DIA (Dot Immuno Assay)*

HIV 1+2

Dot Immunoassay for the detection of antibodies to the Human Immunodeficiency Viruses (HIV-1, HIV-1 group O and HIV-2)

SUMMARY

The Human Immunodeficiency Viruses (HIV-1 and HIV-2) that cause AIDS, are mainly transmitted by sexual contact, exposure to blood or contaminated products derived from blood. In individuals infected with these viruses, antibodies appear as a response from the immunological system to the viral infection. These antibodies neither protect nor grant immunity; yet since they are early markers of the infection, their detection constitutes the basis for the screening of the disease. This kit has been developed to detect anti-HIV-1, anti-HIV-1 group O and anti-HIV-2 antibodies.

PRINCIPLE

The diluted sample is placed in contact with the comb-shaped solid support, which has immobilized HIV-1 and HIV-2 transmembrane synthetic peptides. If the sample has antibodies anti-HIV-1, anti-HIV-1 group O or anti-HIV-2 an immune complex will be formed and it will be bound to the support. After a wash, the support is incubated with a protein A-colloidal gold conjugate (Signal Reagent). Protein A is bound to the Fc fragment of the antibodies. The presence of a reddish dot in the place where the synthetic peptides are immobilized, indicates the formation of the immunocomplex, thus, indicating the presence in the sample of antibodies anti-HIV-1, anti-HIV-1 group O and anti-HIV-2.

PROVIDED REAGENTS

Antigen: comb shaped solid support with immobilized HIV-1 and HIV-2 transmembrane antigens synthetic peptides on each "tooth" (dull side of the comb).
Sample Diluent: saline solution with non-ionic surfactant, pH 7.3.
Concentrated Wash Buffer: 1.4 mol/l sodium chloride in 100 mmol/l phosphate buffer and 0.1 g/l non-ionic surfactant.
Signal Reagent: Staphylococcus aureus A protein with gold colloidal.
Positive Control: dilution of inactivated serum, reactive for anti-HIV antibodies.
Negative Control: dilution of inactivated serum, non reactive.

INSTRUCTIONS FOR USE

Antigen: ready to use. When opening the foil pouch be careful not to cut the combs or the pouch with desiccant.
Sample Diluent: ready to use.
Wash Buffer: at low temperature the reagent component may precipitate. If this happens, place in water bath at 37°C for a few minutes, then mix by inversion. Dilute 1+7 with distilled water (1 part of Concentrated Wash Buffer + 7 parts of distilled water). Take into account that when using this technique the Wash buffer is prepared only once and is used for the two washings. Then, the solution should be discarded, previously adding 5 ml sodium hypochlorite at 5%.
Signal Reagent: ready to use.
Positive Control and Negative Control: ready to use.

WARNINGS

- All samples from patients should be handled as capable of transmitting infection. Controls are inactivated; however they should be handled as if they were infectious materials.
- The Control sera have been tested and found to be non-reactive to HBsAg.
- Dispose of all materials used to perform the test in order to ensure inactivation of pathogenic agents. The recommended method of disposal is autoclaving at 121°C for 1 hour. Liquid waste can be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- If not all the microtitration plate wells are used, discard the liquid from the used wells in a biohazard disposal container having 5% sodium hypochlorite. Then tap the microtitration plate several times on absorbent paper, which will be discarded as biohazardous waste, and wash it with a 50% alcohol solution. Dry the plate and seal the used wells with adhesive tape to prevent re-utilization.
- Do not exchange reagents from different kits with one another.
- Do not use reagents from other sources.
- Reagents are for “in vitro” diagnostic use.
- Wash buffer contains sodium azide.

STABILITY AND STORAGE INSTRUCTIONS

The Provided Reagents are stable in refrigerator (2-10°C) until the expiration date shown on the box.
Wash buffer: prepare at the time of use.
Antigen: the combs with immobilized antigen are provided sealed and with desiccant. Do not open the foil pouch until ready to use and not before they have reached room temperature, to prevent the moistening of the content. The unused combs should be kept in the foil with desiccant, sealed with adhesive tape and at 2-10°C. The combs kept under these conditions may be used within the next 2 months, provided that they do not elapse the kit's expiration date.

SAMPLE

Serum or plasma
a) Collection: obtain the sample in the usual way.
b) Additives: use any common anticoagulant of common

use in case plasma is used as sample.
c) Known Interfering substances: hemolysis may cause erroneous results.
d) Stability and Storage Instructions: undiluted samples can be kept at room temperature no longer than 4 hours and up to 7 days in refrigerator (2-10ºC). To preserve them for longer periods they should be frozen at -20ºC or less. The samples that have been frozen and thawed repeatedly may produce false positive and false negative results.
If samples should be moved, pack them according to the legal specifications concerning the shipment of infectious materials.

REQUIRED MATERIAL

1- Provided

- microtitration plates
- container for washing

2- Non-provided

- 100 ul micropipette
- timer or stopwatch
- adequate volumetric material

ASSAY CONDITIONS

- Total reaction time: 20 minutes
- Reaction temperature: room temperature (higher than 22ºC). If room temperature is lower than 22ºC, perform reaction in incubator at 37ºC.
- Sample volume: 100 ul.

PROCEDURE

Bring the reagents and the samples to room temperature before testing. Once the test has begun, it should be completed without interruption. Process 1 Positive Control (in the right end of the comb tooth) and 1 Negative Control (in the left end of the comb´s tooth) simultaneously. Then proceed as follows:

Material Preparation:

Place 2 drops of Sample Diluent in each well of the sample row to be used. Place 100 ul of Sample or Controls in each of these wells. Prevent contamination of the adjacent wells by placing the sample or controls on the bottom of the wells. Gently mix the sample and diluent using the micropipette several times, to achieve correct homogenization. In the following row place 3 drops of Signal Reagent in each well to be used.
Prepare the Wash Buffer according to the INSTRUCTIONS FOR USE. Place the Wash Buffer in the wash tray supplied, trying not to make foam. The teeth should be completely immersed.

Performing the test:

Open the foil carefully and remove the necessary combs to perform the test. Preserve the remaining combs as indicated under STABILITY AND STORAGE INSTRUCTIONS. Write the ID# of each sample on the corresponding tooth of the comb.

Note: Do not touch the comb reactive zones because this may lead to erroneous results.
Place the comb into the plate wells containing the samples and controls dilutions and incubate for 10 minutes at room temperature.
Remove the comb from the wells and carefully drain off any excess sample, avoiding to touch the paper with the reactive teeth.
Holding the comb in a vertical position with the teeth downwards and the reactive zone facing the operator, place it in the Wash Buffer. Gently move the comb ten times through the wash solution back and forth not touching the sides of the tray. Drain off again the tips of the teeth to an absorbent paper. Place the comb in the wells with Signal Reagent and incubate at room temperature for 10 minutes. Keep in mind that the positive reaction is intensified if shaked during reagent incubation, for instance in a Kline shaker. After incubation, repeat washing as indicated above, using the same Wash Buffer as before.
Place the comb on a clean surface with the reactive zones upwards and let dry completely before reading the results. The dot color intensifies when it dies. Read the results under natural or fluorescent light. Do not use incandescent lamp.

STABILITY OF FINAL REACTION

Reaction color is stable indefinitely, therefore the comb may be kept for future references of results.

RUN VALIDATION CRITERIA

The run is valid if the following conditions are accomplished simultaneously:
a) No color appears on the tooth corresponding to Negative Control.
b) The Positive Control is clearly detectable.
If any of these conditions are not accomplished, repeat the run using a new kit of reagents.

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
The kit is designed to be used with undiluted samples. Therefore, diluted samples should not be use to perform the assay.
he presence of anti-HIV antibodies in serum should not be considered a diagnosis of AIDS. Repeatedly reactive results should be confirmed by reference methods such as Western Blot.
A negative result does not preclude the possibility of exposure to or infection with HIV.
False positives may be obtained in the following situations: autoimmune diseases, tuberculosis, systemic lupus erythematosus, pregnancy, hepatitis B vaccination and other immunizations, hemodialysis, hepatic disease and other disorders.

INTERPRETATION OF RESULTS

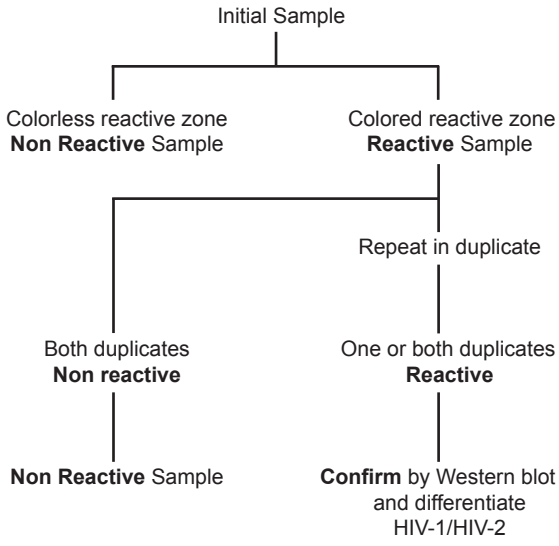
Once the test has been performed and when the comb is completely dry, check the results against a white surface, at eye level under good light.

Reactive sample: the appearance of a colored dot (pink or light red) in the reactive zone of the comb. When this dot is present, even if the color is less intense than the Positive Control, it indicates that the sample is reactive.

Non reactive sample: no color is observed in the reactive zone of the comb.

All initially reactive tests should be retested in duplicate. If either or both duplicates are Reactive, it is assumed that the sample contains antibodies to anti-HIV-1 or anti-HIV-2.
If both duplicates are Non reactive the first result is considered a false positive, assuming that the sample does not contain antibodies to anti-HIV-1 nor HIV-2.
All reactive sample should be tested again using a reference method and differentiating between HIV-1/HIV-2.

INTERPRETATION SCHEME



PERFORMANCE

- In a study directed by the Global Program on AIDS (GPA) belonging to the World Health Organization (WHO) a panel of 600 human sera and 8 panels of seroconversion were evaluated. The obtained data were compared with Western Blot as a reference method, finding a sensitivity of 100%, a specificity of 99.7% and a reproducibility of 99.4%.
- In a study performed on 425 samples of patients from the "Instituto de Infectología Emilio Ribas, São Paulo", was obtained a sensitivity of 99.5%, a specificity of 96.4% and an efficiency of 97.8%.
- In a study performed on two HIV-1 group O samples, B7-2705-0001 and JP8-2707-0001, belonging from Boston Biomedica Inc., were both detected.

WIENER LAB PROVIDES

Kit for 48 assays (Cat. Nº 1723201).

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SYMBOLS EXPLANATION

Antígeno Antigen	**Diluyente** | **Muestra** Sample Diluent
Revelador Substrate	**Buf. Lavado** | **Conc.** Concentrated Wash Buffer
Control | **+** Positive Control	**Control** | **-** Negative Control

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

CE 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:

Xn Harmful

Corrosive / Caustic

Xi Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL − Negative Control

REF Catalog number

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