Welcome

The standard of trust

Establishing Quality Benchmarks: Platform Technology Approach to mRNA Product Quality

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Agenda

- Introduction
- Overview of current mRNA landscape
- mRNA production process
- Building consensus
- Defining and monitoring key PQAs
- Future considerations and next steps
- Resources

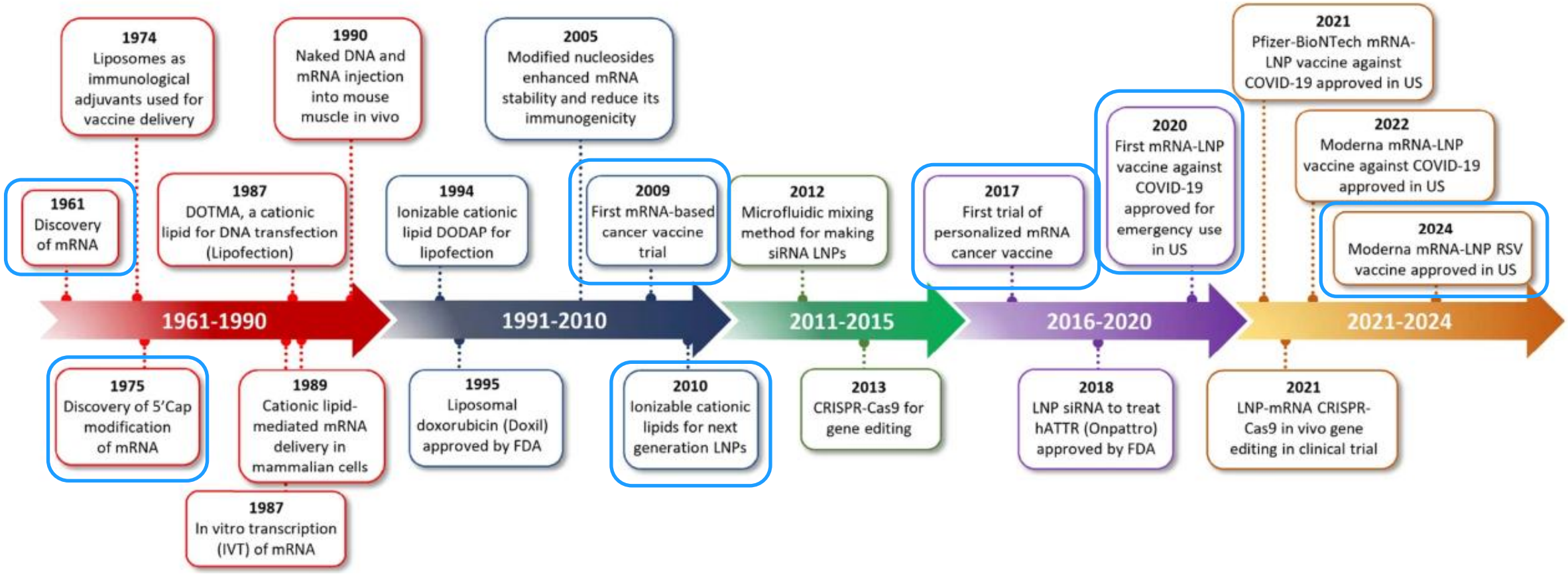
Over 200 Years of Building Trust in The US and Beyond

USP Biologics is expanding standards to support mRNA therapeutics

- For over 200 years the United States Pharmacopeia (USP) has provided public standards for medicines to protect patient safety and improve public health.
- USP is an independent, scientific nonprofit organization focused on building trust in the supply of safe, and quality medicines.
- USP standards are used in over 150 countries and enforced in over 40 countries
- The USP works globally to help ensure medicines are stored, transported, and administered properly.
- The USP works globally to help ensure testing and public standards are available to verify the quality and safety of medicines.

Turning Vision into Reality

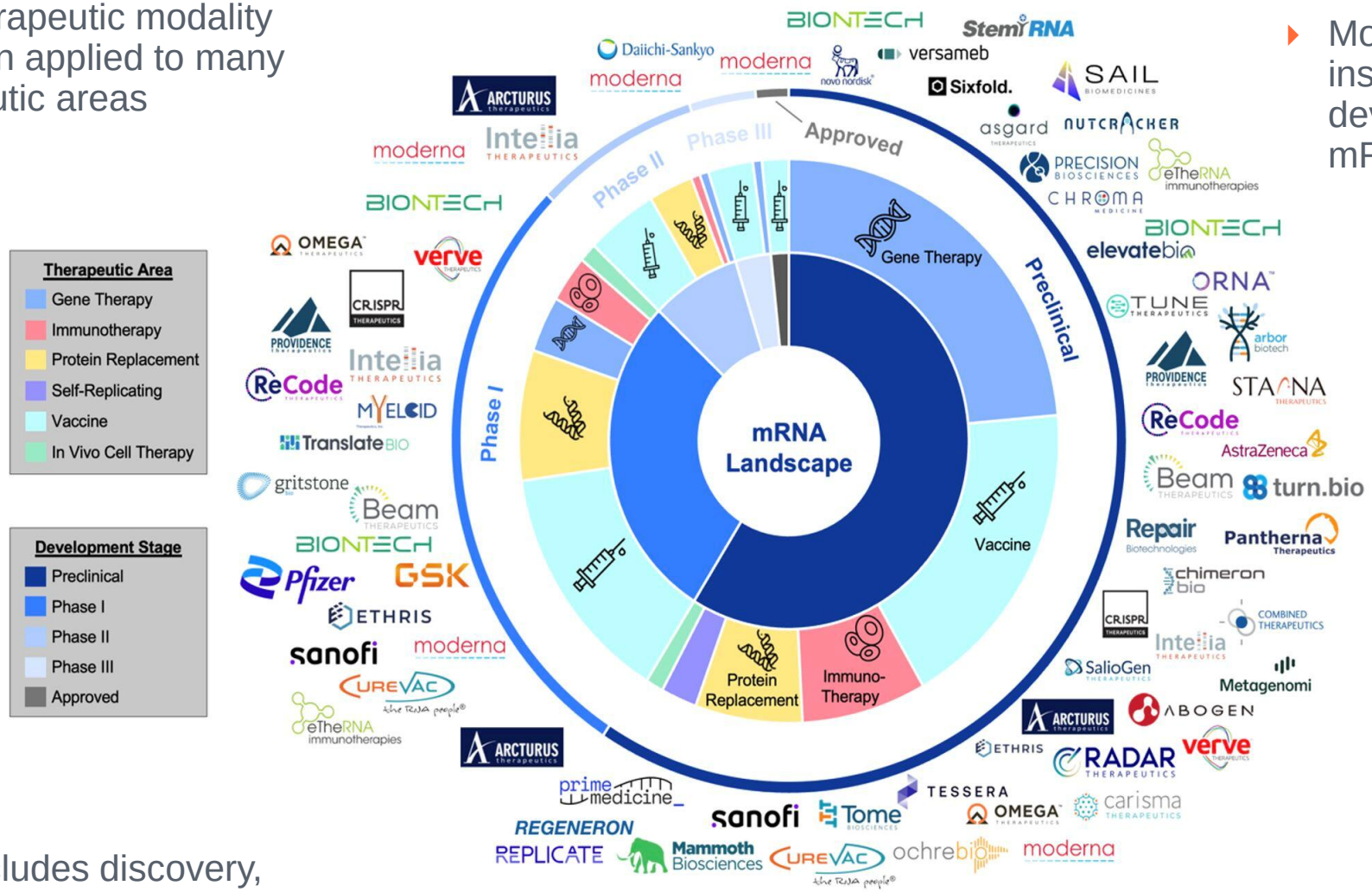
From discovery of mRNA in 1961 to US FDA approval of RSV mRNA-LNP in 2024



Current landscape of mRNA technologies and delivery systems for new modality therapeutics | Journal of Biomedical Science | Full Text

Overview of Current mRNA Landscape

- This therapeutic modality has been applied to many therapeutic areas

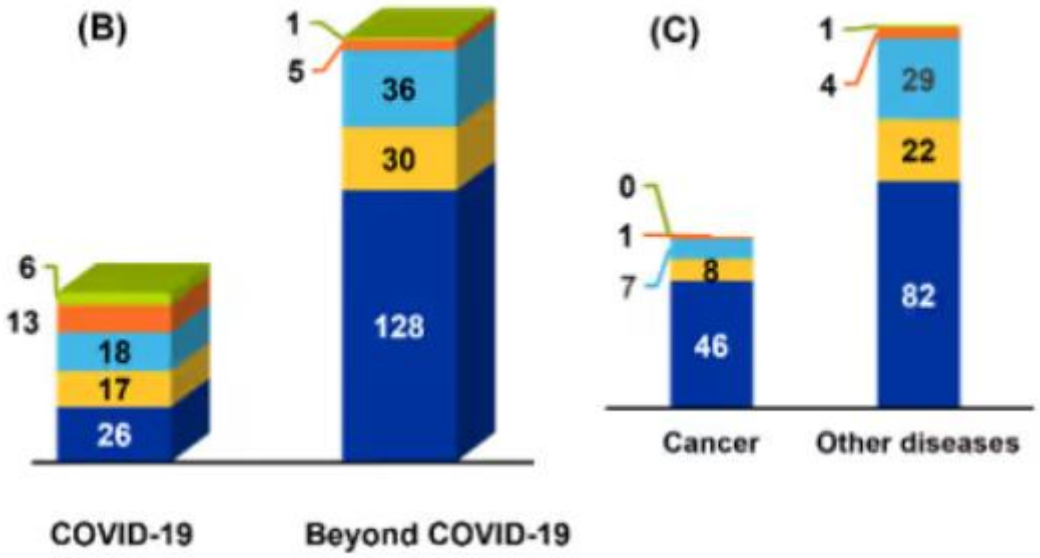


- More than 190 companies and institutes are engaged in the development of more than 310 mRNA vaccines and therapeutics
- Among the active mRNA therapeutics:
  - 57% in discovery, 39% in clinical stages, 1.9% have acquired (pre-) registration and 1.6% approved
- Work includes discovery, preclinical studies, and all states of clinical trials

https://images.app.goo.gl/QSR4hDuYBWnHxUUC7

Global mRNA Pipeline & Market Size

Distribution of mRNA vaccine trails



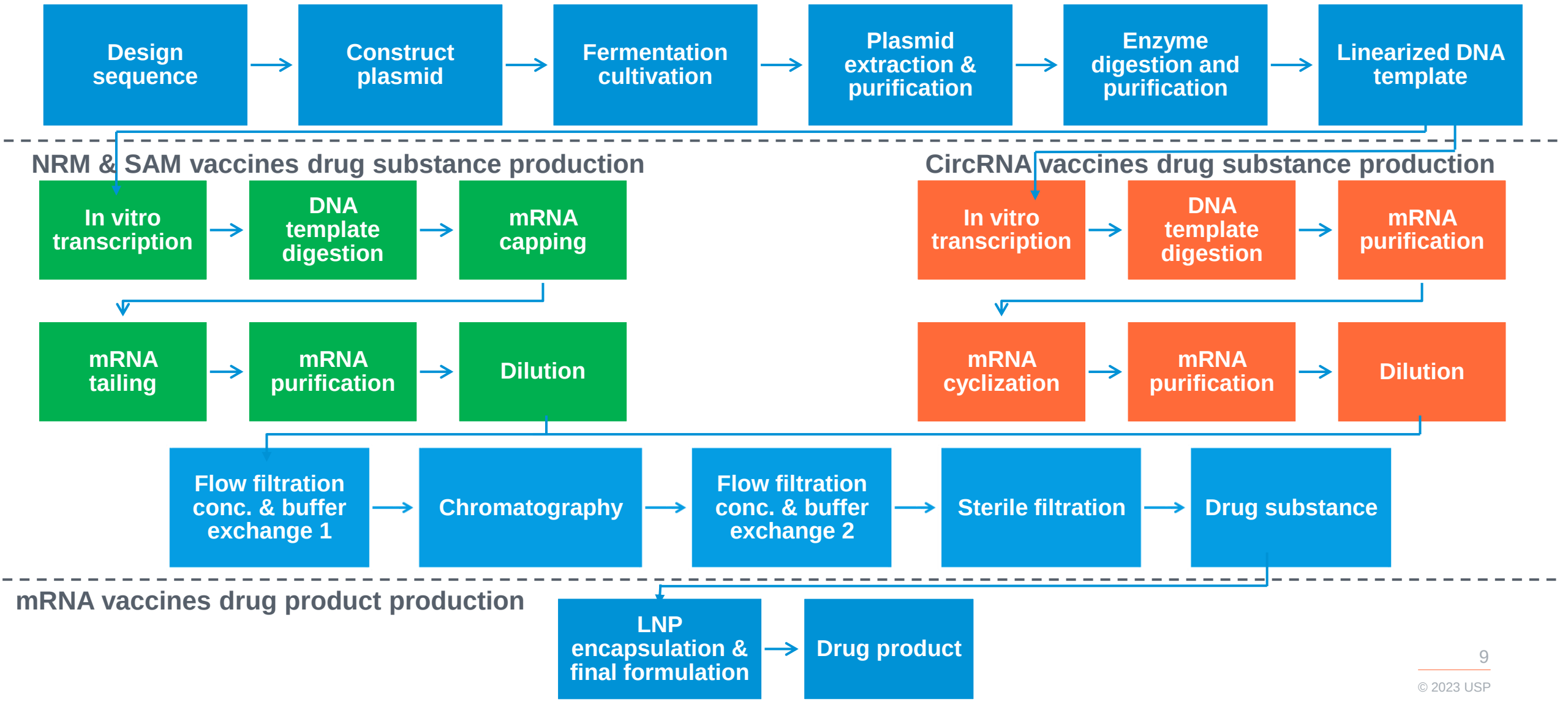
The future of mRNA vaccines beyond COVID-19 | CAS

What is driving the market?

- Rising incidence of chronic and infectious diseases (diabetes, cancer, viral infections, and generic disorders)
- Growing need for therapeutic drugs & vaccinations for viral illnesses (Ebola, influenza, and HIV)
- 14% of the pipeline are accounted for by combination vaccines (COVID, influenza, RSV)
- 31% on cancer
- 69% on other infectious, genetic and immune diseases
- Combination products of mRNA technology with other molecules
  - mRNA and antibodies (conjugates)
  - mRNA and peptides
  - mRNA and CRISPR/Cas9 systems
- Example of R&D initiatives
  - Heart diseases - myocardial ischemia therapy using mRNA

1 mRNA Production Process

mRNA Production Process

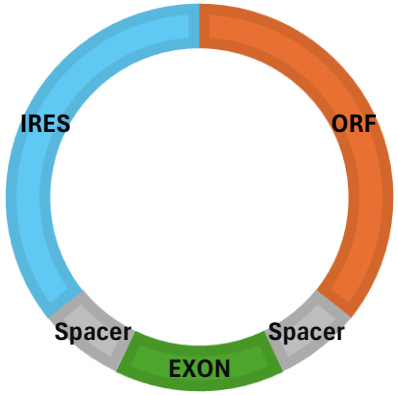


mRNA Structural Differences

Non-replicating mRNA (NRM)

Self-amplifying mRNA (SAM)

Circular mRNA

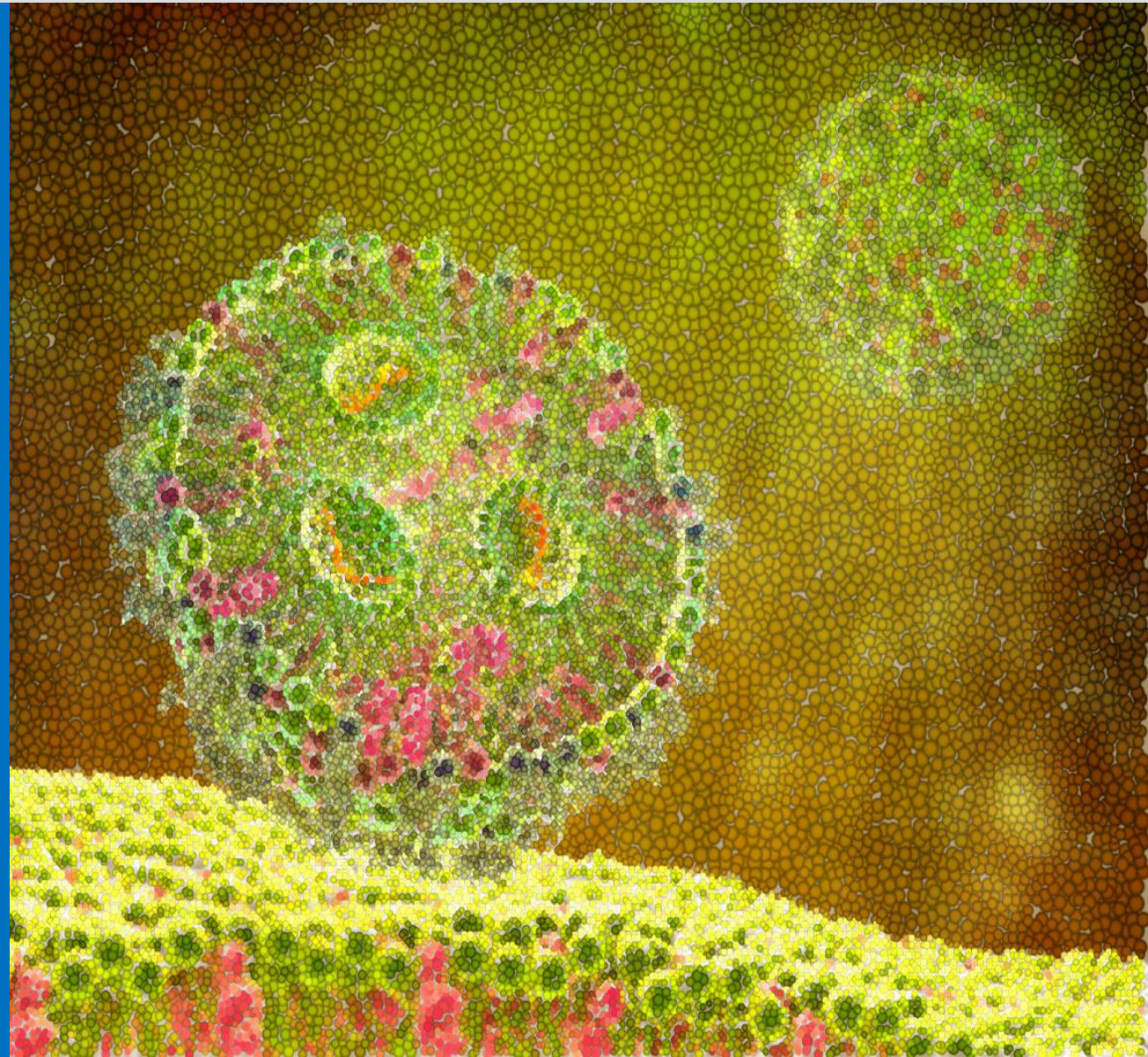


- The major difference between SAM and linear IVT mRNA is incorporation of a **non-structural gene** for the generation of RNA-dependent RNA polymerase
  - Amplifies itself in the cell and induces high level of target protein expression
- Circular mRNA
  - Inserting internal ribosomal entry site (IRES) and sequence of gene of interest are added between exon fragment E1 and E2
  - Exon sequence is flanked by split introns (self-splicing introns)
  - Developed to increase the stability of mRNA and overcome the short half-life of linear IVT mRNA

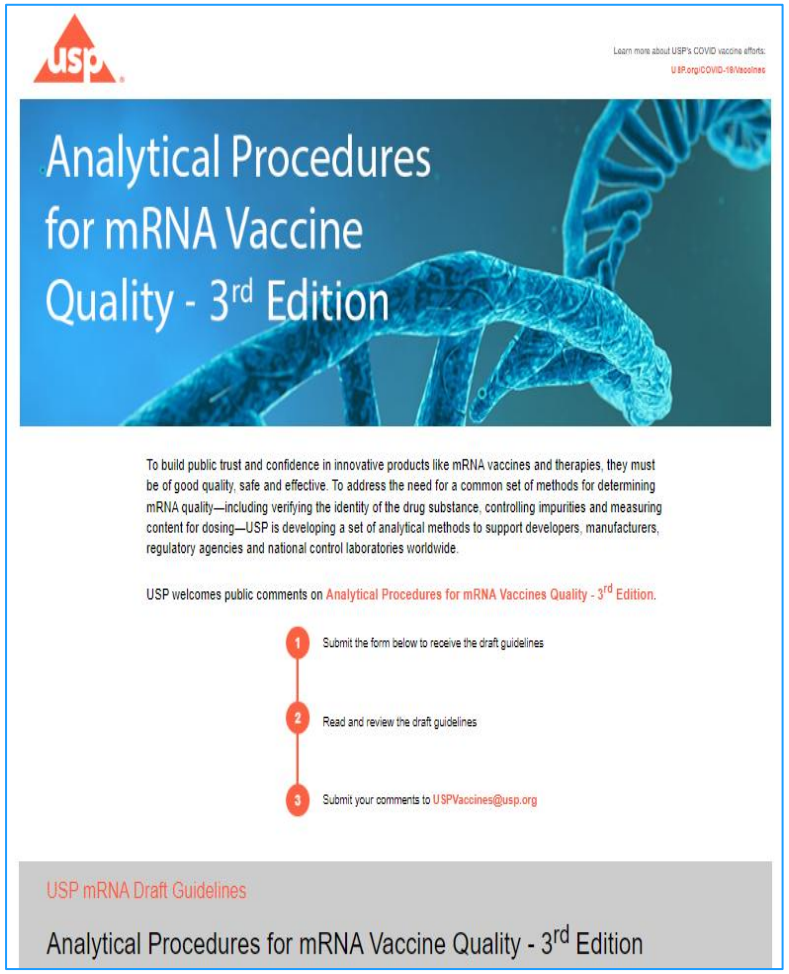
Building Consensus

mRNA Therapeutics: Challenges and Solutions

- **Challenges:**
  - **Diverse** range of product types
  - **Variations** in raw material quality
  - Newly emerging pathogens present **unique challenges** and require rapid development, scalability, and efficacy testing
- **Goals:**
  - Expanding standards development to cover quality testing throughout the product lifecycle
    - **Support** raw materials qualification and biomanufacturing
    - Focus on **analytical tools**, cross-cutting standards and **alignment** with global norms
    - **Standards** to support emerging therapies and new technologies



Building Consensus: Updated Guidelines and Public Outreach



www.usp.org/mrna-quality

Support quality of vaccines by providing analytical procedures for testing common quality attributes

- Applicable across a product class and can be applied to future vaccines
- Provides options for the same attribute - some technology platforms may not be available in all testing labs

Help streamline and accelerate development

- Provides a starting point - can reduce time and resources needed for in-house development
- Allows manufacturers to focus on the disease and unique aspects of their product

Guidelines updated over last 3 years based on public input

- Includes USP verified methods for several CQAs and USP chapter <1040> on DNA plasmid – IN PF49(6)
- Validated methods would provide an additional layer of support - NEXT STEP

https://doi.usp.org/USPNF/USPNF_M17795_10101_01.html

3 Defining and Monitoring Key PQAs

USP's Efforts on mRNA

mRNA manufacturing workflow

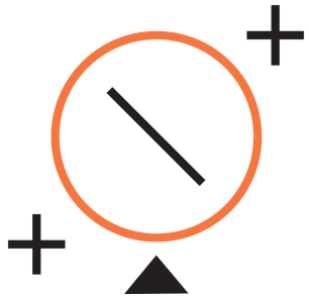
Raw & starting materials → GMP manufacturing → DS Lot release → Formulation → Fill/Finish → DP Lot release → Distribution → Administration

Raw materials

- Manufacturing requires complex raw materials
- Control of raw materials is essential for safety and lot-to-lot consistency

Product Quality Attributes (PQAs)

- Lack of harmonized test methods
- Lack of quality mRNA standards

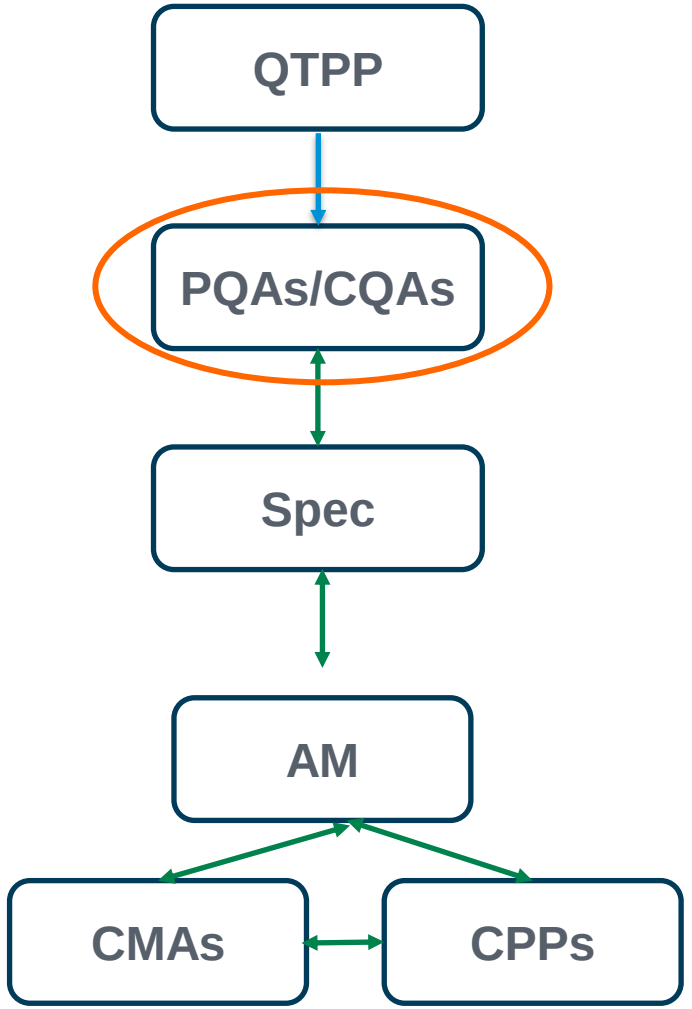


Residuals & Impurities

- Removal of problematic starting materials is critical
  - Nucleotides
  - Enzymes
  - LNP components
  - DNA templates
  - mRNA Fragments

Applying QbD Approach to Establish PQAs/CQAs

- Applying QbD (per ICH Q8 [R2]) to mRNA modality to establish CAQs/PQAs
  - Establish Quality target product profile (QTPP) to identify the drug product quality characteristics that ensure the drug product desired quality, considering safety and efficacy
  - Identify the product quality attributes (PQAs) and critical quality attributes CAQs (DP and DS) to meet the QTPP requirement.
  - Establish the critical material attributes (CMAs) and critical process parameters (CPPs) and how they link to the CQAs/PQAs
  - Apply QbD (per ICH Q14) concept for analytical method (AM) development to establish analytical methods for DP, DS, in process testing and critical material
    - USP <1220> Analytical Procedure Life Cycle
  - Set specifications (per ICH Q6B) for the drug substance(s), excipient(s), and manufacturing process to support the product life cycle. This includes a strategy to manage the CQAs and PQAs lifecycle.



PQAs vs. CQAs

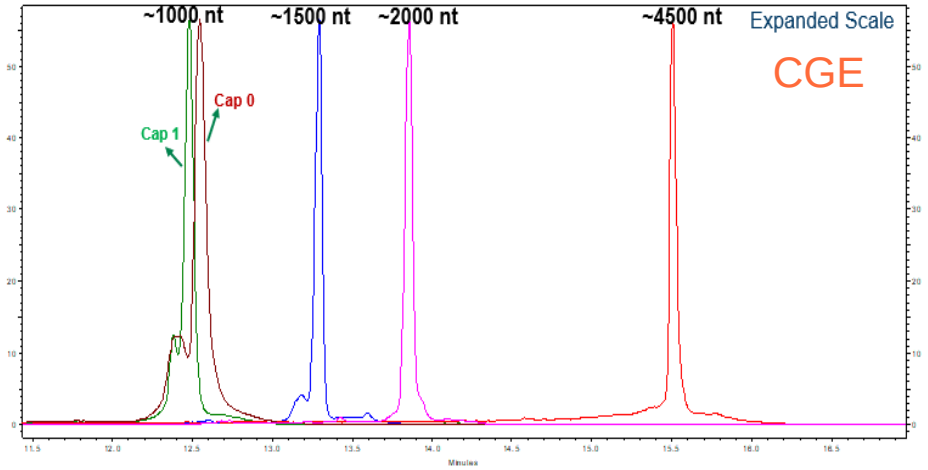
- CQAs are a subset of PQAs, attributes that must be controlled to ensure the product's therapeutic effectiveness and patient safety

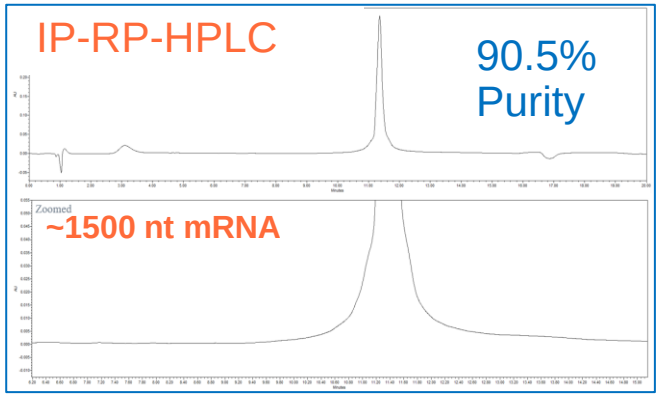
Aspect	PQAs	CQAs
Definition	**General** characteristics that define the **overall** quality of a product	**Specific** attributes that directly impact the **safety and efficacy** of the product
Examples	Appearance, purity, potency, stability, dissolution rate	Concentration of active ingredients, sterility, impurity levels, release testing
Impact on Product	Important for maintaining general quality but may not directly affect safety or efficacy	**Essential** for ensuring the products safety and efficacy
Monitoring and Control	Monitored to ensure **overall quality** standards are met	**Closely monitored and controlled** to ensure critical quality standards are maintained
Identification	Identified based on **general** quality requirements	Identified through **risk assessments and regulatory guidelines**
Regulatory Compliance	Helps in **maintaining** general quality compliance	**Necessary** for meeting specific regulatory requirements for safety and efficacy
Role in Manufacturing	Ensures **consistent quality** across batches	Ensures critical aspects of the product are **within acceptable limits** to guarantee safety and efficacy

Recommended Tests – Target Quality Attributes

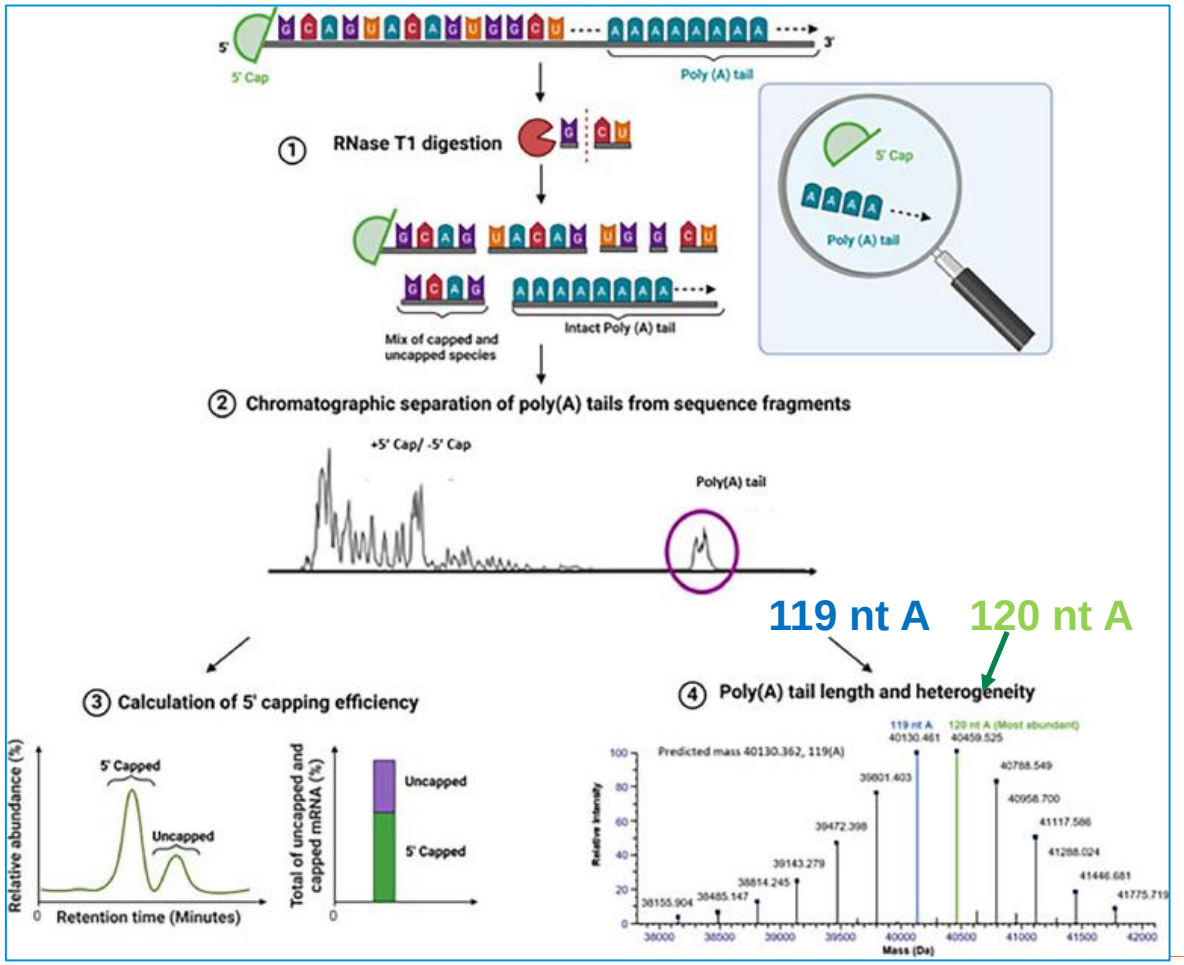
mRNA Drug Substance

- mRNA integrity and purity





- 5’ Capping efficiency
- Poly(A) tail length

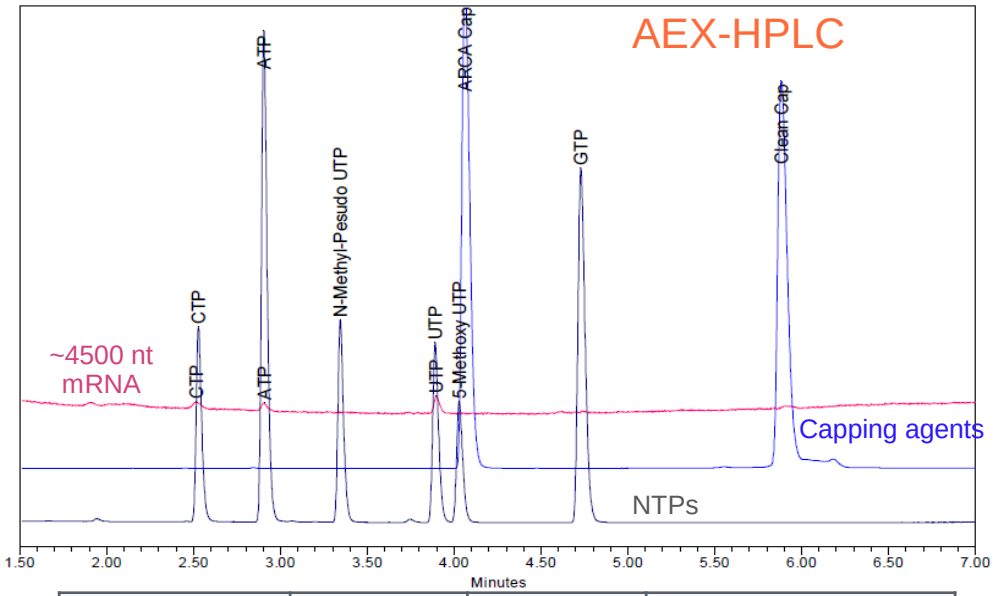


Characterization and analysis of mRNA critical quality attributes using liquid chromatography-based methods - ScienceDirect

Recommended Tests – Target Quality Attributes

mRNA Drug Substance

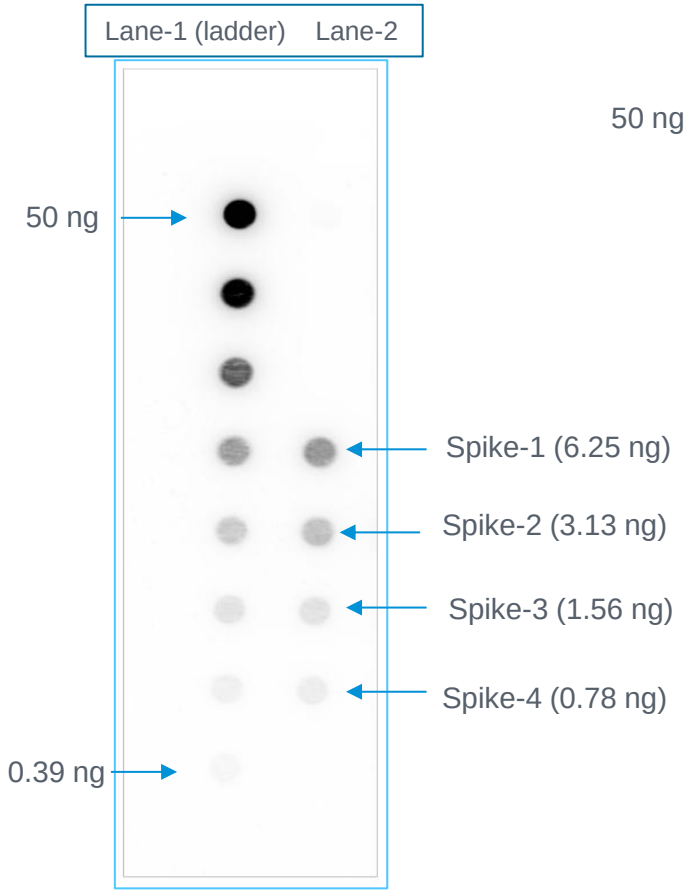
- **Process-related impurities – residual solvents**
  - USP <467>



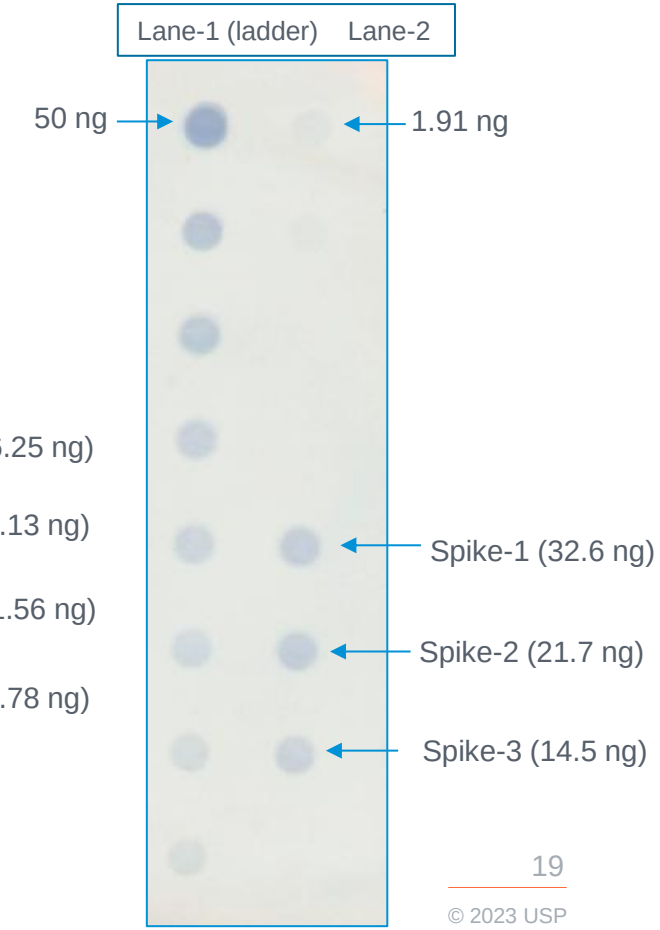
Name of the Sample	Peak ID	Avg Area (AU)	Residual NTP and capping agent (mM) in sample
~4500nt mRNA	CTP	254.0	**1.14E-04**
	ATP	248.0	**4.27E-05**
	UTP	514.5	**2.35E-04**
	GTP	<LLOQ	ND
	CleanCap	<LLOQ	ND

- **Product-related impurities – dsRNA**

Chemiluminescence



Absorbance



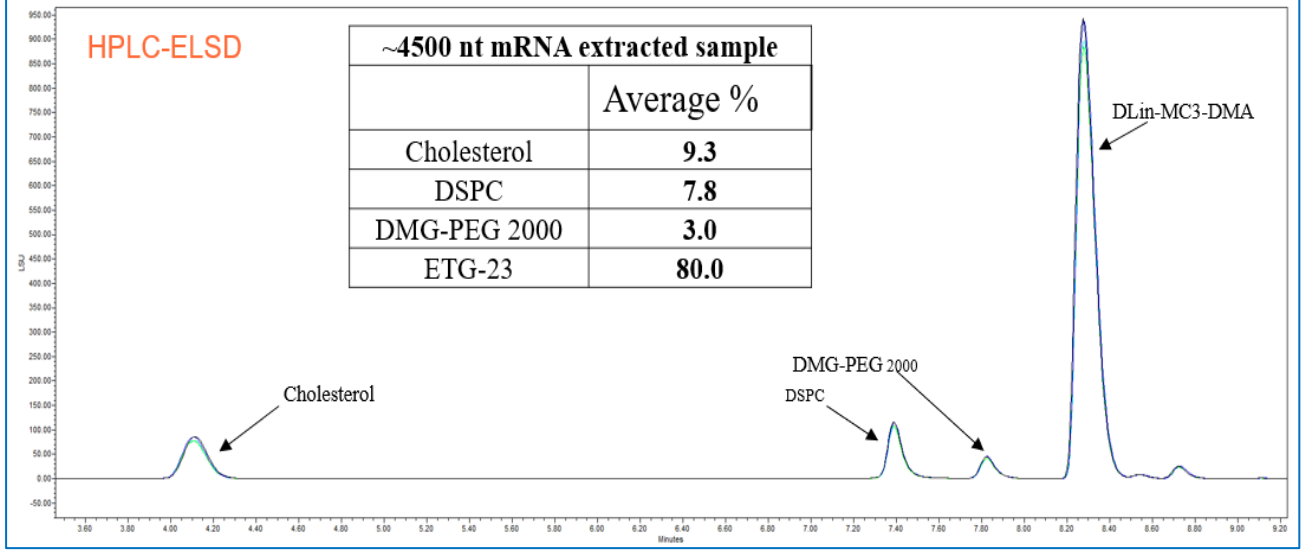
Recommended Tests – Target Quality Attributes

mRNA Drug Product

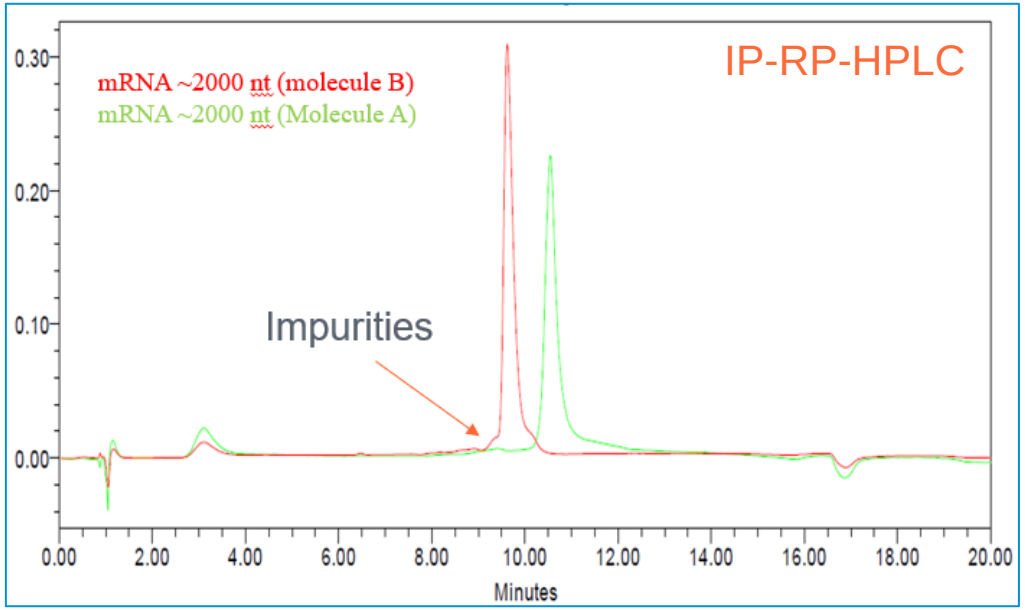
- **Lipid content in LNP**

HPLC-ELSD

~4500 nt mRNA extracted sample	
	Average %
Cholesterol	**9.3**
DSPC	**7.8**
DMG-PEG 2000	**3.0**
ETG-23	**80.0**



- **Purity**

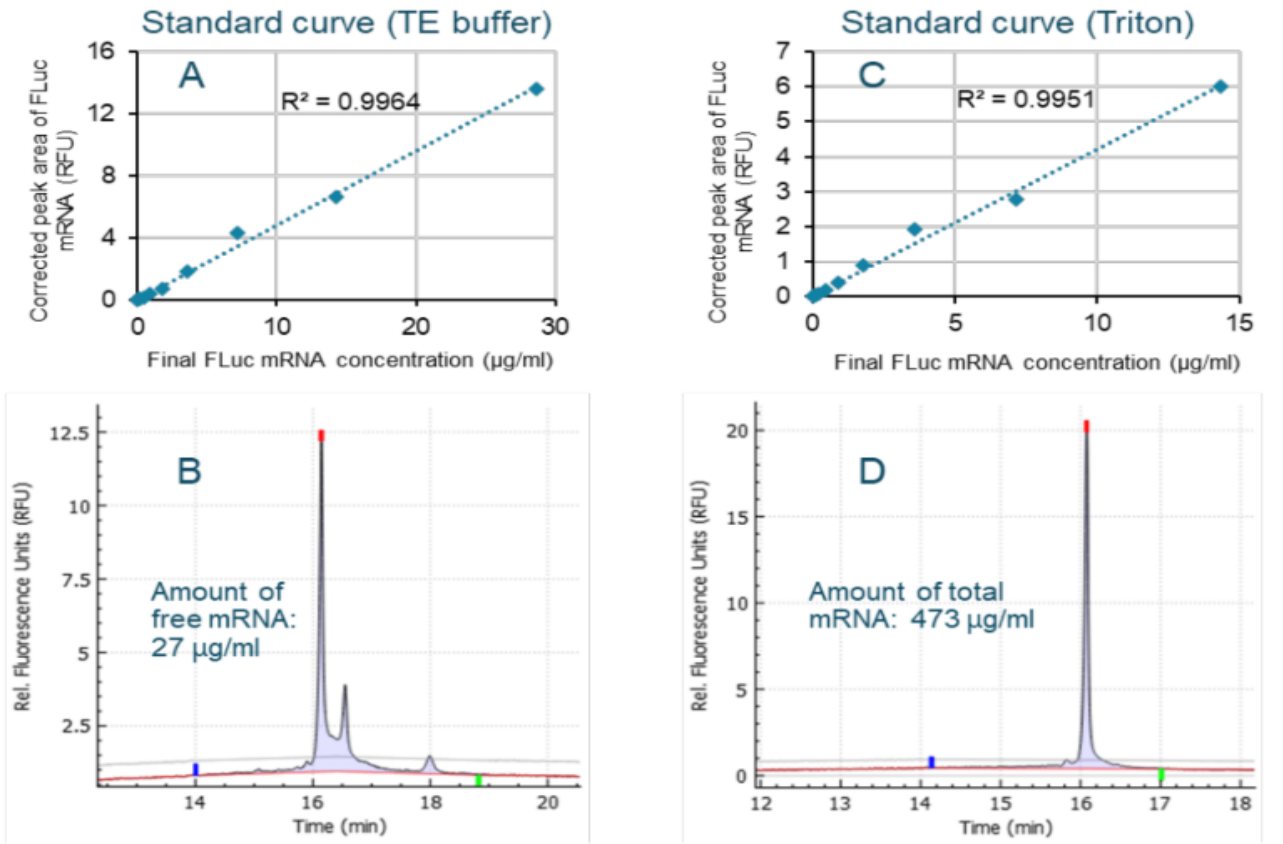


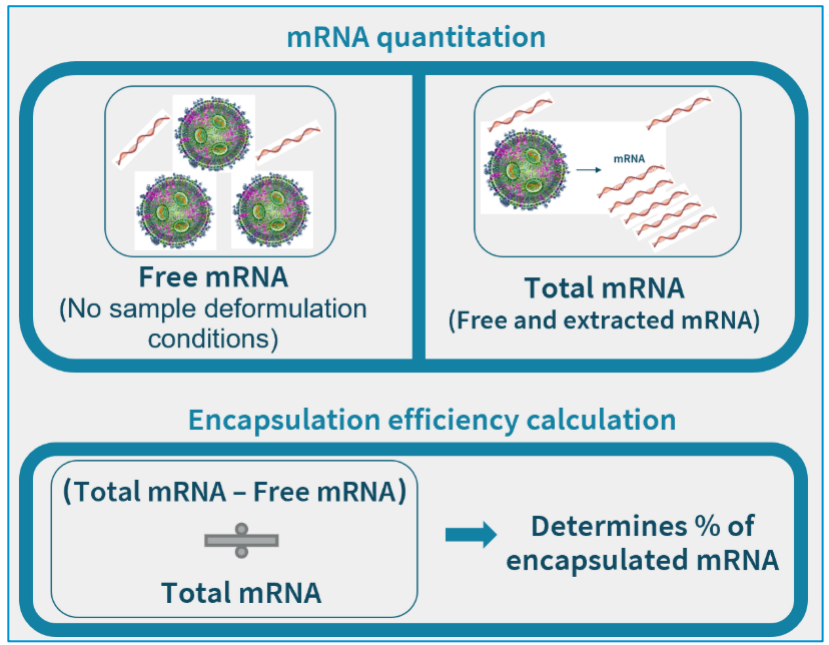
Recommended Tests – Target Quality Attributes

mRNA Drug Product

- **Encapsulation efficiency**

CGE-LIF





94.3% Encapsulated

A new approach to determine encapsulation efficiency of mRNA-lipid nanoparticles (mRNA-LNP) by capillary gel electrophoresis with laser-induced fluorescence detection

Methodologies to Address Analytical Challenges

Quality Attribute	Analytical Methods
RNA Content	UV Spectroscopy, Fluorescence-based assays using fluorescent dye, HPLC
RNA Identity (Sequence)	RT-PCR, Sequencing methods (Sanger, NGS, Microarrays, etc), NGS, Finger printing (LC-MS/MS), base composition
RNA structure Integrity	CE, SEC-MALS, DSC, CD spectroscopy
RNA purity, related impurity	CE, IP-RP-HPLC, EX-HPLC, SEC-HPLC, HILIC-HPLC, Western Blot, FFF-MALS
5' Capping	Digestion or cleavage followed by LC-UV-MS, LC-MS, microarray
Poly (A) length and distribution	Digestion or cleavage followed by CE, RP-HPLC, SEC-HPLC, LC-MS, ddPCR, microarrays, sequencing
dsRNA	Immunoblot, dot blot, ELISA, LC, microfluidics (e.g., electrophoresis, immunoassays, Lab-on-a-Chip)
Residual DNA content	qPCR, fluorescent based assays
Residual enzymes and HCP	BCA, Nano Orange, enzyme specific assays, CE, MS, ELISA
RNA Encapsulation	Ribogreen assay, EX-HPLC, RP-HPLC, CryoEM, SEC-SLS/UV
LNP size, polydispersity	DLS, NTA, MALS, SEC-MALS, CE, FFF-MALS-UV-dRI
LNP Charge	ELS, PALS, CE
Lipid identity, purity and content	HPLC-CAD, LC-MS, FFF-MALS-UV-dRI
LNP morphology	CryoEM, SANS, SAXS, DSC, FFF_MALS-UV-dRI

Platform Analytics

"A platform analytical procedure can be defined as a multi-product method suitable to test quality attributes of different products **without significant change** to its operational conditions, system suitability and reporting structure. This type of method would apply to **molecules that are sufficiently alike** with respect to the attributes that the platform method is intended to measure." USP <1220> and ICH Q2 [R2], Q14

- Leverage prior knowledge, insights, expertise, data
  - Examples: identity testing of mRNA - (defined by the DNA template – same type), platform formulations, same set of raw and starting material used
  - Data obtained from analytical procedure development/validation from existing method
- Implement scientific and risk-based approaches in line with QbD principles throughout analytical life cycle
  - Risk identification, risk scoring and risk management based on prior knowledge and data
  - End-to-End (E2E) approach from development to routine testing
  - Take systematic/holistic approach to mRNA platform methods

True power is built over time – driven by data, sustained by continuous knowledge generation

Platform Analytics

- **Generally Applicable**
  - 5’ Capping – e.g., LC-MS
  - Poly(A) tail length – e.g., LC-MS
  - Sizing – e.g., CGE, RP-HPLC
  - Residual impurities (residual DNA, aggregate, fragmented mRNA, etc) – e.g., AEX-HPLC
  - Plasmid – e.g., topology methods (HPLC, CGE)
  - Enzymes – e.g., Florescence based method
  - Nucleosides – e.g., AEX-HPLC
  - Double-stranded RNA – e.g., dot blot

- **Some Exceptions**
  - Sizing of large mRNAs, including self-amplifying mRNA
  - Degree of circularization of circRNA
  - Potency
    - In-vitro vaccine potency and in vivo vaccine activity
  - When making changes to key components
    - New LNP components
    - Changes in UTRs may impact PCR-based tests

Summary and Future Directions

- **USP is supporting new modalities and technologies**
  - Engagement with stakeholders to build consensus
  - Development of analytical framework and tests for stakeholder comment
  - Collaboration to support method validation, training, and standard development
- **mRNA-based products - unique analytical challenges**
  - New raw and starting materials
  - Novel impurities, requiring new analytical methods
  - Novel delivery systems
- **USP's tool for mRNA quality**
  - Developed analytical toolkits and draft guidelines to support mRNA-based products
  - Working on mRNA size standards for PQAs, dsRNA impurity standard and residual standards (e.g., NTPs and modified NTPs)

USP's Ways and Opportunities to Collaborate

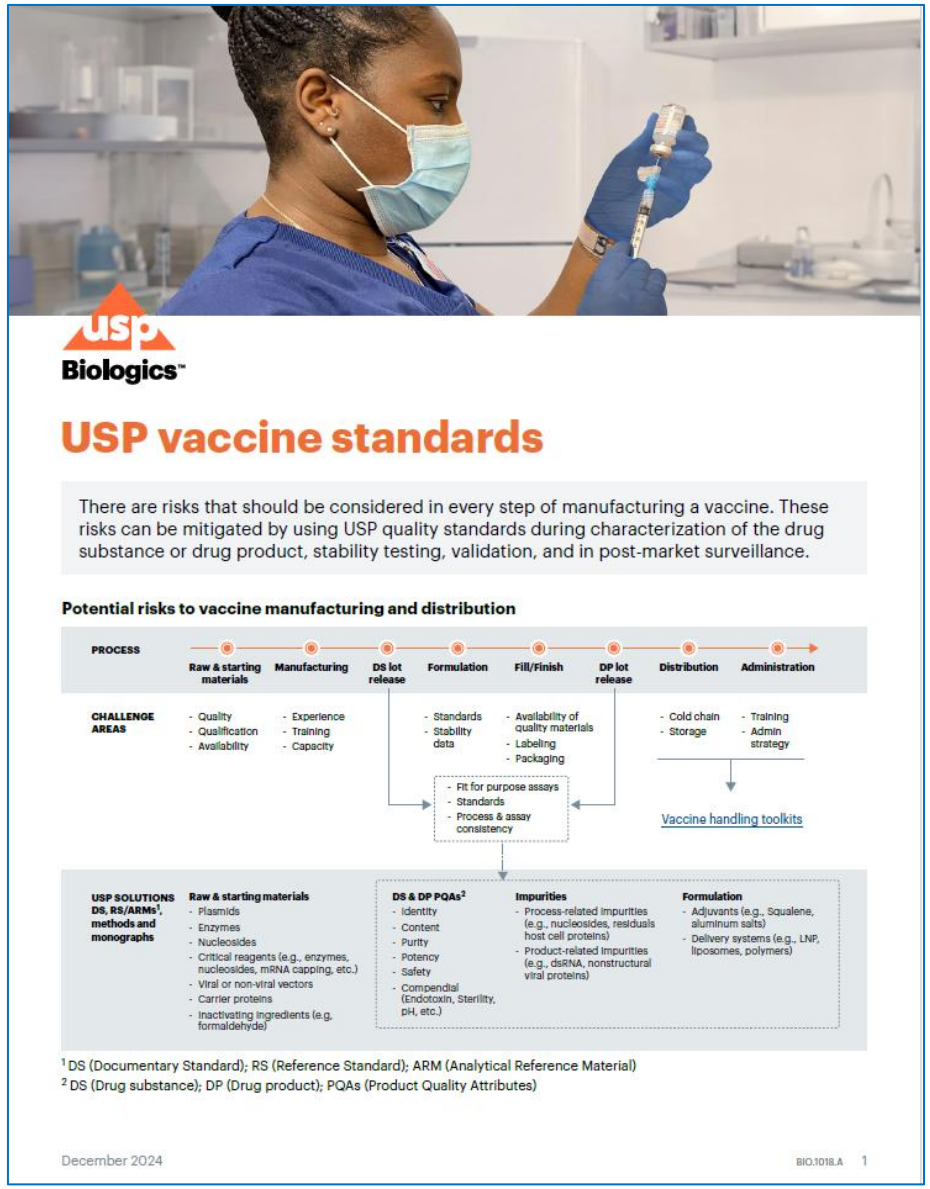
USP is Fueled by Stakeholder Engagement

- Develop a toolbox of platform methods applicable to (most) mRNA products
- Harmonization of analytical methods for assessing CQAs across products/developers
- Development of physical reference materials to support consistent measurement of CQAs
- Build consensus on best practices
- Expert Panel: To draft Chapters on best practices and testing strategies for mRNA vaccines and therapeutics

- Roundtables and working groups
- Donated methods and/or material to support standard development
- Collaborative Testing
- Reviewing Chapters and Stimuli Articles on Pharmacopeial Forum
- Notices posted on USP web site - https://www.usp.org/biologics

USP Resources for Vaccines

- USP's public quality standards are critical tools for ensuring the quality and safety of vaccines
- Many tools provided in the flyer are applicable to mRNA therapeutics
  - Plasmid DNA <1040> PF49(6)
  - Cell banking webinar
  - mRNA guidelines
  - Vaccine toolkits – mRNA
  - Regulatory considerations for vaccines



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USP vaccine standards

There are risks that should be considered in every step of manufacturing a vaccine. These risks can be mitigated by using USP quality standards during characterization of the drug substance or drug product, stability testing, validation, and in post-market surveillance.

Potential risks to vaccine manufacturing and distribution

[1] DS (Documentary Standard); RS (Reference Standard); ARM (Analytical Reference Material)
[2] DS (Drug substance); DP (Drug product); PQAs (Product Quality Attributes)

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Vaccine Standards | Biologics

Thank You

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Questions

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Stay Connected

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