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DETECTION OF HIV AIDS : A RESEARCH STUDY[1]

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INTRODUCTION

In recent years, all other forms of immunodeficiency have been dominated by an epidemic of severe immunodeficiency caused by the infectious agent called Human immunodeficiency virus-1 or HIV-1. (1) Infection with the recently diagnosed human retrovirus, human Immunodeficiency virus (HIV), can be expressed in a great diversity of clinical Manifestation, ranging from asymptomatic infection to sever degeneration of the Central nervous system, or profound immunodeficiency with life taking secondary diseases.

(2) Acquired immunodeficiency Syndrome (AIDS) was first reported in 1981 in San Francisco and New York city of the united states.

HIV is a single stranded RNA virus which selectively infects and is cytotoxic for the sub population of T- lymphocytes with helper/ inducer Phenotype(okt4+lev3a+). After attaching to the T4 receptor molecule on the cell Surface, HIV penetrates the cell membrane and initiates the process of replication by means of reverse transcriptase , an enzyme unique to retroviruses. Here RNA Dependent DNA polymerase permits transcription of viral RNA into DNA. Double stranded proviral DNA sequences are incorporated into the host genome And the normal replication cycle of the host cell produces viral subunit which are Subsequently assembled into mature retroviral particles that bud and are extruded from the cell surface. The viral genetic material remains latent in some infected T -cell, while in those that are immunologically activated, viral replication dominates host cell activity , leading ultimately to cellular destruction.

HIV is transmitted sexually among homosexual men, and from heterosexuals men to their female partners. The heterosexual female also transmit infection to their Male partners. HIV infection is also transmitted by infected blood or blood productes, as occurs among intravenous drug users, transfusion recipients.

The largest group of individuals infected with HIV were exposed to the virus through Unprotected

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sexual contect. Heterosexual intercourse is the predominant mode of Transmission. The virus may infect the mucosal cells of the bowel or the cells of the cervix directly or may gain access to other susceptible cells via injured areas in the mucosal lining of the bowel, vagina or uterus. Sexual transmission of either kind Is enhanced in the presence of the other sexually transmitted disease, such as syphilis etc. In 1988 syphilis patient attended the baltimore city MD, STD clinics, where 9.2% of men and 7.8%were women were found HIV infected.

ASYMPTOMATIC HIV INFECTION

Infection can persist for months to years in subclinical from in individuals Whose cells and body fluids harbor the retrovirus; these patients are thus capable of transmitting infection and are at risk of subsequently developing symptomatic Disease. Patients with Asymptomatic HIV infection may be found to have many of the laboratory abnormalities characteristic of overt disease , including ,depressed T-helper cell number and function and raised immunoglobulin levels. Leukopenia (White blood cells<4800μl) a common finding in HIV infected patients is Generally associated with a normal differential count in early or Asymptomatic Stages.( )

RAPID TEST - I

CAPILLUS

INTRODUCTION

Caltex aggregaton test device for the defection of antibodies to HIV-I/HIV-II in Human whole blood, Serum or plasma.

PRINCIPLE

The trinity Biotech capillus HIV1/2 employs two proteins bound to polystyrene latex beads to form the basis of a direct latex aggregation assay for the detection direction of antibodies to HIV-1/2. in human whole blood, serum & plasma. The assay is performed on a patented capillary slide.

The slide consists of a well for mixing of latex reagent and sample. At one end of the mixing well, there is a capillary flow channel which leads to a viewing window. The latex reagent and test sample are mixed in the mixing well on the silde. The mixed reagents are drawn to the flow channel and the reagents being to flow by capillary action towards the viewing window. Samples with HIV-1 and HIV-2 specific antibodies will cause the antigen coated latex to aggregate. The capillary flow enhances the binding of specific antibodies to the latex and hence promotes aggregation. The reaction is read visually when the latex solution reaches the viewing window. Aggregation in the viewing window should be considered as initially reactive. A smooth milky while appearance is considered non-reactive.

MATERIALS REQUIRED

1. Latex reagent.
2. Positive Control
3. Negative Control
4. Slides
5. Disposable Pipette Tips
6. Pipette

TEST PROCEDURE

1. Allow reagents and patient samples toreach room temperature (18-25^0 C) before use.
2. Record patient sample identification number.
3. Place up to 10 slides on the interpretation station.
4. Mix the latex reagent well by gently agitating the bottle to ensure that the latex suspension is homegenous. Avoid forming of the latex reagent. Also draw latex up and down a few times with the graduated dropper to ensure good mixing before latex is dispensed onto the slide.
5. Draw the latex reagent to the calibration mark (120 ul volume approx) avoid drawing up air bubbles. Dispense the reagent onto the silde at the edge of the mixing well furthest away from the capillary channel. Contact of the graduatd dropper with the slide should be avoided when dispensing the reagent.
6. Using the precalbated piptett, attach a fresh disponsable pipette tip provided in the kit and retrieve the test sample or control (10 ul volume).
7. Dispense the sample directly into the latex solution. Using the pipette, mix the sample and the altex by pumping the mixture in and ouit of the tip three times and stir in a circular motion at least five (5) times.
8. Contnue to use the pipette tip to move the well mixed sample and latex solution to the opening of the channel until the capillary flow begins.
9. Allow the latex mixture to flow through the entire capillary channel and into the viewing

window before interpreting the result. This will require approximately 3-7 minutes.

INTERPRETATION OF TEST RESULTS

Samples demonstrating any latex aggregation should be considered initially reactive. Samples showing no aggregation should be interpreted as non-reactive.

RAPID TEST - II

RETROQUIC

RAPID IMMUNIOCONCENTRATION TEST FOR HIV 1 AND HIV 2 ANTIBODIES

INTRODUCTION

Retroquic$^{(TM)}$ – HIV is a membrane based flow through immunoassay for the detection of antibodies of HIV 1 and HIV 2 in Human serum and plasma. Highly purified synthetic peptides of gp 120 gb 41 (HIV) and HIV 2 in human serum and plasma. Highly purified synthetic peptides of gp 120 and gp 41 (HIV 1) and gp 36 (HIV 2) corresponding to the immundominant regions of the HIV 1 and HIV 2 utilized in the test system assist in visual, qualitative, simultaneous detection and differentiation of antibodies to HIV 1 and 2.

PRINCIPLE

Retroquic-HIV test comprises of a test device stripped with distinct bands of purified gp 120 gp 41 synthetic peptide specific to HIV 1 at test region '1' and gp 36 synthetic peptide specific to HIV 2 at test region '2'. The third band striped at region 'C' corresponds to the assay performance control. First the membrane assembly is hydrated with wash buffer and then the specimen is added. Antibodies to HIV 1 and/or 2 if present, are captured by the respective antigens. After washing with wash buffer, Protein a conjugated gold sol reagent is added to reveal the presence/absence of bound antibodies. Post final wash a positive reaction is visualized by the appearance of purple coloured bands at the test region '1' and/or '2'. The absence of bands at test region '1' & '2' is a negative test result. The appearance of control band serves to validate sample addition, reagent and assay performance.

REAGENTS AND MATERIAL SUPPLIED

Kit components

Retroquic-HIV immunoconcentration test kit for HIV 1 and HIV 2 antibodies involves the following components:

(1) Ready to use individually pouched, flow through test devices striped with HIV 1 specific purified synthetic peptides at test region '1' and HIV 2 specific purified synthetic peptides at test region '2' and a blue dyed protein based control band at region 'C' along with a specimen dropper and desiccant.

(2) Dropper bottle with ready to use wash buffer solution.

(3) Dropper bottle with ready to use protein a Conjugated gold sol solution.

(4) Package insert.

TEST PROCEDURE

1. Bring all reagents and specimen to room temperature (25-30^{0}C) before use. Tighten the wash buffer solution and Protein a gold conjugate dropper bottle caps in a clockwise direction to pierce the respective dropper bottle nozzles. The addition of specimen/reagents must be done at the centre of the reaction port, holding the sample dropper/dropper bottles in a vertical position. Ensure the drops are free falling. Use a new sample dropper for each specimen to avoid cross contamination.

2. Tear open the foil pouches and retrieve the require number of Retroquic-HIV membrane tes devices and label appropriately.

3. Add two drops of wash buffer into the reaction port of the device and allow to soak through completely.

4. Using the sample dropper given, put one drop of the serum/plasma specimen into the reaction port. Let it soak through completely.

5. Put three drops of wash buffer to the reaction prot and allow to soak through completely.

6. Add two drops of protein a gold conjugate to the reaction port and allow to soak through completely.

7. Add two drops of wash buffer and allow the wash buffer to soak through completely.

8. Read and record the results immediately.

INTERPRETATION OF RESULTS

Negative Test Result:

Appearance of only one control band corresponding to control region 'C'.

Positive Test Result:

In addition to the control band 'C', appearance of reactive band at test region '1': Specimen positive for antibodies of HIV 1.

In addition to the control band 'C', appearance of reactive band at test region '2': Specimen positive for antibodies of HIV 2.

In addition to the control band 'C', appearance of reactive band at test region '1' and test region '2': Specimen positive for antibodies of HIV 1 and HIV 2.

INVALID TEST RESULT:

The test should be considered invalid in if neither the test band nor the control band appears. In case of invalid results, test should be repeated using a fresh device.

WESTERN BLOT

Recognition of a specific protein in a complex mixture of proteins can be practiced by a technique known as Western Blotting. It is the confirmatory/supplemental test undertaken to confirm the HIV Infection.

The Quali Code^{TM} H1V 1-2 kit is an in vitro qualitative western blot assay for the detection of IgG antibodies in human serum reactive with HIV1/2 antigens present on Universal Protein Code^{TM} membrane.

PRINCIPLE OF THE TEST

Proteins antigens (HIV-1 viral lysate and recombinant HIV-2 antigen) are disrupted and purified. The HIV-1 viral polypeptides are fractioned according to molecular wt by electrophoresis on a polyacrylamide slab gel (PAGE) in presence of SDS. The separated protein are then transferred from the gel on to a nitrocellulose membrane. A control band and a line of HIV-2 recombinant protein is applied to the membrane. The membrane is then cut into layers for individual sample testing. During the procedure the strips containing the HIV1/2 proteins are reacted with serum specimens & washed to remove unbounded antibodies. Visualization of human immunoglobulins specifically bound to HIV-1 or HIV-2 performed by sequential reaction with goat anti-human immunoglobulin alkaline

phosphatase. Complex of protein-primary antibody secondary antibody can be dectected by using substrature such as NBT (Nitroblue tetrazolium) and BCIP (5-Bromo, 4-Chloro, 3 Indolyl phosphate). Bands corresponding to the position of one or more HIV proteins will be visualized in the strip.

COMPONENTS:-

- HIV 1-2 Negative Control (heat-inactivated normal human serum non reactive with HIV ½ antigen).
- HIV 1-2 Positive Control (heat inactivated human serum containing a high titer of antibodies to HIV 1-2 antigen).
- HIV 1-2 Weakly Reactive Control (Heat inactivated human serum containing a low filter of antibodies to HIV ½ antigens)
- Buffer concentrate
- Anti human IgG conjugate
- Substrate solution.

ADDITIONAL REQUIREMENTS:

- Forceps
- Reference scale
- Incubation trays
- Reference strips

ASSAY PROCEDURE:-

Strip Preparation

- 1 ml. wash buffer was added to cash active channel of the incubation tray.
- Strips were placed individually into wells.
- Incubated for 1 min. on rocking platform
- Aspirated.
- 1 ml. dilution buffer was added to each active channel.

Sample Addition

- 10 µl of test serum was added or HIV ½ controls to appropriate individual active channels.
- Incubated for 2 hrs on rocking platform & aspirated.
- Rocked for 3 min and then aspirated.

Conjugate Addition:

- 1 ml conjugate was added to each channel.
- Incubated for 15 min & then aspirated.
- Rinsed channels with 1 ml wash buffer.
- Rocked for 3 min and aspirated.
- Rinsed with 1 ml distilled water.
- Rocked for 3 min and then aspirated.

Substrate Addition

- 1 ml substrate solution was added to each channel .
- Incubated to initiate colour reaction until the diagnostically significant bands on the HIV ½ weakly Reactive Control strip were clearly visible
- Aspirated the active channels
- Rinsed channels twice with distilled water to stop colour development.
- Air dreid the strips.

INTERPRETATION OF RESULTS

1. Bands on the sample strip were identified by side by side comparision with bands on reference strip. The protein bands on Reference strip were proteins from an HIV-1 viral lysate and from HIV-2 recombinant protein (K1) A control also appears as a dark band indicating the addition of serum.

2. The reactivity of antibodies to HIV ½ in a sample was then determined by comparing the intensity of bands on sample strip with the intensity of equivalent bands on the HIV ½ Weakly Reactive Control strip.

HIV-1 INTERPRETATION

No bands present	NEGATIVE
Any two or more of the following bands must be present: p24, gp41 and gp 120/gp 160 Other HIV-1 protein bands may or may not be present.	POSITIVE
HIV-1 protein bands present, but do not meet criteria for positive	INDETERMINATE

HIV-2 INTERPRETATION

Absence of K-1 band or less intensity of K-1 band than on the HIV ½ Weakly Reactive Control.	NEGATIVE
K-1 band equal to or greater in intensity then the K-1 band line on the HIV ½ Weakly Reactive Control.	POSITIVE

IMPORTANT GUIDELINES ON PROCEDURE & PERFORMANCE

- Sample must be collected in K3 EDTA VACUTAINER.
- Do not store whole blood longer than 48 hours, store at room temperature.
- Do not store whole blood on rocker or other mixing device.
- Do not refrigerate whole blood before preparing.
- Minimize, exposing the reagent tube to light hence they are light sensitive.
- Blood and control beads delivery must be performed by reverse pipetting for accuracy.

- CD4 tube must be run first then CD8 tube.
- After running the samples on the instrument, disperse the tube in accordance with biohazard handling regulations.

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