OnSite® Chikungunya IgM Combo Rapid Test

REF R0066C CE

Instructions for Use

INTENDED USE

The *OnSite* Chikungunya IgM Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of anti-chikungunya (CHIK) virus IgM in human serum, plasma or whole blood. It is intended to be used by healthcare professionals as an aid in the diagnosis of infection with chikungunya virus.

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

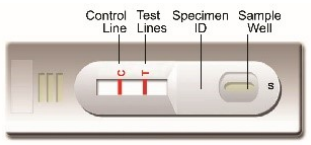
Chikungunya is a rare viral infection transmitted by the bite of an infected *Aedes aegypti* mosquito. It is characterized by a rash, fever and severe joint pain (arthralgias) that usually lasts for three to seven days. The name is derived from the Makonde word meaning "that which bends up" in reference to the stooped posture developed as a result of the arthritic symptoms of the disease. It occurs during the rainy season in tropical areas of the world, primarily in Africa, South-East Asia, southern India and Pakistan[1-2]. However, over the past decade it has also appeared in the South Pacific, southern Europe, and Central America[3].

The symptoms are most often clinically indistinguishable from those observed in dengue fever. Unlike dengue, hemorrhagic manifestations are relatively rare and most often the disease is a self-limiting, febrile illness. Dual infection of dengue and chikungunya is also possible as has been reported in India[4]. Therefore, it is very important to clinically distinguish dengue from chikungunya infection.

Chikungunya is diagnosed based on serological analysis and viral isolation in mice or tissue culture. An IgM immunoassay is the most practical lab test method[5].

The *OnSite* Chikungunya IgM Combo Rapid Test detects Anti-CHIK IgM in serum, plasma or whole blood. The test can be performed within 10 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Chikungunya IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The strip in the test cassette consists of: 1) a colored conjugate pad containing recombinant CHIK antigens conjugated with colloidal gold (CHIK conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with anti-human IgM 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, the specimen migrates by capillary action across the cassette. The anti-CHIK IgM, if present in the specimen, will bind to the CHIK conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a colored T line, indicating an anti-CHIK IgM positive test result.

Absence of the T line 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 the color development on the T line. 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 (5 µL)
3. Sample diluent (REF SB-R0066, 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

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 components from 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 10-15 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside 10-15 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 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 the test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

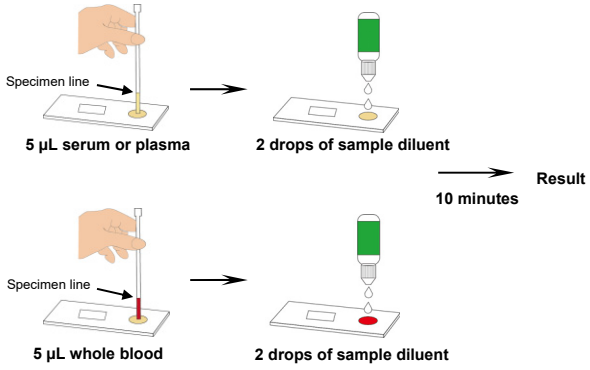
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Fill the capillary tube with specimen (about 5 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 5 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 10 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***However, any results interpreted outside 10-15 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 the specimen and sample diluent. If the C line does not develop, review the whole 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 specimens.
   b. A new lot of kits is used.
   c. A new shipment of kits is used.
   d. The temperature during storage of the kits fall 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 is present, the absence of any color in the test line indicates that Anti-CHIK IgM is not detectable in the specimen. The result is Anti-CHIK IgM negative or non-reactive.

2. **POSITIVE RESULT**: If both the C and the T lines develop, the test indicates the presence of Anti-CHIK IgM in the specimen. The result is Anti-CHIK 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 color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 523 specimens were collected from susceptible subjects and normal healthy subjects in endemic areas and tested by the *OnSite* Chikungunya IgM Combo Rapid Test and by a commercial ELISA test. Comparison for all subjects is shown in the following table:

	OnSite Chikungunya IgM Combo Rapid Test		
ELISA Reference	**Positive**	**Negative**	**Total**
Positive	66	7	73
Negative	9	441	450
Total	75	448	523

Relative Sensitivity: 90.4% (95% CI: 81.5-95.3%)
Relative Specificity: 98.0% 95% CI: 96.2-98.9%)
Overall Agreement: 96.9% (95% CI: 95.1-98.1%)

2. **Cross-Reactivity**

No false positive results in related panels were observed on 10-40 specimens from the following disease states or special conditions, respectively:

Dengue	HAV	HBV	HCV
HIV	*H. pylori*	Malaria	*T. pallidum*
TB	Zika	ANA	HAMA
Pregnant woman	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* Chikungunya IgM 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* Chikungunya IgM Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Acetominophen	20 mg/dL	7. Heparin	3000 U/L
2. Ascorbic acid	20 mg/dL	8. Human IgG	1000 mg/dL
3. Bilirubin	20 mg/dL	9. Glucose	55 mmol/L
4. Creatinine	442 µmol/L	10. Salicylic acid	4.34 mmol/L
5. EDTA	3.4 µmol/L	11. Sodium citrate	3.8%
6. Hemoglobin	2 g/L		

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-CHIK IgM in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Chikungunya IgM Combo Rapid Test is limited to the qualitative detection of anti-CHIK IgM in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-CHIK IgM. However, a negative test result does not preclude the possibility of exposure to or infection with chikungunya.
4. A negative or non-reactive result can occur if the quantity of anti-CHIK IgM present in the specimen is below the detection limit of the assay or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* Chikungunya IgM Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Shah KV, Gibbs CJ Jr, Banerjee G. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: isolation of three strains of Chikungunya virus. Indian J Med Res 1964; 52 :676-83.
2. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. J Gen Virol 2000;81:471-9.
3. Horwood PF, Buchy P. Chikungunya. Rev Sci Tech. 2015 Aug;34(2):479-89.
4. Myers RM and Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science 1967;157:1307-8.
5. Thein S, La Linn M, Aaskov J, Aung MM, Aye M, Zaw A, Myint A. Development of asimple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulinM antibody in serum from patients Following an outbreak of chikungunya virus infection in Yangon, Myanmar. Trans R Soc Trop Med Hyg. 1992 86:438-42.

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	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-R0066C Rev. E2.0
Date released: 2020-09-24
English Version

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