HIV 1+2

ELISA 3ª Generación

Enzyme-linked Immunosorbent Assay (ELISA) for the detection of antibodies anti-HIV-1 and anti-HIV-2 in serum or plasma

SUMMARY

The Human Immunodeficiency Viruses (HIV-1 and HIV-2) are the causative agents of the Acquired Immune Deficiency Syndrome (AIDS). These retroviruses are transmitted by exposure to certain infected body fluids, mainly genital secretions and blood or contaminated products derived from blood and for passage through the placenta.

Serologic evidence of HIV-1 and HIV-2 infection may be obtained confirming the presence of antigens and antibodies in the serum of individuals suspected to have the infection. The antigens may generally be detected in the acute phase and during the symptomatic phase of the disease. The antibodies may be detected throughout the infection, beginning in the acute phase or immediately after it.

The HIV 1+2 ELISA 3ª Generación test is designed to detect antibodies against HIV-1, HIV-1 group O and HIV-2.

PRINCIPLE

The microtitration plate wells are coated with HIV-1 and HIV-2 recombinant antigens. The sample is incubated in the wells. If sample contains antibodies against HIV-1 or HIV-2 they will bind to the antigens present in the wells. The unbound fraction is eliminated by washing. In the next step, is added the conjugate which contains the same antigens than the plate conjugated to peroxidase. They will bind to the antibodies if they were present in the sample. The unbound conjugate is removed by washing.

Then, a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide is added. The reactive samples develop a light blue color that changes to yellow when the reaction is stopped with sulfuric acid.

PROVIDED REAGENTS

Coated microtitration plate: microtitration plate with removable strips and 96 wells, coated with HIV-1 and HIV-2 recombinant antigens.

Conjugate: recombinant antigens bound to peroxidase. Red color.

Substrate: tetramethylbenzidine (TMB) and hydrogen peroxide solution.

Stopper: 2 N sulfuric acid.

Concentrated Wash Buffer: saline buffer with surfactant agent (25x). Green color.

Positive Control: inactivated human serum containing anti-HIV-1 and anti-HIV-2 antibodies. Orange color.

Negative Control: inactivated human serum, non-reactive. Yellow color

NON-PROVIDED REAGENTS

Distilled or deionized water.

NON-PROVIDED REQUIRED MATERIAL

- Micropipettes for measuring stated volumes
- Disposable tips
- Volumetric material for stated dilutions
- Incubator at 37ºC
- Disposable gloves
- Absorbent paper
- Alarm clock or stopwatch
- Sodium hypochlorite
- (Automatic or manual) microtitration plate wash system
- Spectrophotometer for microtitration plate reading

WARNINGS

- The reagents are for “in vitro” diagnostic use.
- All patient samples should be handled as capable of transmitting infection.
- Control sera have been tested and found non-reactive to Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C virus (HCV). However, they should be handled as potentially infectious material.
- All materials used to perform the test should be treated in order to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for 1 hour at 121ºC. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- Do not exchange reagents from different lots.
- Do not use foreign reagents.
- Avoid touching the sides of the wells with the tips.
- Avoid using metallic elements that may be in contact with the reagents.
- Microtitration plates must be incubated in incubator. Do not use water bath. Do not open the incubator during the process.
- Avoid the contact of the microtitration plates with hypochlorite fumes from the biohazard disposal container or other sources, as hypochlorite affects the reaction.
- Avoid contact of the skin and mucous membranes with sulfuric acid (Stopper). H315+H320: Causes skin and eye irritation. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/eye protection/face protection.
- Avoid any spilling of liquids or spraying.

- Do not pipette by mouth. Use disposable gloves and eye protection during handling of the samples and test reagents.
- All reagents and samples must be discarded according to current regulations.

REAGENT PREPARATION

It is important that all the material used for the preparation of reagents is clean and free from detergent and hypochlorite.

Wash Buffer: at low temperature the concentrated reagent components may precipitate. In such case, heat the solution at 37ºC until complete dissolution. To obtain ready to use wash buffer, dilute one part concentrated wash buffer (25x) with 24 parts distilled or deionized water. Example: 20 ml with 480 ml for one microtitration plate.

Coated microtitration plate, Conjugate, Substrate, Stopper, Positive Control and Negative Control: ready to use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable at 2-10ºC until the expiration date stated on the box. Do not freeze.

Concentrated Wash Buffer and Stopper: may be stored at 2-25ºC.

Wash Buffer (1X): once diluted it is stable for up to 3 months at a temperature lower than or equal to 25ºC.

Coated microtitration plate: do not open the pouch before performing the test or until it has reached room temperature, otherwise the surface of the wells can get moistened. Unused well strips should be kept in the pouch with the desiccant, sealed with adhesive tape and stored at 2-10ºC. The strips preserved in these conditions may be used within 4 months as long as the expiration date stated on the package is not exceeded.

SAMPLE

Serum or plasma

a) Collection: obtain the sample in the usual way.

b) Additives: not required for serum. For plasma samples, heparin, citrate or EDTA may be used as an anticoagulant.

c) Known interfering substances: no interferences are observed with bilirubin up to 1500 mg/dl, or hemoglobin up to 270 mg/dl. Samples containing particles should be clarified by centrifugation.

d) Stability and storage instructions: sample should be stored at 2-10ºC. If test is not performed within 72 hours, it should be frozen at -20ºC. Avoid repeated freezing and thawing. This may cause erroneous results. If using frozen samples, they must be homogenized and centrifuged before use.

Heat inactivation can affect the result.

Do not use samples with microbial contamination.

If samples must be transported, they should be packed according to local regulations in force for the shipment of infectious materials.

PROCEDURE

1- Bring reagents and samples to room temperature 30 minutes before initiating the test.

2- Prepare the required diluted wash buffer volume.

3- Place in the strip holder, the number of wells required for the number of determinations to be performed, including two wells for the Positive Control (PC) and 3 for the Negative Control (NC).

4- Dispense the Sample (S) and controls according to the following scheme:

	S	PC	NC
Positive Control	-	50 ul	-
Negative Control	-	-	50 ul
Sample	50 ul	-	-

5- To avoid evaporation, cover the plate with the provided adhesive tape, and incubate for 30 ± 2 minutes at 37ºC ± 1ºC.

6- After incubation, completely discard the liquid from each well. Wash 5 times according to wash instructions (See Washing procedure).

7- Add Conjugate:

Conjugate	100 ul	100 ul	100 ul

8- To avoid evaporation, cover the plate with adhesive tape. Incubate for 30 ± 2 minutes at 37ºC ± 1ºC.

9- Wash 5 times according to wash instructions.

10- Dispense the Substrate transferring to a clean container only the required volume. Do not transfer the remaining Substrate back to the original bottle. Avoid contact of reagent with oxidizing agents.

Substrate	100 ul	100 ul	100 ul

11- Incubate for 30 ± 2 minutes at room temperature (18-25ºC), protecting from light.

12- Add the Stopper:

Stopper	100 ul	100 ul	100 ul

13- Measure the absorbance in spectrophotometer at 450 nm or bichromatically at 450/620-650 nm.

Note: bichromatic reading is recommended. In case the reading is monochromatic, perform a reagent blank that will have to be subtracted from all sample values.

STABILITY OF FINAL REACTION

The reaction color is stable for 10 minutes, thus the results must be read within that period.

WASHING PROCEDURE

Remove the liquid from the wells by aspiration or spilling. The wells are washed with 350 ul diluted wash buffer. When filling the wells make sure not to spill. The wash solution should be in contact with the wells for 30 to 60 seconds. Make sure no residual liquid remains after the final washing step. Perform double aspiration to remove excess buffer. If after such procedure it still persists, invert the plate onto absorbent paper and tap it several times. Otherwise, erroneous results may be obtained.

Note: The washing procedure is crucial for the test result. If excess wash buffer remains in the wells, or if the

wells are not completely filled, erroneous results may be obtained.
Do not let the wells air-dry during the procedure. Automatic washers should be rinsed with distilled or deionized water at the end of the day to avoid obstructions due to the presence of salt in the wash buffer.

SUMMARY OF THE PROCEDURE

STAGE	PROCEDURE	WARNINGS/OBSERVATIONS
Dilution	Prepare Wash solution (1x)	Dissolve salt crystals
Samples	Add 50 ul S, PC and NC	
Incubation	Cover the wells and incubate for 30 ± 2 minutes at 37 ± 1ºC	In incubator
Washing step	Wash each well with 350 ul diluted Wash Buffer (5 times)	Time of contact of the Wash solution from 30 to 60 seconds. Completely remove the residual liquid from the wells
Conjugate	Add 100 ul Conjugate	
Incubation	Cover the wells and incubate for 30 ± 2 minutes at 37 ± 1ºC	In incubator
Washing step	The same washing step as above	
Substrate	Add 100 ul Substrate	Transfer the required Substrate volume to be used. Do not pipette from the original bottle. Discard the remaining reagent. Avoid contact with oxidizing agents.
Incubation	30 ± 2 minutos at 18-25ºC	Mantein the wells protected from light
Stop	Add 100 ul Stopper	
Reading	Measured in spectrophotometer	Read within 10 minutes

ASSAY VALIDATION CRITERIA
The assay is considered valid if the following conditions are simultaneously met:

1. The optical density (OD) average of the Negative Controls should be less than or equal to 0.050.
Example:
Reading 1 = 0.015, Reading 2 = 0.018, Reading 3 = 0.021
Average = (0.015+0.018+0.021) / 3 = 0.018

2. Discard any Negative Control with OD higher than 0.050.

3. If you have removed any Negative Control, recalculate the Negative Control average. A test is valid if at least two Negative Controls are accepted.

4. The OD average of the Positive Controls should be greater than or equal to 1.200.
Example:
Reading 1 = 1.658, Reading 2 = 1.721
Average = (1.658+1.721) / 2 = 1.689

5. The difference between the average OD of Positive and Negative Controls must be greater than or equal to 1.100.

In case one of the above conditions is not met, repeat the assay.
Remember that the readings obtained depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS
a) With optical instrument
The presence or absence of anti-HIV-1 or HIV-2 antibodies is determined by relating the sample absorbance to the Cut-off value.

Cut-off = NC + 0.250

NC: OD average of Negative Control

Example: 0.018 + 0.250 = 0.268

Non-reactive samples: those with absorbance values lower than the Cut-off value.
Reactive samples: those with absorbance values greater than or equal to the Cut-off value.

b) Visual interpretation
If this type of interpretation is chosen, all samples not showing coloration greater than the one observed for the Negative Controls are considered non-reactive. By contrast, a purely yellow sample is considered reactive.

All samples initially reactive should be repeated in duplicate. If one or both replicates are positive, sample must be considered reactive.
An initially reactive sample may be non-reactive in both replicates. This may be due to:

- Cross contamination of a non-reactive well with a reactive sample.
- Sample contamination during dispensation, lack of precision in sample and/or conjugate or substrate dispensation into the well
- Tip reutilization.
- Well contamination with hypochlorite or other oxidizing agents.

In certain cases non-reactive samples may produce falsely reactive reactions, both in the initial analysis and in the replicates. Some probable causes of this effect may be:
- Sample contamination during collection, processing or storage.
- Presence of interfering substances, such as autoantibodies, drugs, etc.
- Ineffective dispensation and/or aspiration of the wash solution (obstructed system).

PROCEDURE LIMITATIONS
- See Known interfering substances under SAMPLE.
- Do not use pooled samples.
- Do not use other body fluids such as saliva, cerebrospinal fluid or urine.
- A negative result does not exclude the possibility of exposure or HIV-1 or HIV-2 infection. Repeatedly reactive results must be corroborated by a confirmatory method, according to current legislation.
- Do not use heat-inactivated samples as they may cause false positive results.

SPECIFIC PERFORMANCE FEATURES
Clinical Sensitivity
a) Clinical sensitivity with commercial seroconversion panels (BBI, Boston Biomedica, Inc.)

Panel name	Sample collection days	Time (days) in wich sample becomes reactive		
		HIV 1+2 ELISA 3ª Gen.	ELISA HIV 3ª Gen. (BBI)	Western Blot (BBI)
PRB 904-D	0, 21, 49, 92, 99	92	92	92
PRB 912-L	0, 9, 14, 16, 28, 30	0	0	9
PRB 916-P	0, 4, 9, 18, 30, 35	30	30	30
PRB 919-S	0, 9, 11	9	9	9
PRB 924-X	0, 2, 8, 10, 26, 33, 35, 40	33	33	35 (undet)
PRB 925-Y	0, 10, 18, 22, 44, 49	44	44	44 (undet)
PRB 930-AE	0, 3, 7, 10	7	7	10 (undet)
PRB 934 AI	0, 7, 11	7	7	ND
PRB 944-AT	0, 2, 7, 9, 14, 16	14	14	14 (undet)
PRB 945 AU	0, 3, 13, 15, 20	15	13	20
PRB 947-AW	0, 9, 11, 20	9	9	20
PRB 949-AY	0, 6, 9, 18, 20	20	20	20 (undet)
PRB 951-BE	0, 2, 8, 11, 15, 19	19	19	Non-reactive
PRB 952-BB	0, 7, 10, 14, 17, 21	17	14	17
PRB 953-BC	0, 3, 7, 10	10	7	Non-reactive
PRB 966	0, 2, 20, 22, 30, 35, 37, 44, 48, 51	48	48	Non-reactive

Undet: undeterminate

b) Clinical Sensitivity in BBI Performance Panels
In a study on different international commercial panels, we obtained the following results:
PRZ-207 (Anti-HIV-1 / 2 Combo Performance Panel) 14 out of 14 reactive samples were detected.
PRZ-206 (Anti-HIV-1 / 2 Combo Performance Panel) 11 out of 12 reactive samples were detected.

c) Clinical Sensitivity in Panels of reactive anti-HIV samples.
In a study of 123 samples from patients with HIV-1 infection confirmed by different methods, all samples were found reactive with the HIV 1+2 ELISA 3ª Generación kit.

b) Specificity
In a study of 3004 serum and plasma samples from five different health centers, 33 samples were found reactive of which 28 were confirmed by other methods, yielding a specificity of 99.83%.
The possible occurrence of cross-reactivity was studied in 272 samples from individuals with different clinical conditions that might be causing nonspecific reactions in the HIV 1+2 ELISA 3ª Generación test. This group includes samples:
- With anti-HAV, HBV, EBV, CMV, HSV, VZV, HCV, HTLV and other viruses
- With antibodies against *Treponema pallidum, Mycoplasma pneumoniae, Toxoplasma gondii, Toxocara canis, Trypanosoma cruzi* and other microorganisms.
- With different autoantibodies (AGA, AMA, ATA, FAN, rheumatoid factor and others)

The specificity in this population was 97.42%.
Another study of 91 plasma samples from pregnant women showed a specificity of 98.9%.

c) Precision
The test accuracy was evaluated following the EP5A protocol recommended by the NCCLS. The tests were performed with samples of different reactivity levels and with controls. Two daily tests were performed to evaluate each sample in duplicate during 20 days.

Sample	Mean (O.D.)	Intra-assay		Inter-assay	
		S.D.	C.V.	S.D.	C.V.
S1	0,354	0,026	7,34%	0,037	10,45%
S2	0,790	0,073	9,24%	0,107	13,54%
C (+)	1,273	0,056	4,40%	0,096	7,54%
C (-)	0,014	0,002	15,22%	0,003	23,19%

n= 80

WIENER LAB PROVIDES

- 96 tests (Cat. Nº 1723096)
- 192 tests (Cat. Nº 1723192)

REFERENCES

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SYMBOLS EXPLANATION

Policubeta | Sensib.
Coated microtitration plate

Buf. Lavado | Conc.
Concentrated Wash Buffer

Conjugado
Conjugate

Control | +
Positive Control

Revelador
Substrate

Control | -
Negative Control

Stopper
Stopper

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP Authorized representative in the European Community

IVD "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL − Negative Control

REF Catalog number

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