CE

CTK Biotech, Inc.
The New Wave of Diagnosis

REF

Catalog Number R0066C

IVD

In vitro Diagnostic

INTENDED USE

The *OnSite* Chikungunya IgM Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of IgM anti-chikungunya virus （CHIK） in human serum, plasma or whole blood. It is intended to be used as a screening test and as an aid in the diagnosis of infection with CHIK. Any reactive specimen with the *OnSite* Chikungunya IgM Combo Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

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].

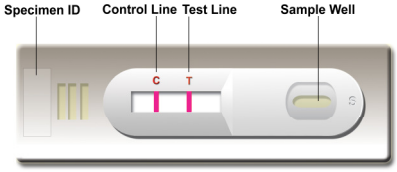
The symptoms are most often clinically indistinguishable form those observed in dengue fever. Indeed, dual infection of dengue and chikungunya has been reported in India[3]. Unlike dengue, hemorrhagic manifestations are relatively rare and most often the disease is a self limiting febrile illness. Therefore it is very important to clinically distinguish dengue from CHIK infection.

CHIK is diagnosed based on serological analysis and viral isolation in mice or tissue culture. An IgM immunoassay is the most practical lab test method[4].

The *OnSite* Chikungunya IgM Combo Rapid Test utilizes recombinant antigens derived from its structure protein[5], it detects IgM anti-CHIK in patient serum or plasma within 15 minutes. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

TEST PRINCIPLE

The *OnSite* Chikungunya IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing CHIK antigens conjugated with colloid gold (CHIK conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with anti-human IgM reagent, and the C band is pre-coated with goat anti-rabbit IgG.



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 IgM antibody to CHIK, 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 reagent, forming a burgundy colored T band, indicating a CHIK IgM positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 30 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One pipette dropper.
   c. One desiccant.
2. Sample Diluent (1 vial, 5 mL)
3. One package insert (instruction for use).

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

1. Positive Control (1 vial, red cap, 1 mL, Cat # R0066-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R0066-N)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

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 gives 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℃) 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 hemolized blood specimen 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 biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. 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℃. The positive and negative controls should be kept at 2℃-8℃. If stored at 2℃-8℃, 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 over 30℃.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2℃-8℃ if not tested immediately.

Store specimens at 2℃-8℃ up to 5 days. The specimens should be frozen at -20℃ 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 on result interpretation.

Blood

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Do not use any hemolized blood for testing.

Whole blood specimens should be stored in refrigeration (2℃-8℃) if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

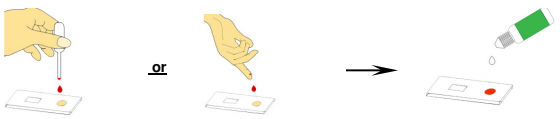
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

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's ID number.

Step 4: **For whole blood test**
Apply 1 drop of whole blood (about 40-50 µL) into the sample well.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

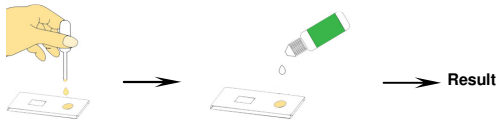


1 drop of whole blood **1 drop of sample diluent**

For serum or plasma test
Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.



1 drop of specimen **1 drop of sample diluent** **15 minutes**

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

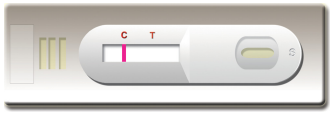
QUALITY CONTROL

Using individual *OnSite* Chikungunya IgM Combo Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

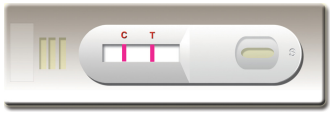
1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2℃ -30℃.
5. The temperature of the test area falls outside of 15℃-30℃.

Expected results are as follows:

Negative Control
Only the C band shows color development. The T band shows no color development.



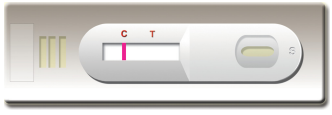
Positive Control
Both C and T bands show color development.



The appearance of any burgundy color in the T band, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is developed, the test indicates that no detectable IgM anti-CHIK is present in the specimen. The result is negative.



2. **POSITIVE RESULT**: If both C and T bands are developed, the test indicates for the presence of IgM anti-CHIK in the specimen. The result is positive.

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

3. **INVALID:** If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

An evaluation study was carried out at Unite de virologie , Institute de Medecine Tropicale de Service de Sante des Armees, Ministere De la Defense, France.

The evaluation specimen panel consisted of 72 recently infected specimens diagnosed by MAC-ELISA and 21 specimens containing 10 from other arbovirus infection, 3 from O'Nyong nyong infection, and 8 negative for all the tests. The evaluation data are showed in the following table.

	OnSite Chikungunya IgM Rapid Test		
MAC-ELISA	**Positive**	**Negative**	**Total**
Positive	65	7	72
Negative	0	21*	21
Total	65	28	93

Relative Sensitivity: 90.3% , Relative Specificity: 100%, Overall Agreement: 92.4%

LIMITATIONS OF TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of IgM anti-CHIK in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Chikungunya IgM Combo Rapid Test is limited to the qualitative detection of IgM anti-CHIK in human serum, plasma or whole blood. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable IgM anti-CHIK. However, a negative test result does not preclude the possibility of exposure to or infection with CHIK
4. A negative result can occur if the quantity of IgM anti-CHIK present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. 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.
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4. Thein S, La Linn M, Aaskov J, Aung MM, Aye M, Zaw A, Myint A.Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M 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.
5. Yamamoto K, Hashimoto K, Ogata T Structural proteins of Chikungunya virus. Simizu B, J Virol. 1984 Jul;51(1):254-8

EC REP **European Authorized Representative:**
CEpartner4U , Esdoornlaan 13, 3951DB Maarn.
The Netherlands. Tel.: +31 (0)6.516.536.26

Manufacturer:
CTK Biotech, Inc.
6748 Nancy Ridge Drive, San Diego, CA 92121, USA
Tel: 858-457-8698, Fax: 858-535-1739,
E-mail: info@ctkbiotech.com

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English Version

Index of CE Symbol

	Attention, see instructions for use
IVD	For *in vitro* diagnostic use only
REF	Catalog #
LOT	Lot Number
	Use by
	Tests per kit
2℃ 30℃	Store between 2-30℃
	Do not reuse
	Manufacturer
	Date of manufacture