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See external label



2°C-30°C



Σ=25 or 50 tests



Cat. #173106P-25

Dengue IgG/IgM**Cat. No. 173106P-25**

For the Differential Detection of IgG and or IgM Antibodies to Dengue Virus in Serum or Whole Blood

For *In Vitro* Diagnostic Use**INTENDED USE:**

The **Cortez Diagnostics Inc.**, Dengue IgM and IgG Combo Rapid Test is a qualitative test for the detection of IgM and IgG antibodies to dengue virus in human serum, plasma or whole blood. The test provides a differential detection of anti-dengue IgM and anti-dengue-IgG antibodies and can be used for the presumptive distinction between a primary and secondary dengue infection. This test is for *in-vitro* diagnostic use only.

INTRODUCTION:

Dengue virus, a virus belonging to the Flavivirus group of viruses, is one of the most significant mosquito-born diseases in the world in terms of morbidity and mortality. Transmitted principally by the mosquito types **Aedes aegypti** and **Aedes albopictus**, the virus is found commonly throughout the tropic and subtropic regions of the world. There are four known serotypes of dengue. Symptoms of dengue fever include high fever, headache, muscle pain and skin rash. The complications often associated with this infection are dengue hemorrhagic fever or dengue shock syndrome.

The immune response to this virus includes the production of IgM antibodies by the 5th day of symptoms, which remain in the circulatory system for 30-60 days. IgG antibodies appear by the 14th day of infection and persist for life. A secondary infection often results in high fever and, in many cases, initiates hemorrhagic events and circulatory failure. A secondary infection also induces an IgM antibody response after 20 days of infection and IgG antibodies rise within 1-2 days after the onset of symptoms. Therefore, patients with secondary infections will have a positive IgG result, usually with a positive IgM result as well. Thus, the use of a reliable and sensitive rapid serological test that can simultaneously detect the presence of anti-dengue IgG and IgM antibodies is of great clinical utility.

The Cortez Diagnostic, Inc rapid test provides an excellent methodology for specifically detecting anti-dengue IgG and IgM antibodies. The presence of high titers of IgG antibodies does not interfere with the detection of IgM antibodies in the sample. By using a mixture of highly purified dengue proteins, the test is able to detect all 4 Dengue serotypes.

TEST PRINCIPLE:

Serum, plasma or whole blood samples may be used with this test. When a specimen is added to the test, IgG and IgM antibodies in the specimen sample react with blue particles coated with dengue envelope proteins. As this specimen/particle mixture migrates along the length of the test, the anti-dengue IgG or IgM antibody particle complex is captured by the relevant IgG and/or IgM test bands located in the test device window causing a pale to dark blue band to form at the IgG or IgM region of the test device window. The intensity of the bands will vary depending upon the amount of antibody present in the sample. The appearance of any color in a specific test region (IgG or IgM) should be considered as positive for that particular antibody type (IgG or IgM). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

PRECAUTIONS:

1. All Specimens should be handled as being potentially infectious. The U.S. Centers for Disease Control (CDC) and the National Institutes of Health (NIH) recommend that all potentially infectious agents be handled at a Biosafety Level 2.
2. Biological decontamination procedures should be followed for all equipment, containers, surfaces, etc. that come in contact with potentially infectious specimens. All disposables that come in contact with these samples should be disposed of as infectious waste.
3. For best results, strict adherence to these instructions is required. Be careful not to touch the tip of the buffer bottle to the sample tube when adding buffer to the tube. This will greatly minimize the likelihood of contaminating the buffer reagent.
4. The buffer contains a low concentration of sodium azide as a preservative (**less than 0.1 %**). Sodium azide is toxic. Do not drink this buffer. High concentrations of sodium azide may also react with lead and copper in plumbing to form explosive compounds. If you dispose of this buffer down a drain, flush the drain with excess amounts of water to minimize the accumulation of potentially explosive metal-azide compounds.
5. Do not use the test devices or wash buffer beyond the stated expiration date marked on the package label.
6. Store the test kits and reagents according to the temperature range stated on the package label.
7. All test devices, rwash buffer and specimens must be at room temperature (**15-30°C**) before running the assay.
8. Do not re-use the test devices or buffer.

STORAGE AND SHELF LIFE OF REAGENTS:

Store the kit between **2°C and 30°C**. Do not store the kit in direct sunlight. Be sure to open only the number of devices to be used. Once the device pouch has been opened, the test device should be used immediately. The test kit may be used until its expiration date, which can be found on the package label.

SPECIMEN COLLECTION:

1. Handle all specimens as being capable of transmitting infectious diseases. Dispose of all materials that come in contact with the specimen as infectious waste.
2. Specimens should be collected aseptically by venipuncture according to the standardized methods such as those recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The use of grossly lipemic or turbid samples should be avoided.
3. Whole blood samples should be used immediately, if possible. NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, HISA. 1990).
4. If Serum or plasma specimens cannot be tested immediately, they should be refrigerated at 2 to 8°C. For storage periods greater than three (3) days, freeze the specimen at -20°C or below.

KIT COMPONENTS:

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label:

- 25 test devices packaged in individual foil pouches.
Each device contains two (2) test lines: one that captures human IgG antibodies and another that captures IgM antibodies. The device also contains a third procedural control line.

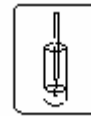
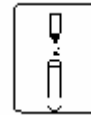
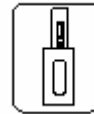
- 1 Product Insert
- 1 Dropper bottle of Dengue Wash Buffer
- 25 - 1 microliter (uL) plastic sample transfer loops.

MATERIALS REQUIRED BUT NOT SUPPLIED:

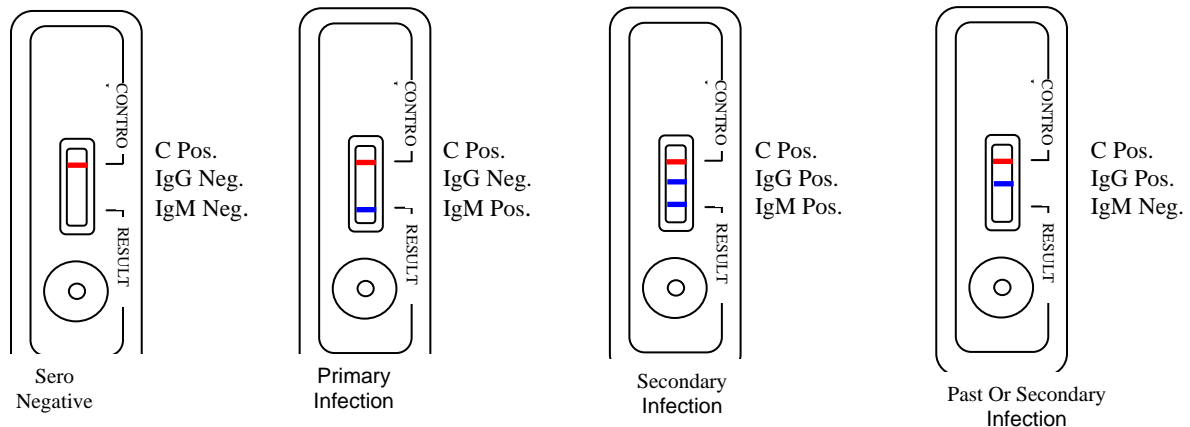
- Timer capable of timing from **0 to 60 minutes**
- Test tubes

TEST PROCEDURE:

- Remove the appropriate number of Dengue Combo Test Device pouches from the kit box. Tear open the foil pouch(es) and remove the device(s). Lay the test device(s) on a clean, flat work surface.
- Add 4 drops (**100 uL**) of Dengue Wash Buffer into a glass or plastic test tube.
- Using a clean, unused **1 uL** plastic loop (provided), dip the circular end of the plastic loop into the specimen, then carefully place the circular end of the plastic loop into the test tube and stir the buffer with the loop. This will add **1 uL** of specimen to the buffer. Remove the loop and dispose of it as a biohazard. Do not reuse the loop. If using a pipetter instead of the loop, add **1 uL** of sample directly to the **100 uL** of buffer in the test tube and vortex. Dispose of the pipette tip as a biohazard.
- Transfer the contents of the tube (the diluted sample) into the sample well of the device.
- Read the test result after 15 to 30 minutes. **Negative results must be confirmed after 30 minutes. Do not read results after 60 minutes.**



INTERPRETATION OF THE RESULTS:



1. The test is not valid if the red control line does not appear, regardless of the presence of a blue IgM or IgG line. Repeat the test with a new device.
2. Specimens with positive IgM antibodies will generate a red line in the control region and a blue line in the IgM region. (a blue line next to the “1”)
3. Specimens with positive IgG antibodies will generate a red line in the control region and a blue line in the IgG region. (a blue line next to the “2”)
4. Specimens with positive IgG and IgM antibodies will generate a red line in the control region and blue lines in the IgG and IgM regions.
5. Positive results may appear as early as 5-10 minutes. **Negative results must be confirmed after 30 minutes.** The results are stable for up to 60 minutes. **Do not read the results after 60 minutes.**

IgM Positive.

The control line (red line) and IgM line (blue line adjacent to the “1”) are visible in the test device window. The test is positive for IgM antibodies. This is indicative of a primary dengue infection (see the “Expected Values and Limitations” sections).

IgM and IgG Positive.

The control line (red line), IgM and IgG lines (both blue lines) are visible in the test device window. The test is positive for IgM and IgG antibodies. This is indicative of a secondary dengue infection (see the “Expected Values and Limitations” sections).

IgG Positive

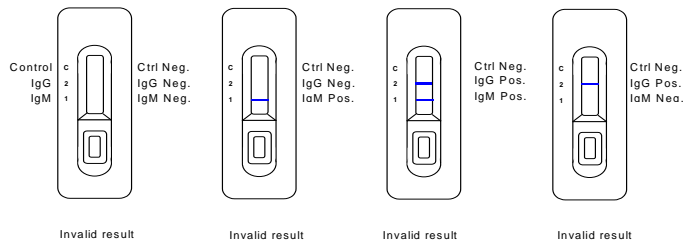
The control line (red line) and IgG line (blue line adjacent to the “2”) are visible in the test device window. The test is positive for IgG antibodies. This may be indicative of a secondary dengue infection (see the limitations section).

Negative Test Result

The control line is the only line visible in the test device window. No IgG or IgM antibodies were detected. The result does not exclude dengue infection. If symptoms persist, a new sample should be drawn from the patient in 3-5 days and then should be retested (see the limitations section).

Invalid Test Result.

If the control line (red line) does not appear in the test device window, the test results are INVALID regardless of the presence or absence of lines in the IgG or IgM region of the device window. Repeat the test using a new device.



EXPECTED VALUES:

Primary dengue is characterized by the presence of detectable IgM antibodies 5 days after the onset of infection.

Secondary dengue is characterized by the elevation of specific IgM antibodies and the elevation of specific IgG antibodies. Usually IgG antibodies will rise within 1-2 days after the onset of symptoms and IgM antibodies will be detectable after 20 days of infection. Therefore, depending on the sampling day, some secondary dengue infections may not have a detectable IgM titer. A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

QUALITY CONTROL:

1. For the assay to be considered valid, the control line must appear. If it does not appear, the test results are not valid and the test must be repeated
2. In addition to your laboratory's standard quality control procedures, the NCCLS recommends that a positive and negative external control be tested at least once within each 25-test kit and by each operator performing testing within a kit. This will verify that the reagents and test devices are working properly and the operator is able to correctly perform the test procedure. Please refer to this NCCLS publication C24-A for recommendations on appropriate Quality Control practices.

LIMITATIONS OF THE TEST:

This test detects the presence of antibodies to dengue in the specimen and should not be used as the sole criterion for the diagnosis of a dengue viral infection. Serological cross reactivity with other flaviviruses is common (e.g., Japanese encephalitis, West Nile, yellow fever, etc.), therefore it is possible that patients with these viruses may show some level of reactivity with this test.

2. In early infections and some secondary infections, detectable levels of IgM antibodies may be low. Some patients may not produce detectable levels of antibody within the first seven to ten days of infection. If symptoms persist, a fresh sample should be drawn from the patient 3-5 days after the first testing date and the new specimen should be retested.
3. As with all diagnostic tests, the result must be correlated with clinical findings. If the test result is negative and a dengue infection suspicion still exists, additional follow-up testing using other clinical methods is recommended.
4. A negative serological result at any time does not preclude the possibility of an early infection of Dengue virus.
5. The use of icteric or lipemic samples should be avoided.

6. Strict adherence to the test procedure is required. Do not re-use negative devices. Do not adulterate the wash solution reagent.
7. This test cannot be used to monitor therapy or to estimate the relative antibody titer.
8. This test should not be used on specimens from immunosuppressed individuals.
9. Negative results must be confirmed at 30 minutes.
10. Results should not be determined after 60 minutes.
11. A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

SENSITIVITY AND SPECIFICITY:

A clinical study using a total 69 serum samples was conducted at various sites in 4 countries. The results of the Cortez Diagnostic Inc., Dengue IgG/IgM combo test were compared with a commercially available ELISA test. The sensitivity and specificity of the IgG and IgM test results are given below:

IgG Results	ELISA (+)	ELISA (-)
Cortez (+)	26	2
Cortez (-)	1	39

Sensitivity = 96% Specificity = 95%

IgM Results	ELISA (+)	ELISA (-)
Cortez(+)	40	1
Cortez(-)	1	27

Sensitivity = 97% Specificity = 95%

CROSS REACTIVITY:

Various samples with known clinical outcomes, verified with PCR, were tested with the Cortez Diagnostic Inc., Dengue IgG/IgM combo test to determine the cross-reactivity of the test. These data are presented in the table below.

	IgM neg (-ve) / total IgM cases	IgG neg. (-ve) / total
JE (5)	5/5 (100%)	5/5 (100%)
Malaria (8)	8/8 (100%)	8/8 (100%)
Thrombocytosis (8) (8 cases)	7/8 (88%)	8/8 (100%)
Negatives (30)	30/30 (100%)	30/30 (100%)

Total (specificity) 50/51 (98%) 51/51 (>99%)

Although the literature suggests that there is serological cross-reactivity within the flavivirus group, i.e., Dengue, Japanese encephalitis and Yellow fever, our test, based on a limited number of samples, did not detect an IgG or IgM response with positive JE samples

STABILITY:

The **Cortez Diagnostic, Inc** Dengue IgG/IgM combo test has been found to be stable for up to 14 months from the date of manufacture when stored between 4 to 30C. The expiration date of each test can be found on the kit box label. No component or reagent of the test should not be used beyond its printed expiration date.

REFERENCES:

1. Sabin, AB and Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice: Science (1945), 101:640.
2. Lam, SK. Dengue haemorrhagic fever. Rev. Med. Micro. (1995), 6:39-48.
3. Innis, BL, Nisalak, A., et.al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trop. Med. Hygiene (1989),40:418-427.
4. CDC/NIH Guidelines. Biosafety in Microbiological and Biomedical Laboratories. 2nd Edition, 1988

Siti-Strong. Diagnosis, prevention, and treatment of tropical disease, 7th ed., Philadelphia, The Ablakiston Company



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