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3.2.S.2.6. COMPARABILITY ASSESSMENT [BIOMAY PLASMID]

For historical purposes, information relevant to Biomay AG is provided; however, the site has been decommissioned and is no longer used for manufacturing of circular plasmid DNA and linear DNA template as well as storage of the relevant cell banks.

3.2.S.2.6.1. Biomay Plasmid and Linear DNA Template Production

3.2.S.2.6.1.1. Introduction - Plasmid and Linear DNA Template Production

A new source of the linear DNA template (Biomay) used as starting material for the drug substance has been established. This section provides comparability information of new material compared with the existing linear DNA template manufactured at Pfizer, Chesterfield.

As demonstrated by showing comparability for RNAs manufactured using PCR-amplified DNA templates (initial clinical development) or with linearized plasmid DNA (characterization studies and commercial supply), 4.2 1st ind [redacted] Accordingly, comparability of the linear DNA template from the new supplier was demonstrated for RNA manufactured using such DNA (see Section 3.2.S.2.3 Control of Materials – Generation of Plasmids - Biomay). In addition, the production processes were compared in the following sections.

3.2.S.2.6.1.2. Cell Bank Comparison

4.2 1st ind [redacted]

Table 3.2.S.2.6-1 lists the release tests performed, acceptance criteria, and the specifications of the MCBs for the two sources of linear DNA template. The side by side comparison shows that for the MCB, similar specifications are applied.

Table 3.2.S.2.6-1. Release Tests for Plasmid MCBs

Test	Description	Acceptance Criteria Pfizer Plasmid	Acceptance Criteria Biomay Plasmid
Culture purity	Demonstrate that the culture exhibits the appropriate colony morphology on selective and non-selective agar and shows no evidence of contaminating organisms.	Typical[a]	*E. coli* typical growth curve (lag, exponential, stationary growth phase)
Bacteriophage – lytic	Detect the presence, if any, of lytic bacteriophages in the MCB.	Absent of lytic bacteriophage	Absence of lysogenic and lytic bacteriophage contamination
Bacteriophage – lysogenic	Detect the presence, if any, of lysogenic bacteriophages in the MCB.	Absent of lysogenic bacteriophage	
Host cell identity (Genotypic testing)	Ensure the host strain is identified as *E. coli*	Identifies organism as *E. coli*	Identifies organism as *E. coli*
Viability (CFU/mL)	Determines an estimated number of viable organisms present in the MCB by plating to enumerate the number of colony forming units present.	Report result	[redacted]
Plasmid retention	Demonstrates retention of the expression plasmid by evaluation of the kanamycin resistance marker.	[redacted]	Report value [redacted]
Restriction map analysis	Confirms plasmid integrity through specific restriction enzyme digest and analysis by agarose gel electrophoresis.	Comparable to reference	Bands visible at approximately [redacted] and [redacted]
Plasmid copy number	Provides assurance that the cells retain the plasmid.	Report result	Report result
DNA sequencing	Demonstrate that the plasmid carrying the gene expression cassette remains unaltered during the cell bank manufacturing and preservation process.	Comparable to reference sequence	Conforms to theoretical sequence

a. Typical: Growth characteristics of *E. coli* species and no evidence of contaminating organisms.
Abbreviations: bp = base pairs

3.2.S.2.6.1.3. Comparison of the manufacturing process

The side by side comparison of the manufacturing process used at both sites is provided in Table 3.2.S.2.6-2. Overall, the key process steps are similar at both sites, [redacted]

It is considered that this leads to a comparable process and product and no impact on the quality and purity of the plasmid manufactured at Biomay is expected.

Table 3.2.S.2.6-2. Process Comparison for Linear DNA Template

Process Step	Process Description	
	Pfizer	Biomay
Shake Flask	4.2 1st ind	
Production Fermenter		
Harvest		
Lysis and Depth Filtration		
Ultrafiltration/ Diafiltration 1		
Anion Exchange Chromatography		

Table 3.2.S.2.6-2. Process Comparison for Linear DNA Template

Process Step	Process Description	
	Pfizer	Biomay
Chromatography II (Hydrophobic Interaction Chromatography / HIC)	4.2 1st ind	[redacted]
Ultrafiltration/ Diafiltration 2		
Filtration		
Linearization		
Chromatography III (Cation Exchange Chromatography)		
Ultrafiltration/ Diafiltration 3		

Table 3.2.S.2.6-2. Process Comparison for Linear DNA Template

Process Step	Process Description	
	Pfizer	Biomay
Filtration	4.2 1st ind	[redacted]

Abbreviations: UFDF = ultrafiltration / diafiltration; HEPES = 2-(4-(2-Hydroxyethyl)-1-piperazinyl)-ethane sulfonic acid

The materials used by both manufacturers of the plasmid are provided in Table 3.2.S.2.6-3. The quality standards used are similar; both manufacturers use Ph. Eur. grade material wherever available.

Table 3.2.S.2.6-3. Raw Materials Used in the Manufacture of Linear DNA Template

Raw Material	Grade	
	Pfizer	Biomay
Acetic Acid Glacial	4.2 1st ind	[redacted]
Agar		
Ammonium Hydroxide (28-30%)		
Ammonium hydroxide solution (25 %)		
Ammonium Sulfate		
Benzyl Alcohol		
Biotin		
Boric Acid		
Calcium Chloride Dihydrate		
Calcium Pantothenate		
Citric Acid		
Cobalt Chloride Hexahydrate		
Copper (II) Chloride Dihydrate		
Cyanocobalamin		
D-glucose anhydrous		
Dextrose, Anhydrous		
DL-Dithiothreitol (DTT)		
4.2 1st ind		
Edetate Disodium Dihydrate (EDTA)		
Ferrous Sulfate Heptahydrate		
Folic Acid		
Glycerol 85 or 87 %		
HEPES		
HEPES Sodium Salt		

Table 3.2.S.2.6-3. Raw Materials Used in the Manufacture of Linear DNA Template

Raw Material	Grade	
	Pfizer	Biomay
Hydrochloric Acid	4.2 1st ind [redacted]	
Kanamycin Sulfate		
L-Isoleucine		
L-Leucine		
L-Valine		
Magnesium Acetate Tetrahydrate		
Magnesium Sulfate Heptahydrate		
Manganese (II) Chloride Tetrahydrate		
Niacinamide		
P(4) - Aminobenzoic Acid		
Peptone from soy meal (papanic digestion)		
Phosphoric acid (85 %)		
Poly(propylene glycol) PPG2000 (antifoam)		
Potassium Acetate		
Potassium Phosphate Dibasic		
Potassium Phosphate Monobasic		
Pyridoxine HCl		
Riboflavin		
SDS		
Sodium Chloride		
Sodium Citrate Dihydrate		
Sodium Hydroxide, 50% (w/w)		
Sodium Hydroxide		
Sodium Molybdate Dihydrate		
Sodium Sulfate		
Thiamine HCl		
Tris Base		
Tris HCl		
Yeast extract		
Zinc Chloride		

HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT = DL-dithiothreitol; EDTA = edetate disodium dihydrate or ethylenediaminetetraacetic acid; NF = National Formulary; USP = United States Pharmacopeia; JP= Japan Pharmacopeia; Ph. Eur.= European Pharmacopeia

3.2.S.2.6.1.4. Comparison of quality attributes

The comparison of the specification used at Pfizer and Biomay are provided in Table 3.2.S.2.6-4. At Biomay, the circular plasmid DNA is evaluated through in-process testing. Some of these tests are carried over into the linear DNA template certificate of analysis. The acceptance criteria applied at Biomay are equal to or tighter than those used at Pfizer. Therefore, it is ensured that the quality of the linear DNA template manufactured at Biomay does not exceed specification limits compared to the template tested at Pfizer.

Table 3.2.S.2.6-4. Comparison of Linear DNA Template Specifications

Analytical Procedure	Quality Attribute	Acceptance Criteria	
		Pfizer	Biomay
Circular plasmid DNA			
Characteristics			
UV260	DNA Concentration	4.2 1st ind	[redacted]
Identity			
Restriction map	Identity	Comparable to reference or Comparable to theoretical	Major bands conform to theoretical restriction fragments (expected band pattern)
Sanger sequencing	Identity of transcribed region Identity of poly A tail	Homology to reference[a]	Conforms to reference sequence
Purity			
Agarose gel electrophoresis	Plasmid topology	4.2 1st ind [redacted]	4.2 1st ind [redacted]
Process-Related Impurities			
HPLC RNA analysis	Residual host cell RNA	4.2 1st ind	4.2 1st ind
qPCR DNA analysis	Residual host cell DNA	4.2 1st ind	4.2 1st ind
Residual kanamycin	Residual kanamycin	4.2 1st ind	[redacted]
Linear DNA Template			
Characteristics			
Appearance (clarity)	Clarity	4.2 1st ind	Clear and colorless solution; free from visible particulates
Appearance (coloration)	Coloration	Not more intensely colored than level 6 of the color standard	
pH	pH	4.2 1st ind	4.2 1st ind
UV260	DNA Concentration	4.2 1st ind	4.2 1st ind
Restriction map	Poly A tail integrity	Bands at 4.2 1st ind [redacted]	Bands detected at expected size

Table 3.2.S.2.6-4. Comparison of Linear DNA Template Specifications

Analytical Procedure	Quality Attribute	Acceptance Criteria	
		Pfizer	Biomay
Purity			
Agarose gel electrophoresis	Linearization Efficiency (Plasmid topology)	4.2 1st ind	<4.2 1st ind
Process-Related Impurities			
Total residual protein by µBCA	Residual protein	4.2 1st ind	4.2 1st ind
Safety			
Bioburden	Bioburden	4.2 1st ind	4.2 1st ind
Endotoxin	Endotoxin	4.2 1st ind	4.2 1st ind

4.2 1st ind

Abbreviations: NTU = Nephelometric Turbidity Unit; CFU = colony forming unit; EU = endotoxin unit

3.2.S.2.6.2. Biomay Linear DNA Template Performance in Drug Substance Process

3.2.S.2.6.2.1. Introduction - Linear DNA Template Performance

A small-scale study was conducted to qualify use of Biomay linear DNA template for use in the BNT162b2 drug substance (DS) process using batch BNTC04-G0121. This batch was the only batch available at the time of the study. This section provides results that confirm Biomay linear DNA template produces DS of suitable quality.

3.2.S.2.6.2.2. Study Design

The Biomay linear DNA template was tested in triplicate using qualified small-scale models (Section 3.2.S.2.6 Manufacturing Process Development - Process Development and Characterization)4.2 1st ind Therefore, the model is considered to be representative of the commercial process.

The Biomay linear DNA template was introduced at the IVT step and forward processed to final DS.4.2 1st ind

3.2.S.2.6.2.3. Biomay Linear DNA Template Qualification Results

The results of the qualification study are provided in Table 3.2.S.2.6-5. The acceptance criteria listed in the table are from Section 3.2.S.4.1 Specification and details on analytical methods can be found in Section 3.2.S.4.2 Analytical Procedures. Additionally, the mean

plus/minus three standard deviation (3 sigma) ranges calculated from 42 historical batches of drug substance are included for attributes amenable to statistical assessment. The 3 sigma ranges provide an added level of stringency in evaluating product quality relative to historical manufacturing experience.

Table 3.2.S.2.6-5. Summary of Biomay Linear DNA Template Performance in the DS Process

Quality Attribute	Acceptance Criteria	3 Sigma Range[a]	Run 1	Run 2	Run 3
4.2 1st ind	l.2 1st ind				

a. Range or limit calculated from 42 historical batches.
b. The 3-sigma range is 4.2 1st ind

All runs produced DS meeting acceptance criteria and all attributes are within the 3 sigma ranges of the historical batches. Biomay linear DNA template is deemed acceptable for use in commercial-scale DS production.

3.2.S.2.6.3. Conclusion

The comparison of the banking system showed that Biomay is 4.2 1st ind using a MCB whereas Pfizer is using an MCB/4.2 1st ind set-up. 4.2 1st ind

The manufacturing process performance at both sites is highly comparable 4.2 1st ind

The quality attributes used for the testing of the linear DNA template are identical and the acceptance criteria applied at Biomay are equal or tighter than those used at Pfizer.

The small-scale drug substance manufacturing evaluation showed that the Biomay linear DNA template consistently produces DS meeting the approved specification.

Therefore, it can be concluded that the plasmid manufactured at Biomay is comparable to the plasmid manufactured at Pfizer and produces drug substance of similar quality.