

Chikungunya Antigen Rapid Test Cassette (Serum/Plasma/Whole Blood)

Package Insert

A RAPID TEST FOR THE QUALITATIVE DETECTION OF CHIKUNGUNYA ANTIGEN IN HUMAN SERUM/PLASMA/WHOLE BLOOD.

For Professional In Vitro Diagnostic Use Only.

INTENDED USE

The Chikungunya Antigen Rapid Test Cassette is a lateral flow chromatographic immunoassay for qualitative detection of E protein antigen in human serum or plasma. This kit is intended to be used as a screening test and as an aid in the diagnosis of infection with Chikungunya virus.

Any reactive specimen with the Chikungunya Antigen Rapid Test Cassette 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 Pakistan¹⁻².

The symptoms are most often clinically indistinguishable from those observed in dengue fever. Indeed, dual infection of dengue and chikungunya has been reported in India³. 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.

The Chikungunya Antigen Rapid Test Cassette is a rapid test that utilizes a combination of anti- Chikungunya E protein antibodies coated colored particles for the detection of Chikungunya E protein antigen in human serum, plasma or whole blood within 15 minutes. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

PRINCIPLE

The Chikungunya Antigen Rapid Test Cassette is a lateral flow chromatographic immunoassay based on the principle of the double antibody–sandwich technique. The test Cassette consists of: 1) a red colored conjugate containing CHIKV antibody conjugated with latex (CHIKV antibody 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 non-conjugated CHIKV antibody, 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 antigen to CHIKV if present in the specimen will bind to the CHIKV conjugates. The immune complex is then captured on the membrane by the pre-coated CHIKV antibody, forming a burgundy colored T band, indicating an antigen 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 immune complex of goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless the presence of any antigens to CHIKV. Otherwise, the test result is invalid and the specimen must be retested with another Cassette.

REAGENTS

The test contains CHIKV antibody pre-coated on the surface of the membrane. And CHIKV antibody conjugated latex.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test must remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use if pouch is damaged.

- 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 being tested.
- Humidity and temperature can adversely affect results.
- The used test should be discarded according to local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (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 beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

1. The Chikungunya antigen Rapid Test Cassette (Serum/Plasma/Whole Blood) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
2. To collect **Finger stick 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.
 - Touch the end of the dropper to the blood until filled to approximately 20µl. Avoid air bubbles.
 - Place the bulb onto the top end of the dropper, then squeeze the bulb to dispense the whole blood to the specimen area of the test Cassette.
3. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
4. Testing should be performed immediately after specimen collection. 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.
5. 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.
6. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

MATERIALS

Materials provided			
• Test Cassette	• Dropper	• Buffer	• Package Insert
Materials required but not provided			
• Specimen collection containers	• Timer		
• Lancets (for fingerstick whole blood only)	• Centrifuge		

DIRECTIONS FOR USE

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

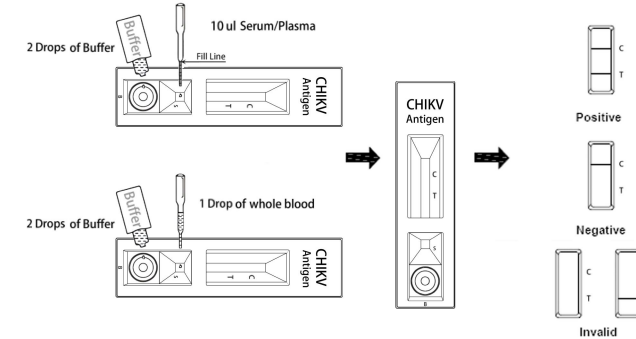
1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Place the test cassette on a clean and level surface.

For **Serum or Plasma** specimens: Hold the dropper vertically and draw the serum or plasma up to the fill line as shown in illustration below (approximately 10µL). Transfer the specimen to the specimen well (S), then add 2 drops of buffer (approximately 80µL) to the specimen well (B), and start the timer. See illustration below.

For **Venipuncture Whole Blood** specimens: Hold the dropper vertically and transfer 1 drop of venipuncture whole blood (about 20µL) to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µL) to the specimen well (B), and start the timer. See illustration below.

For **Finger stick Whole Blood** specimens: To use a dropper: Hold the dropper vertically and transfer 1 drop of Finger stick Whole Blood (about 20µL) to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µL) to the specimen well (B), and start the timer. See illustration below.

3. Wait for the colored line(s) to appear. **Read results at 15 minutes.** Do not interpret results after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE: * Two lines appear. One colored line should always appear in the control line region (C) and another one apparent colored line should be in the test line region (T).

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

NEGATIVE: One colored line appears in the control line region (C). No apparent colored lines appear in any of the test line region (T). This indicates that the concentration of antigen are below the minimum detection levels.

INVALID: Control line (C) 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

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking 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 Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antigen to CHIKV in serum/plasma/whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Chikungunya Antigen Rapid Test Cassette is limited to the qualitative detection of the antigen to CHIKV in human serum/plasma/whole blood. The intensity of the test band does not linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable CHIKV. However, a negative test result does not preclude the possibility of exposure to or infection with CHIKV.
4. A negative result can occur if the quantity of the CHIKV antigen present in the specimen is below the detection limits of the assay, or the antigen 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 antigens 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.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity

The Chikungunya Antigen Rapid Test Cassette (Serum/Plasma/Whole Blood) has been evaluated with a leading commercial Chikungunya Antigen EIA test using clinical specimens. The results show that relative to leading PCR tests, the Chikungunya Antigen Rapid Test Cassette (Serum/Plasma/Whole Blood) exhibits 95% sensitivity and 98.8% specificity .

Chikungunya Antigen Test vs. EIA				
Method		PCR		Total
Chikungunya Antigen Test	Results	Positive	Negative	Results
	Positive	138	2	140
	Negative	4	198	202
Total Results		142	200	342

Relative Sensitivity: 97.18% (95%CI*: 92.74%~99.14%)*

Relative Specificity: 99.0% (95%CI*: 96.19%~99.96%)*

Accuracy: 98.25% (95%CI*: 96.13%~99.29%)*

*Confidence Interval

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of three specimens:a negative, a low positive,and a high positive.The negative,low,positive,and high positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same three specimens,a negative,a low positive,and a high positive.Three different lots of the Chikungunya Antigen Rapid Test Cassette(Serum/Plasma/Whole blood) have been tested over a 3-day period using negative,low positive,and high positive specimens.The specimens were correctly identified >99% of the time.

Cross-reactivity

The Chikungunya Antigen Rapid Test Cassette(Serum/Plasma/Whole blood) has been tested with specimens positive for: Deugue, ZIKA, Yellow fever, HAV, HBV, HCV, HEV, HIV and TP. No cross-reactivity was observed, indicating that the performance of the Chikungunya Antigen Rapid Test (Whole Blood/Serum/Plasma) is stable in presence of these factors.

Interfering Substances

The Chikungunya Antigen Rapid Test Cassette(Serum/Plasma/Whole blood) has been tested for possible interference from visibly hemolyzed and lipemic specimens, as well as serum specimens containing high bilirubin levels. Results indicate that no interference was observed in specimens containing up to 1000 mg/dL hemoglobin; up to 1000mg/dL bilirubin; and up to 2,000 mg/dL human serum albumin.

BIBLIOGRAPHY

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SYMBOLS

Symbol	Meaning	Symbol	Meaning
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	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer		Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse		Consult instruction for use
	Batch code		Meet the requirements of EC Directive 98/79/EC

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