ATLAS ANTI-HIV(1+2) ELISA

(Double-antigen Sandwich)

INTENDED USE
ATLAS ANTI-HIV(1+2) ELISA is an enzyme-linked immunosorbent assay for the qualitative determination of antibodies to human immunodeficiency virus (HIV) in serum or plasma. It is indicated for screening blood donors and for aid in the diagnosis of clinical conditions related to infection with HIV-1 and/or HIV-2, e.g. acquired immunodeficiency syndrome (AIDS).

INTRODUCTION
The human immunodeficiency viruses type 1 and type 2 are etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions. HIV has been isolated from patients with AIDS, AIDS related complex (ARC) and from healthy individuals at high risk for AIDS. Infection with HIV is followed by an acute illness that has flu-like characteristics. This phase may remain unnoticed and the relationship to HIV infection may not be clear in many cases. The acute phase is typically followed by an asymptomatic carrier state, which progresses to clinical AIDS in about 50% of infected individuals within 10 years after seroconversion.

Serological evidence of HIV infection may be obtained by testing for HIV antigens or antibodies in serum of individuals suspected of HIV infection. Antigen can generally be detected during the acute phase and during the symptomatic phase of AIDS only. Antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the total infection period, starting at or shortly after the acute phase and lasting till the end stage of AIDS. Therefore the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection.

Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Therefore all donations of blood or plasma should be tested for the risk of HIV transmission. This can effectively be achieved by testing for antibodies to HIV-1 and HIV-2 using a highly sensitive ELISA.

PRINCIPLE OF THE PROCEDURE
ATLAS anti-HIV (1+2) Elisa is based on a one-step“Sandwich”Principle. A mixture of HIV-antigens coupled to horseradish peroxidase (HRP) serves as the conjugate while tetramethylbenzidine (TMB) and peroxide as the substrate. Upon completion of the test, the development of color suggests the presence of antibodies to HIV-1 and/or HIV-2, while no or low color development suggests the absence of antibodies to HIV-1 and HIV-2.

Specifically, microwells are coated with a mixture of HIV-antigens: recombinant HIV-1 p24, HIV-1gp41, gp120 and recombinant HIV-2 gp-36. The antigens coated in each microwell contain the same epitopes as the HRP-labeled conjugate.

With the presence of antibodies to HIV-1 and/or HIV-2 a solid phase antigen/anti-HIV/enzyme labeled antigen complex is formed. Following a wash procedure and incubation with TMB, color develops which turns yellow when the reaction is stopped with sulfuric acid. If anti-HIV-1 and/or anti-HIV-2 is present in the sample, an intense color develops. However, when the sample is free of anti-HIV, no or low color forms with the addition of substrate.

REAGENTS PROVIDED IN THE KIT

For in vitro diagnostic use only.

Store all reagents not required at 2~8 .

All reagents beyond the indicated Expired Date should not be used.

1. Microwell plate 1 plate
   Blank, white or yellowish microwell plate.
   12×8 wells per plate. Each well contains recombinant antigens to HIV.
   The microwell strips can be broken to be used separately. Place unused wells in the plastic sealable Storage bag provided and return to 2~8 .
2. Negative control Serum 1 vial
   Yellowish liquid filled in vial with green screw cap.
   Normal human serum nonreactive for anti-HIV. Preservatives: 0.1% ProClin 300.
   Ready to use as supplied.
3. Positive control Serum-1 1 vial
   Red color liquid filled in vial with red screw cap
   Normal human serum containing monoclonal anti-HIV 1. Preservatives: 0.1% ProClin 300.
   Ready to use as supplied.
4. Positive control Serum-2 1 vial
   Red color liquid filled in vial with yellow screw cap
   Normal human serum containing monoclonal anti-HIV 2. Preservatives: 0.1% ProClin 300.
   Ready to use as supplied.
5. HRP-Conjugate Reagent 1 bottle
   Red liquid filled in white vial with red screw cap.
   Horseradish peroxidase-conjugated recombinant HIV antigens.
   Ready to use as supplied.
6. Stock wash buffer 1 bottle
   Colorless liquid.
   50ml per bottle.
   PH 7.2 10 × PBS.
   The concentration must be diluted with distilled/deionized water before use.
7. Chromogen solution A 1 bottle
   Colorless liquid filled in white vial with green screw cap.
   Urea peroxide solution.
   Ready to use as supplied.
8. Chromogen solution B 1 bottle
   Colorless liquid filled in the black bottle with black screw cap.

TMB solution. Tetramethylbenzidine in citric acid.
Ready to use as supplied.

9. Stop solution 1 bottle
Colorless liquid filled in white vial with yellow screw cap.
Dilluted sulfuric acid solution (2.0M H_2SO_4).
Ready to use as supplied.

9. Plastic sealable bag 1 unit
For enclosing the strips not in use.

10. Plate sealer (disposable) 3 sheets
Clear, papery adhesive sheet to be used to the reactive microplate wells during each incubation to prevent evaporation or contamination of the wells.

ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves.
3. Timer.
4. Appropriate waste containers for potentially contaminated materials.
5. Disposable V-shaped troughs.
6. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
7. Absorbent tissue or clean towel.
8. Dry incubator or water bath, 37±0.5 .
9. Microshaker for dissolving and mixing conjugate with samples.
10. Microwell reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
11. Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

1. Collection: Serum or plasma samples may be used. Blood collected by venipuncture should be allowed to clot naturally. Care should be taken to ensure that the serum samples are fully clotted. Any visible particulate matter in the sample should be removed by centrifugation. Plasma samples collected into EDTA, sodium citrate or heparin may be tested.
2. Storage: Store samples at 2-8 . Samples not required for assay within 3 days should be stored frozen(-20 or lower). Avoid multiple freeze-thaw cycles.

ASSAY PROCEDURE

Step1 Reagents preparation Allow the reagents to reach room temperature **(18-25°C)** Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer 1:19 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls **(e.g.B1, C1, Dl),** four Positive controls (two for HIV1 and two for HIV2 controls- **e.g. El, Fl, G1, H1**) and one Blank (**Al**, Neither samples nor HRP-Conjugate should be added into the Blank well). Use only number of strips required.

Step3 Adding Samples: Add **100** μl of Positive controls, Negative controls, and Specimen into their respective wells **Note: Use a separate disposable pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.**

Step4 Incubating: Cover the plate with the plate cover and incubate for **30 minutes at 37°C.** It is recommended to use water tank to assure the temperature stability and humidity during incubation. If dry incubator is used, do not open the door frequently.

Step5 Washing: After the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the strips plate down onto blotting paper or clean towel, and tap the plate to remove any remainders.

Step6 Adding HRP-Conjugate: Add **100 μl** HRP- Conjugate
to each well except in the Blank.

Step7 Incubating: Cover the plate with the plate sealer and
incubate for **30 minutes** at 37°C.

Step8 Washing: After the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer as in **Step5.**

Step9 Coloring: Dispense 50μl of Chromogen A and 50μl Chromogen B solution into each well including the **Blank,** cover the plate with plate cover and mix by tapping the plate gently. Incubate the plate at 37°C for **15 minutes avoiding** light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HIV 1/2 Positive sample wells.

Step10 Stopping Reaction: Remove and discard the plate cover. Using a multichannel pipette or manually add 50 μl Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HIV 1/2 Positive sample wells.

Step11 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at **450nm.** If a dual filter instrument is used, set the reference wavelength at **630nm.** Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction)

Note:

1. Do not touch the bottom exterior surface of the wells. Fingerprints or scratches may interfere with microwell reading.
2. Shake the reagents gently before use.
3. The prepared diluted wash buffer should be stored at room temperature less than 15 days. It is necessary to store it at 2~8 for longer time and note the date of producing and expiring.

4. Calibrate the pipette frequently to assure the accuracy. Use a separate disposal pipette tip for each specimen and different reagents in order to avoid cross-contamination.
5. It's necessary to assure the temperature is 37 during incubation. It is suggested to use water tank and the microwell plate should float on water. If using dry box, do not open the door frequently.
6. Avoid touching bleaching powder or disinfector in the procedure, it will affect the result of the assay.

INTERPRETATION OF RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

Normally, the individual absorbance value of negative control is lower than or equal 0.10, while of positive control is higher than or equal 0.80. The values of the negative control will be used for Cut-off calculation and negative / positive interpretation. The values of the positive control will show whether it is a successful assay. If either value of the positive controls lower than 0.80, the run is invalid and should be retested.

1. Calculation of cut-off value
   $\overline{Nc}$ = the mean absorbance value for three negative controls.
   Cut-off value (C.O.) = $\overline{Nc}$ + 0.12
2. Interpretations
   S = the absorbance of the specimen
   Positive: S/C.O. ≥1
   Negative: S/C.O. < 1

Note: Specimens showing a reactive result should be retested in duplicate. The specimens which are not repeatably reactive in duplicate testing should be considered as negative. All repeatably reactive results should be confirmed with an appropriate method.

Example :

1. calculation of $\overline{Nc}$:

Well No.	Absorbance
C5	0.032
D5	0.031
E5	0.027
Total	0.090

$\overline{Nc}$ = 0.090 / 3 = 0.030

2. calculation of Cut-off (C.O.)
   C.O. = 0.030 + 0.12 = 0.150

3. Some data from Lab-testing:

Positive control	Negative sample 1	Negative sample 2	Negative sample 3	Weak positive 1	Weak positive 2	Strong positive 1	Strong positive 2
2.465	0.028	0.008	0.042	0.543	0.845	1.864	2.514

SAFETY PRECAUTIONS

1. Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
2. Do not pipette solutions by mouth.
3. Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands throughly at the end of each assay.
4. All samples, biological reagents used in the assay should be considered as potientially infectious materials. They should therefore be disposed off in accordance with established safety procedures.
5. The stop solution (2M H_2SO_4) is a strong acid. Wipe up spills immediately.

STORAGE INSTRUCTION

1. Store at **2-8** .
2. Valid for 12 months from the date of manufacturing.

REFERENCES

1. Barbe, F.et al., (1994) Early detection of anti bodies to HIV-1 by a third generation enzyme immunoassay. Ann. Biol. Clin. (Paris), 52: 341-345
2. Barre-Sinoussi, F et al., (1984) Islolation of a T-lymphotropic retrovirus from a patient at risk for acquired immuno-dificiency syndrome (AIDS), Science, 220: 868-871
3. Clave, F. et al. (1991) Solution conformation preferences of immunogenic peptides derived from the principal neutralization determination of the HIV-1 envolope glycoprotein gp120. Biochemistry. 30: 9187-9194
4. Constantine,. N., T. et al., (1993) Serologic test for the retroviruses: approaching a dacade ofevolution. AIDS, 7: 1-13
5. Gnann JW et al. (1987) Science; 237: 1346-1349
6. Ecker B et al. (1996) Overpression and purification of a recombinant chimeric HIV type 2 / HIV type 1 envelope peptide and application in a accelerated immunobased HIV type 1 / 2 antibody detection system (AIBS) : A new rapid serological screening assay. AIDS Res Hum Retroviruses. 12 (12): 1081-1091
7. Hashida S. et al., (1996) Shortening of the window period in diagnosis of HIV-1 infection by simultaneus detection of p24 antigen and antibody IgG to p17 and revers transcriptase in serum with ultrasensitive enzyme immunoassy, J. Virol. Meth. 6243-6253
8. Kalyanaraman VS et al. (1998) AIDS research and human retroviruses. 4:319-329
9. Kontermann RE et al., (1997 Jun) Enzyme immnunoassays using biospicific diabodies. Immunotechnology; 3(2): 137-144
10. Mevis RJ. Et al., (1988) The gag gene products of human immunodificiency virus type 1: alignment within the gag open reading fram identificantion of posttranslational modifications, and evidence for alternative gag precursors. J. Virol, 62: 3993
11. Pair JP, Chadwick MD. (1992) The biologic and clinical basis of infectious diseases, eds Shulman ST, Phair JP, Somments HM. Philadelphia: WB Saunders,:383
12. Xu J. Y. et al., (1991) Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodificency virus type 1, using ten human monoclonal antibodies, J. Virol. Sep., 4832-4838

MANUFACTURED BY:
ATLAS MEDICAL
William James House, Cowley Rd,
Cambridge, CB4 4WX, UK
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524

PPI129A01
Revision B (26.06.2005)