AccuDiag™ HIV 1, 2 ELISA

REF 1516-12

IVD See external Label 2°C 8°C Σ 96 Tests

Test	HIV 1, 2
Method	Enzyme Linked Immunosorbent Assay
Principle	Double antigen Sandwich ELISA
Shelf Life	12 Months from the manufacturing date

INTENDED USE

The Diagnostic Automation Inc. HlV l+2 Antibody ELISA is an enzyme- linked immunosorbent assay (ELISA) for the qualitative determination of antibodies to human immunodeficiency virus type 1 and/or 2 in human serum or plasma.

SUMMARY AND EXPLANATION

Human immunodeficiency viruses (HIV) are retroviruses transmitted via sexual contact., parenteral and perinatal pathways or the placenta. HIV-I and HIV-2 were respectively isolated in 1983 and 1985 in patients with AIDS (Acquired immunodeficiency Syndrome).
Serological evidence of HIV infection may be obtained by testing for HIV antigens or antibodies in serum of individuals suspected of HIV infection. Antigens can generally be detected during the acute phase and during the symptomatic phase of AIDS only. Antibodies to HIV-1 and 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.

TEST PRINCIPLE

The wells of the polystyrene microplate strips are coated with purified recombinant gp120, gp4 l (including group O) of HIV-1 and gp36 of HIV-2 antigens. Human serum or plasma, diluted in sample diluent, are incubated in these coated wells. HIV specific antibodies, if present, will bind to the solid phase H1V antigens. The wells are thoroughly washed to remove unbound materials and HIV antigens labeled with horseradish peroxidase is added to the wells. These labeled antigens will bind to the antigen-antibody complexes previously formed and excess unbound labeled antigens are removed by washing. Substrate solution containing hydrogen peroxide end 3,3'.5,5'-tetramcthylbenzidine(TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of a blue color after substrate addition. Reaction is terminated by addition of sulphuric acid. The intensity of the color is measured spectrophotometrically at 450 nm and is proportional to the amount of antibodies present in the specimen.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Microplate 1 plate (96 wells). 12 strips per plate, 8 wells per strip coated with a mixture of HIV-I gpl20, gp41, group O antigen and HIV-2 gp36.
2. Positive Control 1 vial (0.5ml). Inactivated Human serum containing antibodies to HIV antigens. Preservative: Bronidox (2ml/L).
3. Negative Control I vial (0.5ml). Inactivated Human serum without antibodies to HIV antigens. Preservative: Bronidox (2ml/L).
4. Conjugate I vial (6ml). The solution contains HRP- labeled HIV-I gpl20, gp41, group 0 antigen and H.IV-2 gp36, with PBS (0.0 l mol/L) and stabilizing proteins, ready to use.
5. Sample Diluent I vial (6ml). Contains stabilizing proteins and detergent, Preservative: Bronidox (2ml/L).
6. Wash Buffer (30x) 1 vial (25ml). Diluted 30-fold in distilled water as described in section of Preparation of Reagent.
7. TMB Color reagent 1 vial (10ml).
8. Stop Solution 1 vial (6ml). 2N sulphuric acid.
9. Instruction Manual I copy

Materials required but not provided

1. Disposable absorbent benchtop paper and paper towels.
2. Graduated pipettes: 10ml.
3. Multichannel pipettor capable of delivering 50µ1 and 100µ1.
4. Disposable pipette tips.
5. Reagent reservoirs (troughs) with a capacity of 25ml.
6. Distilled water, reagent grade quality.
7. Flasks: 500ml, 1 liter.
8. ELISA Microplate Washer. Alternatively, Washing can be performed manually.
9. A 37C incubator.
10. ELISA Microplate Reader.
11. Sodium hypochlorite (5%) solution or liquid household bleach.

HANDLING PRECAUTIONS

1. Optimal assay performance requires strict adherence to the assay procedure described in this Instruction Manual. Deviations from the procedure may lead to aberrant results.
2. Do not substitute reagents from one kit lot to another. Controls, conjugate and microplates are matched for optimal performance. Use only the reagents supplied with the kit.
3. During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.
4. Avoid repeatedly opening and closing the incubator door during incubation steps.

PREPARATION OF REAGENT

1. All reagents should be allowed to reach room temperature (25± 3°C) before use.
2. Dilute 1 volume of wash buffer (30x) with 29 volumes of distilled water before use.

ASSAY PROCEDURE

1. Add 100µ1 of **sample diluent** to wells Al and Bl as **blank control**.
2. Dispense 50µ1 of **sample diluent** into the other wells.
3. Add 50µ1 of negative control to wells Cl and DI, and add 50µ1 of positive control to wells El and Fl.
4. Add 50µ1 of specimen to the assigned well, starting at well G1.
5. Carefully cover microplate with a plate cover provided to prevent evaporation during incubation.
6. Incubated for 45 minutes at 37°C.
7. Wash the microplate with diluted wash buffer.

- ELISA Microplate Washer- Wash 5 times with at least 300 µl /well/wash.
- Manual Microplate Washer-Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. Be careful not to scratch the inside of the well surface. Fill the entire plate with at least 300 µl/well, then aspirate in the same order. Perform this cycle 5 times.

8. Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry.
9. Add 50µ1 of conjugate to each well (except for the blank control), apply another plate cover.
10. Incubate for 30 minutes at 37°C.
11. Repeat the wash procedure as in Step 7 and Step 8.
12. Add l00µ1 of TMB color reagent to each well.
13. Incubate for 10 minutes at 37°C , away from direct or intense light .
14. Stop the reaction by adding 50µ1 of stop solution to each well.
15. Determine the absorbance for each well at 450nm with a microplate reader.

SAFETY PRECAUTIONS & DISPOSAL

1. Handle assay specimens, Positive and Negative controls as potentially infectious agents. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
2. Autoclave all used and contaminated materials at 121°C, 15 psi. for 30 minutes before disposal. Alternatively, decontaminate material s in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.
3. Wipe any spills promptly with 1% sodium hypochlorite solution.
4. Sulphuric acid can cause severe bums. Avoid contact with the reagent. If the reagent came into contact with skin, wash thoroughly with water.

QUALITY CONTROL

1. The Blank Control, Negative Control and Positive Control should be assayed (ONE WELL AT LEAST) in duplicate on each plate with each run of specimens.
2. Blank value must have an absorbance of < 0.100.
3. Negative Control values must have an absorbance of ≤.100 after subtracting the Blank.
4. Each of the 2 positive Control values must have absorbance of ≥0.600 after subtracting the Blank.
5. For the assay to be valid. the difference between the mean absorbance of the Positive
   Control and the Negative Control (PCx- NCx) would be 0.500 or greater. If not, technique may be suspected and the assay must be repeated. If PCx-NC:< is consistently low, deterioration of reagents may be suspected.

RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless s of the number of plates concurrently processed. **The absorbance, ,values of the blank control must be subtracted from both the controls' and the specimens' absorbance values before interpretation of results**. The presence or absence of antibodies specific for HIV 1+2 is determined by relating the absorbance of the specimens to the CUT-OFF Value of the plate.

The CUT- OFF Value for HIV I+ 2 Antibody ELISA is calculated as O.13 + the Mean Absorbance of the Negative Control (If the mean OD<0.05, CUT-OFF Value =0.13+0.05).

CALCULATION OF RESULTS

1. **Calculation of Negative Control Mean Absorbance (NCx)**.

   Individual Negative Control values should be less than or equal to 0.100 unit. All individual Negative Control values must meet the criteria or the assay is invalid and must be repeated.

2. **Calculation of Positive Control Mean Absorbance (PCx)**
   Individual Positive Control values must be greater than or equal to 0.600 unit. If one Positive Control value does not meet either of the above criteria, the assay is invalid and must be repeated.

3. **Calculation of the difference between PCx and NCx**
   For the assay to be valid., the PCx-NCx value should be 0.500 or greater. If not, improper technique or deterioration of reagents may be suspected and the assay should be repeated.

4. **Calculation of CUT-OFF value**
   CUT-OFF Value= O. l 3+NCx (If the NCx< 0.05, CUT-OFF Value =O. l 3+0 .05)

INTERPRETATION OF RESULTS

1.Specimens with absorbance values **less than** CUT-OFF value are considered Negative by the HIV 1+2 Antibody ELISA.
2.Specimens with absorbance values **greater than or equal to th**e CUT-OFF value are considered initially positive by the criteria of the HIV 1+2 Antibody ELISA and should be retested in duplicate before interpretation.
3.Specimens found positive on retesting are be interpreted to be **repeatedly positive** for antibodies to HIV by the criteria of the HIV 1+ 2 Antibody ELISA.
4.Initially positive specimens which are Negative on retesting are considered negative by the criteria of the HIV 1+2 Antibody ELISA

.LIMITATIONS OF PROCEDURE

1. Repeatedly positive results from the HIV 1+ 2 Antibody ELISA is presumptive evidence of antibodies to H1V in the specimen. Negative result from the HIV I +2 Antibody ELISA indicates the likely absence of detectable antibodies to HIV in the specimen. A negative result does not exclude the possibility of exposure to or infection with HIV.

2. Falsely positive results can be suspected with a test kit of this nature. The proportion of false positives will depend on the sensitivity and the specificity of the test kit. For most screening assays, the higher the prevalence of antibody in a population, the lower the proportion of falsely positive samples.

STORAGE

Store the kit and its components at 2- 8°C when not in use. The kit should not be

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used past the expiry date, which is printed on the carton.

REFERENCES:

1. Pair JP, Chadwick MD. The biologic and clinical basis of infectious diseases, eds Shulman ST , Phair JP, Sommers HM. Philadelphia: WB Saunders. 1992 :383.
2. Kalyan Raman VS et al. AIDS research and human retroviruses. 1988; 4:319 - 329.
3. De Leys RJ. Vanderborght M, Vanden Haesevelde Mer al. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of West-Central African origin. J Virol 1990 ; 64:1207-1216 .
4. . Vanden Haesevelde M. Decourt JL, De leys RJ et al. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. J Virol 1994; 68:1586-1596.
5. Zaaijer H.L., Exel-Oehlers P.V. et al. Early detection of antibodies to HIV-1 by third generation assays. The Lancet, 1992; 340:770-772

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