OnSite® HIV 1/2 Ab Plus Combo Rapid Test

REF R0011C

Instructions for Use

INTENDED USE

The *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of anti-HIV-1 and anti-HIV-2 antibodies (IgG, IgM, IgA) 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.

SUMMARY AND EXPLANATION OF THE TEST

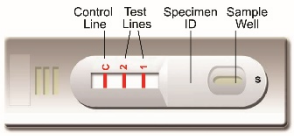
Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped, single-stranded, positive-sense RNA viruses. The causative relationship between HIV-1 and HIV-2 virus and acquired immunodeficiency syndrome (AIDS) has been established over several decades. HIV-1 has been isolated from 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 detects and differentiates anti-HIV-1 and anti-HIV-2 (IgG, IgM, IgA) in serum, plasma or whole blood. The test can be performed within 15 minutes by minimally skilled personnel and without the use of 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 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. Anti-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 colored line at test line 1, indicating an anti-HIV-1 antibody positive or reactive test result. Lack of color development on test line 1 suggests an anti-HIV-1 antibody negative or non-reactive result.

Anti-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 colored line at test line 2, indicating an anti-HIV-2 antibody positive or reactive test result. Lack of color development on test line 2 suggests an anti-HIV-2 antibody negative or non-reactive result.

The test contains an internal control (C line), which should exhibit a 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. Instructions 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. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could 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 15-20 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.
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 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/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and 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.

Whole Blood

Step 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 (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.

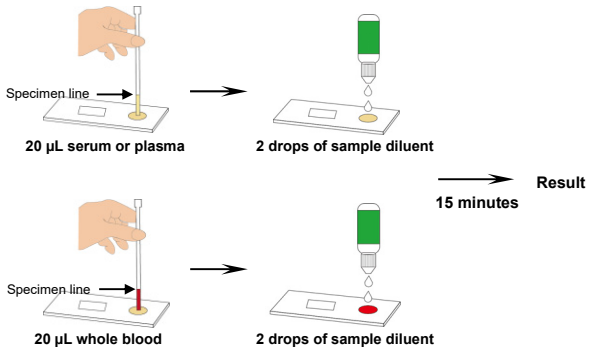
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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: Read results at 15 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, 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.***

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 develops the test indicates that there is no presence of HIV antibodies in the specimen. The result is anti-HIV-1 and anti-HIV-2 antibodies negative or non-reactive.

2. POSITIVE RESULT:

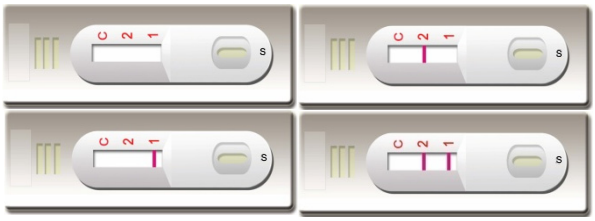
2.1 If both the C line and test line 1 develop, the test indicates that the specimen contains anti-HIV-1 antibodies. The result is HIV-1 positive or reactive.

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

2.3 If the C line and both test lines (1 and 2) develop, the test indicates that the specimen is HIV positive or reactive. To differentiate the type of HIV infection, dilute the sample with sample diluent 1:50 or 1:100 and begin the test again in a new test cassette. (See Limitations of Test section, No. 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.

3. INVALID: If no C line develops, 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

A total of 1,503 samples were collected from susceptible subjects and tested with the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test and with a commercial HIV 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	346	0	346
Negative	0	1157	1157
Total	346	1157	1503

Relative Sensitivity: 100% (95% CI: 99.2-100%)
Relative Specificity: 100% (95% CI: 99.8-100%)
Overall Agreement: 100% (95% CI: 99.8-100%)

2. Cross-Reactivity

No false positive anti-HIV-1 and anti-HIV-2 results were observed on 3-19 specimens from the following disease states or special conditions, respectively:

HBsAg	HAV	HCV	Dengue	Syphilis
TB	*H. pylori*	ANA	HAMA	RF (up to 8400 IU/mL)

3. 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 (negative, weak positive and strong positive) of anti-HIV-1 Ab and anti-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.

List of potentially interfering substances and concentrations tested:

1. Bilirubin	20 mg/dL	5. Salicylic acid	4.34 mmol/L
2. Creatinine	442 µmol/L	6. Heparin	3,000 U/L
3. Glucose	55 mmol/L	7. EDTA	3.4 µmol/L
4. Albumin	60 g/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 anti-HIV-1 or anti-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 negative or non-reactive result for an individual subject indicates absence of detectable anti-HIV-1 or anti-HIV-2 antibodies. However, a non-reactive or negative test result does not preclude the possibility of exposure to or infection with HIV-1 or HIV-2.
4. A negative or non-reactive result can occur if the quantity of the anti-HIV-1 or anti-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 anti-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. Infection may progress rapidly. If symptoms persist while the result from the *OnSite* HIV 1/2 Ab Plus Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
7. **Unusually high titers of heterophile antibodies or rheumatoid factor in specimens may affect expected results. False positive results can be obtained due to high levels of HAMA, RF or other unknown factors in the specimens. This may occur in less than 0.3% of tests performed.**
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, et al. The origin of HIV-1 isolate HTLV-IIIB. Nature (1993) 3/363:466-9.
2. Arya, SK, Beaver, B, Jagodzinski, L, et al. 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, 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.

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
2°C 30°C	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

PI-R0011C Rev E3.0
Date released: 2020-07-17
English version

For Export Only, Not For Re-sale in the USA.