VIDAS® Anti-HBc Total II (HBCT)

IVD

VIDAS Anti-HBc Total II (HBCT) is an automated qualitative test for use on the VIDAS family instruments, for the detection of total antibodies against the hepatitis B core antigen (anti-HBc) in human serum or plasma, 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 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 and 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

Total antibodies against the hepatitis B core antigen (anti-HBc IgM and IgG) can be detected in the serum of patients with acute or chronic hepatitis B or in recovered patients. Anti-HBc total antibodies therefore act as an epidemiological indicator of current or former HBV infections.

In cases of acute hepatitis, anti-HBc antibodies (IgM and IgG) are generally detected 2 to 4 weeks after HBs and HBe antigens appear (3). Whereas anti-HBc IgM are transitory and progressively decrease whether infection develops towards recovery or chronicity, high anti-HBc IgG titers can be detected during the infection and after recovery (2). Anti-HBc IgG can persist for several years, and even throughout the patient's lifetime.

In cases of chronic hepatitis, only titration of anti-HBc IgM antibodies can indicate an active phase of the disease (4). Anti-HBc antibodies do not provide protection. Only the presence of anti-HBs antibodies can confirm immunity.

PRINCIPLE

The VIDAS Anti-HBc Total II assay is an enzyme-linked fluorescent immunoassay (ELFA) based on an inhibition principle.

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, the sample is incubated in the SPR. The anti-HBc antibodies (IgM and IgG) present in the sample bind with the recombinant HBc antigen coating the interior of the SPR. Unbound sample components are eliminated during the washing steps.

The solid phase is then incubated with the conjugate: alkaline phosphatase-labeled monoclonal anti-HBc antibody. This conjugate binds to the solid phase HBc antigen sites that have not bound to serum antibodies. Unbound conjugate is removed by washing.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. 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 inversely proportional to the quantity of anti-HBc antibodies present in the sample. Results are analyzed automatically by the instrument and are expressed as an index calculated using a standard.

CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS:

60 HBCT Reagent Strips	STR	Ready-to-use.
60 HBCT SPRs 2 x 30	SPR	Ready-to-use. Interior of the SPR coated with recombinant HBc antigen.
HBCT Positive control 1 x 1.5 ml (liquid)	C1	Ready-to-use. Human* serum containing anti-HBc antibodies + 0.2 g/l gentamicin sulfate + 1 g/l sodium azide. Index strictly inferior to 1.
Negative control 1 x 1.9 ml (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin+ preservatives. MLE data indicate the index: confidence interval ("Control C2 (-) Test Value Range).
HBCT Standard 2 x 2 ml (lyophilized)	S1	Human* serum containing anti-HBc antibodies + 0.2 g/l gentamicin sulfate. The standard must be reconstituted with 2 ml of distilled sterile water (measured exactly). Allow to dissolve for at least 20 min and then mix using a vortex. After reconstitution, the standard can be kept for up to 1 month at 2-8°C. Aliquoted and frozen at -25 ± 6°C, it can be stored 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 code 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 HBs surface antigen, and antibodies to HIV1, HIV 2 and HCV. However, since no known test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR is coated during production with recombinant HBc antigen. Each SPR is identified by the "HBCT" 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 HBCT strip

Wells	Reagents
1	Sample well
2	Sample diluent: TRIS buffered saline (50 mmol/l) pH = 7.4 with protein and chemical stabilizers + 0.9 g/l sodium azide (300 µl).
3	Wash buffer: TRIS buffered saline (50 mmol/l) pH = 7.4 with protein and chemical stabilizers + 0.9 g/l sodium azide (600 µl).
4 - 6 - 7 - 8 - 9	Wash buffer: TRIS buffered saline (50 mmol/l) pH = 7.4 with protein and chemical stabilizers + 1 g/l sodium azide (600 µl).
5	Conjugate: alkaline phosphatase-labeled anti-HBc monoclonal antibody (mouse) + 0.9 g/l sodium azide (400 µl).
10	Optical Cuvette with substrate: 4-Methyl-umbelliferyl phosphate + diethanolamine DEA* (0.62 mol/l or 6.6%) pH 9.2 + 1 g/l sodium azide (300 µl).

* 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 2 ml and 150 µl.
- Powderless, disposable latex 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 optical cuvette with substrate (well 10) contains an irritant agent (diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or 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 VIDAS User's Manual).

STORAGE CONDITIONS

- Store the VIDAS Anti-HBC Total II kit at 2-8°C.
- **Do not freeze SPRs and strips.**
- **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 non-contaminated sera (plain tube, separator gel) or plasma (validated anticoagulants: EDTA, lithium heparin, citrate).
None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin: 0 to 340 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 450 µmol/l).

Do not decomplementize samples.

Specimen stability

Samples containing impurities must be clarified by centrifugation prior to testing. Samples can be stored at 2-8°C in stoppered tubes for a maximum of 7 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C (**freeze once only**). 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 standard provided in the kit, must be performed upon receipt of a new lot of reagents 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 standard, identified by S1, must be tested **in triplicate** (see User's Manual). The standard 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 "HBCT" strip and one "HBCT" SPR for each sample, control or standard 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 "HBCT" code on the instrument. The standard must be identified by "S1", and tested **in triplicate**. 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. Mix the standard, control(s) and sample(s) using a Vortex-type mixer (for serum or plasma separated from the pellet).
5. **For this test, the standard, control, and sample test portion is 150 µl.**
6. Insert the "HBCT" SPRs and "HBCT" strips into the instrument. Check to make sure the color labels with the three letter assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to the required temperature after pipetting.
9. The assay will be completed within approximately 90 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. 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 and test values are expressed as an index calculated using a standard.

The patient RFV is interpreted by the VIDAS system as follows:

i = test value = patient RFV / S1 standard RFV

A report is printed which records the test value and the interpretation. The following table shows the thresholds and the interpreted results:

Index	Interpretation
i < 1	Presence of anti-HBc antibodies
1 ≤ i < 1.4	Equivocal result
i ≥ 1.4	Absence of anti-HBc antibodies

All equivocal results must be confirmed using a second sample.

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

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS HBCT 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 against reagent components. For this reason, assay results should be interpreted taking into consideration the patient history, and the results of any other tests performed.

PERFORMANCE

Studies performed using VIDAS Anti-HBc Total II gave the following results:

1. Specificity for blood donor population

The results obtained from 4 blood transfusion centers, where 5064 blood donor samples were tested, are as follows:

	Final interpretation: other EIA techniques / other markers	
VIDAS Anti-HBc Total II	Positive	Negative
Positive	47	3
Equivocal	0	8
Negative	0	5006

The eight samples giving an equivocal result were not included in the performance calculation.

Relative specificity with VIDAS Anti-HBc Total II for this population: 99.94%

(95% confidence interval 95%: 99.82%- 99.98%.)

2. Sensitivity:

a) Results obtained using the SNTS 1996 panel in 2 blood transfusion centers:

Characteristics	Panel sections	VIDAS results	Samples
Positive anti-HBc antibodies: concordant screening for the 8 EIA kits studied	7	7 pos.	14, 15, 16, 17, 18, 19 and 20
Anti-HBc antibodies: positive anti-HBc plasma dilutions, anti-HBs and/or anti-HBe positive	5	2 pos. (1 and 2), 2 equiv. (3 and 4) 1 neg. (5)	1, 2 to 5 (sample 1 - increasing dilutions)
	4	2 pos. (21 and 22), 1 equiv. (23) 1 neg. (24)	21 to 24 (same sample - increasing dilutions)
	4	2 pos. (25 and 26), 1 equiv. (27) 1 neg. (28)	25 to 28 (same sample - increasing dilutions)
	4	3 pos. (29, 30, 31), 1 neg. (32)	29 to 32 (same sample - increasing dilutions)
Anti-HBc antibodies: discrepant screening for the 8 EIA kits studied	4	1 pos. (8) 1 pos./equiv. (9) 2 neg. (10,12)	8, 9, 10, 12
Absence of anti-HBc antibodies	1	1 neg.	6

b) Clinical study: 3 studies were performed:

Study 1: 228 documented positive anti-HBc samples were tested in comparison with another reference technique. Discrepant results between the 2 techniques were confirmed using a 3rd reference technique.

Clinical category	Positive VIDAS Anti-HBc Total II
Acute hepatitis B virus N = 8	8 (100%)
Chronic HBV carriers with replication marker (positive HBe Ag and/or positive viral DNA) N = 49	49 (100%)
Treated chronic HBV carriers (anti-viral treatment or anti-HBs immunoglobulins) N = 47	47 (100%)
Chronic HBV carriers without standard replication marker (negative HBe Ag, viral DNA by negative hybridization) N = 42	42 (100%)
Subjects who have acquired natural immunity against HBV N = 56	54* (100%)
"Isolated anti-HBc" (negative Ag HBs and anti-HBs) N = 26	25 (96%)

* Two uninterpretable samples after confirmation (equivocal with VIDAS Anti-HBc Total II, negative and positive with other tests) were excluded from the performance calculation.

Relative sensitivity with VIDAS Anti-HBc Total II: 99.56% (95% confidence interval: 97.46% - 99.9%).

Study 2: study performed using 130 documented positive anti-HBc samples.

	VIDAS Anti-HBc Total II		
Sample category	Positive	Equivocal	Negative
Positive	129	1*	0

* Sample excluded from sensitivity calculation.
Clinical sensitivity = **100%**
(95% confidence interval: 96.99% - 100%)

Study 3: study performed using 87 documented positive anti-HBc samples.

	VIDAS Anti-HBc Total II		
Sample category	Positive	Equivocal	Negative
Positive	85*	0	0

* Two uninterpretable samples after confirmation were excluded from the performance calculation.
Clinical sensitivity = **100%**
(95% confidence interval 95%: 95.75% - 100%)

c) Diagnostic sensitivity

33 fresh samples with a positive status (collection < 24 hours) were tested, 31 were found to be positive, 1 was found to be equivocal and 1 was found to be negative with VIDAS anti-HBc Total II. 31 fresh negative samples (collection < 24 hours) were tested and found to be negative with VIDAS anti-HBc Total II.

3. Precision

Precision was evaluated using a positive sample, an equivocal sample and a negative sample. Each sample was tested in duplicate in two different runs per day for twenty days on three sites.
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 12-4.

The combined results for the three sites are given below:

a) Within-run reproducibility:

Sample	N	Mean index	CV (%)
Negative	240	2.569	4.36
Equivocal	240	1.180	5.65
Positive	240	0.082	7.53

b) Between-run reproducibility:

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

Sample	N	Mean index	CV (%)
Negative	240	2.569	7.42
Equivocal	240	1.180	8.10
Positive	240	0.082	9.25

4. Cross-reactivity

	VIDAS Anti-HBc Total II (positive)
VHC + (Anti-HBc -)	0 / 10
EBV + (Anti-HBc -)	0 / 10
HIV + (Anti-HBc -)	0 / 10
FR + (Anti-HBc -)	0 / 21
ANA + (Anti-HBc -)	0 / 20
Rubella IgG + (Anti-HBc -)	0 / 11
CMV IgG + (Anti-HBc -)	0 / 11
CMV IgM + (Anti-HBc -)	0 / 10
Pregnant women (Anti-HBc -)	0 / 24
Vaccinated: Anti-HBs + (Anti-HBc -)	1 / 20
HSV + (Anti-HBc -)	0 / 6
Patients who have received VIII (Anti-HBc -)	0 / 15

WASTE DISPOSAL

Dispose of used or 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 type 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

1. MILICH D.R., Immune response to the hepatitis B virus: infection, animal models, vaccination, VIRAL HEPATITIS, 1997, 3, 63 -103.
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3. KURSTAK E., Viral Hepatitis, Current Status and Issues, Springer-Verlag Wien New York, 1993, 11, 93-104.
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.

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	09577G	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS
2015/06	09577H	Technical	CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS INSTRUCTIONS FOR USE
2016/05	09577I	Technical	CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS

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