon GC as preservative. The Calibration Curve is blue alimentary dye colour coded. *Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.* |
| Control Serum | One vial with lyophilized reagent. To be reconstituted with EIA grade water as reported in the label. It contains human HBcIgM positive plasma at about 20 ±10% PEI U/ml. 4% BSA enzyme free, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. |

*Important Notes*
*1. The volume necessary to reconstituted the content of the vial may vary from lot to lot. Please use the right volume reported on the label .*
*2. Even if plasma has been chemically inactivated, handle this component as potentially infectious.*

Wash buffer 20X concentrate: One vial containing 60 ml of 20X concentrated solution. Once diluted, the working solution contains 10 mM phosphate buffer pH 7.0±0.1, 0.05% Tween 20 and 0.1% Kathon GC.

Enzyme Conjugate (Immunocomplex): One vial containing 16 ml of ready to use and red colour coded immunocomplex solution formed by a monoclonal antibody to HBcAg linked with a purified recombinant HBcAg and labelled with Horseradish Peroxidase (HRP). MOPS buffer pH 6.2 - 6.7. Contains bovine protein preserved with 0.02% methylisothiazolone and bromonitrodioxane, 20 ppm Proclin 300

Chromogen Substrate: One vial contains **20** ml of ready to use 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methylbenzidine (TMB) and 0.02% hydrogen peroxide ($H_2O_2$).
*Note: To be stored protected from light as sensitive to strong illumination.*

Sample Diluent: Two vials containing 60 ml each of ready to use, blue color coded 100 mM Tris buffer solution pH 7.4±0.1 to dilute samples, together with 0.5% Tween 20, 2% casein, 0.1% Kathon GC and 0.09% sodium azide as preservatives.

Stop Solution: One vial contains **20** ml of ready to use 0.3 M $H_2SO_4$ solution.

Adhesive sealing foils

## OTHER MATERIALS REQUIRED

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

## STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2/8°C.
The expiry date is printed on each component and on the box label.
Reagents have a limited stability after opening and/or preparation.

Microplate Use: open the package on the opposite end from the code which is useful for identification purposes, remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the dessiccant, expell the air and seal by pressing the closure.

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

Control Serum Reconstitute 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 Serum after dissolution is not stable. Store frozen in aliquots at –20°C.

Wash buffer concentrate The whole content of the concentrated solution has to be diluted 20X with EIA grade water up to 1200 ml 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. 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 containers.

Sample Diluent Ready to use. Mix well on vortex before use.

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.

Stop Solution Ready to use. Mix well on vortex before use.

## WARNINGS AND PRECAUTIONS

For in vitro Diagnostic Use Only.

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 (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 has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 3 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. 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.

16. 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 $\pm$2%.

17. The ELISA incubator has to be set at +37°C (tolerance of $\pm$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.

18. 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 350 µl/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 section "QUALITY CONTROL". 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.

19. Incubation times have a tolerance of $\pm$5%.

20. The microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 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.

21. When using ELISA automated workstations, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "QUALITY CONTROL". The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect 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.

## SPECIMEN COLLECTION

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 to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.

4. Haemolysed (red) and lipemic ("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 well 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 sample should not be frozen/ /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 0.2-0.8 um filters to clean up the sample for testing.

## PROCEDURE

### PREASSAY PROCEDURE

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 equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

### ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.
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 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 µl 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 and B1 wells empty for blanking purposes.
4. Pipette 100 µl of the Calibrators in duplicate (CAL1 0 U/ml; CAL2 5 U/ml; CAL3 10 U/ml; CAL4 20 U/ml; CAL5 50 U/ml; CAL6 100 U/ml), 100 µl reconstituted 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.
6. When the first incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS")

7. In all the wells except A1+B1, pipette 100 µl Enzyme Conjugate. Incubate the microplate for 60 min at +37°C.
8. When the second incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS" point 20)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1 and B1 included.
10. Incubate the microplate protected from light at room temperature 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 Stop Solution 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 "WARNINGS AND PRECAUTIONS" using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

**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 µl 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 CAL1 0 U/ml in duplicate, 100 µl CAL3 10 U/ml in duplicate and 100 µl CAL6 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.
6. When the first incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS")
7. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Incubate the microplate for 60 min at +37°C.
8. When the second incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS")
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.
10. Incubate the microplate protected from light at room temperature 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 Stop Solution 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 "WARNINGS AND PRECAUTIONS" using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

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

**IMPORTANT NOTES**

1. *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.*
2. *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.*
3. *Do not expose to strong direct light. as a high background might be generated.*
4. *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.*
5. *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.*

**QUALITY CONTROL**

A validation check is carried out on the controls any time the kit is used in order to verify whether the expected $OD_{450nm}$ or S/Co values have been matched in the analysis.

Control that the following data are matched:

| | |
|---|---|
| Blank well (Bw) | $OD_{Bw} < 0.100$; |
| Calibrator 1 (CAL1; 0 PEI U/ml) | $OD_{CAL1}$ after blanking < 0.150; CV% < 30% |
| Calibrator 2 (CAL2; 5 PEI U/ml) | $OD_{CAL2} > OD_{CAL1}$ + 5 S.D. and anyway: $OD_{CAL2} > OD_{CAL1}$ + 0.100 |
| Calibrator 3 (CAL3; 10 PEI/ml) | $OD_{CAL3} > OD_{CAL1}$ + 0.200 |
| Calibrator 6 (CAL6; 100 PEI/ml) | $OD_{CAL6} > 1.000$ |
| Control Serum (CS) | $OD_{CS} = OD_{CAL4} \pm 10\%$ |

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

**TROUBLESHOOTING**

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

| Problem | Check |
|---|---|
| Blank well: $OD_{Bw} > 0.100$ | 1. that the Chromogen/Substrate solution has not become contaminated during the assay. |
| Calibrator 1 (0 U/ml) $OD_{CAL1}$ after blanking > 0.150<br><br>Coefficient of Variation > 30% | 1. that the washing procedure and the washer settings are as validated in the pre qualification study;<br>2. that the proper washing solution has been used and the washer has been primed with it before use;<br>3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of Cal1; 0 U/ml);<br>4. that no contamination of the Cal1 (0 U/ml), or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate;<br>5. that micropipettes have not become contami- |

| | |
|---|---|
| | nated with positive samples or with the enzyme conjugate<br>6. that the washer needles are not blocked or partially obstructed. |
| Calibrator 2 (5 U/ml)<br>$OD_{CAL2} < OD_{CAL1} + 5S.D.$<br>$OD_{CAL2} < OD_{CAL1} + 0.100$ | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during its distribution;<br>3. that the washing procedure and the washer settings are as validated in the pre qualification study;<br>4. that no external contamination of the calibrator has occurred. |
| Calibrator 3 (10 U/ml)<br>$OD_{CAL3} < OD_{CAL1} + 0.200$ | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during its distribution;<br>3. that the washing procedure and the washer settings are as validated in the pre qualification study;<br>4. that no external contamination of the calibrator has occurred. |
| Calibrator 6 (100 U/ml)<br>$OD_{CAL6} < 1.000$ | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during the distribution of the calibrator;<br>3. that the washing procedure and the washer settings are as validated in the pre qualification study;<br>4. that no external contamination of the calibrator has occurred. |
| Control Serum<br>Different from Calibrator 4 (CAL4; 20 U/ml) | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong sample);<br>3. that the washing procedure and the washer settings are as validated in the pre qualification study;<br>4. that no external contamination of the standard has occurred. |

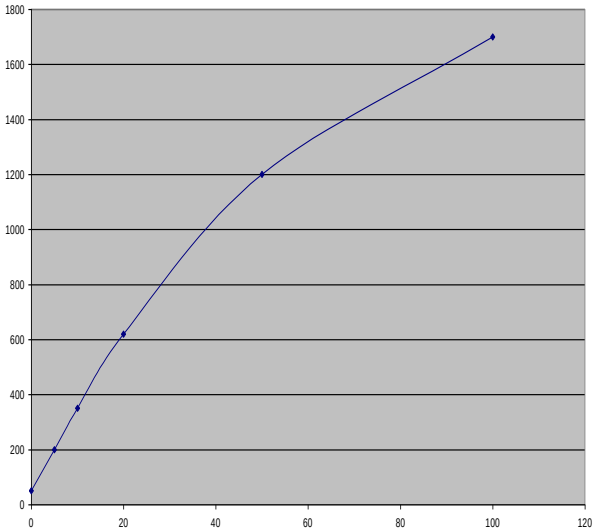
If any of the above problems have occurred, report the problem to the supervisor for further actions.

## RESULTS

### 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 450 nm (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.




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

### Qualitative method

In the qualitative method, calculate the mean $OD_{450nm}$ values for the Calibrators 1 (0 U/ml) and Calibrator 3 (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 1 (0 U/ml):* | *0.020 – 0.024 $OD_{450nm}$* |
| *Mean Value:* | *0.022 $OD_{450nm}$* |
| *Lower than 0.150* | *Accepted* |
| *Calibrator 3 (10 U/ml):* | *0.350 – 0.330 $OD_{450nm}$* |
| *Mean Value:* | *0.340 $OD_{450nm}$* |
| *Higher than Cal 0 + 0.200* | *Accepted* |
| *Calibrator 6 (100 U/ml)* | *2.845 $OD_{450nm}$* |
| *Higher than 1.000* | *Accepted* |

## INTERPRETATION OF RESULTS

### Qualitative results

For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of anti-HBc IgM ≥ 10 PEI U/ml.
Test results are therefore interpreted as a ratio of the sample $OD_{450nm}$ and the $OD_{450nm}$ of the CAL3 (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 |

### 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 grey-zone.

In the follow up of chronic hepatitis, however, values higher of 5 PEI U/ml may be considered positive for anti-HBc IgM, when in presence of other clinical signs.
Samples with a concentration higher than 10 PEI U/ml are considered positive for anti-HBc IgM.

*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.*

## PERFORMANCE CHARACTERISTICS

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).

**Limit of detection**

The limit of detection of the assay has been calculated by means of :

1. the anti-HBc IgM Reference Preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on which the Standard Curve has been calibrated.
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:

| HBcAb IgM | Lot # 0103 | | Lot # 0103/2 | | Lot # 0303 | |
|---|---|---|---|---|---|---|
| PEI U/ml | $OD_{450nm}$ | S/Co | $OD_{450nm}$ | S/Co | $OD_{450nm}$ | 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

| HBcAb IgM | Lot # | 0103 | Lot # | 0103/2 | Lot # | 0303 |
|---|---|---|---|---|---|---|
| BBI 113 | $OD_{450nm}$ | S/Co | $OD_{450nm}$ | S/Co | $OD_{450nm}$ | 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 |

**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 |

**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.

**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:

HBcAb IgM: 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 |

HBcAb IgM: 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 |

HBcAb IgM: 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 |

**LIMITATIONS OF THE PROCEDURE**

Frozen samples containing fibrin particles or aggregates may generates 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.

**REFERENCES**


1. Loccarini SA, Gust ID. Hepadnaviridae: hepatitis B virus and the delta virus. In: Balows A et al. editors. Laboratory diagnosis of infectious diseases: principles and practices. New York Springer-Vertag, 1988:750-96.
2. Follett EAC. Diagnosis of hepatitis B infection. In Young H, McMillan A, editors. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker, 1988:433-49.
3. Hollinger FB, Dienstag JL. Hepatitis B and D viruses. In: Lennette EH et al. editors. Manual of clinical microbiology; 6th ed. Washington D.C.: American Society for Microbiology, 1995:1033-49.
4. World Health Organization (WHO) documents.
5. Nowicki MJ, Balistreri WF. Hepatitis A to E: building up the alphabet. Contemporary Peds 1992:118-28.
6. Zuckerman AJ et al. Hepatitis B virus and hepatitis D virus. In: Principles and practice of clinical virology. 2nd ed. New York: John Wley & Sons, 1992:153-72.
7. Perillo RP,Aach RD. The clinical course and chronic sequelae of hepatitis B virus infection. Semin. Liver Dis. 1981; 1:15-25.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