# 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.
5. Do not smoke, eat or drink in laboratory area.
6. Prevent splashing or spilling of reagents.
7. Extreme care should be taken to avoid microbial contamination of reagents.
8. Do not use Combs and reagents from kits of different batch numbers.
9. Use only microtest wells which have not been used previously.
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, if 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 Comb against a white surface.

### QUALITY CONTROL PROCEDURE

Positive and Negative Controls are provided to check the performance characteristics of the reagents. Positive Control should produce two pink coloured spots / dots at the upper "Control area" and lower edge "Test area" of the tooth of the Comb.
Negative Control should always produce only one pink coloured spot / dot in the "Control area" of the tooth of the Comb.
Every sample should always produce pink coloured spot / dot in the "Control area" with recommended test specimen irrespective of its Positive or Negative status. Controls provided

**One Step Rapid Test for detection of anti-HIV in Human Serum/Plasma/Whole blood**

# MERISCREEN HIV 1-2 WB

Meril Diagnostics

| Product code | Pack Size |
|---|---|
| HVWRPD-05 | 50T |

IVD For in vitro diagnostic use
Read this pack insert thoroughly before use

## INTENDED USE:

MERISCREEN HIV 1-2 WB Test is a qualitative, screening, *in-vitro* diagnostic immunochromatography assay for detection of antibodies specific to HIV-1 and HIV-2 in human serum, plasma and whole blood. The test is intended for use by trained competent person.

## INTRODUCTION:

Acquired Immunodeficiency Syndrome (AIDS) is caused by two types of Human Immunodeficiency Virus, HIV-1 and HIV-2. Transmission of infection is mainly by exposure to certain infected body fluids e.g., blood and blood products, genital secretions etc. and by transplacental route. Infection by HIV-1 has been reported worldwide; HIV-2 infection has been reported as occurring mainly in West Africa and some European countries. Both these viruses show substantial antigenic cross reactivity in their core proteins, but the envelope glycoproteins are least cross reactive. Detection of antibodies against envelope proteins of both viruses ensures detection of antibodies against both types of viruses following infection. The earliest specific antibody response following infection by HIV may be of immunoglobulin M (IgM) followed by a response in immunoglobulin G (IgG). Maximum sensitivity for detection of anti-HIV sero-conversion is achieved by assays which respond to both IgM and IgG.

## 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 and gp36) gold conjugate will form a coloured band in the test region '1' and test region '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 band(s) at test region(s). 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. The intensity of control band has nothing to do with intensity of test band(s).

## REAGENTS AND MATERIALS PROVIDED:

Each kit contains:

1. Pouches; each contains Test device with one desiccant
2. Assay Buffer bottle
3. Positive Control
4. Negative Control
5. Capillary tubes
6. Lancets
7. Alcohol swabs
8. Pack insert

## MATERIALS REQUIRED BUT NOT PROVIDED :

1. Specimen collection containers
2. Centrifuge (for serum/plasma specimen only)
3. Timer
4. Protective gloves
5. Specimen and test waste container
6. Pipettes

## STORAGE AND STABILITY:

All reagents are ready to use as supplied. Store the kit at 2-8°C. Test device has to be brought to room temperature before opening the pouch. In case, the dessicant pouch changes colour from blue to light pink or colourless, the device should not be used. The unopened test device is stable up to the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 8°C.

## PRECAUTIONS:

1. For *in-vitro* diagnostics and professional use only.
2. Allow all reagents and sample(s) to attain room temperature (18°C to 30°C) before use.
3. Test Device is sensitive to humidity; hence use the Test Device immediately once pouch is opened.
4. Do not use the kit contents beyond the expiry date.
5. Do not touch the nitrocellulose part of the device. Finger print or scratch on nitrocellulose membrane may give erroneous results.
6. Test Devices and assay buffers of different lot must not be mixed and used.
7. Do not re-use accessories like capillary tubes for testing purpose.
8. Perform the test by using kit's assay buffers. Performing the test with any other buffer is not valid.
9. Follow the assay procedure and storage instructions strictly. Deviation will lead to erroneous results.
10. Do not use haemolysed or lipemic specimen for testing.
11. Use sufficient volume of sample for testing.
12. Do not re-use the Test Devices and capillary tubes from the procedure; this may lead to aberrant results.
13. Do not pipette reagents by mouth and do not smoke, eat or drink while handling specimens and performing a test.
14. Avoid contact of reagents with eyes and skin.
15. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed. Avoid re-using gloves or use of washed gloves.
16. Handle sample(s) and used materials as if it is capable of transmitting infection.
17. Follow standard Lab procedure and bio safety guidelines for handling and disposal of potentially infective material. All remnants of sample(s), used materials, pipette tips etc. should be disposed in suitable biohazard container. Materials should be autoclaved at 121°C for 30 minutes or dipped in 10% hypochlorite solution for 30 minutes prior to disposal.
18. Clean up spills thoroughly using an appropriate disinfectant.

## SPECIMEN COLLECTION AND PREPARATION:

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures. **Sample:** Serum/Plasma/Whole Blood collected through venipuncture or fingerprick.

## SAMPLE COLLECTION :

### WHOLE BLOOD COLLECTION THROUGH VENIPUNCTURE:

1. Collect the whole blood into collection tubes (containing anticoagulants such as heparin, EDTA or sodium citrate) by venipuncture. These specimens may be stored at 2°C - 8°C for upto 7 days.

### WHOLE BLOOD COLLECTION THROUGH FINGER PRICK:

1. Choose a finger to be pricked and clean the area with an alcohol swab and allow it to dry.
2. Using a sterile lancet, prick the area of the skin. Wipe away the first drop of blood and use the second drop of blood for collection using the capillary tube.

### SERUM OR PLASMA :

1. Collect the whole blood into collection tube containing no anticoagulants through venipuncture and centrifuge it to obtain serum.
2. Meanwhile for plasma, collect the whole blood into collection tube containing anticoagulants such as EDTA, Heparin or Sodium Citrate and centrifuge it to obtain plasma.

## TEST PROCEDURE:

1. Bring the test components, reagents and specimens to room temperature if refrigerated.
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
3. Add 10µl of control (Negative or Positive) to the sample well (S) using pipette.
4. Add 10µl of serum/plasma using pipette and for whole blood, add 20µl of the sample using capillary tube.
5. Add the sample to the sample well (S). Dispose off used pipette tips or capillary tubes as a bio-hazard waste.
6. Add three drops of the Assay Buffer to the sample well (S).

**Interpret the test results at the end of 20 minutes. Do not read the results after 30 minutes.**

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Rapid Test for Differential Detection of Antibodies to HIV-1 & HIV-2 in Human Serum/Plasma.

# TREDRO™ HIV 1-2 Ab

Meril Diagnostics

| Product Code: | Pack Size: |
|---|---|
| HVDRPT - 01 | 20T |
| HVDRPT - 02 | 20T |

IVD For in vitro diagnostic use
Read this pack insert thoroughly before use

**INTENDED USE:**
TREDRO™ HIV 1-2 Ab is a Single use rapid test, flow through in-vitro qualitative assay for the detection of antibodies to HIV-1 and HIV-2 in human Serum/Plasma specimens. The test is intended for used by trained personnel in Medical facility and Clinical laboratory as a screening test for HIV antibody detection.

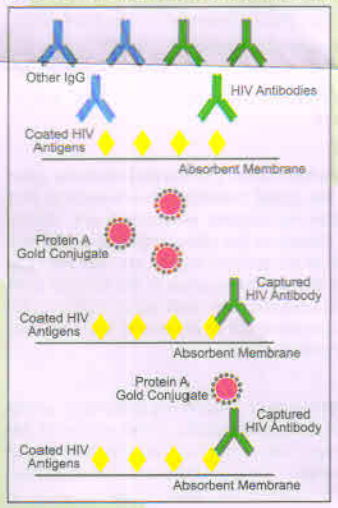
**INTRODUCTION:**
Acquired Immunodeficiency Syndrome (AIDS) is caused by two types of Human Immunodeficiency Virus, HIV-1 and HIV-2. Transmission of infection is mainly by exposure to certain infected body fluids e.g., blood and blood products, genital secretions etc. and by transplacental route. Infection by HIV-1 has been reported worldwide; HIV-2 infection has been reported as occurring mainly in West Africa and some European countries. Both these viruses show substantial antigenic cross reactivity in their core proteins, but the envelope glycoproteins are least cross reactive.

HIV Test kits are classified in terms of Generations by WHO as:

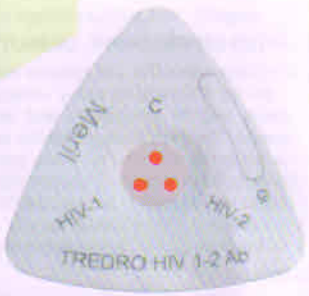
1) 1st Generation Assays: Using Viral Lysate Antigens
2) 2nd Generation Assays: Using Recombinant or Synthetic peptides
3) 3rd Generation Assays: Detects IgM ,IgG and IgA antibodies together
4) 4th Generation Assays: Detects p24 Antigen and Antibodies to HIV together.

TREDRO™ HIV 1-2 Ab is designed to detect the antibodies to envelope glycoprotiens of HIV-1 and HIV-2 by using unique combination of synthetic and recombinanat antigens in the same test device differentially. Consequently potential infectious samples of serum and plasma samples can be identified.

**PRINCIPLE:**
The TREDRO™ HIV 1-2 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 sample 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. (Fig. 1a & 1b).




(Fig. 1a)




(Fig. 1b)

**REAGENTS AND MATERIALS PROVIDED:**
Each kit contains:

| Sr. | Component | Description | Preparation |
|---|---|---|---|
| 1. | TREDRO™HIV 1-2 Ab device | Individual pouch | Bring to R.T before use, cut & open the pouch |
| 2. | Assay buffer | Buffer containing chemicals and stabilizers | Bring to R.T before use |
| 3. | Gold conjugate | Protein-A colloidal gold conjugate | Bring to R.T before use |
| 4. | Positive Control | Inactivated human serum reactive for anti-HIV 1 and 2 antibodies containing preservatives | Bring to R.T before use |
| 5. | Negative Control | Inactivated normal human serum non reactive for anti-HIV 1 and 2 antibodies containing preservatives | Bring to R.T before use |
| 6. | Plastic dropper | Plastic dropper for sample addition | Always ensure to use new dropper |

**STORAGE AND STABILITY:**
The sealed pouches in the test kit to be stored between 2-8°C till the duration of the shelf life as indicated on the pouch. DO NOT FREEZE.

**MATERIAL REQUIRED BUT NOT PROVIDED:**
Hand Gloves, Syringes, Blood collection tubes, Bio-hazard container, Absorbent cotton balls for blood collection, 0.1% Hypochlorite.

**PRECAUTIONS:**

1. For *in-vitro* diagnostics and professional use only
2. Allow all reagents and sample(s) to attain room temperature (18°C to 30°C) before use.
3. Do not use the kit contents beyond the expiry date.
4. Do not touch the nitrocellulose part of the device. Finger print or scratch on nitrocellulose membrane may give erroneous results.
5. Test Devices and reagents of different lot must not be mixed and used.
6. Perform the test by using kit's reagents. Performing the test with any reagents may give erroneous results.
7. Follow the assay procedure and storage instructions strictly. Deviation will lead to erroneous results.
8. Do not use haemolysed or turbid or hazy specimen for testing.
9. Use sufficient volume of sample for testing and add entire 40µl or one drop of the patient sample at once on the reaction membrane.
10. Do not pipette reagents by mouth and do not smoke, eat or drink while handling specimens and performing a test.
11. Do not reuse the test devices or sample dropper from the procedure as it may lead to aberrant results.
12. Avoid contact of reagents with eyes and skin.
13. Wear protective clothing such as laboratory coats and disposable gloves and eye protection when specimens are assayed. Avoid re-using gloves or use of washed gloves.
14. Handle sample(s) and used materials as if it is capable of transmitting infection.
15. Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. Remnants of sample(s), used materials, pipette tips etc. should be disposed in suitable biohazard container. Materials should be autoclaved at 121°C for 30 minutes or dipped in 10 % hypochlorite solution for 30 minutes prior to disposal.
16. Clean up spills thoroughly using an appropriate disinfectant.
17. Sodium Azide is present at 0.1 % in all assay reagents, which can react with lead and copper plumbing to form highly explosive meta azides. If needed to be discarded in to a drain, flush a large amount of water to prevent azide build- up.

**SPECIMEN COLLECTION AND PREPARATION:**
Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

**PLASMA:**

1. Collect blood specimen into collection tube containing EDTA, Citrate or Heparin.
2. Separate the plasma by centrifugation, 1500 RPM for 10 Minutes.
3. Carefully withdraw the plasma into new pre-labelled tube.

**SERUM:**

1. Collect the blood specimen into a collection tube containing no anticoagulants.
2. Allow the blood to clot.
3. Separate the serum by centrifugation, 1500 RPM for 10 minutes.
4. Carefully withdraw the serum into a new pre-labelled tube. Test the specimens as soon as possible after collection.

Stored separated serum/plasma specimens at 2-8°C up to 3 days can be used for testing. Serum/Plasma specimens should be frozen at -20°C for longer storage. If the sample is frozen, completely thaw the sample prior to testing. Avoid repeated freezing and thawing of specimens.

**TEST PROCEDURE:**
Bring the specimen and test components to room temperature.
It is important to ensure sequential addition of reagent as recommended and also allow the reagents to soak completely before addition of consequent reagent.

**MIX THE SPECIMEN WELL PRIOR TO ASSAY:**

1. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface. Label the patient/sample identity details legibly with a marker.
2. Add **3 drops** of Assay buffer onto the untested device and allow it to absorb completely.
3. Add **1 drop** or 40 µl 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.
4. 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.
5. Add **2 drops** of Gold Conjugate and allow it to absorb completely. The gold conjugate binds specifically with the Fc portions of the patient antibodies captured on the membrane.
6. 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.

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