

CMC Review - Ixiaro

MEMORANDUM

Date: October 27, 2008 and final revised on March 24, 2009
Li Yu, M.D., Ph.D.

From: Regulatory Review Officer
OVRP/DVP/LVVD (HFM-451)
Lewis Markoff, Review Committee Chair, STN 125280
Richard Daemer, Regulatory Coordinator, OVRP/DVRPA/VVB

To: Jerry Weir, Director of Division of Virus Products/OVRP
Robin Levis, Acting Deputy Director of Virus Products/OVRP
Office of Vaccine Research and Review (OVRP)

Through: Lewis Markoff, M.D.
Chief, LVVD/DVP/OVRP
CMC review of BLA 125280/00
STN 125280/0 Section 2.2, 2.3, 2.4, 3.2S, 3.2P, 3.2A, 3.2R, and 4.2.1.1
STN 125280/0.2 (amendment)
STN 125280/0.4 (amendment)
STN 125280/0.6 (amendment)

Subject: STN 125280/0.7 (amendment)
STN 125280/0.12 (amendment)
STN 125280/0.13 (amendment)
STN 125280/0.16 (amendment)
STN 125280/0.20 (amendment)
STN 125280/0.28 (amendment)

Sponsor: Intercell AG

Product: Inactivated Japanese encephalitis virus vaccine, IXIARO

RECOMMENDED ACTION: The data submitted to support the chemistry, manufacturing and control of this product were reviewed and found to support the quality and proposed shelf life of this product. Based on this review, I recommend approval of this product.

Review Summary and Conclusion: All drug substance and drug product data in support of biological activity (immunogenicity), purity, stability, potency, and sterility were reviewed. The CMC information provided in this submission suit the requirements of the "Guidance for Industry for Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product". Process procedures (including virus propagation, purification, inactivation, formulation, micro filtration, and filling), in-process controls and validations, manufacturing consistency, and control of bioburden meet the acceptance criterion.

The sponsor shows data ensuring that virus master seeds and virus working seeds used in the production of the vaccine are sterile and free of extraneous agents. (Cell substrates used to manufacture the virus seeds and those used to manufacture the product have been reviewed by Dr. Barry Falgout.) The sponsor also presents results showing that the -----(b)(4)----- throughout the process is consistently higher than -----(b)(4)----- content in commercial bulks is -----(b)(4)----- . The vaccine manufacturing process is robust, and the titers achieved are highly consistent. The sponsor performs in-process testing at different stages of production to ensure that the product meets specifications and is consistent. Testing for Lot Release of final containers (inactivated JE virus) includes: appearance, -----(b)(4)----- , identity, sterility, potency, aluminum, -----(b)(4)----- , and pyrogenicity . The sponsor performed (and is performing) studies demonstrating the stability of the inactivated vaccine for 18 months at the recommended storage condition of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

There were some minor issues and critical deficiencies identified during the review of the original BLA and during the on-site prelicensing inspection. Each of these was communicated with the sponsor. During the BLA review process, the sponsor submitted Amendments in which they provided data and modified several acceptance criteria in response to FDA 483 letter and to CR letter. These include:

- The introduction and validation of an -(b)(4)- for in-process and for identity testing in lot release.
- Adjusting the specification of the -----(b)(4)----- from -----(b)(4)-----.
- Defining hold time limits of each process step in manufacturing, and thus the maximum duration from -----(b)(4)-----.
- Introducing new assays and acceptance criteria for process impurities and indicating that the remaining concentration of full length Protamine Sulfate in drug substance is below the LOD (limit of detection) of -----(b)(4)----- -- and of -----(b)(4)-----.
- Using -----(b)(4)----- assay to replace -----(b)(4)----- assay for drug substance and for drug product in lot release.
- Revising operator training module to better training operators for critical process steps (preparation of sucrose gradient and fraction collection) to improve the consistency of the process; correcting the deficiency of cleaning validation of -----(b)(4)-----.

Since the sponsor has corrected those critical deficiencies, as shown in Amendments and in response to 483 Letter, there were no critical CMC issues that might impact for approval of the BLA.

Review - CHEMISTRY, MANUFACTURING, AND CONTROLS

I have reviewed CMC section of the submission and summarized as following. The review summary is organized as following content list:

1. Drug Product Profile
2. Characterization of the JE Virus SA14-14-2

3. Manufacturing
4. Manufacturing Consistency
5. Process Control
6. Specification
7. Validation
8. Containers and Closure System
9. Stability

The review of Vero cell seed, clinical data, and manufacturer facility is not included in this summary.

1. Drug product profile

The Intercell USA, Inc. (NC, USA) submitted the Biologics License Application (BLA) for its product JE-IC51 (JE-PIV or IXIARO as indicated on labels) on December 18, 2007. The active ingredient of the product is inactivated Japanese encephalitis virus (JE); strain SA14-14-2, formulated with aluminum hydroxide. The purified inactivated JE virus is a neurovirulence-attenuated and mutant virus derived from wild type JEV SA14 strain.

The JEV SA14-14-2 virus is produced on Vero cells, purified with sucrose gradient -----(b)(4)----, inactivated with -(b)(4)- formalin, and formulated with aluminum hydroxide -(b)(4)- in PBS. The final vaccine product contains ~ 6 µg of inactivated JEV antigen in 0.5 ml, supplied as single dose in a pre-filled syringe. The composition of JE- PIV per dose is shown in following Table.

Manufacturing formula for Final Vaccine Lot (pre-filled syringes of JE-PIV)

Component	Quantity per dose (0.5-ml)
Active Substance: JE-PIV antigen	6 µg -(b)(4)- /0.5 ml (protein concentration)
Excipients: Aluminum Hydroxide, ---- (b)(4)---- mg/ml Al) Phosphate Buffered Saline (PBS) --- (b)(4)---- (PO ₄)	-(b)(4)- (vol/vol) to 0.57 ml (extractable volume)

The vaccine is stored at 2-8°C. The biological activity of the drug product is characterized by its immunological properties (plaque reduction neutralization test- PRNT and clinical studies). JE-PIV is to be injected intramuscularly twice at a four week interval to achieve optimal protection against JEV, and is indicated for active immunization for persons aged 18 years and older.

2. Characterization of The JE virus SA14-14-2

The attenuated strain of JEV strain SA14-14-2 (Gene Bank # --(b)(4)--) was derived from its pathogenic parent SA14 strain, which was originally isolated from a pool of *Culex pipiens* larvae following 11 passages in mouse brain. Attenuation of JEV SA14-14-2 was performed by a series of passages in primary hamster kidney cells, mice, hamster, and suckling mice with plaque purification of clones between passages. The attenuated strain was then adapted first to primary canine kidney cells (PDK) and then to Vero cells. The details of passages that gave rise to the attenuated strain SA14-14-2 are outlined in Figure 2.3.S-3 in this submission.

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

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----- (b)(4) -----

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----- (b)(4) -----

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3. Manufacturing

3. 1. Manufacturer

Intercell Biomedical Limited (Livingston, Scotland) is responsible for drug substance and final bulk vaccine manufacture and release as well as final vaccine lot release. The inactivated virus drug substance and the aseptic formulation of drug substance with -(b)(4)- Aluminum Hydroxide to produce JE-PIV final bulk vaccine take place at Intercell plant in Scotland:

Intercell Biomedical Limited
Oakbank Park Road , Livingston
EH53 OTG, Scotland, UK.

While aseptic filling of the final vaccine lot and labeling, packaging and storage of the pre-filled syringes for commercial market take place at:

------(b)(4)-----

-(b)(4)- has been in the market as an independent specialist in the aseptic production of prefilled application systems. -(b)(4)- commercial manufacturing handles the entire process from compounding and aseptic filling to the final packaging of a product. Intercell provided the information of GMP compliance certificate for -(b)(4)- manufacturer.

Quality Control testing is either performed on the Intercell site or contracted out. The list of contract laboratories is provided by the applicant in Section 3.2.P.3.1. See the Establishment Inspection Report for the review of Intercell manufacturing facility and site inspection. These are not summarized here.

Comments: Whether there are additional drug substances or drug products that are produced in the same area or utilized for the same facility (equipments) is not described in the original BLA. The sponsor indicated, during the site inspection and in Amendment 125280/0.2, that no other drugs were produced in this facility during manufacturing the JE-PIV vaccine .

3. 2. Materials and Chemicals Used in Manufacturing

Raw materials and reagents used in manufacturing are documented and summarized below. Appropriate specifications have been established for all raw materials and reagents. Reagents used during vaccine production comply with USP/NF and/or Ph. Eur. PBS , considered as an excipient of the drug product for which there is no reference, complies with in-house specifications (see section 2.3.P.4, Table 2.3.P-16).

3. 2. A. Vero cells

------(b)(4)-----

-----.

3. 2. B. Virus source

A development history of JEV SA14-14-2 in P. R. China was described in section 2.3.S-3 and 3.2.S.2.3.1.2 in this submission. SA14-14-2 was adapted first to primary canine kidney (PDK) cells and then to Vero cells ------(b)(4)-----
------. Manufacturing of both MVSB and WVSb has been described in detail in Section 3.2.S.2.3 and summarized below. Comparing the nucleotides (NT) of envelop (E) gene revealed that five NT and four amino acids of the E were different between SA14-14-2 (PDK) and SA14-14-2 (Vero) viruses (reference, Hong et al., 2001).

----- (b)(4) -----

**20 pages determined to be not releasable:
(b)(4)**

5. Process Control

5. 1. In-Process Controls

In-process tests monitor the performance of the main manufacturing steps. All in-process acceptance criteria were selected based on previous process optimization and qualification. The acceptance criteria of the in-process tests are included in following tables.

----- (b)(4) -----

[--(b)(4)--]

**Four (4) pages determined to be non
releasable: (b)(4)**

----- (b)(4) -----

5. 2. Quality of the Final Bulk Vaccine

The quality of the final bulk vaccine is monitored by following parameters: appearance, -(b)(4)-, sterility, ----- (b)(4) -----.

Controls performed on the final bulk vaccine

Test	Method	Acceptance criteria
------	--------	---------------------

--(b)(4)-----	--(b)(4)-----	--(b)(4)----- -- ----- ----- -----
-(b)(4)-	--(b)(4)----- ----- -----	-(b)(4)-
Sterility	--(b)(4)----- --	Sterile
--(b)(4)----- --	--(b)(4)----- ----- -----	--(b)(4)----- -----

Controls performed during filling of final vaccine lot

Test	Method	Acceptance criteria	Method suitability/ Justification of Specification
--(b)(4)----- --	--(b)(4)----- --	--(b)(4)----- -- -----	--(b)(4)----- - ----- ----- -----

**One (1) page determined to be not
releaseable: (b)(4)**

[--(b)(4)--]

5. 3. Quality Control of Materials

The analysis information and certificates for quality control of raw materials and reagents have been provided by the sponsor and/or by vendors (See section 3.2.S.2.3 appendix11). They comply both with USP/NF and with Ph. Eur. requirements. The sponsor also described the methods that were used for testing the materials such as -----(b)(4)-----
-----.

5. 4. Microbial Controls

Summaries of microbial controls are listed in following tables:

Microbial controls performed on biological materials

[-- (b)(4)--]

Microbial controls performed on starting materials

[--(b)(4)--]

Microbial tests performed during the process

[--(b)(4)--]

5. 5. Viral Adventitious Agents

With respect to viral contamination, controls are performed during manufacturing process of the drug substance. They are summarized in following table:

Virological tests performed during the process

[--(b)(4)--]

5. 6. Quality Control-Filters

Quality control of filters required for manufacture of the drug substance is summarized in following table.

Quality control- membrane and filters

[--(b)(4)--]

5. 7. Release Tests

Acceptance criteria, testing results, and characterization data for batch release was submitted (see Table 4.4. “Batch analysis results” on page 24).

Release tests for drug substance and for drug product are listed in the table as shown in following “6. 1. Drug Substance Specifications and 6. 2. Drug Product Specifications” (page 39-40).

Comments: A Lot Release Protocol was submitted in Amendments 125280/0.20 and 0.28 after consulting with CBER at telecon on August 7, 2008, in which the sponsor proposed to use JEV---(b)(4)-- method as identity test for lot release. The appropriate documentation supporting the use of this assay as a measure of product identity, however, was not submitted until October 1, 2008 after CBER issued a CR letter on September 24, 2008. In response to the CR letter, Intercell submitted validation report of the JEV---(b)(4)-- method, and proposed to implement it into release testing for all final lots released post-licensure in the US (Amendment 125280/0.12) . Thus, the release specifications were modified to be suitable not only for identity but also for -----(b)(4)----- (see comment on page 33-34) .

5. 8. Final Lot Quality

Component	Quantity per dose (0.5-ml)	Function	Reference to standards
Active Substance JE-PIV	6.0 µg - (b)(4)- µg (Total protein)	Antigen	In-house specifications
Excipients Aluminum Hydroxide, ---(b)(4)-- Phosphate Buffered Saline (PBS)	-(b)(4)- (vol/vol) to 0.57ml	Adsorbent/adjuvant pH buffering agent/ drug substance solvent	--(b)(4)-- In-house specifications

5. 9. Sterility

JE-PIV is supplied as a sterile product and the formulation does not contain any preservative. All reagents and solutions in house-prepared were sterilized either by -----(b)(4)-----.

Sterility test is carried out on the -----(b)(4)-----, on the final bulk vaccine and on the final vaccine lot, as part of release testing. The rabbit pyrogenicity test has been carried out on all phase 3 lots and on the three consistency lots. All lots tested were negative. Clinical data also indicates that the product is not intrinsically pyrogenic. The pyrogenicity test is not included in product release of commercial lots, however, -----(b)(4)----- test is retained.

Following virus inactivation step with formaldehyde, the resulting intermediate product is formulated to a -----(b)(4)----- and sterilized by aseptic filtration (-----(b)(4)-----). The drug substance is aseptically adsorbed onto -----(b)(4)----- aluminum hydroxide adjuvant. Final bulk vaccine is then aseptically filled into single dose syringes.

5. 10. Cleaning Procedures

A description of the cleaning procedures and cleaning reagents used in manufacturing process was provided. These included use of -(b)(4)- solution in cleaning -----(b)(4)----- system and

sanitize gloves and package surface with (b)(4)-. A report for cleaning validation of the -----
 -----(b)(4)----- was provided. More detailed cleaning procedures and potential
 cross contamination issues for facility and equipment have been reviewed in site inspection.
 Some 483 issues of cleaning procedures have been identified and been responded by the sponsor
 to correct.

6. Specifications

6. 1. Drug Substance Specifications

Brief descriptions of the procedures and specification of drug substance release test are listed
 below.

Drug substance release test methods and specifications

[--(b)(4)--]

[--(b)(4)--]

* Sponsor has formally agreed in the context of a Post-Marketing Commitment that the ----
 --(b)(4)-----
 -----.

6. 2. Drug Product Specifications

Final vaccine lot release test methods and specifications

Description	Method	Specification
Appearance	Visual examination	----- (b)(4) ----- ----- A white, cloudy liquid/suspension forms upon agitation
(b)(4)-	--(b)(4)----- -----	--(b)(4)----
--(b)(4)----- -----	--(b)(4)-----	--(b)(4)----

Sterility	--(b)(4)----- -----	Sterile
--(b)(4)----- -----	--(b)(4)----- ----- -----	--(b)(4)----- -
Potency	--(b)(4)----- -----	-(b)(4)-
Aluminum	--(b)(4)----- ----- ----	--(b)(4)----- -----
--(b)(4)----- -----	--(b)(4)----- ----- -----	--(b)(4)----- ----- ---
General Safety Test (mice and guinea pigs)	21CFR 610.11; --(b)(4)-----	Satisfactory
Identity	--(b)(4)-- -----	--(b)(4)---

Comments: To confirm the quantity of JE protein in final product, sponsor agreed to use the -(b)(4)- (telecon of August 7, 2008) and the previously validated -----(b)(4)----- (in telecons of February 3 and March 9, 2009) in lot release. Lot ICB05 (run A), which was used in phase 3 clinical trials, is to be used as a reference standard in these tests.

6. 3. Justification of Specifications

All specifications and their justification are listed in the table below.

6. 3. A. Justification of drug substance specifications

[--(b)(4)--]

[--(b)(4)--]

The actual amounts of -----(b)(4)----- were shown in Table 4.4, *Batch Analysis-in Commercial Process* (page 24).

6. 3. B. Justification of final vaccine lot specifications

Assay	Specification	Justification
Appearance	-(b)(4)----- ----- A white, cloudy liquid/suspension forms upon agitation	-(b)(4)----- ----- -----
-(b)(4)-	-(b)(4)--	-(b)(4)----- ----- -----
-(b)(4)-----	-(b)(4)---	-(b)(4)----- ----- ----- -----
-(b)(4)----- --	-(b)(4)-----	-(b)(4)----- ----- ----- ----- -----

		<div></div> <div></div>
Sterility	Sterile	The product is intended for parenteral injection
Potency -(b)(4)-	-(b)(4)-	-(b)(4)- <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div>
Aluminum	-(b)(4)-	-(b)(4)- <div></div> <div></div> <div></div>
-(b)(4)- <div></div>	-(b)(4)- <div></div>	-(b)(4)- <div></div> <div></div> <div></div>
Pyrogenicity		Not pyrogenic

-(b)(4)-

--(b)(4)---

**Comments: (1) The -----(b)(4)----- of vaccine protein to ---(b)(4)---
- obtained from phase 3 clinical trial lots was --(b)(4)-- (shown in Table 4. 4.
Batch Analysis Results). As noted previously in this review, the “-(b)(4)-”
specification in the original BLA and shown in the above Tables was a
typographical error (meeting on August 7, 2008). In collaboration with
CBER DPQC, Sponsor and CBER mutually agreed to a specification of -
(b)(4)- (reference to Amendments 125280/0.12, 0.20 and 0.28).**

**(2). See more comments regarding product identity test in final vaccine lot on
page 33-34 and 38 and also refer to Dr. McCormick, William’s review of the -
(b)(4)- for use as an ID assay for lot release .**

6. 4. Impurity Profile

Process-related impurities and drug substance-related impurities

[--(b)(4)--]

[--(b)(4)--]

------(b)(4)----- is determined as an in-process control at the stage of sucrose gradient
purified material (where its -----(b)(4)----- was expected).

No step of the JE-PIV process is claimed to be capable of inactive/removal of TSE agents. TSE
safety is based only on the safety of biological materials derived from TSE-relevant animal
species (-(b)(4)-, of which was reviewed as described in above Section 3.2.C.). Assessment
reports of TSE risk were provided in Section 3.2.A.2.2.1-Appendix 7, 8, and 9.

**Comments: In their original submission, sponsor had failed to specify the ----
------(b)(4)----- and --(b)(4)- in the final product
and to set upper limits on the amount of each per dose of vaccine. CBER
notified the sponsor (in the context of the Inspection review form 483, and
during the telecon of August 7, 2008) that these substances must be specified
for drug substance . High concentrations of -(b)(4)-- may cause side effects
such as muscle soreness at the injection site (due to osmolality). The relative
amounts of -----(b)(4)----- and of host cell protein in the final lot may**

affect measurements of the quantity of the inactivated JEV protein, because the tests do not distinguish viral proteins from -----(b)(4)----- . In addition, it was remotely possible that excessive amounts of -----(b)(4)----- --- could affect the potency.

In response to CBER requests, the sponsor provided testing results, the specification (----- (b)(4) -----), and the validation data of -----(b)(4)----- in Amendment (125280/0.16, 0.20, and 0.28), showing that the majority of -----(b)(4)----- was removed during -----(b)(4)-----, and only residual amounts of full length -----(b)(4)----- could be detected in virus containing ----(b)(4)---- fractions. The remaining concentration of full length -----(b)(4)----- in drug substance is below the LOD (Limit of Detection) of -----(b)(4)----- and -----(b)(4)----- . The degradation fragments of -----(b)(4)----- in drug substance were calculated to be in the range of the LOQ of the - (b)(4)- method --(b)(4)----- . The acceptance criteria of ----- (b)(4)----- ----- was established on the levels of consistency batch production, with which the potency was not affected. Thus, the level of -----(b)(4)----- - in purified materials and in final lots is within acceptable limits (also see comment on page 31).

6. 5. Test Specifications for Viral Adventitious Agents

(See above 5.4 on page 34-36)

6. 6. Animal studies and Potency

There is ample evidence in the literature to support the concept that efficacy of a JE vaccine is dependent upon its ability to elicit a neutralizing antibody response. (For a detailed summary, see L Markoff. 2000. Vaccine 18 :26-32.) Regulatory authorities in conjunction with the WHO have therefore adopted the Plaque Reduction Neutralization Test (PRNT) as a surrogate for efficacy of JE vaccines. The PRNT measures the titer of virus-neutralizing antibodies in a test serum by observation of the ability of the serum to prevent formation of plaques resulting from infection of a tissue culture monolayer (Vero cells) with a known number of “plaque-forming units” (pfu) of JE virus. For statistical reasons, a 50% endpoint in this assay, i.e., the dilution at which the number of plaques is reduced by 50% in comparison to the number of plaques detected in a negative control plate, is chosen. The potency of the vaccine has been extensively evaluated in pre-clinical studies which were reviewed in the context of the pre-IND and IND phases of development. Validation of the PRNT50 assay used in clinical trials to assess efficacy was reviewed by Dr. Jeff Roberts in the context of his review of the clinical data in this BLA. Validation of the PRNT50 used in animal studies and for potency submitted in the BLA under section 3.2P.5.3. was reviewed (see page 55-56.). The following is a brief summary of the pre-clinical data to be found in Section 3.2P.5.3.App2 of the BLA:

(1) The 50% lethal dose (LD 50) for three disparate virulent JE viruses was determined in 6-week-old female -(b)(4)- mice. These viruses and mice of this type and age were then used in challenge experiments.

(2) IC-51 (also known as JE-PIV or Ixiaro) was shown to produce high levels of neutralizing antibodies in rats and rabbits.

(3) Sera from humans who had been vaccinated with either Ixiaro or JE-VAX were used in passive protection studies in mice. Mice treated to damage the blood-brain barrier and who either did or did not receive human immune serum were then challenged with 50LD 50 (a dose predicted to be fatal to 100% of mice) of two different virulent JE viruses by the intra-peritoneal route; passively immunized mice but not controls were protected. This study demonstrated that Ixiaro was at least as good as JE-VAX in eliciting an immune response in humans that could protect mice. Titration of mouse serum samples obtained prior to challenge suggested that a neutralizing titer in the PRNT50 of <1:10 was protective.

(4) A potency assay based on the mouse model was devised. Briefly, groups of about 10 mice each are immunized with ascending doses of vaccine, e.g., 5ng, 20ng, 50ng, and 100ng. Two subcutaneous injections of 0.2 ml of JE-PIV on day 0 and day 28 are administered, and sera are collected on day 42 and evaluated for neutralizing antibody by PRNT50. The amount of vaccine protein (ng) needed to seroconvert 50% of mice is then calculated. The results of the potency assay demonstrated that the vaccine was able to induce protective levels of JE antibodies in -(b)(4)- mice in a dose-dependent manner. This bioassay is well known to give highly variable results, but a similar assay (using protection as an endpoint, rather than the antibody response) was used to assess potency of JE-VAX. The potency assay was validated in studies conducted at ------(b)(4)----- (using -(b)(4)- sourced mice). Inter-assay precision was determined by testing -(b)(4)- lots of vaccine -(b)(4)- times each over a pre-determined time frame. Reproducibility was evaluated by conducting the nearly identical assays at Intercell AG (using mice supplied by -(b)(4)-). Results show that the assay was more reproducible at the --(b)(4)-- facility than at Intercell, but for two lots (------(b)(4)-----) there was good inter-assay and inter-facility agreement. Lot --(b)(4)-- was selected as the standard for comparison for the potency of future lots of Ixiaro, because it was used in phase 3 clinical trials. The upper limit of potency (expressed as the ng of vaccine protein necessary to seroconvert 50% of mice or ID50) was set at -(b)(4)- , which represents the statistical average of the result obtained for lot -----(b)(4)---- plus three standard deviations.

(5) Active protection studies in mice demonstrated a dose-related increase in protection against lethal JEV challenge. The challenge results are summarized in the following table, in which Ixiaro is referred to as “IC51” and/or “JE-PIV”. The term “JE-VAX” refers to a mouse-brain-derived, formalin-inactivated JE vaccine that was licensed for use in the US but has been withdrawn from the market. JE-VAX was therefore used as a positive control in these studies. IC51 was at least as protective as the licensed JE vaccine in the study below.

IC51 (JE-PIV) - active protection in mice

Vaccine Lot #	Use	Dose	%	Protection
---------------	-----	------	---	------------

		(ng)	Protection	ED50 (95% CI)
Non- adjuvanted Bulk	Manufacture of	1500	100% (10/10)	
---(b)(4)---	Final Vaccine	150	100% (10/10)	2.6 ng
	Lot # 0574	15	80% (8/10)	(0.02 – 7.6)
		1.5	40% (4/10)	
IC51 (JE - PIV)	Final Lot Phase 1 trial	40	60% (12/20)	
---(b)(4)---	Lot # 0737	4	60% (12/20)	6.7 ng
(Final Vaccine)		0.4	15% (3/20)	(1.8 - 6.9)
		0.04	15% (3/20)	
		600	100% (10/10)	
		60	100% (10/10)	1.5 ng
JE - VAX®	JE - VAX®	6	90% (9/10)	(0.6 - 3.7)
		0.6	20% (2/10)	

6.7. Safety

Ixiaro contains proteins of JE virus strain SA14-14-2 which was received from China by scientists at WRAIR. In China, strain SA14-14-2 has been used as a live vaccine in children for about two decades. A controlled short term safety study, published in the Journal of Infectious Diseases (Liu ZL, Hennessy S, Strom BL, Tsai TF, et al. J Infect Dis. 1997 Nov;176(5):1366-9) showed that this vaccine yielded no serious adverse events in ~13,000 Thai schoolchildren over the first 30 days post-immunization. There were no cases of encephalitis. At WRAIR, the virus was passaged several times, first in Primary Dog Kidney (PDK) cells ---(b)(4)----- . Nucleotide sequencing of the virus received from China as compared to the Vero cell isolate showed only one amino acid difference in the envelope (E) gene segment, the major determinant

of virulence (see Huong et al, 2001[reference in BLA]). Nevertheless, it was possible that tissue culture passage had altered the attenuation phenotype. Therefore, a safety study was done in mice. Results showed that the Vero cell adapted virus was not different from the Chinese isolate in its level of attenuation. Although no formal safety study of the vaccine virus used in Ixiaro was ever attempted in humans, it is quite likely that the virus is attenuated based on results in the mouse model. Moreover, the actual vaccine contains only formalin-inactivated (killed) virus. Each lot is safety tested for its ability to form plaques on Vero cell monolayers. The release criterion is zero plaques, i.e., no evidence of any infectious virus particles. Further, a test report of general safety (mice and guinea pigs) was provided (in 3.2.R.3.P-Appendix 7).

7. Validation

7. 1. Process Validation and/or Evaluation

7. 1. A. Process evaluation

Evaluation of the consistency of manufacture of the drug substance has been carried out from the results obtained with three consistency batches (summarized in above table, in section 4) and from commercial batches.

7. 1. B. Validation of critical process steps

In addition to evaluation of the process consistency from the three consistency lots, specific studies have been undertaken with respect to validation of the most critical steps, i.e.:

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

Three (3) pages determined to be not releasable: (b)(4)

----- (b)(4) -----

----- . As shown in section 2.3.P.5.2.8, the sponsor did not complete the evaluation of the ----- (b)(4) ----- , and did not perform potency assay to determine the correct up limitation of ----- (b)(4) ----- . Therefore, this validation is deficient.

This issue was resolved by direct communication with sponsor at the telecon meeting on August 7, 2008 and collaboration with CBER DPQC (see above page 43). Sponsor also proposed use -(b)(4)- to detect virus protein in --- (b)(4)--- assay and submitted validation data in Amendment 125280/0.12, which CBER reviewed and accepted.

Validation of shipment: The shipment method validated by the applicant is the method that was used for shipment of the 3 consistency batches. Although this shipment method was satisfactorily validated, the method was not found to be totally appropriate. A more robust (and secure) method would be used for commercial batches, placing the ----- (b)(4) ----- and maintained at -(b)(4)- during transportation using validated refrigerated vehicles. Calibrated data loggers would be used to monitor the temperature inside the container and inside the vehicle.

Comments: In their original submission, sponsor had failed to specify the ---- (b)(4)----- and --(b)(4)- in the final product and to set upper limits on the amount of each per dose of vaccine. CBER notified the sponsor (in the context of the Inspection review form 483, and during the telecon of August 7, 2008) that these substances must be specified for drug substance . High concentrations of Intercell's response to inspection provided by Destry Sullivan.)

Validation of ----(b)(4)---- of filling: The critical parameters for a successful filling operation are ----- (b)(4)----- of the filled syringes and aseptic processing to ensure the sterility of the complete fill. The qualification of the filling operation at -(b)(4)- will be completed with satisfactory completion of medium fills and placebo fills at the required volumes and satisfactory execution (JEVIVP2-02 December 2006 Page 22 of 26 of) filling of the

----- (b)(4) -----

----- (b)(4) -----

7. 4. Potency Assay Validation

Potency of the final vaccine lot is determined using (b)(4)-----

-----.

----- (b)(4) -----

-----.

----- (b)(4) -----

----- (b)(4) -----

7. 5. Validation of Analytical Procedures

Validations studies for analytical methods have been performed in accordance with relevant ICH Guidelines, while general analytical methods follow pharmacopoeial procedures. A list of

validation of analytical procedures is showed in following table and the results of validation studies are summarized below:

[--(b)(4)--]

**One (1) page determined to be not releasable:
(b)(4)**

(b)(4)-----

Comment: The sponsor notes that “This method is not considered fully satisfactory for determining -(b)(4)- concentration” (-(b)(4)-, which is close to the expected -(b)(4)- concentration in drug substance (----- (b)(4)-----), resulting in a low precision of about $\pm 20\%$ at this -(b)(4)- level consequently. Validation of the ----- (b)(4)----- assay has been done. This assay should be implemented into control of critical steps and to lot release to accurately detect -(b)(4)- level in diluted preparation such as drug substance and drug product, since this method is more sensitive than --- (b)(4)----- assay. As commented above, sponsor has agreed to replace the ----- (b)(4)----- assay with -(b)(4)- (see page 31), thus this issue was resolved.

----- (b)(4)-----

-----.

**One (1) page determined to be not releasable:
(b)(4)**

----- (b)(4) -----

--

125280/0.4, 0.8, and 0.12. (Please refer to the review of Intercell’s response to inspection provided by Destry Sullivan.)

7. 8. Validation of Adventitious Agents

----- (b)(4) -----

7. 8. B. Viral clearance studies

A validation study assessing the inactivation of -(b)(4)- model viruses and of the JE virus itself following the formaldehyde treatment of the JE vaccine production process was performed.

----- (b)(4) -----

----- (b)(4) -----

[--(b)(4)--]

The kinetics of viral inactivation showed that (b)(4)- was completely inactivated by Day (b)(4)- (within the limitations of the assay), as opposed to chemical inactivation of (b)(4)- and (b)(4)- for which the reduction index was still increasing with time by Day (b)(4)- . JEV was shown to be inactivated by Day (b)(4)- (within the limitations of the assay). The (b)(4)- reduction achieved (----(b)(4)-----) and the kinetics of inactivation, showed that the formaldehyde treatment was effective at inactivating potential contaminating viruses.

7. 8. C. *In-Vitro* Assay for the evaluation of -----(b)(4)----- viruses

- -----(b)(4)-----

This test is performed to determine the absence of extraneous agents (adventitious viruses). The assay complies with -----(b)(4)-----.

7. 8. D. Validation studies of steps ensuring microbial safety

The following validation studies have been carried out on steps contributing to the aseptic process:

- Validation study of the sterile filtration and aseptic handling at the end of the manufacturing process of the drug substance (Section 2.3.S.2.5)

-Validation of aseptic handling during final bulk vaccine formulation and Media fill runs to validate the aseptic filling of the final vaccine lot (Section 2.3.P.3.5).

7. 9. Validation of Materials

----- (b)(4) -----

8. Container and Closure System

A brief review of the container system is summarized as following. Detailed information of the system has been reviewed during the site inspection.

-----**(b)(4)**-----

-----**(b)(4)**-----

-(b)(4)- are sterilized with -----**(b)(4)**----- (at a contract sterilization facility). A minimum dose of -(b)(4)- is administered with the maximum dose of ----**(b)(4)**------. The efficiency of the minimum dose of --(b)(4)-- has been validated according to -----**(b)(4)**--

-----.

The ---**(b)(4)**--- of the -(b)(4)- are closed with sterile end-caps -----**(b)(4)**-----
-----, all within a Grade -(b)(4)- environment. Results obtained to date during stability studies for -(b)(4)- content and -----**(b)(4)**----- indicated -----**(b)(4)**----- to container closure system.

8. 2. Container Closure System for the Final Vaccine Lot

Pre-filled syringe: The vaccine is supplied as a pre-filled syringe (containing a 0.5-ml single dose). The container closure system of pre-filled syringes is summarized in the table below. Suitability of material used for the manufacture of syringe barrels with Luer tips and tip caps and of plunger stopper is documented in the conformity certificate provided by the supplier -----
-----**(b)(4)**-----.

Description of pre-filled syringe

Component	Description
Syringe	1.25 ml Syringe made of Type I ----- (b)(4) -----

	----- -----
Plunger Stopper	Chlorobutyl rubber , -(b)(4)-, black , tested according to ----- ----- ----- ----- Commercially available

8. 3. Quality Control and Specification for Container system

------(b)(4)-----

------(b)(4)-----

[--(b)(4)--]

[--(b)(4)--]

------(b)(4)-----

[--(b)(4)--]

Aseptic connection between the ------(b)(4)----- and filling line is made using a ---
(b)(4)-- connector, which is -----(b)(4)----- and supplied -----(b)(4)-----

8. 3. B. Syringe

------(b)(4)-----

[--(b)(4)--]

----- (b)(4) -----

One (1) page determined to be not releasable: (b)(4)

8. 4. B. Syringe

Leaching and extractable : Absence of leaching has been confirmed by the Extraction testing that has been performed by a study conducted by the applicant. Filled syringes from a technical fill of final bulk vaccine, batch ----- (b)(4) -----, were stored -----
(b)(4) -----.

Integrity: the integrity of the container/closure system was verified using a - (b)(4) ----- test by ----- (b)(4) ----- . This study demonstrated that the -----
--- (b)(4) ----- with - (b)(4) - and the --- (b)(4) ----- plunger/stoppers provides a secure barrier to potential contamination.

Comments: As mentioned above, there were some deficient issues regarding the container and closure system that well identified during the site inspection. The sponsor has taken steps to correct these issues in response to 483 Letter showing in Amendment 125280/0.4, 0.8, and 0.12 . (Please refer to the review of Intercell's response to inspection provided by Destry Sullivan .)

9. Stability

9. 1. Drug Substance Stability

9. 1. A. Stability batch information

----- (b)(4) -----

[--(b)(4)--]

Batch information of stability -primary consistency batches

Batch	Size	Date of	Product	Container/Closure
-------	------	---------	---------	-------------------

No.	(kg)	Manufacture	Description	
IC51/07E/ 006A-11	- (b)(4)-	---(b)(4)---	Bulk drug substance	----- (b)(4) ----- -----
IC51/07E/ 007A-11	- (b)(4)-	---(b)(4)---	Bulk drug Substance	----- (b)(4) ----- -----
IC51/07F/ 008A-11	- (b)(4)-	---(b)(4)--- ----	Bulk drug substance	----- (b)(4) ----- -----

9. 1. B Stability-indicating parameters

The stability-indicating tests described in the table below were included in the stability studies.

[--(b)(4)--]

9. 1. C. Analytical procedures

Except for -----(b)(4)-----, the analytical methods, including validation information and specifications, are the same as described in the section “5. 2. Quality of the Final Bulk Vaccine”. For -----(b)(4)-----, test specification is presence of the viral specific --(b)(4)-. The validation data of the method was presented by the sponsor in 3.2.S.7.3-Appendix 1 (page 17-21). However, this procedure was not performed on the consistency batches and not used in stability studies.

Comments: Regarding the -----(b)(4)----- , t he sponsor explained, at a teleconference on July 17, 2008, that the aluminum-formulated protein interfered with the results of --(b)(4)--- assay and that, for this reason, the --- (b)(4)---based assays were no longer used in stability study as well as in lot release, which CBER accepted .

9. 1. D. Drug substance stability data

The stability results generated thus far indicate the following:

Supporting stability data show that the Drug Substance stored in -----(b)(4)----- remains stable up to --(b)(4)-- when stored at --(b)(4)-- and up to --(b)(4)-- when stored at ----(b)(4)----.

Primary stability data show that the Drug Substance stored in -----(b)(4)----- remains stable up to -(b)(4)- when stored either at -----(b)(4)-----.

The applicant considers: “For commercial manufacture, drug substance is stored at -----
------(b)(4)----- before further aseptic formulation to produce
final bulk vaccine. Therefore this maximum holding time is supported by the available stability
data”.

9. 2. Drug Product Stability

9. 2. A. Stability batches

Stability data for determination of shelf-life have been generated from three batches of final bulk
vaccine manufactured at Intercell according to the Phase 3 clinical batch process. The final bulk
vaccine is stored in -----(b)(4)----- . To reduce the quantity of material required for stability
studies, stability samples from phase 3 final bulk vaccine were stored into ---(b)(4)---- containers
made from the same material as the -----(b)(4)-----.

[--(b)(4)--]

[--(b)(4)--]

9. 2. B. Stability-indicating parameters and analytical methods

Test	Reason for Use as Stability-Indicating Parameter	Method	Specification
pH	--(b)(4)----- ----- ----- -----	--(b)(4)----- ----- -----	-(b)(4)-
Appearance	--(b)(4)----- ----- -----	--(b)(4)----- -	--(b)(4)----- ----- ----- -----

			----- ----- -----
--(b)(4)----- 	--(b)(4)----- ----- -----	--(b)(4)----- ----- ----- ----	--(b)(4)-----
--(b)(4)----- ----- -----	--(b)(4)----- ----- -----	--(b)(4)----- ----- -----	--(b)(4)- -
Potency	Used to determine the potency of the product, a change in the potency of the samples with time is indicative of sample deterioration.	-(b)(4)- ----- -----	-(b)(4)-
Sterility	Performed to indicate that the samples for inclusion in the study have been prepared free of contamination and that they remain free of contamination throughout the study.	--(b)(4)----- ----- -----	-(b)(4)-
--(b)(4)----- -----	--(b)(4)----- ----- -----	--(b)(4)-- ----- ----- ----- -----	---(b)(4)---

The -----(b)(4)----- assay has been used to evaluate stability of the phase 3 products. It will be carried out as part of the release testing for commercial final vaccine lot and be included in the stability studies involving commercial lots. A specification will be reviewed as soon as more production and stability data will be available.

Analytical methods and specifications are the same as those used for release.

9. 2. C. Stability data of drug product

[--(b)(4)--]

One (1) page determined to be not releasable: (b)(4)

As shown in above tables, the stability results generated thus far indicate the following:

Stability data consist of supportive data generated on final bulk vaccine and final vaccine lots of drug product manufactured to the phase 3 clinical batch process.

The stability data obtained to date support to refrigerated storage for -----(b)(4)----- and final vaccine lot. An 18-month shelf-life is proposed for the ---(b)(4)--- vaccine when stored at the recommended storage condition of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Supportive stability data obtained from Lot -----(b)(4)----- and Lot ---(b)(4)--- (of which, no full manufacturing process data was provided in this submission) showed that the final vaccine lot has remained within specifications at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ condition for ---(b)(4)---, whereas, There were no potency results from final vaccine Lot -----(b)(4)----- and -----(b)(4)----- at ---(b)(4)--- time point.

The stability data of three consistency lots (-----(b)(4)----- -) manufactured according to commercial process are limited and not available at 9, 12, 18, and - (b)(4)-- time point at the recommended storage condition of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

Comments: (1) The updated stability data was submitted in Amendment 125280/0.6. The available data are adequate to support an 18-month shelf life. Sponsor plans eventually to provide completed potency results from final vaccine lot -----(b)(4)----- and -(b)(4)----- at the - (b)(4)- time point and to provide updated stability data on three consistency (commercial) lots in support of a ---(b)(4)-- shelf life .

(2) The samples in stability studies were pre-filled products used in clinical trials and pre-filled products of three consistency lots. Thus the date of manufacture shall be defined as the date of filling of the final containers.