abia HBs Ag

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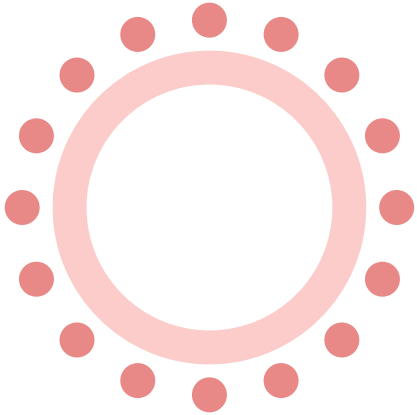
IVD

CE 0483

Note: Changes highlighted ★



abia

Intended use

abia HBs Ag is an enzyme immunoassay for the qualitative detection of wild types and mutant variants hepatitis B surface antigen (HBsAg) 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

Detection of hepatitis B surface antigen (HBsAg), which is the main marker of the disease and which is revealed long before the clinical signs of the disease, is of decisive importance for hepatitis B diagnostics by the use of specific tests.

The high sensitivity of abia HBs Ag allows to reduce the period of diagnostic (serological) window of viral hepatitis type B. That will enhance the quality of hepatitis B diagnostics, allow to reduce the quantity of latent infections both with the presence of complementary markers of anti-HBs and with the absence of any markers and it may also correct the therapy of double infection.

High sensitivity of the test-system allows a more effective detection of mutant variants of HBsAg.

The use of a highly-sensitive test improves the quality of donor blood screening, allows reducing the risk of post transfusion hepatitis B infection.

Principle of the test

abia HBs Ag is a "sandwich" assay based on microwells coated with antibodies against HBs antigen. The conjugate is a biotin-labeled antibodies against HBs antigen and HRP-labeled streptavidin.

Serum or plasma samples are added to the wells and if HBs antigens are present in a sample, they form stable complexes with antibodies immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of:
(1) biotinylated antibodies and
(2) HRP streptavidin 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 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 HBs antigens in the specimen and can be read at 450 nm or 450 and 620–680 nm.

Kit contents

	S	XL	
HBs Ab coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with anti-HBs mAb
Conjugate 1 (concentrated 11-fold)	1 × 1.4 ml	1 × 3.5 ml	mixture of biotinylated mAb to HBsAg; transparent or slightly opalescent violet liquid
Conjugate 2 (concentrated 11-fold)	1 × 1.4 ml	1 × 3.5 ml	mixture of HRP labeled streptavidin; transparent or slightly opalescent blue liquid
Positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready to use; purified recombinant HBsAg in human plasma; red liquid
Low positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready to use; purified recombinant HBsAg in human plasma; orange liquid
Negative control (inactivated)	1 × 2.5 ml	2 × 2.5 ml	ready to use; negative human plasma; green liquid
Conjugate 1 diluent	1 × 14 ml	2 × 18 ml	casein hydrolysate, goat serum; green liquid
Conjugate 2 diluent	1 × 14 ml	2 × 18 ml	casein hydrolysate, bovine serum albumin; transparent yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	solution containing TMB; colorless liquid
Substrate buffer	1 × 25 ml	1 × 70 ml	citric acid and sodium acetate solution, containing H_2O_2; colorless liquid
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
Protective film	3	15	
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: <=0.1 %

Materials and equipment required but not provided

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

Safety notes

- human origin material used in the preparation of the negative control, positive control and low 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 1: mix the reagents thoroughly until diluted, avoid foaming **Note:** before use keep the working solution of conjugate 1 at least within 10 min at 18–24 °C **Stability:** 12 hours at 18–24 °C in a dark place												
Conjugate 1 (concentrated 11-fold), ml	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.7
Conjugate 1 diluent, ml	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	7.0
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	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.7
Conjugate 2 diluent, ml	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	7.0
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

Attention! Incubation is possible as two alternative procedures. During test performance follow the same incubation mode. The combination of incubation modes is not supposed.

	Procedure 1 - microplate incubator	Procedure 2 - microplate thermoshaker
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 HBsAg positive control in well A1, add 100 µl of low positive control in well B1, add 100 µl of negative control in well C1, D1 and E1. Add 100 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. 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.	Without incubation (step 3), go to step 4
4	Remove the protective film slowly and carefully to prevent splashes. Without removing the contents of the wells and washing the wells, add 50 µl of working solution of conjugate 1 into each well.	Add 50 µl of working solution of conjugate 1 in each well.
	The color should change to grey. The color may change to yellow in case of specimens with acid pH, or not change in case of specimens with neutral pH.	
5	Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with protective film. Incubate in microplate incubator for 60 minutes at 37.0 ± 1.0 °C.	Incubate during 60 minutes in shaker at 500 rpm at 37.0 ± 1.0 °C without protective film.
6	Remove the protective film slowly and carefully to prevent splashes. Without removing the contents of the wells and washing the wells, add 50 µl of working solution of conjugate 2 into each well.	
7	Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with protective film. Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.	Incubate during 30 minutes in shaker at 500 rpm at 37.0 ± 1.0 °C without protective film.
8	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.	
9	Add 100 µl of substrate mixture to all the wells. Keep the plates in a dark place for 20 minutes at 18–24 °C.	
10	Add 150 µl of stopping reagent into each well.	
11	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 3 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 0.120.
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 HBsAg positive control should be greater than 1.000.
The OD/cut-off value of low positive control should be in the range between 1.000 and 4.500..

Calculate cut-off value

Mean OD value of the negative control = (OD value C1 + OD value D1 + OD value E1)/3
Cut-off = mean OD value of negative control + 0.120

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 HBs Ag test.

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

No “grey zone” is contemplated.

Performance characteristics

The performance of the abia HBs Ag has been determined by testing samples from random blood donors, from patients with confirmed acute and chronic hepatitis B, native mutants samples and patients in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.
HBsAg sensitivity limit has been tested using WHO 3rd International Standard for HBsAg (NIBSC Code: 12/226).

Diagnostic sensitivity	Number of tested samples	Sensitivity, %
HBsAg positive samples	2 492	100.00
incl. fresh samples	25	100.00
BBI PHA807, HBsAg sensitivity panel	10	100.00
BBI PHA808, HBsAg sensitivity panel	10	100.00
BBI PHA106, low titer performance panel	15	100.00
BBI PHA205, mixed titer performance panel	25	100.00
Native HBsAg mutants	48	100.00
Recombinant HBsAg mutants panel	13	100.00

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

Diagnostic specificity	Number of tested samples	Specificity, %
Unselected blood donors	10 353	99.83
Pregnant women	477	99.37
Hospitalized patients with non-infectious diseases	285	98.95
Patients with infectious diseases (HIV, hepatitis A, C, syphilis, chlamydiosis, herpetic and cytomegalovirus infections)	1 929	99.33
Patients with rheumatoid factor	182	99.45

Analytical sensitivity

The analytical sensitivity was evaluated with WHO International Standard "Third International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2)" (NIBSC Code: 12/226) and defined at 0.02 IU/ml.

Precision

The repeatability within one plate was evaluated by testing 3 positive samples 70 times each. The CV did not exceed 10 %.
The reproducibility between different lots, operators, days, laboratories was evaluated by testing 3 positive samples 16 times. The CV did not exceed 10 %.

Limitations of test

- a sample should not be defined as positive for HBsAg 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 hepatitis B virus doesn't allow to exclude the possibility of false negative results. No known test method can offer complete assurance that the hepatitits B virus is absent.

References

1. Hofer M., Joller-Jemelka H.I., Grob P.J.et al. Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only. Swiss HIV Cohort Study. Eur. J. Clin. Microbiol. Infect. Dis. 17:6-13, 1998.
2. Liang T.J., Blum H.E., Wands J.R. Characterization and biological properties of a hepatitis B virus isolated from a patient without hepatitis B virus serological markers. Hepatology. 12:204-212, 1990.
3. Schuttler C.G., Fiedler N., Schmidt K. et al. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. J. Hepatol. 37:855-862, 2002.
4. Thiers V., Nakajima E., Kremsdorf D. A. et al. transmission of hepatitis B from hepatitis-B-sero-negative subjects. Lancet. 2:1273-1276, 1988.
5. Wanger A.A., Loustaud-Ratti V., Chemin I. et al. Double hepatitis B virus infection in a patient with HIV/hepatitis C virus coinfection and «anti-HBc alone» as serological pattern. Eur. J. Clin. Microbiol. Infect. Dis. 24:623-627, 2005.
6. Weinberger K.M., Bauer T., Bohm S., Jilg W. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. J. General. Virol. 81:1165-1174, 2000.

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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