IVD

VIDAS® Anti-HCV (HCV)

VIDAS Anti-HCV is an automated qualitative test for use on the instruments of the VIDAS family, for the detection of IgG antibodies to hepatitis C virus (anti-HCV) in human serum or plasma (heparin) using the ELFA technique (Enzyme Linked Fluorescent Assay). The detection of these specific antibodies, in conjunction with other clinical information, aids in the diagnosis of infection in persons with symptoms of hepatitis and in persons at risk for hepatitis C infection.

SUMMARY AND EXPLANATION

The Hepatitis C virus (HCV) discovered in 1989 using advanced molecular biology techniques, was rapidly found to account for the majority of those patients with non-A non-B hepatitis. HCV represents a major worldwide public health problem requiring global action for the diagnosis, treatment and prevention of this infection (1).
HCV is primarily parenterally transmitted through direct blood-to-blood contact between two people: use of unsterilized injection devices and transfusion of unscreened blood or blood products (2). The disease frequently progresses to chronic hepatitis C (80%), exposing patients to a greater risk of hepatic complications such as cirrhosis or hepatocellular carcinoma. (3).

The current standard of treatment for HCV is a combination of two drugs: pegylated interferon and ribavirin, but due to the high genetic variability of HCV (4), it is still only partially effective: viral eradication in less than 50% of patients infected with genotype 1 hepatitis C virus against approximately 80% of patients infected with genotype 2 or 3. New therapeutic options are under study to offer more effective and safer personalized treatments (5,6).

Diagnosis of patients infected with HCV can be performed using two categories of virological tests: indirect tests, and direct tests (7). Indirect serological tests are third-generation enzyme immunoassays that detect antibodies to HCV. The antigens used in the tests to detect antibodies are from the structural and non-structural regions of the HCV (8) (capsid, protein, cofactors, polymerase, etc.). The presence of anti-HCV antibodies indicates that an individual may have been infected with HCV in the past or may have an ongoing HCV infection. To confirm the presence of active HCV infection, a positive serological test can be completed using direct tests (e.g.: molecular assays that detect RNA genomes). The results will be used to guide patient management and determine the optimal duration of treatment.

The VIDAS Anti-HCV assay is a third-generation test using antigens corresponding to the HCV core, NS3 and NS4 proteins for the qualitative detection of anti-HCV antibodies.

PRINCIPLE

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).
The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and are 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.

During the first step, the sample is diluted, and then cycled in and out of the SPR several times. The anti-HCV antibodies present in the sample will bind to the antigens representing the HCV core, NS3 and NS4 proteins coated on the interior of the SPR. Unbound sample components are washed away.
During the second step, mouse monoclonal anti-human IgG antibodies in Fab form, conjugated to recombinant alkaline phosphatase (yeast) are cycled in and out of the SPR several times and will bind to the human Ig bound to the molecules on the solid phase. Further wash steps remove unbound components.
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 antibody present in the sample. At the end of the assay, the results are automatically calculated by the instrument in relation to the Standard S1 stored in memory, and then printed out.

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

60 Strips HCV	STR	Ready-to-use.
60 SPRs HCV (2 x 30)	SPR®	Ready-to-use. Interior of SPRs coated with antigens representing the HCV core, NS3 and NS4 proteins.
HCV Positive control 1 x 1.9 ml (liquid)	C1	Ready-to-use. Pooled human serum or plasma* containing anti-HCV IgG in a phosphate buffer + BSA + preservatives. 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. MLE data indicate the index: confidence interval ("Control C2 (-)Test Value Range).
Standard 1 x 1.9 ml (liquid)	S1	Ready-to-use. Pooled human serum or plasma* containing anti-HCV IgG in a phosphate buffer + BSA + preservatives. MLE data indicate the confidence interval in "Relative Fluorescence Value (RFV)" ("Standard (SX) RFV Range")..
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 and HIV2. The product has been inactivated. 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 the antigens representing the HCV core, NS3 and NS4 proteins. Each SPR is identified by the code "HCV". 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 HCV strip

Wells	Reagents
1	Sample well.
2	Sample diluent: TRIS buffered saline + Polysorbate 20 + BSA + preservatives (600 µl)
3 – 4 – 5 – 7 - 8	Wash buffer: TRIS buffered saline + Polysorbate 20 + preservatives (600 µl)
6	Conjugate: mouse monoclonal anti-human IgG antibodies conjugated to recombinant ALP in Phosphate buffered saline + protein stabilizer + preservatives (400 µl)
9	Reactive diluent*: Phosphate buffered saline + preservative (400 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: **WARNING**

Hazard statement

H315 : Causes skin irritation.
H319 : Causes serious eye irritation.

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.

** 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 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 SPR®s 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 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.
- 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-HCV 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.
- **To maintain stability of the remaining SPRs, carefully reseal the pouch after use with the desiccant inside 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

Human serum or plasma
Types of tubes validated:
– Plain tube,
– Tube with lithium heparin,
– Tube with sodium heparin,
– Tube with lithium heparin and separation gel,
– Plastic tube with clot activator,
– Plastic tube with clot activator and separation gel.
The use of heat-inactivated sera has not been validate.

Note: Depending on the manufacturer, blood collection tubes may contain materials and additives that could generate different test results.
It is the responsibility of each laboratory to validate use of these tubes in accordance with the manufacturer's recommendations for use.

Specimen preparation

Plain tubes: wait for samples to coagulate and **centrifuge** according to the tube manufacturer's recommendations to eliminate fibrin.
Other tubes: follow the tube manufacturer's recommendations for use.
Frozen-stored samples: after thawing, these samples must be homogenized before analysis.

Sample-related interference

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 30 mmol/l equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 220 mg/ml or 376 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.

Specimen stability

Serum and plasma samples separated from the clot, can be stored at 2-8 °C in stoppered tubes for 7 days; if longer storage is required, freeze the sera or plasma at -25 ± 6°C.
Do not exceed 3 freeze/thaw cycles.
A study performed on samples frozen for 12 months, showed that the quality of results is not affected.

INSTRUCTION FOR USE

For complete instructions, see the User's Manual.
Reading VIDAS® Protocole Test Change (PTC) protocol data and MLE data

When using the assay for the first time:

With the external instrument barcode reader,
1. Scan the PTC barcode(s) at the end of the package insert. or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data on the box label.

Note: If the MLE data have been read before the VIDAS® PTC protocol, read the MLE data again.

When opening a new lot of reagents:

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 28 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. They can be used immediately.**
2. Use one "HCV" strip and one "HCV" 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 "HCV" 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 is 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 "HCV" SPRs and the "HCV" 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 40 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. The patient RFV is interpreted as follows:
Test value = (patient RFV / standard RFV).
This test value and the interpretation are also included on the result sheet. The interpretation depending on the test value is as follows:

Test value (TV)	Interpretation
<1.00	negative
≥1.00	positive

All positive patient results must be verified in duplicate. If at least one of the repeat values is positive, the patient result is considered as positive. In that case, additional tests should be performed (another immunoassay or HCV marker) on the same sample or on a second one.

Note: In all cases, refer to current national guidelines concerning HCV diagnosis.

Interpretation of VIDAS Anti-HCV test results should be made taking into consideration the patient history and the results of any other tests or hepatitis C markers.

QUALITY CONTROL

One positive control and one negative control are included in each VIDAS Anti-HCV 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

For the diagnosis of HCV infection, the serological results should be used and interpreted taking into account the patient history, the clinical record, and further tests.
A negative test result does not exclude the possibility of exposure to HCV or infection with HCV. In particular, the results between 0.80 and 1.00 must be interpreted with caution. Anti-HCV antibodies may be undetectable in some stages of the infection (acute phase of hepatitis or presence of a serological scar) and in some clinical conditions (immunosuppression) (7,9).
Interference may be encountered with certain sera containing antibodies against reagent components.
This test has not been validated for use with any specimen matrices other than human serum or plasma.

RANGE OF EXPECTED VALUES(1)

Hepatitis C has a worldwide prevalence of 2-3% that varies according to country:

Region of the world	Anti-HCV prevalence (%)
Europe	2.3
Africa	3.2
Americas	1.5
Australia & Oceania	1.2
Asia	2.1
Middle East	4.7
Total	2.4

PERFORMANCE

The following study results demonstrate the conformity of VIDAS Anti-HCV to the Common Technical Specifications of 98/79/CE Directive:

1. Specificity for blood donor population:

5104 blood donor samples (including 2904 fresh samples with a negative status collected ≤ 24 hours previously) obtained from 2 blood transfusion centers, were tested using the VIDAS Anti-HCV assay.

	Status	
VIDAS Anti-HCV	Positive	Negative
Positive	0	20
Negative	0	5084

Diagnostic specificity of the VIDAS Anti-HCV assay on this population: 99.61%
(95% confidence interval: 99.40% - 99.76%)

2. Clinical specificity for hospitalized patients

200 samples with a negative status were tested using the VIDAS Anti-HCV assay.

Diagnostic specificity of the VIDAS Anti-HCV assay on this population: 99.50%.

(95% confidence interval: 97.25% - 99.99%)

3. Diagnostic sensitivity

439 samples with a positive status, including 102 fresh samples (collected ≤ 24 hours previously), were tested using the VIDAS Anti-HCV assay.

Genotypes 1 to 6 were tested:

Genotype	Number tested
1	21
2	21
3	23
4 (including non-a sub-types)	22
5	6
6	2

Results on tested populations:

Population	Positive VIDAS Anti-HCV /total tested	Diagnostic sensitivity observed (95% confidence interval)
HCV/HIV-negative patient	254/254	100% [98.56% - 100%]
HCV/HIV-positive patient	60/61*	98.36% [91.20% - 99.96%]
Patient with unknown HCV/HIV status	124/124	100% [97.07% - 100%]
Total HCV population	438/439*	99.77% [98.74% – 99.99%]

* The HCV/HIV coinfected patient who was not detected using VIDAS Anti-HCV had an index value > 0.8. This same sample was found to be either positive (index value close to the cutoff value) or negative using equivalent methods.

4. Sensitivity for seroconversion panels

Testing of 30 seroconversion panels demonstrated the precocity of detection of the VIDAS Anti-HCV assay. The results are comparable to those obtained using the most sensitive methods.

5. Precision

The repeatability and reproducibility were determined at two sites and calculated according to the recommendations of the CLSI® documents EP5-A2 / EP12-A2.
Four human samples were tested in duplicate using two lots of reagents. Testing was performed twice a day for 10 days on three instruments at one experimental site (N=120). Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table:

Sample ID / Target value	Sample 1		Sample 2		Sample 3		Sample 4	
	0.26		0.93		1.08		1.19	
	Standard deviation	CV (%)	Standard deviation	CV (%)	Standard deviation	CV (%)	Standard deviation	CV (%)
Repeatability	0.01	5.6	0.04	4.3	0.05	4.8	0.06	4.7
Reproducibility	0.07	26.9	0.05	5.9	0.07	6.3	0.07	5.8

6. Cross-reactivity

273 samples from patients with a physiological status that can potentially interfere with the detection of hepatitis C antibodies, were tested using VIDAS Anti-HCV. All of the samples were found to be negative with another EIA method (except one CMV IgG+ sample). No disease-related interference was observed for VIDAS Anti-HCV.

	VIDAS Anti-HCV
HSV +	0/10
VZV +	0/10
EBV +	0/10
HIV +	0/10
CMV IgG +	1/11*
LYME Ig+	0/10
HAV IgG +	0/10
HVB (HBcT +)	0/8
HVB (Ag HBs +)	0/10
Syphilis	0/10
Rubella IgG +	0/10
Toxoplasmosis IgG +	0/10
Rheumatoid factor	0/10
Anti-Nuclear Antibody	0/10
Anti-E. coli antibody	0/10
Anti-Pichia Antibody	0/10
Pregnant women**	0/114

* The reference EIA method also showed one false positive sample, but on a different sample.
** including 10 multipara.

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

1. LAVANCHY D. Evolving epidemiology of hepatitis C virus - Clin Microbiol Infect 2011; 17: 107-115.
2. ALTER MJ. Epidemiology of hepatitis C virus infection, World J Gastroenterol 2007; 13(17): 2436-2441.
3. GURTSEVITCH VE. Human Oncogenic Viruses: Hepatitis B and Hepatitis C Viruses and Their Role in Hepatocarcinogenesis, Biochemistry (Moscow), 2008, 73(5): 504-513.
4. SIMMONDS P. et al. Consensus Proposals for a Unified System of Nomenclature of Hepatitis C Virus Genotypes, HEPATOLOGY 2005;42: 962-973.
5. VERMEHREN J, SARRAZIN C. New HCV therapies on the horizon, Clin Microbiol Infect 2011; 17: 122–134.
6. WEBSTER D.P, KLENERMAN P., COLLIER J., JEFFERY K. JM. Development of novel treatments for hepatitis C, Lancet Infect Dis 2009; 9: 108-17.
7. CHEVALIEZ S. Virological tools to diagnose and monitor hepatitis C virus infection, Clin Microbiol Infect 2011; 17: 116–121.
8. PENIN F, DUBUISSON, REY F-A, MORADPOUR D, ET PAWLOTSKY JM. Structural Biology of Hepatitis C Virus, HEPATOLOGY 2004; 39: 5-19.
9. THIO CL, NOLT KR, ASTEMBORSKI J, VLAHOV D, NELSON KE et THOMAS DL. Screening for Hepatitis C Virus in Human Immunodeficiency Virus-Infected Individuals, Journal of clinical microbiology, Feb. 2000, 38(2): 575-577.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue 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

bioMérieux disclaims all warranties, express or implied, including any implied warranties of MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. bioMérieux shall not be liable for any incidental or consequential damages. IN NO EVENT SHALL BIOMERIEUX'S LIABLITY TO CUSTOMER UNDER ANY CLAIM EXCEED A REFUND OF THE AMOUNT PAID TO BIOMERIEUX FOR THE PRODUCT OR SERVICE WHICH IS THE SUBJECT OF THE CLAIM.

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	9300913E	Administrative	REVISION HISTORY INDEX OF SYMBOLS
		Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS
2015/06	9300913F	Technical change	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS INSTRUCTIONS FOR USE
2016/06	9300913G	Technical change	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS

BIOMÉRIEUX
bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France
673 620 399 RCS LYON
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

CE 0459