

Application Type	Original BLA
STN	125678/0
CBER Received Date	October 25, 2018
PDUFA Goal Date	September 25, 2019
Division / Office	DVP /OVR
Committee Chair	Bharat Khurana
Project Manager	Sudhakar Agnihothram, Josephine Resnick
Priority Review	Yes
Reviewer Name(s)	Lei Huang
Review Completion Date / Stamped Date	
Supervisory Concurrence	Tsai-Lien Lin Branch Chief, Vaccine Evaluation Branch DB, OBE
	John Scott Division Director, Division of Biostatistics OBE
Applicant	Bavarian Nordic A/S
Established Name	Smallpox (Modified Vaccinia Ankara) Vaccine, Live
Trade Name	JYNNEOS
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	Liquid-frozen suspension containing a dose of 0.5 mL with at least 0.5×10^8 Infectious Units of MVA-BN
Dosage Form(s) and Route(s) of Administration	Suspension for subcutaneous injection supplied as a 0.5 mL single-dose vial
Dosing Regimen	Individuals not previously vaccinated against smallpox or monkeypox: Administer two doses (0.5 mL each) 4 weeks apart. Individuals previously vaccinated against smallpox and at continued high risk of exposure to smallpox or monkeypox: Administer as a single 0.5 mL dose.
Indication(s) and Intended Population(s)	Active immunization against smallpox or monkeypox in adults aged 18 years and older

Table of Contents

1. Executive Summary	3
2. Development Report (Doc. 50000037 Amendment 2)	3
3. Validation Report (Doc. 82000202)	6
4. Conclusion	6

1. Executive Summary

This review memo serves as an addendum to my original review dated August 27, 2019 and includes the statistical review of the plaque reduction neutralization test (PRNT), Version (b) (4). I reviewed the development report (Doc. 50000037 Amendment 2) and validation report (Doc. 82000202) submitted to Module 5.3.1.4 in STN125678.0. I identified some issues with precision, linearity, and quantitation limit. Further investigations of these assay characteristics to demonstrate the adequacy of the PRNT, Version (b) (4), may be necessary before firm conclusions can be drawn from the clinical studies in which the PRNT, Version (b) (4), was used.

2. Development Report (Doc. 50000037 Amendment 2)

A total of (b) (4) human serum samples with titers ranging from (b) (4) (Table 1) in addition to positive and negative sera were used for assay development.

Table 1. Human serum samples used in assay development

Sample	Preparation	Nominal GMT	Nominal Log10-titer
(b) (4)			

- (b) (4)
- Nominal GMTs were based on repeated testing of the sample for (b) (4) times.

Source: Summarized by the reviewer based on information provided in Section 2.1 of Doc. 50000037 Amendment 2.

Specificity

A total of (b) (4) sera samples supposed to be negative were tested, and all tested negative. In addition, all (b) (4) human samples that were supposed to be positive showed positive titers (values (b) (4) were considered negative, and (b) (4) were considered positive).

Accuracy

The (b) (4) samples were tested at least (b) (4) times and the applicant showed that all the results were within (b) (4) of the nominal GMT for the (b) (4) samples, within (b) (4) for the (b) (4) sample, and within (b) (4) for the (b) (4) sample on the log-scale.

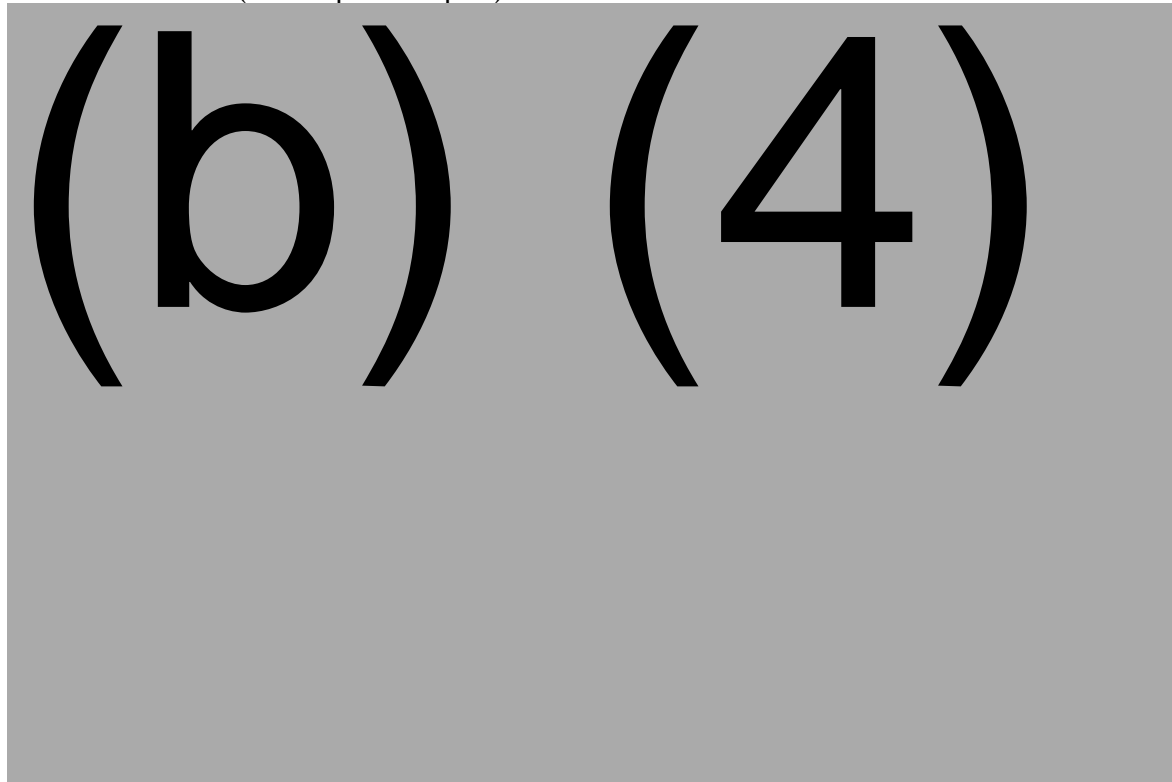
Reviewer Comments

The criteria that the individual titers be within (b) (4) of the nominal GMT on log-scale are too loose. For example, for the (b) (4) sample, the acceptance range was (b) (4) log10-titer, which is roughly equivalent to (b) (4) folds from the nominal GMT. In addition, this assessment of accuracy was more of an assessment of precision since it evaluates how close the individual titer measurement was to the GMT instead of the true concentration. In the absence of an international standard with known concentration, relative accuracy can be assessed through evaluation of linearity.

Precision



(b) (4), inter-batch, inter-day and inter-operator variability was assessed for the evaluation of precision. The experiment and the results are described in Table 2.

Table 2. Precision (Development report)



Source: Summarized by the reviewer based on Tables 4-8 in Doc. 50000037 Amendment 2.
%RSD was calculated by the reviewer based on SD.

Reviewer Comments

1. The applicant calculated %RSD as the (b) (4). This is not the correct formula for computing %RSD. Assuming the (b) (4)  . I calculated the %RSD for each sample using this formula and the results are shown in Table 2.
2. The applicant assessed inter-batch, inter-day, and inter-operator variability by taking the crude sample variance across results collected over (b) (4) runs. Since there are replicates within each run and data are imbalanced across Day and Operator, this approach may dilute the inter-batch, inter-day, and inter-operator variability. A more suitable approach is to apply an Analysis of Variance (ANOVA) model to decompose the variance components. In addition, the applicant assessed inter-batch, inter-day, and inter-operator variability separately but did not assess the overall intermediate precision. I fitted ANOVA models with Day, Operator, and Batch within Day*Operator as random effects to the data provided in Section 8.1 of Doc. 50000037 Amendment 2. The %RSDs for (b) (4) samples from my analyses are (b) (4), respectively, roughly in line with the %RSD calculated by the applicant on the original scale as shown in Section 8.1.

Linearity

Linearity was determined by (b) (4). In the development report, a linear regression was fit for (b) (4). In the Clinical Information Amendment Response to IR 25 Comments 7-8 submitted to STN 125678/0.38, the applicant updated the linear regression of log10-GMT against log10(1/dilution) and reported a slope of (b) (4) and a R-Square of 0.9985.

Reviewer Comments

While the linearity results reported by the applicant in STN 125678/0.38 appear to support linearity of the assay in the range from (b) (4), the data used for this linear regression are different from the data used for the original linearity analysis documented in Doc. 5000037 Amendment 2. To illustrate, the GMT of the neat sample had a concentration of (b) (4) log10-titer in the updated linearity analysis (Figure 2 the Clinical Information Amendment Response to IR 25 Comments 7-8 submitted to STN 125678/0.38), while the neat sample had a sqrt(titer) (b) (4), i.e. titer (b) (4), in the original linearity study as shown in Figure 4 of the development report. It was unclear why the applicant performed the linearity analysis with a different set of data, casting doubt on whether the selection of data to be used for linearity analysis was post-hoc. The data used for the original linearity analysis were not provided; therefore, I cannot perform my own analysis to evaluate linearity.

In addition, data collected for the (b) (4) samples in assay development were used for the updated linearity study and based on that, the (b) (4) samples were prepared by (b) (4). Although the recovery rates for the (b) (4) samples (observed GMT/expected GMT) were likely acceptable (bias (b) (4)), another (b) (4) samples ((b) (4)) which were not included in the updated linearity analysis were also prepared by (b) (4) dilution of the (b) (4), according to the development report. Since the expected titer for the (b) (4) sample is (b) (4), the recovery rates for the (b) (4) samples were (b) (4) based on (b) (4) tests, respectively, indicating substantial variability of the recovery rates for the (b) (4) sample.

Detection Limit and Quantitation Limit

The applicant stated that the DL and QL were calculated based on the “Standard Deviation of the Response and the Slope” method, using a calibration curve generated from titers of the (b) (4) titer samples. The Detection limit (DL) was determined to be a titer of (b) (4) and the Quantitation limit (QL) was determined to be a titer of (b) (4). This estimate has subsequently been validated by the independent analysis of the (b) (4) sample known to be near the detection limit. More than (b) (4) % of the samples were above the detection limit of (b) (4).

Reviewer Comments

It was unclear how the QL was determined based on the calibration curve, and the applicant did not validate the QL by additional experiment. Based on the linearity results, the recovery rates vary substantially at the (b) (4) concentration (GMTs ranging from (b) (4)), indicating that further investigation may be necessary to confirm adequate linearity around this range. The next (b) (4) concentration assessed in the linearity study was (b) (4). Hence, I am not certain that the a QL of (b) (4) is adequately demonstrated.

3. VALIDATION REPORT (DOC. 82000202)

Accuracy

Accuracy was evaluated by (b) (4) operator testing (b) (4) samples ((b) (4)) on (b) (4) on (b) (4) day. The data were analyzed in the same way as in the development report. The applicant concluded that accuracy had been shown since the acceptance criteria were met (b) (4)

(b) (4)

Reviewer Comments

Please refer to Reviewer Comments above for accuracy assessed in the development report – the comments there also apply to the validation report.

Precision

Intra-assay, inter-batch, inter-day, and inter-operator variability were assessed similarly as in the development study. All data were combined, and the applicant calculated the crude sample standard deviation as overall intermediate precision with SDs ranging from (b) (4) for the (b) (4) samples.

Reviewer Comments

- 1. The Reviewer Comments above for the evaluation of precision in the development report apply to the evaluation of precision in the validation report as well.*
- 2. I fitted an ANOVA model with Day, Operator, and Batch within Day as random effects, and the intermediate precision was estimated as (b) (4) % RSD for the (b) (4) samples, respectively. The results are largely different from the intermediate precision results from the development study. The possible causes of this discrepancy were unclear.*

4. CONCLUSION

Collectively, I noted the following issues with the PRNT, Version (b) (4):

- The precision results from the development study and validation study are substantially different, and the potential causes for this discrepancy were unclear.
- Linearity may not be adequate at the (b) (4) titer sample (GMTs ranging from (b) (4)) due to observed varying recovery rates for the (b) (4) samples ((b) (4)).
- It was unclear how the QL of (b) (4) was determined, and additional data near the claimed QL may be needed to support the determination of the QL.

In summary, further investigations of precision, linearity, and QL to demonstrate the adequacy of the PRNT, Version (b) (4), may be necessary before firm conclusions can be drawn from the clinical studies in which the PRNT, Version (b) (4), was used.