OnSite™ Duo HSV-1/2 IgG/IgM Rapid Test

REF R0218C CE

Instructions for Use

INTENDED USE

The *OnSite* Duo HSV-1/2 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 1 (HSV-1) and herpes simplex virus 2 (HSV-2) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals aid in the diagnosis of infection with HSV-1 and HSV-2.

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

Herpes simplex viruses are two types of DNA viruses of the *Herpesviridae* family, 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 is 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 rate of neonatal transmission is higher in mothers with genital HSV-1 than in those with genital HSV-2[4]. Eighty-five to ninety percent of neonatal transmission occurs at the time of delivery with only 5% of infections occurring intrauterine[5]. 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[1].

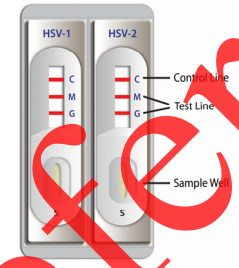
Serology is an effective means of diagnosing HSV because the manifestation of symptoms is transient and the infection is often undiagnosed[1]. Anti-HSV IgM can be detected 9-10 days after exposure and last for 7-14 days, although it may remain detectable for up to 6 weeks[6]. Anti-HSV IgM is often associated 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 and can be achieved by using glycoprotein G1 and glycoprotein G2 as recommended by the CDC[7].

The *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test uses HSV-1 glycoprotein G1 and HSV-2 glycoprotein G2 for the specific detection and differentiation of IgG and IgM antibodies to HSV-1 and 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* Duo HSV-1/2 IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay consisting of 2 cassettes assembled in one panel (left side: HSV-1 IgG/IgM Rapid Test; right side: HSV-2 IgG/IgM Rapid Test).



The HSV-1 IgG/IgM Rapid Test consists of: 1) a colored conjugate pad containing HSV-1 type specific glycoprotein G1 antigens conjugated with colloidal gold (HSV-1 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-1 IgG, the M line is pre-coated with mouse anti-human IgM for detection of anti-HSV-1 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-1 IgG, if present in the specimen, will bind to the HSV-1 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-1 IgG positive test result. Anti-HSV-1 IgM, if present in the specimen, will bind to the HSV-1 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-1 IgM positive test result.

The HSV-2 IgG/IgM Rapid Test 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. An invalid result in one panel does not invalidate the test result in the other panel.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouch containing:
   a. One cassette device
   b. One desiccant
2. 10 µL capillary tubes
3. Sample diluent (REF SB-R0218, 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 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 another test kit to substitute for components of 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. 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 negative and positive controls in the same manner as patient specimens.
12. Read test results 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, e.g. 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.

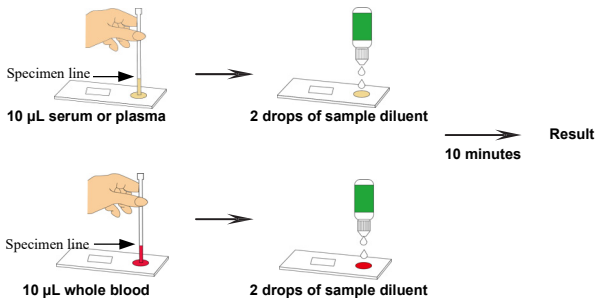
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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.



Step 5: Set up the timer.

Step 6: Result should be read at 10 minutes. Positive results may be visible in as soon as 1 minute. Negative results must be confirmed at the end of 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

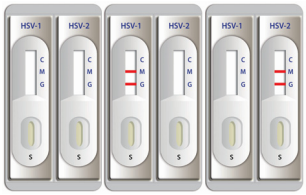
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.
2. **External Control:** Good Laboratory Practice 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 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 neither anti-HSV-1 nor ant-HSV-2 antibodies are not detected in the specimen. The result is both anti-HSV-1 and anti-HSV-2 IgG and IgM antibodies negative or non-reactive.

2. **INVALID:** If no C line develops, the assay is invalid regardless of any color in the G or M lines as indicated below. Repeat the assay with a new device.



3. **POSITIVE RESULT:**

IgM Positive | IgG Positive | IgM/IgG Positive

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

PERFORMANCE CHARACTERISTICS

1. **Accuracy of HSV-1 IgG Detection**

A total of 227 specimens were collected and tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test and by a commercial anti-HSV-1 IgG ELISA. Comparison for all subjects is shown in the following table:

	OnSite Duo HSV-1/2 IgG/IgM Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	174	18	192
Negative	3	32	35
Total	177	50	227

Relative Sensitivity: 90.6%, Relative Specificity: 91.4%, Overall Agreement: 90.7%

2. **Accuracy of HSV-2 IgG Detection**

A total of 214 specimens were collected and tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test and by a commercial anti-HSV-2 IgG ELISA. Comparison for all subjects is shown in the following table:

	OnSite Duo HSV-1/2 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%

3. **Accuracy of IgM Detection**

A total of 107 specimens were collected and tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test and by a commercial HSV-1 IgM ELISA. The overall agreement between the two products was 85.0%.

A total of 21 specimens from the BBI (Boston Biomedica Inc) Anti-Herpes Mixed Titer Performance Panel (PTH202) were tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test. The *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test showed 100% agreement with the panel.

4. **Positive Rate on Random Clinical Specimens**

Ten thousand random, clinical specimens were tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test. The positive rate was 92.8% for anti-HSV-1 IgG and 4.9% for anti-HSV-1 IgM.

Ten thousand random, clinical specimens were tested with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test. The positive rate was 4.6% for anti-HSV-2 IgG and 1.7% for anti-HSV-2 IgM.

5. **Cross-Reactivity**

No false positive anti-HSV-1 or anti-HSV-2 IgG and IgM results were observed on 3-10 specimens from the following disease states or special conditions, respectively:

T. palladium	*H.pylori*	Dengue	Malaria	Typhoid
Toxoplasma	Rubella	CMV	hCG	TB
HAV	HBV	HCV	HEV	HIV
ANA	HAMA	RF (up to 1,000 IU/mL)		

6. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* Duo HSV-1/2 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* Duo HSV-1/2 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

In non–high-risk populations, HSV-1 prevalence tends to increase with age, with acquisition occurring primarily in childhood and adolescence. Prevalence commonly reaches 40% by the age of 15, before increasing to 60-90% in older adults. In a given population and age group, HSV-1 prevalence is nearly always higher than HSV-2 prevalence[8]. Although genital herpes is primarily associated with HSV-2, an increasing proportion of genital herpes is caused by HSV-1, particularly in Europe[9]. Clinical studies report detection of anti-HSV-1 IgM and IgG in 5.9% and 93.2% of patients, respectively[10].

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[8].

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-1 and HSV-2 in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to HSV-1 and 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-1 or anti-HSV-2 antibodies in the specimen.
3. A negative or non-reactive test result does not preclude the possibility of exposure to or infection with HSV-1 or HSV-2. A negative or non-reactive result can occur if the titer of anti-HSV-1 or anti-HSV-2 antibodies present in the specimen is below the level detectable by the assay or if anti-HSV-1 or anti-HSV-2 antibodies were 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-1 or HSV-2. Samples collected too early in the course of an infection may not have detectable levels of IgM.
5. Co-infection with both HSV-1 and HSV-2 can exist clinically[11], however it is rare for a patient to be positive for both anti-HSV-1 and anti-HSV-2 IgM simultaneously. A number of factors can lead to false positive results for anti-HSV-1 and anti-HSV-2 IgM and for this reason. It is recommended when a positive result for both anti-HSV-1 and anti-HSV-2 IgM are seen concurrently on the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test, confirmatory test methods, such as culture or PCR, should be taken.
6. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test is negative or non-reactive, it is recommended to re-test the patient a few days later or test with an alternative test method.
7. The *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test has not been validated on specimens from neonates.
8. Specimens from patients with infectious mononucleosis or high titers of heterophile antibodies, rheumatoid factor (>1,000 IU/mL) may affect expected results.
9. Results obtained with the *OnSite* Duo HSV-1/2 IgG/IgM Rapid Test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of CE 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	EC REP	Authorized Representative		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

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R0218C Rev. AP1.0
Date released: 2020-02-28
English Version

For Export Only. Not For Resale in the USA.