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Detection of Specific Anti-Dengue IgM and IgG Antibodies Differentiate Primary from Secondary Infection

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Abstract

Background: The incidence of dengue fever has increased dramatically worldwide in recent decades. Most cases are asymptomatic or mildly symptomatic and recover with self-management, so the actual number of dengue cases is greater than the number reported. Many cases have also been misdiagnosed as other febrile illnesses.

The number of dengue cases reported to World Health Organization (WHO) has increased more than seven-fold over the past 20 years, from 505,430 cases in 2000 to more than 2.4 million in 2010 and 5.2 million in 2019. The number of reported deaths increased from 960 to 4,032 between 2000 and 2015, mainly affecting younger age groups. Between 2020 and 2021, the total number of cases, as well as the number of reported deaths, appears to have decreased. However, data in this area are incomplete and the COVID-19 pandemic may also have hindered case reporting in several countries.

Clinical presentations range from non-severe dengue fever to severe dengue fever, such as dengue haemorrhagic fever and dengue shock syndrome. Serologically, initial infection is characterized by elevated dengue virus-specific IgM antibodies within 4-5 days after the fever and elevated IgG antibodies 7-10 days later.

Therefore, the diagnosis of primary dengue within the first 5 days requires the application of polymerase chain reaction techniques (RT-PCR) or antigen detection, particularly for NS1 antigen, whereas secondary infections are identified by early elevated IgG antibody titers and lower titer IgM elevations. In addition, the early stages of secondary dengue fever also show PCR and NS1 positivity.

Objective: The main purpose of this evaluation report was to explore the reliability and performance of the CITEST Dengue Rapid Test Cassette 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.

Method: Run a rapid in vitro diagnostic test device for the detection of antibodies (IgG and IgM) to Dengue virus in whole blood, serum, or plasma and compare with a leading commercial Dengue ELISA test for validation of the performance.

Results: The results show that the overall relative sensitivity for the primary and secondary infection of the Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) is 94.3%, the relative specificity is 99.1%, and the relative accuracy is 98.3%.

Conclusion: CITEST Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) 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.

The product is simple to operate and has been validated against an industry leading commercial Dengue ELISA test to give results within 10 minutes of the sample being tested. A comparison of 429 samples showed an accuracy of 98.3% and an excellent specificity of 99.1%. Patients can use this test kit to obtain accurate results and to determine whether they have a primary or secondary dengue virus.

Keywords: Anti-dengue; Primary infection; Secondary infection; Rapid test

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Introduction

About Dengue

Dengue virus is transmitted to humans through the bite of infected Aedes aegypti mosquitoes. (Aedes aegypti or Ae. mosquitoes) mosquitoes. These mosquitoes also transmit the Zika virus, chikungunya virus and others.

Each year, up to 400 million people are infected with dengue fever. Approximately 100 million become ill from the infection and 40,000 die from severe dengue fever. Dengue fever is caused by one of four related viruses: dengue virus 1, 2, 3 and 4. Therefore, a person can be infected with the dengue virus up to four times in his or her lifetime.

Nearly half of the world's population (about 4 billion people) live in areas at risk of dengue fever. Dengue fever is usually the main cause of disease in areas at risk.

Areas with the risk of Dengue

Dengue fever outbreaks occur in many countries around the world, including the Americas, Africa, the Middle East, Asia, and the Pacific Islands. Anyone living in or traveling to an area at risk of dengue fever is at risk of infection.

Since the 1960s, dengue fever has become a global problem. The disease is common in many popular tourist destinations in the Caribbean (including Puerto Rico), Central and South America, Southeast Asia and the Pacific Islands. In the United States, some states with hot, humid climates and Aedes aegypti bites do experience periodic local cases of dengue fever and limited transmission [1].

Primary and Secondary Infections of Dengue

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 [2]. Most Dengue patients in endemic regions have secondary infections [3]. Resulting in high levels of specific IgG antibodies before or simultaneous with IgM response [4]. Therefore, the detection of specific anti-Dengue IgM and IgG antibodies can also help to distinguish between primary and secondary infections.

Secondary dengue fever is a more serious disease than primary dengue fever. It is important to distinguish between the two in the early stages. Important clinical and laboratory differences between the two are identified to allow early differentiation.

Prevention and Diagnosis for Dengue Control

Prevention Method

Eliminate the virus from the root of its transmission route. Prevent Mosquito Bites, and avoid traveling to dengue-prone areas.

Preventing mosquito breeding: prevent mosquitoes from entering egg-laying habitats through environmental management and treatment measures. Methods such as applying appropriate amounts of insecticide to outdoor water storage containers.

Communities also need to be better educated on preventive measures and learn more about the risks of mosquito-borne diseases.

Additional prevention methods include proactive testing of mosquito infestation and viruses, proactive detection and monitoring of vector populations and species composition to determine the effectiveness of control interventions. And prospective testing of virus prevalence in mosquito populations.

Dengue Diagnostic Methods

Dengue infection can be diagnosed in several ways. Different diagnostic methods may be appropriate depending on the timing of the patient's visit to the healthcare provider. Specimens from patients collected during the first week of illness should be tested by two methods [5].

Virus Isolation Methods

During the first few days of infection, the virus can be isolated from the blood. Various reverse transcription-polymerase chain reaction methods can be used, and these are considered the gold standard. However, these methods require specialist equipment and staff training to carry out the test.

The virus can also be detected by testing for a protein produced by the virus (called NS1). Rapid diagnostic tests are commercially available for this purpose, which take only 20 minutes to determine the result and do not require specialized laboratory techniques or equipment.

Serological Methods

Serological methods, such as enzyme-linked immunosorbent assays (ELISA), can confirm the presence of recent or previous infection by detecting anti-dengue antibodies. IgM antibodies can be detected up to 1 week after infection and are detectable for approximately 3 months. The presence of IgG indicates a previous infection.

Evaluation of CITEST Dengue Rapid Test Cassette

Materials and Directions for Use

Materials provided in the kit include a test cassette, buffer, droppers, and a package insert.

The Dengue Rapid Test Kit (whole blood/serum/plasma) is a qualitative membrane-based immunoassay for the detection of antibodies to dengue fever in whole blood, serum or plasma. The test consists of two components, IgG and IgM. In the IgG component, anti-human IgG is encapsulated in the area of the IgG detection line.

As there are three types of samples tested, whole blood, serum and plasma samples are performed in slightly different ways. Regardless of the type of sample, bring the bag to room temperature (15-30°C) before opening it. Remove the test cassette from the sealed bag and use it within one hour.

For Serum or Plasma Specimens

To use a dropper: Hold the dropper vertically, draw the specimen

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up to the Fill Line (approximately 5µl), and transfer the specimen to the specimen well (S) of the test cassette, then add 3 drops of buffer (approximately 120 µl) into the buffer well (B) and start the timer. Avoid trapping air bubbles in the specimen well (S).

To use a micropipette: Pipette and dispense 5 µl of a specimen to the specimen well (S) of the test cassette, then add 3 drops of buffer (approximately 120 µl) into the buffer well (B) and start the timer.

For Whole Blood Specimens

To use 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 (S) of the test cassette, then add 3 drops of buffer (approximately 120 µll) into the buffer well (B) and start the timer.

To use a micropipette: Pipette and dispense 10 µl of whole blood to the specimen well (S) of the test cassette, then add 3 drops of buffer (approximately 120ul) into the buffer well (B) and start the timer.

A colored line will appear and the test result should be read at 10 minutes.

Performance Characteristics

The Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) has been evaluated with specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by a leading commercial Dengue ELISA test (**Tables 1-3**).

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 cases, this is accompanied by elevated levels of IgM [1]. The Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) has been compared with a leading commercial Dengue ELISA test, demonstrating a sensitivity of 83.3% for IgM in primary infection and 98.4% for IgG in secondary infection.

Table 1. Dengue Primary Infection for IgM/Igest results.

Method			ELISA		
Dengue Rapid Test Cassette (Whole Blood/ Serum/ Plasma)	Results		Positive		Negative
			IgM	IgG	
	Positive	IgM	20	0	0
		IgG	4	0	0
	Negative		0	0	0
Relative Sensitivity			83.3%	/	/

Table 2. Dengue Secondary Infection for IgM/IgG test results.

Method			ELISA		
Dengue Rapid Test Cassette (Whole Blood/ Serum/ Plasma)	Results		Positive		Negative
			IgM	IgG	
	Positive	IgM	46	1	0
		IgG	18	63	0
	Negative		0	0	0
Relative Sensitivity			79.1%	98.4%	/

Table 3. Non-Dengue Infection for IgM/IgG test results.

Method			ELISA		
Dengue Rapid Test Cassette (Whole Blood/ Serum/ Plasma)	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%).

Summary

The Dengue Rapid Test Cassette in this evaluation performed satisfactorily in standard experimental conditions. The tests showed both excellent accuracy and specificity.

The results of tested samples demonstrate that the Dengue Rapid Test Cassette (Whole Blood/Serum/Plasma) developed by CITEST Diagnostics Inc. meets the requirements of professional in vitro diagnostic intended use. Thus, a conclusion can be drawn that CITEST Dengue Rapid Test Cassette is capable to be employed in the detection of specific anti-dengue IgM and IgG antibodies to differentiate primary from secondary infection.

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