abia HEV IgG

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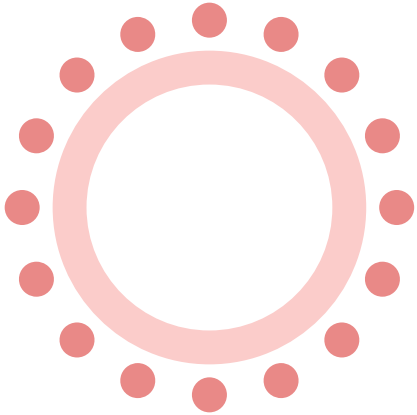
IVD

CE

Note: Changes highlighted ★



abia

Intended use

abia HEV IgG is an enzyme immunoassay for the qualitative detection of IgG antibodies to hepatitis E virus (HEV) in human serum or plasma.

The assay is intended for screening for potentially infectious samples. For professional use only.

Clinical value

Hepatitis E, caused by infection with hepatitis E virus (HEV), is a common cause of enterically-transmitted acute hepatitis in developing countries. Occasional cases of sporadic hepatitis E have been increasingly recognized in developed countries over the past decade. The hepatitis E virus causes around 20 million infections a year. Hepatitis E is particularly dangerous for pregnant women, who can develop an acute form of the disease that is lethal in 20 percent of cases.

Detection of antibodies IgM and IgG to HEV proteins is the most prevalent method of hepatitis E virus (HEV) laboratory diagnostics.

Most patients with acute HEV infection have detectable IgM anti-HEV antibodies by the time clinical symptoms appear. These antibodies persist for a few months, and their presence is taken as an evidence of recent HEV infection.

The IgM antibodies are soon followed by the appearance of specific IgG antibodies.

Detection of IgG anti-HEV antibodies is generally been taken as evidence of prior exposure to HEV. The duration of persistence of circulating IgG anti-HEV antibodies remains unclear. These antibodies have been found in healthy subjects living in all geographical areas, although the prevalence varies widely. In general, prevalence rates are higher in developing countries where hepatitis E is common than in countries where clinical cases due to hepatitis E are uncommon.

Principle of the test

abia HEV IgG is an indirect solid-phase enzyme assay based on microwells coated with recombinant antigens representing ORF2 and mosaic artificial antigen representing immunodominant regions of ORF2 and ORF3. The conjugate is a mixture of HRP-labeled anti-human IgG antibodies.

Serum or plasma samples are added to the wells and if antibodies specific for HEV ORF2 or/and ORF3 immunodominant regions are present in a sample, they form stable complexes with the HEV antigens immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of HRP antibody conjugate.

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 colour which is converted to yellow after the reaction has been stopped with sulphuric acid.

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

Kit contents

	S	XL	
HEV Ag coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with recombinant HEV Ag
Sample diluent	1 × 12 ml	2 × 25 ml	phosphate saline buffer; violet-blue liquid
Conjugate (concentrated 21-fold)	1 × 1.0 ml	2 × 2.0 ml	HRP-labeled polyclonal goat antibodies to human IgG; colourless or pale yellow liquid
Conjugate diluent	2 × 10 ml	3 × 25 ml	urea and sodium chloride; yellow liquid
HEV Ab positive control (inactivated)	1 × 1.2 ml	2 × 1.2 ml	ready to use; human plasma positive for Anti-HEV IgG; red liquid
Negative control (inactivated)	1 × 2.5 ml	2 × 2.5 ml	ready to use; negative human plasma; green liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	phosphate saline buffer; colourless or pale yellow liquid
TMB (concentrated 21-fold)	1 × 2.5 ml	2 × 3.5 ml	solution containing TMB; colourless liquid
Substrate buffer	1 × 25 ml	1 × 70 ml	citric acid and sodium acetate solution, containing H_2O_2; colorless liquid
Stopping reagent 0.75M H_2SO_4	1 × 25 ml	1 × 90 ml	ready to use; 0.75 mol/l sulphuric acid solution; colourless liquid
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
Concentration of preserving agents: <0.2 %

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate incubator 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 and the positive 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)
- 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 undiluted 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 sera or plasma preserved with sodium azide
- 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 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 48 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is 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 400 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
- allow a soaking time of at least 40 seconds 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: mix the reagents thoroughly until diluted, avoid foaming **Note:** before use keep the working solution of conjugate at least within 10 min at 18–24 °C **Stability:** 12 hours at 18–24 °C in a dark place												
Conjugate (concentrated 21-fold), ml	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Conjugate 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 21-fold), ml	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
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 plastic zip-lock bag. Do not remove desiccant bag.
2	Add 100 µl of HEV Ab positive control in well A1. Add 100 µl of negative control in well B1, C1 and D1. Add 90 µl sample diluent and 10 µl of samples to be tested in rest of the wells. Depending on system and number of stripes used, it is possible to modify the position of controls or the order of distribution. The total time should not exceed 15 min. Violet-blue colour of the sample diluent should change to blue-green when samples were added. Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with protective film.
3	Incubate in microplate incubator for 30 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 400 µl of working washing solution into each well. Allow a soaking time of at least 40 seconds and aspirate. Perform this procedure 4 times.
5	Add 100 µl of working solution of conjugate in each well. Mix the contents of the wells by careful tapping on the edge of the plate, 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 400 µl of working washing solution into each well. Allow a soaking time of at least 40 seconds 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 50 µl of stopping reagent into each well.
10	Read the optical density at 450/620-680 nm using a plate reader. Reading the absorbance at 450 nm only is possible. Test results remain stable for reading within at least 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 HEV Ab positive control should be greater than 0.600.

Calculation of the 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 + 0.200

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 HEV IgG 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 HEV IgG test.

No “grey zone” is contemplated.

Performance characteristics

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

Analytical sensitivity

The analytical sensitivity was evaluated with "WHO reference reagent for Hepatitis E virus antibody, human serum" (NIBSC Code: 95/584) and defined at 0.25 U/ml.

Diagnostic sensitivity	Number of tested samples	Sensitivity, %
HEV positive samples	36	100.00

9 commercial seroconversion panels (Biomex) were evaluated and compared against a commercially available CE marked assay.

Diagnostic specificity	Number of tested samples	Specificity, %
Unselected blood donors	1 288	98.30
Pregnant women	20	100.00
Hospitalized patients with non-infectious diseases	44	95.50
Patients with infectious diseases (Hepatitis A, B, C)	29	100.00
Patients with rheumatoid factor	28	92.90

Precision	Mean, OD/ cut-off	SD	CV, %
Intra-assay, sample 1	10.82	0.27	2.50
Intra-assay, sample 2	9.87	0.36	3.65
Intra-assay, sample 3	10.77	0.38	3.53
Inter-assay, sample 1	10.76	0.35	3.25
Inter-assay, sample 2	10.12	0.44	4.35
Inter-assay, sample 3	11.12	0.50	4.50

Limitations of test

- a sample should not be defined as positive for anti-HEV IgG based on a single reactive result. Reactive results should be re-tested; and in case of repeated reactive result confirmed by supplemental assays.
- 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 HEV virus doesn't allow to exclude the possibility of false negative results. No known test method can offer complete assurance that the HEV virus is absent.

References

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Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue 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
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components ★

Warning

Stopping reagent

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

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