

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

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1. Executive Summary

Bavarian Nordic A/S (BN) submitted the original Biologics License Application (BLA 125678) for a proprietary strain of the orthopox virus Modified Vaccinia Ankara Bavarian Nordic (MVA-BN) as a live, highly-attenuated, non-replicating viral vaccine for protection against smallpox disease. MVA-BN has been developed to address the unmet medical and public health need for attenuated smallpox vaccines for people contraindicated to replicating vaccines. MVA-BN is indicated for active immunization against smallpox in adults aged 18 years and older. The vaccine may be used for both primary vaccination of smallpox vaccine-naïve adults and for revaccination of smallpox vaccine-experienced adults.

The clinical assays were validated and reviewed under IND 11596. A series of information requests (IR) were conveyed to the applicant during IND review. Not all IR questions regarding the primary assay (plaque reduction neutralization test, referred to as PRNT) used in the clinical studies were addressed to satisfaction before the submission of the BLA. One major concern was that the applicant determined the lower limit of quantification (LLOQ) based on extrapolation with no supporting data. The review of the appropriateness of the LLOQ continued during the BLA review and is documented in this memo. The applicant eventually agreed to raise the LLOQ of the PRNT assay from (b) (4) to 20 based on an extended linearity study and to recalculate the immunogenicity results that will be included in the package insert to reflect the updated LLOQ. In the July 5, 2019 IR response, the applicant also provided selected analyses for clinical data from Phase 3 Studies POX-MVA-006 and POX-MVA-013 with the hypothetical increase of LLOQ to 20, and showed that the results with a LLOQ of 20 are reasonably similar to those provided in the Clinical Study Reports (with a LLOQ of (b) (4)) and the overall conclusion stays the same. I consider the applicant's response acceptable.


It was noted in the June 7, 2019 IR response that an earlier version of PRNT (hereafter referred to as Version (b) (4)) was used in clinical studies POX-MVA-005, POX-MVA-023 and POX-MVA-011. Therefore, Version (b) (4) PRNT was also reviewed in this memo. I identified some issues and/or inconsistencies with the linearity and precision results, including that linearity was not established down to the claimed LLOQ (20), and the substantial difference in precision results from the pre-validation study and the validation study. Consequently, I consider the Version (b) (4) PRNT to be likely inadequate and additional investigations are warranted to draw conclusions from the clinical studies in which this version was used.

Overall, since the (b) (4) PRNT and the (b) (4) PRNT were used in the pivotal clinical studies and I consider these (b) (4) versions acceptable after adjusting the LLOQ, I recommend approval of this BLA.

2. Regulatory Background

Vaccinia-specific neutralizing antibodies are determined using a PRNT. The PRNT determines the level of virus neutralizing antibodies by measuring the plaque-reducing effect of the test serum after incubation with a constant amount of vaccinia virus. Briefly, (b) (4)

(b) (4)



Multiple versions of the PRNT have undergone development and validation. This review of clinical assays initially focuses on the (b) (4) PRNT and the (b) (4) PRNT, because these (b) (4) versions were the latest versions and were used to test clinical samples in the pivotal clinical studies. The (b) (4) PRNT was used for testing samples from trials POX-MVA-027 and POX-MVA-013, while the (b) (4) PRNT was used for testing samples from trials POX-MVA-006 and POX-MVA-037.

It was later noted in the June 7, 2019 IR response that an earlier version of PRNT (referred to as Version (b) (4) hereafter) was used in clinical studies POX-MVA-005, POX-MVA-023 and POX-MVA-011. Therefore, Version (b) (4) PRNT was also reviewed

3. SOURCES OF DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

3.1 Review Strategy

This statistical review focused on the IR responses regarding CBER's comments on the LLOQ of the PRNT. Version (b) (4) PRNT was also reviewed.

3.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

BLA 125678/0.0 Submitted 10/25/2018

Module 5.3.1.4 Methods Protocols and Reports – Study No. Methods Protocols and Reports STF

PRNT_Dev Report_50000111_Ed.01

PRNT_Dev Report_50000201_Ed.01

PRNT_Val Report_82000478_Ed.01

PRNT_Val Report_82000994_Ed.01

PRNT_Val Report_82000141_Ed.01

PRNT_Val Report_82000128_Ed.01

PRNT_Dev Report_50000037_Ed.01_Amendment3

BLA 125678/0.21 Submitted 02/26/2019

Module 1.11.3 Clinical Information Amendment

Clinical Information Amendment_Response to IR 13

BLA 125678/0.38 Submitted 06/07/2019

Module 1.11.3 Clinical Information Amendment

Clinical Information Amendment_Response to IR 25_Comments 7-8

BLA 125678/0.42 Submitted 07/05/2019

Module 1.11.3 Clinical Information Amendment

Clinical Information Amendment_FU Response to IR 25

BLA 125678/0.47 Submitted 07/31/2019

Module 1.11.4 Multiple Module Information Amendment

Response to Request for Information #30

4. LLOQ OF THE PRNT

4.1 The February 26, 2019 IR Response

FDA Comments are repeated here for completeness:

- A. The detection limit (DL) and LLOQ were set at (b) (4), respectively. You claimed that these limits were set following the ICH Q2(R1) guideline. However, we do not agree that the determination of the limits conforms with the ICH guideline. We agree that the detection limit is likely (b) (4), based on the nature of titer determination of the PRNT. However, setting the LLOQ at (b) (4) was unacceptable for the following reasons:
- 1) Although we acknowledge that in Response to Comment #5 on Serial 0310 to IND 11596, you pointed out that the ICH guideline explicitly mentions “extrapolation”, we do not agree that you followed the extrapolation approach as outlined in the guideline, because the guideline clearly states, and you quoted, that “..., this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.” You did not perform an independent validation to confirm the detection limit or the LLOQ by preparing samples near the limits. Although you stated that you have tested a number of supposedly negative samples, these negative samples may not be anywhere near the detection limit or the LLOQ. Instead, we recommend that you use positive samples diluted down to the range below and near the detection limit to validate the LLOQ and LOD.
 - 2) Even if the extrapolation method is applicable to setting the LLOQ, your determination of the LLOQ at (b) (4) may be incorrect. In the Response to Comment #5 on Serial 0310 to IND 11596, you stated that (b) (4), which may not be true unless the dose response curve passes the origin. The dose-response curve is not likely to always pass the origin due to the nature of the curve. Therefore, there does not exist a simple proportional relationship between the LLOQ and DL on the log scale, which implies that the relationship that $LLOQ = (b) (4)$ may not hold.
 - 3) We recommend that you set the LLOQ based on adequate relative accuracy and precision data. In the linearity study, the (b) (4) was diluted down to a titer of (b) (4). It is unclear if relative accuracy is acceptable at a titer of (b) (4). In addition, it is also unclear if the assay is adequately precise around a titer of (b) (4). We suspect that precision at this range is likely inadequate, because it appears that the assay is not adequately precise near the range of (b) (4), as discussed in Comment B below.
- B. In the precision analyses, you claimed that several (b) (4) samples resulted in a titer of (b) (4) (below QL) and were excluded from analyses. We do not agree with the exclusion of these samples. Firstly, the number of samples resulted in a titer of (b) (4) is substantially more than “several”. Based on Tables 6-20 of the validation report, a total of (b) (4) tests of the (b) (4) sample reported a titer of (b) (4), which accounts for (b) (4) of all the tests. Secondly, results below the LLOQ, if properly defined (refer to Comment 1), should be included for assessing precision across a wide range and confirming that

the assay is precise above the LLOQ. Excluding samples with a titer of (b) (4) will decrease the estimated variability of the assay around VL range and may result in an incorrect decision that the assay is sufficiently precise within this range. In fact, samples with a titer of (b) (4) may even have an actual titer below (b) (4). Including these samples with an assigned titer of (b) (4) in the analyses would have underestimated the true assay variability at this range, not to mention analyses excluding these samples completely. Our calculation shows that the variability for each of the (b) (4) batches with these samples included (as titers of (b) (4)) is (b) (4)

(b) (4) % RSD, respectively, strongly indicating that the assay is not sufficiently precise in the (b) (4) range (titer of (b) (4)). Please comment.

- C. The ULOQ was not evaluated at all for the PRNT. In the response to FDA comment 6 on Serial 0310 to IND 11596, you stated that the highest result from all samples tested from clinical trials was (b) (4), which was diluted from (b) (4) (by a factor of (b) (4) as compared to standard dilution from (b) (4)). You concluded that a ULOQ of (b) (4) can be set because the (b) (4) linearity study demonstrated that the PRNT is linear down to dilution with a factor up to (b) (4). We do not agree that your rationale supports a ULOQ of (b) (4), because no information on relative accuracy and precision at a titer of (b) (4) was provided. Hence, it is unclear if the PRNT is adequately accurate and precise at this range. Please comment.

Applicant response:

The applicant illustrated how the DL and LLOQ were determined based on extrapolation of the linear trend observed in the dilutional linearity experiment and claimed that this was done following the ICH Q2(R1) guideline. The applicant also stated that the fact that the variance on the log-scale stays constant (and that the slope is 1) strongly supports extrapolating further towards the natural lower bound.

With respect to precision, the applicant clarified that titers below the QL (b) (4) were assigned the value of the DL (b) (4) if titers were larger than the DL, and pointed out that the calculation of precision with imputed values of (b) (4) would be an overestimate of the assay variability. In addition, the applicant provided a QQ plot to demonstrate that it is expected that (b) (4) % of the observations will fall below the QL (b) (4) assuming that log-transformed data follow a Normal((b) (4)) distribution, where (b) (4) are the sample mean and standard deviation from the log-transformed titers with observed values equal or above (b) (4). The applicant further pointed out that the actual observed percentage of titers below (b) (4) was (b) (4) %, which is in good agreement with the expectation. Hence, the applicant concluded that this confirmed that titers down to (b) (4) can be reliably measured.

Regarding the ULOQ, the applicant pointed out that (b) (4) samples with a titer of (b) (4) was tested in the linearity study, and it is not possible to conduct the same study with real clinical sample due to the rareness of subjects inducing such high titers. In fact, only (b) (4) of samples in Studies POX-MVA-013 and POX-MVA-006 resulted in a titer (b) (4), respectively.

Reviewer Comments

1. I do not agree that the applicant has followed the ICH guideline to extrapolate the linear trend without collecting additional data to demonstrate that linearity extends to

the claimed LLOQ. The applicant also claimed that the constant variance on log-scale strongly supports further extrapolation towards the natural lower bound. I do not agree with this rationale. The constant variance on log-scale is a property of the log-normal distribution. Data having a log-normal distribution does not necessarily indicate extrapolation.

2. For precision, I agree that calculating assay precision by assigning a value of (b) (4) for titers falling between DL and QL is a conservative approach and is therefore likely to overestimate the variability. However, I do not agree with the conclusion that titers down to (b) (4) can be reliably measured because the probability of having a measurement falls below (b) (4) based on the calculated sample mean and standard deviation (SD) from titers (b) (4) is in good agreement with the actual measured percentage. Excluding those values of (b) (4) will apparently underestimate assay variability and overestimate the mean. The fact that the predicted percentages matched the observed percentage indicates that titers above (b) (4) were likely measured inaccurately or imprecisely. Otherwise, the predicted percentage theoretically should not match the observed percentage. It is unclear whether assay precision and accuracy are acceptable without additional information. In addition, it is not uncommon to use the observed values to assess precision during assay validation, even though the observed values may be below the provisional LLOQ. Therefore, I recommend that values (b) (4) be included in the precision analysis using the actual values instead of being imputed by (b) (4).
3. The applicant's response regarding the ULOQ appears acceptable. Even if accuracy were in doubt, the impact on the analyses of clinical data is likely small due to the rareness of samples of such high titers.

These comments were communicated to the applicant on May 23, 2019 as an IR.

4.2 The June 7, 2019 IR Response

On June 7, 2019, the applicant responded to the May 23, 2019 IR. The IR questions are not repeated here for conciseness.

Applicant response:

The (b) (4) PRNT

The lowest GMT of the sample in the linearity study was (b) (4), while the claimed LLOQ was (b) (4). The applicant agreed to extend the linearity study down to the titer of (b) (4) and submit the data by July 5, 2019.

The method of variance component analysis was adapted, including Operator×Day (=Assay) as a random effect, to assess precision on the (b) (4) sample (GMT=(b) (4)) using the data in Tables 6-20 in the validation report Doc. No. 82000994 Ed. 01. The estimated covariance parameters for Operator×Day and Residual are (b) (4), respectively. The SD of the Assay log₁₀ titer is (b) (4). The applicant further claimed that if the SD of a titer of (b) (4) is similar to the SD of the (b) (4) sample (GMT=(b) (4)), a titer of (b) (4) is 1 (b) (4) SDs above 0 (=log₁₀(1)), indicating a good precision of the assay around a titer of (b) (4).

The applicant also recalculated the SD of the (b) (4) sample without (b) (4) to (b) (4) for values between (b) (4). The CV for the (b) (4) sample is (b) (4) %.

Reviewer comments

1. I do not agree with the applicant's rationale that a titer of (b) (4) being (b) (4) SDs above 0 can support the conclusion of adequate precision at a titer of (b) (4). This rationale only shows that a sample with a titer of (b) (4) is likely to test positive (with a titer (b) (4)), and has no indication of the assay variability near a titer of (b) (4). Nevertheless, since the GMT of the (b) (4) sample is reasonably close to (b) (4), assay precision near a titer of (b) (4) may be acceptable if the assay variability is acceptable for testing the (b) (4) sample.
2. The same (b) (4) sample ((b) (4)) was also evaluated in the development study (BN-QCD-2017.003-DR) and the GMT was (b) (4) based on (b) (4) tests, which is (b) (4) % higher than the GMT of the (b) (4) sample in the validation study. This may indicate that the PRNT is not adequately accurate and/or precise at this range. Nevertheless, since the applicant agreed to provide an extended linearity study, I defer the evaluation of the LLOQ until the extended linearity study is completed.

The (b) (4) PRNT

The lowest GMT of the sample in the linearity study was (b) (4), while the claimed LLOQ was (b) (4). Due to expiry and depletion of reagents used in this assay version, no further experiments can be made to support the gap between the titers (b) (4) formally. The applicant stated that the assay characteristics (specificity, precision, linearity, detection and quantitation limit) were comparable between the (b) (4) PRNTs. The applicant considers the dilutional testing that will be done for the (b) (4) assay to be representative for the (b) (4) assay.

The method of variance component analysis was adapted, including Operator×Day (=Assay) as a random effect, to assess precision on the (b) (4) sample (GMT=(b) (4)) using the data in Tables 5-19 in the validation report Doc. No. 82000478 Ed. 01. The estimated covariance parameters for Operator×Day and Residual are (b) (4), respectively.

The (b) (4) PRNT was used for the evaluation of one primary endpoint in clinical Study POX-MVA-013 (lot-consistency). The serum groups resulted in GMTs well above the quantitation limit, while the placebo group resulted in a GMT below the detection limit. As such, the applicant concluded that the partially missing information regarding precision and linearity exactly at the quantitation limit acceptable.

Reviewer Comments

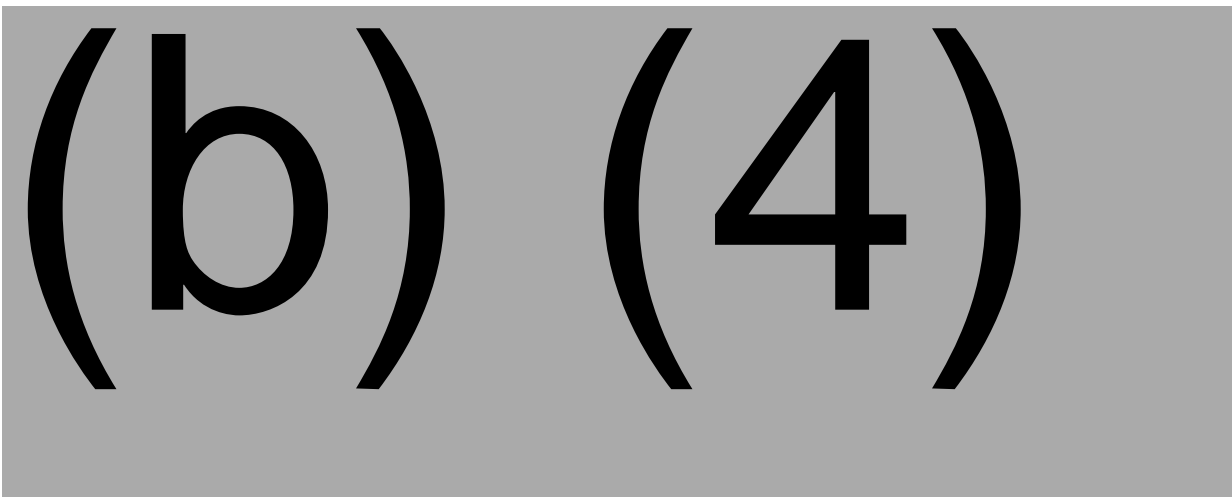
I defer to the product reviewer regarding the acceptability of the assumption that the dilutional linearity results for the (b) (4) PRNT is representative for the (b) (4) PRNT.

4.3 The July 5, 2019 IR Response

The applicant submitted the results of an extended linearity study for the (b) (4) PRNT down to titers at the claimed LLOQ of (b) (4). In this study, (b) (4) was (b) (4) - fold serially diluted and tested neat, as well as in dilutions from (b) (4) (a total of (b) (4) dilutions). A total of (b) (4) measurements were performed for each dilution. Additional dilution series were performed where only (b) (4) dilutions was tested since this dilution has an expected titer being close to (b) (4), resulting in a total of (b) (4) titer determinations for this dilution.

In an initial experiment, the dilution series was expanded by (b) (4) more dilutions up to (b) (4). From dilution (b) (4) onwards, all results were negative (i.e., a titer of (b) (4)). Therefore, these dilutions were skipped in the following experiments and not included in the analysis.

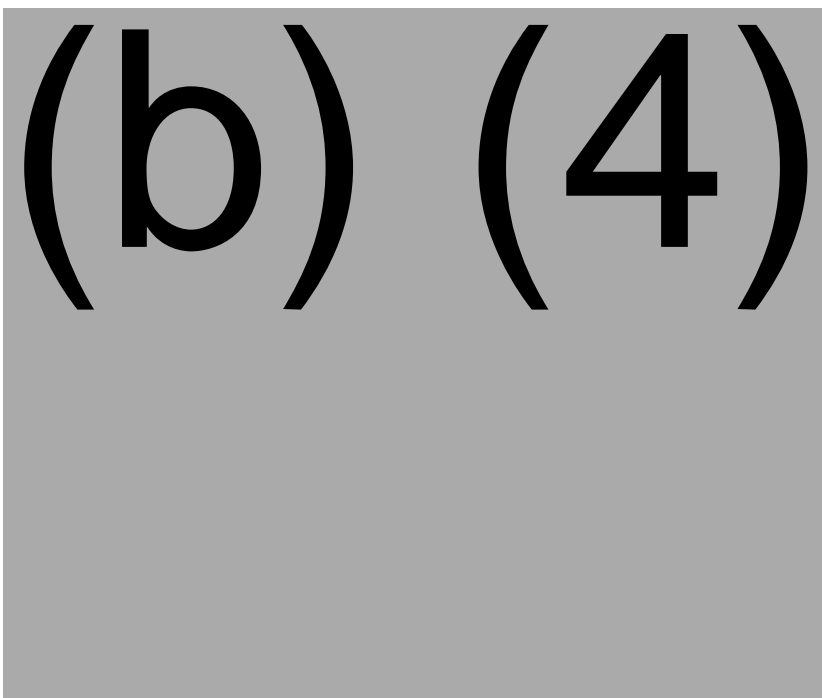
The results are summarized in Table 1. The highest dilution (first row in Table 1) is the antilog10 of the median of the log10-titers, whereas the data for the (b) (4) lowest dilutions are the GMTs of the respective dilution.



1. For the (b) (4) dilution the antilog10 of the median of the log10-titers is presented, whereas for neat to (b) (4) dilution the GMT is presented.

Source: Table 1 in Clinical Information Amendment_FU Response to IR 25 submitted in STN125678/0.42.

Figure 1 shows the data as log10-titers plotted against the log10(1/Dilution). The regression line is made from the average log10 titers against the log10(1/Dilution) of the (b) (4) dilutions from (b) (4) to neat. The slope of the regression line is (b) (4) (SD of slope: (b) (4)). The applicant pointed out that the expected value for the (b) (4) dilution is about (b) (4) (~half of (b) (4) based on Table 1), but the value is only about (b) (4). Thus, the applicant concluded that the PRNT is less precise in this part of the range.



Source: Figure 1 in Clinical Information Amendment_FU Response to IR 25 submitted in STN125678/0.42.

The applicant also provided selected analyses for clinical data from Phase 3 Studies POX-MVA-006 and POX-MVA-013 with the hypothetical increase of the LLOQ to 20, and showed that the results with a LLOQ of 20 are reasonably similar to those provided in the Clinical Studies Reports (with a LLOQ of (b) (4)) and the overall conclusion stays the same.

Reviewer Comments

1. *It is unclear why the applicant used the median to assess linearity for the (b) (4) dilution instead of the mean as for other dilutions. Since the raw data were not submitted, I am not able to evaluate the difference between the mean and the median. Nevertheless, the median results provided by the applicant do not appear to support linearity at the (b) (4) dilution, hence I did not request for the raw data for further analysis.*
2. *The applicant concluded that the assay is less precise at the (b) (4) dilution since the expected value is (b) (4) while median titer was (b) (4). I do not agree with this assessment because*
 - a. *The expected value of (b) (4) was calculated based on the observed (b) (4) dilution (b) (4). This approach is not ideal for the assessment of cumulative bias because any bias accumulated up to the (b) (4) dilution was not accounted for. A more appropriate approach is to calculate the expected value based on the GMT of the neat sample (b) (4). The expected value for the (b) (4) dilution is (b) (4), while the median of observe values was (b) (4), representing a (b) (4) % bias (i.e., larger than (b) (4)-fold difference).*

- b. The linearity analysis where expected values and observed values are compared is an assessment of relative accuracy instead of precision. Given the substantial bias at this dilution, the conclusion should be that linearity/relative accuracy is not adequate for the PRNT at this range.*
- 3. Overall, I recommend that the LLOQ be set at a titer of ~20 given that the linearity and precision results at this range are adequate for the (b) (4) PRNT.*
- 4. Since no extended linearity study can be done for the (b) (4) PRNT, I revisited the development and validation reports for the (b) (4) PRNT (submitted in IND 11596.311). Linearity was assessed by diluting neat (b) (4) samples (with a GMT of (b) (4)) up to (b) (4), with an expected titer of ~20; precision was assessed with the VL sample (with a GMT of (b) (4)). Based on an internal discussion with the product reviewer, it is considered acceptable to set the LLOQ at 20 for the (b) (4) PRNT as well.*
- 5. An IR was sent to the applicant on July 15, 2019 requesting that the LLOQ be set at 20 based on linearity and precision results. The applicant agreed and will recalculate the immunogenicity results that will be included in the package insert to reflect this change.*

5. VERSION (b) (4) PRNT

The review of the Version (b) (4) PRNT focused on the pre-validation report (Doc. 82000141), validation report (Doc. 82000128), and Amendment 3 of the development report (Doc. 50000037 Amendment 3).

5.1 Pre-Validation (Doc. 82000141)

Table 2 provides a summary of the pre-validation results.

Table 2. Pre-Validation Results (Version (b) (4) PRNT)

Parameter	Method	Results
Specificity	(b) (4)	(b) (4)
Accuracy		
Repeatability		
Intermediate Precision		
Linearity		
DL and LLOQ		

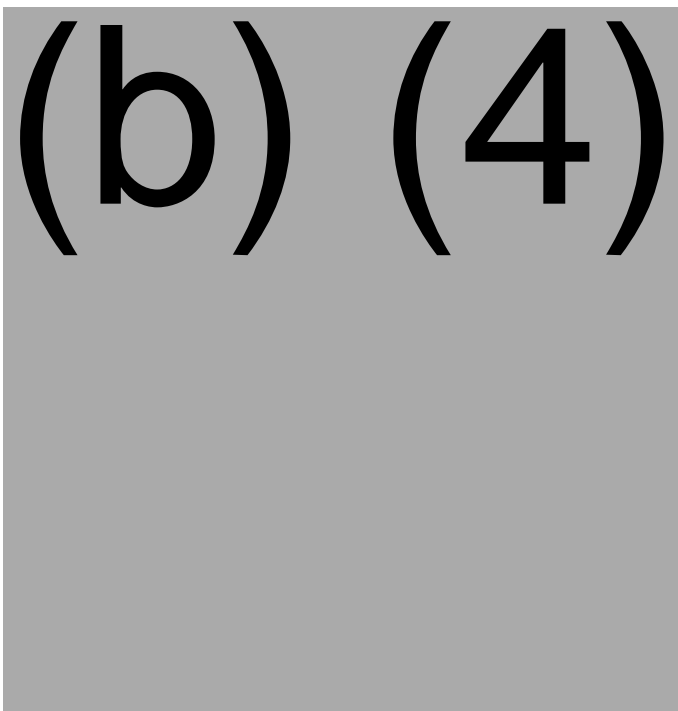
Source: Summarized by reviewer based on pre-validation report Doc. 82000141.

Reviewer Comments

1. The applicant's assessment of accuracy was more of an assessment of precision, because the true concentrations of the (b) (4) samples are unknown, and the applicant's assessment evaluated how close the individual measurements were to the center of the measurements. In addition, the analysis for accuracy did not

provide a meaningful evaluation because the acceptance ranges were too wide. For example, the mean log10 titer for the (b) (4) sample was (b) (4), and within (b) (4) % accuracy (log10 titer) is roughly equivalent to within (b) (4)-fold of the GMT. Hence, the result that 100% tests were within (b) (4)-fold of the GMT does not provide any meaningful assessment of closeness of individual titers to the GMT.

2. The applicant reported the overall precision ranging from (b) (4) % RSD for the (b) (4) samples. However, the calculation of %RSD was incorrect as it appears that the applicant calculated %RSD by (b) (4).
(b) (4) I performed an ANOVA with the precision data using a mixed model including Experiment, Operator and Batch as random effects, and calculated the intermediate precision as (b) (4) % RSD for the (b) (4) samples, respectively.
3. Linearity was assessed by fitting a linear regression with observed titers as the dependent variable and 1/dilution as the explanatory variable. However, the analysis was performed on the original scale instead of on the log-scale, and the regression coefficients cannot be construed as an assessment of linearity/relative accuracy. In addition, the applicant provided a figure for the regression analysis only, and it is impossible to discern whether any bias exists at the lower end of the assay range due to the scale of the Y-axis. In the June 7, 2019 IR response, the applicant provided an additional analysis with log10 transformed data (Figure 2). It should be noted that the applicant performed the linear regression with $\log_{10}((b) (4) \times \text{GMT})$ as the dependent variable. Nevertheless, this does not change the interpretation of the slope. The R^2 was high (0.9997) and the slope was reasonably close to 1. It should also be noted, however, that there were only (b) (4) dilutions included in the analysis. The applicant stated that the lowest GMT included in the linearity analysis was 31 in the June 7, 2019 IR response, while the LLOQ was claimed to be a titer of 20.



Source: Figure 1 in Clinical Information Amendment_Response to IR 25_Comments 7-8 submitted in STN125678/0.38.

4. The applicant set an LLOQ of (b) (4) based on acceptable precision and accuracy results in the pre-validation. It was pointed out to me by the product reviewer that the applicant has applied a multiplicative factor of (b) (4) to Version (b) (4) PRNT titers to harmonize Version (b) (4) PRNT to another method, effectively changing the LLOQ to 20, based on an IR response submitted to IND 11596.369. Since linearity/relative accuracy was not established below a titer of (b) (4) as the applicant stated, it may not be appropriate to set the LLOQ below (b) (4) without collecting additional information.

5.1 Validation (Doc. 82000128)

Accuracy

(b) (4)

. The applicant concluded that accuracy has been shown since each sample fulfilled the acceptance criteria (b) (4)

Precision

Precision was assessed in a similar way as for the pre-validation. The SD for log-transformed data and %RSD for the overall intermediate precision were reported by the applicant as (b) (4) % for the (b) (4) sample, (b) (4) % for the (b) (4) sample, (b) (4) % for the (b) (4) sample, and (b) (4) % for the (b) (4) sample, respectively.

Other assay performance parameters, e.g. linearity, specificity, limits of quantitation, detection limit, etc., were not evaluated during the validation study.

Reviewer Comments

1. *As I commented for the pre-validation report, the accuracy assessment performed by the applicant was more of an evaluation of precision instead of accuracy as it assesses how close a set of measurements were in the absence of the true concentration of the sample. In addition, the acceptance criteria were too lenient. In fact, the GMT for the (b) (4) sample in the accuracy study was (b) (4), representing a (b) (4) % increase from the nominal titer, but the results passed the acceptance criteria still.*
2. *The calculation of %RSD was incorrect, as I commented for the pre-validation report. Nevertheless, there appeared to be a substantial improvement on assay precision compared to the pre-validation results, as evidenced by apparently much smaller SDs. Since the precision study was unbalanced (some operators performed more runs and some operators performed just one run), I performed an ANOVA with the precision data using a mixed model including Operator and Day as random effects, and calculated the intermediate precision as (b) (4) % RSD for the (b) (4) samples, respectively. It was unclear what were the causes for the substantial improvement on precision as compared to the pre-validation results, casting doubt on the assessment of precision.*

5.1 Development Report Amendment 3 (Doc. 50000037 Amendment 3)

This amendment was made for the purpose to correct erroneous calculations from the original development report (Doc. 50000037) and the original validation report (Doc. 82000128).

Re-calculation of Precision (Doc. 82000128)

The applicant re-calculated the precision results using a corrected formula for %RSD. The overall precision results were (b) (4) % for the (b) (4) samples, respectively.

Reviewer Comments

The recalculated precision results were roughly in the range of (b) (4) % RSD, in line with my calculation. The applicant's results appeared to be slightly smaller than mine, likely because the applicant calculated the crude variability with the combined data from all operators and ignored the unbalance of the study design.

Recalculation of Accuracy (Doc. 82000128)

Data obtained in the original validation were re-evaluated on the anti-log₁₀ scale with regard to their actual deviation from the expected value. The (b) (4) titers differed by (b) (4) %, respectively, from the expected titer.

The applicant stated that the accuracy data for the (b) (4) sample were generated by the same operator at the same day. Since inter-operator and inter-day variability contributed to the

overall variability at a considerably higher extent than the intra-assay variability, the GMT of the accuracy data can be considered as a single measurement of a measurement series. Assuming an SD of (b) (4) of the log-titer for the (b) (4) sample (based on the validation study), this (b) (4) GMT was still within the 99% quantiles. Therefore, the applicant considered that accuracy was acceptable.

Reviewer Comments

I do not agree with the applicant's rationale that the (b) (4) % increase in titer for the (b) (4) sample was just one unfortunate measurement from a measurement series, I calculated the GMT from all runs by all operators in the validation study as (b) (4), a (b) (4) % increase from the nominal titer. Hence, it appears that there was a consistent upward shift in the titers for the (b) (4) sample.

Re-evaluation of Linearity (Doc. 50000037)

The linearity data in the development report Doc. 50000037 were re-analyzed on log scale. The (b) (4) sample was diluted with dilution factors of (b) (4). The (b) (4)-fold dilution did not generate a titer, and hence was excluded from the analysis. The R-square was 0.961 with a slope of (b) (4) and a SD of (b) (4) (Figure 3). The applicant concluded that linearity was demonstrated because 1 was within the 95% CI for the slope.

(b) (4)

Reviewer Comments

Based on an internal discussion with the product reviewer, the linearity data for Doc. 50000037 were generated using rabbit anti-vaccinia antibody and may not support linearity for human antibody. Hence, linearity results from this study was not considered towards the validation of the PRNT.

6. CONCLUSION

Based on the July 5, 2019 IR response with the extended linearity study, linearity is demonstrated to be adequate up to the (b) (4) dilution, but not at the (b) (4) dilution. The results do not support a LLOQ of (b) (4) as claimed by the applicant. The applicant provided selected analyses for clinical data from Phase 3 Studies POX-MVA-006 and POX-MVA-013 with the hypothetical raise of the LLOQ to 20, and showed that the results with a LLOQ of 20 are reasonably similar to those provided in the Clinical Studies Reports (with a LLOQ of (b) (4)) and that the overall conclusion stays the same. The review team requested the applicant to set the LLOQ of the PRNT to 20 for the (b) (4) PRNTs. The applicant agreed and recalculated the immunogenicity results to be included in the package insert to reflect the updated LLOQ. I consider the applicant's response acceptable.

Version (b) (4) PRNT was used in clinical studies POX-MVA-005, POX-MVA-023 and POX-MVA-011. I identified some issues and/or inconsistencies with the linearity and the precision results, including that linearity was not established down to the LLOQ (20), and the substantial difference in precision results from the pre-validation study and the validation study. Consequently, I consider that the Version (b) (4) PRNT is likely inadequate and additional investigations are warranted to draw conclusions from the clinical studies in which this version was used.

Overall, since the (b) (4) PRNT and the (b) (4) PRNT were used in pivotal clinical studies and I consider these (b) (4) versions acceptable after adjusting the LLOQ, I recommend approval of this BLA.