VIDAS® HBc IgM II (HBCM)

IVD

VIDAS HBc IgM II is an automated quantitative test for use on the VIDAS family instruments for the determination of anti-hepatitis B virus core antigen IgM (anti-HBc IgM) in human serum or plasma (EDTA, citrate and lithium heparin), using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

The hepatitis B virus is responsible for acute and chronic hepatitis infections. Acute hepatitis can be asymptomatic or present symptoms of varying severity which may progress to fulminant hepatitis in 0.1 to 0.5% of cases. Chronicity occurs in 5 to 10% of cases in adults, but in up to 90% of cases in infants following perinatal transmission. Currently, approximately 300 million people worldwide are chronic carriers of the virus (1). Chronic hepatitis may be asymptomatic or lead to liver lesions of varying severity, possibly evolving to cirrhosis, with an evolution in 5% of cases to hepatocellular carcinoma (2). The hepatitis B virus can be transmitted by parenteral or perinatal pathways or through sexual contact. Persons most at risk are health workers, drug addicts, those with multiple sexual partners, multiple transfusion or hemodialysis patients, close friends and family of an infected subject, and newborns of an infected mother (2).

CLINICAL SIGNIFICANCE

Anti-HBc IgM antibodies are indirect markers of viral replication, reflecting the host's response to intra-hepatic HBc antigen (3).

Diagnosis of acute hepatitis relies on the detection of HBs antigen and anti-HBc IgM. The level of infectivity, and infection evolution are assessed using other markers, which also enable determination of patients requiring screening. In cases of acute hepatitis, anti-HBc IgM titers are generally high (> 100 PEIU/ml), then progressively decrease over a 6-8 month period whether infection develops towards recovery or chronicity. However, total anti-HBc antibodies (mainly IgG) persist after recovery. Expression of HBs antigen can be limited in cases of fulminant hepatitis. Anti-HBc IgM can then indicate active viral replication (2).

In cases of chronic hepatitis, the appearance of anti-HBc IgM reflects hepatic cytolysis, revealing an active phase of the disease (4). As with serum HBe Ag and HBV DNA, a positive result for anti-HBc IgM is of considerable assistance in the diagnosis of chronic hepatitis, but a negative result does not necessarily exclude this possibility.

The diagnosis should therefore be made using both histological and serological criteria. In patients with a significant response, titration of anti-HBc IgM enables anti-viral treatments to be monitored by a specific marker of HBV.

Quantitative and **ultra-sensitive** tests enable patients to be monitored from the time HBs Ag appear up to the appearance of anti-HBs Ab, by titering the anti-HBc IgM, and also a distinction to be made between healthy carriers and those with active chronic hepatitis. These tests are also useful for monitoring chronic hepatitis and anti-viral therapy.

PRINCIPLE

The assay principle combines a two-step enzyme immunoassay method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After dilution of the sample, the serum IgM bind with the anti-µ chain antibodies coating the interior of the SPR. Unbound serum components are washed away. IgM antibodies directed against the core antigen of the hepatitis B virus (HBc) bind to an immunocomplex composed of recombinant HBc antigen and alkaline-phosphatase-labeled monoclonal anti-HBc antibody. Unbound components are eliminated by washing steps. A fluorescent substrate, 4-Methyl-umbelliferyl phosphate is introduced into the SPR and reveals the amount of bound immunocomplex. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.

The intensity of the fluorescence is proportional to the concentration of anti-HBc IgM present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS

30 HBCM strips	STR	Ready-to-use.
30 HBCM SPRs 1 x 30	SPR	Ready-to-use. Interior of the SPR coated with anti-µ antibody (goat).
HBCM positive control 1 x 2 ml (liquid)	C1	Ready-to-use. Inactivated human* serum containing anti HBc IgM + 0.2 g/l gentamicin sulfate + 1 g/l sodium azide. MLE data indicate the confidence interval in PEIU/mL (Paul Erlich Institute unit per milliliter) (“Control C1 (+) Dose Value Range”).
Negative control 1 x 1.9 mL (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin+ preservatives.
HBCM calibrator 2 x 1ml (lyophilized)	S1	Inactivated human* serum containing anti HBc IgM + 0.2 g/l gentamicin sulfate + preservatives. The calibrator must be reconstituted with 1 ml of distilled sterile water (measured exactly). Allow to dissolve for at least 15 min and then mix using a vortex. After reconstitution, the calibrator can be stored at 2-8°C for up to 4 weeks. Aliquoted and frozen at -25 ± 6°C, the calibrator can be kept for up to 6 months. Avoid repeated freeze/thaw cycles.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or MLE bar codes printed on the box label.		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for antibodies to HIV1, HIV2 and HCV. It has been heat inactivated. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious and usual safety procedures should be observed when handling.

** This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious and usual safety procedures should be observed when handling.

The SPR

The interior of the SPR is coated during production with anti-human µ chain antibody. Each SPR is identified by the HBCM code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening**.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the HBCM strip

Wells	Reagents
1	Sample well
2	Sample diluent: buffer containing Tween 20 (pH = 7.4) + protein and chemical stabilizers + 1 g/l sodium azide + products of human origin (400 µl).
3 - 4 - 5 - 8 - 9	Wash buffer: TRIS buffer (50 mmol/l) (pH = 7.4) + protein and chemical stabilizers + 0.9 g/l sodium azide (600 µl).
6	Conjugate: alkaline phosphatase-labeled anti-HBc monoclonal antibody (mouse) + 1 g/l sodium azide + inactivated negative human** serum (300 µl).
7	HBc recombinant antigen (*E. Coli*) + 1 g/l sodium azide + inactivated negative human** serum (300 µl).
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine*** (DEA) (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

** This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

***Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 1 ml and 100 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- VIDAS family instrument.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS HBc IgM II kit at 2-8°C.
- **Do not freeze reagents, with the exception of the calibrator after reconstitution.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SPECIMENS

Specimen type and collection:

Use human sera (plain tube) or plasma (validated anticoagulants: EDTA, citrate, lithium heparin). Sera containing impurities should be clarified by centrifugation or filtration before analysis.
It is recommended that each laboratory checks the compatibility of collection tubes used.
None of the following factors have been found to significantly influence this assay.
- hemolysis (after spiking samples with hemoglobin: 0 to 320 µmol/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 5 mg/ml equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 560 µmol/l).

However, it is recommended not to use samples that are clearly hemolyzed or lipemic and, if possible, to collect a new sample.
Do not inactivate samples.

Specimen stability

Samples can be stored at 2-8°C in stoppered tubes for up to 7 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C. Avoid successive freezing and thawing.
A study performed on frozen samples over a period of 2 months, showed that the quality of results is not affected.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested **in duplicate** (see User's Manual). The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one "HBCM" strip and one "HBCM" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "HBCM" code on the instrument. Indicate the number of determinations to perform. The calibrator must be identified by "S1", and tested in **duplicate**. If the positive control is to be tested, it should be identified by "C1". If the negative control needs to be tested, it should be identified by C2.
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator, controls and samples using a vortex-type mixer (for serum or plasma separated from the pellet) (5).
6. **For this test, the calibrator, control, and sample test portion is 100 µl.**
7. Insert the "HBCM" SPRs and "HBCM" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
8. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to the required temperature after pipetting.
10. The assay will be completed within approximately 55 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
11. Dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR.

The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistics model). The concentrations are expressed in PEIU/ml according to the anti-HBc IgM standard of the Paul Erlich Institute (n°84).

Test Value (PEIU/ml)	Interpretation
< 5	Negative
≥ 5 and < 10	Equivocal *
≥ 10	Positive

* It is advised to follow up the evolution of anti-HBc IgM titers in this zone.

Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS HBc IgM II kit.
These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified by C1 and C2. Results cannot be validated if the control values deviate from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history, and the results of any other tests performed.

PERFORMANCE

Studies performed using VIDAS HBc IgM II gave the following results:

Measurement range

The measurement range of the VIDAS HBc IgM II kit extends up to 200 PEIU/ml.

Sensitivity - Specificity for clinical population.

Sensitivity and specificity were determined by comparison with 2 other anti-HBc IgM screening tests. Final interpretation of samples was based on the "2 out of 3" rule (2 tests with the same interpretation take precedence over the other test). Sera which cannot be classed using this rule (a positive test, an equivocal test and a negative test) were not included in the performance calculation.
Samples which gave an equivocal result using the VIDAS HBc IgM II kit were not included when calculating test performance.

a. Sensitivity

247 documented samples were tested:

- 97 positive samples for anti-HBc IgM, including 5 samples from patients with acute hepatitis.
- 150 sequential sera from 20 subjects with chronic hepatitis B most of whom had received anti-viral therapy.

	Sera classified according to 2/3 rule*	
	Positive	Negative
VIDAS HBc IgM II Positive	194	7**
VIDAS HBc IgM II Negative	0	15

* 24 samples gave results which could not be interpreted between the different techniques (positive with VIDAS equivocal and negative with other tests) and 7 samples gave an equivocal result with VIDAS HBc IgM II. All these results involved chronic HBV carriers with active viral replication, possibly reduced by anti-viral therapy.

** Follow-up samples for chronic HBV carriers with active viral replication, possibly reduced by anti-viral therapy. Four of these patients were positive for HBV DNA.

Relative sensitivity for VIDAS HBc IgM II: 100% (95% confidence interval: 97.98% - 100%)

Out of the 152 documented samples which were positive for HBV DNA or for HBe Ag, the positive screening percentage for anti-HBc IgM using the VIDAS test was 79%. This value was 65.8% and 50.7% using the other two kits in the comparison.

Out of the 5 acute hepatitis cases tested, 4 patients showed anti-HBc IgM titers greater than 200 PEIU/ml and one patient had a titer of 95 PEIU/ml.

In chronic hepatitis cases, the evolution of anti-HBc IgM titers is useful for patient monitoring (natural or therapeutic evolution):

Example: patient with chronic hepatitis B with HBV replication, HBe Ag negative:

Date	VIDAS HBc IgM II	Interpretation	Other viral DNA marker
03/1996	43	Positive	ADN VHB +
End of March 1996: start of anti-viral therapy			
05/1996	> 200	Positive	ADN VHB -
06/1996	115	Positive	Not tested
09/1996	13	Positive	Not tested
01/1997	6	Equivocal	ADN VHB -
07/1997	1	Negative	ADN VHB -

b. Specificity

351 serum samples sent to the laboratory for diagnosis of viral hepatitis were tested.

	Sera classified according to 2/3 rule*	
	Positive	Negative
VIDAS HBc IgM II Positive	20	14**
VIDAS HBc IgM II Negative	0	297

* 3 samples gave an uninterpretable result and 17 samples gave an equivocal result with VIDAS HBc IgM II.

** A study of other markers and case histories showed that 6 of the 14 "false-positive" samples corresponded to chronic hepatitis cases with active replication (detection of viral DNA by branched DNA or by PCR).

Relative specificity for VIDAS HBc IgM II on a clinical population: 95.50%
(95% confidence interval: 92.51% - 97.33%).

Specificity for blood donors:

601 unselected samples of blood donors were tested. Two samples found to be positive and one sample found to be equivocal were confirmed to be negative with the other 2 anti-HBc IgM screening tests.

The equivocal result was not included in the performance calculation.

Relative specificity of the VIDAS HBc IgM II kit for this population is 99.67% (95% confidence interval: 98.77% - 99.91%).

Sensitivity on patients with acute hepatitis B:

The VIDAS HBc IgM II reagent detected anti-HBc IgM in all 40 samples tested.

Precision

Within-run reproducibility (intra-assay precision) and between-run reproducibility (total precision) were calculated according to the recommendations of the NCCLS Document EP5-T2,volume 12 number 4.

Precision was evaluated with a negative sample and two positive samples distributed on the measurement range. Each sample was tested in duplicate in two different runs per day, over a 14-day period on one site.

The combined results are given below:

Within-run reproducibility:

Sample	N	Mean titer in PEIU/ml	CV %
Negative	56	3.98	3.36
Positive no. 1	56	49.54	3.22
Positive no. 2	56	112.34	3.10

Between-run reproducibility:

Regarded as total precision taking into account all sources of variation.

Sample	N	Mean titer in PEIU/ml	CV %
Negative	56	3.98	3.36
Positive no. 1	56	49.54	4,35
Positive no. 2	56	112.34	5.01

CROSS REACTIVITY AND RELEVANT INTERFERENTS

Potentially interfering samples

2 equivocal samples were not taken into account in this study.

	VIDAS HBc IgM II positive
anti HIV Ab	0/19
anti EBV Ab	1*/10
anti HCV Ab	0/15
anti HAV IgM	0/15
anti CMV IgM	0/15
anti-nuclear Ab	0/15
Rheumatoid factors	1*/10
Pregnant women	0/20
anti HTLV Ab	2**/14
anti-*E.coli* Ab	0/10

*these samples were found to be positive with an HBs Ag screening test.

**these samples were found to be negative with an HBs Ag screening test.

RANGE OF EXPECTED VALUES

The reported incidence of hepatitis B cases in Europe is approximately 20/100,000, ranging from 1/100,000 in Scandinavian countries to 60/100,000 in Central Europe. In Europe, endemic cases increase North to South and West to East. In South-East Asia, China, Black Africa or South America, endemic prevalence can exceed 10%.

In cases of acute hepatitis, anti-HBc IgM titers are generally high (> 100 PEIU/ml), then progressively decrease over a 6-8 month period whether infection develops towards recovery or chronicity.

WASTE DISPOSAL

Dispose of used and unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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3. SJOGREN M. A, DUSKEIKO G. M., KEW M. C. AND SONG E. - Hepatitis B virus infection and hepatocellular carcinoma: correlation between IgM antibody to hepatits core antigen, hepatitis B e antigen, and hepatitis B DNA. Am. J. Trop. Med. Hyg.,1988, 39 (6), 582-585 (8 - 082).
4. BRUNETTO, M.R., CERENZIA M.T., OLIVERI F., PIANTONI P., RANDONE A., CALVO P., MANZINI P., ROCCA G., GALLI C. and BONINO F., Monitoring the natural course and response to therapy of chronic hepatitis B with an automated semi-quantitative assay for IgM anti-HBc, Journal of Hepatology, 1993, 19, 431-436.
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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	09433F	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE
2016/05	09433G	Technical	CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS

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