abia HIV AgAb

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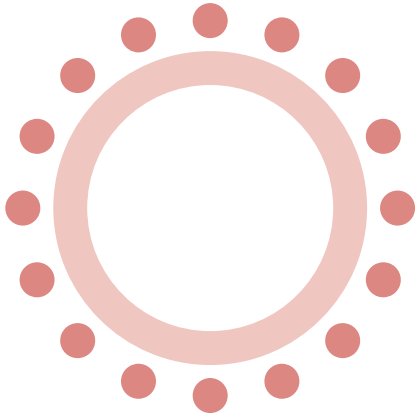
IVD

CE 0483

Note: Changes highlighted ★



abia

Intended use

abia HIV AgAb is an enzyme immunoassay for the qualitative detection of antibodies to human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), HIV-1 group O and HIV-1 p24 antigen 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 antibodies to HIV proteins or simultaneous detection of antibodies and p24 core protein is the most prevalent method of HIV laboratory diagnostics.

HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and the infection.

Use of a highly sensitive kit for simultaneous detection of antibodies and p24 antigen allows reducing a phase of serological window on the average to 4-6 days due to the detection of the earliest marker of HIV infection p24 antigen.

The diagnostic significance of p24 antigen is important also at last stage of an infection, when functional ability of immune system is low.

Principle of the test

abia HIV AgAb is a "sandwich" assay based on microwells coated with recombinant antigens representing immunodominant regions of HIV-1, HIV-1 (O), HIV-2 proteins and antibodies to HIV-1 p24 (p24 mAb). The conjugate is a mixture of biotin and HRP-labeled viral epitopes and biotin-labeled antibodies against p24.

Serum or plasma samples are added to the wells and if p24 antigen and/or antibodies specific for HIV-1, HIV-1 (O) and HIV-2 are present in a sample, they form stable complexes with the HIV antigens or antibodies immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of:
(1) biotinylated antigens and antibodies and
(2) HRP streptavidin conjugate and HRP labeled antigens (for minimization of „hook effect").

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 HIV antigens/antibodies in the specimen and can be read at 450 nm or 450/620 nm.

Kit contents

	S	XL	
HIV AgAb coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with recombinant HIV Ag and p24 mAb
Conjugate 1 (concentrated 11-fold)	1 × 1.2 ml	1 × 2.5 ml	mixture of biotinylated recombinant HIV Ag and p24 mAb; colourless or pale yellow liquid
Conjugate 2 (concentrated 11-fold)	1 × 1.4 ml	1 × 3.5 ml	mixture of HRP labeled recombinant HIV Ag and streptavidin; colourless or pale yellow liquid
HIV Ag positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready to use; purified recombinant HIV-1 p24 Ag in human plasma; crimson-red liquid
HIV Ab positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready to use; human plasma positive for HIV Ab; orange liquid
Negative control	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
Conjugate 1 diluent	1 × 12 ml	1 × 25 ml	sodium caseinate, NaCl; orange liquid
Conjugate 2 diluent	1 × 14 ml	2 × 18 ml	phosphate saline buffer; blue liquid
TMB (concentrated 11-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 0.2M H_2SO_4	1 × 25 ml	1 × 90 ml	ready to use; 0.20 M/L sulphuric acid solution; colourless liquid
Protective film	2	10	
Plastic dish	2	–	
Zip-lock plastic 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 450/620–680 nm filters

Safety notes

- human origin material used in the preparation of the negative control and Ag 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)
- human origin material used in the preparation of the Ab positive control has been tested by CE-marked tests and found nonreactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1 and antibodies to hepatitis C virus
- as no known test method can offer complete assurance that infections agents are absent, handle reagents and samples as if capable of transmitting infections 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; test 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 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 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
- repeat 4 times
- 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	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.5
Conjugate 1 diluent, ml	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2.7	3.0	3.3	5.0
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 zip-lock plastic bag. Do not remove desiccant.	
2	Add 30 µl of working solution of conjugate 1 in each well.	
3	Add 70 µl of HIV Ab positive control in well A1. Add 70 µl of HIV Ag positive control in well B1. Add 70 µl of negative control in well C1, D1 and E1. Add 70 µl of samples to be tested in rest of the wells. Depending on the used system and the number of used strips, it is possible to modify the position of controls or the order of distribution. The total time should not exceed 15 min. Orange colour of the working solution of conjugate 1 should change to pink. The colour may change to yellow in case of specimens with acid pH, or not change in case of specimens with neutral pH. Mix the contents of the wells by careful tapping on the edge of the plate, then cover the plate with protective film.	
4	Incubate in microplate incubator for 60 minutes at 37.0 ± 1.0 °C.	Incubate during 45 minutes in shaker at 500 rpm at 37.0 ± 1.0 °C.
5	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. The pink colour should change to green. 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.	Incubate during 20 minutes in shaker at 500 rpm 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 150 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 HIV Ab positive control should be greater than 0.800.
The absorbance (OD) of HIV Ag positive control should be greater than 0.800.

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 + 0.400

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 HIV AgAb 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 HIV AgAb test.

No "grey zone" is contemplated.

Performance characteristics

The performance of the abia HIV AgAb has been determined by testing samples from random blood donors, from patients with confirmed HIV-1 (including group O) and HIV-2 infection and patients in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.
HIV Ag sensitivity limit has been tested using WHO International Standard (NIBSC Code: 90/636).

Diagnostic sensitivity	Number of tested samples	Sensitivity, %
HIV-1 positive samples	497	100.00
incl. identified subtypes	95	100.00
HIV-2 infected patients	132	100.00
WWRP 302 (M), world wide performance panel	28	100.00
1st International reference panel, NIBSC code: 02/210	6	100.00
BBI PRZ206, anti-HIV 1/2 combo performance panel	11	100.00
BBI PRB204 (M), anti-HIV 1 mixed titer performance panel	23	100.00
BBI PRB601, HIV 1 incidence/prevalence performance panel	15	100.00
BBI QRZ761, anti-HIV 1/2 qualification panel	5	100.00

56 commercial seroconversion panels (Seracare, ZeptoMetrix) were evaluated and compared against a commercially available CE marked 4th generation assay.

Diagnostic specifity	Number of tested samples	Specificity, %
Unselected blood donors	12 776	99.70
Pregnant women	364	100.00
Hospitalized patients with non-infectious diseases	273	100.00
Patients with infectious diseases (Hepatitis A, B, C, syphilis, chlamydiosis, herpetic and cytomegalovirus infections)	182	99.45
Patients with rheumatoid factor	182	100.00

Analytical sensitivity

The analytical sensitivity was evaluated with "HIV-1 p24 ANTIGEN 1st International Reference Reagent" (NIBSC Code: 90/636) and defined at 1.0 IU/ml.

Precision

The repeatability within one plate was evaluated by testing 3 positive samples 25 times each. The CV did not exceed 5 %.
The repeatability between different plates was evaluated by testing 3 positive samples 25 times each using 3 different lots. The CV did not exceed 5 %.
The reproducibility between different lots, operators, days, laboratories was evaluated by testing 3 positive samples 25 times each using 3 different lots. The CV did not exceed 5 %.

Limitations of test

- a sample should not be defined as positive for anti-HIV-1,2 or antigen p24 HIV-1 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 HIV virus doesn't allow to exclude the possibility of false negative results. No known test method can offer complete assurance that the HIV virus is absent.

References

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3. Andre Tehe. Quantification of HIV-1 by a highly improved ELISA: An alternative to HIV-1 RNA based treatment monitoring in patients from Abidjan, Cote d' Ivoire. Journal of Clinical Virology 37: 199-205, 2006.
4. Ulf-Hakan Stenman. Immunoassay Standardization: Is It Possible, Who Is responsible, Who Is Capable. Clinical Chemistry 47: 815-820, 2001.
5. G. Stevens. Evaluation of Two Commercially Available, Inexpensive Alternative Assay Used for Assessing Viral Load in a Cohort of Human Immunodeficiency Virus Type 1 Subtype C-Infected Patients from South Africa. Journal of Clinical Microbiology 43: 857-861, 2005.
6. Feredoun Mahboudi. A serological screening assay of human immunodeficiency virus type 1 antibodies based on recombinant protein p24-gp41 as a fusion protein expressed in Escherichia coli. Journal of Biotechnology 125: 295-303, 2006.

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.

Danger!

TMB

H317	May cause an allergic skin reaction.
H360D	May damage the unborn child.
P201	Obtain special instructions before use.
P281	Use personal protective equipment as required.
P308 + P313	IF exposed or concerned: Get medical advice/attention.

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