VIDAS®HAV IgM (HAVM)

IVD

VIDAS HAV IgM is an automated qualitative test for use on the VIDAS family instruments for the detection of IgM directed against the hepatitis A virus (HAV) after immunocapture, in human serum or plasma (heparin or EDTA), using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

Hepatitis A is caused by the hepatitis A virus, belonging to the picornavirus family. It is transmitted by fecal-oral contact and was therefore considered to be a generally asymptomatic disease, occuring mainly in children. The fall in prevalence observed over the past few years has led to an increase in symptomatic cases in older patients (1). Fulminant hepatitis, which is often fatal, remains rare (0.1 to 0.2% of cases) (2).

Direct diagnosis is difficult since virus excretion in stools is early and generally ends before clinical signs appear (3). The viremia stage is short-lived, and the detection of viral RNA of little significance (4). Furthermore, the early and rapid development of IgG makes it difficult to detect the time of seroconversion by titrating the IgG. This explains why the only reliable method is the detection of anti-HAV IgM, especially after immunocapture. IgM can be detected as soon as the first symptoms appear, and generally persist for 2 to 4 months. In rare cases, residual IgM have been detected 6 to 12 months after the start of the infection (5).

In cases of active prevention, anti-HAV IgM can be detected in patient sera within two weeks following the first injection of the vaccine (6).

Detection of anti-HAV IgM aids in the diagnosis of acute hepatitis, but is not sufficient to validate post-vaccine immunity.

PRINCIPLE

The assay principle combines a 2-step enzyme immunoassay after immunocapture, 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 diluting the sample, the serum IgM bind with the anti-µ chain polyclonal antibodies coating the interior of the SPR. Unbound sample components are removed by washing.

The anti-HAV IgM are specifically detected by an immune complex, formed of inactivated viral antigen and an alkaline phosphatase-labeled anti-HAV monoclonal antibody. The unbound immune complex 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 proportional to the concentration of anti-HAV 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 HAVM strips	STR	Ready-to-use.
30 HAVM SPRs 1 x 30	SPR	Ready-to-use. Interior of SPRs coated with anti-human µ chain polyclonal antibodies (goat).
HAVM positive control 1 x 1 ml (liquid)	C1	Ready-to-use. Human* serum with anti-HAV IgM + protein stabilizer + 1 g/l sodium azide. MLE data indicate the index: confidence interval ("Control C1 (+)Test Value Range).
Negative control 1 x 1.9 mL (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin+ preservatives.
HAVM standard 1 x 1.2 ml (liquid)	S1	Ready-to-use. Human* serum with anti-HAV IgM + protein stabilizer + 1 g/l sodium azide.
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 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.

The SPR®

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

The reagent 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 HAVM strip

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (0.05 mol/l) pH 7.4 + Tween + protein and chemical stabilizers + 1 g/l sodium azide (400 µl).
3 - 4 - 5 - 8 - 9	Washing buffer: TRIS buffer (0.05 mol/l) pH 7.4 + protein and chemical stabilizers + 1 g/l sodium azide (600 µl).
6	Inactivated hepatitis A antigen + protein and chemical stabilizers + 1 g/l sodium azide (300 µl).
7	Anti-hepatitis A monoclonal antibody (mouse) conjugated with alkaline phosphatase + 1 g/l sodium azide (300 µl).
10	Reading 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).

* 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 100 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS® family.

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 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 User's Manual).

STORAGE CONDITIONS

- Store the VIDAS HAV IgM kit at 2-8°C.
- **Do not freeze reagents.**
- **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.

SPECIMENS

Specimen type and collection

Serum or plasma (lithium hepatinate or EDTA).
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 270 µmol/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 2 mg/ml equivalent in trigycerides),
- bilirubinemia (after spiking samples (naturally icteric plasma) with bilirubin: 0 to 500 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.
Freeze once only.
A study performed on samples frozen for 12 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 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 standard, identified by S1, must be tested **in duplicate** (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 "HAVM" strip and one "HAVM" 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 "HAVM" code on the instrument. The standard 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 standard, controls and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
6. **For this test, the standard, control, and sample test portion is 100 µl.**
7. Insert the "HAVM" SPRs and "HAVM" 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. Restopper the vials and return them to 2–8°C after pipetting.
10. The assay will be completed within approximately 60 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
11. Dispose of the used SPRs and reagent 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 patient RFV is interpreted by the VIDAS system as follows:

i = Test Value = Patient RFV / Standard RFV

This Test Value and the result interpretation are both mentioned on the report.
The Test Value is interpreted as follows:

Test Value	Interpretation
i < 0.4	Negative
i ≥ .4 and i < 0.5	Equivocal**
i ≥ 0.5	Positive

** It is advisable to control equivocal results by performing a new test 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 HAV IgM 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 history, and the results of any other tests performed.

PERFORMANCE

Studies performed using VIDAS HAVM gave the following results:

Precision

Within-run reproducibility:

3 samples were tested 15 times in a same run.

	Number	Mean RFV	CV (%)
Negative	15	74.5	4.05
Weak positive	15	994	1.79
Strong positive	15	2627	1.8

Between-run reproducibility:

3 samples were tested in triplicate in 3 different runs on 3 sites.

	Number	Mean Index	CV (%)
Negative	27	0.05	7.63
Weak positive	27	1.01	2.07
Strong positive	27	3.45	2.4

Specificity

Out of the 544 blood donors who were found to be negative with another EIA technique; 538 were found to be negative and 3 were found to be positive with VIDAS HAV IgM, giving **a relative specificity of 99.44%*.**
*Equivocal results were not included in the specificity calculation.

Study of clinical trials

The sensitivity was tested on sera corresponding to samples from patients with clinically and biologically documented hepatitis A or from patients at risk with anti-HAV IgM, detectable using another EIA technique.

1) Acute hepatitis A or positive anti-HAV IgM antibody in patients presenting either an increase in transaminase levels or a risk factor (travel, contact with infection etc.):

Of the 205 sera tested:
- 201 were found to be positive with both VIDAS HAV IgM and the comparative EIA technique,
- 4 were found to be equivocal.

2) Monitoring of 8 clinically and biologically documented cases of acute hepatitis A:

Of the 55 sera tested:
- 23 were found to be negative with VIDAS HAV IgM. Discrepancies were found for six sera with the EIA technique which found them positive.
- 30 were found to be positive with both VIDAS HAV IgM and the comparative EIA technique,
- 2 were equivocal.

The 6 discrepant sera were from two patients who were being monitored for hepatitis A. They probably correspond to residual IgM which were later detected using the comparatvie EIA technique.

CROSS REACTIVITY AND RELEVANT INTERFERENTS

Tested sera	Number of positives/total
Anti-HBc + IgM	0 / 14
Anti-CMV + IgM	0 / 28
Anti-VCA + IgM	1 / 19 *
Automimmune hepatitis +	0 / 10
Cryoglobulinemia +	0 / 22
Rhumatoid factor +	0 / 20
Heterophilic + antibody	0 / 10
Anti-nuclear + antibody	0 / 6
Anti HCV + antibody	0 / 3

* The serum that was found to be positive with VIDAS HAV IgM was confirmed to be positive with the other EIA technique, used as reference.

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 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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6. JILG W., BITTNER R., BOCK H. L., CLEMENS R., SCHÄTZL H., SCHMIDT M., ANDRE F. E. and DEINHARDT F.: Vaccination against hepatitis A: comparison of different short-term immunization schedules.Vaccine, 1992, 10, Suppl. 1, S126 - S128.

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
2015/06	07206Q	Technical	CONTENT OF THE KIT (30 TESTS) - RECONSTITUTION OF REAGENTS INSTRUCTIONS FOR USE
2016/05	07206R	Technical	CONTENT OF THE KIT (30 TESTS) - RECONSTITUTION OF REAGENTS

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