Rapid Response™

HIV Ag/Ab (Antigen/Antibody) 4th Generation Test Cassette

(Whole Blood/Serum/Plasma)

REF HIV1/2/P-13C40L Product Insert

A rapid test for the qualitative detection of antibodies (IgG, IgM, IgA) to anti-HIV-1 (including O) and HIV-2 virus and HIV-1 p24 antigen in human serum, plasma or whole blood.

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette is a lateral flow immunoassay for the qualitative detection of anti-HIV-1 (including O) and anti-HIV-2 virus antibodies (IgG, IgM, IgA), and HIV-1 p24 antigen in human serum, plasma or whole blood. It is intended to be used by healthcare professionals to aid in the diagnosis of infection with HIV.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

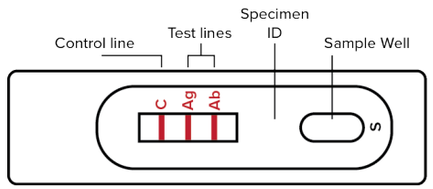
Introduction

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 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 an HIV p24 antigen test 6 days earlier than an antibody test[5].

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

Principle



The Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette is a lateral flow immunochromatographic assay. The test strip in the cassette consists of: 1) a 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 a control antibody conjugated with colloidal gold, 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 a control line 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 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 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 colored line of the immunocomplex of control antibodies regardless of the presence of any colored test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

Precautions

- For professional *in vitro* diagnostic use.
- Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
- Do not open the sealed pouch until ready to conduct the assay.
- Do not use after the expiration date.
- Bring all reagents to room temperature (59-86°F; 15-30°C) before use.
- Do not use components from any other type of test kit as a substitute for the components in
- this kit.
- Do not use hemolyzed blood for testing.
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-bornepathogens.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
- If using, handle the negative and positive controls in the same manner as patient specimens.
- The test result should be read 15-20 minutes after a specimen is applied to the sample well of the device. Reading the test result after 15-20 minutes should be considered invalid and must be repeated.
- Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.

Materials

Materials provided

- Individually packed test cassettes
- Sample diluent (REF SB-R0018, 5 mL/bottle)
- Product insert
- Lancets (for whole blood)
- 20 µL capillary tubes

Materials required but not provided

- Centrifuge (for serum or plasma)
- Timer

Storage and Stability

All reagents are ready to use as supplied. Store unused test devices unopened at 35.6-86°F (2-30°C). If stored at 35.6-46.4°F (2-8°C), 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 or expose the kit to temperatures above 86°F (30°C).

Collection and Storage of Specimens

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

Plasma

1. Collect blood specimen by venipuncture into a collection tube containing EDTA, citrate or heparin.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen by venipuncture into a collection tube containing no anticoagulants.
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. If not tested immediately, store specimens at 35.6-46.4°F (2-8°C) for up to 5 days. The specimens should be frozen at -4°F (-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 to avoid interference with result interpretation.

Blood

1. Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

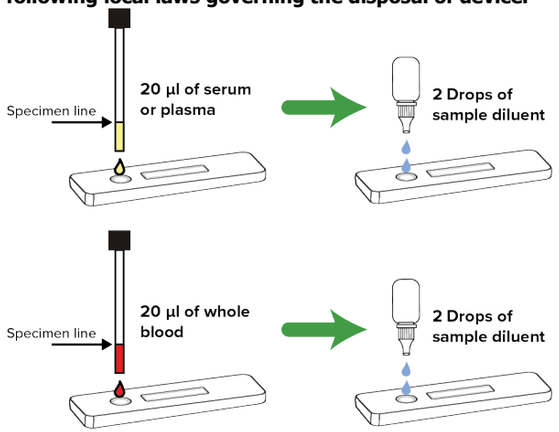
Whole blood specimens should be stored in refrigeration 35.6-46.4°F (2-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

Test Procedure

Allow the test, specimen, buffer and/or controls to reach room temperature (59-86°F; 15-30°C) prior to testing.

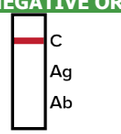
1. Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.
2. When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.
3. Label the device with the specimen's ID number.
4. Fill the capillary tube 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 capillary tube 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.
5. Set up the timer.
6. Results should be read in 15-20 minutes. Positive results may be visible as soon as 1 minute.

Negative results must be confirmed at the end of the 20 minutes only. **Any results interpreted outside 15-20 minutes should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.**



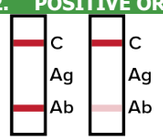
Results Interpretation

1. NEGATIVE OR NON-REACTIVE RESULT:

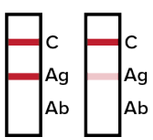


If only the C line develops, the absence of any color in both test lines (Ab and Ag) indicates that neither HIV antibodies nor HIV p24 antigen is detected in the specimen. The result is negative or non-reactive.

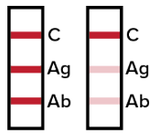
2. POSITIVE OR REACTIVE RESULT:



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



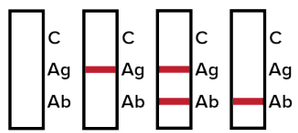
2.2 In addition to the presence of the C line, if the Ag line develops (including faint line), the test indicates the presence of HIV p24 antigen in the specimen. The result is HIV p24 antigen positive or reactive.



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

<u>Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a final diagnostic decision is made.</u>

3. INVALID:



If no C line develops, the assay is invalid regardless of any color development in the test lines as indicated below. Repeat the assay with a new device.

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:
   1. New operator uses the kit, prior to performing the testing of the specimens.
   2. A new lot of test kits is used.
   3. A new shipment of kits is used.
   4. The temperature used during storage of the kits falls outside of (35.6-86°F; 2-30°C).
   5. The temperature of the test area falls outside of (59-86°F; 15-30°C).
   6. To verify a higher than expected frequency of positive or negative results.
   7. To investigate the cause of repeated invalid results.

Limitations

1. The Procedure and the Interpretation of 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 lead to inaccurate results.
2. The Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette is limited to the qualitative detection of antibodies to HIV-1 and/or HIV-2 and HIV p24 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 negative or non-reactive result for an individual subject indicates absence of detectable anti-HIV-1, anti-HIV-2 and/or HIV p24 antigen. However, a negative or non-reactive test result does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2.
4. A negative or non-reactive result can occur if the quantity of the anti-HIV-1/anti-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. Infection may progress rapidly. If the symptoms persist while the result from Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette is negative ornon-reactive, it is recommended to test with alternative test methods.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

Performance Characteristics

1. Clinical Performance

A total of 350 clinical samples were collected and tested by the Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette and by an SFDA licensed HIV 1+2 Ab reference kit. Comparisons for all subjects are shown in the following table:

	Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette		
Reference	**Positive**	**Negative**	**Total**
Positive	105	0	105
Negative	0	245	245
Total	105	245	350

Relative Sensitivity: 100% (95% CI: 97.5-100%)
Relative Specificity: 100% (95% CI: 98.9-100%)
Overall Agreement: 100% (95% CI: 99.2-100%)

2. Specificity

The specificity of the Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette was evaluated with 1,000 specimens from a normal population and 200 specimens from pregnant women. No false positive results were detected.

3. Boston Biomedica Inc (BBI) Seroconversion Panel

The performance of the Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette 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	Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette	
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

4. Cross-Reactivity

No false positive antibodies to HIV-1 and/or HIV-2 or HIV p24 antigen test results were observed on 4-20 specimens from the following disease states or special conditions, respectively:
positive anti-HIV-1 and anti-HIV-2 results were observed on 3-19 specimens from the following disease states or special conditions, respectively:

HAV	HBV	HCV	HEV	*H. pylori*
TB	Syphilis	ANA	HAMA	RF (up to 2,500 IU/mL)

5. Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette. This was studied by spiking these substances into three levels of HIV Ag and HIV Ab standard controls (negative, weak positive, strong positive). The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the Rapid Response™ HIV Ag/Ab 4th Gen. Test Cassette.

List of potentially interfering substances and concentrations tested:

Bilirubin	20 mg/dL
EDTA	3.4 µmol/L
Glucose	55 mmol/L
Hemoglobin	2 g/L
Human IgG	150 mg/dL
Heparin	3,000 U/L
Salicylic acid	4.34 mmol/L
Sodium citrate	3.8%

Bibliography

1. Chang, SY, Bowman, BH, Weiss, JB, et al. The origin of HIV-1 isolate HTLV-IIIB.Nature (1993) 3/363:466-9.

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4. Janssen, RS, Satten, GA, Stramer, SL, et, al. 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.
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.

Glossary of Symbols

Consult instructions for use	Test per Kit	Do Not Reuse
Store between 35.6°F to 86°F	Use by	Catalogue #
Lot Number	For *in vitro* diagnostic use only	Manufacturer
Do not use if package is damaged		

BTNX Inc.
722 Rosebank Road,
Pickering, ON L1W 4B2
Canada
Technical Support: 1-888-339-9964