OnSite® HIV/Syphilis Ab Combo Rapid Test

REF R0035C

Instructions for Use

INTENDED USE

The *OnSite* HIV/Syphilis Ab Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of antibodies to HIV-1, HIV-2 and *Treponema pallidum* (*Tp*) 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 and syphilis.

Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on 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

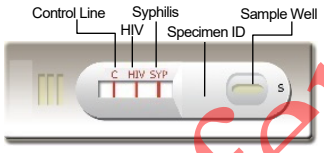
Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped, single-stranded, positive-sense RNA viruses. The two types of HIV have significant variation in sequence. HIV-1 has been divided into three groups: group M (for major), including at least ten subtypes (A through J); group 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). Worldwide, most HIV infections are HIV-1, whereas HIV-2 has largely been confined to persons in or from West Africa. HIV-1 and HIV-2 have the same routes of transmission with both causing acquired immunodeficiency syndrome (AIDS)[1-2].

Tp is a spirochete bacterium causing the sexually transmitted disease syphilis, rates of which have been increasing according to the CDC. In 1995, WHO reported 12 million new cases of syphilis[3]. Recent evidence suggests that STDs increase HIV shedding in the genital tract of HIV positive individuals making early syphilis detection of great importance.

Serological tests detecting antibodies to HIV viruses and *Tp* are commonly used by clinical laboratories as evidence of infection to aid in the diagnosis of AIDS and/or Syphilis. Given the increasing rates of Syphilis among HIV-infected patients, periodic annual screening (2 to 4 times yearly among high-risk groups, e.g. MSM) is strongly recommended[4]. HIV testing is critical for all patients with a new diagnosis of syphilis[4].

The *OnSite* HIV/Syphilis Ab Combo Rapid Test can simultaneously detect antibodies to HIV-1, HIV-2 and *Tp* in patient serum, plasma or whole blood within 15 minutes. The test can be performed by personnel with minimal training without cumbersome laboratory equipment.

TEST PRINCIPLE



The *OnSite* HIV/Syphilis Ab Combo Rapid Test is a lateral flow immunochromatographic assay. The test cassette consists of: 1) a colored conjugate pad containing HIV 1+2 antigens conjugated with colloidal gold (HIV 1+2 conjugates), recombinant *Tp* antigens conjugated with colloidal gold (*Tp* conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (IV line and SY line) and a control line (C line). The IV line is pre-coated with HIV 1+2 antigen, the SY line is pre-coated with recombinant *Tp* antigens 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 cassette, it migrates by capillary action across the cassette. HIV-1 or HIV-2 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV 1+2 conjugates. The immunocomplex is then captured on the membrane by the pre-coated HIV 1+2 antigens forming a colored IV line, indicating a HIV 1+2 positive or reactive test result. Absence of the IV line suggests an HIV-1 and HIV-2 antibody negative or non-reactive test result.

Similarly, if anti-*Tp* antibodies are present in the specimen, they will bind to the *Tp* conjugates. The immunocomplex is then captured on the membrane by the pre-coated *Tp* antigen forming a colored SY line, indicating a *Tp* antibody positive test result. Absence of the SY line suggests a *Tp* 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. Plastic droppers
3. Sample diluent (REF SB-R0035, 5 mL/bottle)
4. Instructions for use

MATERIALS MAY BE REQUIRED AND AVAILABLE FOR PURCHASE

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.
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 specimens 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 patient specimens.
12. The test results should be read 15-20 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device 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 temperature 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 thawed, mix the specimen well prior to performing the assay.

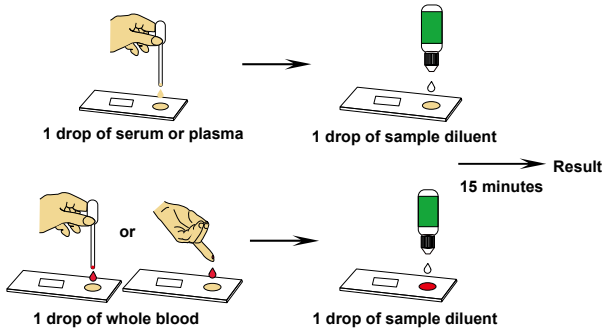
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

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

Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drops of serum/plasma (about 30-45 µL) or 1 drop of whole blood (about 40-50 µL) into the center of the sample well, making sure that there are no air bubbles.

Immediately add 1 drop (about 35-50 µL) of sample diluent into the center of the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Results should be read at 15 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

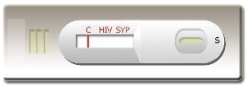
QUALITY CONTROL

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 whole procedure and repeat 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 kits is used.
   d. The temperature 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 neither anti-HIV nor anti-*Tp* antibodies are present in the specimen. The result is negative or non-reactive.



2. **POSITIVE OR REACTIVE RESULT:**

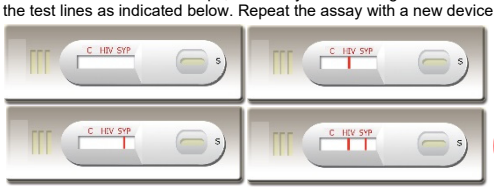
2.1 If both the C and the HIV lines develop, the test indicates the presence of anti-HIV antibodies in the specimen. The result is anti-HIV antibodies positive or reactive.

2.2 If both the C and the SYP lines develop, the test indicates the presence of anti-*Tp* antibodies in the specimen. The result is anti-*Tp* antibody positive or reactive.

2.3 In addition to the presence of the C line, if both the HIV and the SYP lines develop, the result is both anti-HIV antibody and anti-*Tp* antibody positive or reactive.

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

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

1.1 **For the HIV 1+2 Ab Test**

A total of 410 specimens were collected from susceptible subjects and normal healthy control subjects and tested by the *OnSite* HIV/Syphilis Ab Combo Rapid Test and by a commercial HIV1+2 Ab rapid test as reference. Comparison for all subjects is shown in the following table:

	OnSite HIV/Syphilis Ab Combo Rapid Test		
HIV 1+2 Ab Reference	**Positive**	**Negative**	**Total**
Positive	110*	0	110
Negative	0	300	300
Total	110	300	410

*100 HIV-1 positive and 10 HIV-2 positive
Relative Sensitivity: 100% (95% CI: 96.6-100%)
Relative Specificity: 100% 95% CI: 98.7-100%)
Overall Agreement: 100% (95% CI: 99.1-100%)

1.2 **For the Syphilis Ab Test**

A total of 400 specimens were collected from susceptible subjects and normal healthy control subjects and tested by the *OnSite* HIV/Syphilis Ab Combo Rapid Test and by a commercial Syphilis Ab rapid test as reference, and verified by leading ELISA kit. Comparison for all subjects is shown in the following table:

	OnSite HIV/Syphilis Ab Combo Rapid Test		
Syphilis Ab Reference	**Positive**	**Negative**	**Total**
Positive	103	0	103
Negative	3	294	297
Total	106	294	400

Relative Sensitivity: 100% (95% CI: 96.4-100%)
Relative Specificity: 99.0% (95% CI: 97.1-99.7%)
Overall Agreement: 99.3% (95% CI: 97.8-99.7%)

2. **Cross-Reactivity**

No false positive results in related panels were observed on 10 specimens from the following disease states or special conditions, respectively:

Dengue	HAV	HBV	HCV
HEV	HIV	*H. pylori*	*T. pallidum*
TB	ANA	HAMA	RF (up to 8400 IU/mL)
Pregnant women			

3. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* HIV/Syphilis Ab Combo Rapid Test. This was studied by spiking these substances into negative, weak positive, and medium positive specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of each panel member of the *OnSite* HIV/Syphilis Ab Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Bilirubin	20 mg/dL	5. Heparin	3000 U/L
2. Creatinine	442 µmol/L	6. Human IgG	1000 mg/L
3. EDTA	3.4 µmol/L	7. Salicylic Acid	4.34 mmol/L
4. Glucose	55 mmol/L	8. Sodium Citrate	1.5%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of anti-HIV 1+2 and anti-*Tp* antibodies in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* HIV/Syphilis Ab Combo Rapid Test is limited to the qualitative detection of anti-HIV and anti-*Tp* antibodies in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable anti-HIV and/or anti-*Tp* antibody. However, a negative test result does not preclude the possibility of exposure to or infection with HIV and/or *Tp*.
4. A negative result can occur if the quantities of the anti-HIV and/or anti-*Tp* antibodies present in the specimen are below the detection limits of the assay or the antibodies are not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptoms from any of the 4 individual infections persist, even if the test results from the *OnSite* HIV/Syphilis Ab Combo Rapid Test were negative or non-reactive, it is recommended to test with an alternative test method for that particular infection.
6. The *OnSite* HIV/Syphilis Ab Combo Rapid Test has not been validated on specimens from neonates.
7. Some specimens contain unusually high titers of heterophile antibodies or rheumatoid factor, which 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. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever. The emergence of a global health problem. Emerg Infect Dis. 1995;1(2):55–57.
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3. Gerbase, A. C., J. T. Rowley, D. H. Heymann, S. F. Berkley, and P. Piot. Global prevalence and incidence estimates of selected curable STDs. Sex. Transm. Infect 1998; 74:S12-S16.
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5. Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines.*cMMWR Recomm Rep*. 2006:55:1-94
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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	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-R0035C Rev. D3.0
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English version

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