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STUDY OF VARIOUS TECHNIQUES USED IN DIAGNOSIS OF HIV INFECTION[1]

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INTRODUCTION

HIV is a retrovirus with morphological, molecular and biological characteristics, that have led to its proposed classification with the pathogenic animal lentivirus HIV are classified in the family *retroviridae,* genus *lentivirinae* by virtue of their physicochemical, molecular and biologic characteristic. Electron Microscopy of the HIV reveals a sphere with a 72 spikes and cone-shaped core. Virions measure 80 to 130 nm in diameter and have a unique three- layered structure.

As of late 2003, an estimated 38 million individuals have been infected With HIV worldwide . In 1992 a total of 484 ,163 adult AIDS cases were reported to the WHO. HIV is transmitted by three routes: (1) by intimate sexual contect ,(2) by exchange of contaminated blood and/ blood product ,and (3) from mother to fetus or infant. Transmission most likely occurs via virus infected cells. Since relatively little free virus is found in plasma or body fluids. The cell free virus titer in saliva, Tears, urine, and milk is at least 10 fold less then that in plasma .plasma usually Contain between 1 and 5000 particles per ml, a number that differs depending on the Stage of infection.

HIV causes disease by sexual different machanism. HIV infection and cumulative destruction of the helper/ inducer subpopulation of T -lymphocytes leads in its severest form to a progressive and ultimately irreversible immunodificiency. Cell fusion involving the CD4 marker by means through which even uninfected CD4 positive lymphocytes can be incorporated into HIV -infected syncytia, with characteristic Progression to cell death. In the infected patient, peripheral lymphopenia usually accompanies the immune dysfunction. The ratio of helper to suppressor T lymphocytes is markedly reduced , due both to a reduction in the number of helper/ inducer T - lymphocytes and to an increase in suppressor/ cytotoxic T –lymphocytes. The decrease in absolute numbers is accompanied by defects in the functional capacity of those helper cells that remain. This defects result in depressed cell mediated immunity as measured by the failure to respond to antigenic and mitogenic stimulation in vitro, partial or complete absence of delayed hypersensitivity to depressed helper function.

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ACUTE HIV INFECTION:

Initial infection with HIV may be asymptomatic or it may manifest as a self limited febrile illness. After an incubation period of about two to six weeks, a Mononucleosis like syndrome can occur, chracterized by fever, arthralgias, myalgias, Diarrhea, and maculopapular rash. Virus specific antibody starts appearing in the blood from three to twelve weeks after exposure, antibodies to the Major protein (gp120) envelope precursor (gp160) and major core protein (p24). Followed by the appearance of antibodies, to other viral antigen, including p41 (transmembrane protein).

DIAGNOSIS OF HIV INFECTION

Diagnosis of HIV antibodies is still the most useful and most common way to Determine whether an individual has been exposed to HIV and to screen blood and Blood products for this infection agent. A test is considered positive when assays Such as the Enzyme- Linked Immunosorbent Assay(ELISA) and Western Blots are considered reactive. A positive test result indicates exposure and outside of the Perinatal and neonatal periods, is presumed to indicate infection by the virus. In the worldwide epidemic, it is estimated that 75% of the cases of HIV Transmissions are attributable to heterosexual contact. HIV infection is greatly enhanced by the presence of other sexually transmitted disease like SYPHILIS.

ELISA: ENZYME LINKED IMMUNOSORBENT ASSAY

MICROLISA$^{(TM)}$ -HIV : It is developed to detect anti-HIV ENV (envelop) antibodies to HIV-1 and/or HIV-2 with equal reactivity.

PRINCIPLE OF THE TEST

Microlisa -HIV test is a enzyme immunoassay based on Indirect Elisa. Recombinant proteins gp 41, Cterminus of gp 120 , and gp 41 for HIV-1 and HIV-2 representing immunodominant epitopes are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to HIV-1 and HIV-2 if present in the specimen, will bind to the specific antigens captivated onto the surface of the wells.

Horseradish peroxidase (HRP) conjugated antihuman IgG is added to each well . This conjugate will bind to HIV antigen-antibody complex . Finally substrate solution containing chromogen and hydrogen peroxidase is added to the wells and incubated. A blue color will devlop in proportion to the amount of HIV-1 and/or HIV-2 antibodies present in the specimen. The enzyme substrate reaction is interpreted by EIA reader for absorbance at a wavelength of 450 nm. If the sample does contain HIV-1 and HIV-2

antibodies then enzyme conjugate will not bind and the solution in the wells will be either colorless or only faint background color develops.

REAGENTS AND MATERIAL USED

Microlisa-HIV strip plates, Sample diluent.

Enzyme conjugate concentrate(100X),Conjugate dilutent

Wash buffer concentrate(25X),TMBconjugate(100X),

Substrate(TMB,Diluent)

Negative control,Positive control

Stop solution,Plate sealers

ADDITIONAL REQUIREMENTS

Micropippets and disposable pipette tips, Timer, EIA reader, EIA washer Incubator at 37°C, Vortex mixer ,Glassware, Distilled water

TEST PROCEDURE

Fitted the strip holder with the required number of microlisa-HIV strips . Assay control wells were so arranged so that well A-1 was the reagent blank. From well A-1 arranged all controls in a horizontal or vertical configuration.

1. To add 100 µl of the sample diluent to A-1 as well as blank.
2. To add 100 µl Negative Control in each well No.B-1, C-1 respectively.
3. To add 100 µl Positive Control in D-1,E-1 & F-1 wells.
4. To add 100 µl of each sample diluted in sample diluent (1:11)in each well, starting from G1well.
5. To apply cover seal.
6. To incubate at 37°C±2°C for 30 min.±2 min
7. After incubation to wash the wells 5 times with working wash solution .
8. To add 100 µl of working conjugate solution in each well .
9. To apply cover seal.

10 To incubate 37°C±2°C for 30 min.±2 min.

11 After washing to add 100µ of working substrate solution to each well.

12 Incubate at room temperature (20-30) for 30 min in dark .

13. Add 50µl of stop solution.

14. Read absorbance at 450 nm within 30 minutes in ELISA READER

after blanking A-1 well.

CALCULATION OF RESULTS

Abbreviations:

NC -absorbance of the negative control

NCx - Mean negative control

PC -Absorbance of the positive control

PCx - Mean positive control.

TEST VALIDITY:

Blank must be<0.100 in case of differential filter being used.

Negative control acceptance criteria:

NC must be ≤ 0.150.

Positive control acceptance criteria:

1. PC must be ≥ 0.50

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CUT OFF VALUE: CUT OF VALUE = NCx+PCx / 6

Absorbance

NC - 0.042 B1 Well PC - 1.412 D1 Well

- 0.040 C1Well - 1.392 E1Well

Total: 0.082 2 Wells - 1.407 F1 Well

Total: 4.211 3Wells

NCx=0.082/2= 0.041 PCx= 4.211/3= 1.403

CUT OF VALUE = NCx+PCx / 6

INTERPRETATION OF RESULTS

1. Test specimens with absorbance value less then the cut off value are **non - reactive** and may be considered as negative.
2. Test specimen with absorbance value greater than or equal to the cut off value are **reactive** by microlisa-hiv.
3. The O.D. for crystal **clear negative** samples can be in minus and the value could be in the range of (-)0.00 to (-) 0.10.
4. Test specimen with absorbance value within 10% below the cut off should be considered **suspect** for the presence of antibodies.

Specimen with absorbance value equal to or greater then the cut off value are considered **initially reactive** by the criteria of micro-elisa -HIV

RAPID TEST

Dot immunoassay for the defection of antibody to HIV-1 and or HIV-2 in serum or plasma. Cobaids-rs test kit is an in vitro immunoassay intended for the qualitative detection of IgG/IgM antibodies to the HIV-1 and HIV-2 in human serum/plasma.

Principle

This test implies the same principle as enzyme immuno assay (EIA) where by immunobilized antigen antibody complex is visualized by color producing (chromogenic) reaction.

In cobaid-rs the colred endpoint is developed by a colloidal gold protein a signal reagent. Each arm of the comb is spotted with a circular spot near the tip, by optimally standardized blackened of HIV-1 and HIV-2 recombinant antigens and synthetic peptides.

When incubate with a specimens containing HIV ½ antibodies, these antibodies bind specifically to the peptides antigens. The antibodies bind specifically to the peptides antigens. The antibodies bind specifically to the peptides antigens. The antibodies peptide complex is directly visualized after incubation with the protein a colloidal gold signal reagent, a positve result is indicated by the presence of magenta red colured do not the surface of the comb where peptide have been spotted.

Sample – seum / plasma

Components

- Washing buffer (95x)
- Colloidal gold signal reagent
- Sample diluent
- Negative control
- Positive control
- Antigen coated combs
- Control combs

Additional requirements

- Timer
- 5% sodium or calcium hypochiorite

- Measuring cylinder
- Wash reservoir
- Micro test plates
- Micropipettes (100 microtiter)
- Seal bag
- Disposal plastic droppers
- Rubber teats
- Adhesive strip cover

Assay procedure

- Concentrated wash buffer was diluted in the ratio 1:5 with distilled water.
- 2 drops of sample diluent was added into each microtiter well
- 0.1 ml sample/ control was pipetted into microtiter well.
- Combs were placed into the respective wells.
- Plates were incubated at room temperature for 10 minutes.
- **4 drops of colloidal gold signal reagent was added in the microtiter welt.**
- **Combs were washed 10 times by moving forward and backward direction in the washing solufion.**
- Combs were placed in the microtiter well containing colloidal gold signal reagent.
- Plates were incubated at room temperature for 10 minutes.

- The combs were washed as in above steps.
- Combs were allowed to air dry and color development was observed on the tip of arm of the comb.

INTERPRETATION

Positive result is indicated by the presence of magenta red coloured spot/dot near the tip of the arm of the comb.

Negative result is indicated by the absence of the magenta red coloured spot] dot near the tip the arm *of* the comb.

- Incubated to initiate *colour* reaction until the diagnostically significant bands on the HIV 1/2 weakly reactive control strip were clearly visible.
- Aspirated the active channels.
- Rinsed channels twice with distilled water to stop colour development.
- Air dried the strips.

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