Welcome

The standard of trust

USP Biologics

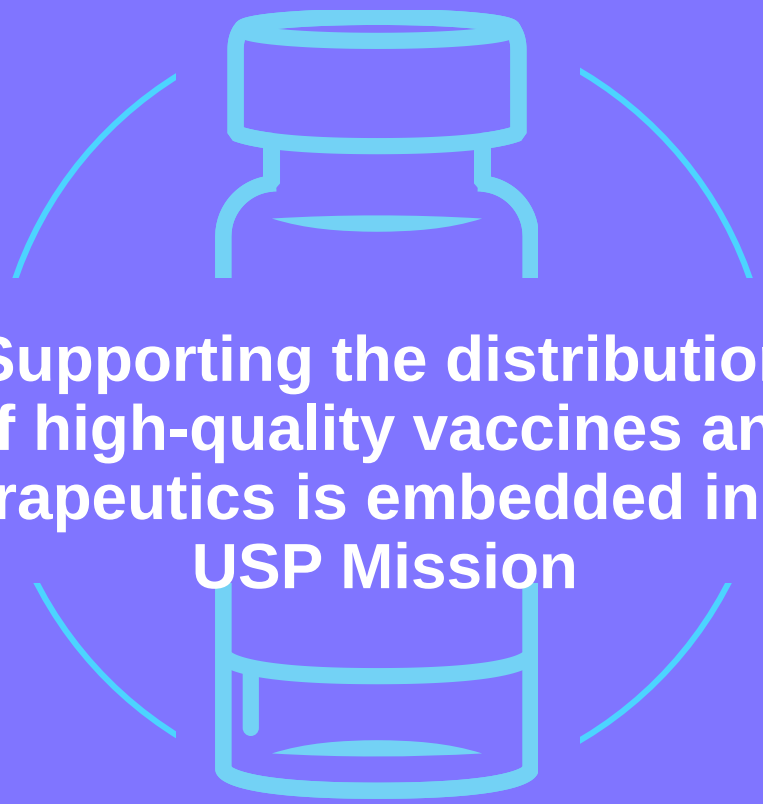
mRNA Open Forum

Collaborating to Pave the Way for mRNA-based Vaccines and Therapeutics Quality

Feb. 28-29, 2024
9:00 a.m. - 1:00 p.m. (EST)
Virtual

Fouad Atouf,
Senior Vice President, Global Biologics
February 28th, 2024

USP Mission



Supporting the distribution of high-quality vaccines and therapeutics is embedded in the USP Mission

- For over 200 years the United States Pharmacopeia (USP) has provided public standards in medicine, dietary supplements, and food to protect patient safety and improve public health.
- USP is an independent, scientific nonprofit organization focused on building trust in the supply of safe, quality medicines.
- As the USP continues to adapt, grow and evolve with science and medicine, we strive for a world where everyone trusts the medicines, we rely on to save lives.
- The USP works globally with our collaborators to help ensure vaccines and therapeutics are stored, transported, and administered properly.
- The USP works globally with our collaborators to help ensure testing and public standards are available to verify the quality of vaccines for patient safety.

Overarching Goals- e.g., Vaccines

Support development, manufacturing, and global distribution of vaccines

Expand and make consistently available standards needed to address quality issues and build awareness on broader supply chain topics by:

1. **Developing** standards, publications, and other guidance supporting potential vaccines and treatments
2. **Expanding** collaborations to provide these tools and facilitate global access to quality vaccines
3. **Supporting** analytical and regulatory capabilities of our partners

- Collaborate with stakeholders to build awareness and consensus on quality
- Utilize innovative approaches to gather feedback, methods and materials
- Leverage science and global reach to maximize impact

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Supporting Quality of mRNA

Potential risks to manufacturing and distribution

Raw & starting materials

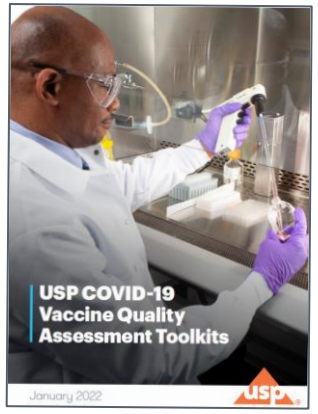
- Quality
- Qualification
- Availability

GMP manufacturing

- Experience
- Training
- Capacity

DS Lot release

- Plasmid
- Enzymes
- Nucleosides
- Capping and other reagents



Formulation

- Standards
- Stability data

Fill/Finish

- Availability of quality materials
- Labeling

DP Lot release

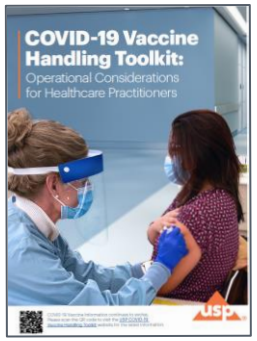
- Fit for purpose assays
- Standards
- Process & assay consistency

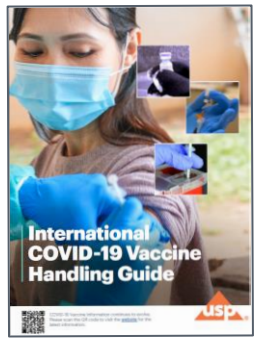
Distribution

- Cold chain
- Storage

Administration

- Training
- Admin Strategy





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Assessing mRNA Quality and Consistency with Analytical Tools

Sarita Acharya
USP Global Biologics
February 28th, 2024

Agenda

- Cell-free manufacturing process
- Critical raw materials
- Building consensus on CQAs and test methods
- Platform methods and standards

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mRNA Manufacturing

Cell-free manufacturing vs.

Cell-based biotherapeutics manufacturing

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Considerations for Cell-free Manufacturing

	Raw Materials	Drug Substance Quality Attributes	Drug Product/ Delivery Systems	Impurities
Cell-based manufacturing	Cells Media and supplements	Protein heterogeneity from cell-based production, especially post-translational modifications Size variants, charge variants, glycosylation etc.	Liquid or lyophilized format with common formulations to support solubility and stability	Host cell DNA Host cell protein
Cell-free manufacturing	Plasmids, enzymes, nucleosides, capping reagent	Physiochemical properties of mRNA that impact stability and translation in patient cells 5’ capping, Poly-A tail length, truncations, modifications	Lipid nanoparticle – has its own set of raw materials and CQAs, some of which are not well understood	Residual plasmids, enzymes, nucleosides, reagents Double stranded RNA

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A New Paradigm

- mRNA cell-free production system has advantages in terms of flexibility and speed, but also generates a need for new analytical tests for:
  - Novel set of raw and starting materials
    - Many not easily sourced in compliance with cGMP
  - Novel delivery systems
  - Unique impurities
- Most tests will be common across mRNA products and therefore are amenable to platform-associated Reference Standards

- "New" raw and starting materials
  - Plasmid – high quality DNA template
  - Enzymes and reagents
    - T7 polymerase, DNAse, restriction enzymes
    - 5' capping and poly(A) tail reagents
  - Nucleosides
    - Native and modified nucleosides
- New delivery systems (e.g. LNPs)
  - Raw materials
  - Size and heterogeneity
  - Lipid content and purity
  - Encapsulation efficiency

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Raw and Starting Materials

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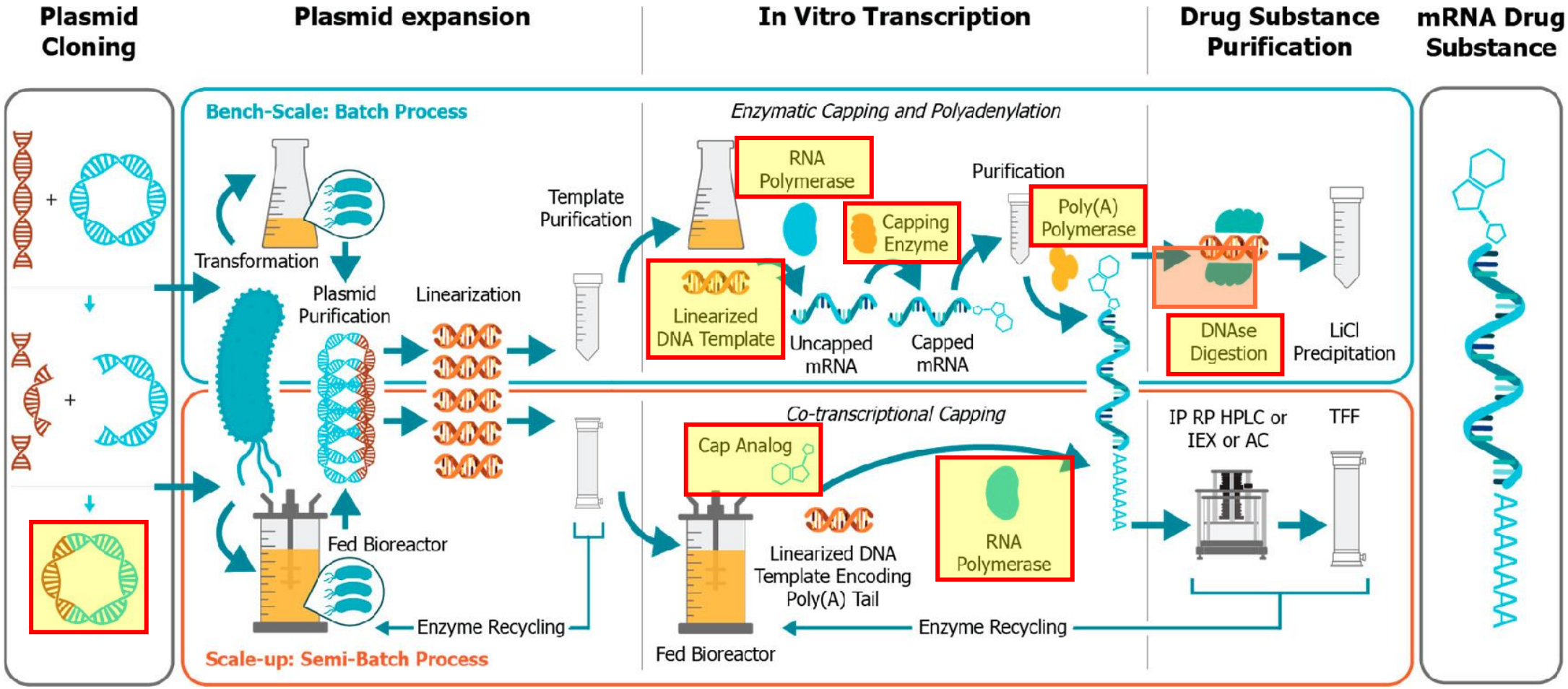
Overview of Raw and Starting Materials



Mol. Pharmaceutics 2022, 19, 4, 1047−1058 https://doi.org/10.1021/acs.molpharmaceut.2c00010

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Proposed testing for DNA plasmid prior to release

Quality	Attribute	Method
Identity	Sequence	Sequencing
	Restriction map	Restriction enzyme analysis with agarose gel electrophoresis
Concentration	Plasmid concentration (A_{260})	Ultraviolet spectroscopy (UV)
Purity	Plasmid purity ($A_{260/280}$)	Ultraviolet spectroscopy (UV)
	% Supercoiled	Capillary electrophoresis (CE) or High-performance liquid chromatography (HPLC)
	Residual host RNA	High-performance liquid chromatography (HPLC) or agarose gel electrophoresis
	Residual host DNA	Quantitative PCR (qPCR)
	Residual protein	SDS-PAGE or Bicinchoninic acid assay (BCA)
	Host cell protein	Enzyme-linked immunosorbent assay (ELISA)
	Residual kanamycin	Enzyme-linked immunosorbent assay (ELISA)
Safety	Endotoxin	USP <85>
	Bioburden	USP <61>
	Sterility	*USP <71>
Other	Appearance	<790>
	pH	USP <791>
	Osmolality	USP <785>
	**Mycoplasma	USP <63>

from *Analytical Procedures for mRNA Vaccine Quality: Draft Guidelines* www.usp.org/mrna-quality

Other USP resources:

<1040> Quality Considerations of Plasmid DNA as a Starting Material for Cell and Gene Therapies

- proposed general chapter published for public comment in PF 49(6)

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Assessing Quality and Consistency: T7 Polymerase

- Critical raw material that transcribes DNA into mRNA, which impacts
  - Transcription efficiency and yield
  - Fidelity
  - Prevalence of incomplete transcripts
- PQAs included on CoA vary across vendors
- Different vendors use different assays to define activity
  - 3 different activity assays across 4 vendors
    - ^{32}P nucleoside incorporation
      - Radioactive assay – not supported in many industry labs
    - Fluorescent assay
      - Proprietary assay
    - Digoxigenin labeling
  - Activity may be buffer-dependent

Quality Attribute	Vendor 1	Vendor 2	Vendor 3	Vendor 4
Appearance		☑		
Activity/ concentration	☑	☑	☑	☑
Exonuclease	☑		☑	☑
Endonuclease	☑	☑ (2 assays)	☑	☑
Nicking activity		☑		☑
DNase	☑			
RNase	☑		☑	☑
Purity	☑	☑		☑
Promoter Specificity	☑			☑
Residual DNA				☑
Endotoxin				☑

- *Common definition of activity units and alignment on quality attributes are needed*

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Critical Quality Attributes

Building consensus on CQAs and relevant test methods

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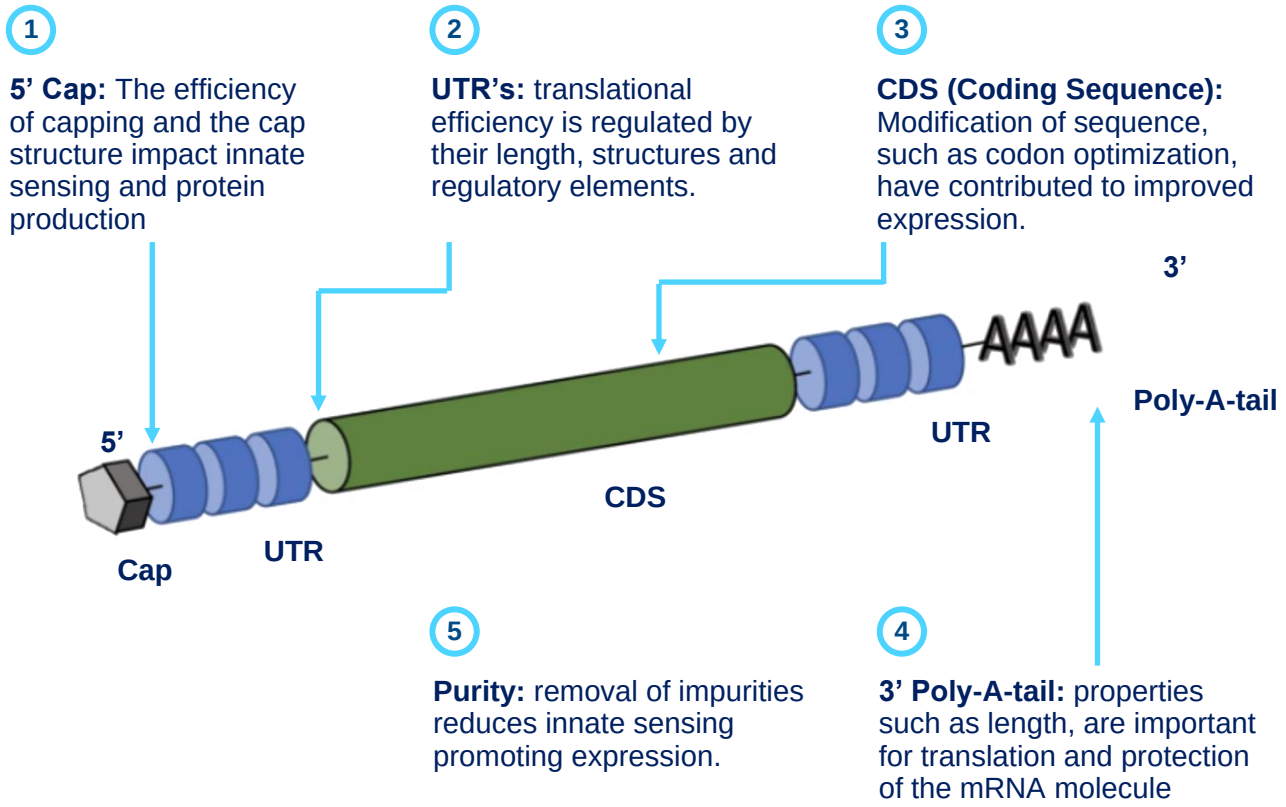
Quality Attributes for mRNA Vaccines

Identity, Purity, Stability, Immunogenicity and Homogeneity



Source: npj Vaccines 5, 11 (2020). https://doi.org/10.1038/s41541-020-0159-8

- Common PQAs make mRNAs amenable to platform analytical methods
  - 5' Cap, poly-A tail
  - Similar size range
  - Aggregates
- "New" but common impurities
  - Residual starting materials (e.g. Plasmid, nucleosides, enzymes)
  - Residual reagents, solvents
  - ds RNA, mRNA fragments, misincorporation

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USP Approach to mRNA Vaccine Guidelines

Specific methods identified and adapted from public sources

New rapid response process – not direct pathway to the compendia

+

Draft reviewed and refined by vaccine experts

USP Expert Committee
USP Vaccine Advisory Group

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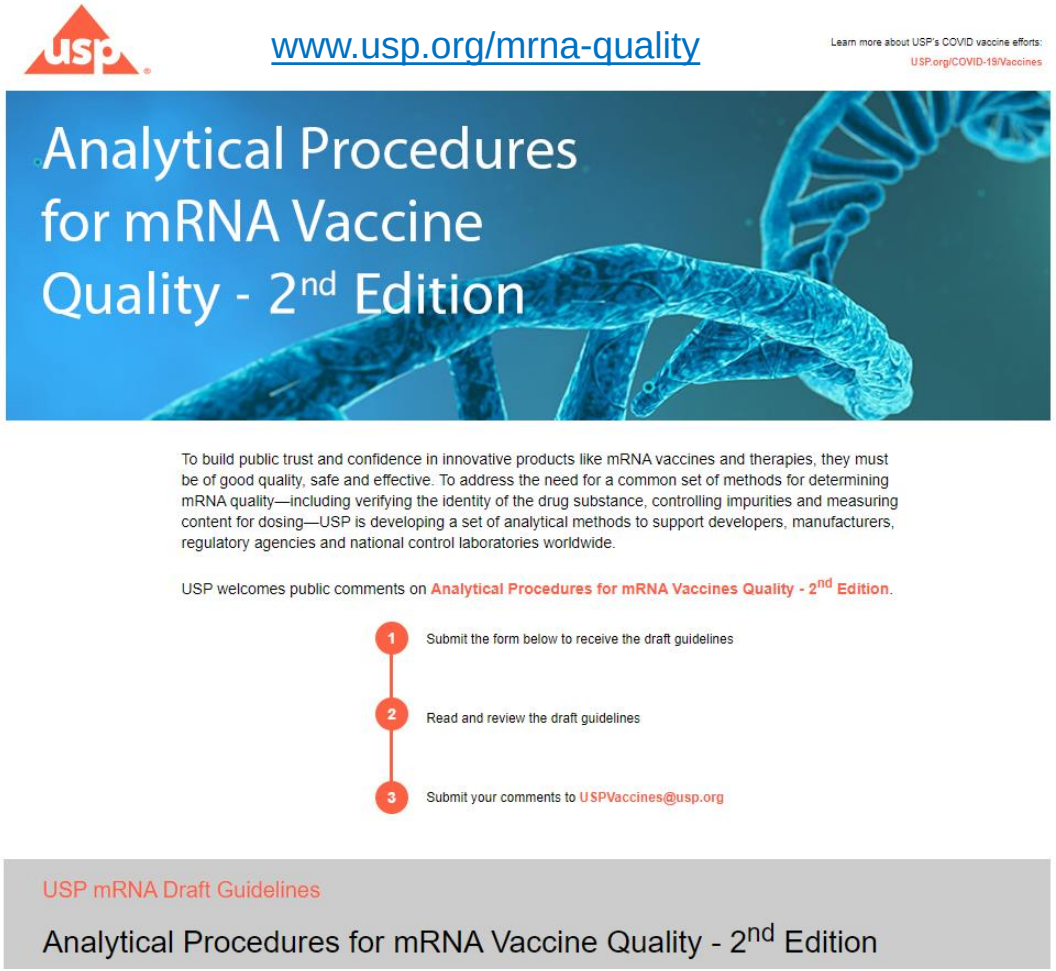
Building Consensus: Updated Guidelines and Public Outreach



- **1st Edition**: Guideline shared for public comments in February 2022
  - Over 300 comments received and evaluated including:
    - Suggestion to add methods for drug product, identification of LNP components
    - Addition of methods for other impurities, nucleosides, capping reagents
    - Guidance on testing plasmid
    - Alternate methods received for several CQAs
    - Technical suggestions to improve methods (column, buffer, etc)
- **2nd Edition**: Updated guideline published in April 2023
  - Over 300 comments received and evaluated:
    - Alternate methods suggested for several CQAs
    - Technical suggestions to improve methods (column, buffer, etc)
- **3rd Edition**: **COMING SOON**
  - Will include USP verified methods for several CQAs

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Proposed Testing for mRNA Drug Substance

Quality	Attribute	Method
Identity	mRNA sequence identity confirmation	High throughput sequencing (HTS)
		Sanger sequencing
		Reverse Transcriptase – PCR (RT-PCR)
Content	RNA concentration	Quantitative PCR (qPCR)
		Digital PCR (dPCR)
		Ultraviolet Spectroscopy (UV)
Integrity	mRNA intactness	Capillary electrophoresis[R]
		Capillary gel electrophoresis (CGE)[R]
		Agarose gel electrophoresis
Purity	5′ capping efficiency	Reverse-phase liquid chromatography mass spectroscopy (RP–LC-MS/MS)[R]
		Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)
	3′ poly(A) tail length	Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)
	Product related impurities - dsRNA	Immunoblot
		Enzyme-linked immunosorbent assay (ELISA)
	Product related impurities - aggregate quantitation	Size exclusion-high-performance liquid chromatography (SEC-HPLC)[R]
	Product related impurities - percentage of fragment mRNA	Reversed-phase HPLC (RP-HPLC)[R]
	Process related impurities-residual DNA template	quantitative PCR (qPCR)
	Process related impurities - quantitation of free/ non-incorporated nucleosides	Reverse-phase liquid chromatography mass spectroscopy (RP–LC-MS/MS)[R]
	Process related impurities - residual T7 RNA polymerase content	Enzyme-linked immunosorbent assay (ELISA)

Quality	Attribute	Method
Potency	Expression of target protein	Cell-based assay
Safety	Endotoxin	USP <85>
	Bioburden	USP <61>, <62>, <1115>
Other	Appearance	USP <790>
	Residual solvents	USP <467>
	pH	USP <791>

- Included multiple options for testing the same attribute where possible to accommodate differences in available equipment

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Proposed Testing for mRNA Drug Product

Quality	Attribute	Method
Identity	mRNA sequence identity confirmation	Sanger sequencing
		Reverse Transcriptase – PCR (RT-PCR)
	Identity of lipids	Reversed-phase high-performance liquid chromatography with charged aerosol detector (RP-HPLC-CAD)
Content	RNA concentration/RNA encapsulation efficiency	Fluorescence-based assay
	Lipid content	Reversed-phase high-performance liquid chromatography with charged aerosol detector (RP-HPLC-CAD)
Integrity	LNP size and polydispersity	Dynamic light scattering (DLS)
	RNA size and integrity	Capillary gel electrophoresis (CGE)[D]
Purity	Product related impurities - aggregate quantitation	Size exclusion-high-performance liquid chromatography (SEC-HPLC)[D]
	Product related impurities - percentage of fragment mRNA	Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC)[D]
Potency	Expression of target protein	Cell-based assay

Quality	Attribute	Method
Safety	Endotoxin	USP <85>
	Sterility	USP <71>
Other	Appearance	USP <790>
	Residual solvents	USP <467>
	Osmolality	USP <785>
	Subvisible particles	USP <787>
	Residual solvents	USP <467>
	Extractable volume	USP <1>, <698>
	Container closure integrity	USP <1207>
	pH	USP <791>

[D] Donated methods

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Considerations for Platforming

Analytical Methods

and Reference Standards

21

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Platform Analytics

- Generally Applicable
  - 5’ Capping
  - Poly A tail length
  - Sizing
  - Residual impurities
    - Plasmid
    - Enzymes
    - Nucleosides
    - Double-stranded RNA

- Some Exceptions
  - Sizing of large mRNAs, including self-amplifying mRNA
  - Potency
  - When making changes to key components
    - New LNP components
    - Changes in UTRs may impact PCR-based tests

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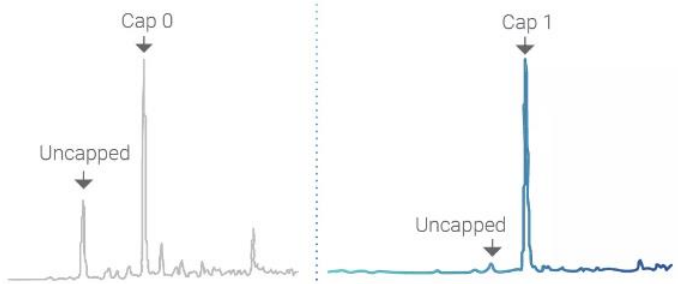
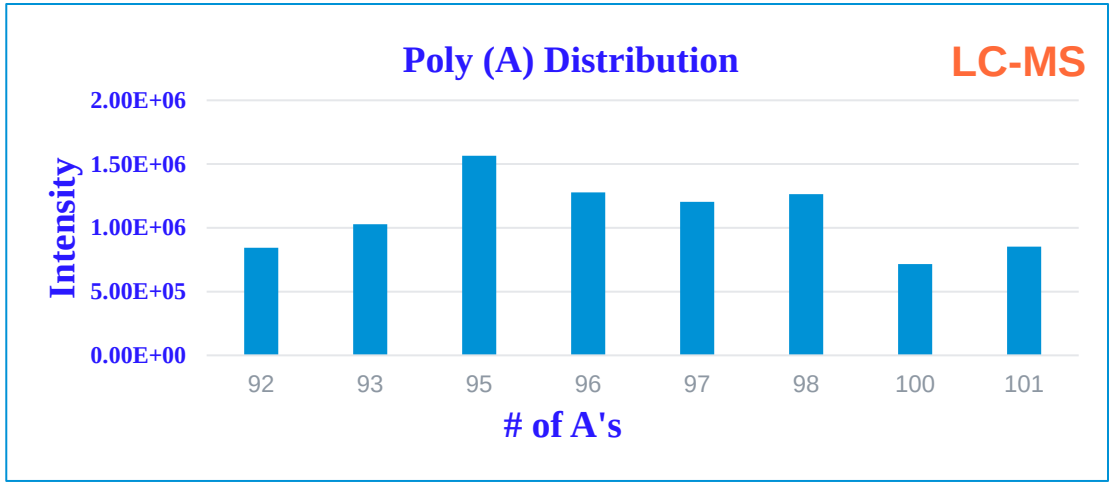
Reference Standards to Support Platform Methods

- Well-characterized reference materials help
  - Establish System Suitability
  - Serve as positive controls
  - Support sizing and quantitation

5’ Capping



Poly A Tail Length



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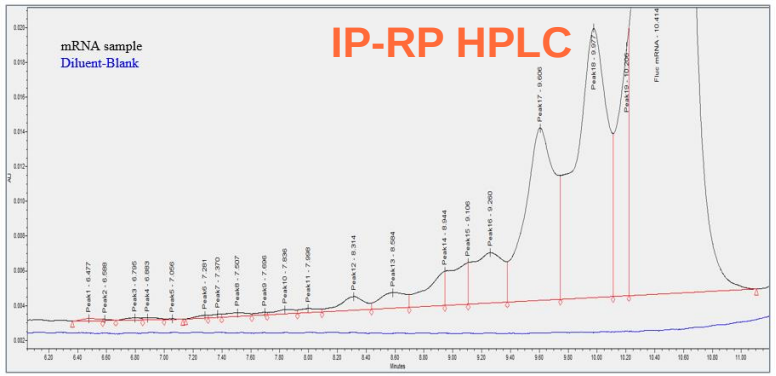
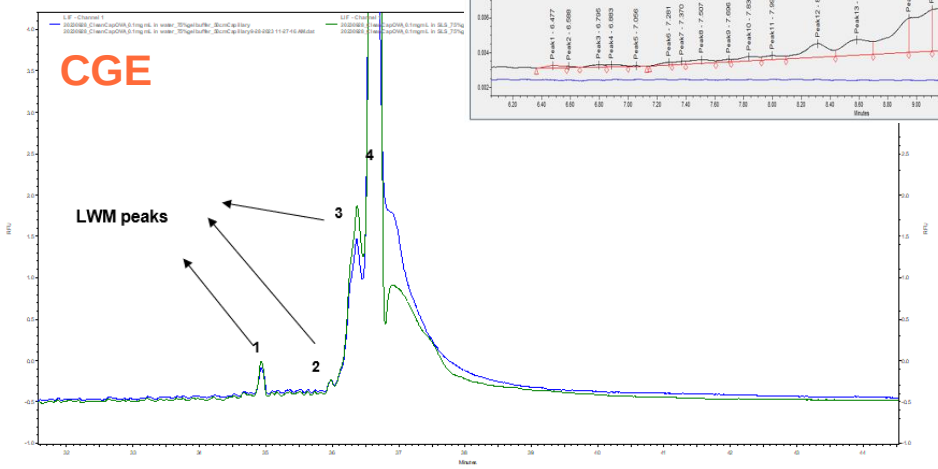
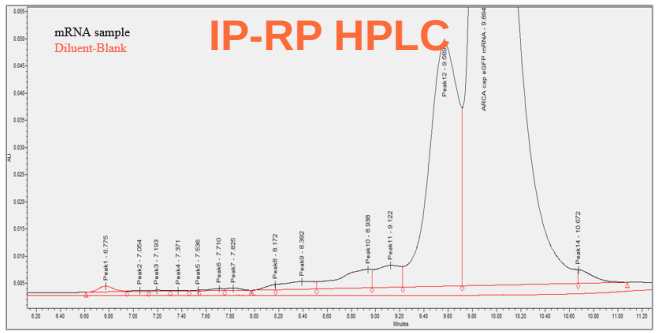
Example Applications and Data

Fragmentation/ Degradation

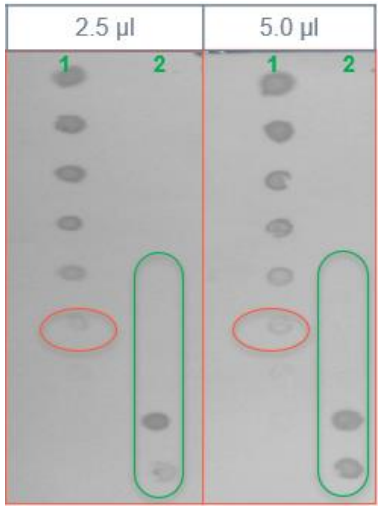




Purity



dsRNA



Dot blot
Lane 1 –Standard
Lane 2 - Sample

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USP Goals for mRNA Vaccine Quality

New guidelines can support vaccine quality by:

Establishing a common understanding of quality attributes for both Drug Substance and Drug Product

Providing testing methods that can be used as a starting point to assess quality attributes (e.g., identity, quantity, purity and safety)

Providing multiple options for testing of the same attribute

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Next Steps for mRNA Vaccine Standards

Opportunities for Collaboration

Continue to engage with mRNA community to build consensus on PQAs and methods

- Encouraging additional comments
- Recruited Expert Panel to draft General Chapter on mRNA
- Seeking donations of methods and validation packages

Evaluate selected analytical procedures included in mRNA Vaccine Guidelines

- Underway now
- Requesting samples for testing to ensure method suitability

Identify standards and controls needed to support methods

- First round of materials in development now
- Seeking bulk materials to develop standards and controls to support analytical methods

Expand global trainings and tools

- Seeking global partners to support training/ tech transfer for receiving laboratories

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Thank You

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