abia HCV Ab

REF DK.067.01.3
REF DK.067.05.3

Σ 96
Σ 480

IVD

CE 0483

Note: Changes highlighted ★

abia

Intended use

abia HCV Ab is an enzyme immunoassay for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum or plasma.

The assay is intended for screening for potentially infectious samples to prevent their use as donor materials. For professional use only.

Clinical value

Hepatitis C is an infectious disease affecting the liver, caused by the hepatitis C virus (HCV). The infection is often asymptomatic, but once established, chronic infection can progress to scarring of the liver (fibrosis), and advanced scarring (cirrhosis) which is generally apparent after many years. In some cases those with cirrhosis will go on to develop liver failure or other complications of cirrhosis, including liver cancer[1].

The hepatitis C virus is an enveloped RNA positive sense virus belonging to the Flaviviridae family. First line hepatitis C screening is third-generation ELISA immunoassay for detecting anti-HCV antibodies. These tests are successfully used for long time for blood donors screening.

Principle of the test

abia HCV Ab is an indirect solid-phase enzyme assay based on microwells coated with recombinant antigens representing immunodominant regions of HCV structural (core) and nonstructural (NS3, NS4, NS5) proteins.

In the first stage antibodies specific for HCV core, NS3, NS4 or/and NS5 present in the sample binds with HCV recombinant antigens coated on the wells and at the same time with biotinylated recombinant HCV core Ag (conjugate 1). The unbound components are removed by washing.

In the second stage HRP-labeled anti-human IgG and streptavidin binds to any human IgG and biotin labeled core Ag captured on the well in the first stage. The unbound components are removed by washing.

After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of HCV antibodies in the specimen and can be read at 450 nm or 450/620 nm.

Kit contents

	S	XL	
HCV Ag coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with recombinant HCV Ag (core, NS3, NS4, NS5); preservatives: sodium azide 0.01 %
Conjugate 1	1 × 10 ml	2 × 25 ml	ready-for-use; Trizma solution (pH 7.0) with biotinylated recombinant HCV core Ag; pink-crimson liquid; amorphous sediment may form which dissolves at shaking; preservatives: ProClin 300 0.1 %
Conjugate 2 (concentrated 11-fold)	1 × 2.0 ml	2 × 3.5 ml	phosphate saline buffer (pH 7.3-7.6) with mixture of HRP labeled mouse anti-human IgG and streptavidin; transparent or slightly opalescent colorless or pale yellow liquid; preservatives: ProClin 300 0.04 %, gentamicin sulfate 0.001 %
Conjugate 2 diluent	2 × 10 ml	3 × 25 ml	saline solution containing urea (8.3%) and casein hydrolysate (0.13%); transparent or slightly opalescent yellow liquid; amorphous sediment may form which dissolves at shaking; preservatives: ProClin 300 0.1 %, gentamicin sulfate 0.001 %
HCV Ab positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready-for-use; phosphate saline buffer (pH 7.3-7.6), heat inactivated human plasma positive for antibodies to HCV antigens; red liquid; preservatives: ProClin 300 0.04 %, sodium azide 0.2 %
Negative control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready-for-use; phosphate saline buffer (pH 7.3-7.6), heat inactivated negative human plasma and goat serum; green liquid; preservatives: ProClin 300 0.04 %, sodium azide 0.2 %, gentamicin sulfate 0.001 %
Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	phosphate saline buffer with Tween 20 (pH 7.5-7.9); colorless or pale yellow liquid; without preservatives
TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	solution containing TMB; colorless liquid; without preservatives
Substrate buffer	1 × 25 ml	1 × 70 ml	citric acid (0.64%) and sodium acetate solution (pH 4.1-4.3), containing H_2O_2 (0.008%); colorless liquid; preservatives: ProClin 300 0.04 %
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	1 × 90 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid; without preservatives
Protective film	2	10	
Plastic dish	2	–	
Plastic zip-lock bag	1	5	

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package
The device is available in two different variants; DK.067.01.3 with 96 determinations (S) and DK.067.05.3 with 480 determinations (XL).

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate incubator set at 37.0 ± 1.0 °C
- automatic microplate washer
- microplate reader equipped with 450 nm or with 450/620–680 nm filters

Safety notes

- human origin material used in the preparation of the negative control has been tested by CE-marked tests and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- human origin material used in the preparation of the Ab positive control has been tested by CE-marked tests and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1 and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke, or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use heparin/EDTA/citrate plasma or serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum or plasma as soon as possible to avoid any haemolysis
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- pooled specimens must not be used since the accuracy of the test with such specimens has not been validated
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than three freeze/thaw cycles are allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (18–24 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section "Washing procedure"
- for the description of test procedure with the automated analyzers see section "Automated analyzers"

Washing procedure ★

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 350 µl per well is used. When using a microplate washer for which this is not possible, ensure that the well is completely filled with a slight positive meniscus without overflow before aspiration
- perform this procedure 4 times in total
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0
Working solution of conjugate 2: mix the reagents thoroughly until diluted, avoid foaming **Note:** before use keep the working solution of conjugate 2 at least within 10 min at 18–24 °C **Stability:** 12 hours at 18–24 °C in a dark place												
Conjugate 2 (concentrated 11-fold), ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Conjugate 2 diluent, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
Substrate mixture: mix the reagents thoroughly until dilution **Note:** substrate mixture should be colorless! **Stability:** 10 hours at 18–24 °C in a dark place												
TMB (concentrated 11-fold), ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Substrate buffer, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

Test procedure

1 Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in a plastic zip-lock bag. Do not remove desiccant.

2 Add 60 µl of conjugate 1 in all to be used wells.
Add 40 µl of HCV Ab positive control in well A1.
Add 40 µl of negative control in well B1, C1 and D1.
Add 40 µl of samples to be tested in rest of the wells.
Depending on system and number of strips used, it is possible to modify the position of controls or the order of distribution.
The total time should not exceed 15 min. Pink-crimson color of the conjugate should change to yellow when samples were added.
Mix the contents of the wells by gentle pipetting, then cover the plate with protective film.

3 Incubate in microplate incubator for 60 minutes at 37.0 ± 1.0 °C.

4 Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant).
Add not less than 350 µl of working washing solution into each well and aspirate. Perform this procedure 4 times.

5 Add 100 µl of working solution of conjugate 2 in each well, then cover the plate with protective film.

6 Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.

7 Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant).
Add not less than 350 µl of working washing solution into each well and aspirate. Perform this procedure 4 times.

8 Add 100 µl of substrate mixture to all the wells. Keep the plates in a dark place for 20 minutes at 18–24 °C.

9 Add 150 µl of stopping reagent into each well.

10 Read the optical density at 450/620-680 nm using a plate reader not before 2 - 3 minutes after stopping reaction. Reading the absorbance at 450 nm only is possible.
Test results remain stable for reading within 10 minutes.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section “Test procedure” and ensure all requirements described in section “Precautions” are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of each negative control should be less than 0.200.
If one negative control does not respect this norm, disregard and recalculate the mean value using the two remaining values. Only one value may be eliminated by this way.
The absorbance (OD) of HCV Ab positive control should be greater than 2.000.

Calculation cut-off value

Mean OD value of the negative control = (OD value B1 + OD value C1 + OD value D1)/3

Cut-off = mean OD value of negative control + 0.240

Interpretation of the results

Non-reactive sample: sample OD value < cut-off
Samples with absorbance values less than the cut-off value are considered to be negative by the abia HCV Ab test.

Reactive sample: sample OD value ≥ cut-off
Samples with absorbance values more than or equal the cut-off value are considered to be positive by the abia HCV Ab test.

No “grey zone” is contemplated.

Performance characteristics

The performance of the abia HCV Ab has been determined by testing samples from random blood donors, from patients with confirmed HCV infection and patients in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.

Diagnostic sensitivity	Number of tested samples	Sensitivity, %	95% CI, %
HCV Ab positive samples	466	100.00	99.18 - 100.00
incl. genotyped samples of genotype 1	26	100.00	87.13 - 100.00
incl. genotyped samples of genotype 2	25	100.00	86.68 - 100.00
incl. genotyped samples of genotype 3	35	100.00	90.11 - 100.00
incl. genotyped samples of genotype 4	24	100.00	86.20 - 100.00
incl. genotyped samples of genotype 5, 6	6	100.00	64.97 - 100.00
incl. samples with different patterns	82	100.00	95.52 - 100.00

32 commercial seroconversion panels (Seracare, ZeptoMetrix) were evaluated and compared against a commercially available CE marked assay.

Diagnostic specifity	Number of tested samples	Specificity, %	95% CI, %
Unselected blood donors	5.060	99.82	99.66 - 99.91
Pregnant women	270	100.00	98.64 - 100.00
Hospitalized patients with non-infectious diseases and infectious diseases (HIV, hepatitis B, chlamydiosis, herpetic and cytomegalovirus infections)	732	99.44	98.56 - 99.78

Precision

The repeatability within one plate was evaluated by testing 3 positive samples 7 times each. The CV did not exceed 8 %.
The reproducibility between different lots, operators, days was evaluated by testing 3 positive samples 7 times each using 3 different lots. The CV did not exceed 15 %.

Limitations of test

- a sample should not be defined as positive for anti-HCV based on a single reactive result. Reactive results should be re-tested; and in case of repeated reactive result confirmed by supplemental assays[2].
- the results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture[3-9].
- non-reactive results can occur if the concentration of marker, present in the sample, is below the detection limit of the assay, or if the marker to be detected is not present during the stage of disease when a sample has been collected.
- the variability of hepatitis C virus doesn't allow to exclude the possibility of false negative results. No known test method can offer complete assurance that the hepatitis C virus is absent.
- the divergence in results at testing the same samples in different assays is maybe connected by primary structure of used recombinant antigens[10].
- false reactive test results can be expected with any test kit. False reactive test results have been observed due to nonspecific interactions.

References

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Hazard and precautionary statements for certain kit components

Warning

Conjugate 1

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.

Danger

TMB

H317	May cause an allergic skin reaction.
H360D	May damage the unborn child.
P201	Obtain special instructions before use.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.

Conjugate 2 diluent

H317	May cause an allergic skin reaction.
H373	May cause damage to organs through prolonged or repeated exposure.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.

Danger

HCV Ab positive control (inactivated), negative control (inactivated)

H373	May cause damage to organs through prolonged or repeated exposure.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280 (for all)	Wear protective gloves/protective clothing/eye protection/face protection.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Batch code
 YYYY-MM-DD	Expiry date
 2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
 n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

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