TEST 1: COMBAIDS

Principle: Dot immunoassay employs the same principle as Enzyme Immuno Assay (EIA) whereby the immobilized antigen-antibody complex is visualized by means of color producing (chromogenic) reaction. In EIA the colour is developed by a coupled reaction between enzyme, substrate and chromogen whereas in combaids HIV 1+ 2 immunodot test kit the coloured end- point is developed by colloidal Gold-Protein - A Signal Reagent. Ech tooth of the Comb is spotted with a circular spot, one near the tip with an optimally standardized blend of HIV 1 and HIV 2 recombinant antigens and/or synthetic peptides and the other spot, a little above the first spot is spotted with "Control Reagent". When incubated with a specimen containing HIV 1 and /or 2 antibodies ,thease antibodies bind directly to the HIV antigens present in the "Tese area" on the tooth of the Comb.The immune complex is directly visualized after incubation with the Colloidal Gold- Protein - A signal reagent. A positive result is indicated by the presence of pink coloured spot/dot in the "Test area" near the tip of the tooth of the comb where antigens are spotted. Built in control is visulised separately in the upper part of the tooth where control; Reagent has been spotted,serving as the procedural control. A pink coloured spot/dot will always appear at the "Control area" during the test after application of test sample detecting presence of human immunoglobins (IgG) , irrespective of the presence or absence of HIV specific antibodies in the specimen.

Procedure:

1. Bring all the reagents at room temperature (+15ºC to 30ºC)
2. Take out the required number of combs and mark them on the front side.
3. Dilute the washing buffer.
4. Add 2 drops (0.1mL) of sample dilute in microtest wells.
5. Add 2 drops (0.1mL) of samples and controls into each microtest well containing samples diluent.
6. Place the combs into respective wells.[Gently rock the comb back and forth 2-3 times at the beginning, at middle (5^{th} minute) and at the end of incubation in the well]
7. Incubate for 10 minutes at room temperature (+15ºC to 30ºC)
8. Add 4 drops (0.2mL) of signal reagent in the required number of microtest wells.(samples and control)
9. Wash the combs by moving the comb forward and backward 10 times in the washing solution in the wash tray.
10. Place the combs into microtest wells containing signal reagent. [Gently rock the comb back and forth 2-3 times the beginning, at middle (5^{th} minute), and at the end of incubation in the well].

11. Incubate for 10 minutes at room temperature. (+15ºC to 30ºC)
12. Wash the comb.
13. Allow the comb to air dry and note the colour development on the spotted area on the tip of teeth of the comb for reactivity as well as for control spot/dot appearance.

TEST 2: MERISCREEN

Principle: The Meriscreen HIV 1- 2 WB Rapid test kit contains a membrane strip, which is pre-coated with HIV-1 antigens (gp41 and gp120) & HIV-2 antigen (gp36) on test region '1' and test region '2' respectively. Recombinant antigen (gp41, gp120, gp36) gold conjugate will form a coloured band in the test region '1' and '2' of result window. As the test sample flows through the membrane after addition of Assay buffer, the antigen gold conjugate complexes with anti-HIV antibodies. The complex moves further on the membrane towards the test region, where HIV antigens are coated and leads to formation of reddish purple bands at test region. Absence of test bands indicates a negative test results.
The control band is used for procedural control and should always appear if the test procedure is performed correctly.

Procedure :

1. Bring the specimen and test components if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
3. Take sample up to the marking (of 10ul) on capillary tube. For serum/plasma take it one time ad or whole blood take it two times.
4. Add sample to the sample well (S) using capillary tube. Dispose off used capillary tube as a bio-hazard waste.
5. Add three drops of the Assay buffer to the sample well (S)
6. Interpret the test results at the end of 20 minutes. Do not read the results after 30 minutes

TEST 3: TREDRO

Principle: The Tredro HIV1-2 Ab is a HIV rapid screening test kit containing a nitrocellulose membrane which is precoated with recombinant antigens as the test samples pass through the membrane, HIV antibodies if present in the samples reacts with antigens coated on the membrane at their respective regions. Protein A gold conjugate binds with the Fc regions of the antibodies bound on the membrane and forms a pinkish red coloured dot.

Procedure:

1. Bring the specimen and test components at room temperature
2. When ready to test, open the pouch at the notch and remove devices. Place the test device on a clean flat surface. Label the patients/sample identity details legibly with a marker.
3. Add 3 drops of Assay buffer onto the untested device and allow it to absorb completely.
4. Add 1 drop or 40ul of patient serum/plasma with a dropper or micro pipette. Ensure quick and complete addition of the sample at a single instance to allow proper exposure of the sample on the membrane.
5. Allow the sample to absorb in and add 3 drops of assay buffer onto the membrane to wash any non specific binding over the membrane and allow it to absorb completely.
6. Add 2 drops of Gold conjugate and allow it to absorb completely. The gold conjugate binds specifically with the Fc portions of the patients antibodies captured o the membrane.
7. Add 3 drops of the assay buffer to allow proper washing of the unbound gold conjugate from the membrane and allow it to absorb completely.