CTK BIOTECH SIMPLIFYING DIAGNOSTICS

REF

Catalog Number R0011C

IVD

In vitro Diagnostic

INTENDED USE

The *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of HIV-1 and HIV-2 antibodies (IgG, IgM, IgA) in human serum, plasma or whole blood. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HIV. Any reactive specimen with the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test must be confirmed with alternative testing method(s) such as ELISA or PCR.

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 virus and acquired immunodeficiency syndrome (AIDS) has been established over several 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].

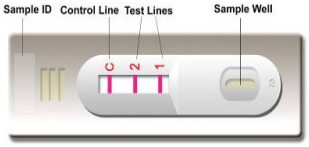
The two types of HIV have significant variation in sequences. HIV-1 has been divided into three groups: group M (for major) including at least ten subtypes (A through J); group O (for outlier); and group N (for non-M, non-O). Similarly, HIV-2 has been classified into at least five subtypes (A through E). Some HIV-1 variants share up to 50% homology in their envelope genes with the sequences of more common prototype strains.

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

The *OnSite* HIV 1/2 Ab Plus Combo Rapid Test was developed to detect and differentiate anti-HIV-1 and anti-HIV-2 (IgG, IgM, IgA) in serum, plasma or whole blood. The test can be performed by minimally trained personnel and without cumbersome laboratory equipment.

TEST PRINCIPLE

The *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant HIV-1 antigen conjugated with colloidal gold (HIV-1 conjugates), recombinant HIV-2 antigen conjugated with colloidal gold (HIV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (1 and 2) and a control line (C). Test line 1 is pre-coated with HIV-1 antigen for the detection of antibodies to HIV-1, test line 2 is pre-coated with HIV-2 antigen for the detection of antibodies to HIV-2, 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 strip. HIV-1 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-1 conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-1 antigen forming a burgundy colored line at test line 1, indicating a HIV-1 antibody positive or reactive test result. Lack of color development on test line 1 suggests an HIV-1 antibody negative or non-reactive result.

HIV-2 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-2 conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV-2 antigen forming a burgundy colored line at test line 2, indicating a HIV-2 antibody positive or reactive test result. Lack of color development on test line 2 suggests a HIV-2 antibody negative or non-reactive 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

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Capillary tubes (20 µL)
3. Sample diluent (REF SB-R0011, 5 mL/bottle)
4. Package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test

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 unless ready to conduct the assay.
3. Do not use expired devices or components.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood 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 bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as the patient specimens.
12. The test result should be read 15 minutes after a specimen is applied to the sample well of the device. Reading the test 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.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 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 the kit or expose the kit to temperatures above 30°C.

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 a 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. The specimens can be stored at 2-8°C 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 samples 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.

Whole blood specimens should be stored in refrigeration (2°C-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 thawed, mix the specimen well prior to performing the assay.

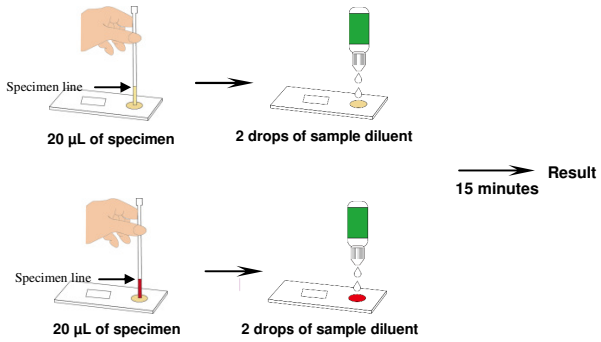
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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

Step 4: Fill the capillary tube with specimen (about 20 µL) not to exceed the specimen line as shown in the images below. **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 (60-80 µL) of sample diluent to the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Result should be read in 15 minutes. Positive results may be visible in as soon as 1 minute.

Do not 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 specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

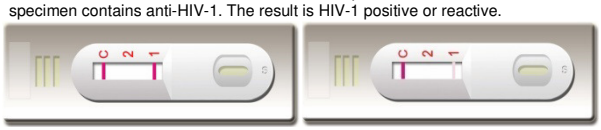
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30°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 RESULT:
If only the C line is developed, the test indicates that the level of anti-HIV-1 and anti-HIV-2 in the specimen is undetectable. The result is negative or non-reactive for HIV-1 and negative or non-reactive for HIV-2.

2. POSITIVE RESULT:

2.1 If both the C line and test line 1 are developed, the test indicates that the specimen contains anti-HIV-1. The result is HIV-1 positive or reactive.

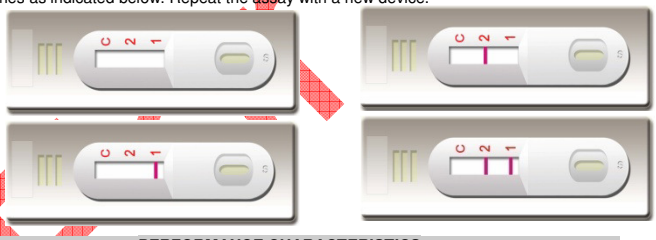


2.2 If both the C line and test line 2 are developed, the test indicates that the specimen contains HIV-2 antibodies. The result is HIV-2 positive or reactive.

2.3 If the C line and both test lines (1 and 2) are developed, the test indicates that the specimen contains anti-HIV-1 and anti-HIV-2. The result is HIV-1 positive or reactive and HIV-2 positive or reactive. *For differentiation of the type of HIV virus infection, see Limitations of Test section, Number 5.*

Samples with reactive results should be confirmed with alternative testing method(s) such as PCR or ELISA and clinical findings before a final diagnostic decision is made.

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



PERFORMANCE CHARACTERISTICS

1. Clinical Performance for HIV-1 Ab Test
A total of 1,308 samples from susceptible subjects were tested with the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test and with a commercial HIV-1 Ab EIA. Comparison for all subjects is shown in the following table.

	OnSite HIV 1/2 Ab Plus Combo Rapid Test		
EIA	**Positive**	**Negative**	**Total**
Positive	326	0	326
Negative	0	982	982
Total	326	982	1308

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

2. Clinical Performance for HIV-2 Ab Test
A total of 195 samples from susceptible subjects were tested with the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test and with a commercial HIV-2 Ab EIA. Comparison for all subjects is shown in the following table.

	OnSite HIV 1/2 Ab Plus Combo Rapid Test		
EIA	**Positive**	**Negative**	**Total**
Positive	20	0	20
Negative	0	175	175
Total	20	175	195

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

3. Cross-Reactivity
Cross-reactivity with specimens from other infectious diseases:

Specimen	Sample Size	HIV-1 Ab Reactivity	HIV-2 Ab Reactivity
HBsAg Positive Serum	10	Negative	Negative
HAV Positive Serum	10	Negative	Negative
HCV Positive Serum	10	Negative	Negative
Dengue Positive Serum	10	Negative	Negative
Syphilis Positive Serum	10	Negative	Negative
TB Positive Serum	10	Negative	Negative
H. pylori Positive Serum	10	Negative	Negative
ANA Positive Serum	8	Negative	Negative
HAMA Positive Serum	19	Negative	Negative
RF Positive Serum	3	Negative	Negative

4. Interference
Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test. This was studied by spiking these substances into three levels of HIV-1 Ab and HIV-2 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 *OnSite* HIV 1/2 Ab Plus Combo Rapid Test.

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

Potential Interfering Substances Spiked	HIV-1 Ab Reactivity			HIV-2 Ab Reactivity		
	Negative	Weak Positive	Strong Positive	Negative	Weak Positive	Strong Positive
Control	-	+	+++	-	+	+++
Bilirubin 20 mg/dL	-	+	+++	-	+	+++
Creatinine 442 µmol/L	-	+	+++	-	+	+++
Glucose 55 mmol/L	-	+	+++	-	+	+++
Albumin 60 g/L	-	+	+++	-	+	+++
Salicylic Acid 4.34 mmol/L	-	+	+++	-	+	+++
Heparin 3,000 U/L	-	+	+++	-	+	+++
EDTA 3.4 µmol/L	-	+	+++	-	+	+++

LIMITATIONS OF TEST

1. The Assay Procedure and Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to HIV in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
2. The *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is limited to the qualitative detection of HIV-1 or HIV-2 antibodies in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
3. A non-reactive result for an individual subject indicates absence of detectable HIV-1 or HIV-2 antibodies. However, a non-reactive test result does not preclude the possibility of exposure to or infection with HIV-1 or HIV-2.
4. A non-reactive result can occur if the quantity of the HIV-1 or HIV-2 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. As illustrated in the Interpretation of Assay Result, Section 2.3, all three test lines (1, 2 and C) may develop when tested with samples containing high titers of HIV-1 antibodies. To differentiate and to resolve antibody cross-reactivity, dilute the test specimen with sample diluent 1:50 or 1:100, then re-test the diluted specimen with a new test device. Only test line 1 and the C line will appear if the specimen contains antibodies to HIV-1. If test line 1, test line 2 and the C line all appear, the test indicates presence of antibodies to both HIV-1 and HIV-2.
6. If symptoms persist while the result from the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is non-reactive, it is recommended to re-sample the patient a few days later or to test with an alternative test method.
7. Unusually high titers of heterophile antibodies or rheumatoid factor in specimens may affect expected results.
8. The results obtained with this test should only be 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.

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
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