Analytical Method Release and Stability Platform for RNA Drug Substance

As a leader in cell and gene therapy (CGT) manufacturing and testing for 25 years, Aldevron has built superior analytical capabilities and expertise to support characterisation, release, and stability testing of internally manufactured products. Aldevron has evolved with this growing industry and expanding regulatory landscape. In addition to this internal knowledge growth, Aldevron has made strategic hires to further enhance its analytical knowledge base. Aldevron uses a life cycle approach to analytical method development and validation as outlined in several recent guidance documents, including USP <1220> , ICH Q2 , and ICH Q14.

Offering analytical testing at the same site as manufacturing provides a key strategic asset supporting the contract development and manufacturing organization (CDMO), helping fill an extensive and growing analytical testing shortage in the CGT industry. Our integrated manufacturing and testing services provide quality and speed – robust and unique advantages for our clients.

Aldevron offers comprehensive analytical support in the Research & Development space to aid the development of commercial products. This end-to-end support includes:

- Method development
- Method validation and transfer
- Process characterisation testing
- In-process testing
- Process validation testing
- GMP release testing in a QC (Quality Control) environment

Aldevron's Quality Control laboratory offers a high level of compliance. As a fully GMP laboratory, clients can be assured that testing is performed under the highest level of good documentation practices, analyst training, instrument qualification and software validation. ALCOA+ principles are applied across the analytical space, especially for method validations, stability, and release testing. Data integrity is assured through document control, archiving, and audit trail review.

With Aldevron now part of the Danaher group of companies, the opportunities to optimise and advance our analytical capabilities are enhanced through close working relationships with industry leaders such as Precision NanoSystems Inc. (PNI), IDT, SCIEX, Molecular Devices, and Beckman Coulter. These collaborative relationships further expand Aldevron's expertise and scope of services and technologies that includes next generation plasmids, such as Nanoplasmids and RNA backbones, process development and manufacturing, and in vitro transcription and gene editing enzymes for gene and cell therapies. This unity under one roof provides solutions and support for our customers to meet their specific needs. Putting the client first, Aldevron is positioned to assist through the full process of end-to-end manufacturing that improves the customer journey from the clinical trial phase all the way through to commercialisation.

Analytical Characterisation for Drug Substance

The mRNA drug substance process utilises enzymatic reactions that include different 5'-cap structures (i.e., Cap0 vs Cap1), variable 3'-poly(A) tail length, and truncated mRNA transcripts. This can produce a mixture of product variants and other notable impurities that include double-stranded RNA (dsRNA) molecules and residual plasmid DNA templates. Despite purification steps to remove these unwanted byproducts, the risk of carrying a small portion of impurities remains. Therefore, such product quality attributes should be characterised during in-process and lot release testing of the purified mRNA.

Aldevron Recommended Drug Substance Release Methods

The analytical method validation team at Aldevron is co-located with the Quality Control laboratory, which optimises speed from development through release. Quality attributes expected for release of biologicals are outlined in Q6B Specifications. The general recommendation is to test for Appearance, General Tests, Content/Quantity, Identity, Purity/Impurities, and Potency/Activity to cover all quality attributes. The recommended panel outlined in Table 1 (below) includes at least one of each of the recommended quality attributes, with the exception of potency/activity. This panel also covers assay types recommended in the USP draft guidance, "Analytical Procedures for mRNA Vaccine Quality," to create a comprehensive testing strategy for mRNA products.

Methods shown in Tables 1 and 2 are currently available and have been validated using a matrix approach following Quality by Design (QbD) principles. This means that if a client product is within the defined design space, little or no sample qualification is needed. This offers speed and affordability, especially for early-stage projects. For example, the concentration method has been validated for a range of RNA sizes (2 kb to 17 kb), formulations (TE, Tris, Water for Injection, and citrate) and concentrations (0.5 to 4.0 mg/mL). This comprehensive testing panel will ensure a high-quality product with high confidence for regulatory filing approval. Using the recommended platform provides shorter turnaround times for qualifications of new products.

Stability-indicating Methods

Certain methods can be considered stability-indicating based on historic knowledge and characteristics demonstrated during method development. Monitoring product stability is a regulatory expectation and is needed to assign the shelf life. Table 1 indicates methods that are suitable for stability. In general, purity/integrity methods are the best methods

Attribute	Method	Assay Category	Stability-Indicating
Visual Appearance	Clarity and Color (Ph.Eur. 2.2.1 and Ph.Eur. 2.2.2)	General Test (Compendial)	Y
pH	pH (USP<791>, Ph.Eur. 2.2.3)	General Test (Compendial)	Y
Concentration	UV spectrophotometry by SoloVPE	Quantity/Content	Y
Identity	RT-Sanger Sequencing	Identity	N
Identity	Capillary electrophoresis (Fragment Analyzer)	Identity (Separation occurs though a gel matrix and Identity is confirmed relative to size markers)	N
Purity/Integrity	Capillary electrophoresis (Fragment Analyzer)	Purity/Integrity (Separation occurs though a gel matrix and purity are calculated as % intact/% total)	Y
Residual pDNA Process-related impurity	qPCR	Process-related impurity (Amplification occurs using product-specific primers and probe)	N
dsRNA Process-related impurity	ELISA	Process-related impurity (ELISA-based method using antibody (J2) specific to dsRNA)	N
Residual Impurity – Protein	NanoOrangeTM	Process-related impurity (NanoOrange reagent binds to protein. Relative levels quantitated by fluorescence detection)	N
Endotoxin	Kinetic Chromogenic LAL	Process-related impurity	N
Bioburden	Membrane filtration or pour plate (USP<61>,PhEur2.6.12)	Safety (Compendial) (Recommended for non-sterile products)	N

Table 1. Recommended Core Assay Panel for mRNA
Current release and stability panel for RNA testing methods recommended by Aldevron to ensure quality and compliance.

for monitoring the stability of the product over time. Purity/integrity methods measure intact products and, as the product degrades, the relative percentage of the intact product will decrease and degradation products will increase. Product-related impurities such as degradants and multimers are also appropriate indictors of stability and are often reported using the same method as those used for purity. Either intact product (purity/integrity), degradation products (impurity), or both can be reported out by the assay and trended over time. As products degrade, the sample colour or clarity could be altered and appearance can change. RNA concentration by SoloVPE will be included in the stability panel. While not a true stability indicating method, since degraded product will still absorb UV, concentration is included in stability studies to provide a baseline value over time.

Identity is not a stability-indicating method. Once the identity of the material placed on stability is confirmed, the identity will not change over time. Residual process-related impurities should not be considered for stability studies. Examples include residual plasmid DNA, residual protein, and residual dsRNA. These are parts of the manufacturing process that are cleared by the purification process down to exceptionally low levels. Once release testing confirms that these have been sufficiently removed, there is no future opportunity for them to be introduced into the product over time. Similar rationale applies to not including contaminant testing on the release panel.

5′ Cap and 3′ poly(A) Tail Methods

The 5′ Cap and 3′ Tail methods have been qualified as platform methods, however each new product will require some level of development activity to assess sequence-specific and matrix variation. Given the complexity of the 5′ Cap and 3′ poly(A) tail methods, longer lead times are needed for these product-specific qualifications, which require some custom

Attribute	Method	Example Acceptance Criteria	Assay Category	Stability-Indicating
Purity-Cap integrity	5' Cap IP-RP-HPLC – Identification of enzymatic fragments	% Capped	Purity/Integrity	Y
Purity/Integrity-Poly(A) efficiency	3' Poly A Tail (RP-HPLC) – Identification of enzymatic fragments	% Polyadenylated and/or Relative poly(A) tail length	Purity/Integrity	Y

Table 2 provides a summary of the 5'Cap and 3' poly(A) methods.
Table 2. 5' Cap and 3' polyA Tail methods

development work for each new construct. To reduce cycle times, these methods can be considered as characterization methods for engineering and early phase GMP lots. These can be performed as release methods as the product moves forward in the life cycle. Note that even when testing is performed as characterisation, the results will be suitable for inclusion in a regulatory filing, as good documentation and traceability will be utilised.

Most poly(A) tails are encoded, and the size of the poly(A) tails is uniform. Therefore, Aldevron has a method available that focuses primarily on confirmation of the relative length of the poly(A) tail by comparison to synthetic poly(A) fragments of known length. The assessment of relative tail length can be useful for assessment of stability. Reporting as percent tailed can also be accommodated with the current procedure.

Experience and Capabilities in Action

Client-specific Methods
While Aldevron offers a comprehensive testing panel for RNA, we understand that certain client products may have unique features or activities that require more specialised analyses. In cases where the client needs a custom assay, Aldevron has considerable breadth of expertise internally to accommodate a wide variety of method types. Client-specific methods can either be developed internally based on client needs or transferred in from the client. Aldevron's internal procedures for assay transfers are aligned with regulatory expectations, particularly USP General Chapter <1224> "Transfer of Analytical Procedures."

Specifications
Aldevron provides analytical support for many clients in multiple therapeutic areas at various stages of clinical development. This experience has provided Aldevron with a solid background in setting phase-appropriate specifications to ensure quality and compliance, as directed by regulatory authorities. Specifications can be set more widely for early phase projects, relying heavily on prior knowledge and functional requirements. Aldevron's extensive experience allows for setting meaningful, phase-appropriate specifications that ensure product quality while allowing flexibility for the innovation that regulatory authorities expect for new products. As product testing and manufacturing knowledge expands, the specifications will be appropriately updated. Justification of specifications is documented so that it is available for filing support.

Regulatory Support
We are not only focused on meeting the unprecedented market demand, where testing queues are at all-time highs, but we also are at the forefront of the requirements for regulatory authorities. This enhances speed to market by expediting the path to approval, by providing customers with advanced analytical methods and platform assays to demonstrate potency, purity, identity, and safety. Since Aldevron's platform methods are developed, validated, and documented in a systematic, compliant manner, relevant analytical information is easily converted to filing-ready content in CTD (Common Technical Document) format. Therefore, support for authoring analytical sections 3.2.S.4 is efficient and seamless. These efforts are supported by analytical subject matter experts as well as an internal regulatory affairs team.

Capacity
The mRNA manufacturing process is extremely sensitive to degradation and should be separated from other cell-based processes. To eliminate any risk, a dedicated manufacturing environment for mRNA is necessary to proceed to the next stage of drug manufacturing. Furthermore, if there is a need to produce multiple mRNA products in the same manufacturing line, the risk of crossover and contamination is amplified and needs to be mitigated. Aldevron has expanded its campus in area, with multiple sites using state-of-the-art modular cleanrooms with integrated building management systems and environmental monitoring for cell banking, inoculation, and fill/finish. This capacity allows the production of clinical and commercial products in GMP quality grades for multiple clients with the highest quality and purity for drug products.

Strategic Partnership
Under the Danaher Operating Company umbrella, Aldevron works closely with PNI, IDT, SCIEX, Molecular Devices, and Beckman Coulter to enhance mRNA manufacturing and analytical capabilities and streamline the end-to-end workflow for their clients. The collection of technologies and expertise helps streamline the process for manufacturing, meeting regulatory compliance, and CMC (Chemistry, Manufacturing, and Control) filing. With insight gained through this collaboration, the focus is on improving and innovating new methods and technologies needed to provide high-quality products to customers.

Conclusion
Aldevron has embraced the life cycle approach and is well-

positioned to maintain a leadership role in the industry. Our internal procedures are already in compliance with the recently effective USP<1220> and the recent draft guidance ICH Q14. As part of the life cycle approach to analytical methods, the assay panel is optimised throughout all phases including continuous improvement. Therefore, Aldevron is well-positioned to support release and stability testing for mRNA drug substance to meet customer specific requirements. With strong commitment to assay life cycle management, we are also dedicated to continuously improving methods and adding innovative technologies. Therefore, strategic partnership expedites products to market with advanced support to demonstrate potency, purity, identity, and safety.

Aldevron

Aldevron is a premier manufacturing partner, producing high-quality plasmid DNA, proteins, enzymes, and other key components for the development of vaccines, gene and cell therapies, immunotherapies, veterinary medicines, agricultural biotechnology, and molecular diagnostics. Headquartered in Fargo, North Dakota, Aldevron supports scientists who are developing revolutionary, lifesaving treatments for millions of people. To learn more about how Aldevron is advancing biological science, visit www.aldevron.com;

Experience Counts

From concept to commercial supply, industry leaders rely on Aldevron

An experienced CDMO can help you avoid potential rework and support your project from early phase research, through clinical trials, and commercialization. Since 1998, it has been Aldevron's mission to help our clients make meaningful contributions to patients' lives. Here are just a few of the ways we support your objectives:

- pDNA, RNA & protein custom manufacturing
- Scale for commercial applications
- Research-grade & cGMP material
- Standardized, in-stock products
- Process development
- Technology transfer
- Analytical methods & assays
- Regulatory support

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The basis for breakthroughs

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