

Serological Testing Prior to Dengue Vaccine Administration
Committee on Immunization
Pediatric Infectious Disease Society of the Philippines
13 March 2017

In May 2016, the PIDSP Committee on Immunization reviewed available evidence on the safety and efficacy of the licensed dengue vaccine, Dengvaxia® (Sanofi). Based on this review, the committee concluded that the live attenuated tetravalent dengue vaccine appears to be effective and safe and can be given to children aged 9 years and older. Aside from providing protection for individuals ≥ 9 years, the data also showed that the vaccine was more effective in those who were already exposed and are positive for dengue antibodies.

Concerns raised over the long-term safety of CYD-TDV, particularly among individuals who are seronegative when first vaccinated, have prompted consideration of serologic testing prior to immunization. In this document, the PIDSP Committee on Immunization reviews current literature to provide information for the clinician.

Efficacy of the Dengue Vaccine

Vaccine efficacy varied by serologic status at the time of receiving the first dose, by serotype, severity of disease, and by age. Evidence suggests that the vaccine provides better protection against severe dengue for older children ≥ 9 years, and for those who were already exposed and are positive for dengue antibodies at the time of first vaccination. Vaccine efficacy was also shown to be lower against serotypes 1 and 2 than against serotypes 3 and 4.¹

Focusing on the study by Capeding et al (the CYD14 trial), the overall efficacy of the vaccine within the first 25 months after the first dose in children aged 2-14 years old was 80% (95% CI 52.7 - 92.4%) against severe dengue and 67.2% (95% CI 50.3 - 78.6%) against hospitalized dengue.¹

During the third year of follow-up in the same study, vaccine efficacy against hospitalization for dengue was 81.6 % (95% CI 60.7 - 92.0%) among participants who were ≥ 9 years, but was lower among those under 9 years old, at 56.1% (95% CI 26.2 - 74.1%).² During this same period, vaccine efficacy against development of dengue hemorrhagic fever was 80.8% (95% CI 70.1 - 87.7%) among participants who were ≥ 9 years of age and 66.7% (95% CI -4.7 - 90.2) among those under 9 years old.²

Safety of the Dengue Vaccine

During the first 2 years of the CYD14 Asian Study, there was no difference in the incidence of non-serious systemic adverse events. However, there was one case of acute disseminated encephalomyelitis post-varicella infection occurring 7 days after the first injection as well as 4 deaths, all unrelated to vaccination: three traffic accidents and one tracheal injury.¹ No immediate hypersensitivity or allergic reactions, and no cases of viscerotropic or neurotropic disease were reported.¹

The results of an extended hospital-based observation study by Hadinegoro et al,² however, showed that by the third year following vaccination, receipt of the vaccine was associated with a 7.45 times increased risk of hospitalization for dengue of any severity in the 2-5 years age group. There was no evidence of increased risk in the 6-11 year old and 12-14 year old groups.²

In addition, the supplementary appendix of the Hadinegoro study provided data on hospitalization for severe dengue over the same period.³ Only for subjects in the CYD14 study, a 5.5 times overall risk for hospitalization was seen among those who were vaccinated with the dengue vaccine (RR 5.50, 95% CI 0.71- 42.6). Further analysis showed that the increased risk was seen in those less than 9 years old. During the year 4 follow-up phase of the same study, this risk was

shown to have decreased compared to the year 3 data (RR 1.19, 95% CI 0.65 to 2.28).⁴

Because of the safety signal of increased risk of hospitalized and severe dengue identified in the 2 to 5 year age group, the current dengue vaccine, Dengvaxia® is not licensed for children under 9 years of age.

Effect of Baseline Dengue Serologic Status on Vaccine Efficacy and Safety

In the CYD14 study, approximately 70% of all participants 2 to 14 years old were seropositive for dengue at baseline, based on the plaque reduction neutralization test (PRNT). Among those \geq 9 years of age, approximately 80% were seropositive at the time of the first dose of vaccine.³ In this age group, vaccine efficacy was higher among seropositive (79.2%, 95% CI 47.2 - 92.7%) than among seronegative participants (61.6%, 95% CI -21.1 - 88.1%).²

The clinical data on seronegative vaccine recipients in the older age group are insufficient for drawing definite conclusions. As in other vaccines, longer follow-up periods and continued surveillance will be required before any definite conclusions can be made regarding the safety of the vaccine when used on dengue-naïve individuals of any age.⁵

A number of interconnected mechanisms involving interactions between the infecting virus, host age, pre-existing immunity and vaccine-induced immunity have been proposed to explain the results, although none have been proven conclusively to explain differences in efficacy and safety.⁶ Data from continued surveillance and safety monitoring of dengue vaccine is important to determine the long term relative risks of all of the relevant outcomes based on serologic status and age at the time of vaccination.

With the above summary to serve as a background, the following practical questions may be helpful for the clinician to consider:

What are the current recommendations on serologic testing prior to vaccination?

Rapid diagnostic tests could be used to screen potential vaccine recipients, with only seropositive individuals being vaccinated. This targeted vaccination strategy, as recommended by some experts on dengue, would reduce the potential risks and maximize the benefits of dengue vaccination. This may be optimal in situations where the resources and infrastructure are in place to conduct the screening prior to vaccination^{5,8}

On the other hand, the WHO SAGE working group advised against screening for serostatus prior to vaccination, pointing out the unavailability of rapid, point-of-care tests to establish serostatus at the time of vaccination, logistical challenges in implementing a screening test prior to vaccination, as well as a lack of demonstrated harm in the older age group.⁸ Rather, based on considerations of superior efficacy and, possibly, the safety and duration of protection in seropositive individuals, SAGE recommended a seroprevalence threshold of 70% or higher in the age group targeted for vaccination as the best population-level strategy^{7,8} -

What is the seropositivity rate of dengue in those \geq 9 years old in the Philippines?

There is currently no national data documenting dengue seroprevalence in the Philippines. However, in one study involving 1,066 Filipino children aged 2-16 years, dengue seropositivity rates as determined by plaque-reduction sero-neutralization assay were found to increase with increasing age: 58% in those age 2-4 years, 74.9% in those 5-8 years, 88.5% in those 9-12 years, and 93% in those 13-16 years.⁹

Subsequently, a prospective longitudinal cohort study conducted in Cebu City among 1,008 children and adults starting from age 6 months and older showed that >98.3% of all those > 15

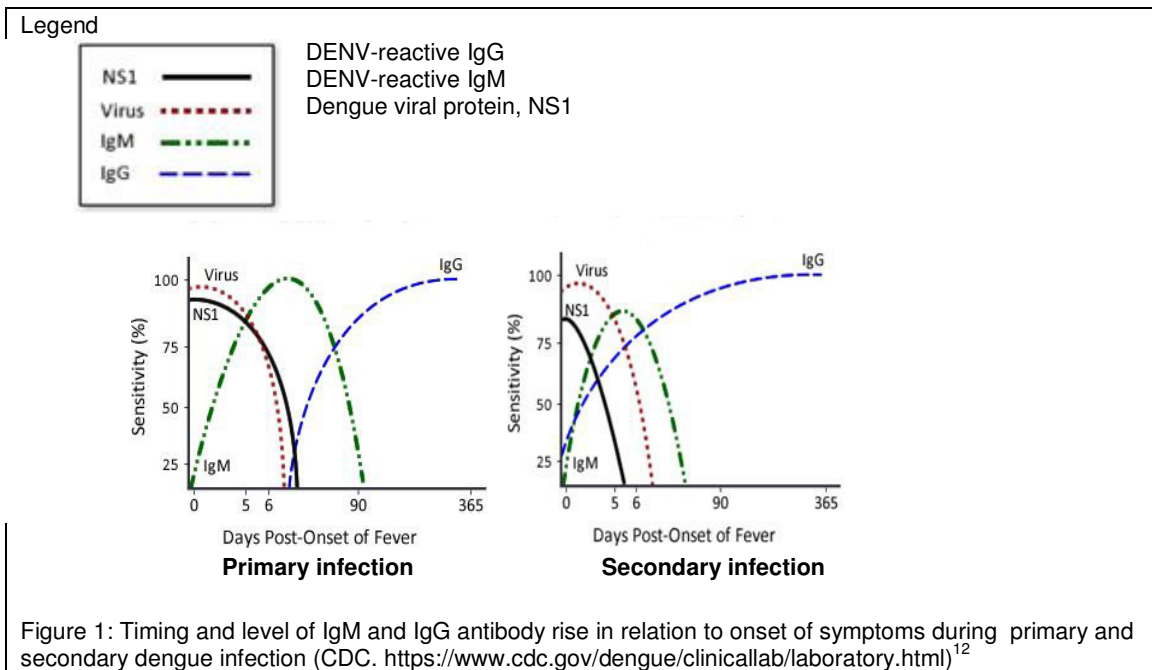
years developed evidence of multi-typic dengue HAI antibodies during the 12-month study period. However, only 17.5% of dengue infections that occurred were symptomatic; 82.5% developed subclinical infection.¹⁰

In the absence of population-based serologic data, the WHO suggests the use of epidemiologic information (incidence, morbidity and mortality rate among infectious diseases) as an indicator.

What do dengue serological tests measure?

Dengue serological tests measure IgM and IgG antibodies against dengue. Serologic testing facilitates diagnosis and helps distinguish primary from secondary dengue infection.

In most infected individuals having primary infection, IgM is detected 5 or more days after the onset of illness while IgG is detected from 10–15 days. During secondary infections, IgM appears earlier or in the same time frame but occurs at lower titers. IgG that has been present since primary infection on the other hand shows rapid increase in titers.¹¹ Figure 1 below shows the timing of detection of IgM and IgG during primary and secondary dengue infection.



What are the locally available dengue serologic tests?

The basic principles of the commonly used dengue serological tests that are locally available are presented in **Table 1**.

Table 1. Basic Principles of Dengue Serological Tests

Test	Principle/Description of the test	Remarks
Hemagglutination inhibition (HAI), test	Based on the ability of dengue antigens to agglutinate red blood cells, this test measures the amount of anti-dengue antibodies in sera that can inhibit agglutination ¹³	No longer used except for research
Enzyme-linked immunosorbent assay (ELISA)	<p>IgM antibody capture ELISA (MAC-ELISA) format is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody, followed by the addition of dengue virus specific antigen (DENV1-4) derived from the virus envelope protein¹²</p> <p>IgG ELISA is used for the detection of a past dengue infection and utilizes the same viral antigens as the MAC ELISA. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups. A negative IgG in the acute phase and a positive IgG in the convalescent phase suggests primary dengue infections. A positive IgG in the acute phase and a 4 fold rise in IgG titer in the convalescent phase (with at least a 7 day interval between the two samples) is a secondary dengue infection¹²</p>	Cross reactivity between other circulating flaviviruses is the major limitation. ¹²
Plaque reduction neutralization test (PRNT)	Currently considered to be the “gold standard” to characterize and quantify circulating levels of neutralizing antibody against dengue. It is the most serologically virus-specific and serotype-specific test among dengue viruses with good correlation between serum levels and protection from virus infection. Newer tests measuring virus neutralization are being developed, but PRNT remains the laboratory standard against which these tests will need to be validated ¹⁴	This test is labor intensive, time-consuming ¹⁴ and is currently available only at the RITM Processing time: 4 weeks
Immunochromatographic Test (ICT)	<p>ICTs for the detection of dengue antibodies are in the form of either a lateral flow cassette that allows the flow of sample in a horizontal plane or a wick-style test that is performed in a tube and draws the sample vertically by capillary action.</p> <p>These rapid diagnostic tests use a combination of dried antigens and colloidal gold-labeled monoclonal antibodies on a pad at the head of a nitrocellulose strip that is impregnated with antibody lines. Test sample and running buffer are added to the pad releasing the colloidal gold that facilitates mixing of the sample with the gold complex, and the migration of reagents and sample by capillary action along the nitrocellulose strip towards the antibody lines. Appearance of maroon bands in the location of antibody lines signifies</p>	This is a commonly used rapid test in local laboratories. It is easy to use, gives rapid results and requires no specialized equipment or training making this test ideal for low-technology environments. Limitations include subjective reading by the operator as well as some cross reactivity with other

	presence of antibody ¹⁵	members of the Flaviviridae family ¹⁵
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How accurate are commercial serologic tests (point-of-care rapid tests)?

Locally available rapid diagnostic tests are indicated for the diagnosis of acute dengue infection through high levels of IgM and IgG during acute and convalescent phase. These rapid tests may give false negative or false positive results due to cross reactivity to other flaviviruses (refer to Table 2 below), malaria, rheumatoid factor, or SLE, and are not intended for the evaluation of serostatus prior to vaccination.

It is important to note these rapid tests have not been validated for the purpose of evaluation of prior exposure to dengue before vaccination. These tests are not being promoted or marketed for this purpose.

Table 2: Sensitivity and Specificity Values of Dengue ELISA and Rapid Diagnostic Tests (RDTs)

Reference	Dengue Test			Sensitivity	Specificity	False positive
	Target antibody/ marker	Type of test (format)	Specific Brand (Company)			
Hunsperger (2014) ¹⁷	IgM	ELISA	Venture	Acute Phase: 98% Convalescent Phase: 97%	Overall specificity: 84%.	False positive reactions: 18-50% observed against other flaviviruses (*SLEV, JEV, WNV,CKV, Hanta virus); Lepto 5-18% Malaria 5-25% Lyme: 10% scrub typhus 5-18% RF 25-90% SLE 100% Pregnancy 5%
			Rapid test	Abon		
		CTK		Acute Phase: 46% Convalescent Phase: 53%		
		Orgenic		Acute Phase: 95% Convalescent Phase: 82%		
		SD Duo IgM	Acute Phase: 89% Convalescent Phase: 98%			
WHO (2009) ¹⁸	IgM and IgG	Rapid test	Dengue Duo Casette (PanBio)	77.8	90.6	Malaria 10-45% RF 31.6-35% Lyme: 5% Other flaviviruses: (JE, WNV, YF, SLVE); hanta virus: 5-20%
			SD Biotline IgG/IgM (Standard Diagnostics)	60.9	90	
			Dengucheck-WB (Zephyr Biomedicals)	20.5	86.7	
Hunsperger (2009) ¹⁹	IgM	ELISA	Panbio	99	84.4	Malaria: 4-35% RF: 40-65% Lepto: 5% Other flaviviruses:
			Standard	97.6	86.6	
	IgM	Rapid test	Panbio	77.8	90.6	
			Standard	60.9	90	
			Pentax	97.7	76.6	
Zephyr	20.5	86.7				

						(JE, WNV, YF, SLEV): 3-45: hanta virus: 10-18%
Blacksell (2011) ²⁰	IgM	Rapid test	Dengue fever IgG, IgM combo device (Merlin)	72.7	73.8	Malaria: 1.1-5.9% Lepto: 9.4-18.1%
			Immunoquick Dengue fever IgG, IgM (Biosynex)	79.8	46.3	Bacteremia: 7.1-9.4% Scrub typhus: 4.8-15.6%
			Panbio Dengue Duo Cassette (Inverness)	70.7	80	Q fever: 2.3-9.4% TB: 3.1-11.8%
			Standard Diagnostics	79.2	82.3	UTI: 2.3% RMSF: 1.1-3.1 CKV: 46.9-59.5

*St. Louis encephalitis virus (SLEV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Chikungunya virus (CKV), Rheumatoid factor (RF), Systemic lupus erythematosus (SLE), tuberculosis (TB), urinary tract infection (UTI), Rocky mountain spotted fever (RMS)

What is the recommendation regarding currently available serologic testing prior to dengue vaccination?

In light of the limitations of the currently available rapid serologic tests, and the difficulty in interpretation of results, no recommendations can be given for serologic testing prior to dengue vaccination using currently available tests at this time.

It is suggested that the clinician use the above data to discuss options for testing and vaccination with individual patients.

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