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Strategies for Setting Patient-Centric Commercial Specifications for Biotherapeutic Products

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ABSTRACT

Commercial specifications for a new biotherapeutic product are a critical component of the product's overall control strategy that ensures safety and efficacy. This paper describes strategies for setting commercial specifications as proposed by a consortium of industry development scientists. The specifications for some attributes are guided by compendia and regulatory guidance. For other product quality attributes (PQAs), product knowledge and the understanding of attribute criticality built throughout product development should drive specification setting. The foundation of PQA knowledge is an understanding of potential patient impact through an assessment of potency, PK, immunogenicity and safety. In addition to PQA knowledge, the ability of the manufacturing process to consistently meet specifications, typically assessed through statistical analyses, is an important consideration in the specification-setting process. Setting acceptance criteria that are unnecessarily narrow can impact the ability to supply product or prohibit consideration of future convenient dosage forms. Patient-centric specifications enable appropriate control over higher risk PQAs to ensure product quality for the patient, and flexibility for lower risk PQAs for a sustainable supply chain. This paper captures common strategic approaches for setting specifications for standard biotherapeutic products such as monoclonal antibodies and includes considerations for ensuring specifications are patient centric.

Abbreviations: ADC, antibody drug conjugate; ADCC, antibody dependent cellular cytotoxicity; ADCP, antibody dependent cellular phagocytosis; CDC, complement dependent cytotoxicity; CE-SDS, capillary electrophoresis sodium dodecyl sulfate; CIEF, capillary isoelectric focusing; CQA, Critical Quality Attribute; CZE, capillary zone electrophoresis; DS, drug substance; DP, drug product; ELISA, enzyme-linked immunosorbent assay; EOSL, end of shelf life; FcRn, neonatal Fc receptor; HILIC, hydrophilic interaction chromatography; ICH, International Council for Harmonization; IEC, ion exchange chromatography; IgG, immunoglobulin G; mAb, monoclonal antibody; PQA, product quality attribute; SEC-HPLC, size exclusion chromatography high-performance liquid chromatography; PK, pharmacokinetics; PD, pharmacodymanics; QbD, Quality by Design; RTRT, Real time release testing; RP-HPLC, reversed phase high-performance liquid chromatography.

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Introduction

Biotherapeutics provide significant benefit to patients across many disease areas and represent a growing proportion of molecules in development as well as amongst those approved.[1,2] The commercial release and stability specifications for a new biotherapeutic must ensure product safety and efficacy. Establishing the analytical tests and associated acceptance criteria for these specifications requires significant effort. For biopharmaceuticals, the guidance document ICH Q6B - Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products defines specifications as "a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described".[3] For some quality attributes, pharmacopeia provide guidance on specific analytical methods and expected acceptance criteria. However, for most product attributes there are product-specific considerations in determining the final list of methods and acceptance criteria required for product release and end of shelf life stability. The final commercial specifications are a matter of agreement between sponsor and regulatory agencies during the commercial marketing application review process.

For product attributes that are not defined by monographs in pharmacopeia, sponsors must establish specifications based on product and process understanding gained during development. ICH Q6B states that specification acceptance criteria should be established and justified based on data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, data from stability studies, and relevant development data. At the time ICH Q6B was implemented (1999) most biopharmaceutical companies used an empirical development approach; the understanding of attribute criticality and the relationship between process parameters and impact to product quality was limited. Because the potential for attributes to impact clinical safety and efficacy (i.e. clinical performance) was frequently not known, specifications needed to include a comprehensive list of tests, with acceptance criteria linked to process experience. More recently, improved platform processes have led to more consistent clinical manufacturing, often with production of few clinical batches, so a process experience-based approach can lead to tight specifications that overly constrain long-term commercial supply or may prohibit consideration of the development of convenient dosage forms or other innovations that improve the experience of patients; the concept of patient centric specifications has been introduced to emphasize the use of risk-based approaches based on product knowledge.[4]

More recent guidance (ICH Q8, Q9, Q10, Q11) provides a science- and risk-based approach for product development and describes a comprehensive control strategy.[5–8] A control strategy is defined as "a planned set of controls, derived from current product and process understanding that ensures process performance and product quality".[7] The control strategy is the culmination of a comprehensive assessment of the potential for the process to impact quality attributes impacting clinical performance. The integrated control strategy ensures delivery of a product that meets quality expectations throughout the approved shelf life. Drug substance and drug product specifications are only one component of the overall control strategy; the role of specifications is to confirm that other controls effectively deliver product of appropriate quality. Facility controls, raw material controls, and process controls are among other essential components of the control strategy. Specifications and controls for devices are also important for combination products but will not be discussed in this manuscript.[9]

Since implementation of ICH Q8 (R2), industry has adopted a systematic (Quality by Design (QbD)) development approach, is investing in product and process characterization, and is using quality risk and quality management systems.[10–12] The identification of PQAs should link to the Quality Target Product Profile (QTPP) which is based on the intended use of the product in a clinical setting. A scientific understanding of PQAs is developed and includes detailed physicochemical characterization, biological assessment of samples enriched for certain attributes or variants, as well as prior knowledge of relevant products or variants based on development activities and scientific literature. This information can be used as input to determine the potential impact of PQAs upon clinical performance. PQAs with impact to clinical performance are assessed throughout process development and characterization. A systematic, risk-based development approach results in a greater understanding of attribute criticality, method performance, and the potential for the process to impact product quality; this development approach is verified by process characterization, method validation, and process validation. Commercial specification acceptance criteria also take statistical evaluations of historical lot and proposed commercial lot data to assess the ranges a process can deliver. Statistical assessments may also identify areas where further PQA understanding, or process control may be beneficial.

Per ICH Q8 (R2), greater understanding or 'enhanced knowledge' can create a basis for more flexible regulatory approaches and control strategies, including the use of alternative approaches to selecting attributes and associated acceptance criteria for the product specification. Prior communications[10–12] provided considerable guidance on development of a product's overall control strategy, including detailed assessments for product attribute criticality and of the tests needed for release and stability, but with limited information about how to establish suitable acceptance criteria. This paper describes approaches used by industry leaders for establishing commercial specifications for biotherapeutics, with a focus on the use of enhanced understanding of product attributes to justify acceptance criteria linked to clinical performance. The co-authors are industry scientists who participate in the IQ Consortium Biologics Working Group on Phase Appropriate Specifications (International Consortium for Innovation and Quality in Pharmaceutical Development).

Results and Discussion

The focus on this paper is development of commercial specifications and acceptance criteria for a monoclonal antibody, since this is a common modality across industry, however, the principles described here apply to other more complex biologic products. In addition, considerations for antibody drug conjugates (ADCs) are included as a case study. The application of risk management tools and scientific understanding provides an opportunity to establish commercial specifications and acceptance criteria based on the risk associated with an impact on clinical performance. The proposed acceptance criteria may, as a result, be wider than those based either on patient exposure in clinical studies or on limited manufacturing experience at the time of market application. Notably, process experience-based acceptance criteria may present adverse long-term supply constraints,[4] and the challenges are even greater when accelerated product development timelines allow very few batches to be available for patient treatment or to be used for projection of long-term process capability. Thus, the risk-based approach described here enables appropriate control over high risk attributes to ensure product quality for the patient while allowing appropriate flexibility for low risk attributes, for a robust supply chain.

Clinically relevant specifications have been defined by FDA as limits that "identify and reject drug product batches that are likely to perform inadequately in the indicated patient populations ….

based on clinical relevance, instead of process capability or manufacturing process control".[13] For the purposes of this manuscript the term Patient-Centric Specifications has been adopted, with the following definition:

> Patient-Centric Specifications: A set of tests and acceptance ranges to which product quality attributes should conform for the product to be safe and effective when used as labeled. Justifications for acceptance ranges focus on risk-based assessment of the impact to patients. Patient-Centric Specifications may also be referred to as clinically relevant specifications.

A common set of drug substance and drug product tests are defined by ICH Q6B and/or by pharmacopeia requirements. For the purposes of this manuscript, the US Pharmacopeia, European Pharmacopeia, and Japanese Pharmacopeia[14–16] were primarily considered as referenced in ICH tripartite guidelines; however, additional pharmacopeia requirements should be considered for global filings. In practice, varying requirements across pharmacopeia present a significant challenge in global specification setting. The common set of drug substance and drug product tests for a standard monoclonal antibody at the commercial stage, is listed in Table 1. Importantly, while common testing strategies for release and stability testing for final drug substance and drug product are described, advanced control strategies such as at-line and real time release testing are increasingly being considered and may be acceptable if appropriate control and justification are provided. In some cases, testing may be required at a minimum for drug product, but some companies reported that they may choose to test at both drug substance and drug product to mitigate business risk, or for other product or process reasons. Moving release testing to the point of control such as after the final step for clearance of a process related impurity, and the use of in-process tests in lieu of release testing, may also be justified. In addition, alternate/innovative technologies such as the mass spectrometry multi-attribute method (MAM), are being implemented.[17] While specification setting for control strategies that utilize innovative testing approaches and technologies are not specifically discussed in this manuscript, the use of a risk and science-based approach would also apply.

A strategy for establishing patient centric specifications is provided in the sections below. The control of product variants by purity methods is described first. Because product variants are subject to risk assessments for critical quality attribute (CQA) identification,[10] a further patient-centric risk assessment of their safe and effective ranges is a natural extension. Potency is closely linked to safety and efficacy and is described next. Details then follow for the tests for which compendial or other guidance provides direction for setting acceptance criteria. The criteria for these compendial or general tests are consistent with a patient-centric strategy by aligning with guidance and compendia for parenteral drugs in addition to the product's specific quality target product profile, thus ensuring that the product is safe and efficacious.

Purity and Product Variants

Control of purity and product variants requires integrated control strategies based on a focused understanding of each attribute. Scientific understanding is leveraged to establish appropriate tests, testing points, and attribute acceptable ranges for the commercial specification. This approach is consistent with FDA regulatory expectations that "establishment of impurity acceptance criteria should be guided by the totality of the data and consideration of the clinical impact of impurity levels instead of basing the impurity limits solely on the manufacturing process capability".[18] Establishment of a control strategy relies on risk management tools including assessments of quality attribute criticality and process impact to CQAs (ICH Q8,9,10). The degree of control over an attribute should be commensurate with the risk.[19] One goal of the risk evaluations is to provide a reasonable scientific basis for establishing the levels of a product attribute that would be expected, with a high degree of confidence, to have no adverse impact on product quality and hence performance.[20]

The establishment of purity specifications should address two questions, 1) what attributes to test, and 2) what suitable acceptance criteria are. Product related variants commonly tested for monoclonal antibody drug substance or drug product are shown in Table 2. Multiple attributes are often tested collectively as an assay region (for example, acidic variants may include multiple deamidation, glycation, and other acidic variants), but additional tests for product-specific attributes may be needed when the general method is not suitable.

For some attributes, especially those with low criticality, a justification for no testing may be possible based on product and attribute understanding or when the acceptable range is sufficiently controlled by the process and maintained during storage. Data from process design and process validation studies, formulation development and robustness studies, and stability studies are analyzed to determine the testing strategy. These data guide decisions of the testing required for in-process, release, and stability for drug substance and drug product. For example, demonstration of no change from drug substance to drug product, or during stability, may justify that no routine testing is required, or that testing only on drug substance or drug product is appropriate. Case Study 2 describes a risk assessment for low levels of Fc oxidation of an IgG antibody with an outcome that no testing was warranted.

Patient-centric acceptance criteria for purity and product variants consider the potential impact of the proposed range for a given attribute against the same four categories which are typically considered when evaluating the criticality of an attribute. These include biological activity, pharmacokinetics, immunogenicity, and safety. Typical approaches for evaluating attribute ranges for these four categories are described in Table 3.

Attribute knowledge gained through literature, experience with other products, product specific development data or defined studies can be used to inform both attribute criticality assessments as well as in defining an appropriate specification with associated acceptance criteria. Attribute specific data can often be generated by testing a variant that has been isolated or enriched, often referred to as structure-function studies. Alternatively, data from stability and forced degradation studies may be used. Case Study 1 provides an example of the use of stability and forced degradation studies to inform the risk assessment and patient centric acceptance criteria for antibody fragmentation. In the absence of data, it may be appropriate to consider the worst-case potential impact of a given variant. Results from in vitro and in vivo experiments (including but not limited to registrational toxicology and clinical studies), as well as prior knowledge, are combined to build the case for the acceptance criteria. General scientific understanding should be leveraged when possible and the input of subject matter experts in these assessments is critical. Some attributes such as C and N terminal amino acid variations and non-CDR deamidation are typically assessed as low impact to biological activity, PK, immunogenicity, or safety, and as a result their acceptable range may be established based on biological activity impact. Justification of acceptance criteria can come from experience with relevant products in relevant indications and patient populations, either from the sponsor's experience or from peer-reviewed literature. For example, studies of isolated IgG1 charge variants have been shown to have no impact on PK in rats,[22] and can be used to support

Table 1
Common Attributes and Testing Strategies for a Standard Biotherapeutic at Commercial Stage (e.g., Monoclonal Antibody).

Category	Quality Attribute	Release Specification[a]	Stability Specification[b]	Additional Consideration
Appearance and Description	Clarity	DP	DP	Appearance and description include specific tests for color, clarity and particles which are typically expected as tests performed on DP. More limited appearance and description testing is typically performed on DS. For lyophilized DP, an assessment of appearance is performed prior to and following reconstitution. Visible Particles may be categorized under Particulate Matter.
	Coloration	DP	DP	
	Visual appearance	DP	DP	
	Visible Particles	DP	DP	
Particulate Matter in Injections	Subvisible Particles	DP	DP	
Identity	Identity	DS, DP		
Quantity or Strength	Protein Concentration	DS, DP	DS, DP	Although not a stability-indicating assay, protein concentration is frequently included in the stability program.
	Content Uniformity	DP		In-line fill weight checks may be performed in lieu of release testing
Potency	Potency (Biological Activity)	DS, DP	DS, DP	
General Characteristics	pH	DS, DP		The decision to include pH testing on stability should be driven by development data
	Osmolality	DP		Often tested for DS. In process testing may be performed, particularly for DS, in lieu of release testing if justified
	Reconstitution Time	DP	DP	Performed for lyophilized drug products
	Moisture Content	DP	DP	Performed for lyophilized drug products
	Extractable Volume	DP		
Excipients[c]	Polysorbate Concentration	DP		Polysorbate testing for DS is not required but may be considered to mitigate business risk, or in place of DP testing if no formulation change at DP and no change on stability. In process testing for DP could be considered in lieu of release testing.
Purity and Product Variants	Size, Charge, Glycans, product specific PQAs	DS, DP	Attribute specific, based on a data-driven assessment	See Table 3
Process-Related Impurities	Residual Host Cell Protein	The final control strategy for process-related impurities are determined based on process understanding, and novel control strategies may be considered. Process related impurities may be part of the drug substance release specification, an in-process control, or not required if suitable clearance is demonstrated during process characterization and validation.		
	Residual Protein A			
	Residual Host Cell DNA			
Microbiological Attributes[d]	Endotoxin	DS, DP		
	Bioburden	DS		
	Sterility	DP	DP EOSL	Container closure integrity may be performed in lieu of Sterility on stability
	Container Closure Integrity		DP EOSL	

DS = drug substance; DP = drug product; EOSL = end of shelf life.

[a] Suitable Real Time Release testing may substitute for DS and DP testing if justified and negotiated with regulatory agencies.

[b] While a typical approach for stability programs has been captured in this table, if development data demonstrate that a test is not stability-indicating, a data-driven argument may be successful in removing the test from the stability program. Additional considerations for in-use stability, as applicable, should also be assessed.

[c] Excipient controls should be considered holistically; for example, the concentration of some excipients is assured by other tests (e.g., pH and osmolality). Polysorbate typically requires a stand-alone test or controls. Additional controls, including tests, may also be required for certain excipients such as preservatives or antioxidants.

[d] Setting specifications for microbial attributes is out of scope of this manuscript although some considerations for endotoxin and bioburden are described in the process-related impurities.

Table 2
Common Product Variant Tests for Monoclonal Antibody Therapeutics.

Attributes	Test
Size Variants (native conditions): Higher molecular mass species	SEC-HPLC
Size Variants (denaturing conditions): Low Molecular Weight Species including fragments	CE-SDS (reduced and/or non-reduced conditions)
Charge variants: acidic species, basic species, main peak	IEC, CIEF, CZE
Glycans (as justified)	HILIC of released glycans

SEC-HPLC = size exclusion chromatography high-performance liquid chromatography; CE-SDS = capillary electrophoresis sodium dodecyl sulfate; IEC = ion exchange chromatography; CIEF = capillary isoelectric focusing; CZE = capillary zone electrophoresis; HILIC = hydrophilic interaction chromatography.

expanded acceptance criteria for charge variants or the use of action limits if charge variants are monitored as an in-process control for process consistency and not as a specification test. Similarly, studies of IgG oxidation showed that both heavy chains must be oxidized at Met252 to impact FcRn binding,[23] which can be used to justify expanded ranges for Fc oxidation because the double-oxidized antibody is much lower abundance than the peptide map measured oxidation (e.g., calculated via binomial distribution). Additionally, attributes of lower criticality that are demonstrated to be controlled by the process may not require routine release or stability testing. Note that it is important that in vitro and in vivo models, as well as the samples tested, provide meaningful information for evaluation of patient risk and are appropriately justified.

An understanding of the link between attribute variability and potential clinical impact allows the focus of tighter controls on the attributes with the highest risk of impacting clinical performance while allowing greater flexibility for lower risk attributes. For example, formation of both dimers and higher order aggregates could be pathways of degradation. If in such a case, dimers have been assessed to have low safety risk, establishing a separate limit for each population may allow for a comparatively wider allowable range for the dimer population. However larger aggregate species would be controlled with an appropriately tight limit given an assumption that the larger species are potentially more impactful to immunogenicity.

Specifications for glycosylation are based on the criticality of the glycoforms and on their degree of process control. For IgGs, Fc glycosylation is typically the only N-linked site, and mannose-5 through mannose-9 (M5-M9) forms have the primary impact on PK, while sialic acid does not. A specification for oligomannose forms may be necessary if process control is not sufficient. It has been shown for IgG1 that oligomannose levels that vary by up to 10% have no measurable impact on PK.[24] Sialic acid levels can impact PK for non-Fc glycans, including those found on Ig-fusion proteins, and testing of the sialic acid level is often necessary in those cases.[25] Fc effector function, e.g., antibody dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), may be a primary or secondary mechanism of action, in which case the relevant glycans require appropriate acceptance criteria. In some cases, the relevant glycan level is sufficiently well correlated with Fc effector function that a specification for those glycans may be used as a surrogate. When there is no Fc effector function, a glycan specification may not be necessary if other glycoforms have low criticality and adequate process control.

Mass spectrometry-based methods show promise for release testing, though few examples currently exist for testing at the commercial stage. These methods typically use LC-MS measurement of tryptic peptides and may be used to quantify specific attributes such as glycans, or as a more comprehensive MAM that can also measure charge, oxidation, fragments, and others in a site-specific manner. Peptide mapping with UV detection is, to date, more commonly used as a site-specific test, when needed. Specific attribute testing such as MAM or peptide mapping could theoretically replace assays that traditionally report collections of attributes such as charge variants based on product-specific understanding.[17]

Although statistical bounds calculated for quantitative attributes are evaluated when establishing in-process limits and acceptance criteria for release and stability, statistical bounds are not the sole basis for establishing limits or criteria. A statistically derived range should be evaluated and adjusted as needed, based upon product, process, and attribute knowledge to establish a range within which there is a low risk of meaningful impact to the clinical performance. Additional inputs to the risk assessment besides attribute knowledge include disease indication, patient population, route of administration, duration of treatment (acute or chronic), mechanisms of action and toxicology. When determining the level of a variant that has been clinically dosed, consideration of higher doses administered during non-pivotal studies and accounting for stability changes based on the age of the lot may be useful.

Potency

Product potency or biological activity is measured through a biological assay (bioassay) that is designed to reflect the presumed primary mechanism of action. As described in ICH Q6B, “a valid biological assay to measure the biological activity should be provided by the manufacturer.” As defined in 21 CFR 610.10, bioassays are inherently product-specific: “tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically

Table 3
Assessment of Product Quality Attributes.

Category of Impact	Typical Approaches for Assessment
Biological Activity	Testing of isolated or enriched variant samples in relevant bioassays (binding, cell-based potency, Fc-effector function, etc.) to determine the risk that a change in an attribute level will impact the established safety/efficacy profile.
Pharmacokinetics	Assessed through prior knowledge and literature, molecule-specific studies, animal studies, clearance of isolated variants, or time-course analysis of patient serum samples. Additionally, relative binding to FcRn is frequently used as a surrogate for area under the curve (AUC).
Immunogenicity	Because of the variety of factors that can affect immunogenicity, product specific risk assessments are foundational to assessing the immunogenicity potential of quality attributes. The FDA guidance Immunogenicity Assessment for Therapeutic Protein Products is a useful reference describing multiple approaches for assessing immunogenicity risk.[21]
Safety	Because of the variety of factors that can affect safety, product specific risk assessments are foundational to safety assessments. Product characterization, experience with similar products, nonclinical and clinical data may be considered in the safety assessment.

designed for each product so as to indicate its potency".[26] A variety of bioassay methods are available — cellular, immunological, enzymatic, in vivo, etc.; in all cases the selection of a relevant, quantitative assay must be justified by the manufacturer.[3]

Potency acceptance criteria are selected as part of a control strategy that reflects understanding of product variants that can impact potency. During development, bioassay methods are used to support the understanding of the potential clinical relevance of product variants to product potency through in vitro structure-function studies. Structure-function assessments and prior knowledge (e.g. literature, commercial experience with similar products) are used in the assignment of quality attribute criticality and when combined with clinical and non-clinical experience, establish control limits for product variants that may impact potency. Importantly, due to the inherent higher sensitivity and precision of physicochemical methods, acceptance limits established for critical quality attributes from the physicochemical methods limit product variability and degradation to a level that is unlikely to impact potency. The control of product potency is therefore primarily ensured through a combination of process controls and acceptance criteria for physicochemical attributes of the product. Measurement of biological activity provides confirmation that potency has not been impacted. In addition, the bioassay can sometimes provide confirmation of tertiary and quaternary structure of the product, based on the link between activity and proper folding of the molecule. Potency testing is typically included in the stability program. Potency acceptance criteria are typically expressed relative to a reference material/standard (as percent relative potency) or using specific activity (as activity units per mg of product). Due to the inherent variability of bioassay methods, potency acceptance criteria must account for analytical variability, which is typically inferred from a statistical evaluation of release and stability data and supported by method development and validation data. This approach is presented in Case Study 3.

Most biologics are administered based on mass: mg product/kg patient mass, or mg product flat dose. Assurance of accurate patient dosing is provided through control of product content/content uniformity. However, some biologics are administered based on the potency assigned through lot release testing and/or calibration against an international standard. In these cases, potency acceptance criteria should be established with consideration of the product therapeutic index, methodologies used for lot release and product labeling.[27]

In limited instances, physicochemical or biochemical assays may be used as a surrogate measure of potency for products with extensive product knowledge that includes correlation of quality attributes, physicochemical methods and biological assays, and well-established manufacturing and commercial history. Establishing appropriate acceptance criteria for surrogate measures of potency and elimination of a potency assay from the product specification should be handled on a case-by-case basis and is therefore, not discussed in detail in this paper.

Appearance and Description

The tests for appearance and description provide a qualitative product-specific statement describing the physical state (e.g., solid, liquid), color, and clarity of the drug product.[3] This assessment forms a baseline for product consistency and quality and provides an expectation for the appearance of the product for health care providers and patients. Product labeling requires a description of the drug product, and specification acceptance criteria are aligned with the product description in the product label and package insert. Companies differ in practice for whether to perform these tests for drug substance. All ranges for these parameters are typically established and justified based on formulation development and stability studies, which demonstrate the product has acceptable quality when maintained within the proposed ranges.

A visual description of the drug product, including the physical state, color, clarity, and any observable extrinsic or intrinsic particulates is formally assessed as part of the manufacture and release process and for lyophilizates also prior and after reconstitution.[14] The color of a drug product solution is a product-specific parameter which can be influenced by manufacturing and formulation conditions.[28] Assessment of color is conducted according to Ph. Eur. via visual comparison against compendial reference color standards or via instrumental testing that may be able to demonstrate more granular color differences that exist within and between pharmacopeia color standards.[29] Most companies establish objective, quantitative acceptance criteria based on these standards or instrument results. If the color of drug substance and drug product do not change during storage, color testing may not be required in the commercial post-approval stability program.

Clarity of the drug product solution is a product-specific parameter which can be influenced by manufacturing and formulation conditions. Clarity is assessed according to Ph. Eur. visually against opalescence standards or via instrumental methods.[15] Companies normally set objective, quantitative ranges based on these standards or instrument results. While clarity assessment of drug substance is not required, a low inherent turbidity of the drug product is desirable so that intrinsic and extrinsic visible particles are not masked, and 100% visual inspection as well as visual assessment by health care providers and patients is facilitated.

For color and clarity, in addition to formulation development study data, an evaluation of historical data and consideration of method capability are used to establish the acceptance criteria.

In addition to the above listed parameters, appearance and description testing for drug product contains an assessment for visible particles as required by the Ph.Eur. and USP.[14,15] Drug product protein solutions should be specified as "essentially" (USP) or "practically free" from visible particles (Ph.Eur.). For a comprehensive review see Mathonet et al.[30] Testing of visible particles for drug substance is typically not required as the point of control for visible particles is at the drug product, and sterile filtrations quantitatively remove visible particles prior to drug product filling.

Subvisible Particles

Subvisible particulates are monitored at release and on stability predominantly by the light obscuration (LO) method, as outlined in the USP, Ph. Eur., and JP.[14–16] The compendia limits for total particle content are set at 6000 particles $\geq$10 μm and 600 particles $\geq$25 μm per container for small volume parenteral ($\leq$100 mL), and 25 particles $\geq$10 μm and 3 particles $\geq$25 μm per mL for large volume parenteral (>100 mL). These limits are generally accepted by regulatory agencies for specifications, although there are examples of regulatory agencies requesting tightening of the compendia limits. There are reports that have indicated subvisible particles less than 10 μm could elicit immune responses.[31,32] The USP has published chapters, *Subvisible Particulate Matter in Therapeutic Protein Injections* <787> and *Measurement* of Subvisible Particulate Matter in Therapeutic Protein Injections <1787> which focus on the identification and characterization of inherent particles and their impact on safety and product quality. Additionally, USP<1787> acknowledges the potential immunogenicity risk with subvisible particles and has recommended the assessment of subvisible particles below 10 μm.[14] The data for particles below 10 μm is typically collected during development to support definition of an appropriate particulate control strategy and presented in a characterization section in the marketing applications. The USP only requires the reporting

of ≥10 μm and ≥25 μm size ranges for subvisible particles in drug product specifications.

Identity

Identity tests are critical to ensure the molecule is as intended and therefore safe and efficacious for patients. Identity test(s) should be highly specific for the active ingredient in drug substance or drug product to ensure its unambiguous verification for secondary manufacture or certainty of the packaged product content.[3] The specificity is normally based on unique aspects of the molecular structure and/or other specific properties probed by physicochemical, biological, and/or immunochemical tests. Although a single test is adequate in most of the cases for drug product, more than one test may be necessary to establish identity for some products. Commonly used analytical procedures include chromatographic methods, electrophoretic methods, immunochemical, or biological assays and each can provide suitable specificity to demonstrate identity. The identity test is typically qualitative in nature, and the assessment is normally performed by comparing a product with an appropriate reference material. Typically, identity testing strategies consider a comparison of the products manufactured in the same facility and these are assessed during method development and validation.

For biochemical methods that compare profiles such as chromatograms, acceptance criterion often is a statement of confirmation of identity or comparison to a reference standard. Instructions for determining confirmation of identity may be included in the specification, or, more commonly, in the test method. For example, comparison guidance could include peak profile including elution/migration order and numbers of peaks and/or specific peak retention/migration time or peak area. It's worth noting that the comparison to the reference material should focus on the key chromatographic/electrophoretic attributes. The identity may still be confirmed while some minor differences are present. For example, in a HPLC-based peptide mapping approach, a C-terminal lysine cleavage present to some degree different from the reference standard does not affect identification of the product. Identity testing is only required at release because identity does not change during storage.

Quantity or Strength

Delivery of the appropriate dose strength to the patient is a requirement for the drug product; therefore, multiple tests are performed to ensure the delivery of the defined dose. There are standard approaches for these tests that are linked to typical process capabilities, method capabilities, and compendia. However, it is important to consider how the product is dosed and the product's therapeutic window to ensure the standard criteria are aligned with the patient needs. Tighter control may be appropriate for a product that has a narrow therapeutic window, to ensure safety and efficacy for the patient.

The measurement of quantity (strength) of the drug substance and drug product is a regulatory expectation for release testing to ensure safety, efficacy, and to establish lot-to-lot consistency.[3,33] A common acceptance criterion is target ± 10%. Although not typically a stability-indicating assay, protein concentration is frequently included in the stability program for a variety of reasons, including demonstrating a lack of interaction of the protein with the container materials of construction. Quantity is typically determined by an appropriate physicochemical assay measuring protein content and expressed as mass per volume; however, depending on the dosage form or route of administration the units may differ (e.g. mass per vial/device versus mass per volume). Criteria for the drug substance quantity should ensure the appropriate concentration to capably support the drug product manufacturing process. In many cases the same range is set for the drug substance and drug product. Alternately the concentration range could be wider for drug substances where, for example, a dilution step is part of the drug product manufacturing process.

Content uniformity/uniformity of dosage testing is performed to ensure that each unit in a drug product lot has an active ingredient content within a narrow range around the label claim. This confirmation of consistency for dosage units ensures safety and efficacy for the patient. The term 'uniformity of dosage units' is defined by pharmacopeia as the degree of uniformity in the amount of the drug substance among dosage units. The methods should comply with USP, Ph. Eur., and JP compendia requirements. This test is typically only needed for lyophilized formulations or formulations that are dosed with a unit basis. Content uniformity does not change during storage and the method does not need to be included on the stability program.

Extractable volume testing ensures that the drug product delivers the intended drug product volume per label claim. The USP terms "container content for injections", "volume of injection", or "volume in container" are also used interchangeably with the Ph. Eur. and JP term "extractable volume".[14–16] The method should comply with compendial requirements. There are also regional requirements for combination products in devices that are out of scope of this paper. The extractable volume acceptance criterion range is based on the volume (which can also be measured as mass divided by density) to achieve the defined label claim. Volume is not a stability-indicating test and is not typically included in the stability specification.

Content uniformity and extractable volume can each be demonstrated by testing the final drug product with methods that meet compendial requirements. Alternatively, in-line fill weight checks performed during the drug product fill process can be leveraged to demonstrate that the lot conforms to compendial uniformity of dosage and/or extractable volume requirements. The target fill volume is higher than the label claim volume to ensure each vial or syringe meets the label claim. The limit for fill volume must be set to ensure the appropriate dose of drug product can be delivered from the container. The acceptance criterion range is based on the label claim plus the container hold up volume (or excess volume) plus a volume range that is based on product filling capability. The limit is justified by demonstrating that it adequately accounts for the label claim, hold up volume and manufacturing capability. Using in-process fill weight checks in lieu of direct testing for content uniformity and/or extractable volume is typically supported by process validation, process controls, and a drug product protein concentration (or protein content) release specification. This in-process approach is considered real time release testing (RTRT). The RTRT approach provides a statistically relevant sample size and real time evaluation of fill weight across the entire lot. This approach demonstrates a level of control for each drug product lot that exceeds compendia requirements for demonstration of content uniformity and/or extractable volume. Therefore, fill weight testing may be used to justify that direct testing of the final drug product is not required.

pH and Osmolality

The pH and osmolality methods provide assurance that the product has been properly formulated, a requirement for patient tolerability of the injection as well as product stability over the shelf life and in conditions of use in a health care or home setting. The methods should comply with USP, Ph. Eur., and JP compendia requirements as well as specific regional requirements for global

submissions. Drug substance and drug product are typically monitored for pH and osmolality either as an in-process control or specification test. The pH and osmolality of the drug substance and drug product should not change during storage. Testing for pH during development is common to demonstrate that the pH remains stable and provides support that pH does not require monitoring in the commercial post-approval stability program.

The pH range is typically established as the target pH ± 0.x pH units. The osmolality range is typically established as the target osmolality result ± xx mOsm/kg. Data from formulation development and stability studies are often provided to demonstrate and justify the product has acceptable quality when maintained within the proposed pH and osmolality ranges.

Reconstitution Time for Lyophilized Drug Product

The reconstitution time method determines the amount of time it takes for the lyophilized cake to dissolve into solution. The acceptance criterion ensures the lyophilized drug product will completely dissolve after reconstitution within the range specified in the product package insert. Reconstitution time is typically included in the drug product specification. There is potential for reconstitution time to change during drug product storage, and data are therefore provided in the marketing application to evaluate reconstitution time upon storage. If reconstitution time does not change over the shelf life of product when stored at recommended storage condition, inclusion in the post-approval stability program may not be required. Additional data under accelerated and stressed conditions may provide additional support for exclusion of this method from the commercial post-approval stability program.

The acceptance criterion typically requires complete product dissolution within a specified time frame, e.g., ≤ x minutes. The numerical limit is based on results obtained during formulation development studies and process validation and is often slightly narrower than the criteria in the package insert, to provide additional time to ensure enough flexibility for the health care provider.

Moisture Content for Lyophilized Drug Product

The moisture content method determines the level of moisture present in lyophilized drug product cake and is related to the general test requirements in ICH Q6B.[3] Acceptable moisture content ensures product stability and performance over the shelf life. Similar to lyophilized cake reconstitution time, the moisture content method is typically included on the drug product specification and may be considered for inclusion in stability testing based on development and stability studies.

The acceptance criterion for moisture typically requires the moisture content of the lyophilized cake to be below a specified level, e.g., ≤ x.x % (w/w). Results from formulation development studies on lyophilized drug product samples with experimentally elevated moisture values can be used to justify no product quality impact or impact on product stability within the specified range of residual moisture. The acceptance criterion is often set slightly tighter than the highest level tested in the development studies which demonstrated no impact, to provide additional assurance the quality will not be impacted if the specification is met.

Polysorbate Concentration

Polysorbate is included as a surfactant in many biological product formulations to enhance product stability, particularly to stresses encountered during manufacturing operations and sometimes for storage stability. While polysorbate levels may not directly impact safety and efficacy, there is often a relationship to maintaining critical PQAs within their appropriate ranges. Testing for polysorbate concentration is therefore typically included in the control strategy to ensure the product has been formulated appropriately. The testing point for polysorbate is product and process dependent, informed by an understanding of how and where within a process polysorbate is needed for stability combined with process design. For products which do not undergo formulation as a component of the drug product process (e.g. drug substance and drug product formulation are identical), the polysorbate concentration can be confirmed as a component of the drug substance control process or as a drug product specification test. In this situation, if polysorbate concentration is tested at the drug substance stage, drug product characterization/validation data must be provided in the marketing application to demonstrate that the polysorbate concentration is homogeneous across the drug product lot and is not impacted by the drug product process. The drug substance result can therefore be considered representative of the drug product.

The polysorbate range may be established as the target concentration ± x % (w/v), or a lower limit may be established based on the minimum level that is required for the stability of the drug product. In addition to evaluating historical lot data, the allowable range is typically justified based on formulation development and stability studies, which demonstrate the product has acceptable quality when maintained within the proposed ranges. If polysorbate concentration has been determined to be critical for product stability, formulation development studies often include assessment of product stability over the allowable polysorbate range. If it is verified during development that polysorbate levels do not change on stability, polysorbate concentration is not considered as a requirement for stability testing.[34] The assessment should include evaluation of the polysorbate assay performance capabilities as appropriate.

Process Related Impurities and Adventitious Agents

Process related impurities are controlled to ensure the overall safety of the product. Process related impurities generally fall into three categories host cell related impurities, process reagents, and adventitious agents.

Host cell-related impurities such as DNA and host cell proteins (HCP) are generally suitably controlled at the drug substance stage and are not impacted by the drug product process. Testing of the drug product is therefore not required. An acceptable range for DNA is based on the WHO acceptance criteria recommendation of no more than 10 ng DNA/dose.[35] HCPs encompass a broad range of proteins and the appropriate criteria for HCPs may consider prior knowledge, product specific historical data, and the coverage of the HCP reagents (USP <1132>).[36] Information gathered during development about the HCP profile and any specific HCPs of concern should be considered in defining the process and when defining acceptance criteria. Typical ranges for HCP in licensed biopharmaceuticals range between 1 and 100 ng/mg protein.[37] Often, process design provides assurance that both DNA and HCPs are robustly controlled by the process as verified through use of clearance studies and demonstrated process control, such that routine drug substance testing may not be required on the commercial specification.

Non-host cell-related process impurities generally come from cell culture additives, or materials used in downstream processing such as detergents, elemental impurities (ICH Q3D), resins or their ligands (e.g., Protein A) or other process/container closure leachables.[38] Generally, commercial tests for these components are not required. Rather, potential exposures are determined assuming no

process clearance and the toxicity profiles are evaluated. For any components which may present a safety risk, additional specific risk assessments or studies are performed to ensure the components are sufficiently cleared by the process. For example, clearance of residual Protein A ligand is typically studied as part of monoclonal antibody purification process development and clearance is demonstrated as part of process validation. If enough process clearance has not been demonstrated, additional process development or inclusion of an in-process test or drug substance specification should be considered.

Adventitious agent testing for commercial drug substance and drug product release specifications typically focuses on microbiological testing such as bioburden and endotoxins derived from bacterial contamination. Additional testing demonstrating sterility and closure integrity of the final product is performed. Much of this testing has criteria of "not detected" or carry a pass/fail criterion. In the case of bioburden, which is frequently tested in-process and for drug substance release, acceptance criteria are generally established to limit the levels that may be present in processing such that bacterially-derived pyrogen or microbial proteases do not contaminate the final product. In-process bioburden testing ensures that potential microbial contaminant levels are low enough to be cleared by filters during the process.

Bacterial endotoxin limits for the final drug product need to meet monograph standards and calculations must consider the intended patient population as well as any product dilutions.[14–16] As exposure limits are calculated based on patient weights, it is critical to factor in the full range of the potential patient population and the highest dose (using the worst-case scenario) to derive acceptance criteria. Further, drug substance and in-process criteria should be set to ensure the final drug product endotoxin criteria will be met.

Lot Selection and Statistics

Although it is not the only source for setting specifications, statistical analyses to determine process capability is a component requiring consideration when generating the final specifications. Statistical analysis of historical lot data to evaluate the manufacturing experience may be conducted in several ways. In all cases, these calculations use a measure of the mean and the variation in historical data and provide a range that describes the population based on knowledge gathered to date. For such a limit to be meaningful, the estimate of both mean and variation must be reflective of the expected future process and assays. Appropriate statistical methods that may be used to characterize historical data and aid in the establishment of acceptance criteria are described widely in pharmaceutical statistical literature. Dong et al. (2015) describe and compare the performance of limits based on reference interval, (Min,Max) methods, tolerance intervals (TI), and confidence limits (CL) on percentiles.[39] Kurtulik et al. (2007) consider how limits set using mean ± 3SD, TI, and historical minimum/maximum value compare and how these limits are affected by historical sample size.[40] Coffey and Yang (2018) describe extension of such statistical methods by estimating random variance components and incorporating consideration of stability trends.[41] In each of these publications, and most others on the topic, authors emphasize that statistics are not a suitable substitute for scientific rationale when deciding on final specification limits. More recently, Bayesian statistical analysis may be used to compute limits, generating a probability distribution of a future reportable value a combination of prior knowledge and historical data, particularly when data is limited.[42] The specific statistical method used to derive a limit is of secondary importance: understanding of process capability must be derived from data that captures future sources of variation to reduce risk of problems in the future.

One of the key factors for evaluating manufacturing experience is the choice of batches to include in the statistical analysis. Typically, representative batches of the commercial manufacturing process are used to compute statistical ranges. Because relatively few lots may have been manufactured at the time of analysis, examination of all relevant lots is critical. The historical data set should include clinical lots not manufactured at the commercial scale when they are representative of the commercial manufacturing process. If the historical data set is limited or does not contain sources of potential random process variation, the estimate of variation may not be representative of the true process variability. Future process changes (e.g. scale up, manufacturing site change, raw material changes) could introduce new sources of variability that may be perfectly acceptable, but such sources of variation may not be represented in a data set consisting of clinical batches alone. Further, even a relatively large data set may be derived from very few raw materials or process intermediate lots, and therefore underestimate expected sources of random process variability. Therefore, acceptance criteria wider than the limits calculated statistically is justified when there is a low risk of impact to patient safety and product efficacy.

Stability Considerations

Stability acceptance criteria, also termed end of shelf life (EOSL), depend on the product stability profile in the final formulation and final container closure. Data for each product quality attribute should be evaluated to determine if there is a change under recommended storage conditions through the intended final expiry. An assessment should be made to ensure that changes in attribute level do not impact safety or efficacy. In cases where a change is observed, a tiered approach is applied where the release specifications are tighter than stability limits. Based on the amount of change observed over the shelf-life, release specifications should provide assurance that lots within the release acceptance criteria will meet the stability acceptance criteria at expiry. Additional considerations for in-use stability, as applicable, should also be assessed.

Case Study 1- Fragmentation

Case study 1 illustrates the use of prior knowledge and in vitro assessments to support setting patient-centric purity acceptance criteria for fragmentation. For the purpose of this case study, fragmentation is defined as peptide bond hydrolysis that results in large or readily measured fragments. Sequential removal of one or several residues from the N- or C-termini is not included under this definition of fragmentation. Gaining product specific information on the impact of fragmentation on potency or safety can be challenging due to the difficulty of generating highly purified fragment pools, or enriched samples with high levels of fragmentation that do not also include other degradants such as high molecular weight species.

While generating samples with high levels of fragmentation in the absence of other degradants is challenging, information available from the product's stability program and characterization studies can be used to inform on the potential risk that fragmentation within a specific range will impact activity or safety. Identification of a range of fragmentation where there is no impact to the product's activity provides evidence that fragmentation within that specific range, even in the presence of other degradation products, has a low likelihood of having a clinically meaningful impact. Below is an example on how stability and stressed condition data can be

Table 4
Results of Accelerated Stability Data Inform on the Impact of Fragmentation on the Potency Bioassay of mAbX.

Drug Substance Lot	Temperature/Time Point	rCE-SDS HC + LC (Result at T = 0) (%)	Relative Potency (Potency at T = 0) (%)
A	25 °C/6 months	87.5 (97.0)	110 (124)
B	25 °C/6 months	87.4 (96.8)	117 (87)
C	25 °C/6 months	88.4 (96.7)	93 (102)

used to assess the potential clinical risk of fragmentation. In this example, the potency bioassay and ADCC assays used for product assessment met robust method performance criteria and were demonstrated to be suitable for their intended purpose. The potency assay was fully validated in accordance with ICH Q2 (R1),[43] and the ADCC assay was qualified to demonstrate it is suitable for its intended use. Method validation and qualification included criteria for accuracy, precision, specificity, linearity, and repeatability. In addition to the reference standard, an independent control sample is included in each assay to monitor assay performance and all samples must meet pre-determined assay system suitability criteria. These assays were shown to be capable of detecting meaningful changes in potency or ADCC activity which could occur due to product degradation and/or alterations in the glycan profile. For mAbX, fragmentation was monitored by a validated reducing capillary gel electrophoresis with SDS (rCE-SDS) method, reported as heavy chain (HC) plus light chain (LC) peak area results (%). Fragmentation results in a concomitant decrease in the HC + LC peak area by rCE-SDS. Therefore, peak area of HC + LC is directly related to the peak area of minor species that result from fragmentation and can be used as a sensitive measure of fragmentation for many products. For this product, the primary site of fragmentation is in the heavy chain in the Fc-region. Fragmentation therefore has the potential to impact effector function, and therefore an ADCC assay was used for the evaluation in addition to the potency bioassay.

Potency

Results from mAbX drug substance, which is considered representative of the drug product, stored at the stressed storage condition of 25 °C for six months, and under high pH stressed conditions, are shown in Tables 4 and 5 respectively. Fragmentation is observed under recommended and stressed storage conditions, as well as under the high pH conditions. In addition, a comparison of the electropherogram profiles of product stored under recommended and stressed storage conditions, and high pH condition demonstrated similar pathways of fragmentation under all three conditions. The potential impact of fragmentation on product potency observed under these conditions is therefore representative of the potential impact which occurs under recommended storage conditions. Data from the stressed storage condition demonstrate that HC + LC levels as low as 87.4% had no meaningful impact on the potency bioassay (Table 4). Data from the high pH condition demonstrated that HC + LC levels as low as 94.8% have no meaningful impact on either potency or ADCC activity (Table 5). For these data, relative change was based on the difference in activity observed between the T = 0 and the 6 month timepoint in Table 4, and between the control and test sample in Table 5. Since the magnitude of the differences were within expected assay variability, it was concluded there was no meaningful impact to potency or ADCC under the conditions evaluated.

PK and Safety/Immunogenicity

For this product, data from the potency assay directly demonstrate that there is no impact to antigen binding (Fab-region activity) when HC + LC levels are ≥ 95.0%. Because there was no impact to ADCC activity, the data also support there is no significant impact to Fc-structure within this range, and the likelihood that fragmentation would impact FcRn binding and PK within this range is low. Since the data indicate no meaningful change to Fab- or Fc-region structure, the likelihood that the level of fragmentation allowed within that range of HC + LC ≥ 95.0% would result in exposure of a new epitope and impact immunogenicity, is therefore also low.

Proposed Specification Acceptance Criterion

For mAbX, fragmentation was not observed during drug substance or drug product manufacturing and storage at the recommended storage temperature. The range of HC + LC observed in clinical drug product lots was 96.4%–97.3%, and a tolerance interval calculated from drug product release and stability results was 95.921%. Based on the attribute risk assessment that supports a low risk of impacting to safety and/or efficacy at levels of HC + LC peak area of ≥94.8%, a specification limit of HC + LC ≥ 95.0% by rCE-SDS was proposed.

Case Study 2- Fc Region Methionine Oxidation

Case study 2 for a monoclonal antibody (mAb) Fc region methionine (Met) oxidation illustrates a situation where there is a wealth of robust, non-product specific prior knowledge of the attribute which can be leveraged to justify a product specific control strategy based on limited product specific data. While Fc region Met oxidation would not be expected to impact the binding activity of a mAb, forced oxidation studies are typically included in characterization studies and data from these studies can be used to confirm no impact to potency. Data/information available from the literature or from internal proprietary prior knowledge can typically be leveraged to assess the potential impact of Fc region Met oxidation on PK and safety, including immunogenicity. As noted in Case Study 1 the analytical assay, potency and ADCC assays used for product assessment met robust method performance criteria and were demonstrated to be suitable for their intended purpose.

Potency

Oxidation of Met residues in the Fc-region would not be expected to impact the binding activity of mAbs to their target antigens. Lack of impact to antigen binding can be confirmed based on

Table 5
Use of High pH Stressed Condition Data to Inform on the Impact of Fragmentation on the Potency Bioassay and ADCC Activity of mAbX.

Stressed Condition	rCE-SDS HC + LC (%)	Relative Potency (%)	Relative ADCC (%)
Control	96.4	100	107
pH 8.0/25 °C	94.8	110	120

Table 6
Use of Forced Degradation Data to Inform on the Impact of Methionine Oxidation on the Potency Bioassay of an IgG1 Antibody (mAbA).

Time point	Percent Met Oxidation, site 1	Percent Met Oxidation, site 2	% Relative Potency
Control	3	1	122
T1	30	13	124
T2	69	39	117

data from forced oxidation studies which typically assess the sensitivity of Met resides to oxidation and the impact of forced oxidation on potency. Examples from forced oxidation studies for two IgG1 molecules, named mAbA and mAbB for this case study, are provided in Tables 6 and 7. The data demonstrate the Met oxidation at levels well above that in untreated control samples has no meaningful impact on potency.

Met oxidation is also not expected to have an impact on Fc region mediated antibody dependent cellular cytotoxicity (ADCC) activity. ADCC requires Fcγ receptor (FcγR) binding and sites for FcγR binding are in the hinge-proximal region, which is not typically impacted by Met oxidation.[44] Studies have demonstrated that Met oxidation at levels that impact FcRn binding have no meaningful impact on FcγRIIIa binding and hence risk of impacting ADCC activity is low.[45] For mAbB, ADCC was identified as a secondary mechanism of action and forced oxidation studies therefore included assessment of the impact of Met oxidation on mAbB ADCC activity. As provided in Table 7, data confirmed that even high levels of Fc region Met oxidation did not impact ADCC activity.

Met oxidation has been reported to interfere with CDC activity at similar levels as those that impact FcRn binding.[46] The impact to CDC activity was suggested to be due to Fc region Met oxidation altering the local conformation at the CH2−CH3 interface and impacting the formation of the stable mAb hexamers, which leads to a decrease in C1q binding and subsequent loss of CDC activity. For mAb products that mediate CDC activity, the risk of Met oxidation on CDC activity should therefore be evaluated though, similar to the impact on FcRn binding, if data demonstrates Met oxidation is present at low, well controlled levels, the risk of impacting CDC activity is low.

PK

There are two Met residues in the Fc region (heavy chain), Met252 and Met428 (based on Kabat numbering), that are conserved between IgG1 and IgG2 mAbs. Studies have demonstrated that Met252 has a dominant negative binding effect on FcRn binding affinity and subsequent clearance.[23,47] Only doubly oxidized Met252 (on both heavy chains) has a significant impact on pharmacokinetics, in contrast to singly oxidized Met252 which had no impact on PK. While Met252 oxidation would have to be very high (>50%) to observe double chain Met252 oxidized species, a risk assessment for the potential of Met oxidation to impact FcRn binding and clearance is typically performed.

Met oxidation can be minimized by limiting product exposure to factors that promote oxidation such as light, metals and low levels of peroxides found in polysorbate. Factors that promote oxidation are typically well controlled during mAb manufacture. The levels of Met oxidation present on mAbs are therefore generally orders of magnitude below those which impact FcRn binding, and the risk that Met oxidation will reach levels where an impact to the pharmacokinetic profile could be observed is low. Drug substance and drug product characterization studies can be used to confirm robust process control during manufacturing and storage. Demonstration of low occurrence and robust control over process parameters that impact oxidation can be used to justify there is a low likelihood that Met oxidation will impact PK.

Safety/Immunogenicity

It has been suggested that Met oxidation of mAb products has the potential to increase immunogenicity risk due to the change in primary structure. There is currently no published information directly assessing the immunogenicity of mAb Met oxidation. However, a similar immune response is produced against aggregates that contain either high or low levels of Met oxidation suggesting that oxidation poses a low immunogenicity risk.[48,49] The immunogenicity risk of Met oxidation is therefore low, particularly at the levels that are present in most mAb products.

Proposed Control Strategy

Taken together the data/information indicate that Fc-region Met oxidation at the low levels present in these mAb products typically does not pose a risk to efficacy and safety, and in many cases would not require routine monitoring during lot release or stability testing.

Case Study 3- Potency Specifications

Case study 3 describes an example of setting a potency specification for a cell-based bioassay that measures the specific ability of a monoclonal antibody product to inhibit antigen activities in vitro (i.e. receptor binding and intracellular signaling). Potency specification limits were developed for drug substance (DS) and drug product based on the understanding of mechanism of action, product quality attributes and their impact on product potency, and statistical evaluation of release and stability data that accounts for both manufacturing process consistency and analytical variability. As noted earlier, the precision and sensitivity of biochemical methods for purity and product heterogeneity are superior to cell-based and ELISA bioassays; however, a potency assay with acceptance criterion is an expectation for monoclonal antibody products.

Mechanism of Action

The antibody was designed to have only Fab activity and was demonstrated to lack Fc-effector functions (e.g. ADCC, CDC, and ADCP activities). A cell-based bioassay designed to be reflective of

Table 7
Use of Forced Degradation Data to Inform on the Impact of Methionine Oxidation on the Potency Bioassay and ADCC Activity of an IgG1 Antibody (mAbB).

Time Point	Percent Met Oxidation, Site 1	Percent Met Oxidation, Site 2	% Relative Potency	% Relative ADCC
control	3	1	100	107
T1	18	11	102	108
T2	57	43	89	112

the presumed in vivo mechanism of action was selected for commercial release and stability testing.

Quality Attribute Understanding

Structure-function assessments were used in the assignment of quality attribute criticality and establishment of control limits for product variants that may impact potency. Specifically, forced degradation studies were performed to evaluate the effects of thermal degradation, light exposure, deamidation, and oxidation on the quality attributes of the mAb. Changes in product purity and heterogeneity induced by these stress conditions were correlated with a decrease in product potency. However, similar changes were not observed under normal manufacturing or recommended storage conditions (inclusive of the 36-month commercial shelf life). The commercial specification limits for product purity and heterogeneity were set to provide a high level of assurance that the quality of the product, and therefore the potency, remain consistent over time and aligned with clinical experience.

Evaluation of Release and Stability Data

The specification limits were established through analysis of drug substance and drug product data; however, the drug substance acceptance criteria were set to be identical to drug product because potency does not change during the long-term storage of the drug substance and is not affected by the drug product manufacturing process. As such, the statistical evaluation was performed using data from sixteen drug substance lots manufactured during three separate production campaigns at the intended commercial launch site, using the intended manufacturing scale and commercial process. Of these sixteen commercial scale lots, at least one lot from each manufacturing campaign was enrolled in the clinical studies. Therefore, all drug product lots are fully representative of the materials used in clinical studies, justifying pooling of the data for specification setting.

All long-term stability data (i.e., drug product stored at the recommended 5 °C condition) were included in the determination of release and stability specification limits. At the time of commercial specification establishment, stability data up to 36 months were available. A statistical evaluation of stability data demonstrated there was no discernible trend in potency over time; therefore, stability data were combined with release data to obtain a more reliable estimate of analytical variability. In addition, the same acceptance criteria are proposed for release and stability specifications.

As mentioned in the "Lot Selection and Statistics" section, the reference interval (e.g, mean±3 standard deviations, SD) and tolerance interval are two potential statistical methods for assessing manufacturing process consistency and analytical variability. In general, mean ± 3-SD is used when the number of lots/batches is significantly large. In this case study, the number of lot/batches is limited at sixteen, as noted above. When the manufacturing process is the major source of total variability, the certainty of the process variability estimate increases with the total number of lots/batches, therefore a Tolerance Interval based on the number of lots/batches is relatively simple and most appropriate because it accounts for uncertainty of the mean and SD estimates caused by limited sample size. However, as previously discussed, in the case of the potency, the method variability is larger than the process variability. Hence, each stability time point within the same lot/batch is used to obtain a more accurate estimate of method variability (which is reflected as the within-lot/batch variability in the absence of degradation trend). Since method variability is the main source of total variability, the certainty of the method variability estimate increases with the total number of stability time points instead of the number of lots/batches. In other words, the quality of the mean and the total variability estimate for potency can be very good as long as the number of stability time points is large (in this case, n = 7 stability lots * 9 time points per lot + 9 release-only lots * 1 = 72). Therefore, in this case study, mean ± 3-SD is a suitable statistical approach for determining the specification acceptance criterion.

Proposed Specification Acceptance Criterion

The mean ± 3-SD limits were calculated from the combined drug product release and stability data sets at the recommended storage condition. As described in Table 8, the mean ± 3-SD limits are (73.7, 126.0%). For simplicity, the limits are rounded to integers. So, the revised commercial specification limit is (74%, 126%), which is consistent with the range observed on drug product stability performed during clinical development (81%−118%). The 3-SD commercial specification limits are closely aligned with these stability data and provides further evidence the specification is representative of the materials used in clinical studies. Therefore, the proposed commercial specification is clinically relevant and within a range that is not believed to impart any difference in efficacy.

Case Study 4- Considerations for Antibody Drug Conjugates

Case study 4 describes considerations for specification setting for an example antibody drug conjugate (ADC). ADCs are antibody intermediates modified by conjugation with a covalently linked small molecule. The modified antibody is able to deliver the small drug moiety to a target location in vivo. Principles provided here may be extrapolated to most forms of conjugated drug substances. ADCs can be broken down into three primary components. The two "active" components are the protein backbone (antibody, Fc Fragment, etc.) and the small drug moiety. The 3rd component is a linker, which may be described as 'cleavable' or 'stable' and connects the protein backbone to the small molecule moiety.[50,51] All three components play a role in delivery and activity of the drug product. Each component of an ADC is manufactured individually from the other components and is considered a product intermediate, although for some ADCs the drug-linker is a single entity. Product intermediates require specifications that must be met during their manufacture. Selection of assays to include in the intermediate specification are typically based on control of intermediate CQAs that may affect safety and/or efficacy of the drug product or that provide assurance that the manufacturing process is controlled and that the intermediate is acceptable for further processing. While critical for the integrated ADC control strategy, intermediates and their respective control specifications will not be discussed in this case study. Considerations for several ADC CQAs specification setting are found below.

ADC Drug to Antibody Ratio (DAR)

One aspect of conjugation that must be controlled is the number of small drug moieties attached during conjugation, also known as the drug to antibody ratio (DAR). Control of the DAR is directly related to the potency of the drug product and therefore is a CQA. The acceptable range for the DAR is product-specific and is derived from pre-clinical studies, manufacturing history, and process characterization.

ADC Potency

For ADCs, the potency assay is most often a cell-based assay that demonstrates the cytotoxicity mechanism of action. These assays are inherently variable and, given the toxicity of the drug, tight control ranges may be expected. However, demonstrating a correlation between DAR as determined using the more precise DAR

Table 8
Statistical Analysis of Drug Product Release and Stability Test Results.

Quality Attribute	Mean	Standard Deviation	Min	Max	3-Standard Deviation Limits	
					Lower Limit	Upper Limit
Potency/Biological Activity (% RP)	99.8	8.73	81	118	73.7	126.0

assay and potency as determined using the less precise cell-based potency assay may provide justification for broader potency ranges. For example, for an ADC with a target of DAR = 8, a range of DAR 7.5−8.5 was demonstrated to be acceptable for patient safety and efficacy. The cell-based potency assay has a variability of 10%. For this ADC, a correlation was demonstrated between the DAR and the potency result such that Δ0.1 DAR = ~Δ5% potency. Therefore, a DAR range of 7.5−8.5 would be evaluated as 75−125% potency. Assuming the cell-based assay itself has an intermediate precision of 10%, one can deduce that a DAR range of 7.5−8.5 might reasonably have a potency range of ~65−135% when accounting for method variability. Thus, assuming 7.5−8.5 is acceptable for patient safety and efficacy, a broad potency range specification may be justified.

ADC Unconjugated mAb

In most ADCs, a fraction of the protein is not conjugated. This fraction can be seen either as a competitor for the ADC reactive site in vivo or as a non-potent variant/impurity. FDA 1997 Points to Consider states that unconjugated mAb should be monitored and controlled.[52] Setting acceptable ranges for the allowable level of the unconjugated mAb impurity/variant is product-specific and may be justified by manufacturing history, clinical experience, in vitro evaluations of the impact of free mAb on cytotoxicity, and, potentially, relevant xenograft experiments which may demonstrate the level of control needed. The key is to have consistency of the amount of this variant; that may be demonstrated by process control or testing with an acceptance criterion.

ADC Free Drug Related Impurities

Clearance of Free Drug-Related Impurities is typically demonstrated using a suitable routine release assay (FDRI assay). Development and validation studies provide evidence that other process-related impurities are cleared by the same process that clears FDRI. Hence, release testing of FDRI in combination with clearance validation studies can justify that there is no need for routine release testing of other process-related impurities. The FDRI assay may also be used to demonstrate stability of drug-linker attachment on storage. A detailed discussion on setting specifications for free-drug in an ADC is available in Gong et al.[53]

Conclusions

Commercial specifications are a critical component of a biotherapeutic product's integrated control strategy. Patient-centric specifications based on the potential impact of an attribute range on patient safety and efficacy ensure product quality while also enabling a robust supply chain. It is important to note that testing is only one part of the overall product control strategy and that efforts to gain deeper process understanding, innovative approaches for at-line process and product quality assessments, and real time release efforts should continue to be explored as alternatives to end-product release testing. These approaches should improve process performance while maintaining product quality at reduced time and cost, enabling delivery of more medicines.

This manuscript summarized specification setting approaches including case studies illustrating science and risk-based decision making to set specifications. Commercial acceptance criteria based solely on statistical ranges may result in the rejection of acceptable product or, if the process was historically highly variable, the release of batches that may be of unacceptable quality. Additionally, if limits are required to be tightened to manufacturing capability based on limited clinical batch experience, further process improvements or development of patient-convenient dosage forms such as room temperature storage may be impeded. Therefore, risk-based assessments of the impact of PQA on safety and efficacy based on prior knowledge and/or product specific assessments should be the basis for specification setting.

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References

1. Mullard A. 2018 FDA drug approvals. *Nat Rev Drug Discov*. 2019;18:85-89.
2. Walsh G. Biopharmaceutical benchmarks 2018. *Nat Biotechnol*. 2018;36:1136-1145.
3. International Conference on Harmonization Guideline Q6B. *ICH Harmonised Tripartite Guideline Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*. Geneva, Switzerland: ICH; 1999. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6B/Step4/Q6B_Guideline.pdf.
4. Bercu J, Berlam SC, Berridge J, et al. Establishing patient centric specifications for drug substance and drug product impurities. *J Pharm Innov*. 2019;14:76-89.
5. International Conference on Harmonization Guideline Q8. *ICH Harmonized Tripartite Guideline Pharmaceutical Development Q8*. Geneva, Switzerland: ICH; 2009. Available at: www.ich.org. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf.
6. International Conference on Harmonization Guideline Q9. *ICH Harmonized Tripartite Guideline Quality Risk Management Q9*. Geneva, Switzerland: ICH; 2005. Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf.
7. International Conference on Harmonization Guideline Q10. *ICH Harmonized Tripartite Guideline Pharmaceutical Quality System Q10*. Geneva, Switzerland: ICH; 2008. Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf.
8. International Conference on Harmonization Guideline Q11. *ICH Harmonized Tripartite Guideline Development and Manufacture of Drug Substances Q11*. Geneva, Switzerland: ICH; 2012. Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf.
9. Gordon C, Lennard A, Hunter B, et al. *Medicinal Product Incorporating a Drug Delivery Device Component: An Industry Perspective on Developing an Efficient, "end to End" Control Strategy*. European Biopharmaceutical Enterprises reflection paper; 2019. Available at: https://www.ebe-biopharma.eu/wp-content/uploads/2019/06/EBE-Reflection-Paper-Integrated-drug-device-products_Control-Strategy-Final_-12-June-2019.pdf.
10. CMC Biotech Working Group. *A-mab; a Case Study in Bioprocess Development*. Emeryville, CA: CASSS; 2009.

11. Finkler C, Krummen L. Introduction to the application of QbD principles for the development of monoclonal antibodies. *Biologicals*. 2016;44:282-290.
12. Taticek R, Liu J. Definitions and scope of key elements of QbD. In: Jameel F, Hershenson S, Khan MA, Martin-Moe S, eds. *Quality by Design for Biopharmaceutical Drug Product Development*. New York, NY: Springer New York; 2015: 31-46.
13. FDA office of pharmaceutical quality white paper. Available at: https://www.fda.gov/media/91721/download.
14. United States pharmacopeia (USP). Available at: https://www.uspnf.com/.
15. European pharmacopeia (Ph.Eur.). Available at: https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition.
16. Japanese pharmacopeia (JP). Available at: https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0009.html.
17. Rogers RS, Abernathy M, Richardson DD, et al. A view on the importance of "multi-attribute method" for measuring purity of biopharmaceuticals and improving overall control strategy. *AAPS J*. 2018;20:7.
18. FDA Manual of Policies and Procedures OPQ. CDER. Establishing impurity acceptance criteria as part of specifications for NDAs, ANDAs, and BLAs based on clinical relevance. MAPP 5017.2. Available at: www.fda.gov; 2018.
19. FDA Guidance for Industry. Process Validation: General Principles and Practices. 2011. Available at: www.fda.gov.
20. Rosenberg AS, Verthelyi D, Cherney BW. Managing Uncertainty: a perspective on risk pertaining to product quality attributes as they bear on immunogenicity of therapeutic proteins. *J Pharm Sci*. 2012;101(10):3560-3567.
21. FDA Guidance for Industry. Immunogenicity assessment for therapeutic protein products; FDA. 2014. Available at: www.fda.gov. https://www.fda.gov/media/85017/download.
22. Khawli LA, Goswami S, Hutchinson R, et al. Charge variants in IgG1. Isolation, characterization, in vitro binding properties and pharmacokinetics in rats. *mAbs*. 2010;2(6):1-12.
23. Stracke J, Emrich T, Rueger P, et al. A novel approach to investigate the effect of methionine oxidation on pharmacokinetic properties of therapeutic antibodies. *mAbs*. 2014;6(5):1229-1242.
24. Goetze AM, Liu YD, Zhang Z, et al. High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans. *Glycobiology*. 2011;21(7):949-959.
25. Liu L. Antibody Glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. *J Pharm Sci*. 2015;104:1866-1884.
26. Code of Federal Regulations. Title 21 Part 610.10 potency. Accessed at eCFR 2019 https://ecfr.io/Title-21/cfr610_main.
27. Hubbard A. Potency labeling of novel Factor VIII and Factor IX concentrates: past experience and current strategy. *Semin Thromb Hemost*. 2015;41:849-854.
28. Li Y, Polozova A, Gruia F, Feng J. Characterization of the degradation products of a color-changed monoclonal antibody: tryptophan-derived chromophores. *Anal Chem*. 2014;86(14):6850-6857.
29. Swartz TE, Yin J, Patapoff TW, et al. A spectral method for color quantitation of a protein drug solution. *PDA J Pharm Sci Technol*. 2016;70:361-381.
30. Mathonet S, Mahler H-C, Esswein ST, et al. A biopharmaceutical industry perspective on the control of visible particles in biotechnology-derived injectable drug products. *PDA J Pharm Sci Technol*. 2016;70:392-408.
31. Kotarek J, Stuart C, De Paoli SH, et al. Subvisible particle content, formulation, and dose of an erythropoietin peptide mimetic product are associated with severe adverse post marketing events. *Pharm Sci*. 2016;105(3):1023-1027.
32. Fradkin AH, Carpenter JF, Randolph TW. Immunogenicity of aggregates of recombinant human growth hormone in mouse models. *J Pharm Sci*. 2009;98(9): 3247-3264.
33. European Medicines Agency. Guideline on development, production, characterisation and specifications for monoclonal antibodies and related products. Retrieved from ema.wuropa.eu http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003074.pdf; 2008.
34. Jones MT, Mahler HC, Yadav S, et al. Considerations for the use of polysorbates in biopharmaceuticals. *Pharm Res (N Y)*. 2018;35(148).
35. Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: WHO Expert Committee on Biological Standardization. Sixty-First Report. Geneva, World Health Organization.
36. *USP General Chapter <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals*. 2016.
37. Champion K, Madden H, Dougherty J, Shacter E. Defining your product profile and maintaining control over it, part 2: challenges of monitoring host cell protein impurities. *Bioprocess International*. 2005;3:52-57.
38. International Conference on Harmonization Guideline Q3D. *Guideline for Elemental Impurities*. Geneva, Switzerland: ICH; 2019. Available at: https://database.ich.org/sites/default/files/Q3D-R1EWG_Document_Step4_Guideline_2019_0322.pdf.
39. Dong X, Tsong Y, Shen M. Statistical considerations in setting product specifications. *J Biopharm Stat*. 2015;25(2):280-294.
40. Kurtulik P, Tougas T, Tsang P, Warner E, Winter W, Wyvratt JM. Acceptable Analytical practices for justification of specifications. *Pharm Technol*. 2007;31(4):156-168.
41. Coffey T, Yang H, eds. *Statistics for Biotechnology and Process Development*. Boca Raton, FL: CRC Press; 2018.
42. Burdick R, LeBlond D, Pfahler L, et al. *Statistical Applications for Chemistry, Manufacturing and Controls (CMC) in the* Pharmaceutical Industry. Switzerland: Springer; 2017.
43. International Conference on Harmonization Guideline Q2. *ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1)*. Geneva, Switzerland: ICH; 1994. Available at: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf.
44. Liu H, Gaza-Bulseco G, Xiang T, Chumsae C. Structural effect of deglycosylation and methionine oxidation on a recombinant monoclonal antibody. *Mol Immunol*. 2008;45(3):701-708.
45. Bertolotti-Ciarlet A, Wang W, Lownes R, et al. Impact of methionine oxidation on the binding of human IgG1 to FcRn and Fcγ receptors. *Mol Immunol*. 2009;46(8–9):1878-1882.
46. Mo J, Yan Q, So CK, Soden T, Lewis MJ, Hu P. Understanding the impact of methioinine oxidation on th biologicl fucntions of IgG1 antibodies using hydogen/deuterium exchnage mass spectrometry. *Anal Chem*. 2016;88:9495-9502.
47. Gao X, Ji JA, Veeravalli K, et al. Effect of individual Fc methionine oxidation on FcRn binding: met252 oxidation impairs FcRn binding more profoundly than Met428 oxidation. *J Pharm Sci*. 2015;10:368-377.
48. Joubert MK, Hokom M, Eakin C, et al. Highly aggregated antibody therapeutics can enhance the in vitro innate and late-stage T-cell immune responses. *J Biol Chem*. 2012;287(30):25266-25279.
49. Bi V, Jawa V, Joubert MK, et al. Development of a human antibody tolerant mouse model to assess the immunogenicity risk due to aggregated biotherapeutics. *J Pharm Sci*. 2013;102(10):3545-3555.
50. Alley S, Anderson K. Analytical and bioanalytical technologies for characterization antibody-drug conjugates. *Curr Opin Chem Biol*. 2013;17:406-411.
51. Wakankar A, Chen Y, Gokarn Y, Jacobson FS. Analytical methods for physicochemical characterization of antibody drug conjugates. *mAbs*. 2011;3(2):161-172.
52. FDA Points to Consider in the Manufacturing and testing of monoclonal antibody products for human use; FDA. 1997. Available at: www.fda.gov.
53. Gong HH, Ihle N, Jones MT, et al. Control strategy for small molecule impurities in antibody-drug conjugates. *AAPS PharmSciTech*. 2018;19(3):971-977.