Rapid Response™

Chikungunya IgG/IgM Test

(Whole Blood/Serum/Plasma)

REF CKG-13
CKG-13C
CKG-13C40

Product Insert

A rapid test for the qualitative detection of anti-chikungunya IgG and/or IgM antibodies in whole blood, serum or plasma to aid in the diagnosis of chikungunya.

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Chikungunya IgG/IgM Test is a rapid chromatographic immunoassay for the qualitative detection of IgG and/or IgM to chikungunya (CHIK) in whole blood, serum or plasma to aid in the diagnosis of chikungunya infection.

Summary

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, 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.

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. A dual infection of dengue and chikungunya is also possible and has been reported in India. 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.

The Rapid Response™ Chikungunya IgG/IgM Test utilizes recombinant antigens derived from its structure protein; it detects IgM anti-chikungunya in patient serum or plasma within 15 minutes. The test can be performed by untrained or minimally skilled personnel without cumbersome laboratory equipment.

Principle

The Rapid Response™ Chikungunya IgG/IgM Test is a lateral flow chromatographic immunoassay. The test consists of: 1) a conjugate pad containing CHIK antigens conjugated with colored mouse IgG/IgM-gold conjugates (CHIK conjugates), 2) a nitrocellulose membrane strip containing an IgG test line, an IgM test line, and a control line. The IgG line is pre-coated with anti-human IgG reagent, the IgM line is pre-coated with anti-human IgM reagent, and the control line is pre-coated with goat anti-mouse 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 test strip. The anti-chikungunya IgG and/or IgM antibodies, if present in the specimen, will bind to the CHIK conjugates. The immunocomplex is then captured on the membrane by the pre-coated with anti-human IgG and/or anti-human IgM reagent forming a colored IgG and/or IgM line(s), indicating a CHIK IgG and/or IgM positive test result.

Absence of the IgG and IgM line suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line resulting from the immunocomplex of the goat anti-mouse IgG with the mouse IgG-gold conjugate. If the C line does not develop, the test result is considered to be invalid and the specimen must be retested with another device.

Reagents

The test contains Chikungunya antigen coated particles, anti-human IgG and anti-human IgM coated on the membrane.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use test if pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

Materials

Materials provided

- Test cassettes
- Droppers
- Buffer solution
- Product insert

Materials required but not provided

- Specimen collection containers (for fingerstick whole blood)
- Centrifuge
- Timer
- Lancets
- Heparinized capillary tubes and dispensing bulb

Storage and Stability

Store as packaged in the sealed pouch either at room temperature or refrigerated 35.6-86°F (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use after the expiration date.

Specimen Collection and Preparation

The Rapid Response™ Chikungunya IgG/IgM Test can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.

- To collect **Fingerstick Whole Blood Specimens:**
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  - Add the Fingerstick Whole Blood specimen to the test by using a capillary tube:
    - Touch the end of the capillary tube to the blood until filled to approximately 40 µL. Avoid air bubbles.
    - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 35.6-46.4°F (2-8°C) for up to 3 days. For long term storage, specimens should be kept below -4°F (-20°C). Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

Test Procedure

Allow the test cassette, specimen, buffer, and/or controls to reach room temperature (59-86°F; 15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the cassette on a clean and level surface.

For Serum or Plasma specimen:

- Hold the dropper vertically and transfer **1 drop serum or plasma (approximately 40 µL)** to the specimen area, then add **1 drop of buffer (approximately 40 µL)**, and start the timer. See illustration below.

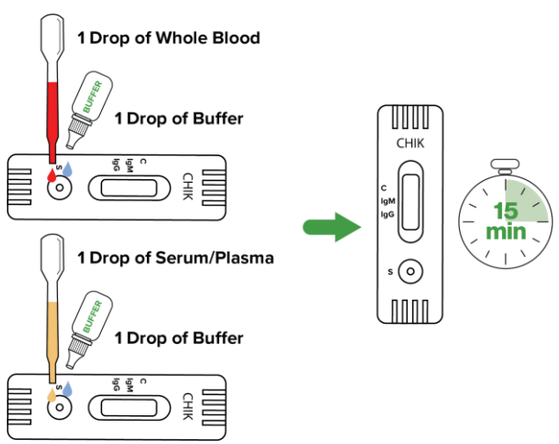
For Venipuncture Whole Blood specimen:

- Hold the dropper vertically and transfer **1 drop whole blood (approximately 40 µL)** to the specimen area, then **add 1 drop of buffer** (approximately 40 µL), and start the timer. See illustration below.

For Fingerstick Whole Blood specimen:

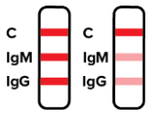
- To use a capillary tube: Fill the capillary tube and **transfer approximately 40 µL of fingerstick whole blood specimen to the specimen** area of test cassette, then **add 1 drop of buffer (approximately 40 µL)** and start the timer. See illustration below.

3. Wait for the colored line(s) to appear. Read results at 15 minutes. Positive results can be visible in as soon as 1 minute. Do not interpret the result after 15 minutes.

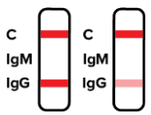


Results Interpretation

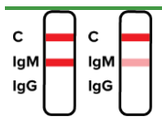
Positive



IgG and IgM:* Three lines appear. One colored line should be in the control line region (C), and two colored lines should appear in IgG test line region and IgM test line region. The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies.



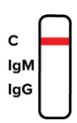
IgG: Two lines appear. One colored line should be in the control line region (C), and a colored line appears in IgG test line region. The result is positive for Chikungunya virus specific-IgG.



IgM: Two lines appear. One colored line should be in the control line region (C), and a colored line appears in IgM test line region. The result is positive for Chikungunya virus specific-IgM.

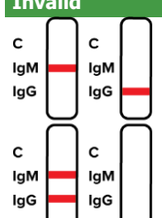
***NOTE:** The intensity of the color in the IgG and/or IgM test line region will vary depending on the concentration of CHIK IgG and/or IgM present in the specimen. Therefore, any shade of color in the IgG and/or IgM test line region should be considered positive.

Negative



One colored line appears in the control line region (C). No line appears in the IgG and/or IgM test line region.

Invalid



Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Quality Control

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms adequate membrane wicking. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Assay Procedure and the interpretation of Assay Result sections must be followed closely when testing for the presence of presence of IgG and/or IgM anti-CHIK in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Rapid Response™ Chikungunya IgG/IgM Test is limited to the quantitative detection of IgG and IgM anti-CHIK 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 result for an individual subject individual absence of detectable IgG and IgM anti-CHIK. However, a negative test result does not preclude the possibility of exposure to or infection CHIK.
4. A negative result can occur if the quantity of IgG and IgM anti-CHIK 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. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor mat affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

Performance Characteristics

Sensitivity and Specificity

The evaluation specimen panel consisted of 10 specimens from recently infected individuals diagnosed by leading commercial test, 6 specimens from infected individuals and 126 negative specimens. The evaluation data are shown in the following table.

Method			Leading commercial Test				Total Result
			Positive		Negative		
Rapid Response™ Chikungunya IgG/IgM Test	Results		IgG	IgM	IgG	IgM	
	Positive	IgG	6	0	0	0	6
		IgM	0	10	0	0	10
	Negative	IgG	0	10	126	0	136
		IgM	6	0	0	126	132
Total Result			12	20	126	126	284

Relative sensitivity: (6+10)/(6+10)>=99.9% (95%CI*: 82.9%~100.0%);
Relative specificity: 126/126>=99.9% (95%CI*: 97.7%~100.0%);
Accuracy: (6+10+126)/(6+10+126)>=99.9% (95%CI*: 97.9%~100.0%).
*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of four specimens: a negative, an IgG positive and an IgM positive. The negative, an IgG positive and an IgM positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same three specimens: a negative, an IgG positive and an IgM positive. Three different lots of the Rapid Response™ Chikungunya IgG/IgM Test have been tested over a 3-day period using a negative, an IgG positive and an IgM positive specimen. The specimens were correctly identified >99% of the time.

Bibliography

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 JMed 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. Myers RM and Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science 1967;157:1307-8.
4. Thein S, La Linn M, Aaskov J, Aung MM, Aye M ,Aye M, Zaw 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.
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Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
Store between 35.6°F to 86°F (2-30°C)
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #
Manufacturer

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