BIOMÉRIEUX

REF 30312

054829 - 02 - 2020-10

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VIDAS® Anti-HAV Total (HAVT)

IVD

INTENDED USE

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).

Combined with VIDAS® HAV IgM, the detection of total immunoglobulins directed against the hepatitis A virus (HAV) with VIDAS® Anti-HAV Total is used for screening of HAV immune status (related to past infection or vaccination) and is an aid in diagnosis of hepatitis A infection in patients with symptoms and/or clinical signs of hepatitis A.

SUMMARY AND EXPLANATION

Hepatitis A is a frequent vaccine-preventable acute inflammatory disease of the liver caused by hepatitis A virus (HAV).[1] Hepatitis A can lead to acute liver failure (fulminant hepatitis) but not to chronic infection.

Interpretation of VIDAS® Anti-HAV Total result is done in conjunction with interpretation of VIDAS® HAV IgM result in order to differentiate current from past HAV infection. A seroconversion of total anti-HAV antibodies with paired samples can suggest recent HAV infection.

For screening of HAV immune status, an assay detecting total anti-HAV antibodies (IgM and IgG) can be used. In this context, a positive VIDAS® Anti-HAV Total result is a marker of a past infection or vaccination.

PRINCIPLE

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

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

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

The anti-HAV immunoglobulin in the sample binds with the inactivated antigen fixed on the SPR device by an antibody.

Unbound components are eliminated during washing steps.

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-Methylumbelliferyl phosphate) is cycled in and out of the SPR device. 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, the results are automatically calculated by the instrument according to the calibration curve stored in memory. The results can then be printed out.

CONTENT OF THE KIT (30 TESTS)

30 HAVT Strips[(a)]	STR	Ready-to-use.
30 HAVT Solid Phase Receptacles 1 x 30	SPR	Ready-to-use. Interior of SPR devices coated with inactivated HAV antigens.
HAVT Calibrator 1 x 2 mL (liquid)	S1	Ready-to-use. Delipidated human* serum with anti-HAV Ig + 1 g/L sodium azide.
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[(b)] 1 x 1.9 mL (liquid)	C2	Ready-to-use. Phosphate buffer + protein stabilizer of animal origin + preservatives.

Specifications for the factory master data required to calibrate the assay: MLE (Master Lot Entry) barcode printed on the box label.
1 package insert 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.

(a) **DANGER** H318 / P280 / P305 + P351 + P338

(b) **WARNING** EUH208 / H317 / P261 / P280 / P302 + P352

Hazard statements

- EUH208: Contains 2-methyl-2H-isothiazolin-3-one. May produce an allergic reaction.
- H317: May cause an allergic skin reaction.
- H318: Causes serious eye damage.

Precautionary statements

- P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.
- P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
- 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, consult the Safety Data Sheet.

The SPR device
The interior of the SPR device is coated during production with inactivated HAV antigens. Each SPR device is identified by the HAVT code. Only remove the required number of SPR devices 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 barcode 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
The strip contains diethanolamine and sodium azide. Refer to the hazard statements “H” and precautionary statements “P” indicated above.

Well	Reagents
1	Sample well.
2	Sample diluent: buffer + protein stabilizer of animal origin and chemical stabilizers + preservative.
3 - 4 - 6 - 7 - 8 - 9	Wash buffer: buffer + preservative.
5	Buffer containing Anti-HAV mouse monoclonal antibody conjugate with alkaline phosphatase + protein stabilizer of animal origin and chemical stabilizers + preservative.
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) + preservative.

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 Manual.

- Instruments of the VIDAS® family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only, by qualified laboratory personnel in clinical laboratories.**
- **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; do not inhale).
- Do not use the SPR devices if the pouch is pierced or if the dot sealing a SPR device has come unstuck.
- Do not use visibly deteriorated strips (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the box 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.
- Refer to the hazard statements “H” and precautionary statements “P” indicated 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 Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (refer to the User Manual for user and preventive maintenance operations).

STORAGE CONDITIONS

- Store the kit at +2°C/+8°C.
- **Do not freeze reagents.**
- **Store all unused reagents at +2°C/+8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPR devices.
- **After use, carefully reseal the pouch with the desiccant inside to maintain stability of the SPR devices, and return the complete kit to +2°C/+8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the box label.

SAMPLES

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, lipemic or icteric and, if possible, to collect a new sample.

Do not inactivate samples.

Sample stability

Samples can be stored at +2°C/+8°C in stoppered tubes for up to 7 days. If longer storage is required, freeze the serum or plasma at -31°C/-19°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 Instrument User Manual.

Reading MLE data

When opening a new lot of reagents

With the external instrument barcode reader, scan the MLE data on the box label before performing the test.

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 Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the MLE data have been entered, and then 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. 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 device for each sample, control or calibrator to be tested. Make sure the SPR pouch has been carefully resealed after the required SPR devices have been removed.
3. The test is identified by the "HAVT" code on the instrument. The calibrator, identified by S1, must be 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" SPR devices and "HAVT" strips into the instrument. Check to make sure the color labels with the assay code on the SPR devices and the Reagent Strips match.
8. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.
9. Close the vials and return them to +2°C/+8°C after pipetting.
10. The assay will be completed within **approximately 90 minutes**. After the assay is completed, remove the SPR devices and strips from the instrument.
11. Dispose of the used SPR devices 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 device is introduced into the substrate.

The second reading is taken after incubating the substrate with the enzyme bound to the interior of the SPR device.

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 Manual), multiply the result to obtain the sample concentration.

Interpretation of test results should be made taking into consideration the patient's clinical 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 control and one negative control are included in each 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 applicable local 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 clinical history and the results of any other tests performed.

PERFORMANCE
Studies performed using the VIDAS® Anti-HAV Total assay 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

Agreement
1437 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.

Positive Percent Agreement: 99.8% (95% Confidence interval: 99.1% - 100%).

Negative Percent Agreement: 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).

Positive Percent Agreement: 99.4% (95% confidence interval: 96.4% -99.9%).

Negative Percent Agreement: 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.

Positive Percent Agreement: 100% (95% Confidence interval: 95.8% - 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

Sample	Dilution factor	Expected concentration (mIU/mL)	Mean measured concentration (mIU/mL)	Mean recovery percentage
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

Potential interference with Rhumatoid factor was studied according to CLSI EP7-Ed3 recommendations.

No significant interference was detected up to maximum concentrations tested [800 IU/mL].

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.

For countries within the EU, It is recommended that all material associated with the test, including material used to clean up spills, contaminated packaging, and/or unused and expired IVD tests, is incinerated.

LITERATURE REFERENCES

1. Ott JJ, Wiersma ST. - Single-dose administration of inactivated hepatitis A vaccination in the context of hepatitis A vaccine recommendations. - *Int J Infect Dis.* - 2013 Nov; 17(11): e939-44.
2. Miller JM, Binnicker MJ, Campbell S, *et al.* - A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. - *Clin Infect Dis.* - 2018 Aug 31; 67(6): e1-e94.
3. Workowski KA, Bolan GA. - Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. - *MMWR Recomm Rep.* -2015 Jun 5; 64(RR-03): 1-137.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer

Symbol	Meaning
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

LIMITED WARRANTY

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

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
2016/05	08447M	Technical	Content of the Kit (30 tests)
2019-10	054829-01	Administrative	Limited Warranty
		Technical	Content of the Kit (30 tests) Warnings and Precautions
2020-10	054829-02	Administrative	Formatting and wording changes.
		Technical change	Intended Use Performance Waste Disposal Literature References

REACH: authorization# - pending EU Commission decision.

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