VIDAS®Anti-HAV Total (HAVT)

IVD

VIDAS Anti-HAV Total (HAVT) is an automated test for use on the VIDAS family instruments for the quantitative measurement of total immunoglobulins directed against the hepatitis A virus (HAV) in human serum or plasma (lithium heparin EDTA and Citrate), using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

The diagnosis of a recent hepatitis A viral infection is usually indicated by a positive anti-HAV IgM serology. Anti-HAV IgM is normally detected when the patient becomes symptomatic. An ELISA technique (e.g. VIDAS HAV IgM-Ref. 30 307 is routinely used for detection).
The appearance of IgG signals recovery and immunity (1).

In developed countries, improved hygiene conditions and water quality have decreased the prevalence of infections by this virus, which is exclusively transmitted by fecal-oral contact. Adults are increasingly affected and outbreaks of severe fulminant, cholestatic or relapsing hepatitis A are observed (2). The recent development of vaccines, which are more efficient than short-lived passive immunization, eliminates the risk of contracting viral hepatitis A in exposed populations (travelers to endemic regions, sewermen, drug addicts, male homosexuals) (2, 3, 4).

Pre-vaccinal screening for anti-HAV total antibody is justified when its prevalence is high (> 35%) in the country of origin of the patient (5). Regardless of the pre-vaccinal strategy followed, the anti-HAV total antibody level measured after the last injection of vaccination will verify the patient's seroconversion (6), the level of which has been determined at 20 mIU/ml by the manufacturers of Hepatitis A vaccine (7).
The detection of anti-HAV total immunoglobulin allows to determine the patient's immune status (6, 8).

PRINCIPLE

The assay principle combines a 2-step enzyme immunoassay competition 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.
The anti-HAV immunoglobulin in the sample binds with the inactivated antigen fixed on the SPR by an antibody.
Unbound components are eliminated by washing.
Antigenic sites which have not reacted with the immunoglobulin of the sample are next saturated with monoclonal antibody conjugated with alkaline phosphatase.
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 concentration of anti-HAV immunoglobulins 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):

30 HAVT strips	STR	Ready-to-use.
30 HAVT SPRs 1 x 30	SPR	Ready-to-use. Interior of SPRs coated with inactivated HAV antigens.
HAVT positive control 1 x 1 ml (liquid)	C1	Ready-to-use. Delipidated human* serum with anti-HAV Ig + 1 g/l sodium azide. MLE data indicate the confidence interval in mIU/ml (milli-International Units 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.
HAVT calibrator 1 x 2 ml (liquid)	S1	Ready-to-use. Delipidated human* serum with anti-HAV Ig + 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 interior of the SPR is coated during production with inactivated HAV antigens. Each SPR is identified by the HAVT 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 HAVT strip

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffer (0.2 mol/l, pH 6.2) + protein and chemical stabilizers + 0.9 g/l sodium azide (300 µl).
3 - 4 - 6 - 7 - 8 - 9	Wash buffer: TRIS HCl buffer (0.05 mol/l, pH 8) + Triton X 100 pH 8 + 8.78 g/l sodium chloride + 0.9 g/l sodium azide (600 µl).
5	Anti-HAV mouse monoclonal antibody conjugated with alkaline phosphatase. Diluent: TRIS buffer (0.05 mol/l, pH 6.3) + Tween 20 pH 6.2 + protein and chemical stabilizers + 0.9 g/l sodium azide (400 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).

* 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 150 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 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 Anti-HAV Total 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 heparin, EDTA and citrate).
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 300 µmol/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 2 mg/ml equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 400 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 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 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 "HAVT" strip and one "HAVT" 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 "HAVT" code on the instrument. 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).
6. **For this test, the calibrator, control, and sample test portion is 150 µl.**
7. Insert the "HAVT" SPRs and "HAVT" 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 **90 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 logistic model). The concentrations are expressed in «mIU/ml».

The patient RFV is interpreted by the VIDAS system. Both the results, expressed in mIU/ml (WHO reference standard 1st Reference Preparation Hepatitis A immunoglobulin) (100 IU/ml), and their interpretation are printed on the result sheet. The results are interpreted as follows:

Concentration	Interpretation
< 15 mIU/ml	Negative
≥ 15 and < 20 mIU/ml	Borderline positive
≥ 20 mIU/ml	Positive

Samples with a concentration > 400 mIU/ml must be retested after dilution of 1/100 in negative human serum.
If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result to obtain the sample concentration.
Interpretation of test results should be made taking into consideration the patient history, and the results of any other tests performed.

Interpretation of borderline positive

Samples with concentrations found between 15 and 20 mIU/ml contain anti-HAV antibodies. Such a concentration does not enable patient immunity to be affirmed; it is recommended to retest the patient after a few days.

QUALITY CONTROL

One positive and one negative control are included in each VIDAS HAVT 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 HAVT gave the following results:

Measurement range

The measurement range of the VIDAS Anti-HAV Total reagent is 15 to 400 mIU/ml.

Precision

Within-run reproducibility

3 samples were tested 30 times in a same run.

Sample	1	2	3
Mean (mIU/ml)	24.2	44.4	231
CV %	8.5	4.2	2.3

Between-run reproducibility

3 samples were tested singly in 29 different runs on the same VIDAS instrument.

Sample	1	2	3
Number	29	29	29
Mean (mIU/ml)	20.8	43.5	212.4
CV %	10.7	3.5	3.3

Specificity

1521 samples were tested in comparison with another commercially available EIA technique in 3 reference laboratories. The discrepant samples were confirmed with a modified RIA technique to obtain a detection threshold close to 10 mIU/ml. Equivocal samples were not used in the performance calculations.

1) Random population

1136 samples from blood donors were tested.

		EIA 1	
		positive	negative
VIDAS	positive	625	0
	negative	1*	510

* This sample was found negative with the RIA confirmation technique.

Relative sensitivity after confirmation: 100%
(95% Confidence interval: 99.4% -100%).
Relative specificity after confirmation: 100%
(95% Confidence interval: 99.2% -100%).

2) Vaccination follow-up samples

200 samples were tested:
- 30 samples before the first injection.
- 60 samples 1 month after the first injection.
- 60 samples 1 month after the second injection.
- 50 samples 1 month after the third injection.

		EIA 1	
		positive	negative
VIDAS	positive	158	0
	negative	1*	35

*This sample was found negative with the RIA confirmation technique.

6 samples tested using the EIA 1 method were excluded from the evaluation:
- 5 were equivocal with this method.
- 1 could not be retested with the confirmation method (insufficient quantity).

Relative sensitivity before confirmation: 99.4%.
Relative sensitivity after confirmation: 100%
(95% confidence interval: 97.5% -100%).
Relative specificity after confirmation: 100%
(95% confidence interval: 89.7% -100%).

3) Acute hepatitis A & natural immunity samples

51 anti-HAV IgM positive samples and 50 natural immunity to hepatitis A samples were tested:

		EIA 2	
		positive	negative
VIDAS	positive	91	10*
	negative	0	0

* These samples were found positive with the RIA confirmation technique.

Relative sensitivity after confirmation: 100%
(95% confidence interval: 96.2% -100%).

ACCURACY

Dilution test

3 samples were diluted in negative human serum and tested singly in 2 runs. The ratio of the mean concentration measured over the expected concentration is expressed as a mean recovery percentage.

Sample	Dilution factor	Expected concentration (mIU/ml)	Mean measured concentration (mIU/ml)	Mean recovery percentage
E1	1/10	-	234.8	-
	1/20	117.4	119	101
	1/40	58.7	59.9	102
	1/80	29.4	32.4	110
	1/160	14.7	17.9	122
E2	1/10	-	210.4	-
	1/20	105.2	98.1	93.3
	1/40	52.6	50	95.1
	1/80	26.3	26.2	99.5
	1/160	13.2	14.9	113
E3	1/10	-	248	-
	1/20	124	130.6	105
	1/40	62	68	110
	1/80	31	35.4	114
	1/160	15.5	19.5	126

CROSS REACTIVITY AND RELEVANT INTERFERENTS

	EIA 2 neg	VIDAS Anti-HAV Total	
		positive	negative
Anti-nuclear antibody (ANA) +	3	0	3
CMV+/EBV+	2	0	2
ANA+/HCV+	35	0	35
HIV +	23	0	23
HCV +	33	0	33
HBV +	5	0	5
Rhumatoid factor +	1	0	1

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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5. VAN DOORSLAER E., THOMAS G., VAN DAMME P. Cost-Effectiveness Analysis of Vaccination Against Hepatitis A in travellers. Journal of Medical Virology, 1994, 44: 463-469.
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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	08447K	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) WARNINGS AND PRECAUTIONS
2015/06	08447L	Technical	CONTENT OF THE KIT (30 TESTS) INSTRUCTIONS FOR USE
2016/05	08447M	Technical	CONTENT OF THE KIT (30 TESTS)

BIOMÉRIEUX

bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France

673 620 399 RCS LYON
Tél. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

CE