Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Package Insert

A rapid test for the qualitative detection of IgG and IgM antibodies to Chikungunya in human's whole blood, serum or plasma specimen.

For professional in vitro diagnostic use only.

INTENDED USE

The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to Chikungunya in human's whole blood, serum or plasma. 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 Chikungunya IgG/IgM Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

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 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].

PRINCIPLE

The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of IgG and IgM antibodies to Chikungunya in whole blood, serum or plasma. The membrane is pre-coated mouse anti-human IgG and mouse anti-human IgM on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with recombinant Chikungunya antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with mouse anti-human IgG or/and mouse anti-human IgM on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test cassette contains recombinant Chikungunya antigen conjugated colloid gold, mouse anti-human IgG and mouse 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.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure 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.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) 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.
  - Add the Fingerstick Whole Blood specimen to the test by using hanging drops:
    - Position the patient's finger so that the drop of blood is just above the specimen area of the test cassette.
    - Allow 1 hanging drop of fingerstick whole blood to fall into the center of the specimen area on the test cassette, or move the patient's finger so that the hanging drop touches the center of the specimen area. Avoid touching the finger directly to the specimen area.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- 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 2-8°C for up to 3 days. For long term storage, specimens should be kept below 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 federal regulations for transportation of etiologic agents.

MATERIALS

Materials provided

• Test cassettes • Droppers • Buffer • Package insert

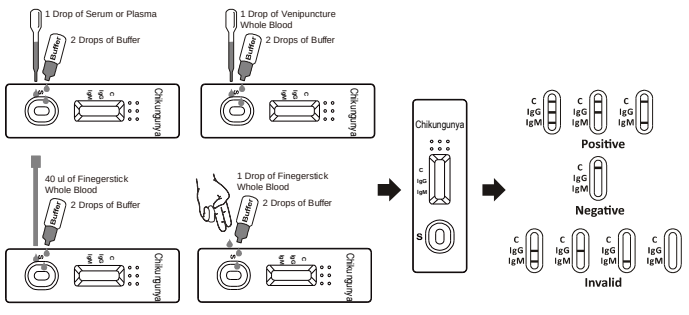
Materials required but not provided

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

DIRECTIONS FOR USE

Allow test cassette, specimen, and/or controls to equilibrate to room temperature (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 of serum or plasma** (approximately 40μL) to the specimen area,then **add 2 drops of buffer** (approximately 80μL),and start the timer, see illustration below.
   For **Venipuncture Whole Blood**specimen: Hold the dropper vertically and **transfer 1 drop of whole blood** (approximately 40μL) to the specimen area, then **add 2 drops of buffer** (approximately 80μL), and start the timer. See illustration below.
   For **Fingerstick Whole Blood**specimen:
   - To use a capillary tube: Fill the capillary tube **and transfer approximately 40μL of fingerstick whole blood specimen** to the specimen area of test cassette, then **add 2 drops of buffer** (approximately 80μL) and start the timer. See illustration below.
   - To use hanging drops: Allow **1 hanging drop of fingerstick whole blood specimen** (approximately 40μL) to fall into the specimen area of test cassette, then **add 2 drops of buffer** (approximately 80μL) and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. Read the result at 15 minutes, do not interpret the result after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

IgG POSITIVE: Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the IgG region.

IgM POSITIVE: Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the IgM region.

IgG and IgM POSITIVE: Three distinct colored lines appear. One color line should be in the control region (C) and another two color lines should be in the IgG and IgM region.

NOTE: The intensity of the color in the test line region (T) will vary depending on the concentration of Chikungunya antibodies present in the specimen. Therefore, any shade of red in the test region should be considered positive.

NEGATIVE: One color line appears in the control region (C). No apparent red or pink line appears in the IgG and IgM 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 cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

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 Direction for Use and the Interpretation of Result must be closely when testing the presence of antibodies to Chikungunya in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is limited to the qualitative detection of antibodies to Chikungunya in human serum, plasma or whole blood. The intensity of the test band does not have linear correlation with antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable Chikungunya antibodies. However, a negative test result does not preclude the possibility of exposure to Chikungunya.
4. A negative result can occur if the quantity of Chikungunya antibodies 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. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

EXPECTED VALUES

The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been compared with a leading commercial Chikungunya IgG/IgM EIA test. The correlation between these two systems is at least 92%.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

A total of 93 samples from susceptible subjects were tested by the Chikungunya IgG/IgM Rapid Test Cassette and by a commercial Chikungunya IgM EIA kit. Comparison for all subjects is shown in the following table.

IgM Results

Method		EIA		Total Result
Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	65	0	65
	Negative	7	21	28
Total Result		72	21	93

Relative sensitivity: 90.3% (95%CI:*81.0%-96.0%)
Relative specificity: ＞99.9% (95%CI:*86.7%-100%)
Accuracy: 92.5% (95%CI:*85.1%-96.9%) *Confidence Intervals

A total of 68 samples from susceptible subjects were tested by the Chikungunya IgG/IgM Rapid Test Cassette and by a commercial Chikungunya IgG EIA kit. Comparison for all subjects is shown in the following table.

IgG Results

Method		EIA		Total Result
Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	33	1	34
	Negative	2	32	34
Total Result		35	33	68

Relative sensitivity: 94.3% (95%CI:*80.8%-99.3%)
Relative specificity: 97.0% (95%CI:*84.2%-99.9%)
Accuracy: 95.6% (95%CI:*87.6%-99.1%) *Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of three specimens: a negative, a Chikungunya IgM middle titer positive, a Chikungunya IgM high titer positive, a Chikungunya IgG middle titer positive and a Chikungunya IgG high titer positive. The negative, a Chikungunya IgM low titer positive, a Chikungunya IgM high titer positive, a Chikungunya IgG low titer positive and a Chikungunya IgG high titer positive values were correctly identified 100% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same five specimens: a negative, a Chikungunya IgM middle titer positive, a Chikungunya IgM high titer positive, a Chikungunya IgG middle titer positive and a Chikungunya IgG high titer positive. Three different lots of the Chikungunya Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested over a 10 days period using negative, a Chikungunya IgM middle titer positive, a Chikungunya IgM high titer positive, a Chikungunya IgG middle titer positive and a Chikungunya IgG high titer positive specimens. The specimens were correctly identified 100% of the time.

Cross-reactivity

The Chikungunya IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Chikungunya negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Aspirin: 20mg/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin 1000mg/dL
Bilirubin: 1g/dL	Oxalic Acid: 60mg/dL
Uric acid: 20mg/dl	Methanol: 10%

None of the substances at the concentration tested interfered in the assay.

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 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.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, 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

Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged				

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