
ANTIBODY TO THE HUMAN IMMUNODEFICIENCY VIRUS

(anti-HIV) (1 + 2) ELISA KIT

Catalog No: E0321

INTENDED USE

The Autobio HIV assay is designed for the qualitative determination of the concentration of antibodies to the human immunodeficiency virus (anti-HIV) (1+2) in human serum or human plasma.

INTRODUCTION

HIV-1 is one of the causes of AIDS (Acquired Immunodeficiency Syndrome). AIDS is the end stage of a drawn out process in which the immune system of an infected person and its ability to control infections or malignant proliferative disorders are progressively destroyed[1]. HIV is mainly transmitted through unprotected sexual intercourse or from mother to child[1]. Most frequently, HIV infection is diagnosed by tests that assess whether an individual's immune system has produced an HIV-specific immune response (antibodies to HIV)[1].

In the USA the standard laboratory test algorithm (set of different tests) may take 48 hours to one week before results may be made available. This algorithm consists of screening with an enzyme immunoassay (EIA) followed by confirmation by Western Blot (WB) or immuno-fluorescent (IFA) methods.

During the last 20 years, HIV infection and severe HIV-related diseases (e.g., AIDS) have become a leading cause of illness and death in the United States. Approximately 800,000-900,000 persons in the United States are infected with HIV and approximately 275,000 of these persons might not know they are infected[2].

Approximately 25 million persons each year in the United States are tested for HIV. Publicly funded counseling and testing programs conduct approximately 2.5 million of these tests each year. In 1995, 25% of these individuals tested HIV positive and 33% of persons tested HIV negative at publicly funded clinics did not return for their test results. Rapid tests to detect HIV antibody can be performed within 20 minutes, enabling healthcare providers to supply definitive negative and preliminary positive results to patients at the time of testing, potentially increasing the overall effectiveness of counseling and testing programs. In comparison, results from enzyme immunoassays (EIAs) currently used for HIV screening often are not available until after 1-2 weeks[3]. Using rapid tests, during 1995, a total of 697,495 more persons would have learned their HIV status[3].

Many advances have been made in HIV/AIDS prevention and treatment, including the development of effective antiretroviral therapies that have reduced HIV-related illness and death. Early knowledge of HIV infection is now recognized as a critical component in controlling the spread of HIV infection[2]. Rapid HIV testing allows clients to receive results the same day in a single visit, which is useful in urgent medical circumstances and settings where clients tend not to return for HIV test results (e.g., some STD clinics)[2]. Advances in these areas have resulted in revised recommendations for HIV screening of pregnant women[4,5] treating opportunistic infections and other sexually transmitted and blood-borne diseases and managing occupational and non-occupational exposures and prophylaxis[6].

PRINCIPLE OF THE TEST

This HIV-1,2 Antibody enzyme linked immunosorbent assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with synthetic HIV polypeptides and recombinant HIV proteins which correspond to highly antigenic epitopes consisting of essential sequences derived from both the envelope and core proteins of HIV-1 and HIV-2. Samples or controls are added to the microtiter plate wells and incubated. HIV specific antibodies, if present, will bind to and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound HIV antibodies and other components of the sample. A standardized preparation of horseradish peroxidase (HRP)-conjugated synthetic HIV polypeptides and recombinant HIV proteins is added to each well to "sandwich" the HIV antibody immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugate and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added

to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain HIV antibody and enzyme-conjugate will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. Samples with absorbance greater than or equal to the Cut-off Value are considered reactive by the criteria of this HIV-1,2 Antibody ELISA Kit.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: microplate with HIV-1, HIV-2 polypeptides and HIV recombinant proteins coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: horseradish peroxidase (HRP) labelled HIV-1 & HIV-2 polypeptides and HIV recombinant proteins in Stabilizing Buffer (1 vial, 7.5ml)
3. Negative Control: inactivated healthy human serum diluted in sample diluent (1 vial, 1.0ml)
4. Positive Control: inactivated HIV-1 & HIV-2 antibody positive human serum diluted in sample diluent (1 vial, 1.0ml)
5. Wash Solution Concentrate: PBS-Tween (1 bottle, 50ml, 20X)
6. Substrate Solution: Hydrogen peroxide (1 vial, 7.5 ml)
7. Chromogen Solution: Tetramethylbenzidine (1 vial, 7.5 ml)
8. Stop Solution (1.0M H₂SO₄) (1 vial, 7.5 ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit:

1. Distilled water
2. Precision pipettes for delivery of 20-200µl, 100-1000µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Microplate ELISA reader with a bandwidth of 10nm or less and an absorbance range of 0-3.5 or greater at 450nm wavelength
4. Magnetic stirrer
5. Vortex Mixer or equivalent
6. Washer for microplates
7. Quality control specimens
8. Incubator
9. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8°C upon receipt and the microplate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable for at least two months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Plasma samples may be used for this assay but serum is the recommended sample type for this assay.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and may be stored up to 48 hours at 2-8°C. For longer storage, freeze the specimens at -20°C and avoid multiple freeze-thaw cycles. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The control sera contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HCV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the control sera and components containing animal substances should still be treated as potentially infectious.

4. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4 , in case of contact, wash thoroughly with water.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Adjust the incubator to 37°C.
3. Prepare Wash Solution: add 50ml of Wash Solution Concentrate to 950ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for two months.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Write down the relative numbers of specimens and wells on the data sheet. Leave 1 well for the blank, 5 additional wells for the controls and 1 well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve one well for blank, add the Negative Control to 3 wells (50µl each), the Positive Control to two wells (50µl each) and 50µl of each sample to each of the other wells.
Notes: use an individual tip for each pipetting to avoid cross contaminations.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate the plate at 37°C for 30 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 6 times with wash solution.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Add 50µl of Enzyme Conjugate Reagent to each well (except the blank well). Mix it gently by swirling the microtiter plate on flat bench.
10. Incubate the plate at 37°C for 30 minutes.
11. Repeat steps 6 to 8.
12. Dispense 50µl of Chromogen Solution into each well.
13. Dispense 50µl of Substrate Solution into each well. Gently mix for 15 seconds.
14. Incubate the plate at +37°C in the dark for 10 minutes without shaking.
15. Stop the reaction by adding 50µl of Stop Solution to each well.
16. Gently mix for 15 seconds. It is very important to make sure that the blue color changes to yellow completely.
17. Read the absorbance at 450nm with a Microplate ELISA reader within 20 minutes.
18. Record the absorbance values on a data sheet, including the kit's lot number, date, operator and any notes about the run. If a printed copy of the absorbance reading is available, it should be attached to the data sheet.

CALCULATION OF RESULTS

Test is valid if:

1. Mean absorbance of negative control is lower than 0.10.
2. Mean absorbance of positive control is equal to or higher than 0.70.
3. Cut-off value = $0.1 + NCx$ (Mean absorbance of Negative Control)
If the absorbance value of a negative control is less than 0.05, it should be recorded as 0.05. If it is more than 0.05, it should be recorded as the actual absorbance value measured.

EXAMPLE OF RESULTS

Example: NCx = 0.032

Cut-off value = 0.1 + 0.050 = 0.150

Any sample, of which the absorbance is equal to or higher than the Cut-off value, is considered positive for HIV. Any sample, of which the absorbance is lower than the calculated Cut-off value, is considered negative for HIV.

PERFORMANCE

A. Sensitivity and Specificity

The evaluation of this kit

The serum wheel contains 500 serum/plasma specimens obtained from (1) 55 specimens from reference laboratories including 30 positive specimens (of which 5 are HIV-2 positive) and 25 negative specimens. (2) 46 national references of anti-HIV (batch code 20061201, purchased from China's national institute for the control of pharmaceutical and biological products) including 20 positive references (of which 2 are HIV-2 positive), 20 negative references and 6 sensitivity references. (3) 399 clinical specimens which are collected by reference laboratories from drug takers and blood donors.

The calculation of the sensitivity, specificity and efficiency

sensitivity = positive/(positive + false negative) × 100%

specificity = negative/(negative + false positive) × 100%

efficiency = (positive + negative)/(positive + false positive + negative + false negative) × 100%

Item	Positive	False Positive	Negative	False Negative	Sensitivity (%)	Specificity (%)	Efficiency (%)
Result	104	1	393	0	100	99.75	99.80

Report from the National Evaluation of Antibody to HIV Diagnostic Products 2007

B. Precision

a. Intra-assay Precision

Intra-assay Precision was determined by assaying each of the 2 control sera in 20 replicates.

Serum	Number	Mean	SD	RSD (%)
High	20	1.105	0.051	4.8%
Low	20	0.354	0.018	5.6%

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of 2 serum pools in 20 separate runs, using a standard curve constructed for each run.

Serum	Number	Mean	SD	RSD (%)
High	20	1.112	0.065	5.9%
Low	20	0.367	0.029	8.6%

LIMITATIONS

The Assay Procedure and the Interpretation of Results sections must be closely adhered to when testing the serum or plasma of individual subjects for the presence of antibodies to HIV. This HIV-1,2 ELISA was designed to test individual units of serum or plasma, thus most data regarding its interpretation was derived from testing individual samples. There is insufficient data to interpret tests performed on other body specimens, pooled blood or processed plasma, and products made from such pools. Testing of such samples is not recommended.

This HIV-1,2 ELISA detects antibodies to HIV-1 and/or HIV-2 in serum and plasma. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV. For most uses it is recommended that repeatedly reactive samples be investigated by supplemental tests. It has been suggested that repeatedly reactive specimens obtained from populations at increased risk and at low risk of HIV infection are more likely to demonstrate the presence of HIV antibodies with supplemental testing, such as Western Blot, immunofluorescence or radioimmuno-precipitation. Reactivity at or only slightly above the cutoff-value is more frequently non-specific, especially from persons at low risk of HIV infection. A person who has antibodies to HIV is presumed to be infected with the virus and appropriate counselling and medical evaluation should be offered. Such evaluation should be considered an important part of HIV antibody testing and should include test result confirmation on a freshly drawn sample.






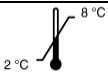


AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically by a physician. ELISA testing alone cannot be used to diagnose AIDS, even if the recommended investigation

of reactive samples suggests a high probability that the antibody to HIV is present. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV. The risk of an asymptomatic person with a repeatedly reactive serum developing AIDS or an AIDS-related condition is not known.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER
	CONSULT INSTRUCTION FOR USE

REFERENCES:

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2. CDC. Revised Guidelines for HIV Counseling, Testing and Referral and Revised Recommendations for HIV screening of Pregnant Women. MMWR 2001;50(19);32-35.
3. CDC. Update: HIV Counseling and Testing using Rapid Tests-United States, 1995. MMWR 1998;47(11).
4. Dabis et al., 6-month efficacy, tolerance and acceptability of a short regimen of oral zidovudine to reduce vertical transmission of HIV in breastfed children in Cote d'Ivoire and Burkina Faso: a double-blind placebo controlled multicentre trial. Lancet 1999;353:786-92
5. Mofenson et al. Advances and research directions in the prevention of mother-to-child HIV -1 transmission. Lancet 2000;355;2237-44
6. Correspondence, Lancet 2000;355:9214

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