

# **CBER CMC BLA Review Memorandum**

**BLA STN 125682**

**Dengue Tetravalent Vaccine, Live  
Dengvaxia**

**Dino Feigelstock, PhD, CBER/FDA**

**1. BLA#:**

STN 125682

**2. APPLICANT NAME AND LICENSE NUMBER:**

Sanofi Pasteur

**3. PRODUCT NAME/PRODUCT TYPE**

Proper name: Dengue Tetravalent Vaccine, Live

Proprietary name: Dengvaxia

**4. GENERAL DESCRIPTION OF THE FINAL PRODUCT**

Dengvaxia is a live attenuated dengue tetravalent vaccine containing four recombinant viruses (CYD-1, -2, -3, and -4) expressing the surface antigens of each of the four dengue serotypes in a yellow fever viral backbone. Each monovalent CYD dengue virus was obtained separately via recombinant DNA technology. The CYD dengue viruses were constructed by replacing the gene encoding the pre-membrane (prM) and envelope (E) proteins of the structural proteins in the attenuated yellow fever (YF) 17D virus genome by the corresponding genes of the four wild type dengue virus serotypes 1, 2, 3 and 4. Dengvaxia vaccine is a sterile and freeze-dried product to be reconstituted before injection with a sterile solution of 0.4% sodium chloride for a 0.5 mL single dose presentation. The vaccine is administered via the subcutaneous route in 3 doses separated at six month intervals and is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age with laboratory-confirmed previous dengue infection and living in endemic areas.

**5. MAJOR MILESTONES**

Filing meeting: October 15, 2018

Advisory Committee meeting: March 6, 2019

Action date: May 1, 2019

**6. CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Dino Feigelstock, OVRR/DVP	Modules 3 (except for facilities and equipment information), 4, and 5 (assays used to assess clinical endpoints)
Lei Huang, OBE, DB	Stability of DP (Section 3.2.P.8)
Tao Pan, OCBQ/DBSQC/LACBRP	Release assays
Noel Biachoo, /OCBQ/DBSQC	Release assays

**7. INTER-CENTER CONSULTS REQUESTED**

Reviewer/Affiliation None	Section/Topic	In agreement with consult recommendations (Yes/No <sup>1</sup> )
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## 8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
31 August 2018	STN 125682/0	Reviewed
01 November 2018	STN 125682/10 (response to IR dated October 18, 2018)	Reviewed
10 December 2018	STN 125682/14 (response to IR dated November 30, 2018)	Reviewed
28 January 2019	STN 125682/20 (response to IR dated January 11, 2019)	Reviewed
11 February 2019	STN 125682/22 (response to IR dated January 11, 2019)	Reviewed
1 March 2019	STN 125682/27 (response to IR dated February 1, 2019)	Reviewed
14 March 2019	STN 125682/29 (response to IR dated February 27, 2019)	Reviewed
18 March 2019	STN 125682/30 (response to IR dated March 1, 2019)	Reviewed
1 April 2019	STN 125682/39 (response to IR dated March 15, 2019)	Reviewed
8 April 2019	STN 125682/42 (response to IR dated April 1, 2019)	Reviewed
16 April 2019	STN 125682/46 (edits to amendment 39)	Reviewed
24 April 2019	STN 125682/48 (response to IR dated April 19, 2019)	Reviewed
29 April 2019	STN 125682/53 (response to IR dated April 25, 2019)	Reviewed
30 April 2019	STN 125682/54 (response to IR dated April 26, 2019)	Reviewed
1 May 2019	STN 125682/55 (response to IR dated April 30, 2019)	Reviewed

## 9. REFERENCED REGULATORY SUBMISSIONS (e.g., IND, BLA, 510K, MASTER FILE, etc.)

## 10.

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<sup>1</sup> In case there is a disagreement with the consult review, the reasons and final resolution of the disagreement should be provided in Section **10. REVIEWER EXECUTIVE SUMMARY and RECOMMENDATION**

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BB-IND 11219	Sanofi Pasteur (initially held by Acambis)	The entire IND	no	Initial clinical development of this vaccine

## 11. REVIEWER SUMMARY AND RECOMMENDATION

### A. EXECUTIVE SUMMARY

Sanofi Pasteur submitted a BLA seeking approval of Dengvaxia on August 31, 2018. I reviewed the CMC section and preclinical studies.

Dengvaxia is a live attenuated dengue tetravalent vaccine containing four recombinant viruses (CYD-1, -2, -3, and -4) expressing the surface antigens of each of the four dengue serotypes in a yellow fever viral backbone. Each monovalent CYD dengue virus was obtained separately via recombinant DNA technology. The CYD dengue viruses were constructed by replacing the genes encoding the pre-membrane (prM) and envelope (E) proteins in the attenuated yellow fever virus (YFV) strain 17D genome by the corresponding genes of each of the four wild type dengue virus serotypes 1, 2, 3 and 4. Each CYD virus is cultured separately in Vero cells under serum-free conditions, harvested from the supernatant of the infected Vero cells and purified by membrane chromatography and ultrafiltration. The purified bulk of each CYD virus is then diluted in a stabilizer solution and (b) (4). The final product is a mixture of the four purified CYD viruses diluted in a stabilizer solution, filtered (0.22 µm), filled into single dose vials and freeze-dried.

Dengvaxia vaccine is a sterile and freeze-dried product to be reconstituted before injection with a sterile solution of 0.4% sodium chloride for a 0.5 mL single dose presentation. The vaccine is administered via the subcutaneous route in 3 doses at six month intervals and is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 16 years of age with laboratory-confirmed previous dengue infection and living in endemic areas. The vaccine contains no adjuvant.

Dengvaxia is approved for use in several countries including Europe. Two clinical trials performed in Asia and Latin America have shown that the vaccine is safe and protected against Dengue disease.

The sponsor shows data ensuring that master cells banks, working cells banks, virus master seeds, and virus working seeds used in the production of the vaccine are free of extraneous agents. The sponsor presented information ensuring safety from TSE concerns.

The final vaccine formulation does not contain any new or hazardous excipients and is therefore considered devoid of any toxicity.

The sponsor presents results showing that the elimination of DNA throughout the process is consistently higher than (b) (4) content in commercial (b) (4) is below (b) (4). There are no antibiotics in the manufacturing process for Dengvaxia.

The vaccine manufacturing process is robust, and the titers achieved are highly consistent.

The sponsor performs testing at different stages of production to ensure that the product meets specifications and is consistent.

Testing for Lot Release of final containers (lyophilized product) includes: Bacterial and fungal sterility test, Virus concentration, Virus identification, (b) (4), Appearance of the freeze-dried product, Residual moisture content, Dissolution time, Appearance after dissolution, (b) (4), and Bacterial endotoxin content.

Testing for Lot Release of final containers (diluent) includes: Color, Odor, Appearance, Identity – Sodium, Identity – Chloride, Sodium Chloride, (b) (4) at release, Vial Volume, (b) (4), Particulate Matter in Injections, Sterility, Bacterial Endotoxin Test, Safety, Major A Defects, and Major B Defects.

Regarding the potency of the vaccine, the sponsor has set an upper limit specification of  $10^{(b)(4)}$  log<sub>10</sub> CCID<sub>50</sub>/dose/serotype which is consistent with clinical studies that have shown safety of the product. Regarding the lower limit specification, the sponsor has set a lower limit specification of  $10^{(b)(4)}$  log<sub>10</sub> CCID<sub>50</sub>/dose/serotype. This release titer, according to calculations made by CBER statisticians, will ensure a titer of 4.5 log<sub>10</sub> CCID<sub>50</sub>/dose/serotype at the end of expiry period of 36 months. Data from clinical studies have shown that the vaccine is immunogenic at a dose close to 4.5 log<sub>10</sub> CCID<sub>50</sub>/dose/serotype, and therefore, a minimum release potency specification of  $10^{(b)(4)}$  log<sub>10</sub> CCID<sub>50</sub>/dose/serotype is acceptable.

Based on the information submitted in the BLAI recommend approval of the product.

## **B. RECOMMENDATION**

### **I. APPROVAL**

#### **a. List of Drug Substance (DS) and Drug Product (DP) manufacturing facilities:**

- Manufacture of DS: Sanofi Pasteur (b) (4).
- Manufacture of DP (lyophilized vaccine): Sanofi Pasteur, (b) (4).
- Manufacture of DP (diluent): Sanofi Pasteur, 1 Discovery Drive, Swiftwater, PA 18370, USA.

**b. List of approvable Comparability Protocols, if applicable**

- Establishment of new (b) (4) reference standard used in the (b) (4) test performed as a release test on the DSs;
- Production of (b) (4) used as critical reagents for the virus concentration and identification tests performed on CYD dengue (b) (4) CYD dengue DP and for the test for extraneous agents using (b) (4) performed on the (b) (4).

Based on approval of these protocols, the sponsor proposes a reduction in reporting category from a CBE-30 to an Annual Report for any future lot-to-lot change of these two substances

**c. List of Post-Marketing Commitments (PMCs)/Post-Marketing Requirements (PMRs), if applicable.**

The following CMC PMCs will be addressed by the applicant as described below and do not need to be included in the approval letter:

- The sponsor will evaluate the impact of (b) (4) during the execution of the virus concentration test. [See section 3.2.S.4.2 and amendment 20, January 28, 2019.
- The sponsor will transfer the (b) (4) test for release of Finished Product (after all labeling activities are concluded) to the Swiftwater site in Q3/Q4 of 2019 before release of the first vaccine batch (amendment 27, sponsor response to IR submitted 2.1.19 regarding the lack of description and validation of the (b) (4) test performed for release of the Finished Product).
- The sponsor will revise the lower limit release (b) (4) specification from (b) (4) in all the eCTD sections and the lot release protocol. The sponsor agreed to change the lower limit release potency, but stated that “In order to comply with Sanofi Pasteur change control process, the revision of the eCTD sections and the lot release protocol will be submitted as a post approval commitment in order to not impact the expected approval due date of May 1st 2019” (amendment 48, sponsor response to comment 1 from IR submitted April 19, 2019). In amendment 53, the sponsor commits to submit the revised lot release protocol and all other documents in the respective eCTD sections that include the new release specification for (b) (4) as an amendment to the BLA by June 30, 2019 at the latest. In addition, also in amendment 53, the sponsor submitted the revised DP specification with the revised lower limit (b) (4) release acceptance criterion changed from (b) (4) that will be included in section 3.2.P.5.1, Specifications, when the eCTD is updated.

**d. Consideration for Inspectional Follow-up (e.g., flagging inspectional issues for future surveillance inspections)**

(b) (5), (b) (7)(E)

**e. Lot release requirements**

The lot release protocol (LRP) is provided (original submission and amended amendment 26) and is acceptable.

**II. SIGNATURE BLOCK**

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Primary Level Review including all CMC reviewers <sup>2</sup>	Concur	
Secondary Level Review (e.g., Branch/Lab Chief)	Concur	
Tertiary Level Review (e.g., Division Director)	Concur	

**Review of CTD**

**Module 3**

**3.2.S Drug Substance (DS)**

**3.2.S.1.1 Nomenclature, Structure and General Properties**

International name: Dengue viruses, live attenuated (serotype 1 to 4)

Internal official name: CYD dengue viruses or CYD-1, CYD-2, CYD-3 and CYD-4 for each virus serotype.

Structure: The CYD chimeric viruses were constructed by replacing the sequence encoding the pre-membrane (prM) and envelope (E) structural ("coat") proteins in YF-17D virus genome by

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<sup>2</sup> The review recommendations as indicated by the reviewer's signature for the CTD sections/subject matter identified in section 6

those encoding for the homologous sequences of the four wild type dengue serotypes 1 ((b) (4) [REDACTED]), 2 ((b) (4) [REDACTED]), 3 ((b) (4) [REDACTED]), and 4 ((b) (4) [REDACTED]).

General properties: CYD-1, -2, -3, -4 chimeric viruses possess the non-structural proteins involved in virus replication and the YFV capsid protein of the attenuated YF-17D, along with the pre-membrane and envelope proteins of each of the 4 wild-type dengue serotypes.

### **3.2.S.2 Manufacture**

#### **3.2.S.2.1 Manufacturer(s)**

Sanofi Pasteur

((b) (4) [REDACTED])

Quality control of the DS

Sanofi Pasteur ((b) (4) [REDACTED])

((b) (4) [REDACTED])

Quality control of the DS and manufacture of DS

#### **3.2.S.2.2 Description of Manufacturing Process**

((b) (4) [REDACTED])





(b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

(b) (4)

### 3.2.P DP

#### 3.2.P.1 Description and Composition of the DP

The DP is the live attenuated tetravalent dengue virus vaccine to be administered by subcutaneous route. The DP contains live attenuated dengue viruses representing each of the four serotypes. It is a white homogeneous freeze-dried product filled in a glass vial.

The diluent used for reconstitution is a 0.4% sodium chloride solution. The reconstituted product is a colorless limpid liquid with possible presence of white to translucent particles (of endogenous nature). The primary packaging materials are a (b) (4) glass, 3 mL vial, a rubber stopper, and an aluminum/polypropylene cap. The composition of the DP after Reconstitution is as follows:

Component*	Quantity (per 0.5 mL dose of reconstituted vaccine)	Function	Reference to quality standards
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CYD dengue virus serotype 1	4.5 - 6.0 log <sub>10</sub> CCID <sub>50</sub> /dose	DS	(b) (4)
CYD dengue virus serotype 2	4.5 - 6.0 log <sub>10</sub> CCID <sub>50</sub> /dose	DS	
CYD dengue virus serotype 3	4.5 - 6.0 log <sub>10</sub> CCID <sub>50</sub> /dose	DS	See 3.2.P.5.1 Specification(s)
CYD dengue virus serotype 4	4.5 - 6.0 log <sub>10</sub> CCID <sub>50</sub> /dose	DS	
Essential amino acids†	0.56 mg	Total quantity stabilizer components: 45.95 mg	Stabilizer
Non-essential amino acids	0.20 mg		Stabilizer
L-arginine hydrochloride	2.50 mg		Stabilizer
Sucrose	18.75 mg		Stabilizer
D-trehalose dihydrate	13.75 mg		Stabilizer
D-sorbitol	9.38 mg		Stabilizer
Trometamol	0.18 mg		Stabilizer
Urea	0.63 mg		Stabilizer
Sodium chloride	2.0 mg		Diluent
Water for injections	Up to 0.5 mL		Diluent

The DP, 0.4% Sodium Chloride Diluent is presented as a solution for reconstitution of freeze-dried vaccines in a unit dose vial and is a sterile, clear, colorless solution that is devoid of foreign matter. Each unit dose vial contains no less than 0.6 mL. Sodium chloride is used for its (b) (4).

Importantly, the sponsor clarifies in amendment 45 of to this BLA that the diluent, with the same contents and container closure, is also licensed for use with ActHIB (STN 103935/5376).

### 3.2.P.2 Pharmaceutical Development

#### 3.2.P.2.1 Components of the DP

##### 3.2.P.2.1.1 DS

The DP (DP) consists of a mixture of four (b) (4) DSs. The four DSs correspond to the four live, attenuated, dengue viral suspensions. The compatibility between the CYD dengue viruses and the chosen excipients is demonstrated by the stability studies performed under normal and accelerated conditions.

##### 3.2.P.2.1.2 Excipients

The excipients are L-arginine hydrochloride, Sucrose, D-sorbitol, Trometamol, Urea, D-trehalose dihydrate, (b) (4), Water for injections, (b) (4), and essential and non-essential amino-acids. A (b) (4) was identified as the best combination to stabilize the freeze-dried product. Presence of trehalose (b) (4)

(b) (4). D-sorbitol further improves stability  
(b) (4) of the CYD dengue virus vaccine. Trometamol was selected as (b) (4)  
(b) (4) which ensures the stability of the live, attenuated, tetravalent dengue virus vaccine. A positive impact was demonstrated with urea on (b) (4) stability of CYD dengue vaccine. Essential amino acids and non-essential amino acids (b) (4) and were selected to maintain the stability of the freeze-dried pharmaceutical form of the vaccine.

Regarding the diluent, Sodium chloride and water for injection (used to prepare the DP, 0.4% Sodium Chloride Diluent) both comply with the (b) (4).

### **3.2.P.2.2 DP**

#### **3.2.P.2.2.1 Formulation Development**

Three main product/process development phases can be distinguished: starting with phase I and continuing through phase III. Phase I process development proposed (b) (4)

(b) (4) From phase II, process development targeted (b) (4)

(b) (4) The choice was a freeze-dried DP (DP) to be reconstituted prior to injection. The development (b) (4)

Since phase III clinical studies, diluent batches used for the reconstitution of the CYD dengue vaccine are the 0.5 mL (b) (4) of 0.4% NaCl solution (also called 072 internally). The compatibility between the phase III DP and its corresponding diluent for reconstitution has been tested. For reconstitution of CYD dengue vaccine intended for US commercial batches, the diluent is a 0.4% NaCl solution (also called 429 internally) filled in vials (0.5 mL) manufactured at the Swiftwater (SWR) site (US). The 0.4% NaCl diluent (429) used for reconstitution of CYD dengue vaccine is already known by CBER as this diluent is currently licensed for the reconstitution of Haemophilus b Conjugate Vaccine, ActHIB (STN 103935). The demonstration of equivalences between 072 and 429 diluents relies on the identical qualitative and quantitative composition of both diluents, the comparison of their respective manufacturing process, and the comparison of their respective release specifications.

Regarding the diluent, the sponsor states that no formulation development has been undertaken, sodium chloride and water for injection being the only ingredients of the finished product.

#### **3.2.P.2.2.3 Physicochemical and Biological Properties**

DP is in a lyophilized form and DS is in a (b) (4) form. The physicochemical and biological properties of the Diluent are determined by the control tests on the Bulk Product and the Filled Product.

#### **3.2.P.2.3 Manufacturing Process Development**

Main manufacturing process changes from phase I to phase III process development are: manufacturing site, FBP scale (from (b) (4) ) and batch size ((b) (4) ), FBP stabilizing solution (see above), pharmaceutical form ((b) (4) versus lyophilized), final sterile filtration ((b) (4) ), then implementation of (b) (4) for commercial batches), diluent (0.4% NaCl solution (b) (4) ), and storage condition (b) (4) 4°C).

Regarding diluent, the sponsor indicates that the 0.4% Sodium Chloride Diluent is a simple inorganic solution consisting of (b) (4) Water for Injection (WFI) and (b) (4) Sodium Chloride (without preservative), and therefore process development was not required.


### **3.2.P.2.4 Container Closure System**

#### **Lyophilized product:**

The container closure systems consist of single-dose glass vial ((b) (4) glass, 3 mL) with a stopper (Halobutyl rubber) and a cap (composed of Aluminum and polypropylene parts). Tests were performed either by the supplier, in-house, or by subcontractors. Tests for (b) (4) Glass Vial are: (b) (4) . Tests for stopper are: (b) (4) . Tests for the cap are: (b) (4) .

#### **Diluent:**

The filled Unit Dose Diluent container closure system is comprised of a 2 mL (b) (4) borosilicate glass vial, a 13 mm latex-free stopper, and 13 mm flip seal cap. When the vials are received on site, they are (b) (4) and depyrogenated prior to use per site procedure. The test performed on vials are: (b) (4)



### **3.2.P.2.5 Microbiological Attributes**

The vaccine is a sterile freeze-dried product in a single-dose vial presentation to be reconstituted with the appropriate diluent prior to injection. Product sterility and container closure integrity were demonstrated through the stability programs (Bacterial and fungal sterility test for freeze and dried product, and sterility and Container Closure Integrity Testing for the diluent)

Regarding the diluent, it is a sterile, clear, colorless and odorless solution that is devoid of foreign matter in a unit dose glass vial presentation. The integrity of the diluent is assured through well controlled manufacturing and filling processes in an aseptic environment. Each lot must successfully pass the predefined criterion for sterility for release of the (b) (4) and Filled Product. Endotoxin levels are also assessed and must not be above a predefined criterion to successfully pass. Product sterility and container closure integrity are demonstrated through the stability programs.

### 3.2.P.2.6 Compatibility

CYD dengue vaccine is a freeze-dried product supplied together with a diluent for reconstitution. The diluent for reconstitution used for the phase II DP was a 0.4% NaCl solution which contained (b) (4). In order to avoid using a (b) (4) component, an investigation using a 0.4% NaCl solution (b) (4) was conducted on phase III DP batches. Since phase III clinical studies, diluent batches used for the reconstitution of the CYD dengue vaccine are the 0.5 mL (b) (4) of 0.4% NaCl solution (also called 072 internally). The compatibility between the phase III DP and its corresponding diluent (072) for reconstitution has been tested and results on the vaccine after reconstitution complied with the acceptance criteria under normal storage condition (+5°C) and under the accelerated storage condition ((b) (4)). No drop in potency (b) (4) values were observed (tested at T0 and end-of-shelf life). For reconstitution of CYD dengue vaccine intended for US commercial batches, the diluent is a 0.4% NaCl solution (also called 429 internally) filled in vials (0.5 mL) manufactured at the Swiftwater site (SWR, US; the 429 diluent is approved for US market for the reconstitution of ActHIB vaccine). For reconstitution of CYD dengue vaccine intended for US commercial batches, the 429 diluent will be used. The equivalence between both diluents, 072 vs. 429, is documented. This equivalence supports that the stability studies of the CYD dengue vaccine reconstituted with the 072 diluent (section 3.2.P.8.1). In the context of the use of the 0.5 mL 0.4% NaCl (429) diluent, (b) (4) stability program will be performed on the CYD dengue vaccine with the 429 diluent.

Regarding the diluent, the primary packaging component for the Final Container Diluent DP, a 2 mL vial made of (b) (4) borosilicate clear glass, is a neutral material commonly used for the containers of preparations for parenteral use. The vial has been determined to be suitable for its intended use. The vial stopper is a 13 mm closure using latex-free rubber. The stoppers are tested by the vendor for biological reactivity. Leachables screening testing was conducted to identify and provide semi-quantitative estimation of any potential leachable compound from the latex-free stopper or glass vial when in contact with the sodium chloride diluent.

Finally, the compatibility of the diluent with its container (in terms of interaction of potential leachables from the packaging materials with the DP) and closure system is demonstrated through the evaluation of stability studies and extractable and leachable studies.

#### **Overall Reviewer's Assessment of Section 3.2.P.2:**

- The information provided is acceptable. Of note, the stability studies of the CYD dengue vaccine were executed with the 072 diluent presentation ((b) (4)) but not

with the 429 diluent presentation, intended to be used in the US market under this BLA. Given the similarities between the two diluent presentations (see above) and the fact that annual stability program will be performed on the CYD dengue vaccine using the 429 diluent, I consider acceptable the use of the 429 diluent.

### 3.2.P.3 Manufacture

#### 3.2.P.3.1 Manufacturer(s)

DP Manufacturers and responsibilities:

Sanofi Pasteur

(b) (4)

Quality control

Sanofi Pasteur

(b) (4)

Manufacturing of DP and quality control

Sanofi Pasteur (b) (4)

(b) (4)

Quality control

Sanofi Pasteur Inc

1, Discovery Drive Swiftwater

Pennsylvania (PA) 18370

United States

Manufacturing of diluent, secondary packaging/labelling of final Product, quality control, and Final packaged product batch release

#### 3.2.P.3.2 Batch Formula

Preparation of the FBP (FBP) involves the formulation of the four CYD dengue viruses with the excipients in order to achieve a homogeneous blend prior to the filling of the final container. The FBP manufacturing formula varies depending upon the virus concentration of each DS batch of CYD dengue virus serotype. An example of typical formula is given in the following table for a (b) (4) batch.

Ingredient	Quantity
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<b>DSs</b>	(b) (4)
CYD dengue virus serotype 1	
CYD dengue virus serotype 2	
CYD dengue virus serotype 3	
CYD dengue virus serotype 4	
<b>Excipients</b>	(b) (4)
FBP stabilizing solution	

The composition of the FBP Stabilizing Solution is as follows

Name of ingredient	Quantity (per (b) (4))
<b>Essential amino acids</b>	(b) (4)
<b>Non-essential amino acids</b>	
<b>Hydrochloric acid concentrated</b>	
<b>L-arginine hydrochloride</b>	
<b>Sucrose</b>	
<b>D-trehalose dihydrate</b>	
<b>D-sorbitol</b>	
<b>Trometamol</b>	
<b>Urea</b>	
(b) (4)	
<b>Water for injections</b>	

The formula of the Bulk Diluent is given in the table below. This batch is for a batch size of (b) (4), for manufacture approximately (b) (4) doses

Raw Material Excipient	Excipient Grade	Factor	Amount per Batch ((b) (4))
Sodium Chloride	(b) (4)		
Water for Injection			

#### Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:

- The information provided is acceptable

#### 3.2.P.3.3 Description of Manufacturing Process

The manufacturing process of the CYD dengue vaccine DP is divided in the following critical steps:

(b) (4)



(b) (4)

The FBP (FBP) manufacturing process includes (b) (4)

the FBP stabilizing solution is 0.2  $\mu\text{m}$  filtrated and then stored at (b) (4). (b) (4)

The FBP is formulated at an equal targeted concentration of 5.0 log<sub>10</sub> CCID<sub>50</sub>/dose for each of the four serotypes. In order to compensate for expected manufacturing losses, a maximum overage of (b) (4) per serotype is applied to the target concentration.

The filling and freeze-drying manufacturing process includes the following steps:

- (b) (4) sterile filtration of the FBP at 0.22  $\mu\text{m}$  ((b) (4) );
- Filling of the FBP (the filling process is conducted aseptically for up to (b) (4) , until stoppering of the vials is complete);
- Freeze-drying and stoppering;
- Crimping;
- Container closure integrity verification ((b) (4) );
- Visual inspection (100%).

Each primary packaging component is (b) (4) before use.

### **Manufacturing process of the CYD dengue vaccine diluent DP**

Bulk diluent formulation activities include: (b) (4)

The filling process includes vial washing, vial depyrogenation, sterile filtration (b) (4) , vial filling into 2 mL vials, stopper insertion (latex free), and capping. An in-process check system is used to (b) (4) . A 13mm cap is crimped onto each vial. After filling, stoppering and capping, there are no reprocess steps in the manufacturing of the Final Container Diluent. The vials can be inspected manually or automated.

After inspection, the diluent filled vials are removed from storage, transported to the packaging area and are labeled. The Bulk Diluent DP can be stored up to (b) (4). The Final Container DP can be stored for twenty-four (24) months from the date of fill.

**Overall Reviewer's Assessment of Section 3.2.P.3.3:**

- The information provided is acceptable.

**3.2.P.3.4 Controls of Critical Steps and Intermediates**

The critical steps are: (b) (4)

Critical Process Parameters (CPPs) are: (b) (4)

For the freeze-drying cycle, the CPP are (b) (4) for each of the (b) (4) steps involved in the process.

The in process control tests are (b) (4)

This condition allows for the run of several formulation batches (b) (4). These operating conditions have been defined during development studies. Development and validation data support the selection and justifications for CPPs, ranges, and in-process controls. An appropriate control strategy is implemented to assure product quality and process consistency.

The following IR was issued on 3.15.19:

The FBP is formulated at an equal targeted content of 5.0 log<sub>10</sub> CCID<sub>50</sub>/dose for each of the four serotypes. In order to compensate for expected manufacturing losses, a maximum overage of (b) (4) per serotype is applied to the target content. Please confirm that this overage is routinely applied so the actual target content is (b) (4) for each of the four serotypes. Please clarify if the (b) (4) target was applied to the Phase 3 and PPQ lots submitted to the BLA and comment or provide an assessment of the overall potency loss during the DP manufacturing steps.

On 4.1.19, the sponsor submitted amendment 39 and responded that for routine production, a maximum overage of (b) (4) per serotype is applied to the target content of 5.0 log<sub>10</sub> CCID<sub>50</sub>/dose ((b) (4)). Historical overages applied to produce phase 3 clinical batches and PPQ batches are presented (target content: (b) (4)). The sponsor did not provide an assessment of the overall potency loss during the DP manufacturing steps but stated that it is assessed on each DP batch produced through the measurement of the virus concentration results at release and stated that this critical quality attribute is monitored through the control charts. Although potency loss was not assessed,

the level of control of potency is acceptable. In addition, during the inspection, the sponsor provided data showing the difference between the expected and observed potencies for each of the 4 CYD viruses in many lots of DP manufactured using (b) (4) DS bulks from each of the 4 CYD viruses.

### **CYD dengue vaccine diluent DP**

The steps identified as critical for the formulation process of the bulk diluent are the following:

(b) (4) The bulk diluent formulation process includes (b) (4) in-process controls: (b) (4). The steps identified as critical for the filling process of the diluent are (b) (4). In-Process Controls for the Final Container Diluent are (b) (4).

The acceptance criteria for fill (b) (4). There is no target fill volume specified in the submission. In section 3.2.P.2.2, the sponsor states that “In order to permit withdrawal and administration of the nominal labeled volume, a (b) (4) volume overfill of the nominal labeled volume (0.5 mL) is applied”. This (b) (4) volume would result in a target volume of 0.6 mL. However, this value is inconsistent with the acceptance criteria for fill (b) (4), which has a minimum of (b) (4). Regarding the upper limit, the sponsor states that it “is controlled for operational purposes and has no impact on the potency of the vaccine as the recommended amount of diluent is removed from the vial for reconstitution of the lyophilized vaccine. However, this information is not consistent with the instructions provided in the package insert. The instructions state to insert the syringe needle into the vial of diluent, withdraw “the entire liquid content”, inject the liquid into the vial of the lyophilized component, and, after swirling and reconstitution, withdraw 0.5 mL of reconstituted product for administration. According to the acceptance criteria, if a diluent vial with (b) (4) were released and all its contents used to reconstitute the vaccine, then 0.5 mL of vaccine would have a significantly lower content of the active component compared to the recommended dose.

The following comments pertain to the diluent (0.4% NaCl):

3) Please indicate what is the target volume (or fill (b) (4)) for the diluent filled into 2 mL final containers.

4) We note that the in-process control acceptance criteria for diluent Fill (b) (4) (3.2.P.3.4). We are concerned that these wide acceptance criteria could result in lack of dose consistency. In Table 4 (3.2.P.3.4) it is indicated that “...The upper limit... has no impact on the potency of the vaccine as the recommended amount of diluent is removed from the vial for reconstitution of the lyophilized vaccine.” However, this justification is not consistent with the instructions for dose preparation for administration in the package insert (2.2 Preparation), where it is indicated to “withdraw the entire content of the diluent vial and inject it into the vial of the lyophilized vaccine.”. Please tighten the acceptance criteria for Fill (b) (4) to ensure consistent dosing or justify the current specification.

On April 24, 2019, the sponsor submitted responses in amendment 48.

The sponsor states that the target fill (b) (4). The sponsor also states that Action and Alert controls are in place for fill (b) (4) as follows:

(b) (4)

Regarding tightening the acceptance criteria for Fill (b) (4), the sponsor maintained the acceptance criteria but has revised the Prescribing Information for withdrawal of diluent at the specified volume of 0.6 mL for reconstitution.

The sponsor responses are acceptable.

**Overall Reviewer's Assessment of Section 3.2.P.3.4:**

- The information provided is acceptable.
- Deficiencies were identified and were resolved:
  - 1) In the original submission, 3.2.P.3.3 page 7 of 15, it is stated that “In these conditions, a maximum of (b) (4) is authorized”. However, data presented in section 3.2.P.2.3 page 28 of 131 does not support this claim. In amendment 20, submitted on 1.28.19, the sponsor amended the corresponding section of the BLA and states that “...This condition allows for the run of several formulation batches (b) (4) possibilities of the DS” and “This study demonstrated that the number of (b) (4) has an impact on the viral concentration of CYD dengue vaccine”
  - 2) The target fill volume is not specified in the submission and the in-process control acceptance criteria for diluent Fill (b) (4). These wide acceptance criteria could result in lack of dose consistency. In amendment 48, the sponsor revised the Prescribing Information for withdrawal of diluent at the specified volume of 0.6 mL for reconstitution. The sponsor's responses are acceptable.




### 3.2.P.3.5 Process Validation and/or Evaluation

The process validation covering the manufacturing steps and parameters studied for CYD dengue vaccine are the following:

(b) (4)



(b) (4)



(b) (4)



(b) (4)

#### **Overall Reviewer's Assessment of Section 3.2.P.3.5:**

- The information provided is acceptable
- No deficiencies were identified

### **3.2.P.4 Control of Excipients**

#### **3.2.P.4.1 Specifications**

The excipients used in the manufacture of the FBP solution are listed previously in this review (section 3.2.P.3.2).

The specifications for L-arginine hydrochloride, Sucrose, D-sorbitol, Trometamol, Urea, D-trehalose dihydrate, (b) (4), and Water for injections are based in (b) (4). Individual amino acids are of (b) (4) grade with in house specifications. Tests for the essential and non-essential amino-acids (b) (4) are (b) (4).

Specification for (b) (4) are in house, and tests are the following: (b) (4).

All the excipients contained in the diluent DP are (b) (4) grade.

#### **3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures**

The analytical procedures used for testing the excipients, for essential and non-essential amino acids (b) (4), and for (b) (4) are performed in compliance with the relevant (b) (4) monographs. The analytical procedures used to control the pharmacopoeial grade excipients (sodium chloride and water for injection) contained in the DP, 0.4% Sodium Chloride Diluent, are those described in the (b) (4) and are therefore not provided.

#### **3.2.P.4.5 Excipients of Human or Animal Origin**

No excipients of human or animal origin are used for the formulation of CYD dengue vaccine (for both, freeze dried and diluent DPs).

#### **3.2.P.4.6 Novel Excipient**

No novel excipients are used for the formulation of CYD dengue vaccine (for both, freeze dried and diluent DP).

#### **Overall Reviewer's Assessment of Section 3.2.P.4:**

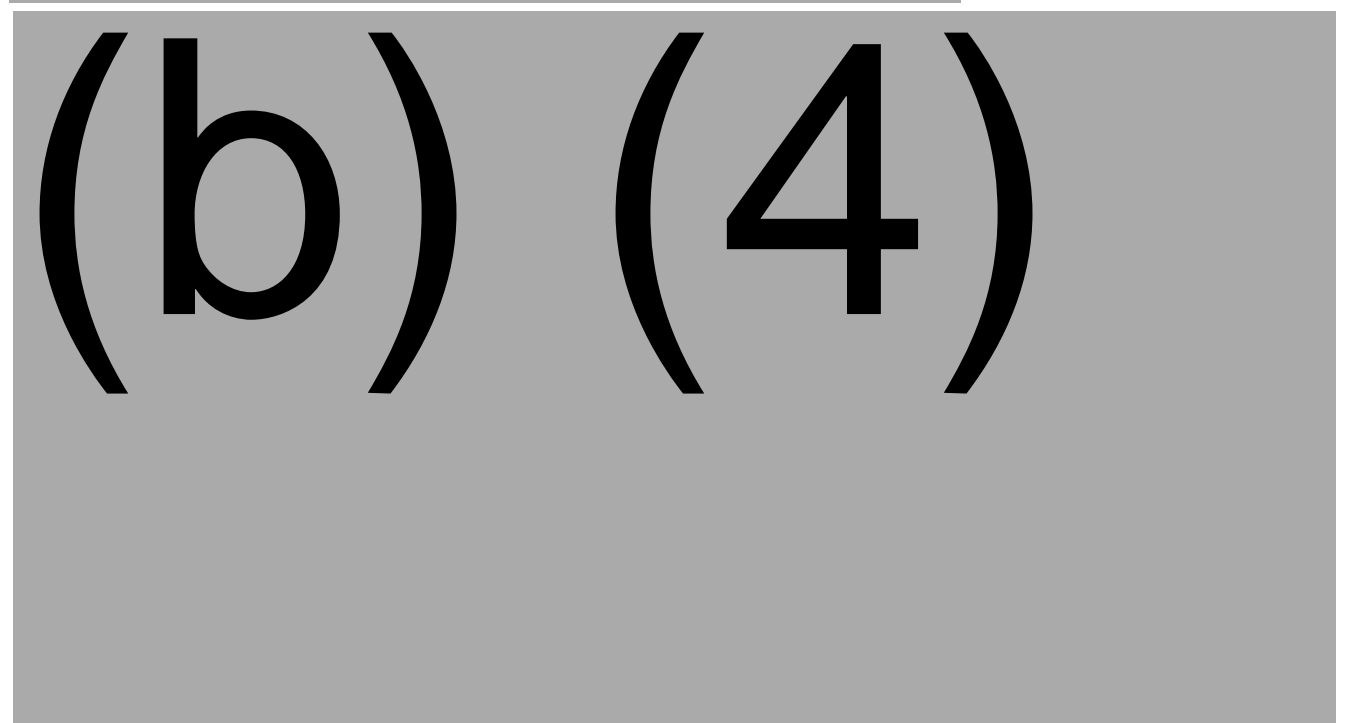

- The information provided is acceptable.

#### **3.2.P.5 Control of DP**


##### **3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)**

##### **Specification for the Lyophilized DP (Unlabeled)**

(b) (4)



(b) (4)







(b) (4)

(b) (4)

#### **Specification for the Final Container Lyophilized Drug Product (Labeled)**

The specification for CYD Dengue Finished Product is “Virus Identification” with an acceptance criterion of “positive for the presence of the 4 dengue Serotypes”. The original submission states that “The identification of viruses in the finished DP is based on a (b) (4) test using serotype specific (b) (4)”. However, the description and the validation of the test was not included. We issued an IR and in amendment 20 the sponsor stated that they commit to provide this information to CBER as soon as available through submission of a CBE-30, before release of the first Dengue vaccine lot for the US market. In response, we issued an IR stating that this information should be included in the BLA. The sponsor provided the SOP and the validation of the identification test in amendment 27. Of note, the sponsor states in amendment 27 that the test will be (b) (4)

The SOP and the validation data presented were reviewed and found to be appropriate. (b) (4)

### Specification for the Final Container Diluent Drug Product (Labeled)

Test	Internal Method Reference	Compendial Reference	Acceptance Criteria
Identity - Sodium	Q_0278221	(b) (4)	Positive
Identity - Chloride			Positive
Identity - (b) (4)			Negative
Identity - (b) (4)			Negative
Identity – Sodium Chloride (b) (4)	Q_0278184	(b) (4)	(b) (4)
(b) (4) Test		Non-Compendial	(b) (4)

(b) (4)

The specification for fill volume is no less than 0.6 mL. No upper limit is specified. The lack of upper limit can result in lack of dose consistency.

On April 19, 2019, The following IR was issued to the sponsor:

5) The acceptance limit for Vial Volume for Final Container Diluent DP (Unlabeled) is (b) (4) mL (3.2.P.5.1). Please include an upper limit or provide a justification.

On April 24, 2019, the sponsor responded (amendment 48) that no upper limit for the vial volume specification is required since the Prescribing Information has been revised to include instructions to withdrawal 0.6 mL of diluent for reconstitution. I consider that the response is acceptable.

#### Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

- The information provided is acceptable.
- Deficiencies were identified.
  - 1) Minimum expiry and release potency specifications (b) (4) log10 CCID50/dose for each of the serotypes).  
 The pivotal clinical trials (CYD14 and CYD15) were conducted with formulations containing more than 5 log10 CCID50/dose for each of the 4 CYD viruses. To justify an expiry potency below 5 log10 CCID50/dose, the sponsor used immunogenicity data collected from clinical trials (CYD12 and CYD17) that, according to the sponsor, were conducted using formulations containing approximately (b) (4) log10 CCID50/dose for each of the 4 CYD viruses. According to the sponsor, the immunogenicity of (b) (4) formulations were comparable to 5/5/5/5 formulations. These clinical trials were

conducted in non-endemic areas. According to the sponsor, the minimum release specification (<sup>(b) (4)</sup> log<sub>10</sub> CCID<sub>50</sub>/dose for each of the serotypes, <sup>(b) (4)</sup> log above the dose considered efficacious) is based on assay variability and stability of the DP. I have the following comments:

a. Study CYD17 was conducted with lots (S4316, S4317 and S4318) that harbored potencies above 5 log<sub>10</sub> CCID<sub>50</sub>/dose for each of the serotypes (3.2.P.2.3, table 17) and the potencies were stable over time (tables 64, 65, and 66).

b. The clinical reviewer determined that the data from study CYD12 are not conclusive to demonstrate that the <sup>(b) (4)</sup> formulation is comparable to the 5/5/5/5 formulation (original submission and amendment 30). Additional data from clinical study CYD23 using vaccine potencies for CYD-1 of <sup>(b) (4)</sup> log<sub>10</sub> CCID<sub>50</sub> (submitted in amendment 30) showed acceptable immunogenicity.

c. The sponsor states that there is no loss of potency during the shelf life (3 years) of the vaccine (section 3.2.P.8). The statistical reviewer Dr. Lei Huang concluded that the data show no evidence against the sponsor's conclusion of no potency loss. However, he indicated that the lack of evidence could be due to the low number of lots analyzed (<sup>(b) (4)</sup> lots/serotype). We issued an IR (11 January 2019) to the sponsor and the sponsor responded in amendment 22 (11 February 2019) that stability data from additional <sup>(b) (4)</sup> lots were analyzed, and no potency loss was observed for CYD-2, -3, and -4, and a <sup>(b) (4)</sup> <sup>(b) (4)</sup> potency loss was observed for CYD-1 for the 3-year shelf life. While the additional stability data show that the product is reasonably stable, calculations made by Dr. Huang and Dr. Phil Krause (OVRD Deputy Director) indicate the need to add approximately <sup>(b) (4)</sup> logs to the end of expiry specification to define the minimum release potency specification to ensure that lots will meet minimum expiry specification at the 3-year shelf-life.

In summary, the data from study CYD23 showed:

- Acceptable immunogenicity data for CYD-1 at a potency between 4.55 and 4.65 log<sub>10</sub> CCID<sub>50</sub> in endemic areas.
- Acceptable immunogenicity data for CYD-4 at a potency of 4.15 log<sub>10</sub> CCID<sub>50</sub> in non-endemic areas
- No data for CYD-2 and CYD-3 viruses showing acceptable immunogenicity at lower doses.

The clinical reviewers considered that the data from CYD-1 and CYD-4 are adequate and representative of CYD-2 and CYD-3.

<sup>(b) (4)</sup>

2) Lack of description and validation of the Virus Identification test performed for release of the Finished Product. The information was submitted in amendments 27 and 54 and is acceptable (see previous sections).

3) For the appearance after dissolution and dissolution time tests in final container, the lyophilized product is reconstituted in diluent which is (b) (4) prior to its use for reconstitution. During the pre-approval inspection, I asked the sponsor the rationale for adding such (b) (4) step, given that the diluent is (b) (4) before its use in routine vaccine administration. The sponsor explained that the (b) (4) step is added (b) (4). I noted that, ideally, the test should be executed under the same conditions used routinely, and the firm agreed. I understand the applicant's justification and consider that the protocol used is acceptable.

4) Specification for fill volume is no less than 0.6 mL, with no upper limit specified. The lack of upper limit can result in lack of dose consistency. An IR was submitted to the sponsor and the sponsor responded that no upper limit for the vial volume specification is required since the Prescribing Information has been revised to include instructions to withdraw 0.6 mL of diluent for reconstitution. I consider that the response is acceptable.

### 3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

DP release methods are summarized above, sections 3.2.P.5.1 and 3.2.P.5.6. Stability test and specification are described in the table below.

Test	Reference method	Acceptance criteria
<b>Appearance of the freeze-dried product</b>	In-house test	White homogeneous freeze-dried product with possible retraction at the basis (ring-shaped cake possible)
<b>Appearance after dissolution</b>	(b) (4)	Colorless limpid solution with possible presence of white to translucent particles (of endogenous nature)
<b>Dissolution time</b>	In-house test	(b) (4)
(b) (4)	(b) (4)	(b) (4)
<b>Residual moisture</b>	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
<b>Bacterial and fungal sterility test</b>	(b) (4)	No bacterial and fungal growth
<b>Virus concentration (CCID<sub>50</sub>)</b>	(b) (4)	(b) (4) log <sub>10</sub> CCID <sub>50</sub> /dose for each serotype and (b) (4) log <sub>10</sub> CCID <sub>50</sub> /dose for each serotype

(b) (4)	(b) (4)	(b) (4)
Abnormal toxicity test†	(b) (4)	No sign of illness or death within 14 days after inoculation
Container closure integrity test	In-house test	Absence of leak

Compendial tests detailed in the European Pharmacopeia were validated in compliance with the requirements of the Ph. Eur. The virus concentration test (not described in the European Pharmacopeia) has been evaluated for specificity, linearity, accuracy and precision. All the acceptance criteria were satisfied.

**Overall Reviewer's Assessment of Sections 3.2.P.5.2 and 3.2.P.5.3:**

- The information provided is acceptable.
- The validations were adequately performed to assure that methods are suitable for their intended purpose.
- Deficiencies were identified and were resolved.

During validation experiments for the potency test, (b) (4)

(b) (4)

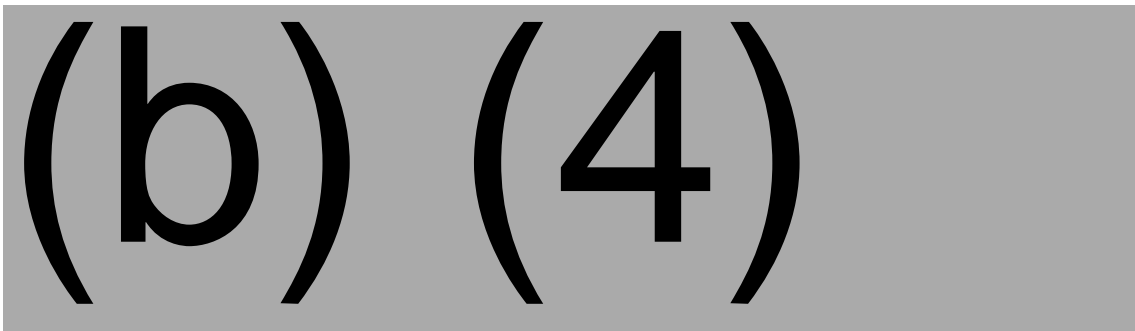
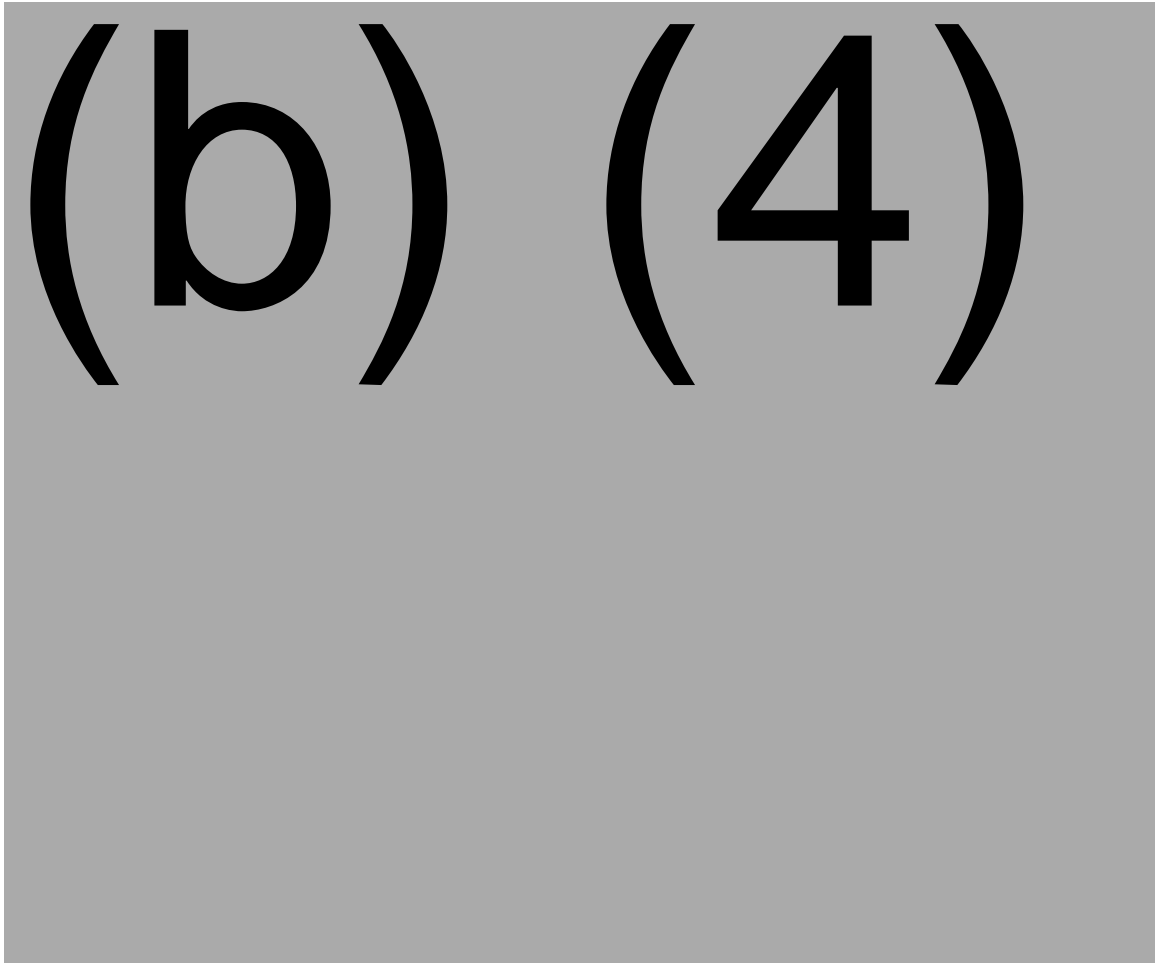
Same validity criterion is applied in the linearity and accuracy experiments (Table 19, page 25). These validity criteria are wider than the variability of the assay, and therefore are not justified. I communicated this concern to the sponsor during the pre-approval inspection and the sponsor agreed that the validity criterion interval is wide. However, the sponsor presented data (expected potencies and obtained potencies) from many drug product lots that were produced from different drug substance lots for each of the 4 CYD viruses. These data showed that the test performs appropriately when using samples harboring titers within the acceptance criteria. I discussed extensively these data with Dr. Phil Krause, deputy Director of OVR. We concluded that the data show that performance of the test is acceptable. Finally, in amendment 20, submitted on January 28, 2019, the sponsor stated that they will revise the validity criteria for future validation experiments.

**3.2.P.5.4 Batch Analyses**

(b) (4) batches of the CYD dengue FBP (batch numbers (b) (4) ) and (b) (4) batches of the CYD dengue freeze-dried product (batch numbers S4316, S4317 and S4318, used in the lot-to-lot consistency clinical trial CYD17) have been manufactured at the industrial scale. (b) (4) complementary industrial scale batches of CYD dengue FBP (batch numbers (b) (4) ) and freeze-dried product (batch numbers (b) (4) ) have been manufactured in order to validate the implementation of the (b) (4) sterile filtration process. All DP batches above mentioned have been manufactured using

DS batches issued from (b) (4) building ((b) (4) ). Additionally, (b) (4) batches of the CYD dengue FBP (batch numbers (b) (4) ) and three batches of the CYD dengue freeze-dried product (batch numbers (b) (4) ) have been manufactured at the industrial scale with DS batches issued from building (b) (4) . These batches were used for batch analysis.

Release Test Results of CYD Dengue Vaccine Freeze Dried Product Batches Manufactured with (b) (4) DS batches are shown in the table below.







(b) (4)

On 27 February 2019, we submitted an IR in relation to the exogenous particles found in DP batch (b) (4) (Section 3.2.P.5.4, page 13 of 18) requesting the investigation report conducted to examine the detection of exogenous particles.

In amendment 29 (submitted 14 March 2019), the sponsor responded to our IR and stated that investigations in the R&D laboratory and in manufacturing were conducted to identify the nature and origin of the filament (a deviation was opened on 6th June 2012 and closed on 26th October 2012). Particles and filaments were analyzed by (b) (4). All filaments were considered to be of exogenous nature and mostly composed of (b) (4). Neither elements nor compounds leading to potential safety issue have been identified during the investigation. Investigations have shown that these particles and filaments do not match with materials used during manufacturing operations. The most probable cause is the contamination of the vials in the R&D QC laboratory during the reconstitution of the freeze-dried product prior to vial visual inspection. It was concluded that there was no impact on the batch. A corrective action was opened to assess (b) (4) different conditions for the reconstitution of vials in the R&D laboratory. Since then operating conditions for the appearance test after dissolution have been optimized to avoid contamination during the reconstitution. Since 2015, for commercial batches, detection of particles is performed at two levels: a) through the release test for appearance after dissolution (test on (b) (4) vials) and b) particle contamination monitoring is systematically conducted for (b) (4) on extended sampling according to the (b) (4). From 2015 to Sep 2018, results are satisfactory: for all test runs for appearance after dissolution, including release and stability test runs, no exogenous particle has been observed; particle contamination monitoring ((b) (4) vials) results conform to acceptance criteria.

On 3.15.19, The following IR was issued to the sponsor:

Question #9: We note that the stabilizing solution (containing essential and non-essential amino acids, (b) (4), L-arginine hydrochloride, sucrose, D-trehalose dehydrate, D-sorbitol, trometamol, urea, and sodium chloride) added during the formulation of the FBP (FBP) does not undergo any testing prior to use and none of the stabilizing components are tested in the FBP (FBP). The following comments pertain to the stabilizing solution used for formulation of the FBP:

a. Please include a description of the manufacturing controls applied to the formulation of the stabilizing solution (such as those described in the briefing package submitted to IND 11219 on

May 1, 2019) in section 3.2.P.3.3 under the section 1.2.1 Preparation of the FBP Stabilizing Solution.

b. Please detail what quality control tests are done on the stabilizing solution. Please include this information in section 3.2.P.3.3 as specified above and in section 3.2.P.4.1 Specifications (for Excipients).

c. The stabilizing solution is stored at (b) (4); however, no information was provided regarding the shelf life of the solution and the container closure system used for storage.

i. Please provide the expiry date for this solution and stability data to support the proposed expiry.

ii. Please provide a description of the container closure system used for storage, as well as material of construction, name of manufacturer, and leachables and extractables information.

d. Please specify which components are considered stabilizing agents (i.e., having activity beyond buffering) and how these will be tested in the final product.

In amendment 39, submitted on 4.1.19, the sponsor provided responses.

Response 9a: the manufacturing controls in place ensure the correct identification and (b) (4) of each of the components of the stabilizing solution. The preparation of FBP stabilizing solution is supported by a validation study. The sponsor states that since all the preparation steps are monitored by the Manufacturing Execution System and the Process Control System, the analytical composition of the stabilizers is not verified on the FBP stabilizing solution nor on the DP. Section 3.2.P.3.3 Description of the Manufacturing Process and Process Controls was updated.

Response 9b: each individual component of stabilizing solution is tested upon receipt in accordance with the existing (b) (4). For the essential amino acid (b) (4) and the non-essential amino acid (b) (4), as there are no specific applicable Pharmacopoeia monographs, they are tested according to in-house specifications (see 3.2.P.4 Control of Excipients). Amino acids (b) (4) specifications encompass appropriate (b) (4) tests. There are no quality control tests performed on the stabilizing solution other than the one performed during manufacture of FBP (see above, 9a). Therefore, the sponsor considers that update of section 3.2.P.4.1 Listing of Excipients is not deemed necessary.

Response 9c-i: The proposed shelf-life for the stabilizing solution is (b) (4). A stability study has been done at (b) (4) on (b) (4) batches, (b) (4), to support the shelf-life. The results of this study are provided. The tests performed are (b) (4). The tests selected are appropriate and all results comply with the acceptance criteria.

Response 9c-ii: the stabilizing solution is stored at (b) (4)

(b) (4)

Response 9d: the main objective of the stabilizing solution formulation development was to achieve a formula which stabilizes the CYD dengue viruses during (b) (4)

. The stabilizing role of each component of the stabilizing solution is summarized in the following table.

**Stabilizing Role of Each Component of the Stabilizing Solution**

Component of the stabilizing solution	Stabilizing role
Essential amino acids	(b) (4)
Non-essential amino acids	
L-arginine hydrochloride	
Sucrose	
D-trehalose dihydrate	
D-sorbitol	
Trometamol	
Urea	

All stability data (on the freeze-dried product stored under long term storage conditions over 36 months and on the reconstituted product over (b) (4) at +5°C after dissolution) demonstrated that the stabilizing solution allows the product to have adequate stability behavior. Since all the preparation steps are monitored by the MES and the Process Control System to secure the

preparation and the correct composition of the stabilizing solution, the analytical composition of the stabilizers is not verified on the FBP stabilizing solution nor on the DP (see above, 9a).

Regarding the diluent, 3 validation batches ((b) (4)) of the Bulk Diluent were manufactured on the Buffer Preparation Suite of building (b) (4) and 3 validation batches ((b) (4)) were filled into 2 mL (b) (4) borosilicate glass vials with latex-free stoppers on Line (b) (4) located on Building (b) (4). Release data for the above lots are provided and comply with specification. It is concluded that all lots of the Diluent DP, 0.4% Sodium Chloride Diluent, Bulk and Final Container, were consistently manufactured to the pre-defined quality standards.

**Release Data for the Final Container Diluent DP, 0.4% Sodium Chloride Diluent**

Test	Test Method	Acceptance Criteria	Results		
			(b) (4)	(b) (4)	(b) (4)
Color	Q_0278663	Colorless	Pass	Pass	Pass
Odor		Odorless	Pass	Pass	Pass
Appearance		Clear solution, No Foreign matter	Pass	Pass	Pass
Identity - Sodium		Conforms	Conforms	Conforms	Conforms
Identity – Chloride		Conforms	Conforms	Conforms	Conforms
(b) (4)		(b) (4)	(b) (4)	(b) (4)	(b) (4)
Sodium Chloride		(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)		(b) (4)	(b) (4)	(b) (4)	(b) (4)
Vial Volume		NLT† 0.6 mL/vial	(b) (4)	(b) (4)	(b) (4)
(b) (4)		(b) (4)	Pass	Pass	Pass
(b) (4)		(b) (4)	(b) (4)	(b) (4)	(b) (4)
Particulate Matter in Injections	Q_0281467	(b) (4)	(b) (4)	(b) (4)	(b) (4)

		(b) (4)	(b) (4)	(b) (4)	(b) (4)
Sterility	Q_0277776	No Growth	No Growth	No Growth	No Growth
Bacterial Endotoxin Test (b) (4)	Q_0233845	(b) (4)	(b) (4)	(b) (4)	(b) (4)

Test	Test Method	Acceptance Criteria	Results		
			(b) (4)	(b) (4)	(b) (4)
Safety	Q_0236167	<ul style="list-style-type: none"> <li>- Animal survived test period</li> <li>- No nonspecific or unexpected responses observed</li> <li>- Final animals weights no less than starting weights</li> </ul>	Meets Requirements	Meets Requirements	Meets Requirements
Major A Defects	Q_0281004	Critical Defects (b) (4) Major Defects AQL **: (b) (4) Minor Defects AQL: (b) (4)	Meets Requirements	Meets Requirements	Meets Requirements
Major B Defects	Q_0277655	(b) (4)	0	0	0
		(b) (4)	0	0	0

\* NMT: Not More Than

† NLT: Not Less Than

(b) (4)

(b) (4)

\*\* AQL: Acceptable Quality Limit

### 3.2.P.5.5 Characterization of Impurities

Process-related impurities and DS-related impurities have been described in 3.2.S.3.2 Impurities. There is no additional impurity due to the manufacturing processes of the CYD dengue FBP and freeze-dried product. The manufacturing process of the CYD dengue FBP consists of the addition of the FBP stabilizing solution to the CYD dengue monovalent of each serotype. The

manufacturing process of the freeze-dried product consists in filling the FBP into vials and freeze-drying. These steps do not result in any additional impurities in the freeze-dried product.

There are no process related impurities for the Diluent DP.

**Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:**

- The information provided is acceptable. Impurities are adequately controlled.
- Deficiencies were identified and were resolved (see above, investigation on batch (b) (4), submitted in amendment 29).
- Of note, the current version of the package insert states under “Preparation and Administration” that “The suspension may develop trace amounts of white to translucent proteinaceous particles” and “Parenteral DPs should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Discard the vial if the solution is cloudy or contains extraneous particles other than trace amounts of white to translucent endogenous particles”. The SOP for the appearance after dissolution test (Q 0143955, submitted in amendment 24) defines criteria to differentiate between endogenous and exogenous particles, and it is expected that health care providers are also trained to differentiate between proteinaceous particles (typical of vaccine suspensions) and extraneous particles.

**3.2.P.6 Reference Standards or Materials.**

Please see above, section 3.2.S.5 Reference Standards or Materials. The only reference standard used to test CYD Dengue vaccine is a Reference Standard for Bacterial Endotoxin Content Assay. Which is an official reference standard ((b) (4) for the bacterial endotoxin content test).

Regarding the diluent, there are no reference standards or reference materials prepared internally and used for the analytical testing of the Diluent DP.

**3.2.P.7 Container Closure System**

The container closure systems consist of single-dose (b) (4) glass, 3 mL vial with a stopper (chlorobutyl rubber) and a cap (Aluminum and polypropylene). Test on the glass vial are (b) (4). Tests on stopper are (b) (4). Tests on cap are (b) (4). The CCS is suitable for protection from (b) (4).

There are no E&L studies submitted for the CCS. The results from stability studies (at storage conditions and accelerated conditions) and the safety profile of this vaccine (Dengvaxia is

currently registered in 20 regulatory authorities across the world, and approximately (b) (4) doses were distributed since its approval) ensures product quality and safety.

DP during shipping: the shipment of the Filled Product from Sanofi Pasteur (b) (4) site ((b) (4)) to Sanofi Pasteur Swiftwater site (US) is performed at controlled temperature. During the entire transportation duration, the shipment conditions are in compliance with the storage conditions of the product. The shipment is qualified between manufacturing sites. The acceptance criteria set to ensure the shipment conformity are sealing of the truck or the transport containers and registered temperature should be in compliance with the storage conditions of product. The temperature is recorded with a temperature recorder. At (b) (4) site, the unlabeled vial containing the freeze-dried product is packaged and loaded into active container with a set temperature at (b) (4). In order to ensure the quality of the CYD Dengue DP during the shipment from (b) (4) to Swiftwater by (b) (4), qualifications were carried out for each type of transportation route ((b) (4)) and showed that equipment and conditions are qualified for routine transportation of the CYD Dengue DP from (b) (4) to Swiftwater.

**Overall Reviewer's Assessment of Section 3.2.P.7:**

- The information provided is acceptable.

### **3.2.P.8 Stability**

#### **3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data**

The proposed shelf-life and storage conditions are 36 months at 4°C. Stability studies were performed to assess the stability of DP formulated with DSs manufactured at (b) (4) site to support the shelf-life of 36 months at +5°C. Stability studies were also initiated on the DP formulated with DSs manufactured at (b) (4) site in the frame of the manufacturing process transfer of CYD dengue DS from (b) (4) site (Building (b) (4)) to (b) (4) site (Building (b) (4)). This stability study was performed on (b) (4) industrial batches to confirm the shelf-life of 36 months at +5°C, and to assess the stability of the vaccine under accelerated storage conditions at (b) (4) over a period of (b) (4) and at (b) (4) over a period of (b) (4) to support possible cold chain break. In addition, the stability study on the reconstituted product is performed for each batch of CYD dengue vaccine over a period of (b) (4) under normal storage conditions (+5°C). For the long-term storage condition study, parameters monitored are Appearance of the freeze-dried product, Appearance after dissolution, Dissolution time, (b) (4), Residual moisture, (b) (4), Bacterial and fungal sterility test, Virus concentration (CCID50), (b) (4), Abnormal toxicity test, and Container closure integrity test. The tests selected to monitor the accelerated stability studies are (b) (4).

Stability study results at +5°C. All results comply with the acceptance criteria over 36 months. No trends are observed. Importantly, no statistically significant decline in potency (virus concentration test) is observed during the duration of storage at +5°C for 36 months. However, almost all potencies at 36 months are lower than at release. The CBER statistician indicated that

the lack of statistically significant decline in potency could be due to the low number of samples analyzed ((b) (4) lots).

The stability study on reconstituted product is completed at +5°C. Samples from T0 and T36 months were tested after reconstitution at 0, ((b) (4)). All the results comply with the specifications over ((b) (4)) after dissolution. However, almost all titers at ((b) (4)) are lower than at release. Dr. Lei Huang, CBER statistician, and Dr. Phil Krause estimated a potency loss of approximately ((b) (4)) after reconstitution. The statistician pointed out the limitation of this estimation, given that the sample size is very small (only ((b) (4)) lots) and only ((b) (4)) time points were tested. However, based on this limited data the applicant agreed to revise the package insert to shorten the hold time between vaccine reconstitution and administration to a maximum of 30 minutes.

Stability study results under Accelerated Storage Conditions ((b) (4))



Stability study results under Accelerated Storage Conditions ((b) (4))



Of note, for residual moisture, in order to improve the robustness of the residual moisture analytical method as regards the sample preparation step, an optimization of the ((b) (4)) method was developed during the course of the stability study. The principle of the analysis remains unchanged ((b) (4)) and the acceptance criterion was not changed.

### 3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

The sponsor commits to place at least one industrial batch of CYD dengue vaccine into the ((b) (4)) stability program ((b) (4)) throughout the shelf-life of the product. The following tests are removed from the ((b) (4)) stability program: ((b) (4)). ((b) (4)) is used to verify the conformity of the ((b) (4)) manufacturing step and as such it is not a stability indicating test. No trend was observed on ((b) (4)) results on the CYD dengue demonstration batches manufactured in the same condition as the consistency batches. ((b) (4)), as



clarified by the (b) (4), should be considered as a vaccine characteristic that provides an indicator of consistency of production in the context of lot release.

**Overall Reviewer's Assessment of Section 3.2.P.8:**

I agree with the proposed shelf-life and storage conditions. The stability data show that all parameters analyzed met specifications when the product is stored at 4°C degrees for 3 years. Regarding potency of the product, a statistical analysis taking into account potency decay and assay variability, concluded that product released at a potency of (b) (4) log10 CCID50/dose/serotype will ensure with 95% confidence that any lot stored for up to 3 years at 4°C degrees will harbor a potency of 4.5 log10 CCID50/dose/serotype. This potency is considered by the clinicians to be efficacious and comparable to the potencies harbored by the lots used in the pivotal clinical trials showing efficacy.

### **3.2.A APPENDICES**

#### **3.2.A.1 Facilities and Equipment**

I did not review this section.

#### **3.2.A.2 Adventitious Agents Safety Evaluation**

See section 3.2.S.2.3 above for assessment of materials of biological origin in DS. No excipients from human or animal origin are used for the formulation of the DP. Regarding animal origin raw materials, the manufacturing process is (b) (4). Only pre-master stages of the CYD dengue vaccine have been manufactured using raw materials of (b) (4) origin. No raw material of human origin has been used in any manufacturing stage.

The safety of the vaccine with regard to viral and non-viral contamination has been assessed through three different approaches: a) Selecting and testing cell lines, seed lots and raw materials of biological origin for the absence of adventitious agents, b) use of appropriate environmental manufacturing conditions and application of good manufacturing practices throughout the production process, and c) testing the product at appropriate stages of the production process for the absence of adventitious agents.

#### **Viral Clearance Studies**

There is no dedicated viral inactivation or clearance step in the manufacturing process as the vaccine is a live vaccine.

**Overall Reviewer's Assessment of Section 3.2.A.2:**

☐ The information provided is acceptable

### 3.2.A.3 Novel Excipients

No novel excipients are used for the formulation of the vaccine

### 3.2.R Regional Information (USA)

#### ❑ Executed Batch Records

The executed batch records were reviewed during the pre-approval inspection.. In addition, SOPs and cGMP reviewed during the inspection were found to be acceptable.

#### ❑ Comparability Protocols

See above, section 3.2.S.5, Reference Standards or Materials.

## Other eCTD Modules

### **Module 1**

#### **A. Environmental Assessment or Claim of Categorical Exclusion**

The sponsor request categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR § 25.31(a). To the applicant's knowledge, no extraordinary circumstances exist that would warrant the preparation of an environmental assessment. The sponsor request is acceptable.

#### **B. Labeling Review**

##### **Full Prescribing Information (PI):**

##### **Dosage Forms and Strengths:**

DENG VAXIA is a sterile suspension for subcutaneous injection (supplied as a lyophilized powder to be reconstituted with the supplied diluent, 0.4% NaCl). A single dose, after reconstitution, is 0.5 mL.

##### **Description:**

DENG VAXIA is supplied as a vial of lyophilized vaccine antigen, which must be reconstituted at the time of use with 0.6 mL of the accompanying vial of diluent. After reconstitution, DENG VAXIA is a clear, colorless suspension (trace amounts of white to translucent proteinaceous particles may be present). After reconstitution, each 0.5 mL dose of DENG VAXIA contains 4.5 - 6.0 log<sub>10</sub> CCID<sub>50</sub> of each of chimeric yellow fever dengue (CYD) virus serotypes 1, 2, 3, and 4. Each 0.5 mL dose is formulated to contain 2 mg sodium chloride and the following ingredients as stabilizers: 0.56 mg essential amino acids (including L-phenylalanine), 0.2 mg non-essential amino acids, 2.5 mg L-arginine hydrochloride, 18.75 mg sucrose, 13.75 mg D-trehalose dihydrate, 9.38 mg D-sorbitol, 0.18 mg trometamol, and 0.63 mg urea. Each of the four CYD viruses was constructed using recombinant DNA technology by replacing the sequences encoding the pre-membrane (prM) and envelope (E) proteins in the yellow fever (YF) 17D204 vaccine virus genome with those encoding for the homologous

sequences of dengue virus serotypes 1, 2, 3, and 4, respectively. Each CYD virus is cultured separately in African Green Monkey kidney (Vero) cells under serum-free conditions, harvested from the supernatant of the Vero cells and purified by membrane chromatography and ultrafiltration. The purified bulk of each CYD virus is then diluted in a stabilizer solution. The FBP is sterilized by filtration at 0.22 µm, filled in vials and freeze-dried. DENGIVAXIA does not contain preservative. The vial stoppers for the Lyophilized Vaccine Antigen and Diluent vials of DENGIVAXIA are not made with natural rubber latex.

### **Clinical Pharmacology:**

Mechanism of Action: Following administration, DENGIVAXIA elicits dengue-specific immune responses against the four dengue virus serotypes. The exact mechanism of protection has not been determined.

Pharmacokinetics: vaccine viremia (measured by genomic amplification methods and virus culture) was observed following vaccination with DENGIVAXIA in 5.6% of subjects, with 90% of these occurrences documented after the first injection. Vaccine viremia was observed 7 to 14 days after vaccination. When observed, vaccine viremia persisted for less than 7 days).

### **How Supplied:**

The vial stoppers for the Lyophilized Vaccine Antigen and the Saline Diluent vials of DENGIVAXIA are not made with natural rubber latex. An outer package of 1 dose (NDC 49281-605-01) contains 1 single dose vial of Lyophilized Vaccine Antigen (NDC 49281-606-58) and 1 single dose vial of Saline Diluent (NDC 49281-546-68).

### **Storage and Handling:**

Store Lyophilized Vaccine Antigen and Saline Diluent in a refrigerator at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect from light. Do not use after the expiration date shown on the vial labels of the Lyophilized Vaccine Antigen and Saline Diluent. After reconstitution, administer DENGIVAXIA immediately or store refrigerated at 2°C to 8°C (36°F to 46°F) and use within 30 minutes. Discard reconstituted vaccine if not used within 30 minutes.

The Full Prescribing Information is acceptable.

### **Carton and Container Label:**

The CMC information provided on primary and secondary container labels is acceptable.

## **Modules 4 and 5**

Immunological and virological assays were used to assess CYD dengue vaccine performance. Assays that have been used in support of primary endpoints and/or have been used consistently throughout clinical development (“core assays”), are the following:

- Dengue PRNT50 (to evaluate vaccine immunogenicity)
- YF qRT-PCR and CYD 1-4 qRT-PCR (to evaluate vaccine viremia)
- DS qRT-PCR, Dengue WT 1-4 qRT-PCR, Simplexa Dengue RT-PCR, and NS1 ELISA (to evaluate dengue wild type (WT) infection).

In addition to the core assays, additional assays were used in the assessment of CYD dengue vaccine performance (immunogenicity, viremia, and dengue WT infection). Supportive assays were either used in a limited number of studies in early clinical development or did not support primary endpoints. Additionally, assays were utilized to detect cellular immunity and measure the immunogenicity of the concomitantly delivered vaccines, and to determine subjects' flavivirus serostatus at enrollment in clinical trials; these assays are also classified as supportive. Assays to measure the immunogenicity of the concomitantly delivered vaccines were also used; these assays were often validated within their respective vaccine program.

### **Dengue PRNT50**

**Principle:** The PRNT for dengue viruses can be used to determine the neutralizing antibody levels following the administration of a dengue vaccine. The infectivity of dengue challenge virus in the assay is reduced as a direct result of the presence of antibody capable of neutralizing infectious virus particles present in the serum. The presence of dengue virus infected cells is indicated by formation of foci. The reported value represents the highest dilution of serum at which  $\geq 50\%$  of dengue challenge virus is neutralized when compared to the mean viral foci count in virus control wells. The dengue challenge viruses used within the assay are the CYD parental strains (dengue serotype 1: (b) (4); dengue serotype 2: (b) (4); dengue serotype 2: (b) (4); dengue serotype 4: (b) (4)).

### **Validation results:**

(b) (4)

(b) (4)

(b) (4)

#### YF qRT-PCR and CYD 1-4 qRT-PCR

The YF qRT-PCR was applied as a screening assay for vaccine viremia. Samples displaying a positive YF qRT-PCR result were subsequently tested in the CYD 1-4 qRT-PCR assay to detect and distinguish between CYD dengue vaccine virus types CYD-1, CYD-2, CYD-3, and CYD-4 and to determine the approximate viral titer present in the sample.

**Principle:** Quantitation of the concentration of the selected templates is achieved using a series of reference standards in the same RT-PCR run where the equipment software calculates the concentration of the unknown sample relative to a linear regression analysis of the reference standards' performance. qRT-PCR methods are highly specific and sensitive, allowing quantification of the nucleic acid in samples when compared to standards processed in the same manner.

#### Validation results YF qRT-PCR

Validation Parameter	Testing Method	Acceptance Criteria	Results	Status
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(b) (4)

**Validation results CYD 1-4 qRT-PCR**

(b) (4)





(b) (4)

**Assays used to Detect Dengue WT Infection: dengue screening qRT-PCR (DS qRT-PCR), Simplexa dengue RT-PCR, WT 1-4 qRT-PCR and NS1 ELISA.**

#### **DS qRT-PCR**

The DS qRT-PCR was applied to acute samples from clinically suspected dengue cases. The DS qRT-PCR targets a specific sequence within the 3' untranslated region (UTR) that is conserved among the four dengue serotypes and can detect all four serotypes of dengue. Positive samples were subsequently tested in the Simplexa dengue RT-PCR or the Den WT 1-4 qRT-PCR assays which provided additional information on WT dengue viremia by confirming the presence of dengue and identifying and quantifying serotypes (if applicable). Both assays use primers that are specific for each of the four dengue serotypes. The Simplexa dengue RT-PCR assay was introduced following observations during the phase II CYD13 clinical study that the Den WT 1-4 qRT-PCR was unable to detect serotype DEN-2 from Latin America.



(b) (4)

**Simplexa Dengue RT-PCR validation results:**

(b) (4)

(b) (4)

**Dengue WT 1-4 qRT-PCR Validation results:**



(b) (4)

#### **NS1 ELISA.**

**Principle:** The dengue NS1 antigen test is a (b) (4) -ELISA based assay that enables detection of NS1 antigen in serum. The dengue NS1 protein is a conserved glycoprotein that is secreted from dengue infected cells in-vitro and is detected within the serum of infected patients. The test uses (b) (4) for capture and revelation. Samples and controls are (b) (4)

#### **Validation and performance assessment results:**

A summary of the validation performed for the kit is provided with the kit product insert. These included assessment of sensitivity, specificity, reproducibility, and cross reactivity. Additional assay performance assessments for sensitivity, specificity and site agreement were performed. The results are summarized below.

(b) (4)

(b) (4)

### Dengue NS1 IgG ELISA

**Background:** A limitation of the interpretation of vaccine efficacy and safety by Dengue serostatus in the Dengue efficacy studies CYD23, CYD14 and CYD15 is that baseline blood samples prior to first vaccination were only obtained in an immunosubset representing approximately 7.5-20% of study subjects. However, as blood samples were collected for all study participants approximately 1 month after the third injection of CYD Dengue vaccine or placebo (month 13, approximately), classification of Dengue serostatus (as a surrogate of prior natural Dengue exposure) of study participants at this timepoint could be used as a baseline for the evaluation of clinical outcomes that occur later. A major challenge for this approach is that seropositivity to Dengue by PRNT can be the result of either prior Dengue exposure or CYD Dengue vaccination. To overcome this challenge, the sponsor has leveraged an assay originally developed by (b) (4) that detects IgG antibodies against the NS1 protein of the Dengue virus by ELISA. The NS1 protein is not highly conserved between Dengue and Yellow Fever virus thus, previous exposure to CYD Dengue vaccine is not expected to induce significant levels of antibody against the Dengue NS1 protein. The sponsor qualified the assay. In addition, samples from clinical studies CYD14, CYD15, CYD47 and CYD51 and from Sanofi Pasteur employee panels were used from subjects with varying flavivirus backgrounds (Dengue +/-, Yellow Fever (YF) +/-, Japanese Encephalitis (JE) +/-), exposed or unexposed to the CYD Dengue vaccine, as well as from subjects who had a virologically confirmed Dengue infection (Document number RED\_00092073, "Assessment of Dengue NS1 IgG ELISA for Dengue Serostatus Classification").

**Principle:** The Dengue NS1 IgG ELISA method is used to quantitate IgG antibodies in human serum against the Non-Structural Protein 1 (NS1) of Dengue virus (Serotypes 1, 2, 3 and 4). The method does not discriminate between serotypes. (b) (4)





(b) (4)

**Overall Reviewer's Assessment of Relevant Sections of Module 4 and 5:**

- ❑ The methods used are suitable for their intended purpose. The parameters and validity criteria selected for the validation studies are adequate. Validation results assure that methods are suitable for their intended purpose. The methods were transferred to different labs and respective validations were conducted and found to be acceptable.
- ❑ Deficiencies were identified in the NS1 IgG ELISA. These deficiencies were extensively discussed with the sponsor during the pre-approval inspection conducted in December 2018. These deficiencies and their resolution are explained in the EIR for the (b) (4) site from Sanofi Pasteur (the EIR can be located in eNSpect). Of note, this assay cross-reacts with anti-ZIKA antibodies, and therefore is qualified and suitable for its intended use for quantifying concentration of IgG antibodies in human serum samples against Dengue NS1 in the absence of antibodies to Zika NS1. Importantly, this assay was used to test for anti-dengue-NS1 antibodies on samples that were collected prior to the current Zika epidemic. In addition, the sponsor tested a subset of those samples (from subjects that received placebo or Dengue vaccine) and found a (b) (4) rate of confirmed Zika samples.

### Pharmacology Studies (section 4.2.1)

The preclinical evaluation of the vaccine has been conducted in (b) (4) monkeys ((b) (4)). While monkeys do not develop symptomatic dengue disease upon dengue virus infection, they do present viremia and develop immunity. Experiments were designed comparing different lots, formulations and immunization regimens. These studies include:

- Cn0901, cn1101, cn1102, and cn1201 (Neutralization of a Panel of Field Isolates by a Pool of Sera from monkeys vaccinated with a Tetravalent Dengue Vaccine)
- den010mk, den011mk, den012mk, den014mk, den016mk, f-im-den010-mk, f-im-den011-mk, f-im-den012-mk, f-im-den014-mk, f-im-den016-mk, and f-im-den020-mk (Evaluation of immunogenicity and viremia in monkeys)
- red00068080 and red00068081 (Genetic stability of CYD Dengue vaccine serotype 4 in human sera after vaccination with CYD Dengue vaccine)
- sbi-1313-88 (A 31-Day Comparative Immunogenicity Study of Six DEN Vaccine Preparations and YF-Vax Administered by a Single Subcutaneous Injection to (b) (4) Monkeys)

The studies were designed to address the following:

- Induction of neutralizing responses against all 4 serotypes (by measuring neutralizing antibodies after each immunization) and evaluation of attenuation and safety (by measuring viremia during the first 10 to 14 days after immunization)
- Protection against wild type dengue infection (by measurement of wild type dengue viremia)
- Assessment of the breadth of neutralization (by measuring neutralizing antibodies against a large panel of recently circulating and representative wild type dengue isolates)
- Evaluation of interferences between dengue vaccine serotypes (by assessing different vaccine schedules).
- Assessment of potential sensitization (by measurement of viremia in case of pre-existing heterologous flavivirus immunity).

Results:

- One or more vaccinations conferred immunity against the four dengue serotypes, with CYD-4 responses being dominant after primary immunization. The immune response induced by the CYD dengue viruses was mostly directed against the envelope protein. Primary immunization with CYD Tetravalent Dengue Vaccine induced short-lived (7 days), low-level viremia (between 3.5 and 5 log<sub>10</sub> GEQ/mL), which was not detectable after subsequent immunizations. The predominant CYD serotype inducing measurable and reproducible viremia after tetravalent CYD immunization was CYD-4. CYD-1, CYD-2, and CYD-3 occasionally induced some viremia, but (b) (4).

- CYD dengue vaccine induced protection after a single dose against a wild type dengue challenge (against each of the four wild type dengue serotypes) performed 6 months after immunization.
- Using a (b) (4) assay, it was shown that CYD dengue vaccine induced neutralizing antibodies against a variety of dengue strains involving all dengue serotypes, and different genotypes, geographical origins and isolation years (82 wild type isolated from 30 countries).
- Different vaccine regimens can mitigate interferences between serotypes and achieve balanced immunity, as follows: (i) simultaneous administration of (b) (4) complementary (b) (4) vaccines at separate anatomical sites; (ii) sequential administration several weeks apart of two complementary (b) (4) vaccines; (iii) pre-immunization against another flavivirus; (iv) reformulation of the tetravalent vaccine with a (b) (4) dosage of the immunodominant virus (CYD-4). Of importance, a third dose administered 1 year after primary immunization and 10 months after the second dose induced a significant response against all 4 serotypes.
- Heterologous flavivirus pre-immunity enhanced CYD dengue vaccine-induced immunity. In particular, the positive effect of YF 17D priming concurs with previous observations in monkeys (and in humans). Heterologous dengue pre-immunity did not sensitize to higher viremia caused by CYD dengue viruses or wild type dengue (as assessed by lack of increased viremia).

Genetic stability *in vivo* was also assessed and indicated that the vaccine virus is stable in monkey sera. The few mutations detected were demonstrated not to modify the attenuation of the viruses in a suckling mouse neurovirulence model.

#### **Biodistribution Study (section 4.2.3.7.7 of the BLA)**


Biodistribution Study of CYD Dengue Vaccine Following One Subcutaneous Administration in (b) (4) Monkey. CIT/Study No. 36762 GEP/CYD Dengue Vaccine/Sanofi Pasteur  
Sponsor Reference Number: SP0056 BD1001

The objective of this study was to evaluate the distribution, as well as the shedding of CYD Dengue Vaccine after a single subcutaneous (SC) administration in 21 males and 21 females seronegative to flavivirus (b) (4) monkeys. Two groups of (b) (4) monkeys were given a single SC injection on day 1 of either the saline control item (6 animals/sex) or the test item CYD Dengue Vaccine (15 animals/sex) at the dose of 5 log<sub>10</sub> CCID<sub>50</sub> per serotype in 0.5 mL. The results showed that one subcutaneous injection of the human dose of CYD Dengue Vaccine to flavivirus-seronegative (b) (4) monkeys was well tolerated during the 21-day observation period. Treatment-related changes were limited to occasional transient and minimal erythema reaction at the injection site, which correlated with minimal to slight inflammatory reaction. All treated monkeys seroconverted against at least one serotype and the level of viral load was low and of short duration in occasional animals. There was no shedding of CYD Dengue Vaccine RNA in body fluids (feces, urine, and saliva). Three or nine days after injection, the distribution data showed CYD Dengue Vaccine RNA of each serotype was initially detected

in the injection site and the draining lymph nodes of almost all animals and of serotype 3 or 4 in the liver of some animals. Nine days after injection, the viral RNA, mainly of serotype 4, distributed also in lymphoid tissues such as distant lymph nodes, spleen, thymus and in adrenals, bone marrow and skeletal muscle in occasional animals. After the 21-day observation period, distribution data showed evidence of clearance with persistence limited to very low level in the injection site and draining lymph node samples in a few animals only.

**Neurovirulence Study (section 4.2.3.7.7 of the BLA)**

(b) (4)



**UNII assignment:** I concur with the list of ingredients for DENG VAXIA as identified by the Substance Registration System (SRS) team.