Article/Artigo

Interpretation of the presence of IgM and IgG antibodies in a rapid test for dengue: analysis of dengue antibody prevalence in Fortaleza City in the 20th year of the epidemic

Interpretação da presença dos anticorpos IgM e IgG nos testes rápidos para dengue: análise da prevalência dos anticorpos da dengue em Fortaleza, no vigésimo ano da epidemia

José Rubens Costa Lima[1,2], Maria Zélia Rouquayrol[1], Maria Roseli Monteiro Callado[2], Maria Izabel Florindo Guedes[3] and Claudia Pessoa[4]

ABSTRACT

Introduction: The diagnosis of dengue and the differentiation between primary and secondary infections are important for monitoring the spread of the epidemic and identifying the risk of severe forms of the disease. The detection of immunoglobulin (Ig)M and IgG antibodies is the main technique for the laboratory diagnosis of dengue. The present study assessed the application of a rapid test for dengue concerning detection of new cases, reinfection recognition, and estimation of the epidemic attack rate. **Methods**: This was a retrospective, cross-sectional, descriptive study on dengue using the Fortaleza Health Municipal Department database. The results from 1,530 tested samples, from 2005–2006, were compared with data from epidemiological studies of dengue outbreaks in 1996, 2003, and 2010. **Results**: The rapid test confirmed 52% recent infections in the tested patients with clinical suspicion of dengue: 40% detected using IgM and 12% of new cases using IgG in the non-reactive IgM results. The positive IgM plus negative IgG (IgM+ plus IgG-) results showed that 38% of those patients had a recent primary dengue infection, while the positive IgG plus either positive or negative IgM (IgG+ plus IgM+/-) results indicated that 62% had dengue for at least a second time (recent secondary infections). This proportion of reinfections permitted us to estimate the attack rate as ≥62% of the population sample. **Conclusions**: The rapid test for dengue has enhanced our ability to detect new infections and to characterize them into primary and secondary infections, permitting the estimation of the minimal attack rate for a population during an outbreak.

Keywords: Dengue. Rapid test for dengue. Immunochromatography test. Epidemic attack rate.

RESUMO

Introdução: O diagnóstico de infecções por dengue e sua diferenciação entre infecções primárias e secundárias são importantes para monitorar a disseminação de epidemias e para identificar riscos de formas graves da doença. A detecção de anticorpos IgM e IgG tem sido o principal mecanismo para o diagnóstico laboratorial. Este estudo visa avaliar a capacidade do teste rápido para dengue para: detectar casos novos da doença, diagnosticar reinfecções e estimar taxas de ataque de epidemias. **Métodos**: Este trabalho consiste de estudo descritivo, transversal retrospectivo, sobre dengue, que utiliza o banco de dados da Secretaria Municipal de Saúde de Fortaleza. Os resultados de 1.530 amostras testadas entre 2005-2006 foram confrontados com dados de estudos epidemiológicos relativos aos surtos de dengue em 1996, 2003 e 2010. **Resultados:** o teste foi capaz de confirmar 52% de infecções recentes entre pacientes com suspeitas clínicas de dengue: 40% das infecções foram confirmadas pela banda IgM e 12% de casos extras foram detectados pela banda IgG reagente em amostras IgM não-reagentes. Resultados IgM reagentes e IgG não-reagentes mostraram que 38% das infecções eram primárias, enquanto resultados IgG reagentes, com ou sem IgM reagente, indicaram que 62% das infecções recentes eram reinfecções. Esta proporção de infecções secundárias permitiu estimar a taxa de ataque como maior ou igual a 62% naquela população amostral. **Conclusões**: O teste rápido para dengue apresentou capacidade aumentada para diagnosticar infecções recentes e de caracterizá-las entre infecções primárias e secundárias, permitindo estimar a taxa de ataque mínima para a população amostral de um surto.

Palavras-chaves: Dengue. Teste rápido para dengue. Imunocromatografia. Taxa de ataque de epidemias.

1. Célula de Vigilância Epidemiológica, Secretaria Municipal de Saúde de Fortaleza, Prefeitura Municipal de Fortaleza. Fortaleza, CE. 2. Hospital Geral de Fortaleza, Secretaria de Saúde do Estado do Ceará, Fortaleza, CE. 3. Laboratório de Bioquímica Humana, Centro de Ciências da Saúde, Universidade Estadual do Ceará, Fortaleza, CE. 4. Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde, Universidade Federal do Ceará. Fortaleza, CE.

Address to: Dr. José Rubens Costa Lima. Célula de Vigilância Epidemiológica/SMS Fortaleza. Rua Antonio Augusto 1571, Aldeota, 60110-317 Fortaleza, CE, Brasil.
Phone: 55 85 3452-6989.
e-mail: rubens@saudefortaleza.ce.gov.br

Received in 18/05/2011
Accepted in 22/09/2011

INTRODUCTION

Dengue is the most important arboviral disease currently affecting humans. It has 4 serotypes, which are transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*[1,2] that are spread throughout the tropical and subtropical areas of the world, transmitting endemic dengue in more than 60 countries.

The diagnosis of dengue and the differentiation between primary and secondary infections are important not only for monitoring the spread of the epidemic but also for identifying the risk of severe forms of the disease. The detection of immunoglobulin (Ig)M and IgG antibodies is the main technique for the laboratory diagnosis of dengue. The present study aimed to establish the effectiveness of a rapid test for dengue.

To better understand the diagnostic strategy, it is essential to know the immune response model for dengue. A few days after the onset of fever, IgM appears as the initial immune response to a primary infection. It suppresses the viremia and is detectable until the end of the second or third month of the disease. Soon after IgM detection, IgG also appears on days 5-7 of the disease, reaching the highest titers during the third week of the disease. In sequence, IgG titers decrease without disappearing, maintaining an immunological memory as a *mark* in the serum or *serological scar*[2,3].

The highest titer of IgG during primary infections is ≤1:1,280[2-4]. In secondary infections, the immune response pattern varies. The IgG titer increases quickly in the first few days of reinfection, being best detected after the seventh day when the titers are higher than in a primary infection, i.e., up to 1:2,560. Conversely, IgM production is lower[2,3,5]. The persistent IgG antibody response is type-specific and does not prevent reinfections by different serotypes in the future[4]. In reinfections, abnormal reactions caused by immunological memory generate more severe forms of the disease, e.g., dengue hemorrhagic fever or dengue shock syndrome, thereby evolving

dengue into a potentially lethal pathology[1]. Therefore, it is of great interest for public health offices to estimate the overall population that is prone to secondary infections.

There have been recurrent outbreaks of dengue fever in Fortaleza City since 1986[6,7], and there is no sign of a reduction in the number of susceptible individuals or vectors. As of September 2006, the 3 serotypes of the dengue virus (i.e., 1, 2, and 3) have circulated simultaneously in multiple outbreaks with alternating dominance, as identified through the surveillance mechanism of the Health Department of the State do Ceará[8]. From 1986 to 1993, only dengue-1 epidemics were detected; from 1994 to 2002, dengue-1 and -2 epidemics occurred simultaneously.[6,7]. In the period 2003-2006, dengue-3 virus was dominant in almost 100% of the infections with positive isolation, although dengue-1 and -2 were also detected[8]. The three simultaneous epidemics (serotypes 1, 2, and 3) maintained a complex epidemiological situation, and the predominance of each virus in the outbreaks has not yet been well described. The only serological survey in the city was performed by Vasconcelos et al. who evaluated the impact of the arrival of dengue virus type 2 in 1994[9]. That study described that 44% of the population of Fortaleza were sensitized by dengue during the outbreak of 1994, 37% had a secondary infection and 7% had a primary infection. On the basis of the data of Vasconcelos et al., following the arrival of dengue virus type 3 in 2003, the Health Municipal Department from Fortaleza estimated that 70% of the city population had been previously sensitized by infections of dengue-1 and/or -2 serotypes, which is a very broad attack rate[7].

As the epidemic unfolded, the private health network brought to the market the so-called *rapid test*, an immunochromatographic test. The rapid test simultaneously detects IgM and IgG antibody titers. The test distinguishes between cases of recent primary and secondary infections[5]. However, the rapid test was not a part of the epidemiological surveillance strategy; it was not recommended or approved by the State of Ceará Health Department or the Brazilian Health Ministry, which questioned the sensitivity of the test for detecting new cases[10]. Nevertheless, in Ceará, rapid test kits were established in the market, being used by the local epidemiological surveillance program to screen cases that were reported to the public surveillance program by a private testing network. Positive test results were repeated via a different method, i.e., the public health laboratory used an IgM-enzyme-linked immunosorbent assay (ELISA). As of 2005, complying with the recommendation of the local health authorities[11], private laboratories began to notify the authorities of all patients who had serological tests for dengue. Consequently, these data became part of the Health Municipal Department database.

Considering that institutional databases are excellent sources of management and scientific information[12], the present study, using the official surveillance records for the results of IgM and IgG rapid serological tests for dengue during the Fortaleza outbreak of 2006, aimed to assess the application of these tests for monitoring the three dengue epidemics with respect to: a) the detection of new cases, b) the identification of reinfections, and c) the estimation of the attack rate of the epidemics.

METHODS

This was as a retrospective, cross-sectional, descriptive study on dengue in the Fortaleza Health Municipal Department database. All of the samples with results in the database were collected from patients who gave their consent to conduct serologic testing for dengue and who had received medical advice to take the test on the eighth day from the onset of fever. We analyzed 1,641 serum samples from 1,530 patients throughout all 6 health regions of Fortaleza from December 2005 to September 2006. The population sample consisted of 684 men and 846 women of all ages, with an average age of 28 years. There were 15 children aged <1 year and 10 patients aged >80 years. There were 1,432 single samples per patient, 87 double samples, 9 triple samples, and 2 quadruple samples (**Table 1**). The test results for the triple and quadruple samples were selected in pairs with the highest clinical outcome per patient, ignoring the others. One record was discarded for its inconsistent results.

TABLE 1 - Sample type and number of patients (n = 1,641 samples).

Sample type	Patients (n)	Samples (n)
Single	1,432	1,432
Double	87	174
Triple	9	27
Quadruple	2	8
Total	**1,530**	**1,641**

Rapid test for dengue

The test utilizes the immunochromatography technique (Dengue Duo Cassette; Panbio, Brisbane, Australia), which highlights the presence of anti-dengue IgM and IgG antibodies in their specific bands and verifies the validity of the kit with a third band. The results of the tests are presented as *reactive* or *non-reactive* to each of the 3 bands, without titration. The reactive results for IgM and/or IgG are more easily found after the viremia that usually disappears by the third day of fever. In the samples tested, antibodies were examined after the eighth day of the disease and thus revealed recent past infections (not active infections). The reactive IgG result is semi-quantitative, showing the presence of antibodies in the serum (with titers ≥ 1:2,560), which are only present in secondary infections[2,3,5,13,14]. This strategic decision of the kit manufacturer was designed to detect only the high serological titers of IgG present in the acute phase of secondary infections, thereby distinguishing them from past infection serological scars (non-recent infections)[5,15,16]. The interpretation of serological results according to the criteria established by the manufacturer, shown in **Table 2**, indicates that the status 4 results, i.e., IgM (-) and IgG (-), correspond to cases without a recent infection. This group consists of dengue-naïve individuals (i.e., with no previous dengue infection) and of individuals with past infections (i.e., non-recent infections), whose low IgG titers are not detected by this kit.

TABLE 2 - Interpretation of serological results for dengue through the dengue duo cassette Panbio rapid test.

	Results		
Status	**IgM**	**IgG**	**Interpretation**
1	(+)	(-)	recent primary infection
2	(+)	(+)	recent secondary infection
3	(-)	(+)	recent secondary infection
4	(-)	(-)	no recent infection

IgM: immunoglobulin M; **IgG:** immunoglobulin G; (+): reactive; (-): non-reactive.
Source: Panbio. New dengue duo cassette. Dengue brochure. Cited on September 14, 2010.

Statistical analysis

The data were analyzed using EpiInfo and Excel in order to evaluate: a) the detection rate of new cases; b) the rate of primary and secondary infections; and c) the attack rate estimation of the dengue epidemic outbreak in 2006.

Ethical considerations

This study was approved by the Ethics Committee of Fortaleza General Hospital under protocol no. 090212/11.

RESULTS

The results of the laboratory tests were classified into 4 categories (Groups A-D) and are summarized in **Table 3**: Group A, individuals with a recent primary infection; Groups B and C, individuals with a recent secondary infection; and Group D, individuals without a recent infection during the exam. Twenty percent of the results were positive for IgM and negative for IgG, indicating that they had a recent primary infection (Group A); and 32% were positive for IgG and positive or negative for IgM, indicating they had a recent secondary infection (Groups B and C). Therefore, these results confirmed the clinical suspicion of dengue in up to 52% of the samples (795 samples). We found that 48% of patients were double-negative for IgM and IgG, i.e., without a recent infection (Group D), corresponding to the total number of individuals without a previous dengue infection up to the moment of the tests plus the cumulatively sensitized individuals since 1986, whose low levels of IgG were not detected by the rapid test used in this evaluation.

TABLE 3 - Dengue serological results (n = 1,530 patients).

Group	Dengue serology IgM	IgG	n	%	Interpretation	Reactive (%)
A	(+)	(-)	301	20.0	recent primary infection	38.0
B	(+)	(+)	308	20.0	recent secondary infection	62.0
C	(-)	(+)	186	12.0	recent secondary infection	
A+B+C			795	52.0	total recent infection	100
D	(-)	(-)	735	48.0	total no recent infection	
Total			**1,530**	**100.0**		

IgM: immunoglobulin M; **IgG:** immunoglobulin G; (+): reactive; (-): non-reactive.

Out of the 795 patients with results that were consistent with a recent infection, 38% were positive for IgM and negative for IgG, indicating that such patients had dengue for the first time (i.e., a recent primary infection). The remaining 62% of the reactive results, which were positive for IgG, showed that those patients had dengue for the second or third time (i.e., recent secondary or tertiary infections, which were indistinctly referred to as recent secondary infections). The latter had already been infected with dengue serotypes 1, 2, and/or 3 during the preceding outbreaks, and this may have sensitized them to the tests.

The confirmation that 62% of patients with a recent infection had serological responses that were consistent with reinfection permitted us to estimate the attack rate at that point of the epidemic in Fortaleza as ≥62%. For a more accurate estimate of the actual attack rate, the percentage of individuals who had been infected in the past, but whose serological scars were not detected by this serologic test for dengue, should be added to this figure.

Out of 97 selected patients with paired results, 85% of the samples were collected with a 14-day interval between the first and second samples; 65% of these samples showed the same result in the second test. Overall, 52% of the samples were initially non-reactive against 48% of the reagents for at least one of the IgGs (**Table 4**).

The 9 initially reactive patients who progressed to non-reactive results in the second test were analyzed separately, as shown in **Table 5**. The results for cases 2, 5, 6, 7, and 8 were difficult to interpret because of the short time between the tests. These 5 patients represent an error rate of 5% (5/97) in the results for the paired samples examined using the rapid test for dengue. Case 2, which was initially positive for IgM and IgG, became non-reactive to both in only 4 days. Cases 5, 6, 7, and 8, which were initially positive for IgG, became non-reactive within 48 h of retesting.

TABLE 4 - Matched tests results (n = 97 patients).

1st test	2nd test	Number	Percentage
Negative (IgG and IgM)		50	52.0
	stable result	35	
	IgG seroconversion	8	
	IgM seroconversion	7	
Positive (IgG and/or IgM)		47	48.0
	stable result	28	
	negative (IgG)	7	
	negative (IgM)	1	
Negative (IgG and IgM)		1	
	IgG seroconversion	7	
	IgM seroconversion	3	
Total		**97**	**100.0**

IgM: immunoglobulin M; **IgG:** immunoglobulin G.

TABLE 5 - Gradual development of the results of patients with a negative response to the second dengue test, in days (n = 9).

Case number	1st Test IgM	1st Test IgG	2nd Test IgM	2nd Test IgG	Interval (days)
1	(+)	(+)	(+)	(-)	9
2	(+)	(+)	(-)	(-)	4
3	(+)	(+)	(-)	(+)	3
4	(+)	(+)	(-)	(+)	20
5	(-)	(+)	(-)	(-)	1
6	(-)	(+)	(-)	(-)	1
7	(-)	(+)	(-)	(-)	2
8	(-)	(+)	(-)	(-)	2
9	(-)	(+)	(-)	(-)	4

IgM: immunoglobulin M; **IgG:** immunoglobulin G; (+): reactive; (-): non-reactive.

DISCUSSION

In the rapid test for dengue, the IgG band combines with IgM to enhance its ability to detect new infections and to characterize them into primary and secondary dengue infections. The IgG band was strategically included in the kit to detect high titers of rebound, i.e., titers ≥ 1:2,560[5], which are only found in secondary infections[2,3,4,5]. A positive result for IgG should not be interpreted as a marker for a serological scar because the titers of IgG as serological scars are below this cut-off point.

The application of the rapid test for monitoring the dengue epidemic outbreak in 2006 showed the following findings.

I) In relation to the detection rate of new cases.

a) The IgM reactivity found in patients from a private testing service indicated a recent infection in 40% of the tested patients, which was equivalent to the 42% obtained through the IgM-ELISA test conducted by the Public Health Laboratory of the Health Department of the State of Ceará among 1,156 samples of suspected dengue patients in Fortaleza in 2010[17]. This demonstrates that the sensitivity of the IgM band test is comparable to IgM-ELISA for the detection of new cases, and it is unlikely that there is a sample selection bias in the patients from private laboratories.

b) The IgG band of the rapid test detected 12% of new cases, identifying 186 cases of secondary infections that were not detected by IgM. This makes the rapid test more sensitive than tests using only IgM by rescuing false-negative cases that are IgM-negative.

II) In relation to the recognition of secondary infections, Vasconcelos et al. and Fortaleza Health Municipal Department estimated dengue attack rates of 44% for 1996[9] and 70% for 2003[7], yet the confirmation of only 32% reactivity to the IgG band demonstrates that the rapid test is unable to detect the low antibodies levels that are characteristic of serological scars. These results indicate that the IgG band test was formulated to distinguish secondary infections from primary infections, which show only IgM band reactivity. Despite the manufacturer's recommendations[5], this interpretation remains poorly known by Brazilian medical and laboratory professionals, who mistakenly conclude that a reactive IgG band result is only indicative of a past dengue infection[18]. Both communities err by not recognizing the IgG band results of this technique as semi-quantitative. The IgG reactive tests are indicative of not only the presence of IgG antibody but, more specifically, also demonstrate the presence of high titers of IgG antibodies, i.e., >1:2,560, which are exclusive to recent reinfections[2–5].

III) In relation to the attack rate estimation of dengue epidemics until the outbreak of 2006, the reactivity of the IgG band test distinguishes secondary from primary infections while also showing the proportion of individuals who already had dengue. Indirectly, this ratio gives an estimation of the lowest attack rate in the population represented by the sample. Among the individuals with negative results for IgM and IgG, many had serological scars that were not detected by the rapid test, which were the results of past infections with dengue-1, -2, or/and -3 viruses during epidemics since 1986.

a) While evaluating only patients with a recent infection (795 with at least 1 reactive result), it was observed that 62% of them were suffering an infection for the second or third time, which in turn permits the estimation of an attack rate for the 2 viral subtypes of ≥62% in this population sample.

b) In 2006, the viral isolations performed at the State of Ceará Health Department[8] identified that dengue serotype 3 had a dominance of 98% during the outbreak. Secondary infections were found in 62% of the cases (previously infected by dengue serotypes 1, 2, or both).

c) The dominant circulation of dengue serotype 3 has been evident since 2003; therefore, a large number of individuals have been sensitized and would not be infected unless a different serotype appeared. Mathematically, to correct the estimation of the dengue attack rate for that year, the total number of dengue serotype 3 cases during the past 4 years, which could not be identified by their serological scar, must be added to the 62% of cases from the population sample.

d) Despite the fact that we assessed a universal sample that included all suspected individuals, the attack rate in the population studied cannot be applied to the whole city because there is a possibility that the infected individuals in the present study had been previously identified.

In 1994, Fortaleza faced a special epidemiological condition after the first outbreak of dengue serotype 2 that affected a dengue-1 serotype-sensitized population. Vasconcelos et al.[9] used the hemagglutination test for dengue and found that 37% of individuals from the sample had a secondary infection (84% of infections) and 7% of individuals had a primary infection (16% of infections).

Considering the random sample nature of the study of Vasconcelos et al., the estimation rate in 1996 could be applied to the whole City of Fortaleza. The 84% of reinfected individuals, who were infected by serotype 2 in 1994, had been previously infected by dengue serotype 1; therefore, the previous attack rate for dengue serotype 1 was 84%. The attack rate cited by Vasconcelos et al. of 44% only represents the attack rate of recent infections by dengue serotype 2.

This meta-analysis using the data of Vasconcelos et al. creates a new conflict in the estimations calculated in this paper. According to the meta-analysis, it is assumed that dengue-1 reached an attack rate of 84% until 1996 in Fortaleza. Including the dengue 2 cases from 1994-2003, even if they occur in the same exposed population groups, would increase this attack rate, involving some new individuals. The attack rate before the appearance of dengue serotype 3 would be ≥84%; however, the findings of this study, which show only 62% of secondary cases, were startling. They suggest that the population sample is less exposed to the dengue mosquito than the general population, which was randomly tested in the study of Vasconcelos et al.

IV) In relation to the reproducibility of the results, the 97 paired results demonstrate that the rapid test for dengue has a good reproducibility, with only 5% inconsistency.

In conclusion, the rapid test for dengue is a 3-band test with good diagnostic ability and increased sensitivity to detect infections through its IgG band in the presence of low levels of IgM. The IgG band, a semi-quantitative test, identifies IgG titers > 1:2,560, thereby distinguishing recent secondary infections from primary ones. The negative IgM and positive IgG results, which are related to recent secondary infections, should not be mistaken as a signal of past infections. While the rapid test identifies primary and secondary cases, it permits the estimation of the proportion of previously sensitized individuals, which is equivalent to the minimal attack rate for a population sample during an outbreak.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239:476-481.
2. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, and control. Geneva: World Health Organization; 1997.

3. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, and control. Geneva: World Health Organization; 1986.

4. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998; 11:480-496.

5. New Dengue Duo Cassette Dengue Brochure (Panbio) [Internet]. [Cited 2010 September 14]. Available from: http://panbio.com/download/Dengue_Brochure.pdf/.

6. Secretaria Municipal de Saúde de Fortaleza. Plano de contingência para prevenção e controle da epidemia de dengue na cidade de Fortaleza, Ceará, em 2010. Fortaleza: Secretaria Municipal de Saúde de Fortaleza; 2010.

7. Secretaria Municipal de Saúde de Fortaleza. Boletim de Saúde de Fortaleza - Dengue. Vol. 7, n. 1. Fortaleza: Secretaria Municipal de Saúde; 2003.

8. Secretaria de Saúde do Estado do Ceará. Informe Semanal da Dengue. Gráfico IV. Percentual de isolamento para dengue por sorotipo viral, Fortaleza: Secretaria de Saúde do Estado; 2010

9. Vasconcelos PFC, Lima JWO, Rosa APAT, Timbó MJ, Rosa EST, Lima HR, et al. Rev Saude Publica 1998; 32:447-154.

10. Ministério da Saúde. Nota Técnica 20/2010 CGLAB/SVS/MS de 20 de setembro de 2010: Uso de testes rápidos IgM/IgG para fechamento de casos de dengue. Brasília: Ministério da Saúde; 2010.

11. Secretaria da Saúde do Estado do Ceará. Ofício Circular 094/2005 Gabinete. Recomenda Notificação de Suspeitas de Doenças Exantemáticas. Fortaleza: Secretaria da Saúde do Estado do Ceará; 2005.

12. Lima JRC. Relação DST x HIV: Estudo do banco de dados do COAS-Campinas, São Paulo, 1994-1997. [Masters Thesis]. [Campinas (SP)]: Universidade Estadual de Campinas; 2001. 158 p.

13. Rivetz B, Siman-Tov D, Ambal E, Jaramillo AC, Ben-Zvi A, Tartakovsky B, et al. New dengue antibody assay with unique differential detection of IgG and IgM antibodies. Clin Biochem 2009; 42:180-184.

14. Inoue S, Alonzo MT, Kurosawa Y, Mapua CA, Reyes JD, Dimaano EM, et al. Evaluation of a dengue IgG indirect enzyme-linked immunosorbent assay and a Japanese encephalitis IgG indirect enzyme-linked immunosorbent assay for diagnosis of secondary dengue virus infection. Vector Borne Zoonotic Dis 2010; 10:143-150.

15. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G capture enzyme-linked immunesorbent assay Panbio kits for diagnostic dengue infections. J Clin Virol 2007; 39:194-198.

16. Vazquez S, Acosta N, Ruiz D, Calzada N, Alvarez AM, Guzman MG. Immunoglobulin G antibody response in children and adults with acute dengue 3 infection. J Virol Methods 2009; 159:6-9.

17. Secretaria Municipal de Saúde de Fortaleza. SIMDA Fortaleza 2010. Positivity of serology for dengue. Fortaleza: Secretaria Municipal de Saúde; 2010.

18. WAMA diagnóstica. Rapid-immuno dengue IgG/IgM. Bula instrutiva do produto MS 10310030096, disponível na embalagem do produto comercializado nos anos 2005-2006.