QUALITY CONTROL

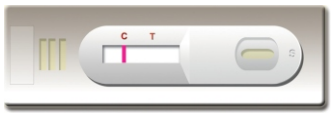
Using individual *Reliable one step* HIV-1&2 Ab$^{3.0}$Rapid Test device as described in the Assay Procedure above, run 1 Positive Control and 1 Negative control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2℃-30℃
5. The temperature of the test area falls outside of 15℃-30℃.

Expected results are as follows:

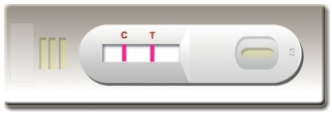
Negative Control

Only the C band shows color development. The T bandshows no color development.



Positive Control

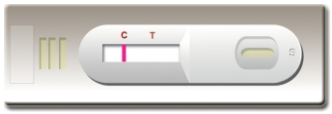
Both C and T band show color development.



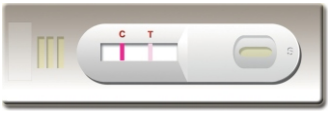
The appearance of any burgundy color in the T band, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT** : If only the C band is developed, the test indicates that no detectable HIV antibodies are present in the specimen. The result is negative.

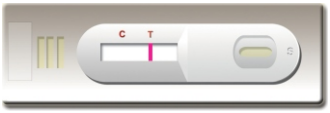


2. **POSITIVE RESULT** : If both C and T bands are developed, thetest indicates for the presence of antibodies to HIV-1, or HIV-2 or both in the specimen; the result is HIV positive.



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 500 samples from susceptible subjects were tested by the *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test and by a Chinese State Drug Administration (SDA) licensed EIA. Comparison for all subjects is showed in the following table:

	Reliable one step HIV-1&2 Ab$^{3.0}$ Rapid Test		
EIA	**Positive**	**Negative**	**Total**
Positive	21	0	21
Negative	2	477	479
Total	23	477	500

Relative Sensitivity: 100% , Relative Specificity: 99.6%, Overall Agreement: 99.6%

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to HIV in serum, plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test is limited to the qualitative detection of antibodies to both HIV-1 and HIV-2 in human serum, plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HIV antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with HIV-1 and /or HIV-2.
4. A negative result can occur if the quantity of the HIV antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should be only interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Chang, SY, Bowman, BH, Weiss, JB, Garcia, RE and White, TJ. The origin of HIV-1 isolate HTLV-IIIB. Nature (1993) 3/363:466-9
2. Arya, SK, Beaver, B, Jagodzinski, L, Ensoli, B, Kanki, PJ, Albert, J, Fenyo, EM, Biberfeld, G, Zagury, JF and Laure, F. New human and simian HIV-related retroviruses possess functional transactivator (tat) gene. Nature (1987) 328:548-550
3. Caetano JA Immunologic aspects of HIV infection. Acta Med Port (1991) 4 Suppl 1:52S-58S
4. Janssen, RS, Satten, GA, Stramer, SL, Rawal BD, O'Brien, TR, Weiblen, BJ, Hecht, FM, Jack, N, Cleghorn, FR, Kahn, JO, Chesney, MA and Busch MP. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA (1998) 280(1): 42-4

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Reliable Pro-detect Biomedicals Pvt. Ltd.

In vitro Diagnostic

INTENDED USE

The *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test device is a lateral flow chromatographic immunoassay for the qualitative detection of IgG anti-HIV-1 and anti-HIV-2 antibodies in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HIV-1 or HIV-2. Any reactive specimen with the *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test device must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

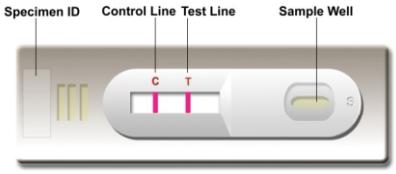
Human immunodeficiency virus type I and type II (HIV-1and HIV-2) are enveloped single strain RNA positive virus. The causative relationship between HIV-1 and HIV-2 virus and acquired immunodeficiency syndrome (AIDS) has beenestablished over decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex, and from healthy individuals with a high risk for developing AIDS[1]. HIV-2 has been isolated from West African AIDS patients and from sero-positive asymptomatic individuals[2].

Both HIV-1 and HIV-2 virus can elicit strong immune responses[3], including the production of anti virus antibodies. Presence of specific anti HIV-1and/or HIV-2 virus antibody in blood, serum and plasma indicates the exposure of an individual to the HIV-1 and/or HIV-2 virus, being a great value of clinical diagnosis[4].

The *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test device utilizes the conserved envelope antigendomains, which allows IgG antibodies to the HIV-1 including O subtype and HIV-2 to be detected.

TEST PRINCIPLE

The *Reliable one step* HIV-1&2 Ab$^{3.0}$ Rapid Test device is a lateral flow chromatographic immunoassay. The test device consists of: 1) a burgundy colored conjugate pad containing mouse monoclonal anti-human IgG antibody conjugated with colloid gold (Human IgG Conjugates) 2) a nitrocellulous membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with recombinant HIV-1 antigen gp120 /gp41 and recombinant HIV-2 antigen gp 36. The C band is pre-coated with goat anti-mouse IgG antibodies.



When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. IgG antibodies to HIV-1, and HIV-2 or the both if present in the specimen will bind to the Human IgG Conjugates. The immunocomplex is then captured on the membrane by the pre-coated recombinant HIV-1 or HIV-2 antigen, forming a burgundy colored T band, indicating a positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG / mouse anti-human IgG conjugates regardless of the presence of any antibody to HIV. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 test devices, each sealed in a foil pouch with two items inside:
   a. One cassette device
   b. One desiccant.
2. Sample diluent (2 bottle, 5 mL)
3. 50 mini plastic droppers.
4. One package insert (instruction for use)

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15℃-30℃) before use.
5. Do not use the components in any other type test kit of as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink,or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 20 minutes after a specimen is applied to the sample well of the device. Read result after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2℃-30℃. The positive and negative controls should be kept at 2℃-8℃ . If stored at 2℃-8℃, ensure that the test device is brought to room temperature before opening.The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30℃.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting Store specimens at 2℃-8℃ if not tested immediately. Store specimens at 2℃-8℃ up to 5 days. The specimens should be frozen at -20℃ for longer storage.

Avoid multiple freeze-thaw cycles. Prior to ,testing bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

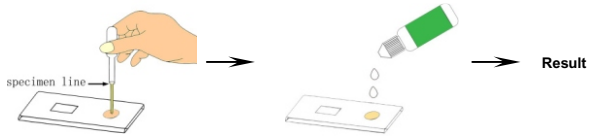
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4. Fill in the mini plastic dropper with the specimen not to exceed the specimen line as showed in the following image. The volume of the specimen is around 5 µL (App. 1 drop) .

Holding the dropper vertically, dispense all of the specimen into the center of the sample well making sure that there are no air bubbles.

Then add 2 drops (about 70-100 µL) of Sample Diluent immediately.



5 µLof specimen (App. 1drop) **2 drops of sample diluent** **20 minutes**

Step 5: Set up timer.

Step 6: Results can be read in 20 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.