Developing Robust Analytical Methods for Early-Stage Biologics

Biologics are a broad class of therapeutic agents, encompassing vaccines, monoclonal antibodies, therapeutic proteins, nucleic acid-based therapies, blood components, tissue therapies, and cellular therapies. Recently, the US Food and Drug Administration (FDA) revised the definition of term "protein" to include peptides containing more than 40 amino acids, therefore categorising them as biologics.[1] The search for new and effective treatments for serious and life-threatening diseases has led to the growing interest in biologics in the last two decades. Biologics have shown promising clinical outcomes compared to traditional small molecules due to their high specificity, profoundly transforming the treatment strategies for cancer and autoimmune disorders. The COVID-19 pandemic has also accelerated the adoption of biologics within the healthcare sector. It is estimated that by 2027, the biologics market will have significant growth to $666 billion from $474 billion in 2023.[2] This remarkable shift can be attributed to several factors, including the rising prevalence of chronic diseases, approval of several Advanced Therapy Medicinal Products (ATMPs), the increasing availability of biosimilars to patients, and the growing recognition of the benefits of biologics over small molecules. Emerging biopharmaceutical companies[1] have also contributed to the growth of the biologics market. Approximately 65% of molecules in the research and development pipeline, including biologics and small molecules, are from emerging biopharmaceutical companies without the involvement of larger biopharmaceutical organisations. This share has seen a steady growth from 34% in 2001 to 50% in 2016, due to increased funding and investments.[3]

Biologics, however, present unique challenges in the journey from discovery to commercialisation due to their inherent complexity, size and charge heterogeneity and susceptibility to changes that could potentially impact efficacy and the patient safety. Therefore, development and implementation of robust analytical methods to monitor critical quality attributes (CQAs) during early-stage is vital for successful biologic development. At early-stage, analytical methods play an important role in candidate screening, process development, formulation screening, stability determination and release testing for first-in-human (FIH) studies. However, developing sensitive and robust analytical methods is challenging in the early-stage of biologics development as there is often insufficient product knowledge. The draft ICH Q14 guideline[4] provides a comprehensive framework based on science and risk-based approaches to overcome some of these challenges and streamlines the post-approval change management of analytical methods. This article primarily focuses on developing analytical methods for release and stability testing of biologics, while developing methods required for characterisation is out-of-scope.

Analytical Target Profile (ATP) – Setting the Analytical Benchmarks

ICH Q14 defines the ATP as a "prospective summary of the performance characteristics of the analytical procedure with anticipated performance criteria to ensure the results are appropriate for the intended use." In other words, the ATP is a pre-defined set of analytical benchmarks that a method should meet to be considered suitable for its intended purpose. The ATP is analogous to the quality target product profile (QTPP) described in ICH Q85 and help guide the selection of analytical technology and the method development process.

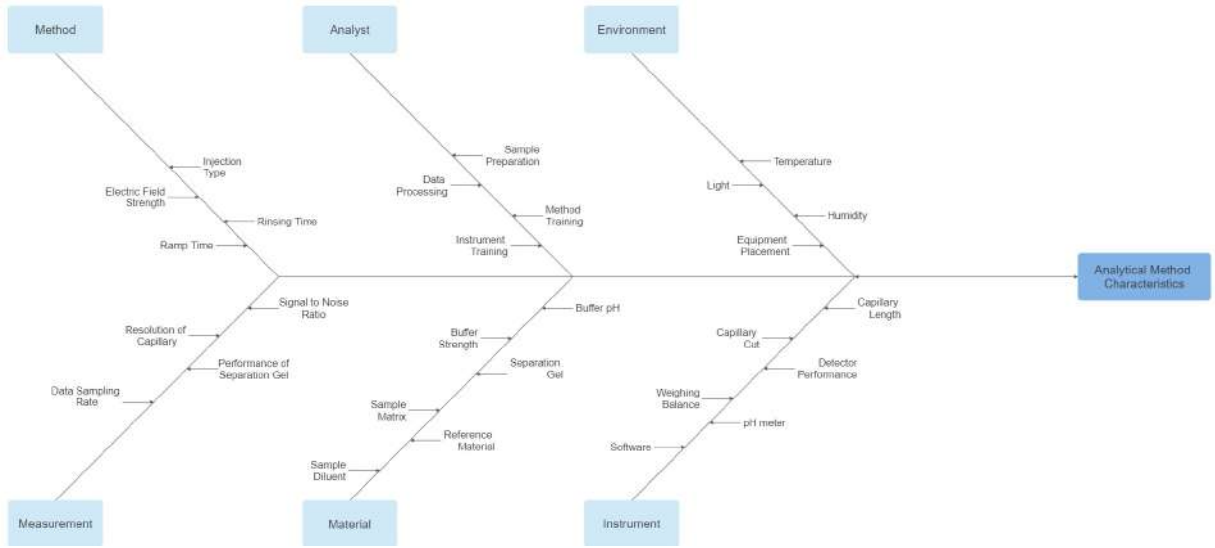
Product Development	Analytical Method Development
Quality Target Product Profile (QTPP)	Analytical Target Profile (ATP)
Critical Quality Attributes (CQAs)	Critical Analytical Method Attributes
Risk Assessment	Risk Assessment
Design Space	Method Operable Design Region (MODR)
Control Strategy	Analytical Method Control Strategy
Continued Process Verification	Continued Analytical Method Verification

Table adapted from reference[6]

The ATP is written based on the product's needs as summarised in the QTPP and the analytical knowledge based on the physical and chemical properties of the drug substance and drug product. However, during the early stages of biologic development, there is often insufficient knowledge about the product and analytical method benchmarks that need to be achieved. As a result, establishing an appropriate ATP can be challenging and will remain as a living document until more knowledge is gathered. Similarly, when changes happen to QTPP warranting improved benchmarks, the ATP should be updated to re-examine method selection. For example, establishing an ATP for CQAs such as dimer and oligomer species is less complicated than determining plasmid DNA isoforms, as there is more knowledge available in the public domain. Therefore, knowledge management becomes a crucial aspect of the method development process – knowledge regarding the physical and chemical properties of the drug substance and drug product is necessary.

Stage of Product – Phase I	
Intended Purpose	
Link to CQA	
Plasmid DNA exists in several topological forms such as supercoiled, linear, and open circular. As supercoiled plasmid shows the highest efficiency in transfecting eukaryotic cells, the content of supercoiled plasmids becomes an important indicator of plasmid quality.	
Characteristics of the reportable result	
Characteristic	**Acceptance Criteria**
Specificity	The analytical method is specific for determination of plasmid DNA isoforms and shows no matrix interference from the formulation components. Any carryover observed should be less than 0.1%
Linearity	The analytical method should exhibit linearity from 50% to 150% of nominal protein concentration
Precision	Supercoiled peak: ≤ 3.0% RSD repeatability Linear or open circular peak: ≤ 0.4 SD repeatability Total analytical error (TAE) ≤ 50% of allowable process capability to achieve Ppk 1.0 in QTPP range (SD 0.4%)
Accuracy	Supercoiled peak: 95.0 – 105% recovery
LOQ	QL ≤ 1.0%

An example ATP for determination of plasmid DNA isoforms



An example Ishikawa diagram for assessing risk determination of plasmid DNA isoforms

Risk Assessment and Technology Selection

Analytical method risk assessments are performed to identify and assess critical method variables and parameters that can impact the ATP. This assessment is highly analogous to identification of critical process parameters (CPPs) during process method development. The structured risk assessments provided in the ICH Q14 include Ishikawa diagrams, Failure Mode Effects Analysis (FMEA) and preliminary hazard analysis.

Technology selection is entirely based on the analytical method performance characteristics drafted in the ATP. If there are several technologies that meet potential requirements, factors including throughput, costs and knowledge of analytical technology should be considered. For the determination of plasmid isoforms, several techniques are available, such as slab gel electrophoresis, HPLC, and capillary electrophoresis. However, capillary electrophoresis is the preferred technique due to the performance characteristics that meet criteria in the ATP.

Method Development Strategies

One of the primary goals of developing analytical methods for early-stage biologics is to determine specific quality attributes of the drug substance and drug product to ensure that they meet applicable standards of identity, strength, quality, and purity as defined in the QTPP. The analytical results generated are then used to support process development, formulation screening, shelf-life determination and demonstrate manufacturing consistency. A well-developed analytical method should achieve the required accuracy and precision in alignment with the Analytical Target Profile (ATP) to support product development.

The traditional (minimal as per ICH Q14) approach largely focuses on the determination of quality attributes the analytical method based on the iterative approach. However, the enhanced approach adopts the principles of Analytical Quality-by-design (AQbD) to identify and minimise factors that could potentially introduce variability into the final reportable result. AQbD begins with building specific objectives through sound–science and quality risk management to achieve the ATP method characteristics.

Many biopharmaceutical companies that develop and manufacture biologics still use the traditional approach to method development because they often lack the resources and knowledge using the AQbD approach.

Method Qualification and Validation

Method qualification is the process of determining whether an assay is fit for its intended purpose. It is typically not a pass/fail process, but rather an iterative process of optimisation until the method meets the performance acceptance criteria defined in the ATP. In general, method qualification is carried out after determining potential analytical method parameters and before method validation. Typically, method qualification differs from validation in that qualification does not have pre-set specifications (acceptance criteria) and may not be carried out in a GMP environment. As a minimum, specificity, linearity, range, precision in terms of reproducibility, needs to be evaluated to ensure the method is scientifically sound. Method qualification is required for early clinical phases while assays intended to ensure patient's safety should be validated.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use. The term "validation" is specifically linked to the product release either lot-release or stability in a GMP environment. It accounts for strict pre-determined acceptance criteria and requires QA approval of parameters in alignment with ICH Q2R1.[7] Validation involves documenting, using specific laboratory investigations, that performance characteristics of the method are suitable for intended analytical applications and are reliable. The acceptability of analytical data corresponds directly to the ATP criteria used to validate the method.

Stability-Indicating Methods for Biologics

Stability studies are an essential and vital part of drug development. A stability-indicating method is an analytical test that can detect meaningful changes in quality attribute(s) during storage. For cell and gene therapies, the assessment of product functionality/potency represents a stability indicating test method. They typically start at the preclinical stage and continue through Phase I–Phase III clinical trials to support formulation development, and to satisfy the regulatory requirements for clinical trials.

During early-stage development of biologics, degradation pathways are elucidated, and the drug substance and drug product are tested under various storage conditions to determine their shelf-life. To ensure reliable data interpretation, robust stability indicating methods are needed, which allow for the detection of physical, chemical, or microbiological changes that affect safety, purity and efficacy of biologics. To demonstrate a method is stability indicating, forced degradation

samples should be assessed during method qualification. If the biologic is found to be stable, spiking of known impurities or variants could be performed to demonstrate method suitability.

Reference Standard Qualification

In accordance with ICH Q6A guidelines, "a reference standard or reference material is a substance prepared for use as the standard in an assay, identification, or purity test. It should have appropriate quality for its intended use". Reference standards are a critical component of a biologic development and are part of analytical control strategy. Due to complexity of biologics, a well characterised reference standard is essential and should be introduced early in the development program. The criteria for selection of reference standards are that they should be stable, uniform and represent the properties of test material with respect to the quality attributes.

Often primary reference standards are not available from an official recognised source and therefore "in-house reference standards" should be established. Recently, National Institute of Standards and Technology (NIST) has released an extensively characterised IgG1k monoclonal antibody called the NISTmAb reference material to represent an industry standard for evaluating the performance of methods that determine physicochemical and biophysical attributes of monoclonal antibodies.[8]

If the reference standards are not available from an official source, they should derive from the toxicology material and evaluated for its intended purpose using extended characterisation techniques along with release methods. However, the primary reference standard is usually manufactured from process performance qualification (PPQ) batches most importantly, from a batch representative of the pivotal batch. Pooling of batches may be used to represent an average of properties (mostly commonly for vaccines).

The qualified reference standard should serve as an analytical control to monitor method performance characteristics as defined in the ATP.

Conclusion

Developing robust analytical methods for biologics during early-stage development is a crucial but a challenging task. ICH Q14 guideline provides a detailed framework for this process. As the landscape continues to evolve for biologics, the principles of ICH Q14 will remain instrumental in advancing analytical methods and facilitating the development of safe and effective biologic therapies.

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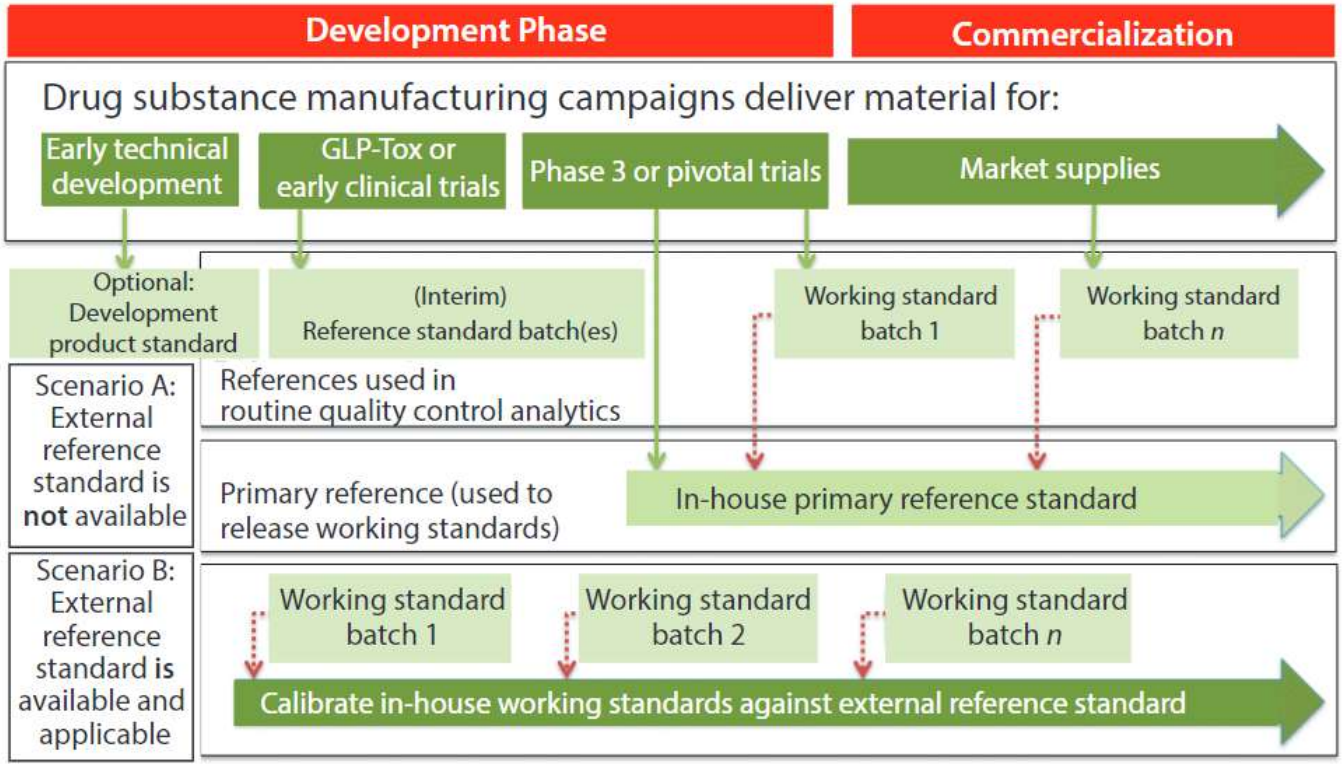

Different types of reference standards used throughout the lifecycle of biologic development, Image adapted from reference 9

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