COMBAIDS® - RS Advantage-ST

HIV 1+2 immunodot test kit

REF 51SP201-48N
Σ 48

IVD +2 °C +8 °C

Dot immunoassay for the detection of antibody to HIV 1 and / or 2 in Whole Blood*, Serum or Plasma.

AIDS (Acquired Immuno Deficiency Syndrome) is an infectious disease with world wide prevalence and a very high fatality rate[1]. The causative agent is a retrovirus, Human Immunodeficiency Virus (HIV)[2]. Although the virus is transmitted via blood and blood products, it is not possible with presently available methods to detect the virus routinely in blood/body fluids. Detection of antibody to HIV in Whole Blood*, Serum or Plasma, however, provides good evidence of the exposure to the virus and can be used to screen blood and blood products prior to transfusion, so as to avoid possible transmission of the infecting agent. Currently available test methods use the viral proteins or some of their constituent peptides as antigens. The specific peptides used in this test are not prepared from the virus itself but produced synthetically or by recombinant technology. These antigens are, THEREFORE, 100% NON-INFECTIOUS and yet highly specific[3]. **Combaids® - RS Advantage**-ST - HIV 1+2 immunodot test kit is an *in vitro,* visually read, Dot immunoassay intended for the qualitative detection of IgG / IgM antibodies to the HIV type 1 and / or 2 in human whole blood*, serum or plasma.

PRINCIPLE

Dot immunoassay employs the same principle as Enzyme Immuno Assay (EIA) whereby the immobilised antigen-antibody complex is visualised by means of colour producing (chromogenic) reaction. In EIA the colour is developed by a coupled reaction between enzyme, substrate and chromogen whereas in **Combaids® - RS Advantage**-ST - HIV 1+2 immunodot test kit the coloured end-point is developed by a Colloidal Gold - Protein-A Signal Reagent[4,5]. Each tooth of the Comb is spotted with a circular spot, one near the tip with an optimally standardised blend of HIV 1 and HIV 2 recombinant antigens and / or synthetic peptides (Test spot), and the other spot, a little above the first spot is spotted with "Control Reagent" (Control spot). When incubated with a specimen containing HIV 1 and / or 2 antibodies, these antibodies bind directly to the HIV antigens present in the "Test area" on the tooth of the Comb. The immune complex is directly visualised 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 visualised separately in the upper part of the tooth ("Control area"), 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 immunoglobulins (IgG), irrespective of the presence or absence of HIV specific antibodies in the specimen.

SAMPLE

Whole Blood*, Serum or Plasma can be used. For short term storage, specimens can be stored at +2 °C to +8 °C. However, they should be stored frozen (-20 °C or lower) for a long term storage. Grossly haemolysed and contaminated samples should not be used.

*** Whole blood should always be used freshly collected in EDTA / Heparin / citrate anticoagulant. The intensity of the control dot is better visualised with fresh samples than stored / frozen samples, however it does not affect the performance and interpretation of the assay.**

REAGENTS / ACCESSORIES (Supplied in the kit)

Reagent 1 : Washing Buffer (5x)
Reagent 2 : Signal Reagent
Reagent 3 : Sample Diluent
Reagent 4 : Negative Control
Reagent 5 : Positive Control
Reagent 6 : Antigen & Control Reagent Coated Combs

ACCESSORIES

1. Wash Reservoir
2. Microwell Strips in frame
3. Disposable Plastic Droppers
4. Rubber Teats
5. Reference Colour Index for SPIA [Solid Phase Immunosorbent Assay]

ACCESSORIES REQUIRED BUT NOT PROVIDED IN THE KIT

1. Timer
2. Dust Cover
3. Absorbent Pad or Paper Towels
4. Bleach (5% Sodium or Calcium Hypochlorite). Alternative disinfectants include 70% ethanol or 0.5% Wescodyne
5. Disposable Gloves
6. Measuring Cylinder - 100 mL
7. Micropipettes (100 µL) and Pipette tips

REAGENT STORAGE AND STABILITY

All kit components are stable until the expiry date shown on the label when stored between +2 °C to +8 °C. Before opening, the pouch containing the Comb should be brought to room temperature (+15 °C to +30 °C). Combs are highly sensitive to moisture. Once diluted, the Washing Buffer is stable for one week if stored at +2 °C to +8 °C.

WARNING

Since no method can totally guarantee the absence of infectious agents therein, all samples for analysis should be considered potentially infectious and handled with care.
Serum used in Positive Control and Negative Control of **Combaids - RS Advantage**-ST - HIV 1+2 immunodot test kit has been inactivated by heating at 60 °C for 1 hour to render it 100% non-infectious.

PRECAUTIONS (Safety Instructions)

1. The kit should be used for *in vitro* diagnostic use only.
2. Remnants of samples, controls, buffer, aspirated reagents and accessories should be collected in a bottle / bag which should be finally autoclaved for 1 hour at 121 °C and 15 psi or treated with sodium / calcium hypochlorite solution for 30 minutes, prior to discard.
3. Wear gloves during the assay and wash hands thoroughly after completion of test.
4. Do not pipette any reagent by mouth.
10. Allow each reagent and sample to fall freely from the dropper tip / dropping bottle by holding them at 90° over the microwells.
11. Do not use the expired kit.
12. Do not use a test, if packaging is broken.
13. **Do not touch the tooth of the Comb.**
14. **Take care that marking of samples is done on front side of the Comb. The desiccant pad on back side of Comb.**

SETTING UP THE TEST

Washing Solution: Dilute (Reagent 1) the concentrated Washing Buffer (5x) to 1:5 with distilled water or Reagent Grade Water (Arkray Product Code 23RR668-85 or equivalent) by adding 2 mL of concentrated Washing Buffer (5x) to 8 mL distilled water, taking care to avoid foaming. Fill the wash reservoir with Washing Solution.
Note: 10 mL working Washing Solution should not be used for washing more than 2 Combs.

ASSAY PROCEDURE

All kit components and samples to be tested should be brought to room temperature (+15 °C to +30 °C) before starting the test. Clearly mark all samples to be tested and record their identity before starting the test.

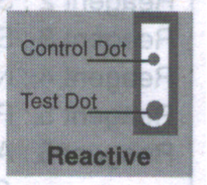
1. Mark the sample numbers on the microtest wells and add two drops (0.1 mL) of Sample Diluent (Reagent 3) to each microtest well that will be used for samples or controls.
2. Add two drops (0.1 mL) of sample with the help of disposable plastic dropper / control to each of the above wells containing Sample Diluent. Mix sample with diluent by repeated aspirating and expelling or stirring with disposable plastic dropper tip. Record the position and identity of samples or controls as they are added.
3. Open zip lock bag and take out required number of blister pack. Peel off the blister pack and carefully remove the Comb (Reagent 6) by holding it from surface having desiccant pad. Store the remaining blister packs in tightly sealed zip lock bag. Mark the sample numbers on front side of the Comb and place it into rows of corresponding microtest wells.
4. Place the Comb into the first row of diluted samples by holding the Comb vertically with the teeth pointing down. Set the timer for 10 minutes and start the timer. [Gently rock the Comb back and forth 2-3 times at the beginning, at middle (5th minute), and at the end of incubation in the well]. Incubate exactly for 10 minutes at room temperature (+15 °C to +30 °C).
5. In the meantime, dispense 4 drops (0.2 mL) of Signal Reagent (Reagent 2) into each of another set of unused microtest wells.
6. Remove the Comb from the sample containing wells and blot the tips of the teeth on absorbent material. DO NOT BLOT THE FRONT SURFACE OF THE COMB. Hold the Comb vertically with tips pointing down and rock them forward and backward in the Wash Solution for a total of ten times. Blot the tips of the arms again.
7. Place the Comb into well containing Signal Reagent (Reagent 2). [Gently rock the Comb back and forth 2-3 times at the beginning, at middle (5th minute), and at the end of incubation in the well]. Incubate exactly for 10 minutes at room temperature (+15 °C to +30 °C).
   After incubation, repeat the washing procedure as described in step-6.
8. Place the Comb on a clean surface, desiccant pad side down. Do not blot or wipe the surface of the Comb. Allow the Comb to air-dry completely before reading the results.
9. Use the Reference colour index for SPIA to compare and interpret the results.

INTERPRETATION

The surface of the Comb should be perpendicular (at a 90° angle) to the eyes.
Do not attempt to read the Comb by viewing it at other angles, as a faint, uncoloured spot / dot may be visible which does not represent true reactivity.

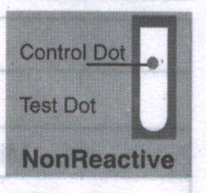
REACTIVE

- Appearance of pink coloured spot / dot on both "Test area" and "Control area" indicate positive result as shown in the picture.
- The positive result on test spot / dot shows either HIV 1 or HIV 2 or both together.
- However, intensity of spot / dot shall be equal to more than 1.0 colour index when compared with Reference colour index for SPIA.



NON REACTIVE

- Absence of pink spot / dot in test area indicate negative result as shown in the picture. However in such case pink spot / dot shall be present in "Control area."

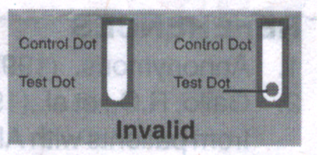


INDETERMINATE RESULT

- The test should be considered as "indeterminate" in case of faint coloured spot / dot in test area having colour intensity between 0.00 and 1.0 colour index. In such cases it is recommended to repeat the test to confirm the result, it the results is still "indeterminant", fresh sample should be drawn often after 4-8 weeks and retested again.

INVALID RESULTS

- The test is to be considered as "invalid" if no pink coloured spot / dot is visible in "Control area" irrespective of presence or absence of pink coloured spot / dot in the test area. In such cases the test should be repeated using a new Comb and fresh specimen.



Notes:

1. Intensity of the colour developed on "Test area" has no direct relation with the nature of reactive sample or the severity of the infection.
2. The control spot / dot is always visible, when the test is performed as indicated in the assay procedure with recommended test specimens.
3. The control spot / dot may not be visible, if the test is run with any fluid or biological specimens other than recommended in the test kit.
4. For reliable performance and proper sensitivity of the test, all Combs and reagents should always be stored properly and used before expiration date.
5. When reading the Combs, examine them in moderate light, preferably by holding the