OnSite™ HSV-2 IgG/IgM Rapid Test

REF R0213C CE

INTENDED USE

The *OnSite* HSV-2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of antibodies (IgG and IgM) to herpes simplex virus 2 (HSV-2) 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 HSV-2.

SUMMARY AND EXPLANATION OF THE TEST

Herpes simplex viruses are two types of DNA viruses of the Herpesviridae family, herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2)[1]. HSV-1 is generally acquired during childhood via non-sexual contact and affects mainly the orofacial area. HSV-2 is nearly always sexually transmitted and is the main cause of genital herpes. HSV-1 and HSV-2 can infect both genital and orofacial areas[1]. Up to 50% of first-episode cases of genital herpes are caused by HSV-1, but recurrences are much less frequent for genital HSV-1 infection than genital HSV-2 infection[2]. HSV subclinical viral shedding are less frequent for genital HSV-1 than genital HSV-2[2]. Genital HSV infection has also been associated with increased risk for sexual transmission of HIV[2,3]. After primary infection, these viruses persist in a latent state for life[1].

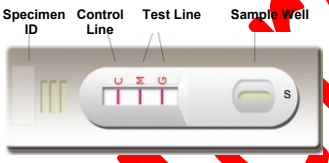
One of the biggest risks associated with HSV is neonatal transmission[1]. The majority of the transmissions occur in the pregnant woman with primary HSV infection. Eighty-five to ninety percent of neonatal transmission occurs at the time of delivery with only 5% of infections occurring intrauterine[4]. Clinical manifestations of neonatal infection with HSV range from local lesions of the skin, mouth, eye or central nervous system to severe, widespread dissemination involving visceral organs and potentially death[5].

Serology is an effective means of diagnosing HSV because the manifestation of symptoms is transient and the infection is often undiagnosed[5]. IgM anti-HSV can be detected 9-10 days after exposure and last for 7-14 days, although it may remain detectable for up to 6 weeks[6]. IgM anti-HSV is often associated with primary infection but may be detectable during recurrence of the disease[6]. IgG anti-HSV can be detected 21-28 days post exposure and detectable titers typically remain for life[6]. Detection of IgM anti-HSV in the absence of IgG anti-HSV can be an effective tool in detecting early stages of HSV infection and as an indicator of potential primary infection.

HSV-1 and HSV-2 infections have different prognoses. Type-specific serological diagnosis is beneficial, which can be achieved by using glycoprotein G1 and glycoprotein G2 as recommended by the CDC[7].

The *OnSite* HSV-2 IgG/IgM Rapid Test uses HSV-2 glycoprotein G2 for the specific detection and differentiation of IgG and IgM antibodies to HSV-2 in serum, plasma and whole blood. The test can be performed in 10 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* HSV-2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing HSV-2 type specific glycoprotein G2 antigens conjugated with colloidal gold (HSV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with mouse anti-human IgG for detection of IgG anti-HSV-2, the M line is pre-coated with mouse anti-human IgM for detection of IgM anti-HSV-2, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen and sample diluent are dispensed into the sample well, the specimen migrates by capillary action across the cassette. IgG anti-HSV-2, if present in the specimen, will bind to the HSV-2 conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a burgundy colored G line, indicating an HSV-2 IgG positive test result. IgM anti-HSV-2, if present in the specimen, will bind to the HSV-2 conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgM forming a burgundy colored M line, indicating an HSV-2 IgM positive test result.

Absence of any test lines (G or M) suggests a 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 (G and M). If no control line (C line) develops, 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. 10 µL capillary tubes
3. Sample diluent (REF SB-R0213C, 5 mL/bottle)
4. One 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 testing

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 expired devices or components.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use components from any other 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 patient specimens.
12. The test result should be read within 10 minutes after a specimen is applied to the sample well of the device. Reading the result after 15 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 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.

Whole 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 (2-8°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.

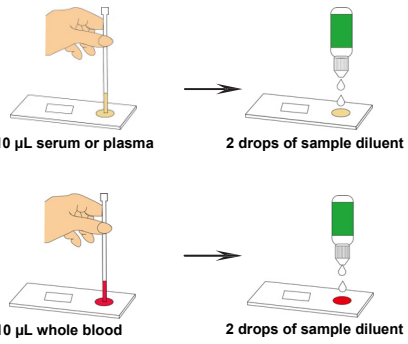
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Fill the capillary tube with specimen not exceeding the specimen line as shown in the images below. The volume of specimen is approximately 10 µL.
For better precision, transfer specimen using a pipette capable of delivering a 10 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.



Step 5: Set up the timer.

Step 6: Result should be read in 10 minutes. Positive results may be visible as soon as 1 minute.

Do not read the result after 15 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 test kits is used.
d. The temperature used during storage of the kits 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 anti-HSV-2 antibodies are not detected in the specimen. The result is negative or non-reactive.

2. POSITIVE RESULT

2.1 In addition to the presence of the C line, if only the G line develops, the test result indicates the presence of IgG anti-HSV-2; the result is HSV-2 IgG positive or reactive.

2.2 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of IgM anti-HSV-2. The result is HSV-2 IgM positive or reactive.

2.3 In addition to the presence of C line, if both the G and M lines develop, the test indicates the presence of IgG anti-HSV-2 and IgM anti-HSV-2. The result is HSV-2 IgG and HSV-2 IgM positive or reactive.

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

3. INVALID

If no C line develops, the assay is invalid regardless of any color development on the test lines (G and M) as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Accuracy of IgG Detection

A total of 214 specimens were collected and tested with the *OnSite* HSV-2 IgG/IgM Rapid Test and by a commercial IgG anti-HSV-2 ELISA. Comparison for all subjects is shown in the following table:

	OnSite HSV-2 IgG/IgM Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	60	4	64
Negative	6	144	150
Total	66	148	214

Relative Sensitivity: 93.8%, Relative Specificity: 96.0%, Overall Agreement: 95.3%

2. Performance on BBI Anti-Herpes Mixed Titer Performance Panel

The performance of the *OnSite* HSV-2 IgG/IgM Rapid Test was evaluated using the BBI(Boston Biomedica Inc) Anti-Herpes Mixed Titer Performance Panel (PTH202). The results are shown in the following table:

BBI Reference Panel	*OnSite* HSV-2 IgG/IgM Rapid Test	
	Number of Panels	Number of Agreement
IgG HSV-2 Positive Only	7	4
IgG HSV-1 Positive Only	10	10
IgG HSV-1 and HSV2 Positive	2	2
IgM HSV-2 Positive	4	4
IgM HSV-1 Positive	3	3

3. Positive Rate on the Random Clinical Specimens

One thousand random, clinical specimens were tested with the *OnSite* HSV-2 IgG/IgM Rapid Test. The positive rate was 4.6% for IgG anti-HSV-2 and 1.7% for IgM anti-HSV-2.

4. Cross-Reactivity

No false positive IgG and IgM HSV-2 results were observed on 3-10 specimens from the following disease states or special conditions, respectively:

CMV	Dengue	HAV	HBV	HCV
HEV	HIV	hCG	*H. pylori*	HSV-1
Malaria	Rubella	TB	Toxoplasma	Typhoid
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 *OnSite* HSV-2 IgG/IgM Rapid Test. This was studied by spiking these substances into negative, IgG positive and IgM positive specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *OnSite* HSV-2 IgG/IgM Rapid Test.

List of potentially inferring substances and concentrations tested:

1. Albumin	60 g/L	6. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Salicylic acid	4.24 mmol/L
4. EDTA	3.4 µmol/L	9. Sodium citrate	3.8%
5. Glucose	55 mmol/L		

EXPECTED VALUES

HSV-2 infects over 500 million people worldwide, with an estimated 23 million new infections annually. Seroprevalence ranges from 3.2% in some Chinese populations to over 80% in some areas of sub-Saharan Africa[8,9]. Seroprevalence in women is up to twice as high as men, and increases with age. Most people are not aware of the infection, and infection is widespread even among people with low or moderate levels of sexual activity[9].

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 HSV-2 in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* HSV-2 IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to HSV-2 in human serum, plasma or whole blood. The intensity of the test line does not have linear correlation with the titer of HSV-2 antibody in the specimen.
3. A negative or non-reactive test result does not preclude the possibility of exposure to or infection with HSV-2. A negative or non-reactive result can occur if the titer of HSV-2 antibody present in the specimen is below the level detectable by the assay or if HSV-2 antibody was not present during the stage of disease in which the sample was collected.
4. A negative result does not rule out a infection with HSV-2. Samples collected too early in the course of an infection may not have detectable levels of IgM.
5. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* HSV-2 IgG/IgM Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method or to re-test the patient a few days later.
6. The *OnSite* HSV-2 IgG/IgM Rapid Test has not been validated on specimens from neonates.
7. Specimens from patients with infectious mononucleosis or high titers of heterophile antibodies, rheumatoid factor (>2,500 IU/mL) may affect expected results.
8. Any use or interpretation of this preliminary test results 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.

REFERENCES

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Index of CE Symbols

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

CTK Biotech, Inc.
10110 Mesa Rim Road
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