INX100594280: PF-07302048 (Comirnaty) Residual DNA Characterization Report

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DOCUMENT REVIEW AND APPROVAL:
The following responsible areas have reviewed and approved this memorandum:

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REVISION HISTORY

Revision Level	Change:	Rationale for change:
1.0	Original Version	N/A

1. EXECUTIVE SUMMARY

This report presents data characterizing the residual DNA template present in the Comirnaty vaccine. The findings presented herein are consistent with the Residual DNA Risk Assessment, which established that the presence of residual DNA in Comirnaty, which is below the internationally recommended threshold levels [4], and the non utilized sequence elements (like SV40 promoter) found at trace levels in the residual DNA, pose no safety risk to vaccinees. The summary findings are as follows:

- Comirnaty drug substance (DS) residual DNA template results are similar across all manufacturing sites and the manufacturing process consistently yields DS that complies with the established specification, which is based on WHO recommendations of not more than 10 ng per dose [4].

- Residual DNA testing on drug product (DP) yields similar or lower residual DNA compared to direct assessment in DS. Thus, testing for residual DNA template in DS provides an appropriate assessment of DNA levels present in DP.

- All Comirnaty DP dosage forms for adult, adolescent and pediatric populations comply with WHO recommendations of not more than 10 ng residual DNA per dose.

- Residual DNA size distribution data demonstrate the Comirnaty DS manufacturing process, which includes an enzymatic digestion of DNA template followed by a 2-step purification, yields short residual DNA template fragments. Most digested fragments are smaller than [redacted] [redacted] Fragments larger than [redacted].

- All Comirnaty DS batches were confirmed positive for SV40 sequence elements, as expected based on the understanding of the residual DNA composition. [redacted]

- [redacted]

CCI [redacted]. The plasmid DNA used in Comirnaty production does not contain genes associated with causing cancer (oncogenes) and is not replication competent in mammalian cells [7], i.e., it cannot amplify in the human body. Therefore, it is not considered to pose a safety risk.

2. BACKGROUND INFORMATION

Comirnaty is an mRNA-based vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 vaccines have prevented approximately 14.4 million deaths, of which Comirnaty contributed a large extent [1]. Following administration of more than 1 billion doses worldwide, Comirnaty has a well-established safety and reactogenicity profile and a confirmed overall favorable benefit/risk. The vaccine's mRNA component is made using a linearized plasmid DNA template enzymatically cut from a circular DNA plasmid. The plasmid DNA contains sequences that support its generation as well as regions important for production of mRNA. CCI [redacted]

In subsequent steps of the vaccine manufacturing process, the bulk of the DNA template is further removed by enzymatic digestion followed by a 2-step purification. The resulting purified mRNA, known as the Drug Substance (DS), is later formulated into lipid nanoparticles (LNP) for the final vaccine Drug Product (DP). No DNA material is used or introduced in the manufacturing process other than the initial use of the DNA plasmid.

The DNA template (circular plasmid and linear DNA) is a well characterized starting material, manufactured under Good Manufacturing Practices (GMP) conditions which ensures a high level of control and quality in the production process. DNA template is released against the globally registered specifications. The mRNA DS is tested for several quality attributes, including residual DNA, which is considered an intrinsic impurity, routinely found in all cell-derived biological products, such as vaccines based on inactivated or attenuated microorganisms, recombinant protein vaccines, DNA plasmid and viral vector vaccines [2, 3]. All Comirnaty doses released for use globally, meet the residual DNA requirements as defined by approved specifications and the World Health Organization (WHO) standards [4].

3. PURPOSE

The purpose of this document is to present data characterizing the residual DNA template and its non utilized SV40 sequence elements that may be present in the vaccine. The data presented in this document address the following:

- Evaluation of residual DNA template batch analysis data from the registered DS manufacturing sites
- Assessment of residual DNA template quantitation in DP samples
- Estimated residual DNA template content in DP
- Characterization of the size distribution of residual DNA template fragments
- Assessment of the presence of SV40 sequence elements in residual DNA template
- CCI [redacted]

4. EVALUATION OF RESIDUAL DNA BATCH ANALYSIS DATA

The World Health Organization (WHO) has established specific acceptance criterion recommendations for residual DNA levels, considered safe for human use in biological products, including mRNA vaccines (using DNA template as starting material). The acceptance criterion for residual DNA in Comirnaty's DS (≤ 330 ng DNA/mg RNA) aligns with WHO recommendations of not more than 10 ng per dose, translating to a total of 9.9 ng or less per 30 µg dose.

Residual DNA is routinely controlled in the DS material using an appropriate validated, quantitative Polymerase Chain Reaction (PCR) assay. The PCR assay is a widely recommended standard for residual DNA testing [5, 6] and approved by regulatory authorities worldwide. The assay used in Comirnaty testing is highly sensitive, enabling detection and quantification of trace amounts of DNA. To date, all commercialized DS batches of Comirnaty meet the regulatory approved acceptance criterion for residual DNA.

Residual DNA release testing results for 236 commercial Comirnaty DS batches were evaluated. The summary of this evaluation is presented in Table 1.

The commercial DS batches were comprised of:

- Batches manufactured ranging from 2020 to 2023
- Batches manufactured at three commercial manufacturing sites: Pfizer Global Supply (PGS) Andover, PGS Grange Castle, and BioNTech Marburg
- Four unique variants (Wildtype/Original and Omicron BA.1, BA.4/BA.5, and XBB.1.5)

Table 1 Residual DNA Batch Analysis Evaluation Summary

Parameter	All Sites Residual DNA ng DNA/mg RNA	PGS Andover Residual DNA ng DNA/mg RNA	PGS Grange Castle Residual DNA ng DNA/mg RNA	BioNTech Marburg Residual DNA ng DNA/mg RNA	Release Specification ng DNA/mg RNA
Minimum	CCI				≤ 330
Maximum					
Average					
Median					
Standard Deviation					

Conclusion:

This evaluation demonstrates residual DNA template results are similar across all manufacturing sites and the Comirnaty DS manufacturing process consistently yields DS which complies with the established specification.

5. ASSESSMENT OF RESIDUAL DNA IN DRUG PRODUCT SAMPLES

Purified DS is forward processed into DP through the combination mRNA DS with lipids to form lipid nanoparticles (LNP), which encapsulate the mRNA. Residual DNA template

present in the DS is expected to be present in the DP as there are no purification steps for further removal of residual DNA within the DP manufacturing process. Therefore, routine quantitation of residual DNA template in DS provides an accurate assessment of residual DNA present in DP. An assessment of residual DNA in DP samples was performed for characterization and to demonstrate the suitability of testing at DS.

Each DP sample was analyzed with and without sample extraction step. Two separate nucleic acid extraction techniques were employed for DNA (and RNA) extraction from the LNPs in three DP samples.

CCI # GN0005 CCI # HD9364Z CCI

[redacted]

CCI # 22-DP-01012 CCI

[redacted]

5.1. Method

The level of residual DNA template in DP test samples was determined CCI [redacted]

5.2. Results and Analysis

Results of the residual DNA analysis in exemplary DP samples are presented in Table 2.

The results demonstrate the qPCR assay is capable of residual DNA template quantification in DP samples, with or without sample extraction treatments. This observation is consistent with other PCR-based methods used in the analysis of DP samples, which do not require RNA extraction prior to analysis CCI [redacted]. Additionally, both manual and automated nucleic acid extraction methods yielded residual DNA template results comparable to analysis without nucleic acid extraction.

The DP results are compared to the residual DNA template results for the corresponding DS batches used in the manufacture of each DP lot. For the two bivalent DP lots (GN0005 and 22-DP-01012), the Wildtype (WT/Original) and Omicron BA.4/5 DS mRNA are mixed at a [redacted] to formulate the final bivalent DP. Therefore, the average residual DNA result for the two DS batches is used for comparison. The residual DNA template measured in DP samples (see Table 2, column 'Residual DNA Measured in DP') are comparable to, or, lower than residual DNA template results measured during DS release testing (see Table 2, column 'Residual DNA for Input DS Batches'). [redacted]

Table 2 Drug Product Residual DNA Analysis

Drug Product Sample	Residual DNA Measured in DP ng DNA/mg RNA	Residual DNA for Input DS Batch(es) ng DNA/mg RNA	% Recovery of Average DS Result
GN0005 (WT / BA.4/5 bivalent) [redacted]	[redacted]	[redacted]	[redacted]
GN0005 (WT / BA.4/5 bivalent) [redacted]	[redacted]	[redacted]	[redacted]
22-DP-01012 (WT / BA.4/5 bivalent) [redacted]	[redacted]	[redacted]	[redacted]
22-DP-01012 (WT / BA.4/5 bivalent) [redacted]	[redacted]	[redacted]	[redacted]
HD9364Z (XBB.1.5) [redacted]	[redacted]	[redacted]	[redacted]
HD9364Z (XBB.1.5) [redacted]	[redacted]	[redacted]	[redacted]

[1]Average of the two input DS batch residual DNA results for bivalent DP batches used for % Recovery calculation.
[2]Monovalent DP batch, one input DS batch only.

Using the residual DNA template batch analysis data presented in Table 1, the estimated residual DNA template content within each DP dose is calculated and summarized in Table 3. All DP dosage forms comply with WHO recommendations of not more than 10 ng per dose. [redacted]

CCI [redacted]

Table 3 Estimated Residual DNA Content per Drug Product Dose

Residual DNA Template in DS ng DNA/mg RNA	Residual DNA per Drug Product Dose		
	3 µg Pediatric	10 µg Adolescent	30 µg Adult
Average (all sites)	CCI [redacted]		
Maximum (all sites)			
Minimum (all sites)			
Acceptance Criterion Limit	330	CCI [redacted]	

Conclusion:

Testing at DP yields a similar but more variable assessment of residual DNA in the vaccine, which is attributed to the requirement for low levels of residual DNA to be liberated from the LNP for detection and quantification. Thus, testing for residual DNA template in DS provides an appropriate assessment of DNA levels present in DP.

All DP dosage forms adult, adolescent and pediatric populations comply with WHO recommendations of not more than 10 ng per dose. As described in the Residual DNA Risk Assessment, the residual plasmid DNA template in Comirnaty is not considered to pose a safety risk.

6. DRUG SUBSTANCE SELECTED FOR RESIDUAL DNA CHARACTERIZATION

Subsequent characterization of residual DNA in Comirnaty is performed using DS samples, which is consistent with the routine batch testing performed for the vaccine. Twelve DS batches were selected to include the three commercial manufacturing sites (PGS Andover, PGS Grange Castle, and BioNTech Marburg), four unique variants (Wildtype/Original, Omicron BA.1, Omicron BA.4/BA.5, and XBB.1.5) and residual DNA template CCI [redacted]

CCI . Table 4 describes the DS batches and characterization analyses.

Table 4 Drug Substance Samples for Residual DNA Characterization

Drug Substance Sample Description				Residual DNA Characterization Analyses		
Batch	**Manufacturing Site**	**Strain**	**Residual DNA[1]** ng DNA/mg R	**Size Dist. by TapeStation**	**SV40 presence by PCR**	CCI
GA5174	Andover	BA.1				
GF1001	Andover	WT				
GH5745	Andover	BA.4/5				
GJ3638	Andover	BA.4/5				
GJ6907	Andover	BA.4/5				
HD1999	Andover	XBB.1.5				
AB00050	Marburg	XBB.1.5				
AB00051	Marburg	XBB.1.5				
AB00060	Marburg	XBB.1.5				
HG3789	Grange Castle	XBB.1.5				
HG4918	Grange Castle	XBB.1.5				
HG4921	Grange Castle	XBB.1.5				

CCI

[1] Residual DNA template measured at DS batch release.
[2] Results from [redacted] DS batches determined to be sufficient and representative CCI

7. CHARACTERIZATION OF RESIDUAL DNA SIZE DISTRIBUTION

7.1. Method

The fragment size distribution of residual DNA template in DS was estimated CCI

[redacted]

CCI [redacted]

7.2. Results and Analysis

CCI [redacted] Based on the FDA guidance for residual DNA size reduction below the approximate size of a functional gene (~200 bp) [8], the CCI assay was used for determining the fragment size CCI [redacted].

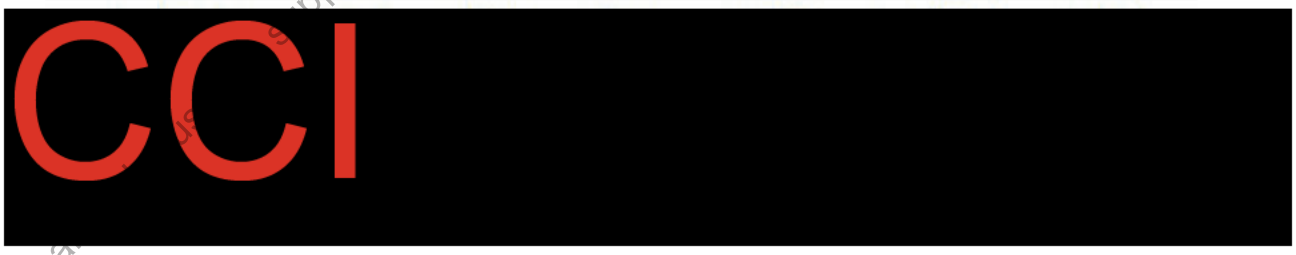
The results of the residual DNA size distribution testing are provided in this section. CCI [redacted] **Figure 1** and **Figure 2** show the relative fragment size distribution

of residual DNA in the tested DS materials as estimated CCI

[redacted]

CCI

[redacted]



CCI

CCI

CCI

Table 5. Percent Region Molarity (pmol/l) of Fragments CCI

DS Batch	% Region Molarity (pmol/l) CCI CCI	% Region Molarity (pmol/l) CCI CCI	% Region Molarity (pmol/l) CCI CCI	Average (%)	Standard Deviation	% RSD
GA5174	CCI					
GF1001						
GH5745						
GJ3638						
GJ6907						
HD1999						
AB00050						
AB00051						
AB00060						
HG3789						
HG4918						
HG4921						

Table 6. Percent Region Molarity (pmol/l) of Fragments CCI

DS Batch	% Region Molarity (pmol/l) CCI CCI	% Region Molarity (pmol/l) CCI CCI	% Region Molarity (pmol/l) CCI CCI	Average (%)	Standard Deviation	% RSD
GA5174	CCI					
GF1001						
GH5745						
GJ3638						
GJ6907						
HD1999						
AB00050						
AB00051						
AB00060						
HG3789						
HG4918						
HG4921						

Conclusion:

The size distribution data demonstrate the Comirnaty DS manufacturing process, which includes an enzymatic digestion of DNA template followed by a 2-step purification, yields short residual DNA template fragments. CCI

CCI [redacted]

8. CHARACTERIZATION OF SV40 SEQUENCE ELEMENTS IN RESIDUAL DNA

The plasmid-derived linear DNA template, serving as a starting material for synthesizing Comirnaty vaccine's DS mRNA, incorporates additional DNA sequence elements common in multipurpose plasmid DNAs such as antibiotic resistance genes and other DNA sequence elements which are not utilized for DS mRNA production. These typical DNA sequence elements include restriction and cloning sites, as well as sequences aiding transcription and polyadenylation in eukaryotic cells such as the CCI [redacted] The properties of each sequence element are well known, and the functional roles have been documented (Section 3.2.S.2.3).

Based on the size distribution data presented in Section 7, the composition of residual DNA template fragments CCI [redacted] This is confirmed using a CCI assay CCI designed to specifically detect CCI [redacted].

8.1. Method

The presence of CCI [redacted] within residual DNA template in DS samples was determined CCI [redacted]

CCI [redacted]

Table 7. PCR Primer Design for Detection of SV40 Sequence Elements

Sequence Element	Origin	Starting Position (bp)	Ending Position (bp)
CCI [redacted]	Backbone cloning vector sequence	CCI [redacted]	

8.2. Results and Analysis

The results of the PCR testing for presence of CCI [redacted] elements are shown in. All DS batches were confirmed positive for these SV40 sequence elements, as expected based on understanding of the residual DNA composition.

Table 8. CCI Results for Detection of CCI

DS Batch/Sample	Result	Mean Ct Value
G3789	CCI	CCI
G4918		
G4921		
AB00050		
AB00051		
AB00060		
GA5174		
GF1001		
GH5745		
GJ3638		
GJ6907		
HD1999		
High Assay Control		
Low Assay Control		

[1]The Low Assay Control is used as a reference to indicate positive or negative result for samples.

Estimation of SV40 Sequence Element Content in Residual DNA:

CCI

Conclusion:

All DS batches were confirmed positive for SV40 sequence elements, as expected based on the understanding of the residual DNA composition. CCI

CCI [redacted]

9. CCI [redacted]

Enzymatic digestion and filtration methods used in the DS process are highly effective at linearizing and removing the majority of DNA. As the approved Linearization Efficiency (Plasmid Topology) acceptance criterion CCI [redacted] (Section 3.2.S.2.3), CCI [redacted] As the plasmid DNA contains sequence elements required for amplification in bacteria, an CCI [redacted] assay is a highly sensitive means of detecting CCI [redacted]

9.1. Method

CCI [redacted]

[redacted]

Table 9. DS Batches Selected for CCI [redacted]

DS Batch	Strain	DS Concentration mg/ml
GA5174	BA.1	CCI [redacted]
GF1001	WT	
GH5745	BA.4/5	
GJ3638	BA.4/5	
GJ6907	BA.4/5	
HD1999	XBB.1.5	

CCI [redacted]

Table 10. CCI [redacted] Experimental Design

Sample Number	DS Batch	DS Volume		Positive (+) Control	TE or Positive (+) Control
1	GA5174	13	µL	[redacted]	
2	GF1001	13	µL		
3	GH5745	13	µL		
4	GJ3638	13	µL		
5	GJ6907	13	µL		
6	HD1999	13	µL		
7	Null (TE)	13	µL		
8	GA5174	13	µL		
9	GF1001	13	µL		
10	GH5745	13	µL		
11	GJ3638	13	µL		
12	GJ6907	13	µL		
13	HD1999	13	µL		
14	Null (TE)	13	µL		

CCI [redacted]

CCI [redacted]

CCI [redacted]

9.2. Results and Analysis

Table 11 CCI [redacted] **Assay Results**

Sample Number	DS Batch	Strain	Positive Control (+) CCI Included?	Plate Counts
1	GA5174	BA.1	CCI [redacted]	CCI [redacted]
2	GF1001	WT		
3	GH5745	BA.4/5		
4	GJ3638	BA.4/5		
5	GJ6907	BA.4/5		
6	HD1999	XBB.1.5		
7	Null (TE)	None		
8	GA5174	BA.1		
9	GF1001	WT		
10	GH5745	BA.4/5		
11	GJ3638	BA.4/5		
12	GJ6907	BA.4/5		
13	HD1999	XBB.1.5		
14	Null (TE)	None		

CCI [redacted]

CCI

[redacted]

[redacted]

CCI [redacted]

[redacted]

Conclusion:

CCI [redacted]

CCI . The plasmid DNA used in Comirnaty production does not contain genes associated with causing cancer (oncogenes) and is not replication competent in mammalian cells [7], i.e., it cannot amplify in the human body. Therefore, it is not considered to pose a safety risk (See Residual DNA – Risk Assessment).

10. CONCLUSION

The safety profile of Comirnaty is well characterized after administration of more than 1 billion doses to individuals worldwide over the last 3 years. The safety profile of Comirnaty is described in the product labeling. The presence of residual DNA in Comirnaty, CCI , is non-infectious, non-oncogenic, and is below the recommended limits set by the WHO guidelines. Additionally, the plasmid DNA used in Comirnaty production is not replication competent in mammalian cells [7], i.e., it cannot amplify in the human body. In summary, residual DNA in Comirnaty does not pose a safety risk to vaccinees.

CCI

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