TRUSTline HIV-Ab/Ag 4th Gen Rapid Test – Cassette

REF AR0018C IVD

for the Detection of Antibodies to HIV 1 & HIV 2 and HIV-1 p24 Antigen in Human Serum / Plasma / Whole Blood

INTENDED USE

The TRUSTline HIV-Ab/Ag 4th Gen Rapid Test is a lateral flow immunoassay for the qualitative detection of antibodies (IgG, IgM, IgA) to HIV-1 and HIV-2 virus and HIV-1 p24 antigen in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with HIV. The test kit is not automated and does not require any additional instrument. Any reactive specimen with the TRUSTline HIV-Ab/Ag 4th Gen Rapid Test must be confirmed with alternative testing methods, such as ELISA/Western blot assay/PCR, and clinical findings.

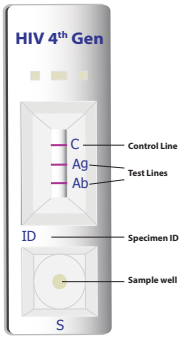
SUMMARY AND EXPLANATION OF THE TEST

Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped, single-stranded, positive-sense RNA viruses. The causative relationship between HIV-1 and HIV-2 viruses and acquired immunodeficiency syndrome (AIDS) has been established 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]. HIV-1 is much more prevalent than HIV-2 worldwide. Recent studies have shown that over 30 million people have been infected with HIV-1.

Both HIV-1 and HIV-2 viruses can elicit strong immune responses[3] including the production of antiviral antibodies. Presence of specific anti-HIV-1 or HIV-2 virus antibodies in whole blood, serum or plasma indicates the exposure of an individual to the HIV-1 or HIV-2 which is of great value for clinical diagnosis[4]. Tests that detect HIV p24 antigen may be useful for the early diagnosis of HIV as p24 antigen is one of the earliest markers of HIV infection. It has been suggested that HIV infection is detectable with a p24 antigen test 6 days earlier than an antibody test[5].

The TRUSTline HIV-Ab/Ag 4th Gen Rapid Test utilizes recombinant gp-120-41, gp36 and anti-p24 antibodies can qualitatively detect antibodies (IgG, IgM, IgA) to HIV-1 or HIV-2 viruses and HIV-1 p24 antigen in patient serum, plasma or whole blood in 15 minutes. The test can be performed without cumbersome laboratory equipment.

TEST PRINCIPLE



The TRUSTline HIV-Ab/Ag 4th Gen Rapid Test is a lateral flow immunochromatographic assay. The test strip consists of: 1) a burgundy colored conjugate pad containing recombinant HIV-gp120-41 and gp-36 antigens conjugated with colloidal gold (HIV conjugates), monoclonal anti-HIV-p24 antibody conjugated with colloidal gold (p24 conjugates) and rabbit IgG-gold conjugates (for control line), 2) a nitrocellulose membrane strip containing two test lines (Ag line and Ab line) and a control line (C line). The Ab line is pre-coated with HIV-gp120-41 and HIV-2 gp-36 antigens for the detection of antibodies to HIV-1 including O or HIV-2, the Ag line is pre-coated with another monoclonal anti-HIV-p24 antibody for the detection of p24 antigen, and the C line is pre-coated with goat anti-rabbit IgG antibody.

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, IgM or IgA antibodies to HIV-1 or HIV-2, if present in the specimen, migrate through the conjugate pad where they bind to the HIV conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-1+2 antigens forming a burgundy colored Ab line, indicating a positive test result. Absence of the Ab line suggests an HIV-1 and HIV-2 antibody negative result.

HIV-1 p24 antigen, if present in the specimen, migrates through the conjugate pad where it binds to the p24 conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-p24 antibody, forming a burgundy colored Ag line, indicating a positive test result. Absence of the Ag line suggests a HIV-p24 antigen negative result.

The test contains an internal control (C line), which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of color development on the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

Materials Provided	Pack Size: 10 Test/Kit	Pack Size: 100 Test/Kit
Individually sealed foil pouches containing: 1 cassette device and 1 desiccant	10 Nos.	100 Nos.
Specimen transfer device	10 Nos.	100 Nos.
Sample diluent bottle	1 No.	3 Nos.
Package insert	1 No.	1 No

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test
3. Alcohol swab
4. Disposable gloves

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 may lead to inaccurate test results.
2. Do not open the sealed pouch until ready to conduct the assay.
3. Do not use the test device if pouch is not intact.
4. Do not use expired devices or components.
5. Bring all reagents to room temperature (15-30°C) before use.
6. Do not use components of different lots and of from any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolyzed blood for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. The test result should be read in 15 minutes after a specimen is applied to the sample well of the device. Reading the result after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.
14. Clean up spills thoroughly using appropriate disinfectant.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store test kit at 1-30°C. If stored at 2-8°C, ensure that all reagents are brought to room temperature before opening. The sample Diluent (Opened and unopened) and unopened test device is stable through the expiration date printed on the label, when stored at recommended temperature. Do not freeze the kit or expose the kit to temperatures above 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

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

Serum

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

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately, for up to 5 days. The specimens should be frozen at -20°C 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 specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Blood

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). Do not use hemolyzed blood for testing. Capillary blood (fingertip puncture) can be used directly without anticoagulant. Collect blood with sample pipette and transfer it to sample well of device.

Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

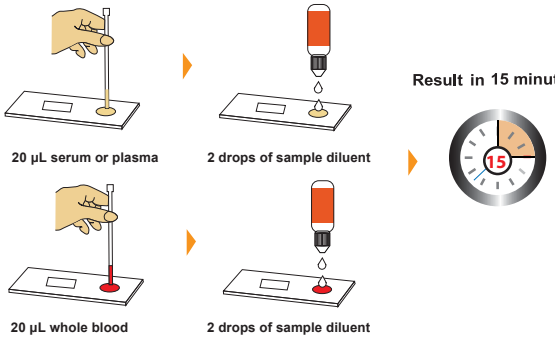
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Fill the specimen transfer device with specimen not exceeding the specimen line as shown in the images below. The volume of specimen is approximately 20 µL **For better precision, transfer specimen using a pipette capable of delivering a 20 µL volume.**

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

Immediately add 2 drops (about 60-80 µL) of Sample Diluent to the sample well with bottle positioned vertically.



Step 5: Set up the timer.

Step 6: Results should be read in 15 minutes.

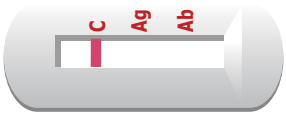
Do not read the result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control**: This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.
2. **External Control**: Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of the specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kits falls outside of 1-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE OR NON-REACTIVE RESULT**: If only the C line is present, the absence of any burgundy color in both test lines (Ab and Ag) indicates that neither HIV antibodies nor HIV p24 antigen is detected in the specimen. The result is negative or non-reactive.



2. **POSITIVE OR REACTIVE RESULT**:

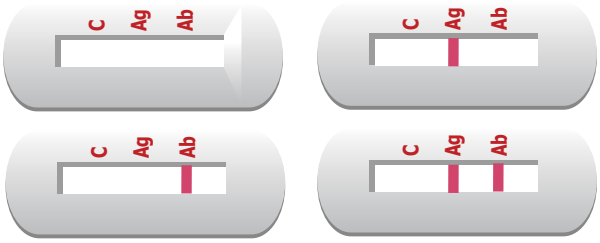
2.1 In addition to the presence of the C line, if the Ab line is developed, the test indicates the presence of antibodies to HIV-1 and/or HIV-2 in the specimen. The result is HIV-1 or HIV-2 Ab positive or reactive.

2.2 In addition to the presence of the C line, if the Ag line is developed (including faint line), the test indicates the presence of HIV-p24 in the specimen. The result is HIV p24 positive or reactive.

2.3 In addition to the presence of the C line, if both the Ab line and the Ag line are developed, the result is both HIV 1+2 Ab and p24 antigen positive or reactive.

Specimens with reactive results should be confirmed with alternative testing method(s) such as Western Blot assay, PCR or ELISA and clinical findings before a diagnostic decision is made.

3 **INVALID:** If no C line is developed, the assay is invalid regardless of any burgundy color in the test line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 755 clinical samples were collected and tested by TRUSTline HIV Ab/Ag 4th Gen Rapid Test with a commercial US-FDA licensed HIV Ag/Ab EIA. Comparisons for all subjects are shown in the following table:

	TRUSTline HIV-Ab/Ag 4th Gen Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	105	0	105
Negative	1	649	650
Total	106	649	755

Relative Sensitivity: 100%, Relative Specificity: 99.84%, Overall Agreement: 99.86%

2. **Boston Biomedica Inc (BBI) Seroconversion Panel**

The performance of the TRUSTline HIV-Ab/Ag 4th Gen Rapid Test was evaluated using BBI seroconversion panel PRB967. The results are shown in the following table:

PRB-967 panel		BioMerieux HIV Ag pg/mL	Abbott HIV1/2 Ab s/co	TRUSTline HIV-Ab/Ag 4th Gen Rapid Test	
Members ID	**Days bleed**			**Ag reactivity**	**Ab reactivity**
PRB967-04	17	>400.0	2.5	Positive	Positive
PRB967-05	19	>400.0	8.3	Negative	Positive
PRB967-06	24	10.5	8.4	Negative	Positive

Note: s/co < 1: Negative, s/co >=1: Positive

3. **Cross-Reactivity**

Cross-reactivity was tested with specimens from other infectious disease, the results are shown in the following table:

Specimen	Sample Size	TRUSTline HIV-Ab/Ag 4th Gen Rapid Test	
		Ag reactivity	**Ab reactivity**
HBsAg Positive	20	Negative	Negative
HCV Positive	10	Negative	Negative
Syphilis Positive	10	Negative	Negative
HAV Positive	10	Negative	Negative
HEV Positive	10	Negative	Negative
H.pylori Positive	10	Negative	Negative
TB Positive	10	Negative	Negative
ANA Positive	6	Negative	Negative
HAMA Positive	4	Negative	Negative
RF Positive (< 2500IU/mL)	10	Negative	Negative

4. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTline HIV-Ab/Ag 4th Gen Rapid Test. This was studied by spiking these substances into three levels of HIV Ag and HIV Ab standard controls. The results are presented in the following table and demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTline HIV-Ab/Ag 4th Gen Rapid Test.

Potential Interfering Substances Spiked	HIV Ag Reactivity			HIV Ab Reactivity		
	Negative	Weak Positive	Strong Positive	Negative	Weak Positive	Strong Positive
Control	-	+	+++	-	+	+++
Bilirubin 20 mg/dL	-	+	+++	-	+	+++
Glucose 55 mmol/L	-	+	+++	-	+	+++
Hemoglobin 2 g/L	-	+	+++	-	+	+++
Salicylic Acid 4.34 mmol/L	-	+	+++	-	+	+++
Heparin 3,000 U/L	-	+	+++	-	+	+++
EDTA 3.4 µmol/L	-	+	+++	-	+	+++
Human IgG 150 mg/dl	-	+	+++	-	+	+++
Sodium citrate 3.8%	-	+	+++	-	+	+++

Note: -: Negative; +: Weak Positive; +++: Strong Positive

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to HIV and/or p24 antigen in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTline HIV-Ab/Ag 4th Gen Rapid Test is limited to the qualitative detection of antibodies to HIV-1 and HIV-2 and/or HIV p24 antigen in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer or antigen level of the specimen.
3. A non-reactive result for an individual subject indicates absence of detectable anti-HIV-1, anti-HIV-2 and/or HIV p24 antigen However, a non-reactive test result does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2.
4. A non-reactive result can occur if the quantity of the HIV-1/HIV-2 antibodies and/or HIV p24 antigen present in the specimen is below the detection limits of the assay or the antibodies/antigen that are detected are not present during the stage of disease in which a sample is collected.
5. False negative results may arise because of hook effect due to very high titer of antibodies/antigen in sample. Repeat the test by using different dilution of same sample.
6. Hemolytic samples may give reddish background even after end of the test time.
7. Infection may progress rapidly. If the symptoms persist while the result from the TRUSTline HIV-Ab/Ag 4th Gen Rapid Test is non-reactive, it is recommended to test with alternative test methods or to re-test the patient a few days later.
8. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
9. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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5. Busch, M.P., Lee, L.L., Satten, G.A., Henrard, D.R., Farzadegan, H., Nelson, K.E., Read, S., Dodd, R.Y and Peterson, L.R. Time course of detection of viral and serogic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion (1995) 35:91-7.

Index of Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
Temperature limit 1-30 °C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	
Do not use if package is damaged	Keep away from sunlight	

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