

Dengue vaccine: reliably determining previous exposure



Dengue fever is the most prevalent and widespread mosquito-borne viral disease, and can only be countered by integrated prevention and control strategies, including sustained vector control programmes, the best evidence-based clinical care, and vaccination.

The first dengue vaccine, CYD-tetravalent dengue vaccine (CYD-TDV) or Dengvaxia (Sanofi Pasteur, Lyon, France), is licensed in 20 countries. Initial findings from two large phase 3 clinical trials have shown good but incomplete protection, in particular against severe dengue and dengue disease that requires hospital admission.^{1,2} In November, 2017, Sanofi Pasteur announced the results from additional studies showing that the vaccine has a differential performance in individuals who have previously been infected by dengue virus (seropositive) versus those without previous dengue virus infection (seronegative).³ Vaccine efficacy against laboratory-confirmed symptomatic dengue virus infection was high among individuals who were seropositive at baseline and aged at least 9 years (76%, 95% CI 63.9–84.0), but much lower among participants who were seronegative at baseline (38%, –0.9 to 62.9). Furthermore, vaccination of individuals who are seronegative increases their risk of severe dengue or dengue that requires hospital admission.⁴ Subsequently, in April, 2018, WHO's Strategic Advisory Group of Experts recommended that in countries considering the introduction of vaccination with CYD-TDV, pre-vaccination screening should be preferred to assess dengue virus serostatus, and only people who are dengue seropositive should be vaccinated. To this end, WHO also encouraged the urgent development of rapid diagnostic tests (RDTs) to establish serostatus.

Because dengue viruses are of the genus *Flavivirus*, composed of genetically, structurally, and antigenically related viruses, such as Zika virus, yellow fever virus, and tick-borne encephalitis virus, antibody detection tests have high cross-reactivity and consequently poor reliability for the diagnosis of past dengue virus infection.⁵ With large flavivirus-exposed populations in Latin America, Asia, and Africa, a rapidly growing population of travellers from and to endemic areas,⁶ and increased vaccination coverage against yellow fever virus, tick-borne encephalitis virus, and Japanese encephalitis virus in travellers and in certain endemic regions, it is evident that establishing serostatus is extremely challenging.

Many commercial immunoglobulin (Ig)G-containing point-of-care tests (POCTs) and dengue IgG ELISA assays are available. For the purpose of pre-vaccination screening, the assay with the highest sensitivity and specificity would be the desirable option. Low sensitivity would result in under-vaccinating individuals who are truly seropositive and who would benefit from the vaccine, whereas low specificity would lead to falsely vaccinating people who are truly seronegative, putting them at risk of severe dengue during the next natural infection with dengue virus.

The plaque reduction neutralisation test (PRNT) is still considered the gold standard for establishing serostatus, but requires specific laboratory and technical capacity, and is labour intensive, costly, and time consuming. Although dengue IgG ELISA correlates reasonably well with the results of a PRNT, the test has a lower sensitivity and specificity than PRNT, although the studies^{7,8} that showed this lower sensitivity and specificity were done before Zika virus became a widespread problem in the Americas. Dengue IgG ELISA requires about 2.5 h of laboratory time, excluding the time needed for sample transportation to the laboratory, batch analysis, and reporting to the clinician, which requires two visits for the vaccinees. Point-of-care testing that uses RDTs provides the vaccine recipient with a result within 15–30 min, and can be done in an outpatient or outreach setting, such as schools and care facilities, using a finger prick sample. Thus, a decision on vaccination eligibility can be made during the same visit, thereby ensuring a reasonable vaccine uptake. POCTs generally have lower sensitivity and specificity than dengue IgG ELISAs. However, this low sensitivity and specificity needs to be weighed up against the faster speed of testing, lower cost, and improved accessibility outside specialised laboratories compared with IgG ELISAs.

As available RDTs were mainly developed for the purpose of diagnosing acute dengue virus infection, further efforts would be justified to fine-tune such RDTs to increase sensitivity and specificity for diagnosing serostatus. To increase the sensitivity of dengue RDTs to detect previous dengue virus infection, several modifications could be contemplated, one of which would be recalibration by changing the concentration

Published Online
June 22, 2018
[http://dx.doi.org/10.1016/S2214-109X\(18\)30295-X](http://dx.doi.org/10.1016/S2214-109X(18)30295-X)

of the IgG capture antigen or detection reagent to lower the limit of anti-dengue IgG detection. To address cross-reactivity with other flaviviruses, particularly Zika, other modifications should be considered to improve specificity.

Diagnostic methods for flavivirus antibody detection use the entire virus particle, recombinant surface E-protein, or NS1 protein as antibody-capturing antigens. Epitopes with high amino acid sequence homology among serotypes or flaviviruses can trigger cross-reactive responses, but type-specific antibodies directed towards unique E and NS1 epitopes are also raised.^{9,11} The art of making specific serological tests lies in the identification, selection, and presentation of those epitopes, either as recombinant protein fragments or peptides that only bind type-specific IgG, instead of using the entire virion or recombinant protein that contains both type-specific and cross-reactive antibody epitopes. Dengue virus antibody tests that use recombinant E-protein to absorb IgG will not be useful anymore for pre-testing purposes or for seroprevalence studies once the dengue vaccine (that uses dengue virus E-protein as an immunogen) is widely spread. Capturing type-specific non-E-protein-directed antibodies could be an alternative approach, as applied in the recently developed Zika virus-specific NS1 blockade-of-binding ELISA.¹⁰ Comparative epitope modelling and genome-wide peptide microarray analysis can help to identify virus-specific epitopes targeted by antibodies in individuals who are flavivirus seropositive. Whereas obtaining type-specific epitopes is challenging but technically feasible, the difficulty might be in retaining sufficiently high sensitivity when aiming to detect specific subsets of antibodies in people who have been exposed to flavivirus. The epitope specificity and the abundance of such subsets of antibodies might also differ between populations and individuals as a result of differences in genetic background, co-circulating pathogens, vaccination, history of natural infection, and timing of sampling. Combining multiple type-specific epitopes and antigen-antibody crosslinking could potentially help

address this issue. Evaluation platforms and access to well characterised samples to accelerate test development and access to market are urgently needed.

*Kevin K Ariën, Annelies Wilder-Smith

Virology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp B-2000, Belgium (KKA); Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium (KKA); Institute of Public Health, University of Heidelberg, Germany (AW-S); and London School of Hygiene and Tropical Medicine, London, UK (AW-S)
karien@itg.be

We declare no competing interests. KKA serves as a consultant on diagnostics to WHO and AW-S as a consultant on arboviral diseases to WHO. KKA also serves as an expert for the Belgian Federal Agency for Medicines and Health Products. The views expressed in this manuscript do not necessarily represent the views of WHO or the Belgian Federal Agency for Medicines and Health Products. KKA is a member of ZikaPLAN and AWS is the coordinator of the ZikaPLAN consortium that received funding from the European Union's Horizon 2020 research and innovation programme, under the ZikaPLAN grant agreement 734584.4.

Copyright © 2018 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

- 1 Capeding MR, Tran NH, Hadinegoro SR, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014; **384**: 1358–65.
- 2 Villar L, Dayan GH, Arredondo-Garcia JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* 2015; **372**: 113–23.
- 3 Sanofi. Sanofi updates information on dengue vaccine. 2017. <http://mediaroom.sanofi.com/sanofi-updates-information-on-dengue-vaccine/> (accessed May 4, 2018).
- 4 WHO. Background paper on dengue vaccines. 2018. http://www.who.int/immunization/sage/meetings/2018/april/2_DengueBackgrPaper_SAGE_Apr2018.pdf (accessed May 4, 2018).
- 5 Goncalves A, Peeling RW, Chu MC, et al. Innovative and new approaches to laboratory diagnosis of Zika and dengue: a meeting report. *J Infect Dis* 2018; **217**: 1060–68.
- 6 Glaesser D, Kester J, Paulose H, Alizadeh A, Valentin B. Global travel patterns: an overview. *J Travel Med* 2017; **24**. DOI:10.1093/jtm/tax007.
- 7 Sanchez-Vegas C, Hamer DH, Chen LH, et al. Prevalence of dengue virus infection in US travelers who have lived in or traveled to dengue-endemic countries. *J Travel Med* 2013; **20**: 352–60.
- 8 Rocha ES, Oliveira JG, Santos JR, et al. Recombinant envelope protein-based enzyme immunoassay for IgG antibodies is comparable to neutralization tests for epidemiological studies of dengue infection. *J Virol Methods* 2013; **187**: 114–20.
- 9 Collins MH, McGowan E, Jadi R, et al. Lack of durable cross-neutralizing antibodies against Zika virus from dengue virus infection. *Emerg Infect Dis* 2017; **23**: 773–81.
- 10 Balmaseda A, Stettler K, Medialdea-Carrera R, et al. Antibody-based assay discriminates Zika virus infection from other flaviviruses. *Proc Natl Acad Sci USA* 2017; **114**: 8384–89.
- 11 Premkumar L, Collins M, Graham S, et al. Development of envelope protein antigens to serologically differentiate Zika virus infection from dengue virus infection. *J Clin Microbiol* 2018; **56**. DOI:10.1128/JCM.01504-17.