Afrigen

Biologics & Vaccines

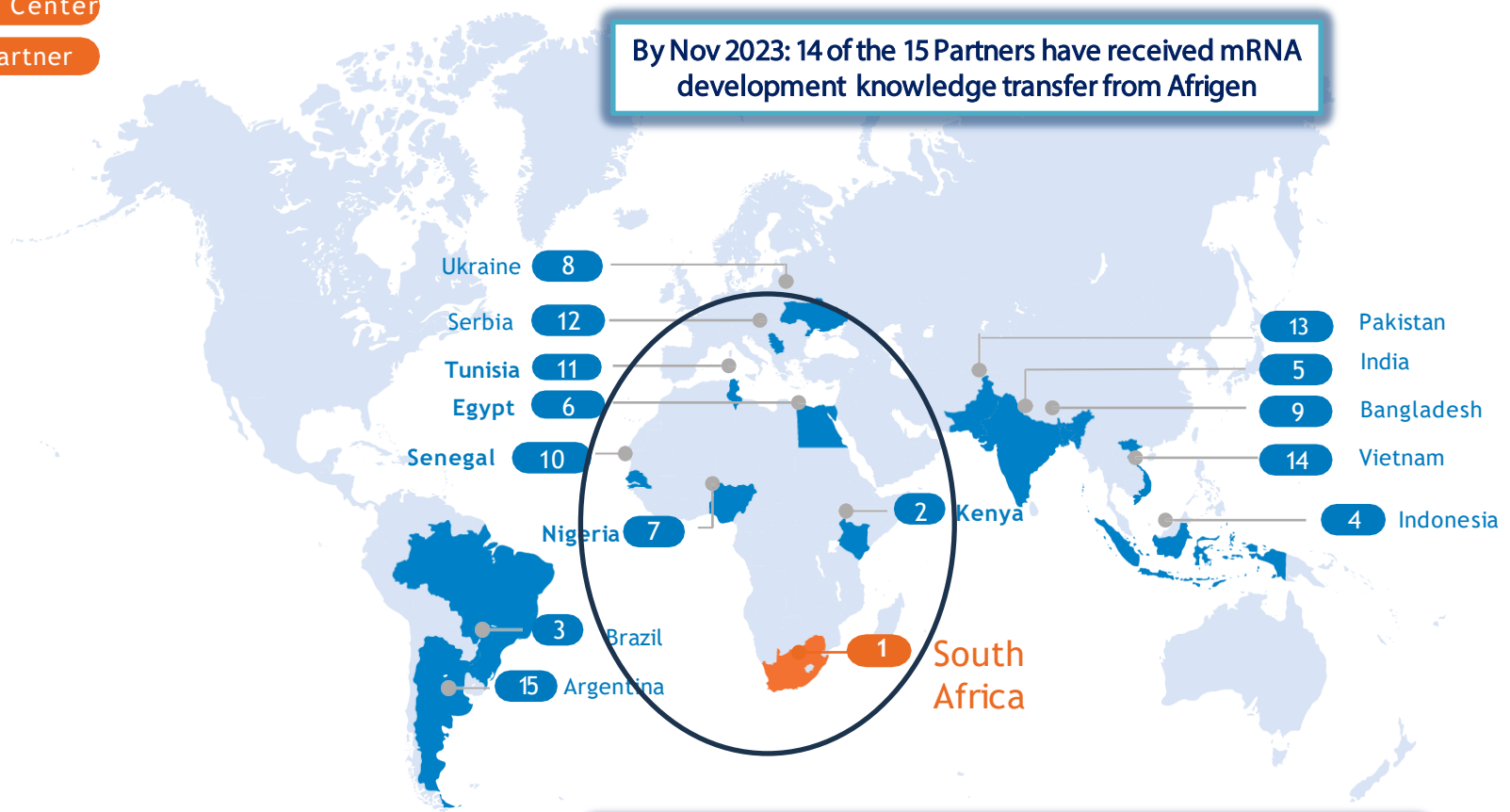
An Avacare Health & IDC Company

# Afrigen's Experience of Analytical challenges in mRNA vaccines: Current solutions and future directions

# WHO and MPP mRNA Technology Transfer Programme

- **Africa** at its Centre
- Building Sustainable Capacity and Capabilities
- A LMIC partnership network straddling 4 continents and connecting 15 countries, representing **>3 Billion people**

| | | |
|---|---|---|
| 1 | **Afrigen** | mRNA Center |
| | Biovac | 1st partner |
| 2 | BioVax Kenya | |
| 3 | Bio-Manguinhos | |
| 4 | Biofarma | |
| 5 | BiologicalE | |
| 6 | BioGeneric Pharma | |
| 7 | Biovaccines Nigeria | |
| 8 | Darnitsa | |
| 9 | Incepta Vaccine | |
| 10 | Institut Pasteur de Dakar | |
| 11 | Institut Pasteur de Tunis | |
| 12 | Institut Torlak | |
| 13 | National Institute of Health, | |
| 14 | Polyvac | |
| 15 | Sinergium Biotech | |




mRNA Technology Transfer Programme

# The Approach

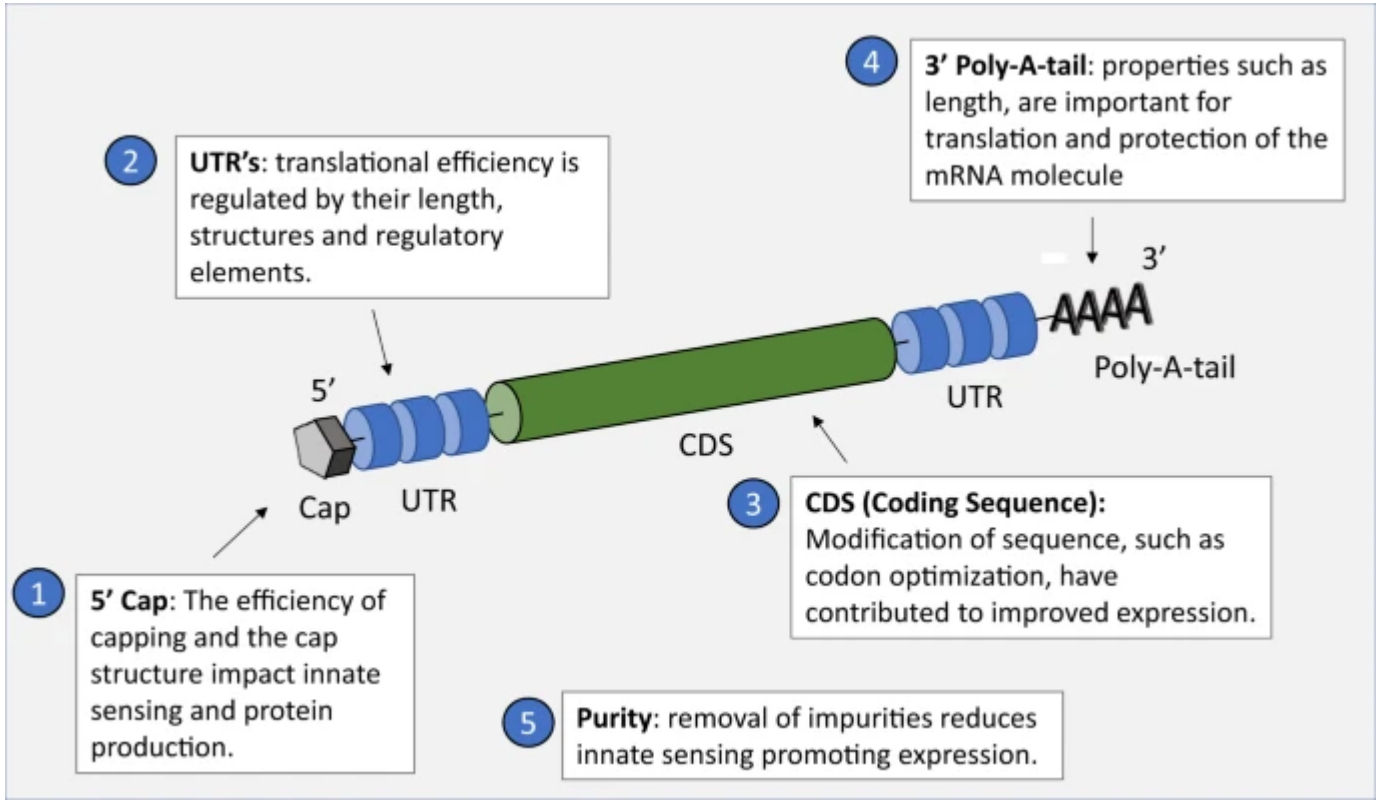
- Follow regulatory guidelines
  - Assay development follows strict regulatory guidelines depending on the stage of development, intended use, and industry requirements. The most widely recognized regulatory guidelines for assay development and validation come from organizations like: ICH & FDA

- Method Qualification
  - A research-based evaluation conducted without a formal protocol, ensuring the method is suitable for use in R&D.

- Method verification
  - An assessment of a compendial method to confirm its suitability for use by evaluating minimal performance characteristics.

- Method transfer
  - The process of transferring an analytical method from a sending unit to a receiving unit, ensuring the assay remains suitable for its intended use through the addition of a formal protocol.

# mRNA Characterization




| 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[D] |
| | | Capillary gel electrophoresis (CGE)[D] |
| | | Agarose gel electrophoresis |
| Purity | 5' capping efficiency | Reverse-phase liquid chromatography mass spectroscopy (RP–LC-MS/MS)[D] |
| | | 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)[D] |
| | Product related impurities - percentage of fragment mRNA | Reversed-phase HPLC (RP-HPLC)[D] |
| | 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)[D] |
| | Process related impurities - residual T7 RNA polymerase content | Enzyme-linked immunosorbent assay (ELISA) |
| 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> |

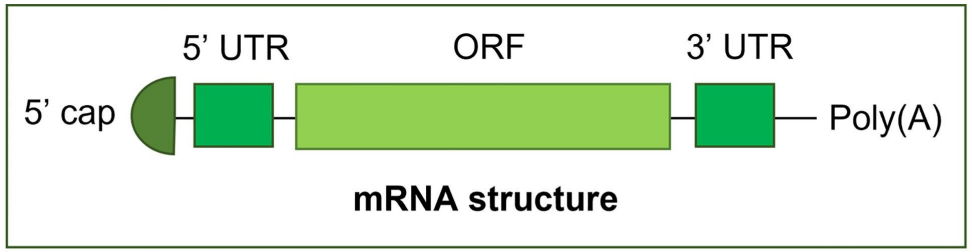
[D] Donated methods

Afrigen Biologics & Vaccines

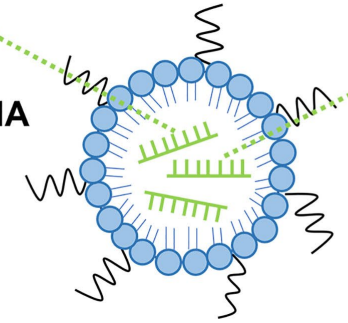
Jackson, N.A.C., Kester, K.E., Casimiro, D. *et al.* The promise of mRNA vaccines: a biotech and industrial perspective. *npj Vaccines* **5**, 11 (2020). https:/ / doi.org/ 10.1038/ s41541-020-0159-8

United States Pharmacopeia" (USP) as the source, specifically mentioning their "Analytical Procedures for mRNA Vaccine Quality" document

# mRNA-LNP Characterisation




**Lipid-based mRNA nanoparticle**



**Components of lipid nanoparticles**
Cholesterol
phospholipid
ionizable lipid
PEG-conjugated lipids

| 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 |
| 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



Front. Immunol., 26 May 2022 Sec. Cancer Immunity and Immunotherapy Volume 13 - 2022 | https://doi.org/10.3389/fimmu.2022.923647

United States Pharmacopeia" (USP) as the source, specifically mentioning their "Analytical Procedures for mRNA Vaccine Quality" document

# Performance Characteristics

- When developing and validating an assay, several key performance characteristics must be assessed to ensure reliability, accuracy, and regulatory compliance. These characteristics depend on the assay type (e.g., quantitative, qualitative, bioanalytical).

- Based on ICH Q2R2;

    - Specificity
    - Sensitivity
    - Precision
    - Accuracy
    - linearity and range
    - System suitability
    - Robustness

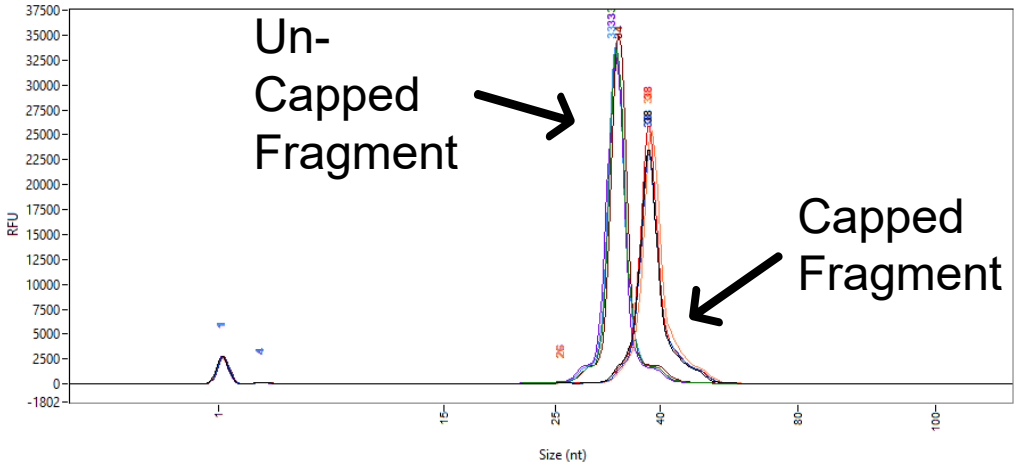
    - Stability

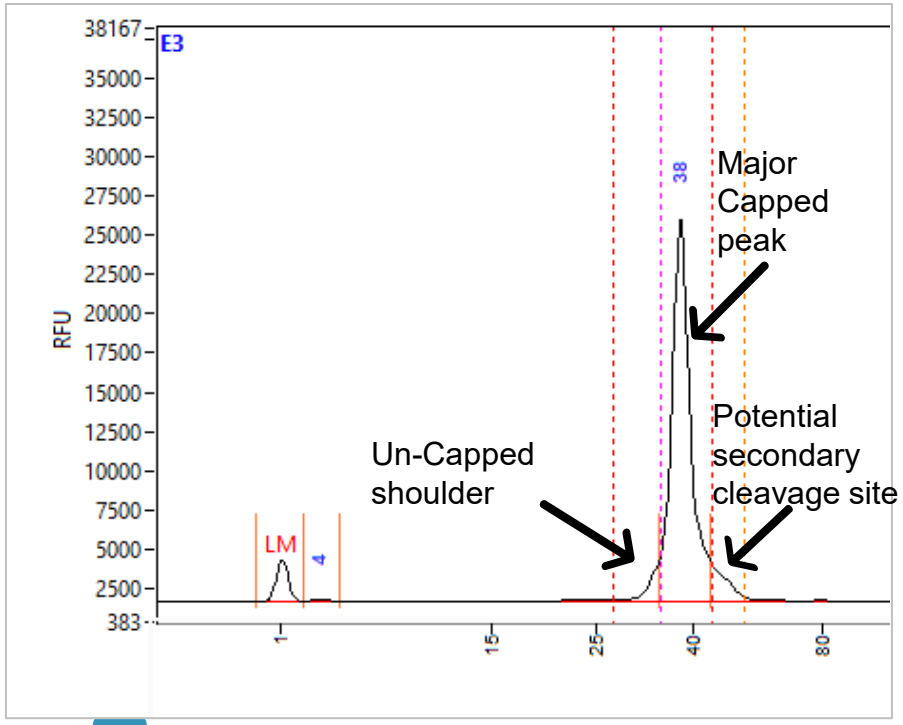
# Afrigen's qualification approach based on ICHQ2 guidelines

| Attribute | Assay | Release / Characterization / IPC / IPT | pDNA / DS / DP | Type of Analytical Procedure (per ICH Q2 guideline) | Qualification Characteristics to be assessed (based on ICH Q2 guideline) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Accuracy | Precision | | Specificity | Detection limit (LOD) | Quantitation limit (LOQ) | Linearity | Range | Robustness |
| | | | | | | Intermediate Precision | Repeatability | | | | | | |
| **pDNA linearization confirmation** | Agarose gel | IPT | pDNA | Qualitative Purity | X | - | X | X | X | - | - | X | - |
| **% Capping** | Capture probe & RNase H treatment +CGE | Release | DS | Quantitative Purity | X | X | X | X | X | - | - | (1) | X (brief) |
| **PolyA tail length** | RNase T1 treatment +CGE | Release | DS | Qualitative Purity | X | X | X | X | X | - | - | - | X (brief) |
| **dsRNA** | dot blot | Release | DS | Quantitative Purity | X | X | X | X | X | X | X | X | X |
| **Residual pDNA - template** | qPCR | Characterization | DS | Quantitative Purity | X | X | X | X | X | X | X | X | - |
| **Residual Protein** | AccuOrange (Fluorescence) | Characterization | DS | Quantitative Purity | X | X | X | X | X | X | X | X | - |
| **mRNA concentration** | A260 (Nanodrop) | Release | DS | Content | X (mRNA of known concentration) | X | X | X | - | X | - | - | - |
| **mRNA integrity** | CGE | Release | DS, DP | Quantitative Purity (Integrity, USP guidelines) | - | X | X | X | - | X | (2) | X | X (brief) |
| **mRNA size** | | Release | DS, DP | | X | X | X | X | X | - | - | X | X (brief) |
| **Identity** | RT-PCR +Sanger Sequencing | Release | DS, DP | Identity Test | - | - | - | X (3) | - | - | - | - | - |
| **mRNA concentration & % encapsulation** | Ribogreen Assay (Fluorescence) | Release | DP | Content | X (for mRNA control only) | X | X | X | - | - | X | X | X (brief) |
| **Particle size and PDI** | Dynamic Light Scattering (DLS) | Release | DP | Qualitative Purity (Integrity, USP Guidelines) | X (only for size standards) | X | X | X (only for size standards) | - | - | - | X | X |
| **Zeta potential** | Dynamic Light Scattering (DLS) | Characterization | DP | | X (only for zeta standard) | X | X | - | - | - | - | X | X |
| **Protein expression** | Western Blot | Release | DP | Protein expression | - | - | - | X | - | - | - | - | - |
| **Lipid identity** | HPLC-CAD | Release | DP | Identity | - | - | - | X | - | - | - | - | - |
| **Lipid concentration** | | Release | DP | Content | X | X | X | X | - | - | X | X | X |

**(1): Sample always tested at the same molar ratios of mRNA:capture probe:beads. Range testing would be changing molar ratios, which is anyway limited because only so many ratios can be tested.**
**PolyA is qualitative: one does not say how much of the mRNA has a certain tail, but rather the average length of the tail on average (assuming all mRNA is capped).**
**(2): Working range tested.**
**(3): Sample matrix. Sodium Acetate interferes with cDNA synthesis - this has been confirmed at Inqaba. Samples are therefore submitted in water.**

The uncapped sample (left) has a single major peak and falls on the shoulder of the capped sample.



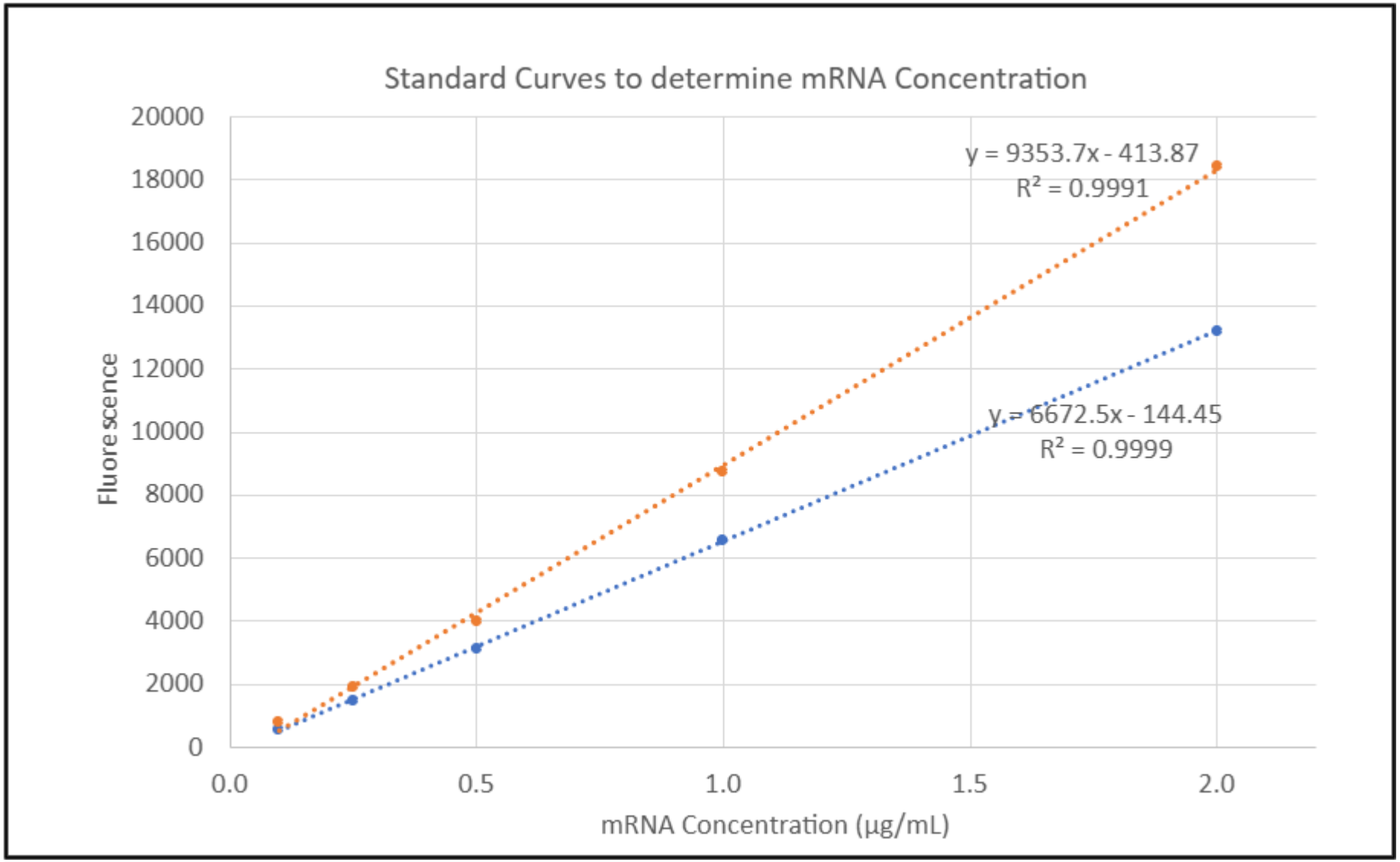

| Range | ng/uL | % Total | nmole/L | Avg. Size | %CV |
|---|---|---|---|---|---|
| 28 nt to 45 nt | 6,5487 | 90,9 | 530,7017 | 38 | 5,78 |
| 35 nt to 45 nt | 6,2084 | 86,2 | 499,5433 | 38 | 4,98 |
| 28 nt to 55 nt | 6,9725 | 96,8 | 554,4972 | 39 | 9,17 |
| 35 nt to 55 nt | 6,6322 | 92,0 | 523,4680 | 39 | 8,69 |

Total region without secondary site
Capped region without secondary site
Total region with secondary site
Capped region with secondary site

Capping Efficiency without secondary cleavage:
86,2/90,9 x 100 = 94,8 %

Capping Efficiency with secondary cleavage:
92,0/96,8 x 100 = 95,0 %

Possible secondary cleavage had no impact on calculated capping efficiency.


Standard Curves to determine mRNA Concentration
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Fluorescence
0.0
0.5
1.0
1.5
2.0
mRNA Concentration (µg/mL)
y = 9353.7x - 413.87
R² = 0.9991
y = 6672.5x - 144.45
R² = 0.9999

# Challenges & Tech Transfer

- Reference material
  - In-house reference material established as no standard industry references available
- Assays fit for purpose
  - In R&D the method performed as intended
- Orthogonal methods e.g. CGE & HPLC & LCMS
  - With developing technology it is important to have orthogonal methods in case systems are done or waiting on material
- Tech transfer to QC and partners
  - On-site training very beneficial, comparative results
  - Equipment
- Acceptance Criteria
  - Establish once processes are locked and over time with data generation

A PROUDLY
AFRICAN BIOTECH

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# THANK YOU!

# Bio

**Astrid Trimmel – R&D Analytics Lead**

Dr. Astrid Trimmel is an accomplished scientist and leader in analytical research and development. She holds a PhD in Chemistry from the University of Cape Town, where her research focused on conjugate vaccines. With a strong background in analytical chemistry and biopharmaceutical development, Astrid currently leads the R&D Analytics team in advancing cutting-edge mRNA and mRNA-LNP assay development. Her expertise lies in optimizing analytical methodologies to support innovative therapeutic solutions. Passionate about scientific innovation and precision analytics, she is dedicated to driving impactful advancements in the field of mRNA technology.

# Abstract

As mRNA vaccines continue to transform modern medicine, maintaining their quality, consistency, and stability remains a critical challenge. This presentation addresses key analytical hurdles in mRNA and lipid nanoparticle (LNP) characterization, focusing on mRNA integrity, encapsulation efficiency, potency, and stability. A range of physicochemical techniques, including high-performance liquid chromatography (HPLC), dynamic light scattering (DLS), and plate-based assays, were employed to assess mRNA purity, structural integrity, cap structure, and LNP encapsulation efficiency.

After applying the typical validation characteristics [ICH Q2(R1)] to the test methods in detecting impurities, evaluating mRNA length distribution, and analysing lipid composition, we found the methods are highly effective. The degree of revalidation required depends on the nature of changes in m RNA (this means that we expect changes to happen and through revalidation these changes will be identified). Furthermore, this work emphasizes the importance of cross-industry collaboration in establishing global benchmarks for mRNA and LNP quality control.

By sharing these insights, we aim to contribute to the development of standardized analytical protocols and encourage meaningful discussions among stakeholders. Such collaborative efforts are essential for advancing the production and accessibility of high-quality mRNA therapeutics worldwide

# Overview of Analytical Methods

| Process Stage | Stage | Quality Attribute | Assay | Method | Compendial | IPC | IPT | Characterization | Release | Stability |
|---|---|---|---|---|---|---|---|---|---|---|
| pDNA | CoA release | Identity | PolyA tail length | AGE/ CGE | | | | | X | |
| pDNA | CoA release | Identity | pDNA sequence | Sanger Sequencing | | | | | X | |
| IVT IPC | In-process | Purity | Confirmation of pDNA Linearization | AGE | | X | | | | |
| IVT IPT | In-process | Content | mRNA crude quantification | Qubit | | | X | | | |
| DS & DP | Bulk and FF | Other | Appearance | Ph. Eur. 2.2.1 and Ph. Eur. 2.2.2, USP <790> | X | | | | X | X |
| DS & DP | Bulk and FF | Other | pH | Ph. Eur. 2.2.3, USP <791> | X | | | | X | X |
| DS & DP | Bulk | Safety | Endotoxin | Ph. Eur. 2.6.14, USP <85> | X | | | | X | |
| DS | Bulk | Safety | Bioburden | Ph. Eur. 2.6.12, USP <61> | X | | | | X | |
| DS | Bulk | Content | mRNA concentration | UV (A260) | | | X | | X | X |
| DS & DP | Bulk | Identity | mRNA sequence | RT-PCR followed by Sanger sequencing | | | | | X | |
| DS | Bulk | Purity | Capping efficiency | Capture probe +RNase H treatment followed by CGE | | | | | X | X |
| DS | Bulk | Purity | PolyA tail length | RNase T1 treatment followed by CGE | | | | | X | X |
| DS & DP | Bulk and FF | Integrity | Size and integrity | CGE | | X | | | X | X |
| DS | Bulk | Purity - Product related impurities | dsRNA content | Immunoblot (dot blot) | | | | | X | |
| DS | Bulk | Purity - Process related impurities | Residual pDNA template | qPCR | | | | X | X | |
| DS | Bulk | Purity - Process related impurities | Residual enzymes and proteins | AccuOrange - Fluorescence | | | | X | X | |
| DP | Bulk and FF | Integrity | Particle size & Polydispersity | Dynamic light scattering using the Zetasizer | | | | | X | X |
| DP | Bulk | Integrity | Zeta Potential | Laser Doppler Electrophoresis using the Zetasizer | | | | X | | |
| DP | Bulk and FF | Content | mRNA concentration & %Encapsulation | RiboGreen Assay - Fluorescence | | X | | | X | X |
| DP | Bulk and FF | Protein expression | Protein expression (size / purity) | Western Blot | | | | | X | X |
| DP | Bulk and FF | Content and Identity | Lipid quantitation | HPLC-CAD | | | | | X | X |
| DP | FF | Other | Container Closure integrity testing | USP <1207> | X | | | | X | X |
| DP | FF | Other | Extractable volume | Ph. Eur. 2.9.17, USP <697> | X | | | | X | |
| DP | FF | Safety | Sterility | Ph. Eur. 2.6.1, USP <71> | X | | | | X | X |
| DP | Bulk and FF | Other | Osmolality | Ph. Eur. 2.2.35, USP <785> | X | | | | X | |
| DP | Bulk and FF | Other | Residual solvent | Ph. Eur. 2.4.24, USP <467> | X | | | X | | |
| DP | FF | Other | Particulate matter | Ph. Eur. 2.9.19, USP <788> | X | | | | X | |