

## OTR-Short Form Report

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**Subject:** ICCR2018-03837 on BLA-125694: [REDACTED] method in AVXS-101 Release Assay

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**Background:** The Office of Tissues and Advanced Therapies (OTAT) of CBER is performing a priority review of the original BLA (125694) as submitted by AveXis, Inc. The drug product Onasemnogene abeparvovec (Zolgensma®), an Adeno-associated virus (AAV) vector AVXS-101, was developed for the gene therapy treatment for Spinal Muscular Atrophy (SMA) Type 1. The AVXS-101 contains (b) (4) of (b) (4) and a (b) (4) (b) (4). The amounts of (b) (4) in the product are critical quality attributes (CQAs), and the applicant has developed an (b) (4) assay to quantitate the relative amounts of the (b) (4). The previous FDA inter-center consult request (ICCR2017-01717) for the IND (15699) version of this (b) (4) assay was documented in FY18-028-DPA-S. In the past year, the assay has undergone additional development and validation in the firm. The BLA containing this assay was received on 10/01/2018 and CBER assigned it as a priority review. The current consult request is a new request for CDER/OPQ/OTR/DPA to provide the expert opinion on the suitability of the improved/validated (b) (4) assay for use as a lot release assay.

### Conclusions:

The proposed (b) (4) protocols as a release assay of BLA-125694 has improved significantly over the previous version in the IND stage. The proposed (b) (4) experimental protocol needs some additional improvements and validations to be qualified as a release specification to ensure the consistent drug quality to patients. The current suggestions to the firm include corrections in more testing on (b) (4) determination, experiment setup, data processing parameters and additional acceptance criteria. Comments to the firm are summarized in the report.

## Results and Discussion:

The firm's documents of experimental procedure SOP-263, specification assessment PRO-590, validation report RPT-592, method transfer report RPT-640 and other supporting materials have been reviewed (**Table 1**). The SOP-263 describes the release protocol for (b) (4)

(b) (4) assay for measuring, (b) (4)

(b) (4) (2) the (b) (4)  
(b) (4), (3) the (b) (4)

virions. The PRO-590 established the acceptance criterion in SOP-263. The validation report RPT-592 determined the specificity, intra-assay precision (repeatability), intermediate precision, limit of detection (LOD), limit of quantification (LOQ), linearity and accuracy. There are (b) (4)

(b) (4) identified in AVXS-101 drug product, (b) (4)

(b) (4) (**Figure 1**). It is not clear if (b) (4)

functional form based on (b) (4) data only. The (b) (4) results of the reference samples of (b) (4)

(b) (4) (**Figure 2**) (b) (4) (**Figure 3**) were available. In addition to the commonly

used (b) (4) data analysis software (b) (4) software has been used for data

integration in RPT-640 which may be acceptable as long as entire data set is included for

analysis. Though the experiments were performed quite differently in RPT-640 and RPT-592,

the results do not show much variation. This might be due to liberal use of fitting parameters for

the (b) (4) data. Nevertheless, more improvements in (b) (4) resolution is suggested to discern

minor virion species that might be present. In general, the results were reasonable.

**Table 1** The list of provided documents from CBER/OTAT.

Document	Owner	Content	Samples tested
SOP-263	Avexis (sponsor)	GMP method description for using (b) (4) to determine the relative amount of (b) (4) virion particles in AAV	AVXS-101, Reference Sample (RS)
PRO-590	Avexis (sponsor)	Establishing acceptable specification in SOP-263	AVXS-101, RS
RPT-592	(b) (4)	Validation of the SOP-263	Full AAV, RS; (b) (4), control sample (CS)
RPT-640	Avexis (sponsor)	Method Transfer report of the SOP-263	AVXS-101, RS (Drug Product, (b) (4))
RPT-691	Avexis (sponsor)	Method Optimization for SOP-263	(b) (4)
RPT-735	Avexis (sponsor)	Establish % (b) (4) Specification for SOP-263	Drug Product, (b) (4)
RPT-779	Avexis (sponsor)	Determining (b) (4) of (b) (4)	AVXS-101, GMP lots; AVXS (b) (4)

2 pages have been determined to be not releasable: (b)(4)

(b) (4)

(b) (4)

### Comments to the Firm

1. In RPT-779, please explain how the (b) (4) was made and why only (b) (4) was tested.
2. The samples were (b) (4) (b) (4) prior to the run. Some additional time is required for instrument setup (b) (4) (probably (b) (4)). The current SOP-263 procedure does not account for the (b) (4) of possibly larger particles during this time. Please confirm that larger virus particles (which could (b) (4) /setup time) were not present in the test solutions. (b) (4) methods like (b) (4) might be useful for measuring larger virion (b) (4). Probably a separate protocol to control (to show nonexistence) larger virions is needed because the current (b) (4) was also too high for their detection.
3. From the data submitted in RPT 592 Figure 10.7 - 10.37, about (b) (4) usable data scans were analyzed, which are an inadequate number of usable scans with respect to the strict (b) (4) parameter input applied (confidence level F-ratio of (b) (4)). In RPT-640, although the experiments were run for (b) (4) scans, only about (b) (4) scans are usable i.e. contain a meaningful particle size information. Using all (b) (4) scans (as shown in current data) for (b) (4) analysis will certainly improve fitting RMSD (flat baseline fit well) but would not yield essential improvement in virion size resolution. SOP 263, section 10.1.5 table mentions scan count of (b) (4). Therefore, A wide disagreement has been found in the number of scans used for (b) (4) data collection. Acquiring more number of **usable** (b) (4) data scans is needed for a complete identification and quantification of various (b) (4) species and the consistent use of number of scan is critical for the drug control.
4. Please analyze only the required number of scans to get a correct representation of fit quality and resolution. Please analyze about (b) (4) scans before the (b) (4) or before (b) (4) (ref.[1]). The suggestion from RPT-691 section 6.1 as well as (b) (4) could be exercised to achieve more scans before (b) (4) is completed. Literature also suggested (b) (4) for higher data resolution (b) (4). Any modification to SOP-263 will affect the (b) (4) results and need to be reported and reviewed by the agency.

5. Higher confidence level (F-ratio) yielded (b) (4) around the mean (reported in RPT-691 section 7.4.3). Since, there is no sufficient resolution and no supporting (b) (4) data to show that samples were (b) (4) virion particles only; a lower confidence level (F-ratio equal to (b) (4)) is recommended to identify all possible virion species present in the solution (b) (4).
6. Large variation in fitted frictional ratios (RPT-592, from (b) (4) - (b) (4)) across the datasets were observed. Frictional ratio is characteristic of shape parameters of the molecule. Please report the range of variation for frictional ratio values. The frictional ratio variation, indicative of virion (b) (4) shape, should be included as an acceptance criterion for lot release.
7. The current acceptance criteria (%RSD of s-value and %Area) were based on the integration of (b) (4) data results using (b) (4) only. (b) (4) is considered as a down-stream software to visualize the (b) (4) fitting results out of (b) (4). Therefore, (b) (4) fitting parameters like confidence level (F-ratio) should be specified in SOP-263 and fitting results like RMSD and frictional ratio (b) (4) need to be included as part of acceptance criteria to ensure consistent fitting quality.
8. The (b) (4) for (b) (4) are about same and is evident from (b) (4) as well (RPT -779 Tables 3,4, and 5). What was the reasoning behind using (b) (4) for the (b) (4) experiments? The firm may adopt the published method of using (b) (4) in quantifying AAV (b) (4) (b) (4) (b) (4). Alternatively, (b) (4) probably does not need to be measured and considered for calculating percent population of (b) (4) (b) (4) will be used as is.
9. The firm has used relative quantification as specification for (b) (4) species. The (b) (4) lot release assay should also include absolute quantification measure as acceptance criterion, because the relative percentage can be kept similar from lot to lot with differences in the total virion (b) (4). The LOD and LOQ, currently specified by (b) (4), should also be specified using absolute quantity measure.
10. For fitted data (b) (4) integrations, where an adequate (b) (4) (b) (4) (Figure 4) is not achieved, (b) (4) integration boundaries (maximum integration range) should be specified in SOP-263. The integration boundary will, in case of (b) (4) results, help to avoid integrating unknown virion species hidden in the (b) (4) to be counted as (b) (4) (b) (4).

(b) (4)

Answers to OTAT/CBER's Specific questions:

1. In SOP-263, are the procedures for setting the meniscus appropriate?  
Yes, the current data provided are analyzed with the appropriate setting for meniscus position.
2. SOP-263 allows two different f-ratios (b) (4). Is this appropriate, do the instructions clearly describe how to select an f-ratio, and has the use of two different f-ratios been appropriately validated?  
No. The use of two different confidence level (F-ratio) has not been validated in the report. Using two different F-ratios is not appropriate unless being demonstrated that there is no difference in the results. The use of F-ratio has been briefly described in RPT-691 page 7, section 7.4. In fact, F-ratio of (b) (4) was recommended in the literature but not used by the firm in this analysis.
3. RPT-640 mentions a known (b) (4) issue for the (b) (4) (b) (4). Has the applicant dealt with this (b) (4) issue appropriately?  
(b) (4) is a newer generation instrument. We do not have detailed information of the (b) (4) issue of this instrument. The firm mentioned that they are in discussion with the instrument manufacturer in order to correct the issue.
4. System suitability in SOP-263 consists of analysis of a reference standard within the past 7 days. Are the system suitability criteria adequate?  
Yes, this should be adequate. System suitability criteria were probably designed by the (b) (4) instrument manufacturer (b) (4). This criterion might have been set for

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instrument used on daily basis. Instrument is stable for at least couple of months under light use (once every couple of weeks).

5. Note: we are unclear on how the extinction coefficients were obtained for (b) (4) (b) (4). We are requesting more information from the applicant, and we will forward this information to the CDER consult reviewer when available.  
We have raised some questions as well above.

**References:**

1. (b) (4)
2. (b) (4)
3. (b) (4)
4. (b) (4)