11 November 2021
EMA/754271/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Dengvaxia

Common name: dengue tetravalent vaccine (live, attenuated)

Procedure No. EMEA/H/C/004171/II/0011

Note

Variation assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

CCDS	Company Core Data sheet
CCID50	cell-culture infectious dose 50%
CDP	Clinical Development Programme
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
NS	Non structural
PDI	Past dengue infection
PRNT	plaque reduction neutralization test
RDT	Rapid diagnostic test
RMP	Risk Management Plan
VCD	virologically-confirmed dengue
VE	Vaccine efficacy
WHO	World Health Organization
YF	Yellow fever

1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, on 20 August 2020 Sanofi Pasteur submitted an application for a variation to the European Medicines Agency.

The following variation was requested:

Variation requested		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I and IIIB

To modify the approved therapeutic indication to include conditions for the eligibility to pre-vaccination serostatus screening. As a consequence, sections 4.1, 4.2 and 4.4 of the SmPC and sections 1, 2 and 3 of the Package Leaflet are updated accordingly.

The variation requested amendments to the Summary of Product Characteristics and Package Leaflet.

Information on paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included (an) EMA Decision(s) P/0065/2020 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-001545-PIP01-13-M02 was completed.
The PDCO issued an opinion on compliance for the PIP EMA/PDCO/172848/2020.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the MAH did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific advice

The MAH did not seek Scientific Advice at the CHMP.

1.2. Steps taken for the assessment of the product

The Rapporteur appointed by the CHMP was:

Rapporteur: Christophe Focke

Timetable	Actual dates
Submission date	20 August 2020
Start of procedure:	12 September 2020
CHMP Rapporteur Assessment Report	10 November 2020
CHMP members comments	30 November 2020
Updated CHMP Rapporteur(s) (Joint) Assessment Report	4 December 2020
Request for supplementary information (RSI)	10 December 2020
CHMP Rapporteur Assessment Report	27 April 2021
CHMP members comments	n/a
Updated CHMP Rapporteur Assessment Report	12 May 2021
Request for supplementary information (RSI)	20 May 2021
CHMP Rapporteur Assessment Report	27 October 2021
CHMP members comments	n/a
Updated CHMP Rapporteur Assessment Report	5 November 2021
CHMP Opinion	11 November 2021

2. Scientific discussion

2.1. Introduction

In the EU, at the time of this application, Dengvaxia was indicated for the *prevention of dengue disease caused by dengue virus serotypes 1, 2, 3, and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas*. The vaccination schedule consists of 3 injections 6-month apart.

Large randomized controlled trials in dengue endemic regions revealed an overall efficacy of Dengvaxia in preventing virologically-confirmed dengue (VCD) over two years following first vaccination, in individuals 9-16 years irrespective of serostatus. Efficacy was variable across the four dengue serotypes following vaccination. Dengvaxia also showed good efficacy in preventing severe dengue disease in that period. However, stratification by serostatus at baseline showed that vaccine efficacy in ≥9years of age individuals was actually lower in those who were seronegative than in those who were seropositive (39% vs. 76%, respectively) at baseline. Follow up of the subjects revealed an increased risk of severe dengue disease in vaccine recipients who were seronegative prior to vaccination. In contrast, vaccine efficacy was sustained in those seropositive at baseline, suggesting benefit of vaccination against more severe disease in this population. This lead in EU to the current indication with a restriction to individuals with prior dengue virus infection.

To maximize the positive impact of the vaccine, while maintaining the risk of vaccinating false-seropositive individuals to the minimum, the MAH proposed updates of the SmPC based on the following:

- Removal from the indication (SmPC section 4.1) of the criterion limiting the use of the vaccine to individuals living in endemic areas or endemic transmission setting. The objective of this update is to address an unmet medical need in populations living in non-endemic areas with high probability of previous dengue infection, such as expatriates or people working for recurrent long-stay missions in endemic areas, who are likely to have been exposed to dengue in the past and could be re-exposed in the future. They would therefore be eligible to screening and vaccination, if tested positive.

- Update of the eligibility criteria for vaccination in SmPC section 4.2, by adding further details.

- Harmonization of the wording of the indication worldwide, limiting the use of the vaccine to individuals who tested positive to dengue.

In their submission, the MAH presented additional knowledge accrued on the performance of existing serotests since the initial MAA, modelling data and a literature review.

2.1.1. Problem statement

Disease or condition

Dengue is an acute, systemic viral infection caused by a virus that is transmitted primarily by the *Aedes aegypti* mosquito bites. The infection may be asymptomatic, cause flu-like illness, and can develop into a potentially lethal complication called severe dengue (including dengue hemorrhagic fever [DHF]/dengue shock syndrome [DSS]).

There are 4 types of closely related but antigenically distinct dengue virus serotypes (1, 2, 3, and 4). Primary dengue virus infection is thought to provide lifelong protection against the infecting serotype and transient cross-protection against heterologous serotypes. Dengue haemorrhagic fever and dengue shock syndrome occur mostly in individuals during secondary dengue virus infection with a different serotype.

Proposed new therapeutic indication

The MAH applied for an extension of the approved indication (SmPC section 4.1) to individuals who tested positive to dengue, living or not in endemic areas:

Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with test-confirmed previous dengue infection ~~prior dengue virus infection and living in endemic areas.~~

To complement the proposed update of the indication, the MAH proposed to also include the following text in section 4.2: '*Dengvaxia should only be administered to individuals with a previous dengue infection. Previous dengue infection must be confirmed by a test, either documented in the medical history or performed prior to vaccination.'*

The MAH considered that these proposed changes were supported by additional knowledge accrued on the performance of existing serotests to screen individuals, as well as on modeling data on the impact of a screen and vaccinate approach on the occurrence of dengue disease based on different dengue endemicity levels and a literature review to further assess the burden of dengue in individuals not living in endemic areas and who had been exposed to the virus while residing or working in endemic areas.

Epidemiology and risk factors

Dengue is the most common mosquito-borne viral disease in humans, spreading globally during the past 30 years as a result of changes in human ecology (Trop. Med. Infect. Dis 2020). The rapidly expanding global footprint of dengue inflicts a significant public health, economic and social burden on the populations of endemic areas. Half of the world's population is now considered at risk of infection by the dengue viruses. Worldwide, an estimated 390 million dengue infections occur every year, of which around 100 million are associated with clinical manifestation of dengue. Around 500.000 hospitalizations are reported each year, and around 20.000 cases result in death.

While geographical expansion of dengue and its vector are evident, the true burden of symptomatic dengue disease is underestimated. The true numbers are probably far worse, since significant underreporting and misclassification of dengue cases have been documented. Constraints inherent to public health surveillance systems and challenges specific to dengue do not allow dengue cases to be fully captured by public health surveillance systems.

The terms 'endemicity' and 'hyperendemicity' are used to indicate the simultaneous circulation of one or several Dengue virus serotypes, respectively (Trop. Med. Infect. Dis 2020). Dengue epidemiology varies across regions and seasons, meaning that simultaneous exposure to all 4 DENV serotypes is unlikely in a natural setting. An endemic region is defined as a region where cases are present over the majority of time during the year. This means that transmission is constantly ongoing. In contrast, an epidemic region is a region where cases are only present during a short period of time. Yearly epidemics can happen, or an epidemic can happen over several years.

In endemic areas, the entire population is at risk of dengue infection. The disease affects all age groups. The age distribution of infected individuals varies between countries and no clear pattern of populations at risk has been identified. Additionally, the population at highest risk can shift over time, as was observed in Colombia and Thailand over the last decade.

Seroprevalence is a proxy for endemicity. During an epidemic 70% of the population could be infected, hence a seroprevalence of 70% could be reached for that circulating serotype, but when there is no transmission in the rest of the year(s), it is not considered an endemic region. Dengue epidemiology is dynamic in serotype prevalence. The seroprevalence of each serotype fluctuates over time. The four dengue virus serotypes are genetically diverse and share limited identity (around 60-75%) at the aminoacid level. Genetic variations between serotypes and clades may be important determinants of differential viral fitness, virulence and epidemic potential.

Geographical distribution

Dengue disease is a major public health concern in more than 128 countries, with the four dengue virus serotypes found in tropical and sub-tropical regions, including some European territories.

Dengue is endemic in Asia, the Pacific area, Africa, and Latin America (including the Caribbean). In 2017, more than 500.000 dengue cases were reported to the WHO South-East Asia office and in 2019 more than 1 million cases were reported to the WHO Western Pacific region main countries for dengue (i.e., Australia, Cambodia, Lao Popular Democratic Republic, Malaysia, Philippines, Singapore and Viet Nam).

A decrease of 75% in number of dengue cases was reported across the Americas in 2017 and 2018, compared to 2016. However, the incidence of disease increased again in 2019 with a total of more than 3 million cases reported for the WHO Americas region.

After decades of absence in the United States of America (US), dengue has recently emerged with cases which were locally acquired.

Sustained transmission of dengue fever does not naturally occur in continental Europe, though sporadic

autochthonous dengue cases had been reported in Croatia in 2010 and in France in 2010, 2013, 2014, and 2015, even if more limited. Dengue, however, is endemic in the overseas territories of some European countries such as France (French Guiana, Martinique and Guadeloupe).

In 2020, dengue continues to affect several countries, with reports of increases in the numbers of cases in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste and Yemen.

Risk factors for severe dengue

Epidemiologic studies have identified young age, female sex, high body-mass index, virus strain, and genetic variants of the human major-histocompatibility-complex class I–related sequence B and phospholipase C epsilon 1 genes as risk factors for severe dengue.

Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock.

Chronic disease (bronchial asthma, sickle cell anaemia and diabetes mellitus) and ethnicity may represent additional individual risk factors that determine the severity of disease.

Secondary infection as risk factor for severe dengue

Primary dengue virus infection is thought to provide lifelong protection against the infecting serotype and transient cross-protection against heterologous serotypes. Dengue haemorrhagic fever and dengue shock syndrome occur mostly in individuals during secondary dengue virus infection with a different serotype. Increased risk in secondary infection is thought to be linked to antibody-dependent enhancement of virus infection in Fc receptor–bearing cells and the generation of a large infected cell mass *in vivo*. The antibody-mediated enhancement of dengue seems to be related with the presence of suboptimal neutralizing heterotypic antibodies (that accelerate the rate of internalization of the virus and infection of host cells), and may also be related to the presence of memory T cells with low affinity for the present infecting virus but high affinity for previous infecting serotype(s).

Clinical presentation and diagnosis

- Clinical presentation

Dengue disease has a wide and unpredictable range of clinical presentations, from asymptomatic to severe diseases. According to CDC, an estimated 1 in 4 dengue virus infections are symptomatic. Symptomatic dengue virus infection most commonly presents as a mild to moderate, nonspecific, acute febrile illness. Approximately 1 in 20 patients with dengue virus disease progress to develop severe, life-threatening disease called severe dengue. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Dengue shock syndrome (DSS) is the most severe form of dengue disease and results from hypovolaemia caused by vascular leakage. Early clinical findings are nonspecific but require a high index of suspicion because recognizing early signs of shock and promptly initiating intensive supportive therapy can reduce risk of death among patients with severe dengue to <0.5%.

- Diagnosis

Diagnostic methods to confirm dengue virus infection may involve detection of viable virus, viral nucleic acid, peripherally circulating viral antigens or host antibodies, or a combination of these techniques. Depending on the time of patient presentation, the application of different diagnostic methods may be more or less appropriate.

For the diagnosis of acute Dengue infection, tests are based on Dengue virus (DENV) isolation, presence of DENV antigens (NS1), detection of viral nucleic acids (RT-PCR), IgM seroconversion and/or 4-fold or greater rise in IgG antibody titre in paired blood samples collected at least 14 days apart. Virus detection and antigen detection are the most accurate diagnostic tools during the 5 first days of illness. IgG and IgM are not produced until 5-7 days after the onset of symptoms in primary infection. IgM levels can become undetectable after 3-6 months, while IgG levels often persist for lifetime and can be used to indicate an individuals' previous infection with DENV. It should however be mentioned that people infected with or vaccinated against other flaviviruses (such as Zika, West Nile, yellow fever, and Japanese encephalitis viruses) may have cross-reactive flavivirus antibodies, yielding false-positive serologic dengue diagnostic test results (WHO, CDC, Verhagen 2014).

Determination of previous dengue virus exposure

Measuring anti-DENV antibodies (IgM and IgG) can be done using conventional ELISA (most widely used), using RDT or by plaque reduction neutralisation testing (PRNT).

The gold standard test for previous dengue virus exposure (and vaccine immunogenicity) is the PRNT. PRNT is the most specific assay currently available, as cross-neutralisation with other flaviviruses is lower than with conventional IgM/IgG ELISAs. Unlike ELISA, the serotype specificity and sensitivity of PRNTs allows monitoring of dengue exposure history and population seroprevalence. PRNTs are costly, labour intensive and require specialized laboratory expertise and equipment. Despite WHO guidance for standardization, variation in assay methodology exists (such as cell line used, viral strain and passage used, read out method etc.). Novel high-throughput pseudovirus-based neutralisation tests for multiple flaviviruses have been develop recently that have reduced the turn-around time and cost of PRNTs (Matsuda 2018). PRNT might be envisaged for pre-vaccination testing/testing strategy in settings where they can be performed, such as traveller setting.

Even if performed correctly, antibody-capture ELISAs have good but not perfect sensitivity and specificity, and these vary between commercial kits. There are two major limitations associated with antibody testing: (i) in endemic regions, IgM persists for about 60 days and IgG persists likely lifelong, meaning that positive results do not distinguish recent from current dengue infections, (ii) cross-reactivity with related flaviviruses increases uncertainty of serology testing in areas where flaviviruses co-circulate and/or where vaccination (such as YF or JEV) is done.

RDTs (such as lateral flow tests) are rapid, simple and cheap tests detecting specific IgM/IgG that can be done at the point-of-care/point-of-vaccination. The short turn-around time of RDTs could increase/maximize vaccine update.

The problem with currently available RDTs is that they have not been validated yet for screening for past dengue infection and may lack sensitivity and specificity to ensure correct serostatus determination. Data on cross-reactivity are lacking. A recent systematic review (Luo 2019) evaluating the sensitivity and specificity of commercially available RDTs used to detect IgG against DENV as a marker of previous infection evidenced that, overall, there are no studies published that directly evaluated the use of RDTs (with an IgG component) for DENV serostatus determination. Since then, two studies evaluating the performance of 4 commercialized RDTs (and 2 conventional ELISAs) for the detection of dengue past-exposure have been published by the MAH (Bonaparte 2019 and 2020) and are part of this variation's dossier. The MAH also provided the last performance of the RDT.

Management and Prevention

There is no specific treatment for dengue disease. The management of dengue disease is supportive,

with rest, control of fever and pain with antipyretics/analgesics, and adequate fluid intake. Supportive intensive care and fluid management are the mainstays of therapy for severe disease.

Up to the end of 2015, the only available prevention of dengue by vector control has proven to be of limited success, very difficult to sustain and costly. Vaccination provides a viable and practical alternative in disease control measures. The only vaccine currently on the market is Dengvaxia.

Since the first marketing authorization obtained in Mexico on 8 December 2015, Dengvaxia has been licensed in 22 countries in total. However, due to a suspension for the license in the Philippines and the non-renewal in Malaysia, at the time of this application the vaccine was registered by 21 regulatory authorities across the world.

Based on EMA's CHMP recommendation, the European Commission has granted the marketing authorization in Europe on 12 December 2018.

In the EU, Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas.

The prequalification by the World Health Organization (WHO) was granted on 25 March 2020.

Despite registration in a certain number of countries, it should be noticed that there is a gap between the availability of Dengavxia and its use.

As an example, in March 2019, the French authorities did not recommend Dengvaxia for people living in La Réunion and Mayotte. For subjects living in Guadeloupe, Martinique or Guyana, vaccination is not recommended but could be proposed if there is a proof of documented virologically-confirmed past dengue infection.

No recommendations were found for the Netherlands (Netherlands Antilles, Aruba, Curaçao, Sint Maarten) and Portuguese (Madeira) populations living in dengue endemic areas.

The WHO recommended a strategy of pre-vaccination screening and vaccination of seropositive persons. No specific assay or assay strategy was listed in the recommendations. To maximize vaccine uptake and applicability in endemic regions, a serological test, and ideally a rapid diagnostic test, is required for serostatus screening.

Medical need in non-endemic area

Dengue can affect tourist travelers, business travelers and expatriates, migrants including those visiting friends and relatives (VFR), and pilgrim; both in adult and pediatric travelers[1]. Vaccination against Dengue could therefore provide a benefit for such specific populations.

2.1.2. About the product

CYD dengue vaccine is a tetravalent, recombinant, live attenuated viral vaccine. The viruses in the

[1] Riddell A: Imported dengue fever in East London: a 6-year retrospective observational study. J Travel Med 2017, 24.
Neuberger A, Turgeman A, Lustig Y, Schwartz E: Dengue fever among Israeli expatriates in Delhi, 2015: implications for dengue incidence in Delhi, India. J Travel Med 2016, 23.
Chen LH, Leder K, Barbre KA, Schlagenhauf P, Libman M, Keystone J et al.: Business travel-associated illness: a GeoSentinel analysis. J Travel Med 2018, 25.
Leder K, Tong S, Weld L, Kain KC, Wilder-Smith A, von Sonnenburg F et al.: Illness in travelers visiting friends and relatives: a review of the GeoSentinel surveillance network. Clin Infect Dis 2006, 43:1185-1193.
Diagne CT, Barry MA, Ba Y, Faye O, Sall AA: Dengue epidemic in touba, senegal: implications for the grand Magal pilgrimage for travelers. J Travel Med 2018.
Rabinowicz S, Schwartz E: Morbidity among Israeli paediatric travellers. J Travel Med 2017, 24.

vaccine consist of the replicative engine of the attenuated yellow fever vaccine virus 17D (coding for the non-structural proteins and capsid), along with the genes coding for the pre-membrane and envelope proteins of each of the 4 wild-type dengue serotypes.

CYD dengue vaccine consists of a sterile, freeze-dried powder formulation that is reconstituted with a sodium chloride solution (0.4% for the single-dose presentation, 0.9% for the multi-dose presentation) before injection and does not contain any adjuvant or preservative. Each dose contains 4.5-6.0 log-10 Cell-Culture Infectious Dose 50% (CCID50) per serotype (as per CCDS and EU Product Information).

After reconstitution, one dose (0.5 mL) is to be administered by the subcutaneous route.

2.1.3. General comments on compliance with GCP guidelines

All clinical studies evaluating the CYD dengue vaccine complied with the guidelines in force during the CDP, such as: the Quality Standards of the International Conference on Harmonization (ICH) guidelines, the Food and Drug Administration (FDA) guidelines for Good Clinical Practice (GCP), EU Directive 2001/20/EC, and the EMA guidelines on clinical evaluation of new vaccines.

2.2. Non-clinical aspects

No new non-clinical data have been submitted in this application, which was considered acceptable by the CHMP.

2.3. Clinical aspects

No new clinical data were submitted with this application, but supportive literature data.

2.3.1. Supportive aspects

2.3.2. Introduction

Since 2002, the MAH undertook an extensive clinical development programme (CDP) in subjects aged 9 months through 60 years in dengue endemic and non-endemic regions, leading to marketing authorizations in 21 countries, including the US, and in the European Economic Area. At the time of submission of the dossier for this procedure, Dengvaxia was the only authorised dengue vaccine.

The CDP was conducted to characterize the vaccine in terms of efficacy, safety and immunogenicity profiles, when assessed in different regions, in different age groups and in populations with various degrees of endemicity, from highly endemic to non-endemic.

There is no established immunological correlate of protection.

Data from 24 clinical studies (5 Phase I studies, 13 Phase II, and 6 Phase III) allowed the obtention of the first marketing authorizations.

The 2 main pivotal Phase 3 studies, performed in 10 countries of southeast Asia Pacific (CYD14) and Latin America (CYD15), were conducted to demonstrate the efficacy of the vaccine. Vaccine efficacy against severe dengue was demonstrated in those with prior dengue infection (PDI), whereas the use of the vaccine in subjects who did not yet acquire a natural immunity against dengue was associated with an identified risk for severe dengue.

In Europe, at the time of submission of this application, Dengvaxia was indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3, and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas.

In several other countries, the vaccination is not limited to individuals with prior dengue virus infection (e.g. Mexico, El Salvador, Costa Rica, Guatemala, Bolivia, Cambodia, Thailand, Venezuela, Honduras, Myanmar, and Dominican Republic, Paraguay, Bangladesh, Singapore).

For countries considering vaccination as part of their dengue control programme, WHO recommended a pre-vaccination screening as the best strategy.

Dengue serological assays are thus required to identify individuals who are eligible for vaccination, except those with documented PDI. There are currently no approved serological tests to identify individuals with PDI.

The MAH undertook the evaluation of existing dengue IgG assays to identify adequate performant test(s). The MAH has also co-developed a RDT, designed for the identification of PDI, in partnership with the diagnostic company CTK Biotech.

2.3.3. Specification of the indication to Previous dengue infection

In the context of the clinical development of the CYD Dengue vaccine (hereafter called Dengvaxia), seropositivity was determined by the antibody-mediated virus neutralization by plaque reduction neutralization test (PRNT50), the reference method according to WHO. Additional analyses were conducted on the efficacy trials using methods, such as PRNT90 and the anti-dengue NS1 IgG enzyme linked immunosorbent assay (ELISA) with a threshold of 9 ELISA Unit (EU)/ml or 50 EU/ml (Th9 and Th50).

The results of the post-hoc analyses classifying the subjects with these various methods (PRNT50, PRNT90 and anti-NS1 ELISA Th9 and Th50), showed a protection against VCD cases, hospitalized and severe cases in seropositive subjects.

The test positivity or seropositivity criterion to determine the eligibility to receive the vaccine was proposed as a predictive, measurable and practical criterion to determine history of past dengue infection (PDI) supporting the indication. Further description of the available tests that can potentially be used in the context of pre-vaccination screening is provided below.

The CHMP noted that Dengvaxia was first intended to be used in endemic countries for vaccinating infants, children and adults. Results of the pivotal clinical trials indicated that the use of the vaccine in subjects previously exposed to dengue virus (seropositives) was associated with protection against dengue. On the other hand, and importantly, the use of the vaccine in subjects who did not yet acquire a natural immunity against dengue virus (seronegatives) was associated with an identified risk for severe dengue, which can be potentially fatal.

In the context of the clinical development of the CYD dengue vaccine, seropositivity was defined by PRNT50 measured in the immunogenicity subset but results were also analysed according to seropositivity classification based on PRNT50 and PRNT90 (measured or imputed) or anti-NS1 IgG ELISA (with thresholds of 9 EU/ml or 50 EU/ml) in the NS1 Supplemental analyses.

The PRNT assay is the gold standard test for identification of previous dengue virus exposure and vaccine immunogenicity. The validated PRNT assay was the core immunologic assay for measuring functional antibodies able to neutralize dengue virus in studies submitted at the initial MAA. This assay measures the serum titre needed for a specified reduction (typically 50%-90%) in virus infectivity of an in vitro cell culture incubated with a serial dilution of a test serum. Although gold standard for determining past

exposure, the assay has limitations, particularly in terms of specificity due to cross-reactivity with other flaviviruses. Serological cross-reactivity amongst members of the *Flaviviridae* family (DENV, Yellow Fever (YF), West-Nile virus (WNV), Japanese Encephalitis virus (JEV) and Tick borne encephalitis virus (TBEV)) is a well-known problem, although to a lesser extent than with conventional ELISAs. PRNT90 is more specific than PRNT50 by decreasing the background serum cross-reactivity among flaviviruses. PRNT90 is therefore associated with less false-positive dengue classification and less cross-reactivity between serotypes.

During the initial MAA, a new quantitative ELISA test based on the detection of dengue NS1-specific IgG antibodies as a biomarker of DENV exposure was used (Nascimento 2018). Since Dengvaxia does not express DENV NS1 but YFV NS1 from the vaccine backbone, measuring DENV anti-NS1-specific IgG could then be utilized to evaluate dengue serological status pre- and post-vaccination with Dengvaxia. A thorough analysis showed that the DENV anti-NS1 IgG ELISA could be a suitable alternative to Dengue PRNT for identifying seronegative subjects when using the 9 ELISA Unit (EU)/mL threshold. This threshold is near the lower limit of quantification (LLOQ) and minimizes the false negative rate, but does result in a relatively high false positive rate. By applying a higher threshold of 50 EU/mL, the false positive rate is minimized and consequently those previously exposed to DENV are more correctly identified by limiting the misclassification of those not previously exposed as seropositive. The real rate of misclassification is however unknown since specificity and sensitivity were established on the basis of samples classified as seropositive or seronegative with the imperfect gold standard PRNT. The cross-reactivity with other flaviviruses remain to be determined, particularly with Zika, West Nile viruses, Ilheus virus, Rocio virus, and Murray Valley virus. Importantly, the levels and stability of anti-dengue NS1 IgG for longer than 3-4 years after dengue infection remain to be determined.

Based on the data from Phase 3 trials, Dengvaxia is indicated for subjects with prior dengue virus infection (approved indication in the EU), and it was clarified in section 4.2 of the SmPC that '*Previous dengue infection has to be assessed before vaccination by laboratory confirmed history of dengue or through an appropriately validated serological test.*'

For countries considering vaccination as part of their dengue control programme, WHO recommended a pre-vaccination screening as the best strategy. The testing to be implemented in order to ascertain previous dengue infection prior to vaccination is in the remit of the local Public Health (PH) authorities. At the time of the initial MAA, there were few possibilities available to them. Knowledge around the performances of available tests that could be used as screening test before vaccinating subjects, such as conventional ELISAs and rapid diagnostic tests (RDTs) were limited.

Thus, there is a gap between the availability of Dengavaxia and its use. As an example, in March 2019, the French authorities did not recommend Dengvaxia for people living in La Réunion and Mayotte. For subjects living in Guadeloupe, Martinique or Guyana, the vaccination is not recommended but could be proposed if there is a proof of documented virologically-confirmed past dengue infection.

To address this knowledge gap around the tests, the MAH further investigated the performance of the available tests that can potentially be used in the context of pre-vaccination screening, and results are presented below. Notably, the recommendations about pre-vaccination screening test or strategy tests is the responsibility of national health authorities.

2.3.4. Eligibility Criteria for Vaccination

Evaluation of currently available serotests for the identification of PDI

Since the initial Marketing Authorisation Application, further knowledge on the ability of available serotests to detect PDI has been accrued, showing high levels of specificity (>98%) and therefore, according to the MAH, allowing to correctly identify individuals who should not be vaccinated, i.e. seronegative individuals, as detailed below.

An evaluation of the performance of commercially available dengue IgG serological tests, including four rapid diagnostic tests (RDTs) and two conventional ELISAs (Bonaparte 2019), was performed by the MAH. The table below presents the diagnostic tests that were evaluated in Bonaparte 2019 (extracted from the publication).

Table 1. Diagnostic tests evaluated (Bonaparte 2019)

Table 1. Diagnostic tests evaluated

Name	RDT Dengue IgA/IgG (RDT)	OnSite Dengue IgG/IgM (RDT)	SD Bioline Dengue IgG/IgM (RDT)	Dengue IgG/IgM Rapid Test (RDT)	Panbio® Dengue IgG Indirect ELISA	Dengue Virus IgG DxSelect (OUS) (Indirect ELISA)
Company	Bio-Rad[a]	CTK Biotech, Inc.[b]	Alere (Abbott)[c]	GenBody/Bahiafarma[d]	Alere (Abbott)[e]	Focus Diagnostics[f]
Test catalogue number	70 701	R0061C	11FK20	HDVAB0	10PE30	EL11500G
Package insert, date	881 105 70 701, 11-2015	PI-R0061C Rev. I1.0, 21-01-2018	11FK20-02-1, 01-2009	CNPJ: 13078518/0001-90 Revision 03, 02-2017	01PE30 Rev. 1, 08-2013	EL1500G Rev. O, 31-03-2011
Assay principle	Lateral flow immunochromatography	Lateral flow immunochromatography	Lateral flow immunochromatography	Lateral flow immunochromatography	ELISA	ELISA
Class of Ig detected	IgG, IgA	IgG, IgM	IgG, IgM	IgG, IgM	IgG	IgG
Specimen type	Whole blood, serum or plasma	Whole blood, serum or plasma	Whole blood, serum or plasma	Whole blood serum or plasma	Serum	Serum
Sample volume required	5 µl	5 µl	10 µl	10 µl[g], 5 µl[h]	10 µl	10 µl
Time to result from blood sample	20–30 min[i]	20–25 min[i]	15–20 min[i]	15–20 min[i]	Minimum 2·5 h	Minimum 2·5 h
Storage temp	2–30°C	2–30°C	Room temperature	2–30°C	2–8°C	2–8°C

[a]Discontinued by manufacturer
[b]Registered in Latin America (Argentina, Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Paraguay, Peru, Venezuela), Asia (Bangladesh, Cambodia, India, Indonesia, Laos, Philippines, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam) and elsewhere (the European Union, Mozambique, Saudi Arabia)
[c]Registered in Latin America (Brazil, Mexico, Paraguay), Asia (Australia, Cambodia, India, Indonesia, Myanmar, Singapore, Taiwan, Thailand) and elsewhere (Ethiopia, the European Union, Russia, United Arab Emirates)
[d]Registered in Brazil
[e]Registered in Latin America (Argentina, Brazil, Cost Rica, Ecuador, Guatemala, Peru), Caribbean (Jamaica), Asia (Australia, India, Indonesia, Singapore, Thailand) and elsewhere (European Union, Israel)
[f]Registered in Latin America (Argentina, Brazil, Colombia, Costa Rica, Panama, Uruguay), Caribbean (Barbados), Asia (Indonesia, Philippines, Taiwan, Thailand) and elsewhere (Canada, the European Union, Israel, Kuwait, Saudi Arabia)
[g]Whole blood
[h]Serum
[i]Serum/plasma needs to be separated by centrifugation before testing adds ~30 min to test.
[j]Based on inclusion of incubation times from the assay protocol and excluding time for sample transportation to the laboratory, batch analysis and reporting to the clinician (two visits for potential vaccine recipients)[9]
ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; RDT, rapid diagnostic test; temp, temperature

Different types of samples were tested to determine the sensitivity and specificity of the tests and are described in the table below (extracted from Bonaparte 2019).

Table 2. Samples panel for assay evaluation (Bonaparte 2019)

Table 2. Sample panel for assay evaluation

Group	No. of samples	Subgroup (verification)	No. of samples	Sample information and source		
				Origin	Age	Source clinical trial
	Specificity panel (negative for prior dengue infection)					
Known dengue $PRNT_{50}$ negative[b], 'non-endemic' region	229	JE-/YF-negative	179	US	18–45 years	NCT01488890 (Sanofi Pasteur 2014)/NCT01943825 (Sanofi Pasteur 2017) Before vaccination[a]
		JE-positive (JE vaccination)	25	US	18–45 years	Employee Serum After JE-VAX® vaccination
		YF-positive (YF $PRNT_{50}$-positive)	25	US	18–45 years	NCT01488890 (Sanofi Pasteur 2014) Before vaccination[a] Prior documented YF vaccination
Known dengue $PRNT_{50}$-negative[b], NS1 IgG-negative (<9 EU/ml), 'endemic' region	305	JE-negative (JE $PRNT_{50}$-negative)	94	Philippines	2–14 years	Capeding *et al.* 2014[20] Before vaccination[a]
		JE-positive (JE $PRNT_{50}$-positive)	12	Philippines	2–14 years	Capeding *et al.* 2014[20]/Dubey *et al.* 2016[21] Before vaccination[a]
		YF-negative (YF $PRNT_{50}$-negative)	167	Colombia, Honduras, Mexico, Puerto Rico	9–16 years	Villar *et al.* 2015[23] Before vaccination[a]
		YF-positive (YF $PRNT_{50}$-positive)	32	Colombia, Honduras, Mexico, Puerto Rico	9–16 years	Villar *et al.* 2015[23] Before vaccination[a]
	Sensitivity panel (positive for prior dengue infection) [c]					
Known dengue $PRNT_{50}$-positive[b], (no dengue infection documented), 'endemic' region	90	Low $PRNT_{50}$ titre	30	Colombia, Mexico, Puerto Rico, Philippines	2–45 years	Capeding *et al.* 2011[19]/Villar *et al.* 2013[22] Before vaccination[a]
		Medium $PRNT_{50}$ titre	37	Colombia, Honduras, Mexico, Puerto Rico	9–16 years	Capeding *et al.* 2011[19]/Villar *et al.* 2013[22] Before vaccination[a]
		High $PRNT_{50}$ titre	23	Colombia, Honduras, Mexico, Puerto Rico, Philippines	2–45 years	Capeding *et al.* 2011[19]/Villar *et al.* 2013[22] Before vaccination[a]
Recent documented VCD infection, 'endemic' region	90	Recent documented VCD infection by PCR[d] ≈0–12 months from first study injection; sample collected ≤ 13 months after first injection	90	Colombia, Honduras, Mexico, Puerto Rico Philippines	2–16 years	Capeding *et al.* 2014[20]/Villar *et al.* 2015[23] Placebo recipients
Remote documented VCD infection, 'endemic' region	90	Remote documented VCD infection by PCR[d] ≈0–12 months from first study injection; sample collected ≈3–4 years after first injection	90	Colombia, Honduras, Mexico, Puerto Rico Philippines	2–16 years	Capeding *et al.* 2014[20]/Villar *et al.* 2015[23] Placebo recipients

[a]Before vaccination with tetravalent dengue vaccine or control
[b]Dengue $PRNT_{50}$-negative samples were negative ($PRNT_{50} < 10$) for all four serotypes. Dengue $PRNT_{50}$-positive samples were positive ($PRNT_{50} \geq 10$) for at least one of the four serotypes tested.
[c]Prior dengue infection (PDI) case was defined as (i) an individual who had virologically confirmed dengue infection in the past (RT-PCR-positive at the time of acute infection) or (ii) an individual who was $PRNT_{50}$-positive at the same sampling time point as the one used for the evaluation of RDT/ELISAs.
[d]Infecting dengue serotype was determined by the Focus Simplexa™ Dengue real-time RT-PCR method (Focus Diagnostics).
Ig, immunoglobulin; JE, Japanese encephalitis; NS1, non-structural protein 1; RT-PCR, reverse transcriptase-polymerase chain reaction; $PRNT_{50}$, 50% plaque reduction neutralisation test; RDT, rapid diagnostic test; VCD, virologically confirmed dengue

The major conclusion of this evaluation, as considered by the MAH, was that all the dengue IgG components of the RDTs and one of the two ELISA tests exhibited high specificity > 98% for identifying PDI, including in samples collected from individuals not living in endemic areas. In addition, low to no cross-reactivity with several flaviviruses was reported in these evaluations (see figures below).

An additional study evaluated two serotests available in Puerto Rico and provided similar conclusion, i.e. high test specificities (≥ 99%) (Bonaparte 2020). The tests evaluated were an IgG/IgM rapid diagnostic test (RDT) (Biocan Diagnostics Inc. Canada) and an IgG enzyme-linked immunosorbent assay (ELISA) (SciMedx, USA). Samples evaluated were from the same studies than those used in the previous study as indicated in the table below. Classification of the samples slightly differs from Bonaparte 2019.

Table 3. Sample panels for assay evaluation (Table 1, Bonaparte 2020)

Sample panels for assay evaluation.

Group (origin)	No. of samples	Subgroup (verification)	No. of samples	Sample source
Specificity panel (negative for prior dengue infection)				
Nonendemic region (USA): Dengue $PRNT_{50}$ negative	125	JE/YF negative	75	NCT01488890 (Sanofi Pasteur 2014)/ NCT01943825 (Sanofi Pasteur 2017) Before vaccination[a]
		JE positive (JE vaccination)	25	Employee Serum After JE-VAX® vaccination
		YF positive (YF $PRNT_{50}$ positive)	25	NCT01488890 (Sanofi Pasteur 2014) Before vaccination[a] Prior documented YF vaccination
Endemic region (Latin America or Asia): Dengue $PRNT_{90}$ negative, and one of the following: (1) $PRNT_{50}$ negative and NS1 IgG negative (<9 EU/mL) or low-positive (≥9 to <50 EU/-ml); (2) $PRNT_{50}$ positive and NS1 IgG negative (<9 EU/mL)	205[b]	JE negative ($PRNT_{50}$ negative)	50	Capeding et al. (2014) Before vaccination[a]
		JE positive (JE $PRNT_{50}$ positive)	15	Capeding et al. (2014)/Dubey et al. (2016) Before vaccination[a]
		YF negative (YF $PRNT_{50}$ negative)	114	Villar et al. (2015) Before vaccination[a]
		YF positive (YF $PRNT_{50}$ positive)	25	Villar et al. (2015) Before vaccination[a]
Sensitivity panels (positive for prior dengue infection)				
Endemic region (Latin America or Asia): Known dengue PRNT positive, (no dengue infection documented)	200	Dengue $PRNT_{90}$ positive	194	Capeding et al. (2011)/Villar et al. (2013) Before vaccination[a]
		$PRNT_{90}$ negative, $PRNT_{50}$ positive and Dengue NS1 IgG ELISA low-positive	6	Capeding et al. (2011)/Villar et al. (2013) Before vaccination[a]
Recent documented VCD infection, *endemic* region (Latin America or Asia)	89	Recent documented VCD infection by PCR[c] ≈0–12 months from first study injection; sample collected ≤13 months after first injection	89	Capeding et al. (2014)/ Villar et al. (2015) Placebo recipients
Remote documented VCD infection, *endemic* region (Latin America or Asia)	90	Remote documented VCD infection by PCR[c] ≈0–12 months from first study injection; sample collected ≈3–4 years after first injection	90	Capeding et al. (2014)/ Villar et al. (2015) Placebo recipients

Ig = immunoglobulin; JE = Japanese encephalitis; NS1 = nonstructural protein 1; PCR = polymerase chain reaction; $PRNT_{50}$ = 50% plaque reduction neutralization test; $PRNT_{90}$ = 90% plaque reduction neutralization test; RDT = rapid diagnostic test; VCD = virologically confirmed dengue.

[a] Before vaccination with tetravalent dengue vaccine or control.

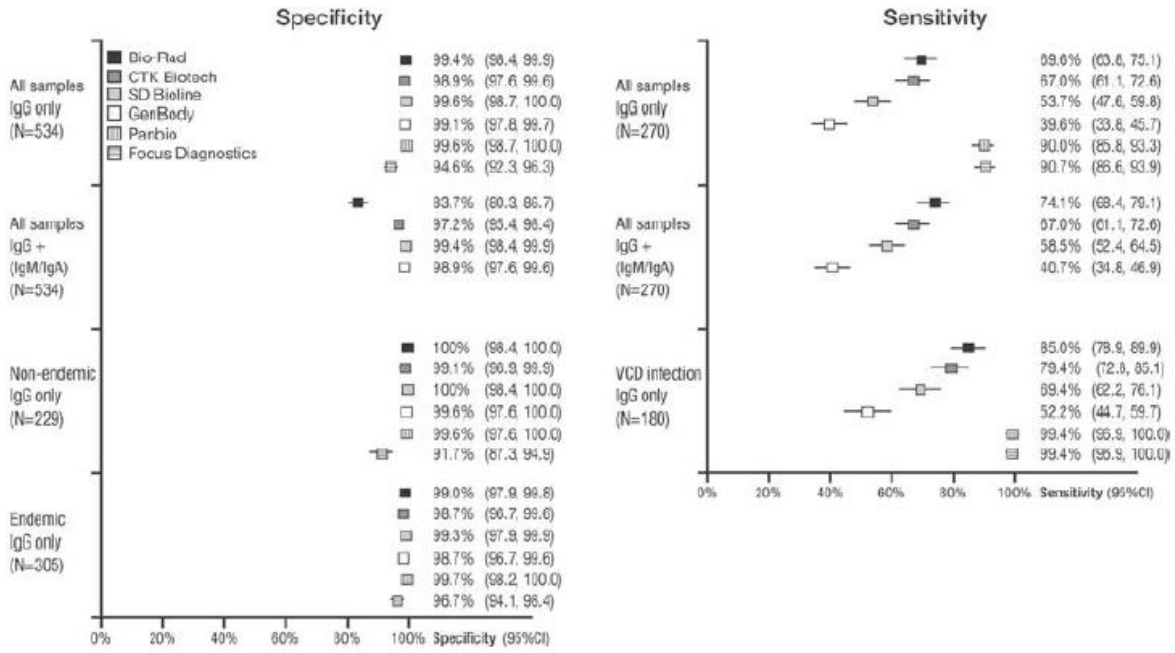
[b] One sample was not tested for either YF or JE.

[c] Infecting dengue serotype was determined by the Focus Simplexa™ Dengue RT-PCR method (Focus Diagnostics).

According to the MAH, these findings (Bonaparte 2019 and 2020) indicate that the use of these immunoassays in pre-vaccination screening would successfully minimize the risk of inadvertently vaccinating dengue seronegative individuals.

Another important conclusion of these evaluations is the modest or moderate performance of RDTs in terms of sensitivity (see figure below, from 39.6 to 69.6%), and more precisely as seen in one of the exploratory analyses performed, their limited capacity for the detection of lower Ab titers ranging from 100-500 in PRNT50 (from 0 to 32%) and even the absence of detection of very low titers below 100 in PRNT50 (Bonaparte 2019, Figure below). These findings indicate that currently-available RDTs are unlikely to detect individuals who have been exposed only once and/or remotely without being boosted naturally, or individuals with basal titers of non-specific cross-reactive immunity to other flaviviruses or different pathogens (Figure below). This also partially explains the low level or even the absence of cross-reactivity reported with RDTs in these evaluations. Although these findings justify the need to improve the performance of these proof of concept (POC) IgG RDTs to detect a larger proportion of seropositive individuals who could benefit from vaccination, they also confirm the low risk of false positive results in low to non-endemic transmission settings where low antibody levels are expected.

Figure 1. Specificity and sensitivity of selected available serological assay on samples from endemic and non-endemic areas



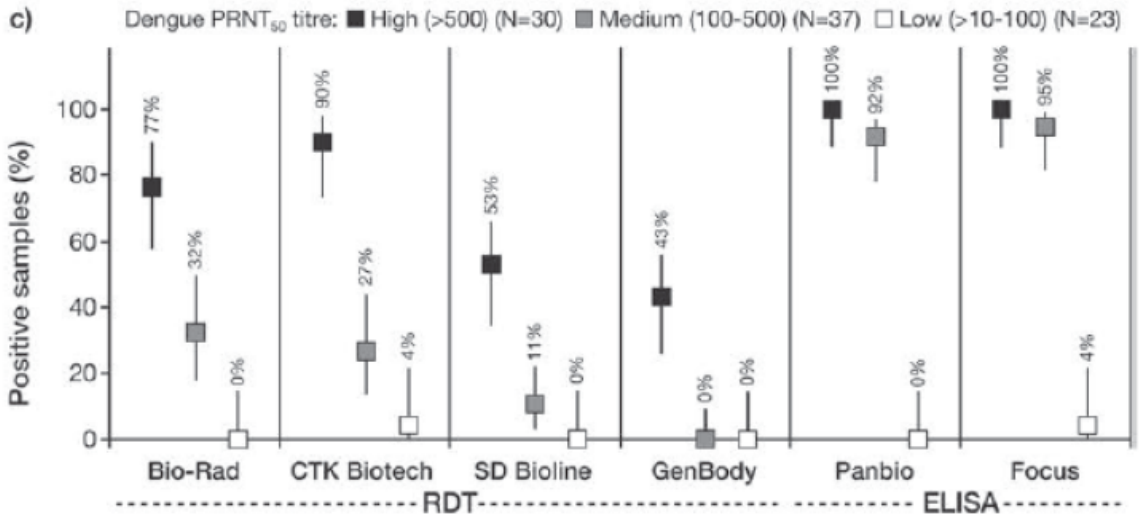
Specificity and sensitivity of RDT/ELISAs for identifying prior dengue infection. Evaluated RDTs were from Bio-Rad (RDT Dengue IgG/IgA), CTK Biotech, Inc. (OnSite Dengue IgG/IgM), SD Bioline now Alere/Abbott (SD Bioline Dengue IgG/IgM) and GenBody (Dengue IgG/IgM Rapid Test).
ELISAs were from Panbio now Alere/Abbott (Panbio® Dengue IgG Indirect ELISA) and Focus Diagnostics (Dengue IgG DxSelect Indirect ELISA). IgM testing was performed for all RDTs except the Bio-Rad RDT, which evaluated IgA antibodies; IgM/IgA testing was not performed for the ELISAs.
VCD is virologically confirmed dengue infection. These samples were obtained from subjects who, in the past, had symptomatic dengue infection confirmed by a positive RT-PCR result

Figure 2. False-positive rate in dengue-negative samples from individuals positive for other flaviviruses

Manufacturer	Test (type)	JE + ive ($n = 37$) [b]	YF + ive ($n = 57$) [c]	WN + ive ($n = 59$) [d]	Zika + ive ($n = 41$) [e]
Bio-Rad	RDT Dengue IgG/IgA (RDT)	0 (0%)	1 (2%)	NA	NA
CTK Biotech, Inc.	OnSite Dengue IgG/IgM (RDT)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Alere (Abbott)	SD Bioline Dengue IgG/IgM (RDT)	0 (0%)	0 (0%)	4 (7%)	3 (7%)
GenBody/Bahiafarma	Dengue IgG/IgM Rapid Test (RDT)	0 (0%)	1 (2%)	4 (7%)	0 (0%)
Alere (Abbott)	Panbio® Dengue IgG Indirect ELISA	1 (3%)	0 (0%)	30 (51%)	14 (34%)
Focus Diagnostics	Dengue IgG DxSelect (Indirect ELISA)	12 (32%)	6 (11%)	ND	ND

a Data presented is the number (percentage) of dengue-seronegative samples that were incorrectly classified as positive by the evaluated assay. b Samples are either JE PRNT50-positive, dengue PRNT50-negative and with dengue NS1 IgG ELISA <9 EU/ml or from JE-VAX-injected subjects and dengue PRNT50-negative. c Samples are YF PRNT50-positive and dengue PRNT50-negative; those from endemic regions are also with dengue NS1 IgG ELISA <9 EU/ml. d Samples are positive with West Nile IgG DxSelectTM ELISA (Focus Diagnostics) and with dengue NS1 IgG ELISA <20 EU/ml. e Samples are positive with Zika

Figure 3. Detection of prior dengue infection cases according to dengue PRNT50 titre levels



Data presented is percentage, with 95% confidence interval, of samples from PDI cases that were correctly classified as positive by the evaluated assay. RDTs were from Bio-Rad (RDT Dengue IgG/IgA), CTK Biotech, Inc. (OnSite Dengue IgG/IgM), SD Bioline now Alere/Abbott (SD Bioline Dengue IgG/IgM) and GenBody (Dengue IgG/IgM Rapid Test). ELISAs were from Panbio now Alere/Abbott (Panbio® Dengue IgG Indirect ELISA) and Focus Diagnostics (Dengue IgG DxSelect Indirect ELISA).
(c) Dengue PRNT50-positive samples were positive (PRNT50 ≥10) for at least one of the four serotypes tested and the titre used was the highest dengue PRNT50 titre obtained among the four serotypes.

As part of the data submitted to support this application, the MAH summarized the performance of the RDT that was co-developed by the MAH and CTK Biotech for the identification of past dengue infection, through the detection of anti-dengue IgG. The clinical evaluation of this new dengue IgG RDT demonstrated, according to the MAH, performance characteristics allowing to reliably and safely identify people who can benefit from dengue vaccination with an assay specificity of 98.0% (95% CI: 95.9, 99.2), a sensitivity of 95.3% (95% CI:91.7, 97.6), a sensitivity for monotypics of 88.1%, a sensitivity of 94.4 % (95% CI: 87.5,98.2) for remote (3-4 years) dengue infection, and with minimal to no cross-reactivity to other flaviviruses (Liberal 2020, poster).

Figure 4. Performance characteristics of the OneSite Dengue IgG RDT (Liberal, extract of the poster presented at the ASTMH 2020 Annual meeting)

Table 5. OnSite Dengue IgG RDT sensitivity by dengue immune profile

Profile	N	Sensitivity, % (No. positive)
Multitypic	171	98.8 (169)
Monotypic (all)	59	88.1 (52)
DV1 monotypic	11	81.8 (9)
DV2 monotypic	24	87.5 (21)
DV3 monotypic	17	94.1 (16)
DV4 monotypic	7	85.7 (6)

Table 6. Cross-reactivity to related flaviviruses

Study panel	Flavivirus	N	Cross-reactivity, % (no. positive)
Clinical study (specificity panel)	YFV	72	1.4 (1)
	JEV	5	0 (0)
Analytical	ZIKV	35	0 (0)
	YFV	42	2.4 (1)
	JEV	36	2.8 (1)
	WNV	32	0 (0)

Table 7. Cross-reactivity to other diseases or special conditions

Disease/ condition	N	Cross-reactivity, % (no. positive)	Disease/ condition	N	Cross-reactivity, % (no. positive)
ANA	13	0 (0)	HSV-1	10	0 (0)
Borrelia (Lyme)	12	0 (0)	HSV-2	11	0 (0)
Chikungunya	19	0 (0)	Influenza A	17	0 (0)
CMV	12	0 (0)	Influenza B	15	0 (0)
SARS-CoV-2	36	2.8 (1)	Leptospirosis	12	8.3 (1)
EBV	13	0 (0)	Malaria	20	0 (0)
Enteroviruses	15	0 (0)	Measles (rubeola)	23	0 (0)
HAMA	15	0 (0)	Parvovirus B19	12	0 (0)
Hepatitis A	11	0 (0)	RF	14	0 (0)
Hepatitis B	16	0 (0)	Rubella	23	0 (0)
Hepatitis C	18	0 (0)	Syphilis	12	0 (0)
HIV 1/2	36	2.8 (1)	Varicella-zoster virus	12	8.3 (1)

ANA, anti-nuclear antibodies; CMV, cytomegalovirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HAMA, human anti-murine antibody; HIV, human immunodeficiency virus; HSV, herpes simplex virus; RF, rheumatoid factor.

The CHMP acknowledged that the MAH performed an evaluation of the performance of 6 commercially available dengue IgG serological tests (of around 20 tests are commercialized worldwide), including four rapid diagnostic tests (RDTs) based on immunochromatography and two conventional ELISAs (published in Bonaparte 2019). Most of these data were previously assessed during the initial MAA (05-09-2018 report). The number of dengue-negative West-Nile- or Zika- positive samples is increased in these final results (n=59 and 41 respectively) when compared to the previous submitted data (n=3 and 4 respectively). The NS1-based IgG assay developed by the MAH was not included in this evaluation. The reason for not considering this test as screening test for the detection of previous dengue infection is unknown.

Several limitations to results interpretation were evidenced during the initial assessment in 2018 and were addressed in the study conducted by Bonaparte 2019 such as a better description of the samples or analyses of the sensitivity and specificity according to the different sets of reference dengue positive samples (virologically-confirmed vs PRNT50, 1-13 months vs 3-4 years after infection) and dengue-negative samples (endemic vs non-endemic).

Specificity was assessed using 534 dengue-negative serum samples from the US (n = 229) and dengue-endemic regions (n = 305). The serostatus was determined by PRNT50 for non-endemic region, and by PRNT50 and anti-NS1 IgG (Th9) for endemic regions. Samples for both non-endemic and endemic regions included JE or YF negative and positive samples.

Sensitivity was assessed using 270 samples from recent (n = 90) or remote (n = 90) virologically confirmed prior dengue cases, and dengue PRNT50-positive samples (n = 90). The latter (PRNT50-positive, no dengue infection documented, endemic region) were divided into 3 groups to better assess the test sensitivity (see table describing the sample characteristics).

Cross-reactivity was assessed in dengue-seronegative samples that were seropositive for YF (n = 57), JE (n = 37), WN (n = 59) or Zika (n = 41).

The CHMP considered that the study results demonstrated that all the dengue IgG components of the RDTs and one of the two ELISA tests (Panbio) exhibited high specificity (> 98%), both in samples collected from individuals living and not living in endemic areas. However, using the 270 dengue positive samples from confirmed recent or remote PDI, the RDTs showed low to moderate sensitivities (40-70%) up to 3-4 years after infection.

Both recent and remote infection were similarly detected but the dengue positivity rates with the RDTs or ELISAs declined as PRNT50 levels decreased. Cross-reactivity with other flaviviruses such as JEV, YFV, WNV and ZIKV was low for RDTs in contrast to both ELISAs. The RDT from CTK Biotech was the only one where no cross-reactivity to any of the 4 flaviviruses was observed.

Although well-conducted, the Bonaparte study (2019) has several limitations, including some that are acknowledged by the authors themselves.

Cross-reactivity with other flaviviruses such as JEV, YFV, WNV and ZIKV was apparently low for all tests, except for the Focus Diagnostics ELISA. For all the tests, this was concluded based on the analysis of a reasonable number of samples. The authors noted that JE-positive samples used in the study were from subjects who received the inactivated JE vaccine (30–60 days before sample collection), and that there was limited information on samples from clinical trials with regard to JE vaccination history or infection. It is therefore possible that cross-reactivity in this study may underestimate the cross-reactivity rate arising from vaccination with the live attenuated JE vaccine or from natural JE infection. Additionally, as discussed by Bonaparte et al., the cross-reactivity of the assays would need to be further defined using sample panels that include patients with other common tropical diseases, including those infected with other viruses or microorganisms transmitted by mosquitoes such as Chikungunya, related flaviviruses Rocio, Ilheus and Murray Valley, or malaria. Cross-reactivity against the common microorganisms such as HIV, HCV, HSV1, CMV, EBV, ParvoB19 would also need to be evaluated (WHO PQ GL). Cross-reactivity to tick-borne encephalitis (TBE) should also have been evaluated since vaccination against TBE is more and more encouraged in Europe, so growing TBE vaccinated population of EU travellers that may benefit (or not) from dengue vaccination when traveling to endemic regions. Since it is known that cross-reactivity increase with more flavivirus exposure, testing sera from multiple exposed individuals (people with multiple different flavivirus infections or people vaccinated for YF, TBE and JE together) would be relevant.

The limited data on viruses/microorganisms cross-reactivity are a limitation for using the tests as pre-vaccination screening, as false-positive results could lead to incorrect serostatus determination and consequently to inappropriate vaccination for dengue.

Sensitivities of the RDTs (40-70%) were lower than those of the ELISAs (≥90 0%). This finding is not surprising because RDTs were developed to diagnose acute and actually ill patients with high IgG titres and were not designed to detect low IgG titres commonly observed with prior dengue infection. The sensitivities of IgG ELISAs reported by Bonaparte et al. were higher than those reported in the publication of Rafaat et al. 2019 (56% for one of the tests and 89% for the other one).

Table 4: Summary of commercially available antibody serology kit diagnostic performance

Antibody	Kit	Sensitivity (%)	Specificity (%)	Reference
IgM	DENV Detect IgM Capture ELISA (InBios International, Seattle, WA, USA), catalogue number: DDMS-1	92	94	[25]
	Panbio Dengue IgM Capture ELISA (Abbott, Macquarie Park, NSW, Australia), catalogue number: E-DEN02M	89	88	[26]
	SD Bioline Dengue IgM ELISA (Standard Diagnostics, Suwon, Korea), catalogue number: 11EK20	85	97	[26]
IgG	Panbio Dengue Virus IgG Capture ELISA (Abbott, Macquarie Park, NSW, Australia), catalogue number: E-DEN02G	56	95	[26]
	SD Bioline Dengue IgG ELISA (Standard Diagnostics, Suwon, Korea), catalogue number: 11EK10	89	64	[26]

The dengue positivity rates (sensitivity) of samples with high PRNT50 values were already low for 3 out of the 4 RDTs (43-77%). Moreover, it was observed that dengue positivity rates (sensitivity) with the ELISA and the RDTs declined as PRNT50 levels decreased. Some samples (n=23) with lower PRNT50 values (10-500) were however also negative by both PRNT90 and anti-NS1 IgG ELISA, indicating possible miss-classification by PRNT50 (false-seropositive samples). Data were not shown, and it is not clear if this applies to samples with PRNT50 values between 10-100 but also to those with values between 100-500. Nevertheless, this shows that currently available tests may miss a high proportion of individuals who have been exposed only once and/or remotely without being boosted naturally, which is highly relevant for the EU setting. Determination of the sensitivity of the tests in true-seropositive subjects with low PRNT50 values is of utmost importance.

Bonaparte et al. indicated that the sensitivity of the tests assessed in the study would be biased towards performance in individuals with documented, symptomatic dengue infection (such population representing 2/3 of the inclusion). However, the majority of true-seropositive individuals that would be vaccinated would have likely experienced asymptomatic infection sometime in the past. It is possible that antibody titres to dengue may be lower in the context of asymptomatic infection.

In addition, Bonaparte et al. reported that the PRNT50 dengue-seropositive reference panel more likely corresponded to samples from individuals who experienced more than one dengue infection and, thus, may have also resulted in some overestimation of the sensitivity than would otherwise be representative of populations with higher proportions of individuals who have experienced only one dengue infection.

Both recent and remote infection were similarly detected but, as indicated by the authors, no conclusions can however be drawn regarding detection of exposure to dengue 5 years or more previously. Even if it is acknowledged that anti-dengue IgG responses are reported to be long lived, the adequate detection of such samples remains to be proven.

A second study retrospectively evaluated the performance of two tests used in Puerto Rico (one ELISA and one RDT) and confirmed the apparent high specificity of these immunoassays. The samples used for these evaluations were similar than those used in the study of Bonaparte 2019.

As indicated by the authors, prospective confirmation of performance characteristics in conditions replicating field use (endemic settings, targeted age) would be important to complement the work.

Rodriguez-Barraquer et al. also argue that, if a key goal of pre-vaccination screening is to minimize harm to seronegative individuals, sensitivity and specificity might not be the most useful target metrics for assay development but, the PPV makes more sense, as this value directly quantifies the probability that a person who tests positive is truly seropositive, or the probability that they have been misclassified (1-PPV).

Because Bonaparte et al. 2019 found that, in general, dengue IgG RDTs were more specific and less cross-reactive than ELISAs (but ELISAs were more sensitive in identifying PDI), the MAH concluded that currently available RDTs could be tools for rapid and safe pre-vaccination screening until improved RDTs with increased sensitivity for low IgG levels become available. This was not fully endorsed but will not be discussed in depth in the context of this assessment, as the choice of the testing strategy relies on local Public Health authorities.

The CHMP acknowledged that with the very high specificity of RDTs, a high PPV could potentially be reached and hence the risk of vaccinating false-seropositives could potentially be low. Nevertheless, as PPV highly depends on the pre-test probability, with a specificity of 98% and a sensitivity of 50%, the risk of inadvertently vaccinating naïve subjects remains high if the pre-test probability is low (for ex. with a pre-test probability of 5%, as much as 43% of the vaccinated seropositive subjects would actually be naïve). Therefore, in contrast with the MAH proposal, if RDTs may be appropriate for highly endemic areas, this is not the case for areas of low endemicity/non endemic where much more specific approaches will likely be implemented by PH authorities, such as using several tests in series, or relying on an history of virologically-confirmed dengue (as illustrated for Guadeloupe, Martinique or Guyana).

Moreover, the current RDTs have sensitivities of approximatively 50% which means that half of those with a prior dengue infection would test negative and thus would be wrongly excluded from vaccination and not be benefiting from the protection it provides (Hunsperger et al. 2019).The cost-effectiveness of pre-vaccination strategy which such test will also enter into consideration before initiation mass campaign vaccination.

Hunsperger et al. also underlined that the low sensitivity of RDTs would produce negative predictive values (NPV) that are highly influenced by the prevalence of prior dengue infection in a given population and hence the seropositivity rate in that population. For example, over 80% of those who tested negative on an RDT with a 50% sensitivity would be incorrect. The impact of the seropositive rate in the NPV also indicates that RDTs produce more informative negative results in dengue endemic settings but with low seroprevalence (approx. 20%) without significant change to the PPV.

The figure (by Hunsperger, 2019) below illustrates the above considerations. The graph represents the PPV and NPV in populations with different baseline seroprevalence rate. Data calculated using a sensitivity of 50% and specificity of 99%, which are representative of the findings of Bonaparte 2019, are shown.

Figure 5: The PPV and NPV of RDTs in populations with different baseline seroprevalence rate. Data calculated using a sensitivity of 50% and specificity of 99%, which are representative of the findings of Bonaparte et al. (in press), are shown

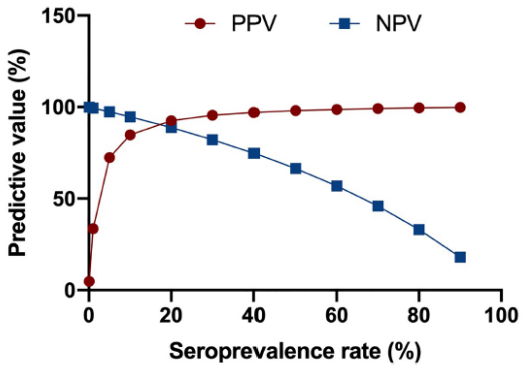


Figure 1. The PPV and NPV of RDTs in populations with different baseline seroprevalence rate. Data calculated using a sensitivity of 50% and specificity of 99%, which are representative of the findings of Bonaparte et al. (in press), are shown

The performance characteristics of the RDT co-developed by the MAH with CTK Biotech were provided. Results were only published in the form of a poster presented at the ASTMH annual meeting 2020, and not (yet) as a manuscript in a peer-reviewed journal. Both sensitivity and specificity were high. Cross-reactivity was characterized on a broader panel of samples than for the other tests. As mentioned for the other tests, cross-reactivity against related flaviviruses Rocio, Ilheus and Murray Valley, or against TBE should also have been relevant.

Post-hoc Evaluation of Vaccine Efficacy in Tested Positive Subjects

These recent evaluations were completed by a post-hoc evaluation of the vaccine efficacy (VE) of the CYD dengue vaccine in seropositive individuals identified using the same dengue IgG immunoassays previously evaluated, except one RDT which was no longer commercialized (Bio-Rad, RDT Dengue IgG/IgA).

The table below extracted from the abstract of DiazGranados 2020 summarizes the characteristics of the different immunoassays used in the study.

Table 5. Characteristics of dengue immunoassays used in the study (DiazGranados 2020)

Table 1. Characteristics of dengue immunoassays used in this study.

Dengue Serotest	OnSite IgG/IgM RDT[1,2]	SD Bioline IgG/IgM RDT[1,3,4]	TellMeFast IgG/IgM RDT[1,2]	Panbio® IgG Indirect ELISA[3]	Euroimmun IgG ELISA[2]
Manufacturer	CTK Biotech	Alere (Abbott)	Biocan	Euroimmun	Euroimmun
Catalog number	R0061C	11FK20	B803C	EI 266b-9601 G	EI 266b-9601 G
Assay format	Lateral flow	Lateral flow	Lateral flow	ELISA	ELISA
Dengue antigen	Recombinant envelope protein	Recombinant envelope antigen	Recombinant dengue antigen	Purified DEN virus antigen	Purified viral particles DEN2
Zika cross-reactivity, % (no. positive/no. tested)[5]	0 (0/41)	7 (3/41)	3 (1/38)	34 (14/41)	8 (2/26)

[1]Readout for the IgG band only was considered in determining the test result for the IgG/IgM RDTs.

Testing was performed at the [2]Global Clinical Immunology Laboratory, Sanofi Pasteur, Swiftwater, PA USA or the [3]Central Virology Laboratory, Israel Ministry of Health, Ramat Gan, Israel.

[4]Two readers were used, with pre-defined plan to score discordant readings (+/-) as a positive test result.

[5]Cross-reactivity sample selection and results for all tests except Euroimmun IgG ELISA were previously published (refs. 6, 7).

The samples used were baseline samples from participants in the immunogenicity subsets of the Phase 3 CYD14 and CYD15 efficacy trials. The table below summarizes the sample classification.

Table 6. Algorithm for classification of reference dengue serostatus (Table 2, DiazGranados 2020)

Table 2. Algorithm for classification of reference dengue serostatus.

Group	Reference test results			Interpretation
	$PRNT_{90}$[1]	$PRNT_{50}$[1]	DV NS1 IgG ELISA[2]	
1	Negative	Negative	Negative	Reference seronegative
2	Negative	Positive	Negative	Reference seronegative
3	Negative	Negative	Low positive	Reference seronegative
4	Negative	Positive	Low positive	Reference seropositive
5	Negative	Any[3]	High positive	Reference seropositive
6	Positive	Positive	Any[3]	Reference seropositive

[1]Positive $PRNT_{50}$ and $PRNT_{90}$ were defined by a titer ≥10 (1/dil) against ≥1 dengue serotype.

[2]Anti-dengue NS1 IgG ELISA results were classified as negative (titer <9 EU/ml), low positive (≥9 to <50 EU/ml) and high positive (≥50 EU/ml).

[3]Any = positive or negative.

The outcomes of the analysis confirmed that subjects aged 2 to 16 years who were identified as dengue seropositive by the immunoassays were protected against VCD following vaccination with the CYD dengue vaccine, with a high VE (over 25 months post-dose 1) against symptomatic VCD across all five immunoassays (83–90%), as well as against hospitalized VCD (73–92%) (over 6 years post-dose 1), and severe VCD (73-100%) (over 6 years post-dose 1). The same high specificity (≥ 98% for all immunoassays apart from one RDT) and modest sensitivity (around 50%) were reported for the evaluated RDTs, as previously assessed.

The CHMP acknowledged the performed post-hoc evaluation of the VE of the CYD dengue vaccine in seropositive individuals identified using the above-mentioned 5 dengue IgG immunoassays, except one RDT (Bio-Rad). Samples used were baseline samples from participants in the immunogenicity subsets of the Phase 3 CYD14 and CYD15 efficacy trials. VE for symptomatic virologically-confirmed dengue (VCD) regardless of severity and serotype in all test(+) subjects 2-16 years of age during active phase FU or the entire 6 years of FU period was calculated. VE was estimated in test(+) subjects using a Cox regression model with the treatment group and study as fixed effects, with corresponding 95% CI calculated by the exact binomial method (Clopper-Pearson method).

Samples were classified according to PRNT and anti-NS1 IgG ELISA results. Both PRNT50 and PRNT90 and both anti-NS1 IgG thresholds of 9 and of 50 were combined to determine the serostatus as much as correctly possible. This reference classification of the samples is endorsed. However, neither the number of subjects classified as seropositives, nor the concordance of each test with the reference classification were presented.

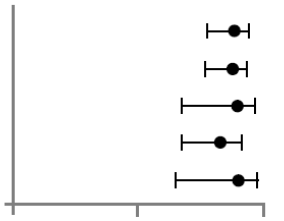
The VE against symptomatic VCD across all five immunoassays (83–90%), against hospitalized VCD (73–92%) (over 6 years post-dose 1), and against severe VCD (73–100%) (over 6 years post-dose 1) was variable, reflecting the difference in the performance of the tests (dependent of the number of subjects included in the analysis). The different VE values were not given for the 2-16 yo subjects

classified as seropositive based on the reference status.

95% CI interval of the VE against symptomatic VCD up to M25 in test(+) subjects 2-16 yoa were wider for RDTs compared to ELISAs. This is probably due to the lower number of subjects tested positive with the RDTs.

Figure 1. CYD-TDV efficacy against symptomatic VCD up to M25 in test(+) subjects 2-16 years of age.

Immunoassay	Density Incidence* (95% CI)		Vaccine Efficacy (95% CI)
	CYD-TDV	Placebo	
Euroimmun-ELISA	0.4 (0.2, 0.6)	3.0 (2.2, 4.0)	88.2 (77.3, 93.9)
Panbio-ELISA	0.4 (0.2, 0.6)	3.0 (2.2, 3.9)	87.6 (76.7, 93.4)
TELL ME FAST-RDT	0.2 (0.1, 0.6)	2.0 (1.2, 3.0)	88.8 (67.0, 96.2)
SD BIOLINE-RDT	0.5 (0.2, 0.8)	2.7 (1.9, 3.8)	82.8 (66.9, 91.1)
OnSite RDT	0.2 (0.0, 0.5)	1.7 (1.0, 2.8)	89.7 (64.6, 97.0)



Data pooled from CYD14 and CYD15 in all ages (2-16 years).
VCD symptomatic virologically-confirmed dengue.
*per 100 person-years at risk.

The lower number of seropositive subjects by the RDTs confirmed the modest sensitivity of the RDTs observed in the Bonaparte studies (2019 and 2020). The One site RDT's sensitivity was lower than the one found in Bonaparte 2019. High specificity (>98.5%) was found for four of the five assays.

The authors conclude that '*These findings indicate that use of these existing serotests for pre-vaccination screening would enable the safe and effective use of CYD-TDV and could thus serve as suitable temporizing tools to inform vaccination decisions until more sensitive, point-of-care tests become available*.'

The CHMP, however, considered that the data provided are not meant to support such conclusion. The data only allow concluding that efficacy varies according to test used, which is expected as the proportion of false positive varies. The data do not provide reassurance on the safe use of Dengvaxia. The PPV may remain too low, depending on the seroprevalence/pre-test probability of past dengue. In addition, because of this very low sensitivity of the RDTs, a large part of the population who might benefit from vaccination will not be detected. Implementation of a screening before vaccination would lead to a vaccination of around only half of the people that could benefit from it, and still with the uncertainty to vaccinate false-seropositive individuals.

2.3.5. Use of the Vaccine in Individuals Not Living in Endemic Area

The condition living in endemic area was initially specified in the indication based on the rationale that the vaccine was developed to be used mainly in endemic countries, knowing that in these countries the level of disease transmission can vary from low to high intensity. After confirmation of a safety signal in seronegative subjects and the demonstration of a positive benefit/risk in seropositive individuals, this limitation was kept in the indication as a risk minimization measure. The reason was the limited knowledge around the performances of available tests to correctly identify eligible individuals to vaccination, i.e. seropositive individuals, and more importantly, to correctly identify individuals who should not be vaccinated, i.e. seronegative individuals.

As detailed above, further knowledge has now been accrued on available serotests which all showed high levels of specificity (>98%) and their ability to correctly identify seronegative individuals who should not

receive the CYD Dengue vaccine, therefore (in the view of the MAH), limiting the risk of inadvertently vaccinating false-positive individuals.

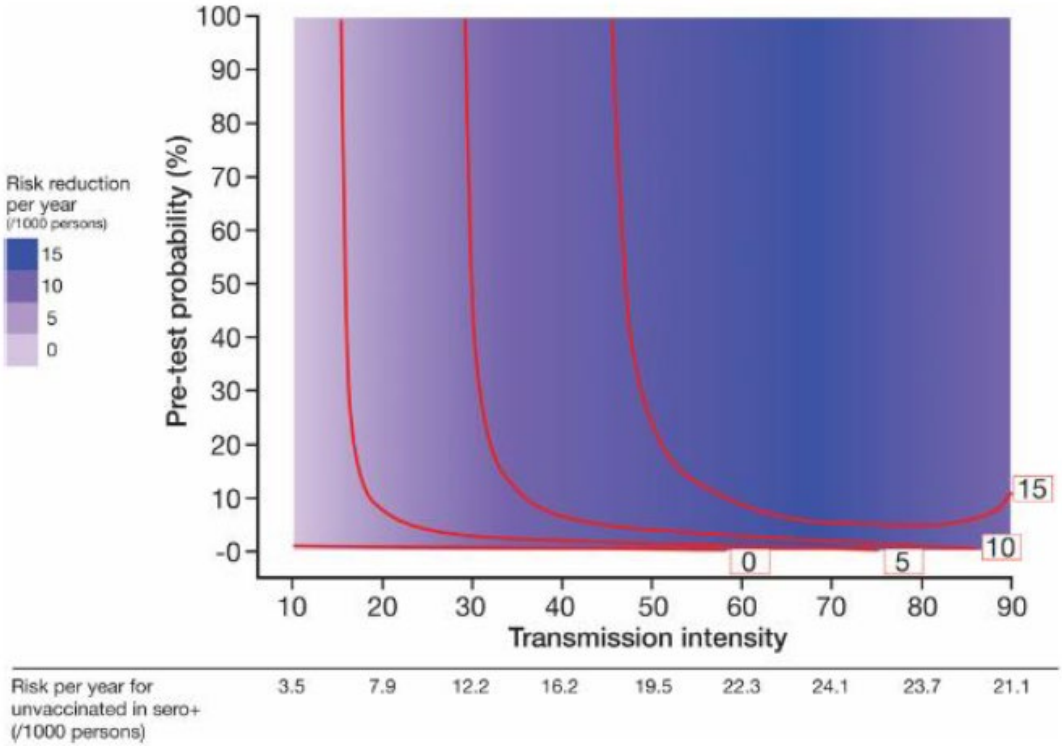
In addition to these data, modelling results were published on the impact of screen and vaccinate approach in different endemic settings, and a literature search was conducted to assess the risk of dengue infection in those not living in endemic areas. These are described in the following sections to further support the use of the vaccine in some individuals not living in endemic areas.

Modelling results of the screen and vaccinate approach

The potential impact of a 'screen and vaccinate' approach was assessed based on a previously reported transmission model and added, for the screening part, three rapid diagnostic tests with identical specificity (99%) but alternative sensitivities (50-70-90%) in the detection of PDI, in several dengue endemic settings, at the population and at the individual levels (Coudeville 2018).

The results show that for a given level of vaccine and test performances, the effect of a screen and vaccinate approach on the expected risk of an individual to develop dengue depends on both previous dengue exposure (i.e. pre-test probability, which is the probability of being seropositive before screening), and on the level of future exposure to dengue (i.e. the transmission level an individual will be exposed to in the future). Figure 6 below shows the risk reduction expressed in terms of the number of dengue hospitalizations avoided, for those vaccinated upon positive screening over a 10-year period for an RDT with a sensitivity at 70%, where it is assumed that there is only direct protection conferred by the vaccine. In the vast majority of cases, where the pre-test probability is >= 1%, regardless of the transmission setting, the risk of hospitalization for individuals tested positive was reduced with the screen and vaccinate approach. Similar results were observed for severe dengue. According to this model no increased risk of symptomatic dengue, regardless of the levels of dengue endemicity, was observed in tested-positive vaccinated individuals (Coudeville 2018).

Figure 6. Absolute risk reduction (per 1000 individuals in dengue hospitalization of a screen and vaccinate strategy per individual vaccinated according to the pre-test probability and level of exposure to dengue.



In the view of the MAH, that these results show that across all settings, when individuals are tested seropositive with a test showing a specificity of 99% and a sensitivity of 70%, the risk of dengue hospitalization and severe dengue is lower if they are vaccinated compared to their risk if not vaccinated. These results apply to a wide range of past exposure to dengue infection rates as low as 1%, showing that even individuals with a very low probability of being tested seropositive, such as those not living in endemic areas but who lived in endemic areas or spent significant time in endemic areas, would benefit from the screen and vaccinate approach. The magnitude of the reduction depends on the future exposure to dengue; therefore, this parameter has to be also taken into account when deciding to screen an individual not living in endemic areas.

The CHMP considered that the results described above were not found in the referenced publication of Coudeville et al. of 2018 but in a paper published in 2020. The referenced study rather described analyses that explored the benefits and risks of dengue vaccination according to baseline serostatus. In the publication of 2020, Coudeville et al. specifically focus on the potential impact of various screen and vaccinate strategies.

The analysis was based on the previously reported age-structured, host-vector and serotype-specific deterministic compartmental transmission model, which assessed the outcomes of dengue vaccine at individual and population levels (Coudeville 2016 and 2018). The analysis also uses data from the Bonaparte's assessment of available RDTs used to determine prior dengue infection. Three RDT characteristic profiles were chosen to reflect both current and possible future test performance (for example an 'RDT 70%' scenario corresponding to a screening test for which the sensitivity was set at 70% and the specificity at 99%). The impact of vaccination considered settings representing different levels of transmission intensity, ranging from 10% (very low) to 90% (very high). Three levels of disease

severity (dengue-related hospitalization, severe dengue, and symptomatic dengue) were considered. The authors considered strategies covering 80% of the age cohort targeted i.e. vaccination is given to the all individuals detected seropositive among the 80% of the age cohort screened for their dengue serostatus. The time horizon considered in these models was 10 years.

The CHMP considered that the study shows that, across all settings, when a subject is detected as being seropositive through screening, their risk of dengue hospitalization and severe dengue is lower if they are vaccinated compared to their risk if not vaccinated. The magnitude of this reduction varies according to the level of future exposure to dengue.

This result is a direct consequence of the assumed high specificity of the RDT used (99%). The interpretation of the findings is thus limited by the above-mentioned limitations on the accuracy of the test performance.

The vaccination of people living in non-endemic area will be considered on a case by-case basis, since pre- and post-vaccination exposure are highly variable from one individual to another and difficult to ascertain.

The CHMP agreed that seropositive subjects, even those living in low endemic areas, might benefit from vaccination. However, this requires an adequately validated test, with very high specificity to ensure a very high PPV (See also below).

Literature Review of People not living in endemic areas and travelling to dengue endemic areas

A literature review was performed to further assess the burden of dengue in those not living in endemic areas and who have likely been exposed to the virus while residing or working in endemic area. The literature search was carried out in May 2020 on Embase, with the following query strategy.

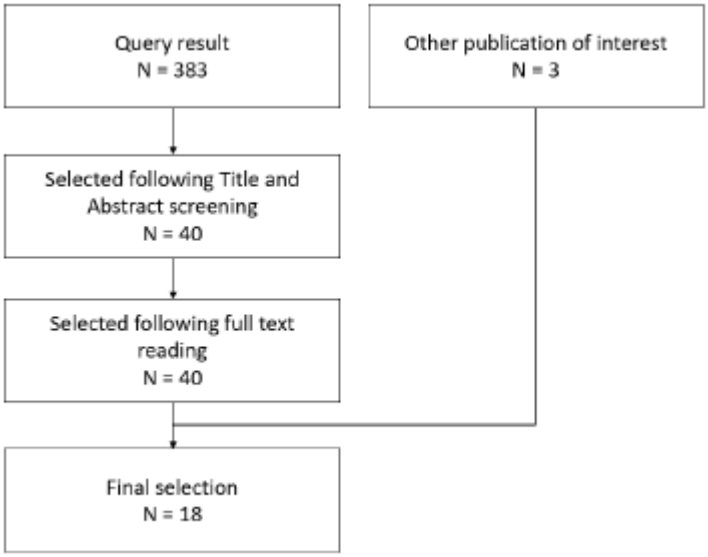
Figure 7. Literature query strategy

Dengue/exp
1. Travel/exp
2. Seroprevalence/exp
3. Screening/exp
4. Antibody/exp
5. Enzyme linked immunosorbent assay/exp
6. Serology/exp
7. 'migrant'/exp
8. oversea*.ti.ab.kw
9. mainland*.ti.ab.kw
10. continental*.ti.ab.kw
11. 'carribbean islands'/exp
12. 'puerto rico'/exp
13. 3 OR 4 OR 5 OR 6 OR 7
14. 2 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13
15. 1 AND 14 AND 15

A year restriction focusing only on papers from 2010 (because of the changing dengue situation change across ages) and on French and English languages was applied.

This search led to 383 publications, to which 3 other publications of interest not found by the query were added. The selection was made based on the following the flow chart:

Figure 8. Flow chart of the selection of publication



The literature search identified 18 publications of interest from 2010 onwards, which allowed to identify an unmet medical need as regards to dengue prevention in these populations.

Among those, individuals who were born in endemic areas and moved to reside in non-endemic countries showed the highest rates of dengue seropositivity or PDI, from approximately 20% up to 95.8% depending on endemicity of the country of origin and depending on the age, which is consistent with seroprevalence generally reported in endemic areas (Humphrey 2019). For instance, a seroprevalence study conducted on a convenience sample of first-generation Surinamese immigrants living in the Netherlands, reported a rate of 81.3% probably comparable to Surinamese inhabitants (Overbosch 2014).

Individuals who were born in non-endemic settings and who resided or stayed without interruption for long periods, several months or years, in endemic areas were also associated with level of seropositivity or PDI comparable to that from endemic countries. For instance, a study conducted by Sanchez-Vegas et al. which enrolled 600 participants in travel clinics of the Boston Area, to assess the prevalence of dengue infection in individuals established in non-endemic settings and who have lived in or travelled to dengue-endemic countries, suggest that dengue pre-test probability can be substantial, especially in individuals who have lived in or travelled for ≥ 1 year to dengue-endemic countries. Anti-dengue virus IgG was identified in 40% of these individuals, versus 51% in the group of people born in endemic countries and 6.9% in the group of people who travelled for ≥ 2 weeks but < 1 year. Overall, 19% of the entire study population had a positive dengue serology. In this study, individuals born in non-endemic countries and/or who have lived in or travelled for ≥ 1 year to endemic settings displayed background rates of past-dengue infection comparable to those seen classically in endemic areas.

Overall, studies addressing the potential risk of PDI in individuals living in non-endemic transmission settings reported rates of IgM and/or IgG dengue serologies in the range of 6-40%, which confirmed that there is an unmet medical need in populations living in non-endemic areas with a probability of having had previous dengue infection, such as expatriates or people working for recurrent long-stay missions in endemic areas (see Table below).

Table 7. Dengue seroprevalence in population from non-endemic countries with long-term stay in dengue endemic region

Group	Period	Duration	Dengue Diagnostic Test(s)	Prevalence	# pos / # tested	Reference
U.S. travelers attending Boston Travel Network Clinic	2008-9	≥1 yr. 2-52 wks.	IgG ELISA (Focus)	40% 6.9%	(12/30) (29/421)	(21)
Long-term **Dutch** travelers to DV endemic regions	2008-11	12-52 wks.	Ind. IgG ELISA (Panbio)	6.5%	(39/600)	(20)
U.S. Army Special Forces	2006-08	'Frequent' deployment to DV endemic region	Microneut assay (MNT_{50})	11.2%	(56/500)	(22)
U.S. military	2008-11	Deployed to DV endemic region (median, 213 d)	Microneut assay (MNT_{50})	7.6%	(76/1000)	(23)
Expatriate and local persons affiliated with an American veterinary school (Saint Kitts and Nevis)	2008-09	Students, 1.6 yrs, Staff 30.5 yrs Faculty 6.6 yrs	Anti-DENV IgG ELISA	44.1% Overall 30.1% in students, 100.0% in staff 57.9% in faculty	(52/118)	(24)
NGO workers in Léogane and Port-au-Prince	2002	Not reported	Anti-DENV IgG ELISA	94% Overall 79% in expatriates, 100.0% in Haitiens	161/173 41/52 121/121	(25)

The MAH concluded that this literature review supports the extension of the use of the vaccine to test-positive individuals not living in endemic areas, who have likely been in contact with dengue virus (e.g. individuals who lived before or had recurrent stay in endemic areas) considering that their level of seropositivity or PDI is comparable to individuals from endemic countries, addressing an unmet medical need.

The CHMP acknowledged the MAH's literature review to further assess the burden of dengue in those not living in endemic areas and who have likely been exposed to the virus while residing or working in endemic area.

The query strategy for the literature review by the MAH was considered overall appropriate and that the findings of the final selected publications were consistent.

Overall, the seropositivity rate in people not living in endemic areas and travelling to dengue endemic areas ranges from 6-40% but can also be higher when focusing in very particular populations such as expatriates with long-term stay. The seropositivity rate in people born in endemic areas and who moved to reside in non-endemic countries ranges from 20% to 96%. This confirms, as indicated by the MAH, an unmet medical need in these types of population.

As already discussed during the initial MAA, it is agreed that vaccination may be beneficial to specific population of individuals not living in endemic areas and travelling regularly to dengue endemic area, such as health care workers or any workers of non-governmental organisations. Not only travellers with laboratory confirmed dengue history could be vaccinated with Dengvaxia, but potentially also travellers with an history of travel in dengue endemic area, even if dengue infection was asymptomatic provided an adequate pre-vaccination testing strategy is applied.

The vaccination of people living in non-endemic area will be considered on a case by-case basis, based on the recommendations from local Public Health authorities. Many variables can influence the previous exposure to dengue (and hence the pre-test probability) such as the country of origin, the number of months/years spent in the endemic area, the age, the seroprevalence of travel area, the frequency and/or duration of the travel. True seroprevalence of an area is difficult to ascertain (highly locally variable, variable over time, variable according to demographic characteristics) and a true estimate of the probability to have been infected by dengue virus several years after might be challenging. All these parameters make the evaluation of the PPV of a test difficult/uncertain for the above-mentioned populations. A test with very high specificity (approaching 100%), and adequately determined, is

therefore required.

Effective strategies could nevertheless be developed to correctly identify travellers eligible for vaccination. A multiple testing strategy resulting in a specificity approaching 100% might be developed and/or novel tests using new technologies allowing for a higher specificity. Test strategies with sequential testing with different RDTs and/or ELISAs and/or PRNT (where possible) to get the highest possible confidence could be developed. Therefore, it is possible that in the future, approaches for testing individuals living in non-endemic countries prior to vaccination may be included in the local Public Health recommendations. Till that time, the Public Health bodies may limit vaccination to those individuals who had previous laboratory confirmed dengue.

Finally, as also already pointed out during assessment of the initial MAA, the current 3-dose schedule (with the current interval between doses) renders the use of Dengvaxia in a travel medicine setting difficult, and the results of studies on alternative schedules would need to be available before this approach becomes more widely available.

2.3.6. Clinical safety

No new safety data were submitted with this application. The CHMP invited the MAH to describe the safety database for individuals living in non-endemic areas (size, age range, serostatus at baseline).

As requested, the MAH provided a description of the safety database for individuals living in non-endemic areas, including size, age range, and serostatus at baseline.

A total of 879 individuals, aged 6-60 years, living in non-endemic areas, were exposed to at least one injection of a tetravalent CYD dengue vaccine (with the final formulation and schedule) in studies CYD12 and CYD51 conducted in the US, and CYD17 conducted in Australia. From these 879 individuals, 71 were seropositive (as assessed by PRNT50), 806 were seronegative, and 2 were undetermined.

The following statement from the MAH was endorsed by the CHMP: 'Although the low number of seropositive subjects living in non-endemic areas does not allow to draw relevant conclusions, no safety signals were detected in this specific group.'

2.3.7. Discussion

The efficacy of the Dengvaxia vaccine against dengue disease was demonstrated in those individuals with prior dengue infection, whereas the use of the vaccine in subjects who did not yet acquire a natural immunity against dengue was associated with an identified risk for severe dengue.

As a result, Dengvaxia is currently indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3, and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas (EU indication).

For countries considering vaccination as part of their dengue control programme, WHO recommended a pre-vaccination screening as the best strategy.

The testing to be implemented in order to ascertain previous dengue infection prior to vaccination is in the remit of the local Public Health authorities. At initial MA, there were few possibilities available to them. Knowledges around the performances of available tests that could be used as screening test before vaccinating subjects, such as conventional ELISAs and rapid diagnostic tests (RDTs) were limited.

Therefore, there is a gap between the availability of Dengvaxia and its use. As example, in March 2019, the French authorities did not recommend Dengvaxia for people living in La Réunion and Mayotte. For

subjects living in Guadeloupe, Martinique or Guyana, the vaccination is not recommended but could be proposed if there is a proof of documented laboratory-confirmed past dengue infection.

To address this knowledge gap around the tests, the MAH further investigated the performance of the commercially available tests that can potentially be used in the context of pre-vaccination screening. The MAH has also co-developed a RDT for the identification of past dengue infection (PDI), through the detection of anti-dengue IgG. The MAH also presented published modelling results on the impact of screen and vaccinate approach (with various assay performance) in different endemic settings. Although important to follow, and as above-mentioned, the recommendations about pre-vaccination screening test or test strategies are the responsibility of the national health authorities. Therefore, these data were not assessed in depth, and are not considered to weigh in the conclusion of this report.

Both studies of Bonaparte et al. demonstrated that, in general, dengue IgG RDTs were more specific and less cross-reactive than ELISAs. ELISAs were more sensitive in identifying PDI than RDTs. Several limitations were evidenced, including with regards to cross-reactivity evaluation. Although better characterized, limitations with regards to cross-reactivity evaluation also applies to the RDT co-developed by the MAH and the CTK Biotech. The identified limitations preclude the use of the tests as pre-vaccination screening, as false-positive results could lead to incorrect serostatus determination and consequently to inappropriate vaccination for dengue.

Overall, a screening test should be both highly sensitive and highly specific to minimize false positives and negatives. Both positive predictive value (PPV) and negative predictive value (NPV) are more meaningful indicators because they combine positive and negative pre-test probability and performance characteristics of a given test. Tests with a given sensitivity and specificity are more likely to misclassify truly seronegatives in a setting where low dengue virus transmission occurs (i.e. low seroprevalence) than in a setting with high transmission, simply because the pre-test probabilities are lower. For example, in populations where the seroprevalence is very low (5%), even tests with very high specificity (98%, sensitivity of 50%) will misclassify 43% of those who test positive. While PPV constraints test accuracy in low prevalence settings, NPV constraints do so for high prevalence settings. Specificity is a higher priority than sensitivity given the identified increased risk of severe and/or hospitalized dengue for inadvertently vaccinated dengue naïve individuals.

These considerations are directly related to the main proposal of the MAH, which was the extension of the indication to individuals with test-confirmed previous dengue infection, i.e. either living in endemic or in non-endemic countries.

The MAH conducted a literature search to assess the risk of dengue infection in those not living in endemic areas.

Overall, the seropositivity rate in people not living in endemic areas and travelling to dengue endemic areas ranges from 6-40% but can also be higher when focusing in very particular populations such as expatriates with long-term stay. The seropositivity rate in people born in endemic areas and who moved to reside in non-endemic countries ranges from 20% to 96%. This constitutes an unmet medical need in these types of populations.

As already pointed out as part of the assessment of the initial MAA, it is agreed that vaccination may be beneficial to specific population of individuals not living in endemic areas and travelling regularly to dengue endemic area, such as health care workers or any workers of non-governmental organisations. Not only travellers with laboratory-confirmed dengue history could be vaccinated with Dengvaxia, but potentially also travellers with an history of travel in dengue endemic area, even if dengue infection was asymptomatic/not detected provided an adequate pre-vaccination testing strategy is applied.

The vaccination of people living in non-endemic area will be considered on a case by-case basis, based on the recommendations from local Public Health authorities.

Many variables can influence the previous exposure to dengue, and hence the pre-test probability, such as the country of origin, the number of months/years spent in the endemic area, the age, the seroprevalence of travel area, the frequency and/or duration of the travel. True seroprevalence of an area is difficult to ascertain (highly locally variable, variable over time, variable according to demographic characteristics) and a true estimate of the probability to have been infected by dengue virus several years after might be challenging. All these parameters make the evaluation of the PPV of a test difficult/uncertain for the above-mentioned populations. A test with very high specificity (approaching 100%), and adequately determined, is therefore required.

Effective strategies could nevertheless be developed to correctly identify travellers eligible for vaccination. Test strategies with sequential testing with different RDTs and/or ELISAs and/or PRNT (where possible) to get the highest possible confidence (approaching 100% specificity) could be developed. Novel tests using new technologies allowing for a higher specificity could also be envisaged. Therefore, it is possible that in the future, approaches for testing individuals living in non-endemic countries prior to vaccination may be included in the local Public Health recommendations. Until that time, the Public Health bodies may limit vaccination to those individuals who had previous virologically-confirmed dengue.

Limited safety data following vaccination with the CYD dengue vaccine (final formulation) of individuals living in non-endemic countries are available from the early phase development and did not raise concern. At the time of the initial MA, it was concluded that the safety profile of the CYD dengue vaccine was acceptable within 6 months post any injection in all the populations studied, i.e. in all age groups and regions (non-endemic, endemic Asia Pacific, or endemic Latin America), and irrespective of gender and dengue, JE or YF status at baseline based on post-hoc analyses.

It is unclear to which extent efficacy, as well as immunogenicity, demonstrated in highly endemic countries can be extrapolated to areas of low endemicity, and to non-endemic areas. Efficacy and immunogenicity levels in subjects living in non-endemic areas but who have been exposed in the past are likely depending on the level of exposure they experienced. Pre-existing immunity to dengue virus and other flaviviruses may affect immunogenicity and efficacy levels (cross-protection). Overall, higher baseline dengue virus-neutralizing Ab GMTs were associated with higher PD3 GMTs. Hence, there is an uncertainty around the level of efficacy and immunogenicity in EU vaccine recipients. Since there is no immune correlate of protection, the clinical relevance of the vaccine–induced immunogenicity is not known.

The long-term efficacy of Dengvaxia in travellers residing in non-endemic areas might also differ from the one derived from endemic area and might vary among the diverse population of travellers. The effect of and adequate timing for a booster dose is unknown. The risk of severe dengue disease due to waning protection against dengue disease over time is included in the safety concern as important potential risk in the RMP.

Finally, the current 3-dose schedule (with the current interval between doses) renders the use of Dengvaxia in a travel medicine setting difficult.

2.3.8. Conclusions

With this application, the MAH proposed a broader indication to vaccinate individual 9-45 yo with prior dengue virus infection regardless of geographical considerations (i.e. living in endemic areas or not). The proposed indication addresses the unmet medical need for specific populations living in non-endemic

areas (such as travellers, expatriates or migrants with high probability of previous dengue infection). The proposed indication is endorsed.

Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with test-confirmed previous dengue infection ~~prior dengue virus infection and living in endemic areas~~.

There is an identified risk of severe and/or hospitalized dengue for individuals not previously infected by dengue virus. The safety of Dengvaxia is highly dependent on the approaches used to identify prior dengue infection before vaccination. Recommendations for screening before vaccination have been added in section 4.2 of the SmPC to minimize the risk of a false positive test. The approaches to be implemented in order to ascertain previous dengue infection prior to vaccination is in the remit of the local Public Health authorities. It is not known how these recommendations will evolve.

There is an uncertainty around the level of efficacy and immunogenicity in EU vaccine recipients.

Persistence of efficacy is even more uncertain. It was shown in endemic area that efficacy decreases starting one year after the last dose, and appears to be low or absent thereafter. In addition, the long-term efficacy of Dengvaxia in travellers residing in non-endemic areas might differ from the one derived from endemic area and might vary among the diverse population of travellers. The effect of and adequate timing for booster dose(s) is unknown.

2.4. Update of the Product information

As a result of this variation, sections 4.1, 4.2, 4.4 and 5.1 (the latter upon request from the CHMP) of the SmPC are being updated to modify the approved therapeutic indication to include conditions for the eligibility to pre-vaccination serostatus screening. The Package Leaflet (PL) is updated accordingly.

Changes are summarised below. New text is shown as **bold underlined** and deleted text marked as strikethrough.

- SmPC

Section 4.1:

*Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with **test-confirmed previous dengue ~~infection~~** ~~prior dengue virus infection and living in endemic areas.~~*

Section 4.2 (under 'Method of administration'):

Dengvaxia should only be administered to individuals with a previous dengue infection. Previous dengue infection must be confirmed by a test, either documented in the medical history or performed prior to vaccination.

~~*Previous dengue infection has to be assessed before vaccination by laboratory confirmed history of dengue or through an appropriately validated serological test (see section 4.4).*~~

Section 4.4:

Traceability

In order to improve the traceability of biological medicinal products, the name and the batch number of the administered product should be clearly recorded.

~~*Prior to vaccination*~~

Hypersensitivity

Appropriate medical treatment and supervision must always be readily available in the event of an anaphylactic reaction following administration of the vaccine.

~~*Vaccination should be preceded by a review of the individual's medical history (in particular, previous vaccinations and possible adverse reactions which occurred after vaccination).*~~

The tip caps of the prefilled syringes contain a natural rubber latex derivative, which may cause allergic reactions in latex sensitive.

Intercurrent illness

Administration of Dengvaxia must be postponed in individuals suffering from moderate to severe febrile or acute disease.

Syncope

Syncope (fainting) can occur following, or even before, any vaccination as a psychogenic response to injection with a needle. Procedures should be in place to prevent injury from falling and to manage syncopal reactions.

~~*Protection*~~

~~*A protective immune response with Dengvaxia may not be elicited in all vaccinees. It is recommended to continue personal protection measures against mosquito bites after vaccination.*~~

~~*Special patient groups*~~

~~*Individuals who have not been previously infected by dengue virus or for whom this information is unknown*~~

Prior dengue infection pre-vaccination screening

Individuals who have not been previously infected by dengue virus ~~*or for whom this information is unknown*~~ *should not be vaccinated because* ***of*** *an increased risk of hospitalisation for dengue and clinically severe dengue* ~~*(predominantly grade 1 or 2 Dengue Hemorrhagic Fever)has been*~~ *observed* ***during the long-term follow up of the pivotal clinical trials*** ***in*** ~~***these***~~ ***vaccinated individuals not previously infected***~~*individuals during the long-term follow up of clinical trials*~~ *(see section 4.8).*

In the absence of documented prior dengue virus infection, previous infection ~~*has to*~~***must*** *be confirmed by* ***a*** *test before vaccination* ***(see section 4.2)****. To avoid vaccination of false positives, only test methods with adequate performance in terms of specificity and cross-reactivity based on the local disease epidemiology should be used* ***in accordance with official recommendations****.*

~~*In non-endemic areas or low transmission settings, the lower the proportion of true seropositive individuals, the higher the risk of false seropositives with any test used to determine dengue serostatus.*~~

~~*Thus, testing performed prior to vaccination should be limited to individuals with high probability of past dengue infection (e.g. individuals who lived before or had recurrent stay in endemic areas) and who are*~~

~~*likely to be exposed to dengue in the future. The objective is to minimize the risk of a false positive test., as in non-endemic areas, the proportion of individuals truly infected by dengue is considered generally very low.*~~

In non-endemic areas or low transmission settings, the use of the vaccine should be restricted to individuals who have high probability of future exposure to dengue.

The lower the proportion of true seropositive individuals, the higher the risk of false seropositives with any test used to determine dengue serostatus. Thus, pre-vaccination testing and vaccination should be limited to individuals with high probability of past dengue infection (e.g. individuals who lived before or had recurrent stay in endemic areas). The objective is to minimize the risk of a false positive test.

Special populations ~~*patient groups*~~

Women of childbearing potential

Women of childbearing potential have to use effective contraception during at least one month after each dose (see section 4.6).

Travellers

~~*There are no safety, immunogenicity or efficacy data to support vaccination of individuals living in non-endemic areas and travelling to endemic areas, therefore vaccination of these individuals is not recommended.*~~ ***There are no clinical data to support vaccination of individuals living in non-endemic areas with low probability of past dengue infection and who only occasionally travel to endemic areas, therefore vaccination of these individuals is not recommended.***

~~*Outbreaks*~~

~~*Dengvaxia should not be used in the context of dengue outbreak in non-endemic regions.*~~

Protection

A protective immune response with Dengvaxia may not be elicited in all vaccinees. It is recommended to continue personal protection measures against mosquito bites after vaccination.

~~*Others*~~

~~*Administration of Dengvaxia must be postponed in individuals suffering from moderate to severe febrile or acute disease.*~~

~~*Vaccination should be preceded by a review of the individual's medical history (in particular, previous vaccinations and possible adverse reactions which occurred after vaccination).*~~

~~*Appropriate medical treatment and supervision must always be readily available in the event of an anaphylactic reaction following administration of the vaccine.*~~

~~*Syncope (fainting) can occur following, or even before, any vaccination as a psychogenic response to injection with a needle. Procedures should be in place to prevent injury from falling and to manage syncopal reactions.*~~

~~*The tip caps of the prefilled syringes contain a natural rubber latex derivative, which may cause allergic reactions in latex sensitive individuals.*~~

~~*Women of childbearing potential have to use effective contraception during at least one month after each dose (see section 4.6).*~~

~~*Dengvaxia must not be administered by intravascular injection under any circumstances.*~~

Dengvaxia contains phenylalanine, sodium and sorbitol

Dengvaxia contains 41 micrograms of phenylalanine in each 0.5 ml dose. Phenylalanine may be harmful for people with phenylketonuria (PKU), a rare genetic disorder in which phenylalanine builds up because the body cannot remove it properly.

Dengvaxia contains less than 1mmol of sodium (23 mg) per 0.5 ml dose, that is to say essentially "sodium-free".

Dengvaxia contains 9.38 milligrams of sorbitol in each 0.5 ml dose.

~~*Traceability*~~

~~*In order to improve the traceability of biological medicinal products, the name and the batch number of the administered product should be clearly recorded.*~~

Section 5.1:

[…]

VE against hospitalized and severe VCD cases in subjects 6 to 16 years of age

In subjects 6 to 16 years of age, dengue seropositive at baseline (immunogenicity subset), two clinically severe VCD cases in CYD14 and one in CYD15 were reported during the 25-month period after the first injection in the control group versus none in the vaccine group. Eight hospitalized VCD cases in CYD14 were reported in the control group versus one in the vaccine group and two hospitalized VCD cases in CYD15 were reported in the control group versus none in the vaccine group. These data are inconclusive due to the low number of cases in the immunogenicity subset.

Efficacy was assessed in moderate-high endemic areas. The magnitude of protection may not be extrapolated to other epidemiological situations.

- Package Leaflet

Section 1

Dengvaxia is given to adults, young people and children (from 9 to 45 years of age) with prior dengue virus infection ***confirmed by a test****~~and who live in endemic areas~~ (also see sections 2 and 3).*

~~*Endemic areas are areas where the disease has been continuously present among the people who live there and where outbreaks or epidemics have occurred.*~~

Section 2

[…] Warnings and precautions

If you or your child have never been infected by dengue virus before vaccination, you may have an increased risk of a more serious dengue illness that may lead to hospitalisation if you are later bitten by a dengue-infected mosquito.

Before the administration of Dengvaxia, your doctor, pharmacist or nurse will check if you or your child have ever been infected by dengue virus, and will tell you if a ~~sero~~test~~ing~~ has to be performed.

Tell your doctor, pharmacist or nurse before using Dengvaxia if you or your child have:

a mild to high fever or acute disease. You will not get Dengvaxia until you or your child have recovered.

ever had any health problems when given a vaccine. Your doctor will carefully consider the risks and benefits of vaccination.

ever fainted from an injection. Fainting, and sometime falling, can occur (mostly in young people) following, or even before, any injection with a needle.

had any allergic reaction to latex. The tip cap of the pre-filled syringe contains a natural rubber latex which may cause an allergic reaction.

Travellers

*Vaccination is not recommended if you **have never** live**d** in **an** area where dengue infections ~~do not~~ regularly occur and if you plan to **only occasionally** travel to an area where dengue infections regularly occur.*

~~Outbreaks~~

~~Dengvaxia should not be used in the context of dengue outbreak (sudden occurrence of disease) in non-endemic regions.~~ […]

Section 3

Previous dengue infection must be confirmed by a test, either documented in the medical history or performed prior to vaccination.

Dengvaxia is given by your doctor or nurse as an injection under the skin (subcutaneous injection) in the upper arm. It must not be injected into a blood vessel.

You or your child will receive 3 injections of 0.5 mL – one every 6 months.

The first injection will be given at the chosen or scheduled date.

The second injection, 6 months after the first injection.

The third injection, 6 months after the second injection.

Dengvaxia should be used according to official recommendations.

~~Previous dengue infection has to be assessed by laboratory confirmed history of dengue or through serotesting according to official recommendations.~~

For the full list of changes, see Attachment 1.

2.4.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the MAH show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

In the EU, at the time of this variation application, Dengvaxia was indicated *for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3, and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas*.

The MAH seeks an extension of the approved indication to individual tested positive to dengue, living or not in endemic areas: '*Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age* *with test-confirmed previous dengue infection* ~~*prior dengue virus infection and living in endemic areas*~~.'

The current vaccination schedule consists of 3 injections 6-month apart.

Dengue is an acute, systemic viral infection caused by a virus that is transmitted primarily by the *Aedes aegypti* mosquito bites. The infection may be asymptomatic, cause flu-like illness, and can develop into a potentially lethal complication called severe dengue (including dengue hemorrhagic fever [DHF]/dengue shock syndrome [DSS]). According to CDC, an estimated 1 in 4 dengue virus infections are symptomatic. Symptomatic dengue virus infection most commonly presents as a mild to moderate, nonspecific, acute febrile illness. Approximately 1 in 20 patients with dengue virus disease progress to develop severe dengue. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Dengue shock syndrome (DSS) is the most severe form of dengue disease and results from hypovolaemia caused by vascular leakage.

There are 4 types of closely related but antigenically distinct dengue virus serotypes (1, 2, 3, and 4). Primary dengue virus infection is thought to provide lifelong protection against the infecting serotype and transient cross-protection against heterologous serotypes. Dengue haemorrhagic fever and dengue shock syndrome occur mostly in individuals during secondary dengue virus infection with a different serotype. Increased risk in secondary infection is thought to be linked to antibody-dependent enhancement of virus infection in Fc receptor–bearing cells and the generation of a large infected cell mass in vivo. The antibody-mediated enhancement of dengue seems to be related with the presence of suboptimal neutralizing heterotypic antibodies (that accelerate the rate of internalization of the virus and infection of host cells), and may also be related to the presence of memory T cells with low affinity for the present infecting virus but high affinity for previous infecting serotype(s).

Dengue is the most common mosquito-borne viral disease in humans, spreading globally during the past 30 years as a result of changes in human ecology. The rapidly expanding global footprint of dengue inflicts a significant public health, economic and social burden on the populations of endemic areas. Half

of the world's population is now considered at risk of infection by the dengue viruses. Dengue disease is a major public health concern in more than 128 countries, with the four dengue virus serotypes found in tropical and sub-tropical regions, including some European territories. Dengue is endemic in Asia, the Pacific area, Africa, and Latin America (including the Caribbean). Sustained transmission of dengue fever does not naturally occur in continental Europe, though sporadic autochthonous dengue cases had been reported in Croatia and in France these last years, even if more limited. Dengue, however, is endemic in the overseas territories of some European countries such as France (French Guiana, Martinique, and Guadeloupe).

3.1.2. Available therapies and unmet medical need

There is no specific treatment for dengue disease. The management of dengue disease is supportive, with rest, control of fever and pain with antipyretics/analgesics, and adequate fluid intake. Supportive intensive care and fluid management are the mainstays of therapy for severe disease.

Up to the end of 2015, the only available prevention of dengue by vector control has proven to be of limited success, very difficult to sustain and costly. Vaccination provides a viable and practical alternative in disease control measures. The only vaccine currently on the market is Dengvaxia.

Since the first marketing authorization obtained in Mexico on 08 December 2015, Dengvaxia has been licensed in 22 countries in total. Based on EMA's CHMP recommendation, the European commission has granted the marketing authorization in Europe on 12 December 2018. The prequalification by the World Health Organization (WHO) was granted on 25 March 2020.

Unmet need

Data of the literature review presented by the MAH demonstrated that, overall, seropositivity rate in people not living in endemic areas and travelling to dengue endemic areas ranges from 6-40% but can also be higher when focusing in very particular populations such as expatriates with long-term stay. The seropositivity rate in people born in endemic areas and who moved to reside in non-endemic countries ranges from 20% to 96%.

Vaccination may thus be beneficial to specific population of individuals not living in endemic areas and travelling regularly to dengue endemic area, such as tourist travellers, business travellers, expatriates or people working for recurrent long-stay missions in endemic area (such as health care workers). Not only could travellers with virologically-confirmed dengue history be vaccinated with Dengvaxia, but also potentially travellers with an history of travel in dengue endemic area, even if dengue infection was asymptomatic, provided an adequate pre-vaccination testing strategy is applied.

3.1.3. Main clinical studies

There were no new clinical data submitted. The available clinical data remain those from studies included in the Clinical Development Programme, and particularly from the main pivotal studies (CYD14 and CYD15) which were assessed at the time of the initial MAA.

The recommendations about pre-vaccination screening and test(s) to be used are the responsibility of the national health authorities. Therefore, data on the performance of existing serotests including the MAH co-developed RDT to screen individuals as well as the modelling results on the impact of screen and vaccinate approach in different endemic settings were not assessed in depth and are not considered to weigh in the benefit/risk balance.

A literature search to assess the risk of dengue infection in those not living in endemic areas was presented in support to the removal from the indication of the criterion limiting the use of the vaccine to individuals living in endemic areas. The literature data documented the unmet need in specific population of individuals not living in endemic areas and travelling regularly to dengue endemic area (see unmet medical need above).

3.2. Favourable effects

Results of the main pivotal Phase 3 studies, performed in 10 countries of southeast Asia Pacific (CYD14) and Latin America (CYD15) demonstrated the vaccine efficacy over one year post dose 3 against dengue, hospitalised and severe dengue in those 9-16 years with prior dengue infection (seropositives).

3.3. Uncertainties and limitations about favourable effects

Efficacy data were generated in highly endemic populations (LatAm and Asia Pacific regions). There is no efficacy data in EU endemic territories or in travellers living in non-endemic areas.

It is unclear to which extent efficacy demonstrated in highly endemic countries can be extrapolated to areas of low endemicity, and to EU travellers who have been previously infected with dengue. Efficacy and immunogenicity levels in subjects living in non-endemic areas but who have been exposed in the past are likely depending on the level of exposure they experienced. Pre-existing immunity to dengue virus and other flaviviruses may affect immunogenicity and efficacy levels (cross-protection). Overall, higher baseline dengue virus-neutralizing Ab GMTs were associated with higher PD3 GMTs. Hence, there is an uncertainty around the level of efficacy and immunogenicity in EU vaccine recipients. Since there is no immune correlate of protection, the clinical relevance of the vaccine–induced immunogenicity is not known.

There is no efficacy data over 16 yo and only limited immunogenicity data in 18-60 yo individuals. Individuals living in non-endemic areas that may benefit from vaccination will be of all ages.

It was shown in endemic area that efficacy decreases starting one year after the last dose and appears to be poor or absent after this period. Long-term efficacy (and immunogenicity) of Dengvaxia in the travellers populations living in non-endemic areas is not known. Persistence of efficacy cannot be extrapolated from endemic areas where pre-existing immunity is higher and regular natural re-boostings of immunity occur, which may contribute to maintain efficacy over time. Long term efficacy might vary among the diverse population of travellers. The effect of and adequate timing for booster dose(s) is unknown.

The current 3-dose schedule (with the current interval between doses) renders the use of Dengvaxia in a travel medicine setting difficult.

3.4. Unfavourable effects

No new safety data has been generated specifically for this application. Briefly, in subjects 9 to 45 years of age, the most frequently reported reactions whatever the dengue serostatus prior to vaccination, were headache (54%), injection site pain (49%), malaise (44%), myalgia (43%), asthenia (34%), and fever (16%). In the paediatric population 9-17 yo, fever has been observed with a higher frequency (very common) than in adults (common).

Overall, the same adverse reactions but at lower frequencies were observed in dengue seropositive subjects.

There is an identified increased risk of severe and/or hospitalized dengue for individuals not previously infected by dengue virus (seronegatives) who are inadvertently vaccinated with Dengvaxia. This risk has been classified as an important identified risk in RMP and listed in the product information with a warning.

3.5. Uncertainties and limitations about unfavourable effects

Limited safety data following vaccination with the CYD dengue vaccine (final formulation) of individuals living in non-endemic countries are available from the early phase development but did not raise concern. At initial MA, it was concluded that the safety profile of the CYD dengue vaccine was acceptable within 6 months post any injection in all the populations studied, i.e. in all age groups and regions (non-endemic, endemic Asia Pacific, or endemic Latin America).

The use of the vaccine in subjects who did not yet acquire a natural immunity against dengue virus (seronegatives) was associated with an identified risk for severe dengue, which can be potentially fatal. The safety of Dengvaxia is highly dependent on the testing approaches used to identify prior dengue infection before vaccination. The recommendations about pre-vaccination screening and test(s) to be used are the responsibility of the local health authorities.

As for individuals living in endemic areas, a risk of severe dengue disease due to waning of vaccine-protection over time is an important potential risk in the RMP.

3.6. Effects Table

Not applicable

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The proposed indication addresses the unmet medical need for specific populations living in non-endemic areas with high probability of previous dengue infection, such as expatriates or people working for recurrent long-stay missions in endemic areas, who are likely to have been exposed to dengue in the past and could be re-exposed in the future.

There is an identified risk of severe and/or hospitalized dengue for individuals not previously infected by dengue virus. The safety of Dengvaxia is highly dependent on the approaches used to identify prior dengue infection before vaccination. The approaches to be implemented in order to ascertain previous dengue infection prior to vaccination is in the remit of the local Public Health authorities.

There is uncertainty around the level of efficacy in EU vaccine recipients. The long-term efficacy and the effect of and adequate timing for booster(s) dose is unknown.

3.7.2. Balance of benefits and risks

Altogether, the benefit of Dengvaxia outweighs the unfavourable effects, linked mainly to reactogenicity.

3.7.3. Additional considerations on the benefit-risk balance

It is considered that the main risk with Dengvaxia is to inadvertently vaccinate individuals who did not yet acquire a natural immunity against dengue virus. The recommendations about pre-vaccination screening and test(s) to be used to avoid a false positive test are the responsibility of the local health authorities. It is not known how these recommendations will evolve, and how far they will be appropriately followed. Some approaches are not associated with a risk (such as vaccination of individuals with a virologically-confirmed prior dengue infection). In contrast, approaches relying on serological assays are associated with a risk of false positives, depending on the epidemiological context and assay performances.

3.8. Conclusions

The overall B/R of Dengvaxia is positive.

The extension of indication is recommended for approval.

4. Recommendations

Outcome

Based on the review of the submitted data, the CHMP considers the following variation acceptable and therefore recommends the variation to the terms of the Marketing Authorisation, concerning the following change:

Variation accepted		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I and IIIB

Update of the PI impacting the Therapeutic Indications section to further detail the conditions for the eligibility to pre-vaccination serostatus screening.

As a consequence, sections 4.1, 4.2, 4.4 and 5.1 of the SmPC and sections 1, 2 and 3 of the Package Leaflet are updated accordingly.

Amendments to the marketing authorisation

In view of the data submitted with the variation, amendments to Annexes I and IIIB are recommended.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk management plan (RMP)

The Marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

In addition, an updated RMP should be submitted:

At the request of the European Medicines Agency;

Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.