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DIAQUICK Dengue IgG/IgM Ab Cassette

(en) English

REF	Content
Z06240	- 30 tests individually packed in foil pouches (30x REF Z06240B) - 30 droppers - 1x 3 mL buffer - 1 package insert

For professional in vitro diagnostic use only.

INTENDED USE

The DIAQUICK Dengue IgG/IgM Ab Cassette is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to Dengue virus in human whole blood, serum, or plasma as an aid in the diagnosis of primary and secondary Dengue infections.

DIAGNOSTIC SIGNIFICANCE

Dengue is a flavivirus, transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. It is widely distributed throughout the tropical and subtropical areas of the world,[1] and causes up to 100 million infections annually.[2] Classic Dengue infection is characterized by a sudden onset of fever, intense headache, myalgia, arthralgia and rash. Primary Dengue infection causes IgM antibodies to increase to a detectable level in 3 to 5 days after the onset of fever. IgM antibodies generally persist for 30 to 90 days.[3] Most Dengue patients in endemic regions have secondary infections,[4] resulting in high levels of specific IgG antibodies prior to or simultaneous with IgM response.[5] Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.
The DIAQUICK Dengue IgG/IgM Ab Cassette is a rapid test that utilizes a combination of Dengue antigen coated colored particles for the detection of IgG and IgM Dengue antibodies in human whole blood, serum, or plasma.

TEST PRINCIPLE

The DIAQUICK Dengue IgG/IgM Ab Cassette is a qualitative membrane-based immunoassay for the detection of Dengue antibodies in whole blood, serum, or plasma. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with Dengue antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region. If the specimen contains IgG antibodies to Dengue, a colored line will appear in IgG test line region. In the IgM component, anti-human IgM is coated in IgM test line region. During testing, the specimen reacts with anti-human IgM. Dengue IgM antibodies, if present in the specimen, reacts with the anti-human IgM and the Dengue antigen-coated particles in the test cassette, and this complex is captured by the anti-human IgM, forming a colored line in IgM test line region.
Therefore, if the specimen contains Dengue IgG antibodies, a colored line will appear in IgG test line region. If the specimen contains Dengue IgM antibodies, a colored line will appear in IgM test line region. If the specimen does not contain Dengue antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENT COMPOSITION

The test cassette contains Dengue antigen conjugated gold colloid particles and anti-human IgM, anti-human IgG coated on the membrane.

MATERIAL REQUIRED BUT NOT PROVIDED

- Specimen collection containers
- Micropipette
- Lancets (for fingerstick whole blood only)
- Centrifuge (for plasma only)
- Timer

REAGENT PREPARATION

The test is ready to use.

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.

WARNINGS AND 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.

SPECIMEN COLLECTION AND STORAGE

- The DIAQUICK Dengue IgG/IgM Ab Cassette can be performed using whole blood, 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 cassette by using a dropper or micropipette measuring 10 µL The dropper provided with the test dispenses approximately 10 µL in one drop even if more blood is aspirated in the dropper.
- 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 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.
- 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.

TEST PROCEDURE

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. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface.

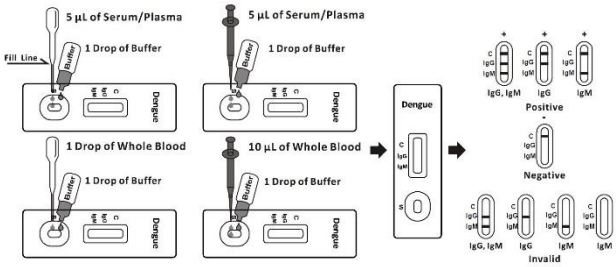
- For **Serum or Plasma Specimens**:

➢ For the usage of a **dropper**: Hold the dropper vertically, draw up the specimen to the Fill Line (approximately **5 µL**), and transfer the specimen to the specimen well of the test cassette, then add **1 drop of buffer** (approximately **40 µL**) and start the timer. Avoid trapping air bubbles in the specimen well.
➢ For the usage of a **micropipette**: Pipette and dispense **5 µL of specimen** to the specimen well of the test cassette, then add **1 drop of buffer** (approximately **40 µL**) and start the timer.

- For **Whole Blood** (Venipuncture/Fingerstick) Specimens:

➢ For the usage of a **dropper**: Hold the dropper vertically, draw the specimen about **1cm** above the Fill Line, and transfer **1 drop of whole blood** (approximately **10 µL**) to the specimen well of the test cassette, then add **1 drop of buffer** (approximately **40 µL**) and start the timer.
➢ For the usage of a **micropipette**: Pipette and dispense **10 µL of whole blood** to the specimen well of the test cassette, then add **1 drop of buffer** (approximately **40 µL**) and start the timer.

3. Wait for the colored line(s) to appear. The test result should be read at **10 minutes**. Do not interpret the result after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)
IgG and IgM POSITIVE:* 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 and IgM antibodies indicated end stage of primary Dengue infection and early stage of secondary Dengue infection.

IgG POSITIVE:* 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 Dengue virus specific-IgG and is probably indicative of secondary Dengue infection.

IgM POSITIVE:* 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 Dengue virus specific-IgM antibodies and is indicative of primary Dengue infection.
***NOTE:** The intensity of the color in the IgG and/or IgM test line region(s) will vary depending on the concentration of Dengue antibodies in the specimen. Therefore, any shade of color in the IgG and/or IgM test line region(s) should be considered positive.

NEGATIVE: **One colored line should be in the control line region (C)**. No line appears in IgG and IgM test line region(s).

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

QUALITY CONTROL AND CALIBRATION

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal valid procedural control, confirming sufficient buffer volume and 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.

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PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The DIAQUICK Dengue IgG/IgM Ab Cassette has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test.
The results show that the overall relative sensitivity for the primary and secondary infection of the DIAQUICK Dengue IgG/IgM Ab Cassette is 94.3%, the relative specificity is 99.1% and the relative accuracy is 98.3%.

Dengue Primary Infection for IgM/IgG test results

Method			ELISA		
DIAQUICK Dengue IgG/IgM Ab Cassette	Results		Positive		negative
			IgM	IgG	
	Positive	IgM	20	0	0
		IgG	4	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%	/	/

Dengue Secondary Infection for IgM/IgG test results

Method			ELISA		
DIAQUICK Dengue IgG/IgM Ab Cassette	Results		Positive		negative
			IgM	IgG	
	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			71.9%	98.4%	/

Non-Dengue Infection for IgM/IgG test results

Method			ELISA		
DIAQUICK Dengue IgG/IgM Ab Cassette	Results		Positive		negative
			IgM	IgG	
	Positive	IgM	0	0	1
		IgG	0	0	3
	Negative		0	0	429
Relative Sensitivity			/	/	99.1%

Relative sensitivity: (20+63)/ (24+64) = 94.3% (95%CI*: 87.2%-98.1%);
Relative specificity: 429/433 = 99.1% (95%CI*: 97.7%-99.7%);
Accuracy: (20+63+429)/ (24+64+433) = 98.3% (95%CI*: 96.7%-99.2%).
*Confidence Intervals

Precision

Intra-Assay

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

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same four specimens: a negative, an IgG positive, an IgM positive and an IgG/IgM dual positive. Three different lots of the DIAQUICK Dengue IgG/IgM Ab Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The DIAQUICK Dengue IgG/IgM Ab Cassette has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, HCV, 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 Dengue negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2 g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin: 1000 mg/dL
Bilirubin: 1 g/dL	Oxalic Acid: 60 mg/dL

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

TRACEABILITY

The DIAQUICK Dengue IgG/IgM Ab Cassette has been compared to a leading commercial Dengue ELISA test.

EXPECTED VALUES

Primary Dengue infection is characterized by the presence of detectable IgM antibodies 3-5 days after the onset of infection. Secondary Dengue infection is characterized by the elevation of Dengue-specific IgG. In most of the cases, this is accompanied by elevated levels of IgM.[5]
The DIAQUICK Dengue IgG/IgM Ab Cassette has been compared with a leading commercial Dengue ELISA test, demonstrating sensitivity of 83.3% for IgM in primary infection and 98.4% for IgG in secondary infection.

LIMITATIONS

1. The DIAQUICK Dengue IgG/IgM Ab Cassette is for in vitro diagnostic use only. The test should be used for the detection of Dengue antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in Dengue antibody concentration can be determined by this qualitative test.
2. The DIAQUICK Dengue IgG/IgM Ab Cassette will only indicate the presence of Dengue antibodies in the specimen and should not be used as the sole criteria for the diagnosis of Dengue.
3. In the early onset of fever, anti-Dengue IgM concentrations may be below detectable levels. For primary infection, an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) showed that 80% of the Dengue patients tested exhibited detectable levels of IgM antibody by the fifth day after infection, and 99% of the patients tested IgM positive by day 10. It is recommended that patients be tested within this time. For the secondary infection, a low molar fraction of anti-Dengue IgM and a high molar fraction of IgG that is broadly reactive to flaviviruses characterize the antibodies.[5]
   The IgM signal may be faint and the cross reaction in the region of IgG line may appear.
4. Serological cross-reactivity across the flavivirus group (Dengue 1, 2, 3 and 4, St. Louis encephalitis, West Nile virus, Japanese encephalitis and yellow fever viruses) is common.[6,7,8] Positive results should be confirmed by other means.
5. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
6. Results from immunosuppressed patients should be interpreted with caution.
7. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
8. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Dengue infection.

WASTE MANAGEMENT

The used test should be discarded according to local regulations.

LITERATURE

1. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue. Rev. Infect. Dis. 1984; 6:251-264
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4. Lam SK. Dengue haemorrhagic fever. Rev. Med. Micro. 1995; 6:39-48
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6. Yamada K, et al. Antibody responses determined for Japanese dengue fever patients by neutralization and hemagglutination inhibition assays demonstrate cross-reactivity between dengue and Japanese encephalitis viruses. Clin Diagn Lab Immunol. 2003 Jul; 10(4): 725-8.
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USED SYMBOLS

Symbol	Description
Cont.	Content

CE IVD 2°C 30°C