***OnSite*®** HSV-2 IgG/IgM Rapid Test

REF R0213C CE

Instructions for Use

INTENDED USE

The *OnSite* HSV-2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of IgG and IgM antibodies to herpes simplex virus 2 (HSV-2) in human serum, plasma or whole blood. It is intended to be used by professionals as a preliminary test result to aid in the diagnosis of infection with HSV-2.

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

Herpes simplex viruses are two types of DNA viruses of the Herpesviridae family, herpes simplex virus-1 (HSV-1) and 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]. Anti-HSV IgM 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]. Anti-HSV IgM is often associated with primary infection but may be detectable during recurrence of the disease[6]. Anti-HSV IgG can be detected 21-28 days post exposure and detectable titers typically remain for life[6]. Detection of anti-HSV IgM in the absence of anti-HSV IgG 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 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 anti-HSV-2 IgG, the M line is pre-coated with mouse anti-human IgM for detection of anti-HSV-2 IgM, and the C line is pre-coated with a control line antibody.
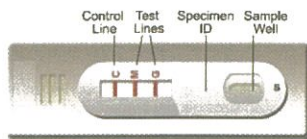
When an adequate volume of test specimen and sample diluent are dispensed into the sample well, the specimen migrates by capillary action across the cassette. Anti-HSV-2 IgG, if present in the specimen, will bind to the HSV-2 conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming a colored G line, indicating an HSV-2 IgG positive test result. Anti-HSV-2 IgM, 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 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 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-R0213, 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 testing

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 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 results should be read 10-15 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 10-15 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. 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/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 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.


10 µL serum or plasma   2 drops of sample diluent

Result
10 minutes

10 µL whole blood   2 drops of sample diluent
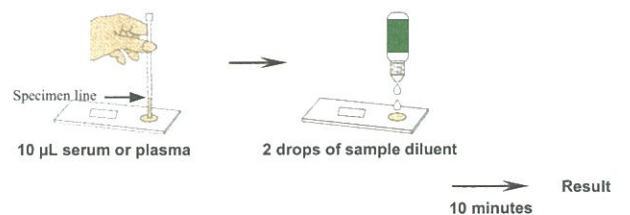
Step 5: Set up the timer.

Step 6: Results should be read at 10 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***Any results interpreted outside of the 10-15 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 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. 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.
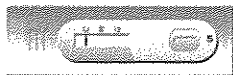
2. **POSITIVE RESULT:**

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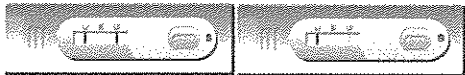
2.2 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-HSV-2 IgM. The result is HSV-2 IgM positive or reactive.


2.3 In addition to the presence of C line, if both the G and M lines develop, the test indicates the presence of anti-HSV-2 IgG and IgM. The result is HSV-2 IgG and HSV-2 IgM 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 any color development on the test lines (G and M) as indicated below. Repeat the assay with a new device.
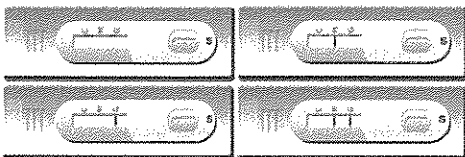
PERFORMANCE CHARACTERISTICS

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 anti-HSV-2 IgG ELISA. Comparison for all subjects is shown in the following table:

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

Relative Sensitivity: 93.8% (95% CI: 84.8-98.3%),
Relative Specificity: 96.0% (95% CI: 91.5-98.5%),
Overall Agreement: 95.3% (95% CI: 91.6-97.7%)

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

4. **Cross-Reactivity**
No false positive anti-HSV-2 IgG and IgM 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[6].

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 anti-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 anti-HSV-2 antibody present in the specimen is below the level detectable by the assay or if anti-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 an 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

1. Kimberlin DW. Neonatal herpes simplex infection. Clin Microbiol Rev 2004. 17(1):1-13.
2. LeGoff J, Péré H, Bélec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. Virol J. 2014. 11(1):83.
3. Laderman EI, Whitworth E, Dumaual E, et al. Rapid, sensitive, and specific lateral-flow immunochromatographic point-of-care device for detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in serum and whole blood. Clin Vaccine Immunol 2008. 15(1):159-63.
4. Brown ZA, Wald A, Morrow RA, et al. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. J Am Med Assoc 2003. 289(2):203-9.
5. Starface G, Selmin A, Zanardo V, et al. Herpes simplex virus infection in pregnancy. Infect Dis Obstet Gynecol 2012.
6. Page J, Taylor J, Tideman RL, et al. Is HSV serology useful for the management of first episode genital herpes? Sex Transm Infect 2003. 79(4):276-9.
7. Workowski KA, Levine WC. Sexually Transmitted Diseases Treatment Guidelines. CDC 2002. 51(RR06):1-80.
8. Clo JY, Lim WWL, Ho DWT, et al. Difference in seroprevalance of herpes simplex virus type 2 infection among antenatal women in Hong Kong and southern China. Sex Transm Infect 1999. 75(2):123.
9. Johnston C, Koelle DM, Wald A. HSV-2: in pursuit of a vaccine. J Clin Invest 2011. 121(12):4600-9.

Index of CE Symbols

[i]	Consult instructions for use	IVD	For *in vitro* diagnostic use only	⌛	Use by
REF	Catalog #	LOT	Lot Number	∇ₙ	Tests per kit
❄30°C 2°C	Store between 2-30°C	EC REP	Authorized Representative	⊘	Do not reuse
🏭	Manufacturer	~	Date of manufacture		