RESPONSE TO FDA COMMENTS ON CMC RECEIVED ON 22 OCTOBER 2021

The Sponsor acknowledges FDA comments on CMC topics (in **Bold)**

ITEM 1:

Regarding determination of Protein Expression from mRNA using (b) (4) (b) (4) **(SOP # 0937):**

a) (b) (4) **is used for determination of protein expression in the** (b) (4) **in-vitro translation assay. However, there is no information provided for the qualification of a new lot of** (b) (4) **to verify its suitability before using in release testing. Please provide the qualification procedure for new** (b) (4) **lots.**

Sponsor Response:

The Sponsor does not have a qualification procedure for new (b) (4) (b) (4). (b) (4) performs (b) (4) test for each lot and provides a Certificate of Analysis.

The Sponsor confirms that each SOP-0937 test includes a positive control, and the positive control must meet system suitability criteria for the test to be valid [(b) (4) (b) (4) (b) (4) Failure to meet this criterion would indicate any issue with the assay or with the lot of (b) (4) used.

ITEM 2:

Regarding determination of % RNA Encapsulation by (b) (4) (b) (4) **(SOP# 1000):**

a) Per SOP 1000, a Response Factor (RF) of (b) (4) **is used for determination of free mRNA. Please provide in detail how the RF was determined.**

Sponsor Response:

The Sponsor performed method development studies to demonstrate that a response factor (RF) (b) (4) was applicable to this assay as shown here in Figure 1. Method development studies performed have evaluated an impact of (b) (4) on the RF and have demonstrated that the RF exhibits little, if any, (b) (4). Figure 1 illustrates the (b) (4) (b) (4) (b) (4) (b) (4) (b) (4) establishes the RF for the assay.

Figure 1: **Principle of the** (b) (4) **for Determination of Free mRNA**

b) Please provide the validation data (Attachments 1 -3) in the validation report QC-MVR-0009.

Sponsor Response:

The validation data (Attachment 1, Attachment 2 and Attachment 3) from validation report QC-MVR-0009 are provided.

ITEM 3:

Regarding release testing sites:

a) If you are planning to perform release testing at Quality Control laboratory Annex (Dedham, MA) or any other site, please provide the method transfer qualification reports.

Sponsor Response:

The Sponsor is performing releasing testing for the following methods at the Dedham site: SOP-0278 (Appearance), SOP-0288 (pH), SOP-0950 (Container Content), SOP-0994 (%PolyA Tail / tailed variant mRNA), SOP-0996 (Purity / Impurities), SOP-0997 (%5' Capped), SOP-0999 (Total RNA Content), SOP-1001 (Lipid ID, Content and Impurities), and SOP-1229 (Container Content). Refer to method transfer reports MQR-0281 and MQR-0310.

ITEM 4:

Regarding SOP-0997 "Determination of (b) (4) (b) (4) **: Since the accuracy and precision of** (b) (4) **reasonably established but the accuracy of measuring** (b) (4) **is not, we recommend calculating the** (b) (4)

(b) (4)

(b) (4)

Please comment.

Sponsor Response:

The Sponsor has taken this recommendation into consideration and agrees with this assessment and will revise the method accordingly during the next revision cycle.

ITEM 5:

Regarding SOP -0998 "Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering" for drug substance (DS) mRNA-1273 LNP and drug product (DP) mRNA-1273 LNP: In order for CBER to perform the test, additional information is needed. Please provide a formula to calculate the (b) (4) **of a sample as described in section 8.10.5.2 or attachment 5.**

Sponsor Response:

The (b) (4) is calculated as follows:

(b) (4)

ITEM 6:

Regarding document QC-MVR-0011 "Validation Report of SOP-0998":

a) Assay accuracy studies were conducted using standards diluted in (b) (4) **. Please demonstrate linearity and accuracy of the assay in the product matrix. Since DS and DP** (b) (4) **, we suggest including a qualified DS or DP lot as control in each sample run.**

Sponsor Response:

(b) (4)

(b) (4)

b) Linearity of the method was not validated, please demonstrate the linearity of method for DS and DP matrix with defined acceptance criteria.

Sponsor Response:

Please refer to the response described for item 6a.

c) Validation studies were performed at your Norwood, MA facility. Please confirm that this is the only site performing this release test. Please note that if you intend for other sites to perform this or other DS or DP release tests, transfer reports need to be submitted to your file.

Sponsor Response:

Currently the Norwood, MA facility is the only testing site for SOP-0998. The Sponsor acknowledges that a transfer report must be submitted to the file prior to performing SOP-0998 testing at any other testing sites.

ITEM 7:

Regarding SOP-0999 "Determination of RNA Concentration in (b) (4)

(b) (4)

a) Attachment 2 provides a typical (b) (4) **standard preparation, in which a** (b) (4) **. Please explain the measures taken, such as** (b) (4) **, to ensure resolution of** (b) (4) (b) (4) **.**

Sponsor Response:

(b) (4)

(b) (4) Refer to below images for representative (b) (4) (Figure 2).

Figure 2: (b) (4)

(b) (4)

(b) (4)

b) Please provide representative (b) (4) **demonstrate** (b) (4) **details. We recommend** (b) (4) **be included in your SOP if they differ from the reference standard** (b) (4) **.**

Sponsor Response:

Refer to (b) (4) (Figure 3 - Figure 6) provided for (b) (4) (b) (4) The (b) (4) do not differ from those of the (b) (4) Reference Standard. However, the sponsor acknowledges that (b) (4) should be added into SOP-0999 and will plan to do so in the next SOP revision cycle.

Figure 3: (b) (4)

(b) (4)

(b) (4)

Figure 4: (b) (4)

(b) (4)

(b) (4)

(b) (4)

Figure 5: (b) (4)
(b) (4)

(b) (4)

Figure 6: (b) (4)
(b) (4)

(b) (4)

ITEM 8:

Regarding document QC-MVR-0008 "Validation Report of SOP-0999":

a) Please provide (b) (4) (b) (4) that were used in the linearity study.

Sponsor Response:

Please refer to below images (Figure 7 – Figure 10) for (b) (4) representing linearity study.

Figure 7: (b) (4) (Linearity Study QC-MVR-0008)

(b) (4)

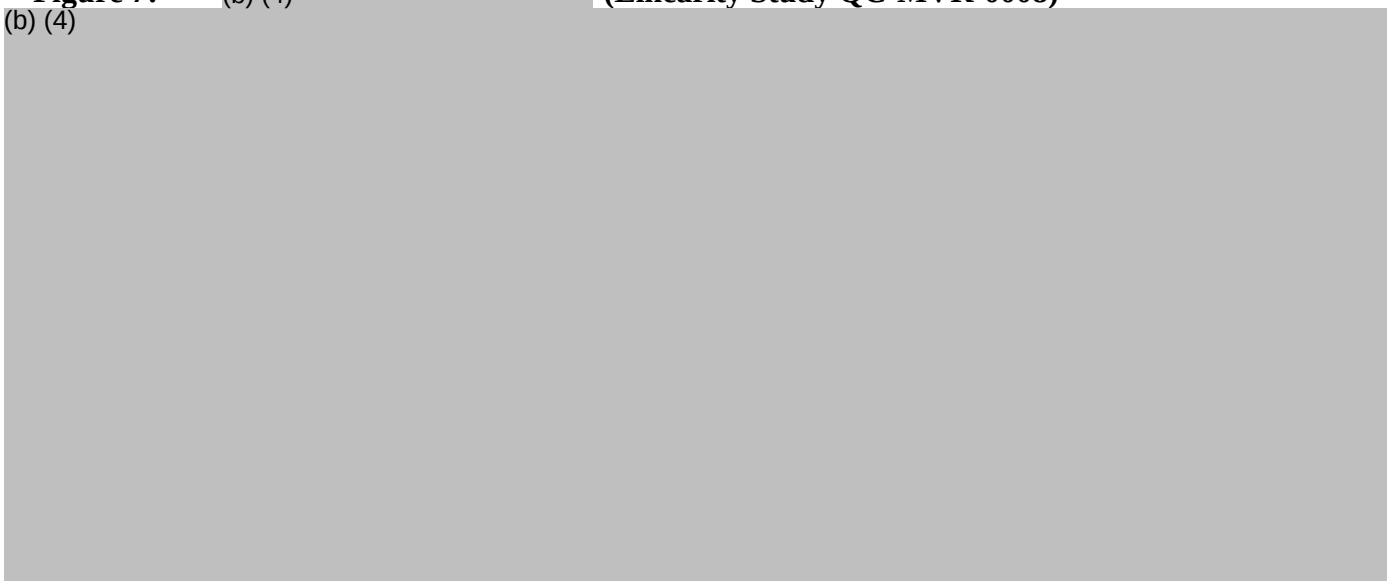

Figure 8: (b) (4) (Linearity Study QC-MVR-0008)

(b) (4)

Figure 9: (b) (4) **(Linearity Study QC-MVR-0008)**

(b) (4)

Figure 10: (b) (4) **(Linearity Study QC-MVR-0008)**

(b) (4)

b) It is not clear how the spiked samples were prepared. To demonstrate the method is suitable for its intended purpose, we expect accuracy and linearity of the method to be validated with samples in DS/DP matrix spiked with known amounts of mRNA, and the theoretical amount of the spiked mRNA measured with an orthogonal method. Please describe how the spiked samples were prepared and if they are not representative of DS and/or DP, provide justification.

Sponsor Response:

The linearity steps are described in detail within the method validation protocol (QC-MVP-0008). Known amounts of CX-024414 mRNA, for which the concentration was measured by orthogonal test method SOP-0995 (mRNA Concentration by (b) (4)), were spiked into (b) (4) (b) (4) (b) (4) This matrix solution is representative of mRNA-1273 samples. Refer to the following details provided below as clarification.

Linearity Dilution Scheme for spiked samples:

Step 1:

(b) (4) (b) (4) Refer to Table 1.

Step 2:

(b) (4) (b) (4). Refer to Table 1.

Table 1: Linearity Stock Dilution Scheme from QC-MVP-0008

(b) (4)

Step 3: (b) (4) (b) (4) **:**

(b) (4)

Table 2: Linearity Stock Dilutions Details

(b) (4)

Step 4:
The prepared samples in Step 3 (Table 2) were diluted in method diluent. Each of the samples was prepared in triplicate (n=3) and tested per SOP-0999 (refer to Table 3).

Table 3: Linearity Sample Dilutions Details

Sample conc. mg/ml	Target conc. (mg/mL)	Sample Volume (µL)	Method Diluent volume (µL)	Preparations per level	(b) (4)
(b) (4)				3	
				3	
				3	
				3	
				3	