Cromatest

LINEAR

Dengue-CHIK cassette CE

CONTENTS			
REF	4274240	Dengue - CHIK	40 Tests
For professional *in vitro* diagnostic use only			

Dengue IgG/IgM-CHIK IgM

A rapid one test for the simultaneous detection and differentiation of IgG anti–dengue virus, IgM anti-dengue virus and IgM anti-chikungunya virus (CHIK IgM) in human serum, plasma or whole blood

ONE STEP

PRINCIPLE

The LINEAR Dengue-CHIK cassette is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG anti–dengue virus, IgM anti-dengue virus and IgM anti-chikungunya virus (CHIK) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with dengue and chikungunya viruses. 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.

The Dengue IgG/IgM test in the left panel of the cassette is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant dengue envelope antigens conjugated with colloidal gold (dengue Ag conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The G line is pre-coated with anti-human IgG, the M line is coated with anti-human IgM, and the C line is pre-coated with a control line antibody.

The CHIK IgM test in the right panel of the cassette is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing CHIK antigens conjugated with colloidal gold (CHIK Ag conjugates) and a control antibody conjugated with colloidal gold, 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 test cassette, the specimen migrates by capillary action across the cassette. IgG anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgG forming a burgundy colored G line indicating a dengue virus IgG positive result. Absence of color development on the G line suggests a dengue virus IgG negative result. IgM anti-dengue virus, if present in the specimen, will bind to the dengue Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a burgundy colored M line indicating a dengue virus IgM positive result. Absence of color development on the M line suggests a dengue virus IgM negative result. IgM anti-CHIK, if present in the specimen, will bind to the CHIK Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a burgundy colored T line indicating a chikungunya virus IgM positive test result. Absence of color development on the T line suggests a chikungunya virus IgM negative test result. Both test panels contain an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies, regardless of color development on the test lines (M, G or T lines). If the C line does not develop in a panel, the test result is invalid, and the specimen must be retested with another device. An invalid result in one panel does not invalidate the test result in the other panel.

PACKAGING CONTENTS

REF	4274240	40 Dengue-CHIK test device
		40 Capillary tubes for Dengue IgG/IgM (5 µl)
		40 Plastic dropper for CHIK IgM
		2 Sample diluent (5 mL)

STORAGE AND STABILITY

Store at 2-30ºC. The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. **Do not freeze the kit or expose the kit over 30°C**. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

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 at 2-8°C if not tested immediately, 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. 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 hemolyzed blood for testing. Stored in refrigeration (2-8°C) if not tested immediately, within 24 hours of collection.

MATERIAL REQUIRED

- Timer

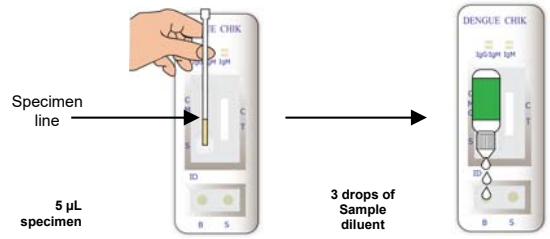
PROCEDURE

Allow the test cassette, urine or serum specimen and/or controls to equilibrate to room temperature (20-30°C) prior to testing.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results the assay should be performed within one hour.
2. Fill the blood transfer device with the specimen (serum, plasma or whole blood).

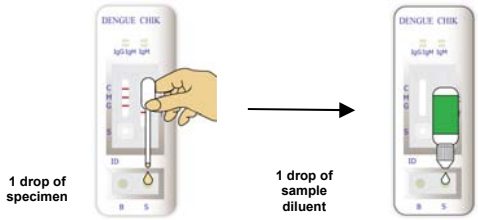
For detection of Dengue IgG/IgM

Hold the capillary tube vertically and make sure that specimen does not exceed the specimen line as shown in the following image. The volume of the specimen is around 5 µL. Dispense the entire specimen into the center of the sample well (S well) making sure that there are no air bubbles. Then hold the diluent bottle vertically and add 3 drops (about 90-120 µL) of sample diluent immediately into the buffer well (B well).



For detection of CHIK IgM

Holding the plastic dropper vertically, dispense 1 drop specimen (30-45 □l serum or 40-50 µl blood) into the sample well (S well), making sure that there are no air bubbles. Then hold the diluent bottle vertically and add 1 drop (abou 30-40 µL) of sample diluent immediately.



QUALITY SYSTEM CERTIFIED ISO 9001 ISO 13485

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3. Set up timer.
4. Results can be read at 20-25 minutes for the Dengue IgG/IgM test and at 10-15 minutes for the CHIK IgM test. Positive results can be visible in as short as 1 minute. Negative results must be confirmed at the end of 25 minutes only for the Dengue IgG/IgM test and at the end of 15 minutes only for the CHIK IgM test.
   Any results interpreted outside the 20-*25-minute window for the Dengue IgG/IgM test and outside the 10-15-minute window for the CHIK IgM test should be considered invalid and must be repeated.*

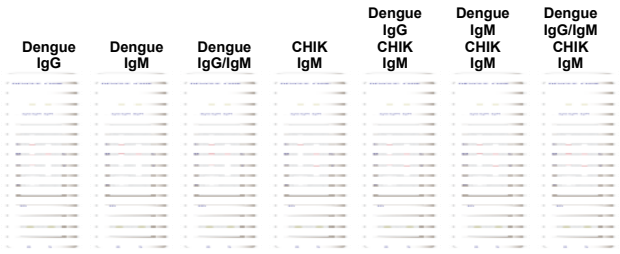
Discard used devices after interpreting the results following local laws governing the disposal of devices.

INTERPRETATION OF ASSAY RESULT

NEGATIVE RESULT.
If only the C line is present, the absence of any burgundy color in the G, M and T lines indicates that neither anti-dengue virus antibodies nor anti-CHIK antibody are detected. The result is negative.

INVALID:
If no C line is developed, the assay is invalid regardless of any burgundy color in the G, M or T lines as indicated below. Repeat the assay with a new device.

POSITIVE RESULT:



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

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 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 good laboratory testing practice to confirm the test procedure and to verify proper test performance.

CLINICAL SIGNIFICANCE

Dengue virus is an enveloped, single-stranded, positive-sense RNA virus that comprises four distinct serotypes (Den 1, 2, 3, 4). Dengue virus is transmitted by mosquitoes of the daytime-biting *Stegomyia* family. Today, more than 2.5 billion people are at risk for dengue virus infection. An estimated 100 million cases of dengue fever and 250,000 cases of life-threatening dengue hemorrhagic fever occur annually on a worldwide basis.[1-3]
Chikungunya (CHIK) is a rare viral infection transmitted by the bite of an infected *Aedes aegypti* mosquito. Chikungunya is characterized by a rash, fever and severe joint pain (arthralgias), which usually last for three to seven days. Chikungunya occur during the rainy season in tropical areas of the world[4-5].The symptoms of Chikungunya are most often clinically indistinguishable from those observed in dengue fever. Unlike dengue, hemorrhagic manifestations are relatively rare in Chikungunya infections, and most often the disease is a self-limiting, febrile illness. Dual infection with dengue and chikungunya is also possible and has been reported in India[10]. Therefore, it is very important to clinically distinguish a Dengue infection from a Chikungunya infection. Serological detection is a common method for the diagnosis of infection with dengue virus. IgM anti-dengue virus starts to appear at 3 days after initial exposure and remains in circulation for about 30-60 days. IgG anti-dengue virus is raised at around 7 days, peaks at 2-3 weeks and persists for life[6-8]. An IgM immunoassay is the most practical lab test method for Chikungunya detection[9].

ANALYTICAL PERFORMANCE

Clinical Performance for Dengue IgM Test. A total of 314 patient samples from susceptible subjects were tested by the Dengue IgG/IgM-CHIK IgM Rapid Test and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue IgG/IgM-CHIK IgM		
IgM EIA Test	**Positive**	**Negative**	**Total**
Positive	31	1	32
Negative	3	279	282
Total	34	280	314

Relative Sensitivity: 96.9%, Relative Specificity: 98.9%, Overall Agreement: 98.7%.

Clinical Performance For Dengue IgG Test. A total of 326 patient samples from susceptible subjects were tested by the Dengue IgG/IgM and by a commercial EIA. Comparison for all subjects is shown in the following table:

	Dengue IgG/IgM-CHIK IgM		
IgG EIA Test	**Positive**	**Negative**	**Total**
Positive	36	1	37
Negative	2	287	289
Total	38	288	326

Relative Sensitivity:97.3%, Relative Specificity:99.3%, Overall Agreement: 99.1%

Clinical Performance for CHIK IgM Test
A total of 93 specimens consisting of 72 recently infected patient samples diagnosed by MAC-ELISA and 21 negative specimens containing 10 samples positive for other arbovirus infection, 3 samples positive for O'nyong nyong infection and 8 clean negative samples. The evaluation data are shown in the following table.

	Dengue IgG/IgM-CHIK IgM		
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%.

Cross Reactivity. No false positive test results were observed on 10 specimens from the following disease states: HAV-HBV-HCV-*H. pylori*-TB-*T. pallidum.*

Interference. Common interfering substances (such as pain and fever medication, blood components) may affect the performance of the cassette. This was studied by spiking these substances into negative and positive standard controls of CHIK IgM and Dengue IgG and IgM. The results are presented in the following table and demonstrate, at the concentrations tested, the substances studied do not affect the performance of the test:
List of potentially interfering substances and concentrations tested:
1. Albumin 50 g/L 2. Bilirubin 20 mg/dL 3. EDTA 3.4 µmol/L
4. Glucose 55mmol/L 5. Heparin 3,000U/L 6. Salicylic acid4.34mmol/L

PRECAUTIONS

1. The insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the 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 the components in 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.
8. 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 as biohazardous waste.
11. Handle the Negative and Positive Controls in the same manner as patient specimens.
12. The Dengue IgG/IgM test results should be read 20-25 minutes and the CHIK IgM test results 10-15 minutes after a specimen is applied to the sample well, respectively. Any results interpreted outside the 20-25 minute window for the Dengue IgG/IgM test or outside the 10-15 minute window for the CHIK IgM test 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.

LIMITATIONS OF TEST

1. The assay is limited to the qualitative detection of antibodies to dengue virus and to chikungunya. The intensity of the test line does not have linear correlation with antibody titer in the specimen.
2. The Test cannot be used to differentiate if the infection is primary or secondary.
3. Serological cross reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile virus, yellow fever, etc.). Therefore, it is possible that patients who were exposed to these viruses may show some level of the reactivity with this test.
4. A negative or non-reactive result indicates absence of detectable antibody. However, this results does not preclude the possibility of exposure to or infection with dengue or chikungunya virus.
5. A negative result can occur if the quantity of the dengue virus antibodies or CHIK IgM present in the specimen is below the detection limits of the assay or if the Dengue antibodies and the CHIK IgM that are detected are not present during the stage of disease in which a sample is collected.
6. Infection may progress rapidly. If the symptoms persist, while the results are negative, it is recommended to test with an alternative test method.
7. Some specimens containing an unusually high titer of heterophile antibodies or rheumatoid factor (such as 2000IU/mL) may affect the results.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

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