RESPONSE TO FDA COMMENTS ON INFORMATION REQUEST#32 for BLA 125752 RECEIVED ON DECEMBER 10, 2021

The Sponsor acknowledges INFORMATION REQUEST#32 dated 10 DECEMBER 2021 in (**BOLD**)

Product: COVID-19 Vaccine, mRNA (SPIKEVAX)

Subject: Analytical method procedure and validation

In reviewing your response to information request (IR) #26, we have an additional IR regarding the endotoxin in the Drug Product using the (b) (4) **LAL procedure.**

ITEM 1:

We would like to clarify that we do not object to the DP release test for endotoxin being performed on a sample that has been extracted with (b) (4) **. However, in addition to those results, we request you measure and report the results from samples that are not treated prior to release testing so that the total endotoxin activity of the product is reported and the specification for the product as a whole, is met. Please state the date by which data will be submitted to support the additional method. Please request a telecon if you would like to discuss this request.**

Sponsor Response:

The Sponsor acknowledges that in principle, it would be expected to evaluate the sample without pretreatment to demonstrate interference prior to application of a pretreatment step. USP <85> contains instructions that indicate when endotoxin recovery is out of the specified range of (50-200%) a treatment may be applied. Early development work with the Sponsor's LNPs determined that testing without pretreatment steps did not provide consistent and satisfactory recoveries. The pre-treatment method was developed with consideration to the known challenges of working with LNPs:

(b) (4)

2. An additional challenge in working with the LNP products results from endotoxins being lipophilic and the potential for them to be become entrapped in LNP (Dobrovolskaia, 2018) (Paul Harmon, 1997). The LAL assay is only able to measure free endotoxin. The drug delivery system utilized by the Sponsor includes encapsulated mRNA. In order to ensure the assay is able to detect any endotoxin that may be present, including entrapped endotoxin, sample pretreatment steps were incorporated.
   a. (b) (4)
   b.

Characterization studies to evaluate the assay performance at three dilutions (b) (4) (b) (4) were performed for mRNA-1273 DP. The assay included the controls as mentioned (b) (4) (b) (4)). The assay continued to show optimal performance at (b) (4)

(b) (4)

The following data describes:

- Table 1. mRNA-1273 0.5 mg/mL Drug Product Endotoxin Method Verification Data (b) (4))
- Table 2. mRNA-1273 Variant Drug Product Endotoxin Method Verification and Characterization Data - (b) (4) (b) (4))
- Table 3 mRNA-4157 1.0 mg/mL Drug Product Endotoxin Method Verification Data ((b) (4))
- Table 4. SM-102 Raw Material Endotoxin Method Verification

Table 1. mRNA-1273 Drug Product Endotoxin Method Verification Data ((b) (4)

(b) (4)

Table 2: mRNA-1273 Variant Drug Product Endotoxin Method Verification and Characterization Data - (b) (4)

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

Table 3: mRNA-4157 1.0 mg/mL Drug Product Endotoxin Method Verification Data (b) (4)

(b) (4)

(b) (4)

Table 4: SM-102 Raw Material Endotoxin Method Verification

(b) (4)

The collective data obtained through the scientific literature, the assessment of samples assessed without treatment support the continuation of the established method to ensure that both the free endotoxin and any potential encapsulated endotoxin are assessed to provide the assay with the greatest sensitivity.

In light of this new information presented in the response, the Sponsor will work with the Agency to assess any additional method development and deliverables as a post-marketing commitment once the BLA is approved.

Bibliography

Christopher W. Lester, J. T. (2019). Bacterial Endotoxins Testing in Lipid-Based Drug Formulations Using Liquid-Liquid Extraction. *American Pharmaceutical Review*.

Dobrovolskaia, B. W. (2018). Considerations and Some Practical Solutions to Overcome Nanoparticle Interference with LAL Assays and to Avoid Endotoxin Contamination in Nanoformulations. In S. E. McNeil, *Characterization of Nanoparticles Intended for Drug Delivery, Methods in Molecular Biology* (pp. 23-32). New York: Springer Nature.

Paul Harmon, D. C.-L. (1997). The Release and Detection of Endotoxin from Liposomes. *ANALYTICAL BIOCHEMISTRY*, 139-146.

ITEM 2:

Please provide the positive product control (PPC) recovery for drug product (beginning, middle and end samples) tested under Associates of Cape Cod (ACC) report numbers# 0121-012TESVR, 0421-290TESCVR, 0421-290TESCVR, 0521-099TESCVR, 0820-082TESVR and 1220-223TESVR.

Sponsor Response:

The positive product control (PPC) recovery for drug products are provided in the following table.

Table 5: Positive Product Control (PPC) recovery

DP Lot Number	ACC Report Number	Maximum Valid Dilution (MVD)	PPC Recovery of Drug Product	PPC Recovery of Challenge Sample
030M20	0121-012TESVR	(b) (4)		
038D21	0421-290TESCVR[(2)]			
939888	0521-099TESCVR			
001H20	0820-082TESVR			
032L20	1220-223TESVR			

1) Lot 030M20 was part of PPQ, so (b) (4)
2) Duplicate data request