



HIV p24 Ag ELISA TEST SYSTEM

INTENDED USE

The Reactiva Search's HIV p24 Ag is an enzyme immunoassay for the detection of p24 antigen of Human Immunodeficiency Virus free or antibody-complexed in human serum or plasma samples. The assay is intended for the diagnosis and the monitoring of HIV infection.

SUMMARY AND EXPLANATION

The HIV is a member of Retroviridae family, Lentivirus genus. It is an enveloped virus with a nucleocapsid containing two molecules of RNA. The RNA template is retrotranscribed to cDNA and integrated into the host genome. The env, pol and gag genes codify for the three major types of structural proteins: gp120 and gp41 envelope proteins, p24 capsid protein, p17 matrix protein and an enzymatic protein for transcription of RNA (reverse transcriptase). Two HIV genotypes designed HIV-1 and HIV-2 are originally identified in American and African patients with AIDS and AIDS related complex (1, 2). The HIV-1 is subdivided according to the divergence in the structure of env gene in M (major) group with subtypes A to J and in a further cluster of heterogeneous viruses named group O (outside the classic HIV-1) primary appearing in West Africa (3). The HIV infection is transmitted through sexual intercourse, contaminated needle, blood transfusion, maternal/newborn route. The pathogenesis is characterized by progressive depression of immune system with developing of opportunistic infections (virus, fungi, protozoa) and neoplasms such as Kaposi's sarcoma and malignant lymphoma. Serological diagnosis of HIV infection is usually based on the detection of antibodies by enzyme linked immunoadsorbent assay (Elisa).

Since its introduction in 1985, the test have been improved in sensitivity and specificity until the recent introduction of p24 antigenemia detection, which could be the only HIV marker in the window phase of infection (4). The Elisa test, able to detect HIV p24 antigen free or antibodycomplexed, allows to obtain an earlier diagnosis, reducing the Risk of HIV transmission by blood donation and monitoring pharmacological treatment (5-7).

PRINCIPLE OF THE TEST

The HIV p24 Ag ELISA Test System is an assay based on "direct sandwich" Elisa principle. The microplate wells are coated with monoclonal antibody (mab) to p24 antigen. In the first incubation the sample, dispensed into the well, reacts with the solid phase; the p24 antigen free or antibody-complexed, if presents, is captured by the monoclonal antibody to p24. After washing out all the other components of the sample, in the second incubation bound p24 antigen is detected by the addition of an anti-p24 polyclonal antibody labelled with horse radish peroxidase (HRP). The enzyme captured on the solid phase, acting on the Chromogen/Substrate solution, generates an optical signal that may be detected by an Elisa reader.

MATERIALS AND COMPONENTS PROVIDED

- Strip Microplate - Microplate of 8 x 12 strips of breakable wells activated with mab anti-p24. The microplates are sealed in an aluminium pouch in presence of desiccant bag. No. of microplates: 1
- Positive Control – Ready to use. Buffered solution of p24 recombinant protein. It contains 0.02 % gentamicin sulfate and 0.09 % Kathon as preservatives. Volume 1.0 ml
- Negative Control - Ready to use. Buffered solution of human serum base not reactive for antibodies to HIV and for p24 antigen. It contains 0.02 % gentamicin sulfate and 0.09 % Kathon as preservatives, and Coomassie brilliant blue as colouring agent. Volume 2.0 ml
- Washing Solution - To dilute before use. Solution 25x concentrated that contains Imidazole buffer and surfactive agent. Volume 50.0ml
- *Conjugate - To dilute before use. Buffered proteic solution, 20x concentrated, that contains specific PAb anti-p24 labelled with HRP, proteic stabilizers, 0.02 % gentamicin sulfate and 0.09 % Kathon as preservatives. Volume 0.8 ml

- *Conjugate Diluent - Buffered proteic solution for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulphate and 0.09 % Kathon as preservatives and Coomassie brilliant blue as colouring agent.

Volume 16.0 ml

- **Chromogen - To mix with Substrate. Solution of 3, 3', 5, 5' tetramethylbenzidine (TMB), activators and stabilizers, in a phosphate/citrate buffer. Note - Store protected from light. Volume 8.0 ml

- **Substrate - To mix with Chromogen. Solution that contains hydrogen peroxide (H2O2), activators and stabilizers, in a phosphate/citrate buffer. Volume 8.0 ml

- Stop Solution - Solution of 0.3 M sulphuric acid. Note: Handle with care. Volume 13.0 ml

- Cardboard Sealer – Transparent plastic sealer to cover Microplates during the incubation at 37 °C. no. of sealers: 2

- Package insert – The present document.

- Symbol information sheet – List of the symbols.

Note - All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
- Vortex mixer and adsorbent papers.
- Distilled water.
- Timer.
- Incubator set at 37 ± 1 °C (dry or moist heat).
- Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
- Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

STORAGE CONDITIONS

- The kit must be stored at 2 – 8 °C and used before the expire date declared on the external label.
- The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
- The diluted Washing solution, at room temperature, is stable for 1 week.
- The Chromogen/Substrate are stable until the expiration of the kit.
- The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any crosscontamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "Washing Instructions" section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation

step or thereagent preparation.

9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.

10. Samples and materials potentially infective have to be handled with care as they could transmit infection. All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 30 minutes. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.

11. Avoid any contact of liquids with skin and mucous membrane. Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.

12. Some reagents of the kit contain sodium azide which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.

13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.

14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.

15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.

16. The color developed in the last incubation is stable for maximum 1hour in the dark.

17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 °C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters. The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

- **Washing Solution** - The concentrated solution to be diluted 25 x with distilled water before use.

- **Conjugate** - Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. The diluted Conjugate is stable for 1 week at 2 – 8 °C, when stored in a sterile disposable container.

- **Chromogen/Substrate** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. This solution is stable for 4 hours at room temperature protected from light.

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results. In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle. Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances. Any case, potentially infective wastes from microplate washing must be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials have to be discarded according to the law as potentially infective wastes.

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TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

1. Leave the A1 well empty for blanking operations. Distribute 100 µl of Controls and Samples according to the scheme. Incubate the microplate sealed at for 60 minutes at 37 °C.
 2. Peel out the plate sealer and wash the microplate according to instructions. Dilute the quantity of concentrated Conjugate you need.
 3. Add 100 µl of the Conjugate to all the wells, but A1.
 4. Cover the microplate with the plate sealer. Then incubate the microplate sealed for 60 minutes at 37 °C.
 5. Peel out the plate sealer and wash the microplate according to instructions. Prepare the necessary Chromogen/Substrate solution mixing 1 volume of Chromogen with a 1 volume of Substrate.
 6. Add 100 µl of Chromogen/Substrate to all the wells, A1 included.
 7. Incubate the microplate for 20 minutes at room temperature, protected from light.
 8. Block the enzymatic reaction by adding 100 µl Stop Solution to all the wells, A1 included. Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on A1 well.
- Note – Read the microplate within 30 minutes after the dispensing of the Stop Solution.

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

Position	Controls/Samples
A1	Blank
B1+C1+D1	Negative Control
E1	Positive Control
F1.....H12	Samples

Reagents	Blank (A1)	Controls	Samples
Controls	-	100 µl	-
Samples	-	-	100 µl
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle			
Dilute the quantity of concentrated Conjugate you need			
Conjugate	-	100 µl	100 µl
Cover with the sealer and incubate for 60 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle			
Prepare the necessary Chromogen/Substrate solution.			
Chromogen/Substrate	100 µl	100 µl	100 µl
Incubate for 20 minutes at room temperature in the dark			
Stop Solution	100 µl	100 µl	100 µl
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the microplate background, then at 450 nm.			
Note – Read the microplate within 30 minutes after the dispensing of the Stop Solution.			

CALCULATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

$$\text{Cut-off} = \text{NC mean} + 0.100$$

Example of calculation

Negative Control mean 0.030 OD 450 nm

Positive Control 1.900 OD 450 nm

$$\text{Cut-off} = 0.030 + 0.100 = 0.130$$

Sample 1 0.050 negative

Sample 2 1.250 positive

Samples with an OD value lower than the Cut-off are classified as negative for p24 antigen.

Samples with an OD value higher than the Cut-off are classified as positive for p24 antigen.

VALIDITY OF THE ASSAY

The assay is considered valid if:

1. the OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination.
2. after blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.200. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section;
3. the OD 450 nm value of the Positive Control is > 1.000. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure. In case that the above data do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

PERFORMANCE CHARACTERISTICS

1. Sensitivity – The analytical sensitivity was evaluated examining the BBI (Boston Biomedical Inc.) p24 Ag panel. The value obtained was 25 pg/ml.
2. Specificity – The specificity of HIV p24 Ag assay testing 500 samples from unselected blood donors was 99.6% and 100% examining 90 hospitalized patients HIV negative with a licensed reference kit. A total of 70 potentially cross-reactive samples including IgM anti-HDV, IgM anti-toxoplasma, IgM anti-rubella, IgM anti-CMV, samples from multiparous females, autoimmune patients, lipemic, haemolytic and icteric samples, and subjects RF positive have been examined. All samples were negative on HIV p24 Ag (specificity 100%).

Specimen	No. examined	False positive	Specificity
Blood donors sera	500	2	99.6 %
Hospitalized patients sera	90	0	100 %
Potentially crossreactive sera	70	0	100 %

3. Reproducibility – Replicates of HIV p24 Ag negative, low positive and high positive sera have been examined with the same HIV p24 Ag lot and with multiple kit lots on multiple days. The results within and between assays are reported in the table.

Specimen	No. replicates	Intra-assay	
		SD	CV%
Negative	24	0.005	11.9
Low +	24	0.022	5.8
High +	24	0.083	4.7

Specimen	No. replicates	Intra-assay	
		SD	CV%
Negative	3	0.007	15.3
Low +	3	0.059	13.6
High +	3	0.126	6.1

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

The test is for research use only.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended

WASTE MANAGEMENT

Please, refer to local legal requirements.

REFERENCES

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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET066-2