Chapter 9

Quality Control Biochemistry

Objectives

This chapter provides an overview of the operating principles in Quality Control Biochemistry for biopharmaceuticals.

After completing this chapter student will be able to:

- describe a Quality Control (QC) operating system and structure in a biotechnology organization.
- define the skills, knowledge, and personnel traits required for QC in a biotechnology organization.
- list the requirements for data management related to QC.
- explain how specifications are used in QC Biochemistry.
- describe the techniques used in Biochemistry Quality Control and their application in the biotechnology industry.
- define the phases of analytical methods, including development, qualification, and validation.
- explain the importance of measurements performed for in-process monitoring, product release, and product stability monitoring.
- list and explain the requirements of a stability program for a typical monoclonal antibody-based therapeutic.
- summarize QC's role in laboratory investigation and the investigation of Out of Specification and Out of Trend results.

Terms

% Relative Standard Deviation: % RSD (or coefficient of variation, % CV) = (Standard deviation/mean) x 100%

% differences: %differences = (Result A – Result B) X 100% / Mean of A & B

Accuracy: demonstrates the closeness of test results obtained by the method to the true value (nominal) or an acceptable reference value

Analytical method: a laboratory procedure used to measure a physiochemical entity or attribute of the entity

Characterization method: a scientifically-sound analytical test method used to evaluate a specific quality attribute; they are used in support of cGMP manufacturing, process development, process characterization, formulation development/optimization/characterization, deviation investigations, comparability, reference standard qualification, process validation, or other studies required for regulatory submissions

Coefficient of Variance (CV) or % Relative Standard Deviation: a statistical measure of variability

Detection Limit (also referred to as Limit of Detection or LOD): the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value

Identity assay: an analytical procedure that confirms the presence of the active product ingredient

Impurity detection assay: an analytical procedure that indicates the presence of degradants and other impurities present with the active product ingredient

Impurity quantitation assay: a quantitative analytical procedure that measures the amount of degradants or other impurities present with the active product ingredient

Intermediate precision: expresses the precision of a method, under the same operating conditions, when there are intra-laboratory variations involving different days, different analysts, and different equipment

Limit of Detection (LOD): the lowest amount of analyte that can be detected but not quantitated as an exact value

Limit of Quantitation (LOQ): the lowest amount of analyte that can be quantitatively determined with suitable accuracy and precision

Linearity: demonstrates a method's ability (within a given range) to obtain test results directly proportional to the concentration (amount) of analyte in the sample

Loading (working) range: an interval of the linear range targeted for sample loading; this range is determined during method development and should provide a proportionate response to main analyte and appropriate sensitivity to other analytes that may be present

Percent differences: measure of the difference from two separate results

Performance characteristics: attributes of a method that are evaluated to assure that an analytical method is suitable for its intended use

Precision: demonstrates the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of the same test article under the prescribed conditions; precision as applied to analytical methods has three components: Repeatability, Reproducibility, and Intermediate Precision; precision can be measured as repeatability (intra-assay variability), intermediate precision (intra-laboratory variability), and reproducibility (inter-laboratory variability). ***Chapter 4 Metrology*** covers precision and the related term *accuracy*

Purity assay: a quantitative analytical procedure used to determine the purity of the active product ingredient

Purity method: a qualitative analytical procedure used to determine the purity of the active ingredient

Qualification: an experimental study demonstrating that an analytical method performs as expected, providing consistent and meaningful data under a defined set of conditions

Qualification Result: the result calculated for a particular performance characteristic tested during a Qualification; the reportable Qualification Result is usually determined by statistical analysis of a specified number of reported values

Quantitation Limit (also referred to as Limit of Quantitation or LOQ): the lowest (Lower Limit of Quantitation or LLOQ) and highest (Upper Limit of Quantitation or ULOQ) amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy; the upper and lower limits define the endpoints of the range

r (the correlation coefficient): R^2 is the coefficient of determination which will be used in validation reports

Range (also referred to as linear or assay range): the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity

Repeatability: expresses the precision of a method under the same operating conditions over a short interval of time, such as performing the test by the same analyst, on the same day, and using the same experiment set ups—measures inherent method variability (short time between measurements)

Replicate: the determination of a single reported value as prescribed by the method Standard Operating Procedure (SOP); multiple replicates are treated as individual samples during Qualification studies

Reported value: the result obtained by following the testing scheme outlined in the method SOP; most often the reported value is the average result calculated from a specified number of replicate or individual measurements

Reproducibility: expresses the precision between laboratories; demonstrates the measurement of inter-laboratory method variability

Robustness: demonstrates the method's ability to remain unaffected by deliberate variations in the method procedure and provides an indication of its reliability under normal operating conditions

Specification: an explicit set of requirements or limits to be satisfied by a product when subject to testing

Specificity: demonstrates the ability of the method to measure an analyte in the presence of components that may be in the sample, including impurities, degradants, and matrix components

Standard Deviation: measure of how widely values are dispersed from the average value (mean)

Standard Operating Procedure (SOP): a written and approved procedure that is version-controlled and used consistently for cGMP-related testing or operations

Validation protocol: defines the scope and procedures necessary to measure the performance of an analytical procedure with respect to specific validation characteristics for its intended use; predetermined acceptance criteria shall be set in the protocol for all studies

Introduction to Quality Control in the Biotechnology Industry

The active substances in biopharmaceutical medicines are typically large protein molecules that are thousands of times larger than conventional chemical drugs. In the manufacture of biopharmaceutical drug substances, the production processes are typically of extended duration, always involve living cells, and involve complex science and elaborate engineering processes. It is essential that the manufacturer verifies through various types of testing that the process is performing as expected and that the product meets expectations during the entire production process (i.e., all activities are in a state of control). Biopharmaceutical Quality Control is based on well-developed scientific methods that make possible relevant measurements of the product stream, the facility, the materials, and the people. Quality Control testing is applied throughout the entire process of the production of biopharmaceuticals.

The disciplines involved in biopharmaceutical QC testing include:

- cell biology
- virology
- microbiology
- inorganic chemistry
- organic chemistry
- analytical chemistry
- molecular biology
- immunology

The evaluation of a biopharmaceutical drug substance coming out of a production run is measured against a specification for that substance. A specification is an explicit set of requirements or limits to be satisfied by a product when subject to testing. For the QC Laboratory this translates into a defined set of tests and the acceptance criteria associated with each test. Quality Control testing is governed by the product specification. Every batch of the product is tested against the specification. Typical specifications for a biopharmaceutical drug substance and a biopharmaceutical drug product are provided in Table 7-4 of ***Chapter 7 Quality Assurance*****.**

Modern analytical technology has allowed Quality Control testing in the biotechnology industry to advance to a sophisticated level. QC in biopharmaceutical manufacturing goes far beyond the common perception of simple one-step checks of the quality of a product. Consequently, those who perform this type of work in the biopharmaceutical industry are highly-trained specialists. This ensures that QC tests are performed properly and to exacting standards. In return, these staff members are given job security, job satisfaction, competitive compensation, and continuing opportunities in career growth.

Introduction to Quality Control Biochemistry

As indicated in the overview to this unit, the term Quality Control (QC) can be used to refer to an activity(e.g., testing versus a specification) as well as to the actual organizational group who execute this work. QC efforts focus on a product-oriented environment in which analytical methods play key roles. In general terms, QC is a system of checks and measures to ensure that produced material meets certain pre-determined standards. Figure 9-1 illustrates that QC in biotechnology involves multifaceted groups that focus on many aspects of a manufactured product. Each group plays a different role in ensuring that each product is produced to a consistent, high level of quality.

Figure 9-1. The many functions of a QC department

Quality Control Responsibilities & Maintaining a State of Control

What are QC's responsibilities?

The primary responsibility of a Quality Control group is to provide scientific evidence to ensure that products are consistently produced to meet the required purity, safety, and efficacy standards. To meet this primary responsibility, Quality Control staff routinely performs the following activities:

- **Maintenance of the cGMP laboratory:** Maintaining a GMP laboratory involves the normal housekeeping activities that would pertain to any laboratory or even to one's home. It also includes ensuring that all equipment is within its calibrated use period; that all solutions, standards, and reagents are labeled, stored appropriately, and within their expiry period; and that all equipment is performing adequately.

- **QC laboratory documentation system:** The work of the QC laboratory involves an abundance of documentation, and various documents such as SOPs, forms, training records, etc., need to be periodically reviewed and updated.
- **Generating testing data and performing data trending**: This is the activity performed at the laboratory bench and includes adhering to SOPs by using calibrated and effectively functioning equipment and appropriate materials to obtain and evaluate results. These results are then reported and evaluated for trends.
- **Conducting QC related investigations**: If laboratory testing indicates a problem with a batch (or test results are unexpected), a formal investigation must be conducted that leads to appropriate corrective actions.

QC also contributes to other groups within an organization; there are few groups with which QC does not work closely. For example, QC works with the research and development groups to ensure methods are transferred and perform as expected. QC also provides data to manufacturing, regulatory affairs, and clinical operations groups for the support of the various information/documentation that these groups will submit for the company (e.g., product licensing applications, responses to queries from governmental agencies regarding the company's activities, etc.). QC also works with outside organizations to transfer methods and facilitate testing. These are only a few examples of the contributions of the QC group, a diverse group in both roles and responsibilities.

The need for a state of control

As outlined in the Introduction to the Quality Section, consumers usually assume the adequate quality of a medicinal product. In effect, one puts his or her trust in pharmaceutical companies and regulatory agencies to ensure that products are both safe and, especially in the case of pharmaceuticals, effective. The safety and effectiveness of all medicines, however, rely fundamentally on both the company having a thorough understanding of a product and the appropriate checks and measures in place to ensure the production of quality materials.

QC testing is a key aspect of the control of processes and procedures and helps to verify the continuity of this control. A lack of such control is a justifiable cause for regulatory agencies to investigate and potentially shut down an organization. One of QC's most important roles, therefore, is ensuring that all operations in the laboratory are carried out in accordance with the requirements of the applicable cGMPs, with a majority of the responsibility involving the management of data. The FDA regulations that provide general or minimum compliance requirements are listed in Table 9-1. For more information, refer to the FDA website *www.fda.gov*.

Table 9-1. Food and Drug Administration regulations

Regulation	Topic
21 CFR parts 210 and 211	Good Manufacturing Practices
21 CFR part 11	Information and Resources
21 CFR part 58	Good Laboratory Practices

In addition, the International Conference on Harmonization (ICH) has generated a series of applicable guidance topics that impact the Biopharmaceutical QC Laboratory's activities (Table 9-2).

Table 9-2. International Conference on Harmonization (ICH) Quality topics

Guidance	Topic
Q1A	Stability Testing of New Drug Substances and Products
Q1C	Guideline on Stability Testing for New Dosage Forms
Q2A	Guideline on Validation of Analytical Procedures: Definitions and Terminology
Q2B	Guideline on the Validation of Analytical Procedures: Methodology
Q3A	Guideline on Impurities in New Drug Substances
Q3B	Guideline on Impurities in New Drug Products
Q5A	Guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
Q5B	Final Guideline on Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products
Q5C	Final Guideline on Stability Testing of Biotechnological/Biological Products
Q5D	Guideline on Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products

Q7A	Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients

The FDA regulations and ICH guidelines shown in these tables determine how an organization sets up its own internal quality-based structures and policies. Although all organizations must meet the mandatory regulatory requirements, they can differ in their specific interpretations.

The reputation of an organization can be destroyed if it loses sight of the importance of quality control. An example involves the automotive industry. Failures in QC can lead to the production of vehicles that have potentially serious safety issues. As a result, an auto manufacturer could be forced to recall hundreds of thousands of vehicles, which can cost millions of dollars and potentially cause irreparable damage to its reputation. Ensuring that products are manufactured to a high level of quality takes constant vigilance; the slightest oversight can cause long term damage in the minds of the consumers and/or even result in injuries or death.

Maintaining a state of control

A vital method for maintaining a state of control involves operating under standard procedures. On any given day there are numerous activities occurring in a cGMP lab. For example, a typical QC Biochemistry lab contains numerous pieces of analytical equipment, buffer preparation areas, environmental chambers, chemical fume hoods, Biological Safety Cabinets (BSCs), and analysts. A fair amount of testing is performed individually; however, some activities (such as the preparation of buffers) are more general and can be used by numerous resources.

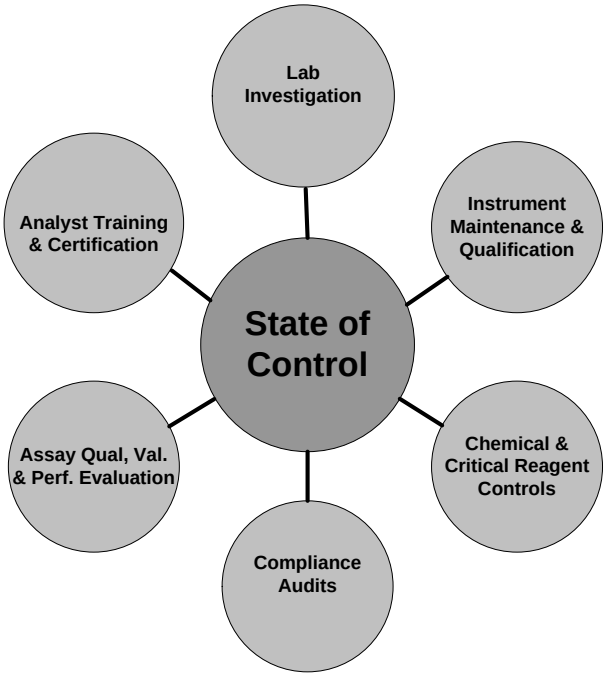

Figure 9-2. Components of the state of control

Numerous policies and procedures are established and documented to govern the work performed in a cGMP-compliant laboratory (figure 9-2), including:

- safety- and assay-specific training for lab workers
- instrument maintenance and qualification
- chemical and critical reagent controls
- internal audits
- lab investigations regarding assay failures (no-tests, out of trend and out of specification results)
- method qualification, validation, and performance evaluation

As the quality system is subject to inspection from both internal and external sources, strict adherence to documented policies and procedures is vital. It is important to remember that these policies and procedures, along with governmental rules and regulations, are instituted and designed to protect the consumer by ensuring that an organization has done everything in its power to consistently produce a safe and effective product.

The role of Quality Control Biochemistry

QC analyst skills and abilities

There are numerous disciplines applied to the QC of biopharmaceutical productions. A QC analyst can assume various roles throughout his or her career. As part of the QC team, a person can play a crucial role in the release of a product, the support of manufacturing groups, and the assessment of product stability. Individuals are expected to have a high level of integrity, attention to detail, trustworthiness, and reliability. Good communication skills are also necessary, as a QC analyst can interact with numerous internal and external organizations. These include, but are not limited to, metrology, validation, facilities, lab services, development laboratories, manufacturing, regulatory affairs, outside vendors/organizations, auditors, and regulatory agents.

Routine release and stability testing

QC is involved in a wide range of testing procedures, including a variety of process intermediates, the **Drug Substance (DS)**, the **Drug Product (DP)**, and samples from product stability studies.

Real-time testing of in-process material is often used to make quick process-related decisions. For example, some purification columns have limits to the amount of material that can be loaded onto them at one time. Therefore, a quick and reliable method is used to determine the concentration of the material to be added to a column to ensure that a column is not overloaded. Additional in-process methods are used to monitor the various steps of the biopharmaceutical manufacturing process. Regardless of the method utilized, all provide a better understanding of the process and allow for trending over time.

Process trending is an important tool, as it shows how a process performs over time. Figure 9-3 represents a Host Cell Protein (HCP) content in Column X over 25 runs. From run to run one

might not notice an upward trend in HCP content. By looking at the entire data set over time, however, one can see that the column is not clearing the HCP content as well as it did initially. Data like these can be useful in understanding the performance of a column over its lifetime.

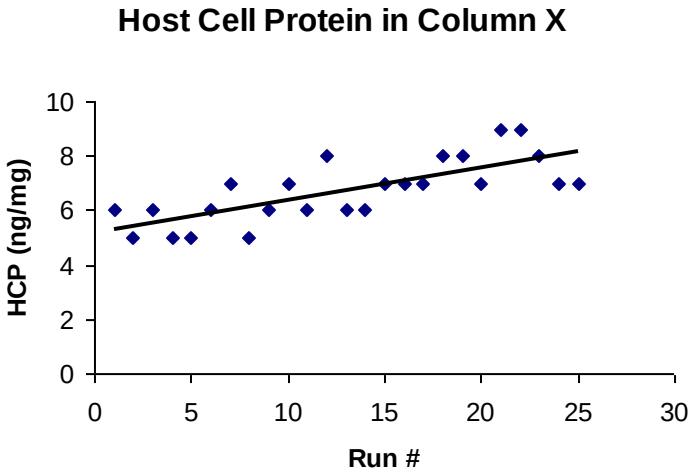

Figure 9-3. Trending graph

In addition to in-process testing, the evaluation of DS and DP is a primary responsibility of QC. These release tests are the last line of defense to ensure that a consistently-produced and safe drug product is delivered to patients. The methods are designed to assess the drug product from many different angles, such as purity, content, identification, impurity levels, and potency. Depending on the type and complexity of the product, additional characterizations and/or structure-indicating methods can be utilized as well. A later topic in this chapter will examine commonly used methods for in-process, release, and stability testing.

Investigations participation

Human error is an undeniable factor in process performance; it is even present in automated processes such as maintenance issues, ineffective monitoring, etc. Inevitably mistakes are made or process variations occur. QC plays a significant role in supporting investigations that review product quality issues/deviations. Various analytical methods are used to assess the impact when issues/deviations to an established process occur. An evaluation that assists in understanding the impact of the deviation on the product can keep batches from being discarded or prevent potentially unsafe products from reaching the consumer.

Basic Overview of a Quality Control Function

In biotechnology, as with most other manufacturing industries (everything from cars to electronics to chemicals), a Quality Control function is established and maintained to verify that products meet expected standards. These standards are typically documented in a **specification**. A later topic in the chapter addresses specifications.

Quality Control and Quality Management

Quality Control is part of:

- Quality Systems, specifically related to setting defined policies and procedures for routine operations
- Laboratory Control Systems, specifically related to executing and deriving testing data, conducting laboratory investigations, and maintaining the state of control of a lab
- Facility/Equipment Systems
- Packaging/Labeling Systems
- Production Systems
- Material Systems, specifically related to raw material controls

A Quality Management System, illustrated in Figure 9-4, ensures that an emphasis on quality is an integral part of all biotechnology manufacturing operations, including engineering, execution, and measurements. Quality Control is an administrative function closely associated with the Quality Management hierarchy in the biotechnology industry.

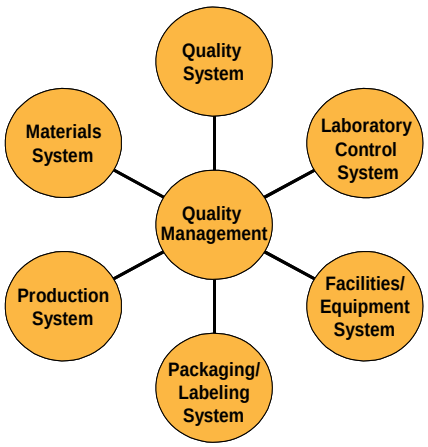

Figure 9-4. Quality Management System

Specifications

As outlined in the overview to this unit, all QC tests have an associated specification or set of limits that allow the analyst to determine if the result obtained for that test meets

"specification" or is outside of "spec." Generally when a result fails the specification it is unexpected; thus the reason for the failure must be thoroughly investigated. When a product lot meets the specification for all the associated tests, the batch "passes" and can be released (distributed to patients). Thus specifications are of great importance in the QC laboratory and drive much of the activity within.

A specification is also used to refer to a document that lists all the tests, respective limits, or acceptance criteria for a product. For biotechnology-produced products, these specifications include:

- raw materials specifications to control the quality of the incoming raw materials
- in-process specifications to control the quality of the in-process intermediates
- release specifications to control the quality of both the **Active Pharmaceutical Ingredients** (API—commonly referred to as drug substance) and the final drug products

The development of a product specification is integrated with the product development. All testing measurements are developed based on scientific principles and the product's intended therapeutical use. There are also regulatory requirements for product quality and stability that must be verified. All testing measurements, therefore, are designed to provide physical, chemical, biological, microbiological, and other related data to demonstrate the product's purity, efficacy, safety, and stability. See Tables 9-3 and 9-4 for hypothetical specifications for the fictitious monoclonal antibody drug substance *WondermAb* and the associated final drug product *WonderProd*.

Table 9-3. Mock drug substance specification for WondermAb

Product Code:	**Storage Conditions:**
MAB001	≤ -65° C

Test	Analytical Method	**Acceptance Criteria**	**Amount Required/ Testing Laboratory**
Appearance	Visual Inspection (Color, Ph. Eur. Section 2.2.2) and Ratio Turbidimetry (Clarity and Degree of Opalescence, Ph. Eur. Section 2.2.1)	Description of color, clarity and detection of particulate matter	mL/Quality Control Biochemistry
pH	pH Electrode (USP <791>)	pH value	
Osmolality	Freezing Point Depression (USP <785>)	mOsm/kg	
Identity	ELISA	Identity confirmed	
Potency	Bioassay	% Relative potency	
Protein Concentration	Concentration by A_{280}	Result in mg/mL	
Purity	SDS-PAGE or CGE	% Purity	
Purity	SEC-HPLC	% Purity Main Peak % Aggregate	
Charge Heterogeneity	IE-HPLC	% Main Peak % Acidic Peaks % Basic Peaks	
Bacterial Endotoxin	Kinetic Turbidimetric (USP <85>)	≤ XXX EU/mg	mL/Quality Control Microbiology
Bioburden	Microbial Plate Count (Membrane Filtration, USP <61>)	≤ XX CFU/XX mL	mL/Quality Control Microbiology
Relevant residual contents	DNA, HCP, or other residual proteins	< a limit	Appropriate quantity

Table 9-4. Mock drug product specification for WonderProd

Product Code	**Storage Conditions**
MAB001	≤ -65° C

Test	**Analytical Method**	**Acceptance Criteria**	**Amount Required/ Testing Laboratory**
Appearance	Visual Inspection (Color, Ph. Eur. Section 2.2.2) and Ratio Turbidimetry (Clarity and Degree of Opalescence, Ph. Eur. Section 2.2.1)	Description of color, clarity and detection of particulate matter	mL/Quality Control Biochemistry
pH	pH Electrode (USP <791>)	pH value	
Osmolality	Freezing Point (USP <785>)	mOsm/kg	
Identity	ELISA	Identity confirmed	
Potency	Bioassay	% Relative potency	
Protein Concentration	Concentration by A_{280}	Result in mg/mL	
Purity	SDS-PAGE or CGE	% Purity	
Purity	SEC-HPLC	% Purity Main Peak % Aggregate	
Charge Heterogeneity	IE-HPLC	% Main Peak % Acidic Peaks % Basic Peaks	
Bacterial Endotoxin	Kinetic Turbidimetric (USP <85>)	≤ XXX EU/mg	mL/Quality Control Microbiology
Bioburden	Microbial Plate Count (Membrane Filtration, USP <61>)	≤ XX CFU/XX mL	mL/Quality Control Microbiology

Analytical methods

An analytical method is a laboratory procedure used to measure a chemical or biological entity or product attribute to verify its structural quality, purity, biological activity, and composition in the formulation. In general terms, analytics involves separating something into its basic components and comparing it against a yardstick or reference. Monoclonal antibodies, for example, are highly complex, and no single analytical measurement will provided all of the information about the molecule. The application of different analytical methods in this example act as the pieces of a puzzle, with each analysis helping to piece together a greater understanding of the molecule or overall picture.

Lifecycle of an analytical method

All analytical methods have a lifecycle, which typically begins in Development and continues through Validation and routine use. As the product goes through clinical development, the expectation for process development and product knowledge increases. Therefore, the requirements for scientific rigor, robustness of the method, and compliance increase in importance. The evaluation of the method does not stop once it is validated, however. Analytical methods are constantly assessed for performance post-validation and are performed consistently by following documented Standard Operating Procedures.

All methods used in QC are developed and implemented through an evolutionary process, and QC works closely with the development professionals during development. The method development process runs nearly parallel to the product development process, ideally running slightly ahead of process development so that all the necessary analytical measurements can be provided to support the process development.

This section describes the expectations of a cGMP method throughout its lifecycle and explores how a method lifecycle (Figure 9-5) corresponds to a product lifecycle.

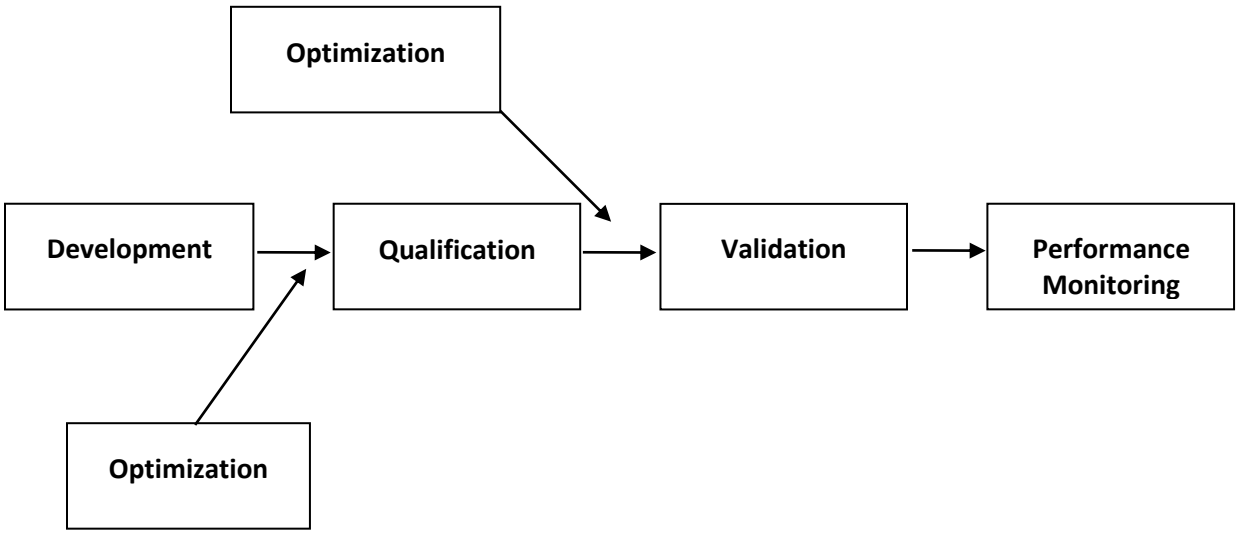

Figure 9-5: Lifecycle of a method

Method development

Analytical method development is a process that generates a sound analytical measurement procedure to verify product quality, purity, or biological activity using scientific principles. The method development must create a measurement scheme that can consistently generate a measured and reliably reproduced result.

Method qualification

Analytical method qualification is a set of experimental studies demonstrating that an analytical method performs as expected, providing consistent and meaningful data under a defined set of conditions.

Method validation

Analytical method validation creates documented evidence that provides a high degree of assurance that a specific analytical method will consistently produce data meeting appropriate analytical performance parameters as they apply to the intended use of the method. These parameters can include accuracy, specificity, reproducibility, precision, **Limit Of Detection** (LOD) and **Limit Of Quantitation** (LOQ), linearity, range, and robustness. Each validation must be executed according to the respective approved validation protocols and predetermined acceptance criteria for each performance parameter.

Types of analytical methods

Analytical methods encompass a variety of functions (Figure 9-6), which vary based on their intended use and technique. Certain methods are required by regulatory agencies to approve

the release of a drug product; the requisite analyses include determination of identity, purity, and potency (bioactivity) and an impurities evaluation.

The characterization method and scientifically sound analytical test method are used to evaluate a specific quality attribute and are vital for both product understanding and the submittal of a Biological Licensing Agreement (BLA). They are not typically required for a product lot release; however, depending on the complexity of a molecule, characterization methods may be deemed necessary.

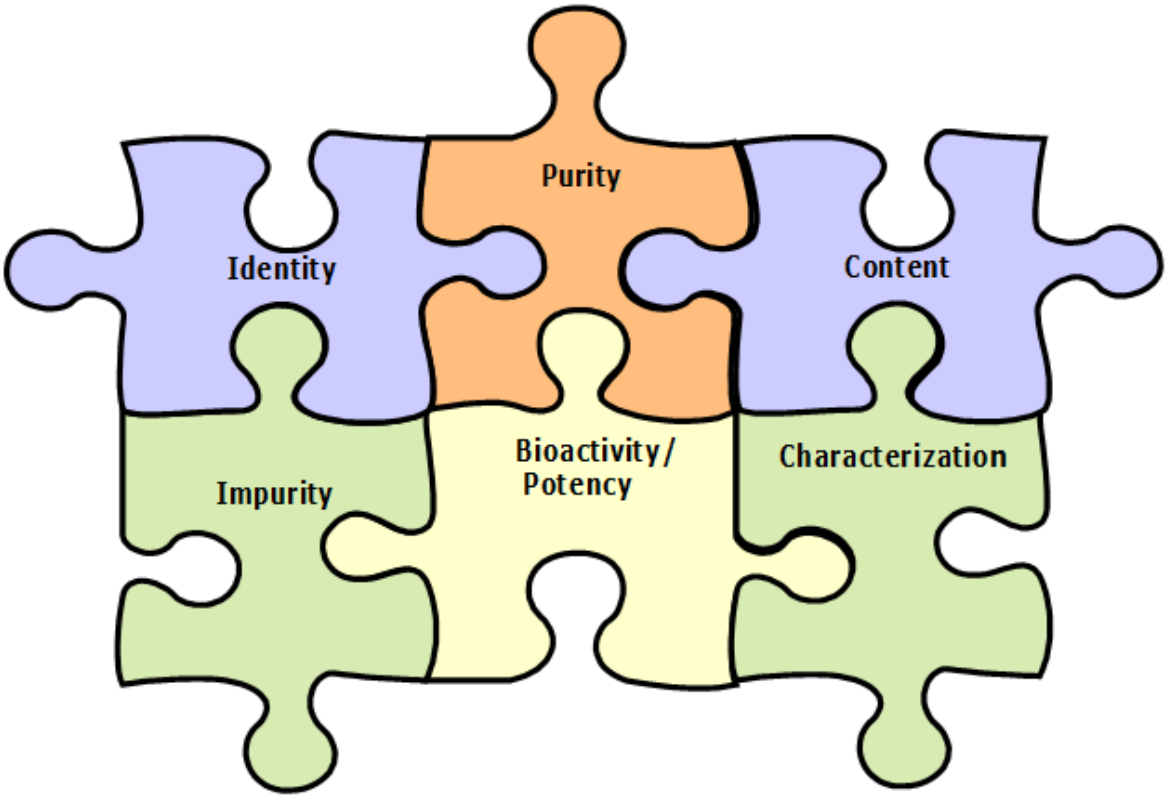

Figure 9-6. Types of methods used to verify the quality, activity, and safety of a product

Some methods assess the product itself, whereas others focus on the matrix or the chemical environment of the product. A broad understanding of both the chemical nature and biological activity of the molecule to be used as a therapeutic, along with its environment, are valuable and necessary.

The following section will examine some of the common methods used in a QC biochemistry lab, including those that are necessary for the release of a final drug product; the ones that are commonly used for the assessment of product impurities and residuals; and stability indicating methods and additional characterization methods (which can be part of a specification depending on the complexity of the protein therapeutic). These methods are fairly standard for the release of a biopharmaceutical-produced monoclonal antibody DS; however, they are

constantly advancing as knowledge in the area of science increases and new analytical techniques are developed. Thus industry standards may change with the adoption of new and/or more advanced methods and procedures.

Compendial test methods

Compendial test methods are standards found throughout the pharmaceutical industry that are published as monographs in pharmacopeia, with the three most commonly used being the United States Pharmacopeia (USP), European Pharmacopoeia (EP), and Japanese Pharmacopeia (JP). Compendial methods include such tests as:

- pH
- osmolality
- appearance (visual inspection)
- content uniformity
- bacterial endotoxin
- subvisible particulate matter
- sterility
- residual moisture content

pH

The measurement of pH follows the USP compendial method <791>. A pH meter is used with National Institute of Standards and Technology (NIST) pH control standards. Typically a pH meter calibration is performed using several standards at a variety of pH values above and below the expected sample's pH. A control standard is then tested to ensure the pH meter is functioning as expected. At this point the pH of the product can be assessed with a high degree of certainty that the pH value provided is appropriate.

Osmolality

Osmolality is defined as the number of osmoles of solute particle per kilogram of pure solvent and is represented in milliosmoles per kilogram (mOsm/kg). An osmometer typically uses freezing point depression to measure osmolality; and the instrument is calibrated and verified using NIST standards. The measurement of Osmolality follows USP <785>.

Appearance (visual inspection)

Appearance (visual inspection) is a qualitative test that assesses color, clarity, and visible particulate matter (Figure 9-7). Along with visually inspecting a vial of product, a turbidimeter, which measures light scattering, can be used to assess the clarity of a compound. Visual inspection or appearance uses the EP Method, section 2.2.1 and 2.2.2.

Figure 9-7. Examples of visual inspection: Fully intact cake, broken cake, and collapsed cake

Content uniformity

Content uniformity is assessed according to USP <905> using 10 random FDP vials. The vials are reconstituted and the concentration for each vial is determined. Concentration differences between the label claim and determined concentration is calculated.

Bacterial endotoxin

The amount of endotoxin in samples is determined according to both USP <85> and Ph. Eur. Section 2.6.14 using an endotoxin analysis system.

Subvisible Particulate Matter

Subvisible particulate matter is assessed for final drug products according to both USP <788> and Ph. Eur. Section 2.9.19 using a light obscuration particle count test.

Sterility

The sterility of the final drug and placebo products is determined according to both USP <71> and Ph. Eur. Section 2.6.1., ensuring there is no growth of bacteria or fungi.

Residual moisture content

Residual moisture content is calculated based on the colorimetric detection of water through the Karl Fischer reaction. The basic reaction consists of an iodide-containing analyte reacting with water to form iodine. When all of the water has been consumed in the titration cell, an excess of iodine is produced and is detected electrochemically, which in turn signals the titration's completion or endpoint.

Non-compendial test methods

Non-compendial methods (i.e. those that do not follow a pharmacopeia) are often developed in-house and are typically specific to a particular product. They do not follow any standards provided by published pharmacopeia.

Identity

Identity methods are used to confirm the presence of what has been defined as the active product ingredient. These methods use the product's unique characteristics to distinguish it from other material. The common identity methods are:

- identity detection using antibody and antigen interaction via **Enzyme-Linked Immunosorbent Assay** (**ELISA**)
- identity detection through the display of unique charge heterogeneity using ion exchange chromatography
- identity detection through the display of the unique peptide fingerprint

Potency

Potency of a biopharmaceutical is demonstrated by its *in vitro* or *in vivo* biological activity. The mechanism of the biological activity measurement is usually related to how the molecule works in the human body. Most of the potency assays for biologics are cell-based assays. In rare cases they involve testing in animals, such as mice or rabbits. The *in vitro* assay explores a dose-response relationship in which the concentration of the biologic is compared against its biological functions in the selected cells. These functions are usually reflected by signals in cell proliferation enhancement or inhibition, cytotoxicity, apoptosis, elevated or decreased enzyme or protein expressions, etc. Figure 9-8 illustrates a typical potency assay dose-response curve.

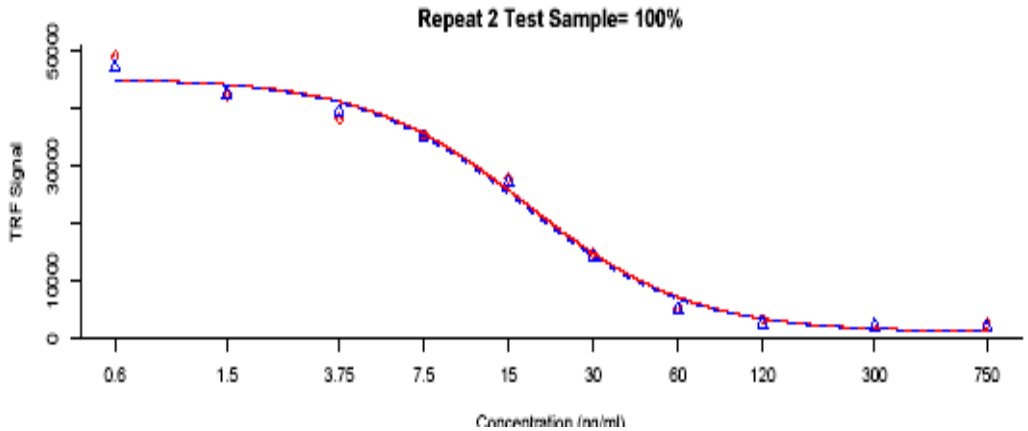

Figure 9-8. Potency assay dose-response curve

Content

The content method is vital to the manufacturing process, as it facilitates all additional testing that is dependent on concentration. Additionally, it is used to assess whether a drug product is at the correct concentration for clinical dosage. Content can be determined using a chromatography method or using protein concentration determination.

Affinity chromatography

Affinity chromatography uses the binding affinity of a protein to a specific gel matrix to separate it from other substances/contaminants. This method is commonly used in purification but requires considerable knowledge of the molecule to be purified (i.e., to what it will bind).

In an analytical lab, affinity chromatography is typically used to purify upstream or "dirty" samples. In this application, material produced from the cell culture process is loaded into the first of several possible purification columns. For the column chromatography process to work effectively and to determine the overall yield of the production process, it is important to know the concentration of the material loaded into the column. Cell culture material, however, usually contains a variety of interfering species that prohibit the use of assays such as concentration by A_{280}. Affinity chromatography is a commonly used method for the assessment of content in upstream samples.

Size-exclusion chromatography (SEC)

Size-exclusion chromatography, or SEC, is also known as gel filtration chromatography and separates molecules based on their size. An SEC column contains particles (resin beads) which have circuitous channels of a defined diameter or pore size passing through them. As a mixture of proteins of various sizes moves through the column, larger proteins move directly through the column by traveling around the resin beads, whereas smaller proteins are forced to travel through a maze of pores within the chromatography resin beads. The general result is that the larger proteins travel faster through the column than smaller ones. As such, SEC can be used to determine the molecular weight of a protein and resolve size-based variants such as aggregates and fragments. Along with being used to purify a product, SEC can also be used to evaluate the stability of a protein when protein aggregates and fragments could result from protein degradation. Figure 9-9 illustrates a mixture of proteins separated by size exclusion column chromatography and taking the absorbance at 280 nm.

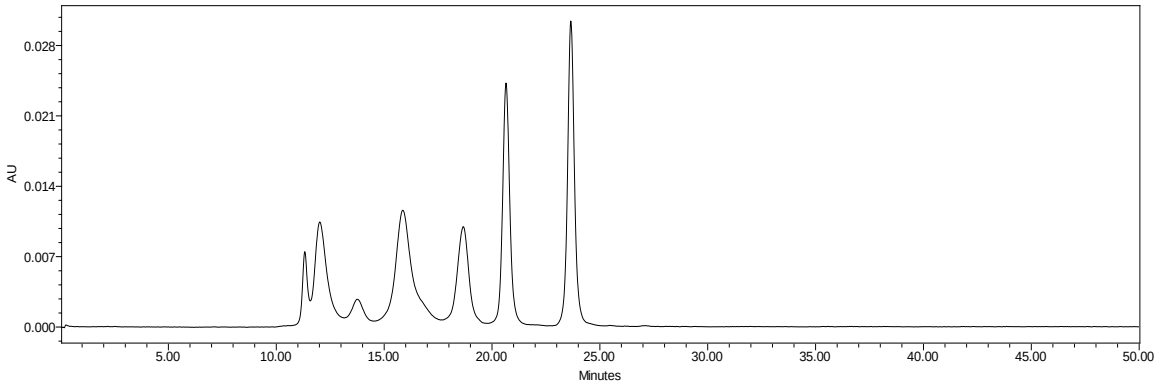

Figure 9-9. A protein mixture separated by size exclusion chromatography

Protein Concentration Determination

The light absorption of protein is strongest between 200 and 300 nm, and the aromatic side chains of certain amino acids show the absorption maximum between 270 and 295 nm. Thus 280 nm is typically used to measure the absorbance of a protein and is a commonly used

method for the determination of protein concentration. The absorbance of the protein depends on the molar absorptivity (extinction coefficient), which is affected by several factors, including:

- the number of aromatic amino acid residues
- the number of disulfide bonds
- the specific microenvironment of the light-absorbing residues

The determination of protein concentration by absorbance at 280 nm is based on the Beer Lambert Law ($A = \varepsilon \times L \times C$). By measuring the absorbance of protein (A) with a known path length (L) and a known molar absorptivity (ε) specific to the protein, the concentration (C) of the protein can be determined. To measure absorbance, a beam of light with intensity I_0 is aimed at a cuvette containing the tested solution. The intensities of the entering beam (I_0) and the emerging beam (I_1) are measured, and the absorbance (A) is calculated from the ratio of the two.

Concentration determination by A280 is a quick and straightforward way to find the concentration of a protein; can be used in many different matrices; and is a useful tool at most every step in a process.

Purity

The purity of an active product ingredient can be assessed by a variety of analytical techniques. The results of purity analyses are impacted by the sensitivity and specificity of the assay itself, so several methods are typically used in determining the product's purity.

Due to the complex molecular characteristics of biotechnology-produced drug products, the drug of interest can potentially include other molecular entities and/or variants. Such variants are included as part of the drug product when they are derived from post-translational modifications. When variants are created during the manufacturing process and have properties that are similar to the desired therapeutic, they are not considered impurities but product-related substances. Commonly used methods for evaluating product purity include high performance liquid chromatography (HPLC) and electrophoresis.

High Performance Liquid Chromatography (HPLC)

HPLC is one of the most commonly-used and most versatile procedures in analytical labs. Similar to low pressure liquid chromatography, HPLC makes use of resin-filled columns to separate molecular components such as proteins from one another. The column is contained within a narrow metal tube containing a stationary phase, or resin, that can separate and purify proteins from other molecules based on inherent molecular characteristics such as size, charge, ligand affinity, hydrophobicity, etc. A high pressure pump moves a mobile phase containing the sample molecules through the column, resulting in their separation. A detector then analyzes the sample as it leaves the column. Detectors come in a variety of packages depending on how the molecule must be evaluated. Since most compounds absorb light at different wavelengths, HPLC units are also frequently equipped with a UV/Vis spectrophotometric detector.

One of the characteristics of HPLC assays is that they are relatively hands-off once the appropriate run parameters have been validated. Following are the basic steps for the analyst:

1. prepare mobile phases, samples, and reference material
2. connect the selected column by attaching it to the HPLC instrument
3. condition (prepare) the column by running the mobile phase buffers through the column prior to the application of samples
4. add (inject) the material to be analyzed into the column
5. press "go"

At this point the analytical software of the instrument processes the data.

A variety of distinguishing chromatographic test methods are utilized to analyze the preparation, with each method providing a different piece of the aforementioned puzzle—that is to say the various HPLC methods can also evaluate purity, heterogeneity, and amount of protein in the preparation.

Polyacrylamide Gel Electrophoresis

Polyacrylamide Gel Electrophoresis (PAGE) involves the migration of charged particles (e.g., proteins) through a gelatinous matrix via an electric field. The gel matrix, which can be cylindrical or (more commonly) cast as slabs, serves as a "molecular sieve," with smaller molecules generally migrating through the gel more rapidly than the larger ones. Molecules of the same species, or at least of the same size, move through the gel matrix and form discrete bands on the gel. The position of each band can be compared with the position of protein standards of known sizes that have been run on the gel concurrently but in separate lanes. In this manner the relative molecular weight of each band on the gel can be ascertained. Depending upon experimental conditions and procedures, gel electrophoresis analyses may be either qualitative or quantitative.

Prior to electrophoresis of proteins, samples are usually treated with Sodium Dodecyl Sulfate (SDS). SDS is a negatively-charged detergent used in commercial laundry detergents, toothpaste, bubble baths, degreasers, etc., and binds very effectively to proteins; the binding of SDS is usually facilitated by heating the samples prior to applying them to a gel. The binding of SDS to proteins causes proteins to stretch out (denature) and assume ellipsoid shapes (Figure 9-10). As a result, the proteins assume a net negative charge that is proportional to their length. The resulting charge-size proportionality allows proteins of different size to be pulled through the gel matrix at different rates by an electrical current, thus separating one protein from another into discrete bands within the gel. The location of protein bands can be determined by a variety of techniques.

It is important to note that the rate at which proteins migrate through the gel is dependent upon the size, shape, and net charge of the molecule; therefore, it is possible for several different proteins to migrate within a single band on a gel. Thus a single band on a gel does not necessarily mean that the sample contains only a single, pure protein. Additional testing should be performed if SDS-PAGE is being used to evaluate product purity. On the other hand, when

evaluating a product of known purity, multiple protein bands on a gel may indicate the presence of structural variants and fragments.

SDS-PAGE can be performed under both non-reducing and reducing conditions. The addition of a reducing agent, such as mercaptoethanol or dithiothreitol, causes the reduction of disulfide bonds, therefore facilitating denaturing of the protein. Solubilization with SDS and reduction of disulfide bonds by low MW thiols enhances the ability to visualize certain size variants.

Figure 9-10. Size separation by SDS-PAGE

Capillary Gel Electrophoresis

Capillary Gel Electrophoresis (CGE) was developed in recent years due to some of the limitations with traditional slab gel SDS PAGE. CGE is similar to SDS-PAGE, as proteins are denatured (to some extent) with SDS. As an electric current pulls them through a gel-sieving matrix coating the capillary wall, they are separated based on their size. The use of capillaries has a number of significant advantages over SDS-PAGE—separations are faster, more efficient, and provide better resolution.

Capillary gels can be run at much higher voltages due to their ability to better dissipate heat, while slab gels tend to build up extra heat which is detrimental to separation. Running capillary gels at a higher voltage increases the resolution of separation, yielding more interpretable and reproducible results. Another significant advantage of CGE over traditional slab gel analysis is automation of the separation and data analysis due to the use of both an autosampler and in-line UV/Protein DNA Analyzer (PDA) detection. Capillary electrophoresis has numerous applications, and as with an HPLC, different detectors can be used to perform a variety of analyses.

Ion-Exchange Chromatography (IEC)

Assessing the heterogeneity of product is another means of assessing quality. Therefore, it is important to understand a product's heterogeneity profile and assess it for lot-to-lot consistency; this can be done using ion-exchange chromatography. Ion-Exchange Chromatography (IEC) can be performed by Low Pressure Liquid Chromatography (LPLC) and High Pressure Liquid Chromatography (HPLC) and involves a protein's net electric charge and the relative local charge of the stationary phase. A column can serve either as an anionic exchanger or a cationic exchanger depending upon the chosen stationary phase. Anionic exchange columns have cations (positive charged groups) covalently attached to the polymeric beads of the stationary phase. Conversely, cationic exchange columns have anionic groups (negatively charged groups) covalently bound to the polymeric beads of the stationary phase. When a protein sample with a strong net positive charge is added to an anionic exchange column, it moves faster through the column (because it is repelled by the positive charge on the polymer beads). Concerning a protein with a negative charge, it will be attracted to the positive charge associated with the resin and thus move through the column more slowly.

IEC is used to assess product purity as well as acidic and basic variants. Therefore, it is often used as a release and stability indicating method, a quantitative test method used to detect impurities. IEC can also be utilized as an identity method, as charge profiles tend to be unique.

Impurity/Residual (contamination) tests

Impurities in a bulk drug product can be either product- or process-related. According to ICH Q6B, product-related impurities are "molecular variants with properties different from those of a desired product formed during manufacture and/or storage."

Process-related impurities in a DS can originate from various sources. ICH Q6B states that these sources include "cell culture media, host cell proteins, DNA, monoclonal antibodies, or chromatographic media used in purification, solvents, and buffer components." ICH Q6B further states that "these impurities should be minimized by the use of appropriate, well-controlled manufacturing processes." The concern with process-related impurities arises since they may present a safety concern for patients.

Host Cell Protein (HCP)

Purified therapeutic biopharmaceutical drug products derived from recombinant DNA technology have the potential to contain host cell impurities, such as Host Cell Proteins (HCPs), DNA, and other cellular constituents. Proteins that are derived from the host cell line may be present in pharmaceutical products manufactured by recombinant DNA technologies. These proteins can pose a safety concern for patients, potentially causing allergic or toxic reactions. HCP is evaluated not only to assess the final product purity but also as a decision-making tool for process development activities.

Widely-used methods to quantify HCP impurities are immunoassays such as an ELISA and Western Blot analysis. These techniques require antibodies raised against an HCP antigen. The antigen mixtures used to generate antibodies are obtained from the cell line used for protein expression.

Residual Host Cell DNA

In addition to HCP, residual host cell DNA can be present in a purified monoclonal antibody therapeutic. The presence of DNA represents a process-related impurity. The FDA and the World Health Organization (WHO) require that such impurities be monitored and checked against product release limits for patient safety.

Residual DNA is typically measured via a limits assay to ensure that it is either at or below a certain amount. Typically DNA is removed throughout the process, so an assay sensitive enough to detect DNA at low levels is required. Methods exist that measure DNA or specific host cell line DNA and relate the total to the host cell line DNA present in high protein-containing samples. Each method has its advantages with respect to: (1) acceptance by regulatory agencies; (2) sensitivity; (3) reliability of the method; (4) throughput; and (5) cost.

Quantitative Polymerase Chain Reaction (qPCR), or real-time PCR, is a technique that is quickly becoming an industry standard for quantifying residual DNA. DNA is initially isolated from protein samples then combined with a probe, primer, polymerase, and reaction buffer in a 96 well plate. The plate is then placed in a qPCR instrument and run through numerous cycles of denaturation, annealing, and elongation. While PCR measures the end reaction, qPCR measures fluorescence while the reaction progresses, yielding faster and more sensitive results.

Stability-indicating methods

Stability-indicating methods should be sufficiently specific to differentiate between unaltered drug and any possible degradation/decomposition products. The method should be specific enough to determine the level of active, molecularly-intact ingredients in the presence of other substances such as closely related drugs, intermediates, impurities, degradation or by-products, or the drug preparation excipients, all of which could reasonably be expected to mask or simulate the analytical behavior of the ingredient. The specificity of these methods must be demonstrated in assay validation prior to use for routine stability testing.

Characterization

Characterization methods can be qualitative, quantitative, or semi-quantitative and are used to support a variety of aspects when a manufacturing process is being created. For example, characterization methods can be used when a detailed understanding of a molecule is needed. They can also be used for comparability, process validation, process characterization, qualification of a reference standard, and formulation or development. These methods are not typically used for release or stability testing; however, depending on the complexity of the protein, they may be required for release of the product. Following are a few of the more common characterization methods used for product characterization and, at times, release of a drug.

Carbohydrate Profile

Carbohydrate analysis of a protein is an important tool in characterization. Changes in the glycan (oligosaccharide) structure of a post-translationally modified protein can have a variety of implications with respect to potency, rate of clearance, bioactivity, stability, and immunogenicity. Capillary Electrophoresis with Laser-Induced Fluorescence (CE-LIF) is one

method used to perform carbohydrate analysis. CE-LIF is run using a Capillary Electrophoresis system, similar to CGE but with a Laser Induced Fluorescence (LIF) detector. An antibody is first digested through use of a proteolytic enzyme. The released glycans are then labeled with a charged fluorophore and separated through a capillary using an electric field. Glycans are separated based on their hydrodynamic size and relative charge. **Peptide mapping**

Peptide mapping can be used as an additional identity test for a protein of interest. The method that is developed is specific to the protein of interest and the result is considered a "fingerprint" of the protein. Digested protein peptides are isolated via HPLC and compared against a reference material. The pattern of the peptide fragments from a protein molecule and its reference material are compared and used to generate a "fingerprinting" profile. Peptide mapping can identify single amino acid changes and thus when compared to a reference standard, shows any alterations in primary structure that would indicate the material is unacceptable.

Phases of Methods (Development, Qualification, and Validation/Post Validation)

The intended use of a given method dictates the pathway it will take through its lifecycle; this is a vital concept to understand. As stated previously, methods support a variety of functions in the development of a product. Methods for cGMP testing will be used for the execution of batch records; compared to a specification for lot release; or performed per a stability monitoring protocol. Therefore, the expectation is that they will be formally validated and monitored to ensure they are performing as expected. On the other hand, methods for characterization are designed for better product understanding and to support the development and manufacture of clinical supplies. The major difference between these two method examples is that characterization methods are not typically required for batch release or stability testing. They are, however, used to support activities where a detailed product understanding is required, including, but not limited to, comparability, process characterization, process validation, reference standard qualification, and formulation development.

Table 9-5. cGMP method requirements compared to characterization methods

Methods	Development	Qualification	Validation
cGMP	X	X	X
Characterization	X	X	NA

The development of a method begins with understanding its intended use. What purpose will the method serve as it moves with the lifecycle of a product? Will it be used for batch release or detailed product understanding during process and product development? Once the intention of the method is established, the progression of its lifecycle can begin. It is important to note

that the intended use of a method can change depending on the complexity of the molecule as well as feedback from regulatory agencies. Although it is situation-dependent, methods can move on and off product specifications if the need permits.

Methods used to support products in early phase clinical development are primarily focused on safety and efficacy, thus they should be scientifically sound, reliable, and appropriate for their intended use. Methods to support cGMP testing for late phase and commercial material will be held to a higher standard than a cGMP method for a Phase 1 product (Figure 9-11). The reason for this is that when a product is considered commercial, there should be a thorough understanding of that product and its process. Control is of utmost importance, thus consistency in the process and resulting product are vital.

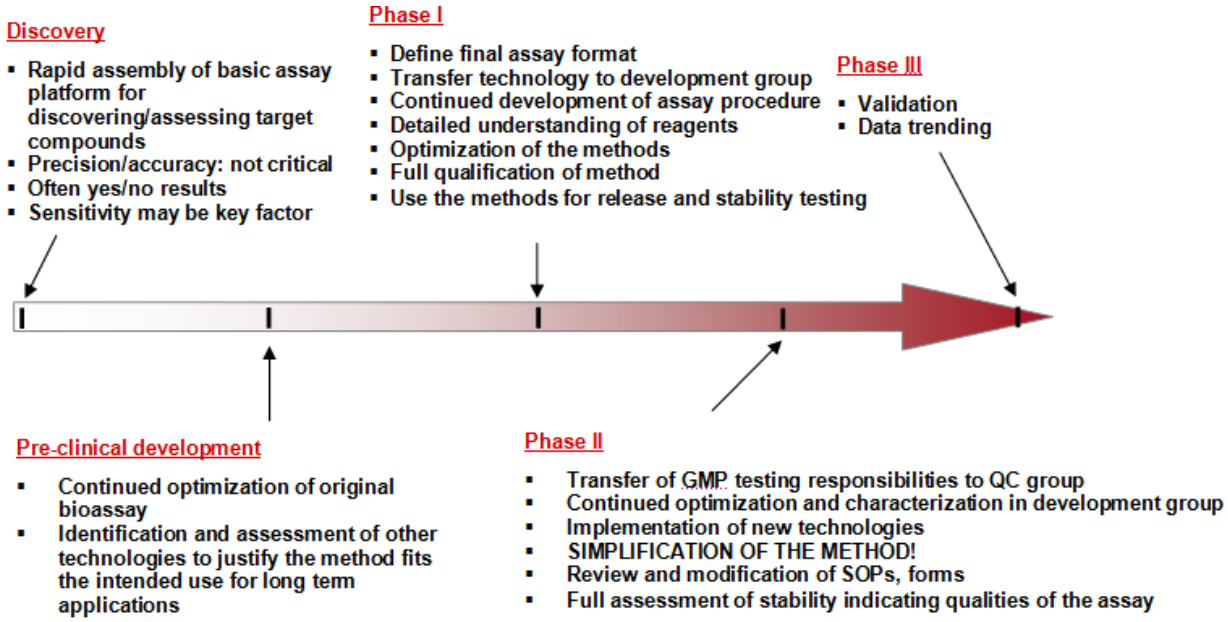

Figure 9-11. The evolution of the QC methods during the lifecycle of a biotechnology product

Data Trending

Statistics are used extensively in QC laboratories. The data generated for each batch or stability study are cumulatively trended. Data-trending provides information such as the consistency performance of the assays, the consistency of production process, the changes on the characteristics at molecular structural state (e.g., forms of oxidation, deamidation, and aggregations), and the changes in biological activities. Data-trending also provides numerical values for acceptance criteria placed in product specifications. Figure 9-12 illustrates an example of a trending chart.

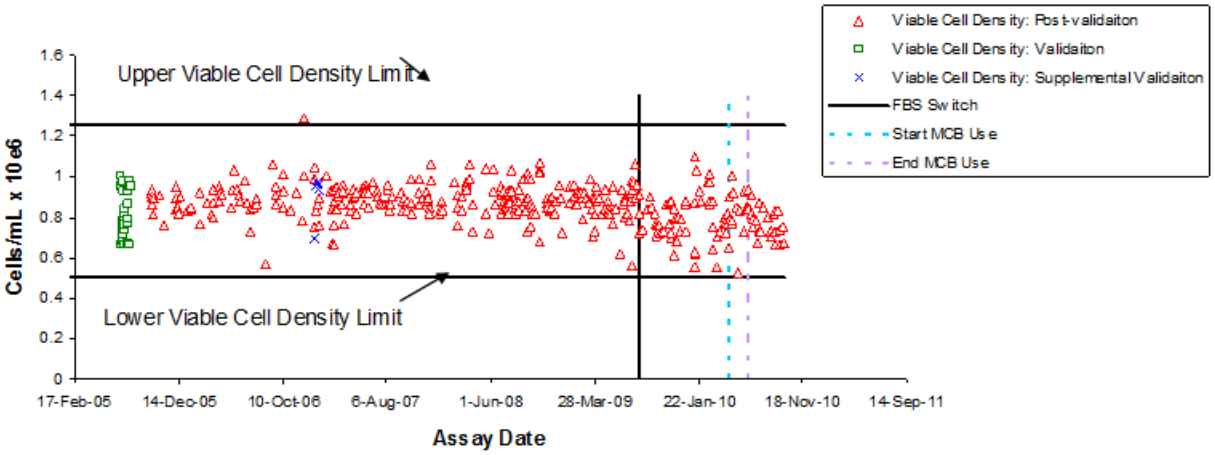

Figure 9-12. Trending of cell viability across different assay runs over time

Check Your Knowledge

1. The 6 components that assure a constant state of control include: _____, _____, _____, _____, _____, _____, and _____.
2. Stability testing assures quality of the product through:

   a) shipment
   b) production processing
   c) packaging
   d) storage
   e) all of the above
3. Release-testing is performed to assure that a consistent, safe, and efficacious product is delivered to patients. a. True b. False
4. What is the difference between a product contaminant and a product impurity? _____.
5. Analytical methods are assessed once in their lifecycle for performance.
   a. True b. False
6. Optimization of an analytical method is performed during validation.
   a. True b. False

Activities

1. In your lab/production environment, describe all the quality systems that are in place, their function, and their purpose. Write a 2–3 page report on your findings.
2. Pick a situation from daily life, monitor it, and plot the data. From the data, develop a trend chart, standard deviation, mean, etc. An example would be the price of gas over time.
3. For a situation from everyday life, identify the quality controls that are used daily to assure the outcome (e.g., preparing dinner, growing plants, etc.). Present a report to your class.
4. Research a current event where a product contaminant posed significant patient impact. Describe the event, its cause, and possible paths to prevent it from recurring. Discuss your information with the class.