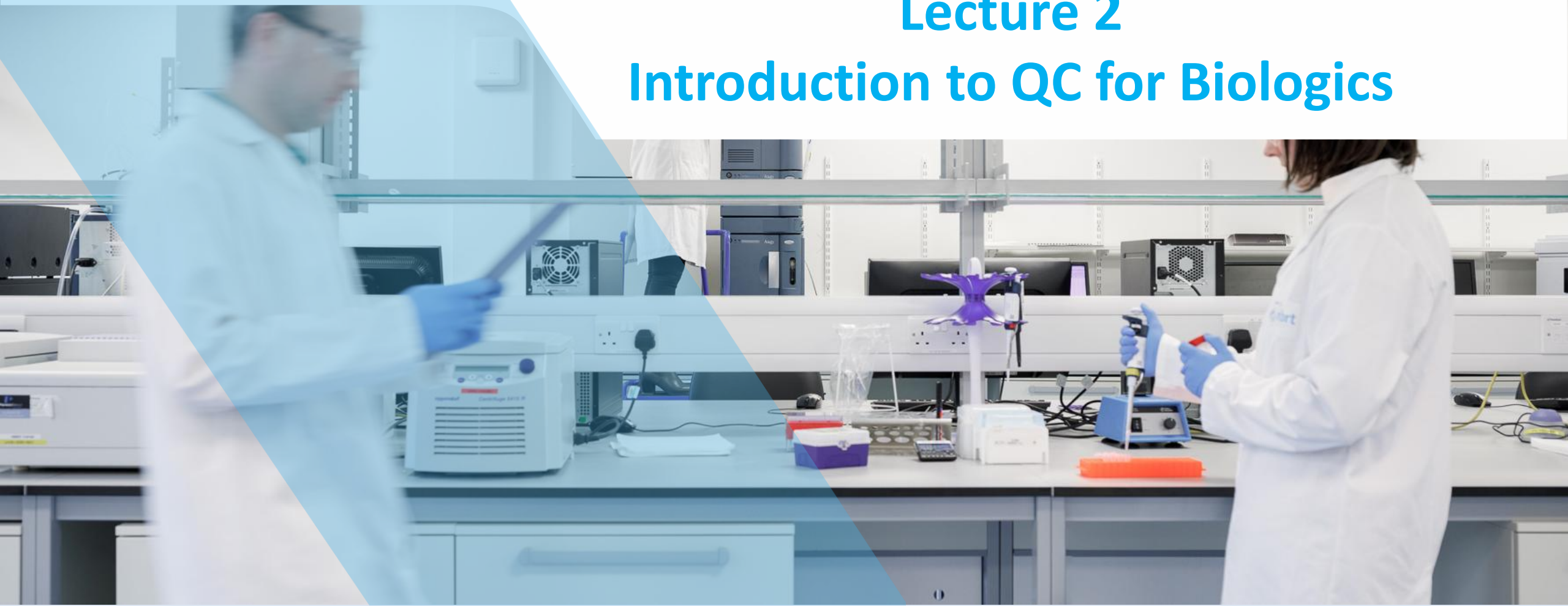
nibrt
National Institute for Bioprocessing Research and Training

Bioanalytical Techniques

Lecture 2

Introduction to QC for Biologics



Reminder

- Have a look at the project titles and put your name down for your preferred project by **12th February**

Learning Objectives

- Discuss the **complexity of biologics testing.**
- Describe the **different types** of biologics testing; product characterisation, stability testing, in-process and product release testing.
- List **critical quality attributes** of biologics
- Identify **key ICH guidelines** for the quality control of biologics

Topics

- Complexity of Biologics
- Introduction to QC Testing
- Key ICH Guidelines

What are Biopharmaceuticals?

- **Biopharmaceuticals** are medicinal products derived from **biological sources**
  - E.g. genetically engineered cells

- **Bioprocessing** is the manufacturing process for biopharmaceuticals

A World of Complexity

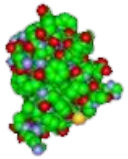
Traditional Medicines

Aspirin
(180.2 Da)

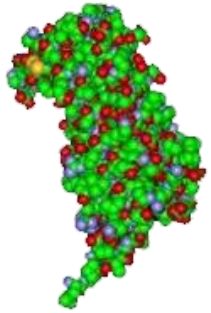
Atorvastatin
(558.6 Da)

Ibuprofen
(206.3 Da)

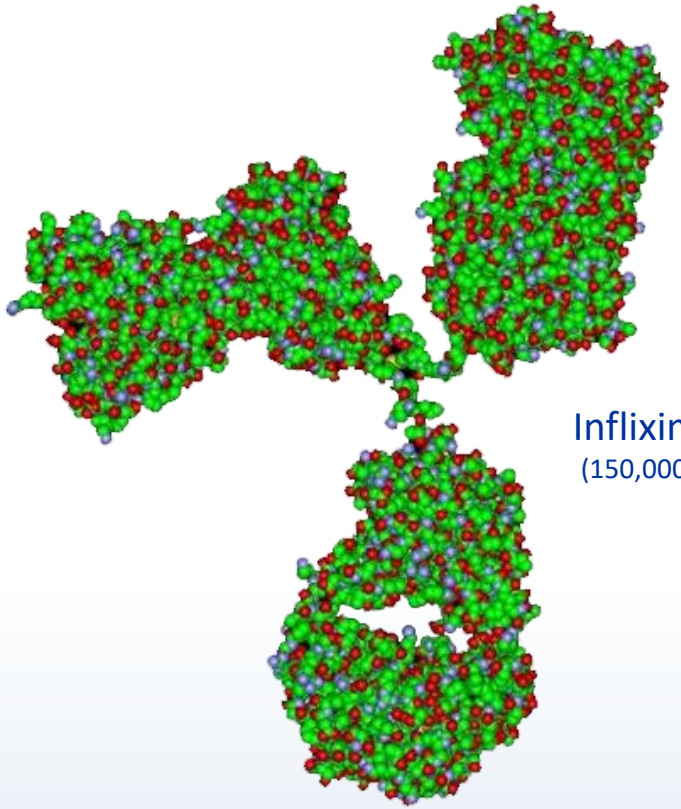
Biopharmaceuticals



Insulin
(5808 Da)



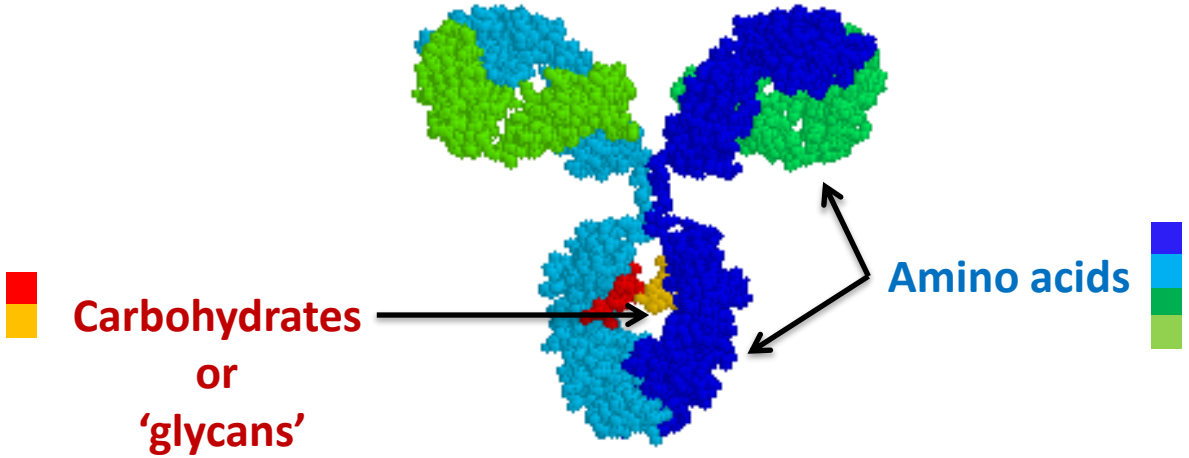
Filgrastim
(18,800 Da)



Infliximab
(150,000 Da)

What makes proteins so complex?

What makes a protein?

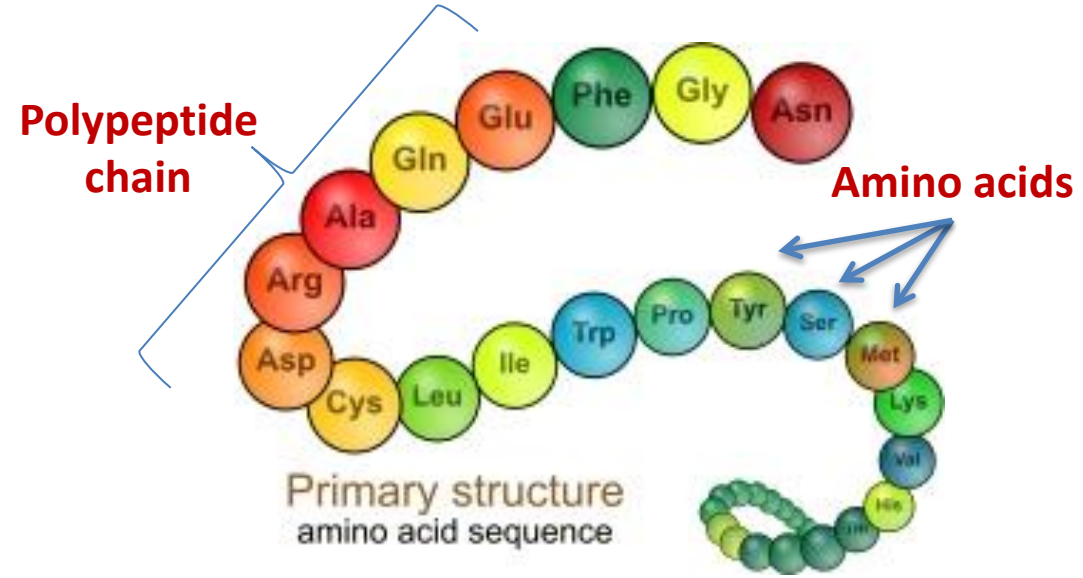


- Proteins are primarily composed of molecular building blocks called '**amino acids**'

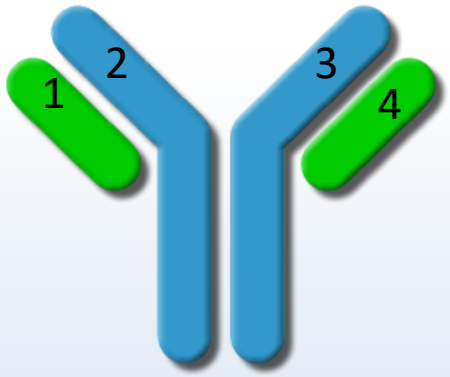
- Many biopharmaceuticals also contain carbohydrates (sugars) called '**glycans**'

What makes a protein?

- The amino acids are arranged in linear chains called **‘polypeptides’** by an enzyme complex in the cell

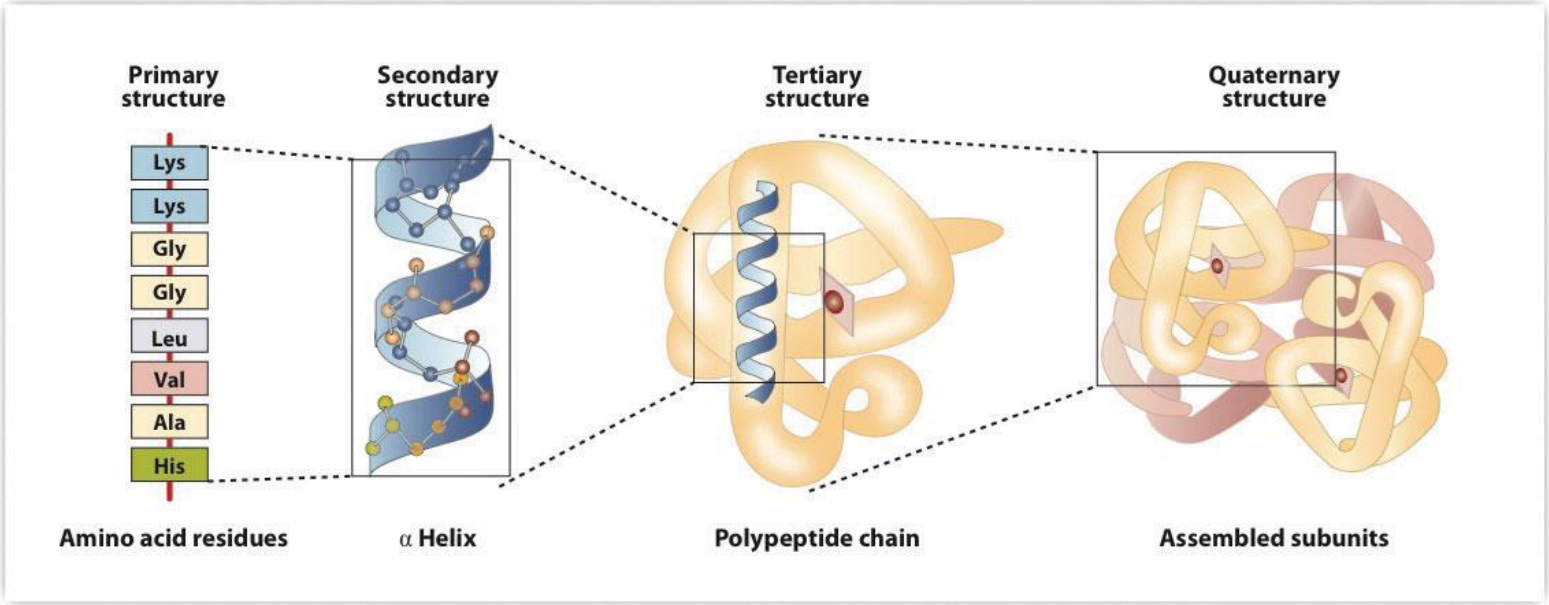


- Some proteins contain more than **one polypeptide chain**



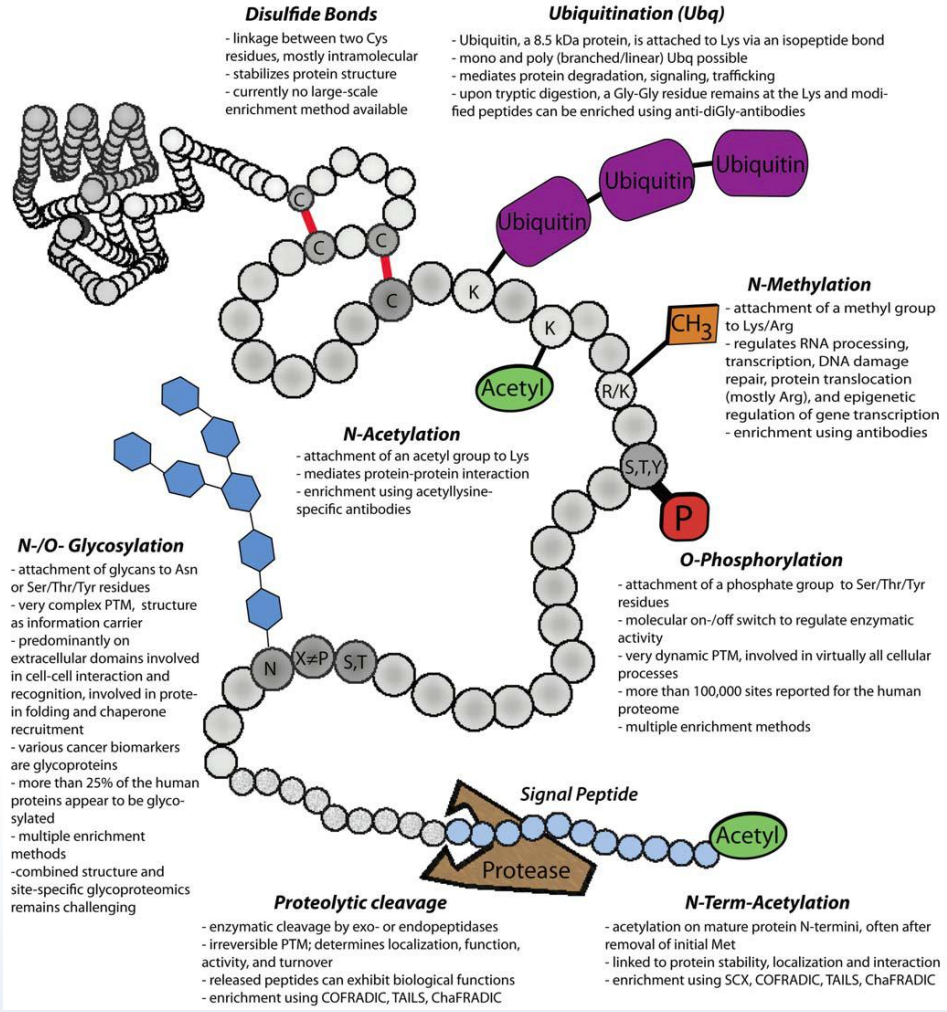
What makes a protein?

- The amino acids are arranged in a **specific sequence** which is dictated by the **gene** for that protein
- The sequence is **extremely important** for the protein to take its **biologically active shape**



Post-translational Modification (PTM)

- Proteins can undergo **100's of additional modifications** within the cell and also during bioprocessing
- These modifications usually significantly impact on **stability and function**
- **Most important PTMs - Glycosylation**

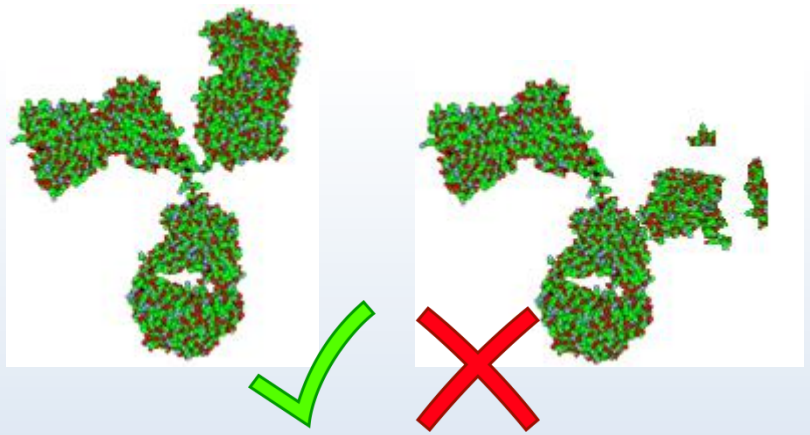


Pagel, Oliver, et al. "Current strategies and findings in clinically relevant post-translational modification-specific proteomics." *Expert review of proteomics* 12.3 (2015): 235-253.

Proteins are not static, rigid structures

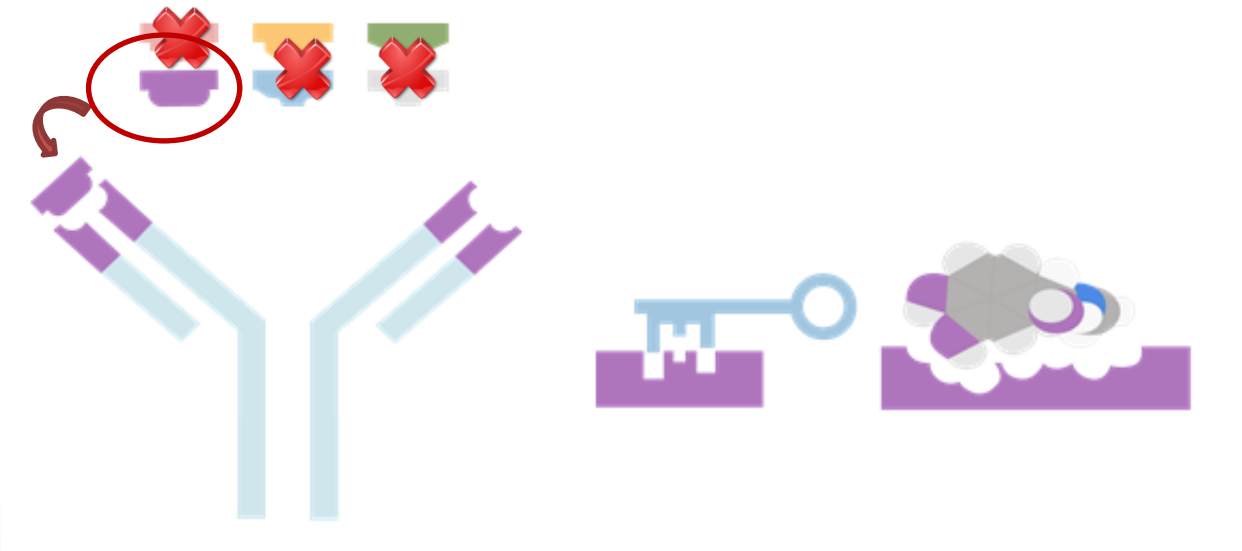
- **The folding of the protein is not fixed!**
- The protein will have a specific conformation ideal for biological interactions: **'active conformation'**
- **Processing conditions** influence this folding
- **Proteins can easily degrade**

  if handled incorrectly



Proteins and targets: Getting the best fit

- A protein binding to its target is like a **key fitting a lock.** If the protein is the incorrect structure, it will not bind the target effectively.

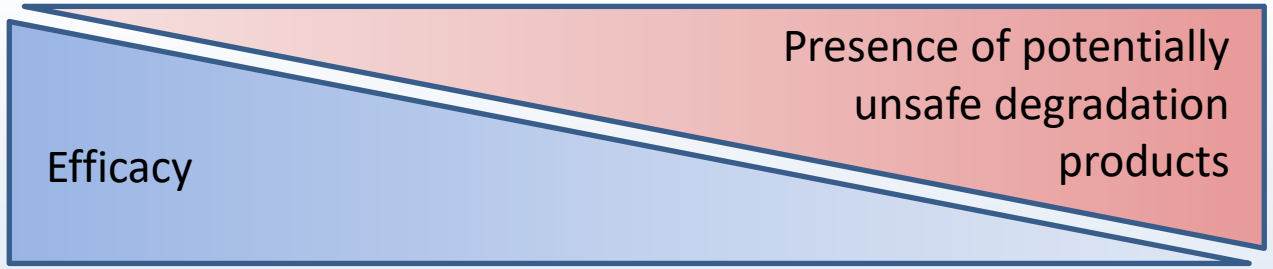


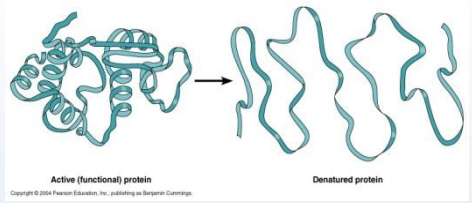
- Preservation of this **biologically active conformation** is what drives the **process design**

Formulation and Stability Issues

- As traditional medicines degrade, major concern is loss of **efficacy**
- As biopharmaceuticals degrade, there can be loss of **efficacy** *and* potentially increased **safety** issues

  - Aggregation
  - Precipitation
  - Fragmentation
  - Hydrolysis
  - Photolysis
  - Deamidation (ASX,GLX)
  - Oxidation (MET)
  - Disulfide Scrambling
  - Deglycosylation (Glycoproteins)

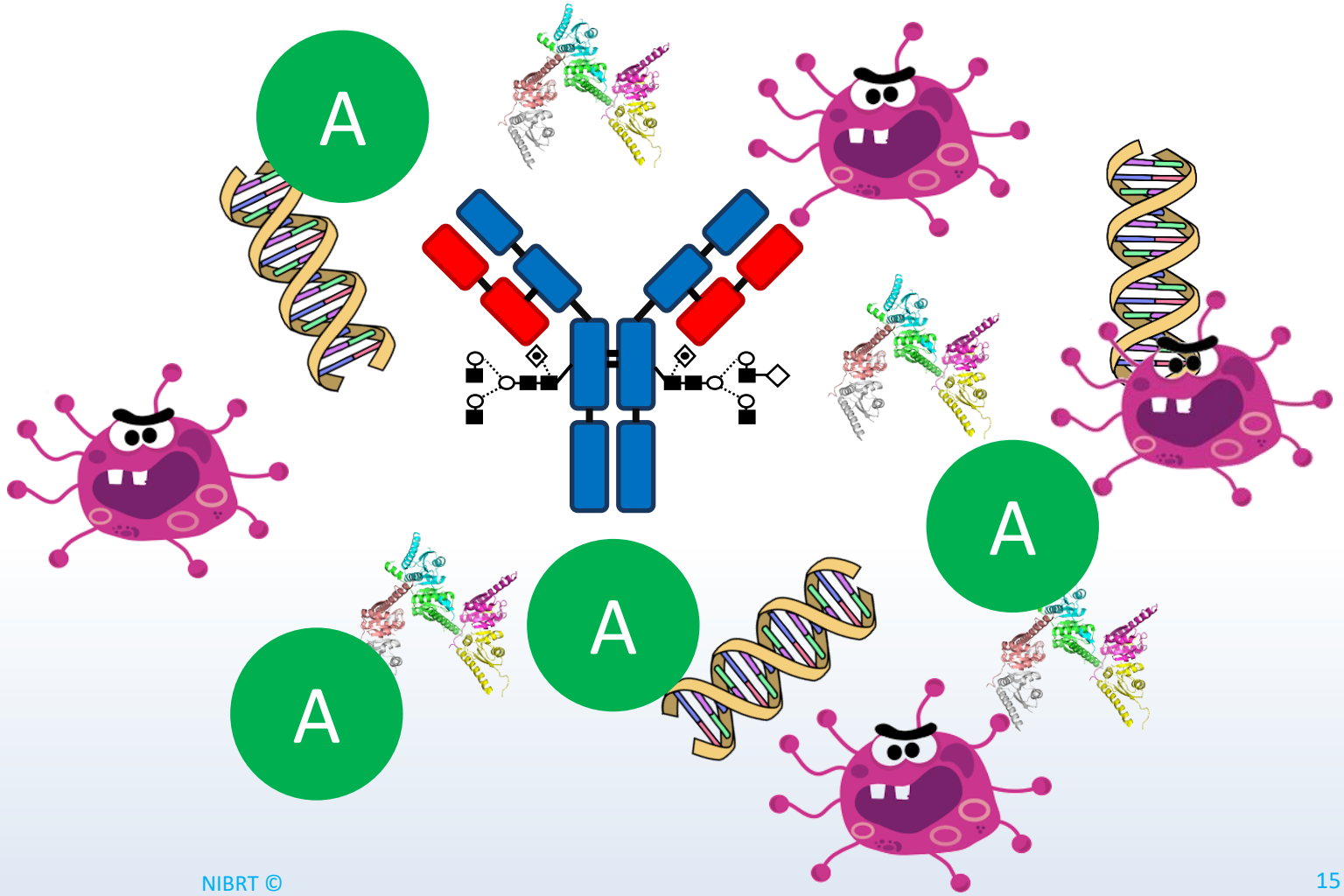




Process-related impurities must also be tackled!

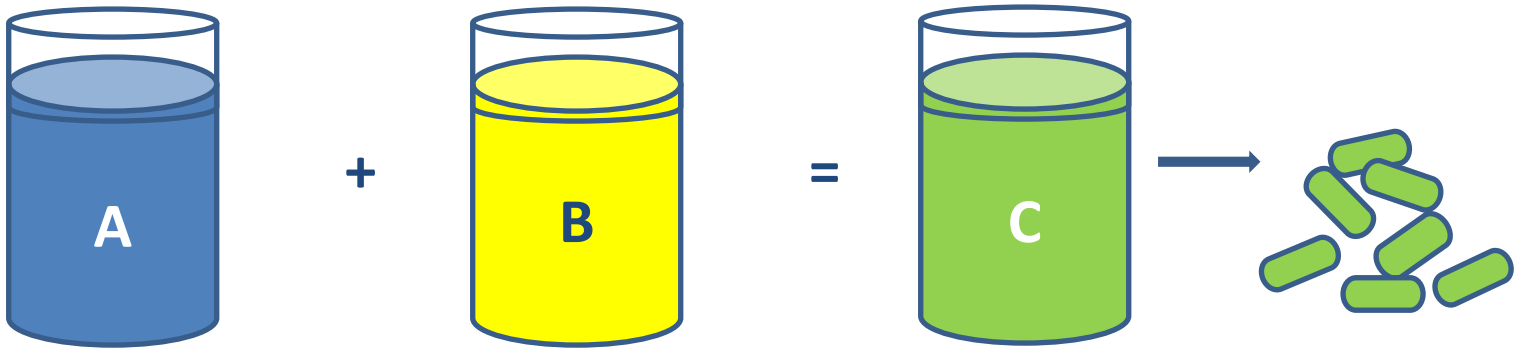
- Detecting and removing **process** related impurities represent another challenge

  - Host Cell Proteins
  - Host Cell DNA
  - Virus
  - Residual Protein A



What about the process?

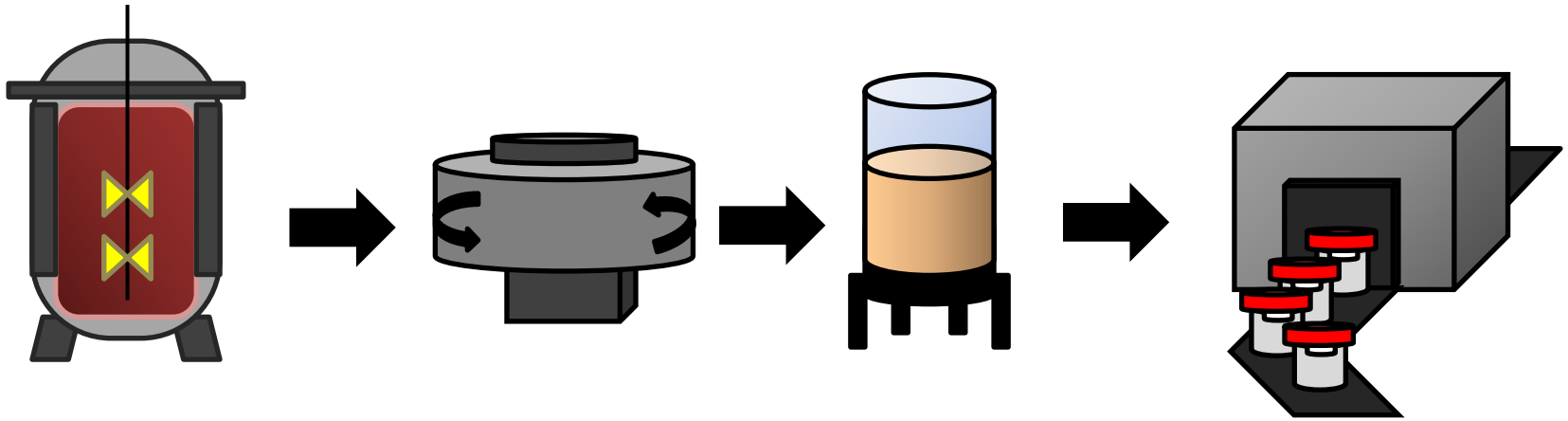
Chemical Synthesis: Follow the Recipe



Traditional pharmaceuticals are made through **chemical synthesis**

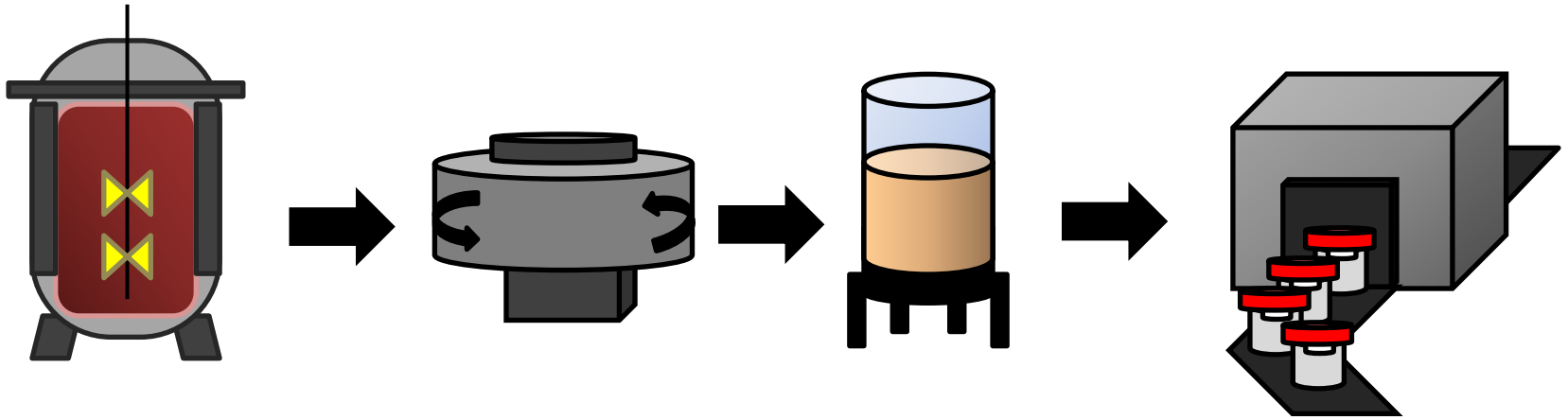
Bioprocessing: Complex and Unique

Many complex **cell culture** and **purification** procedures.



Upstream	Harvest	Downstream	Fill Finish
Cell Culture scale up and protein production	Cell removal	Protein purification	Formulation and Filling

Upstream Variables

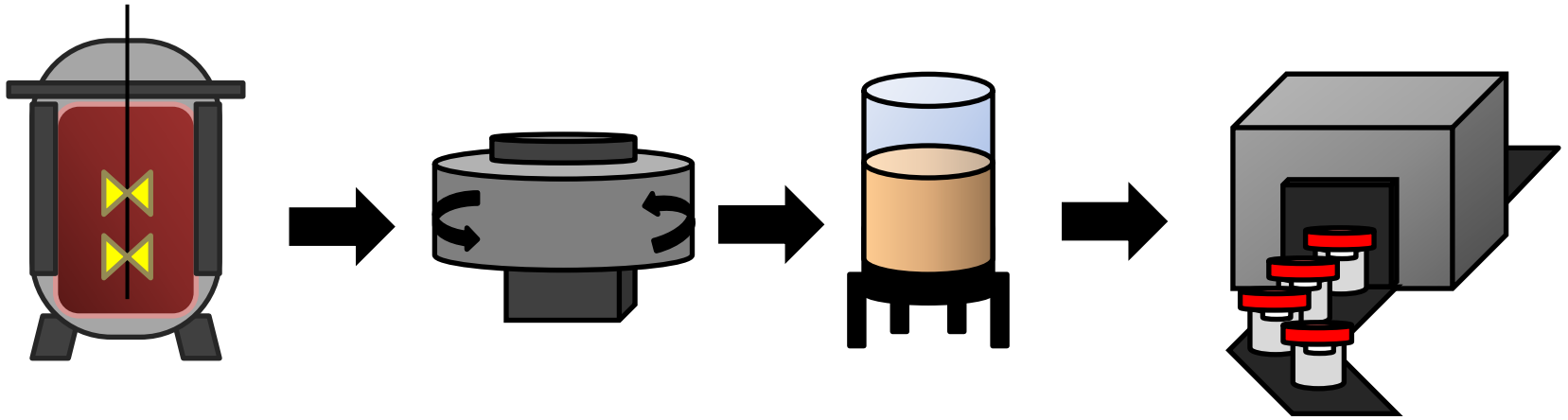


Upstream | **Harvest** | **Downstream** | **Formulation and Fill**

Cells
Mutations
Cell metabolism
Contaminants

Culture conditions
Medium composition
Nutrient availability
Bioreactor scale
pH & temperature

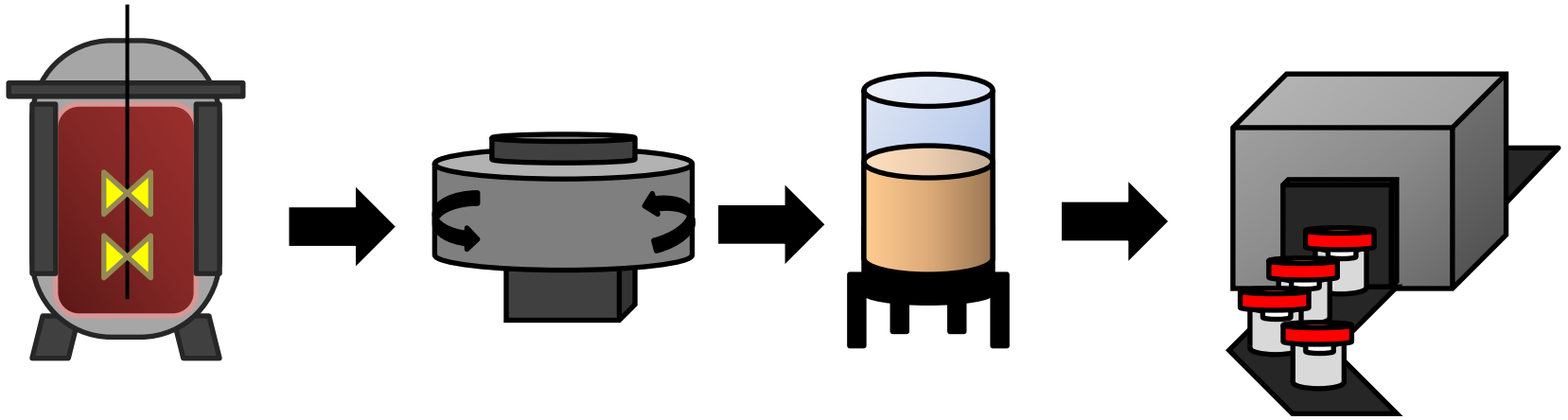
Harvest Variables



Upstream | **Harvest** | **Downstream** | **Formulation and Fill**

Depending on harvest method used, could have increased levels of enzymes leaking from broken cells that will degrade the biopharmaceutical

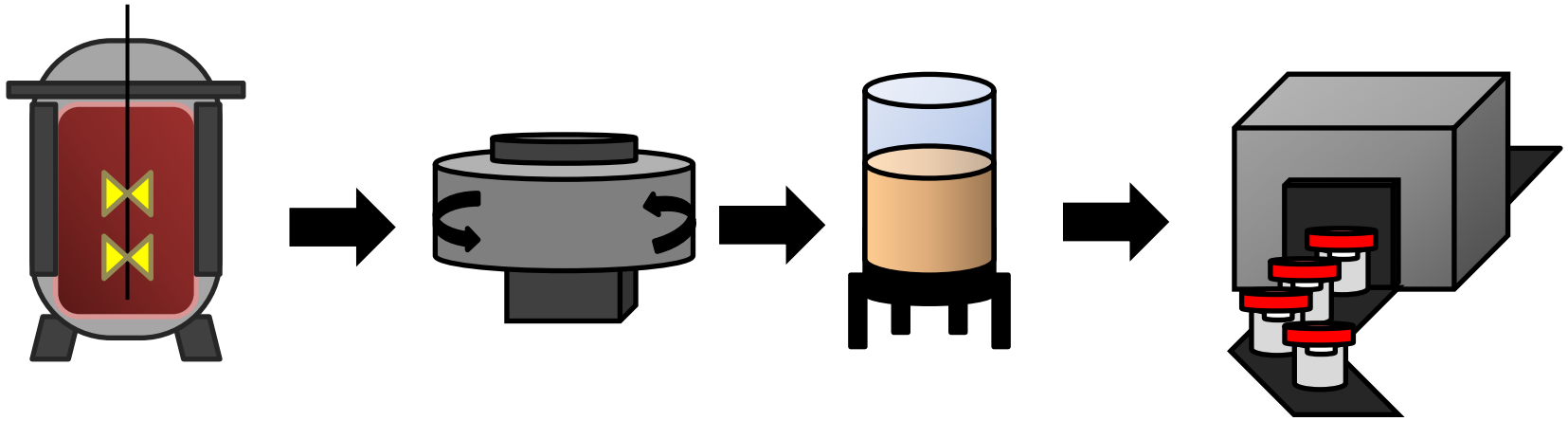
Downstream Variables



Upstream | **Harvest** | **Downstream** | **Formulation and Fill**

Purification columns
Composition of purification solutions (buffers)
Exposure to pH extremes

Fill Finish Variables



Upstream | **Harvest** | **Downstream** | **Formulation and Fill**

Final formulation composition
Vials/syringe components
Contaminants from filling line equipment

Ensure Product Quality with Process Control

With so many variables involved, each step of the process must be controlled through **GMP activities** and monitored using **QC testing**

The Quality Management System (including QC testing) proves the **consistency of the process**

Topics

- Complexity of Biologics
- Introduction to QC Testing
- Key ICH Guidelines

QC testing and Product Quality

- **Quality control testing** aims to ensure the **safety** and **efficacy** of medicines
- Quality **cannot be tested into the product**; must be built into the process
- Thus, the process is monitored by **testing the quality** of:
  - All chemicals and solutions associated with the production of the medicine
  - The drug substance (DS)
  - The drug product (DP)

Quality Control testing in biopharma

- **Product characterisation**
  - Initial in-depth analysis of product. Set quality specifications (proven acceptable ranges)

- **Stability Testing**
  - Determine the shelf life and storage conditions for product

- **In-process testing**
  - Testing bulk drug substance and associated materials
- **Lot release**
  - Ensure final product meets specifications as per product characterisation

Typical QC Testing in Bioprocessing

Buffer/Media Prep
- pH
- conductivity
- Osmolality
- Bioburden
- Endotoxin
- Water quality testing

Raw Materials
- Identity and purity
- HPLC
- Spectroscopy (FTIR, NMR)
- Residual moisture (KF Titration)
- TOC

UPSTREAM
- Cell density and % viability
- pH and gases
- Cell metabolites and substrates
- Biopharmaceutical titre (HPLC)
- Endotoxin and/or Bioburden samples (pre and post-innoculation, also in production)

HARVEST
- Total protein (protein assay)
- Biopharmaceutical titre (HPLC)
- Micro testing: Endotoxin and Bioburden (pre-filtration)
- External testing for mycoplasma and virus pre-filtration

DOWNSTREAM
- Total protein by UV after UF
- pH/conductivity after DF
- Biopharmaceutical titre after every chromo step
- Stability indicating assays (HPLC, electrophoresis)
- Bioburden before each step, i.e. pre filtration, pre chromo etc.
- Endotoxin performed after each step

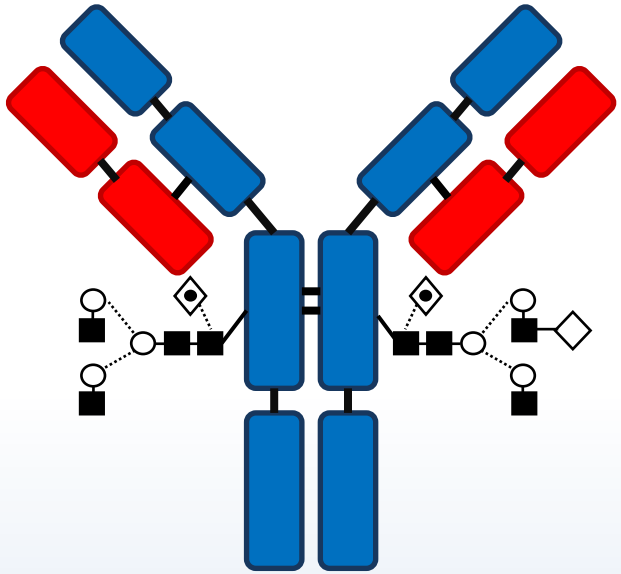
Formulated DS & DP
- Purity, integrity, identity (HPLC, electrophoresis)
- Total protein by UV or Bradford/BCA
- Potency (cell-based assay/bioassay)
- Process related impurities (Western blot, ELISA, HPLC, SPR)
- Product related impurities (HPLC, electrophoresis)
- Peptide mapping (HPLC)
- Glycan analysis (HPLC/Capillary electrophoresis)
- Charge variants (IEF, HPLC)
- Stability testing
- pH
- conductivity
- Osmolality
- Appearance
- Leachables and extractables
- Bioburden
- Endotoxin
- Extractable volume

Critical Quality Attributes (CQAs)

- 'A CQA is a **physical, chemical, biological**, or **microbiological property** or characteristic that should be within an **appropriate limit**, range, or distribution to ensure the **desired product quality**.' ICH Q8 (R2)

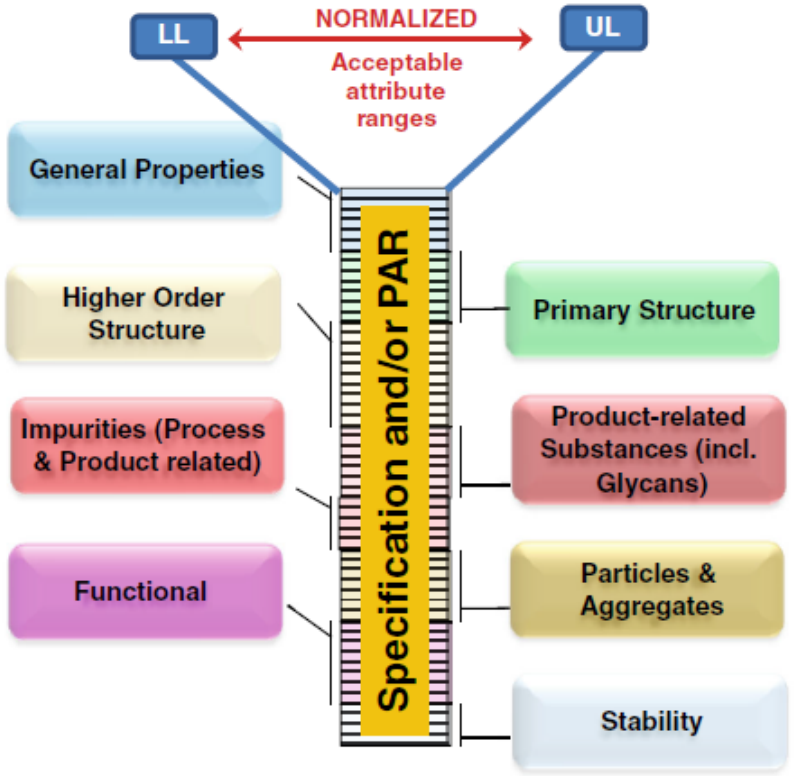
- E.g. molecular weight, potency, charge variants, glycoforms, process and product related impurities

- **Any property that affects:**
  - Biological Activity
  - PK/PD
  - Immunogenicity
  - Safety



http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf

Quality Control testing ensures all CQAs are within specification



A specification is a **proven acceptable range (PAR)** for a given QC test result

Ramanan, Sundar, and Gustavo Grampp. "Drift, Evolution, and Divergence in Biologics and Biosimilars Manufacturing." *BioDrugs* (2014): 1-10.

How does the manufacturer know what tests to use?

- Manufacturer is free to use **any scientifically valid test** for testing of product in their license application

- Information on generally acceptable types of testing:
  - **Pharmacopoeias (EP, USP, etc.)**
  - **FDA Guidance documents**
  - **ICH Guidance documents**
  - **Scientific literature, etc.**

- Also possible to use alternative tests to those prescribed by biological product standards: 21 CFR 610.9/ICH, if justified

- Once approved, **must continue to use the approved tests for all subsequent batches**

Topics

- Complexity of Biologics
- Introduction to QC Testing
- Key ICH Guidelines

ICH

- **International Council for Harmonisation** of Technical Requirements for Registration of Pharmaceuticals for Human Use

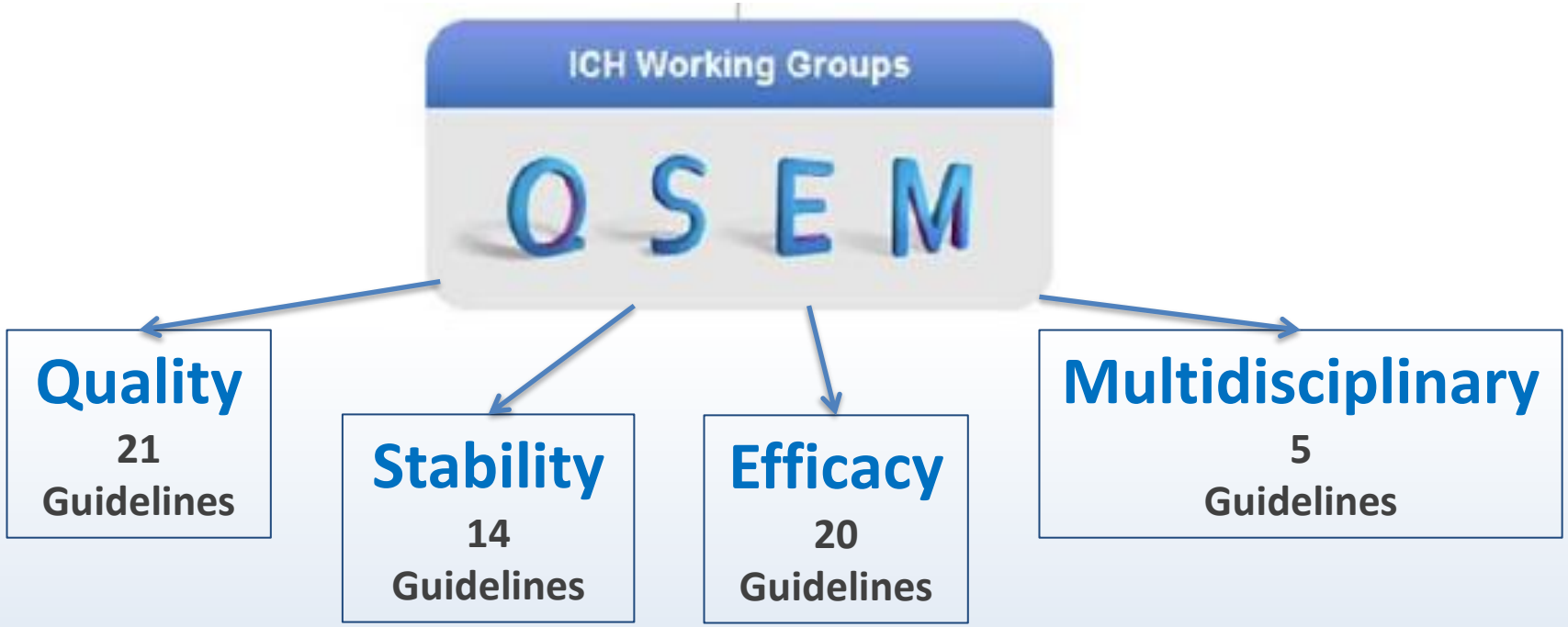
- Unique **harmonisation** project involving the regulators and industry of:
  - US, EU and Japan
  - WHO, Canada, and EFTA are observers

http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html

ICH

ICH harmonisation for better health

ICH Provides **60 Guidelines on technical requirements under 4 Working Groups**



http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html

ICH Guidelines for Quality of Biopharmaceuticals

ICH
harmonisation for better health

Quality Guidelines

Q5A - Q5E Quality of Biotechnological Products

Code	Document Title	Previously coded
Q5A(R1)	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin	Q5A
Q5B	Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products	
Q5C	Stability Testing of Biotechnological/Biological Products	
Q5D	Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products	
Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process	

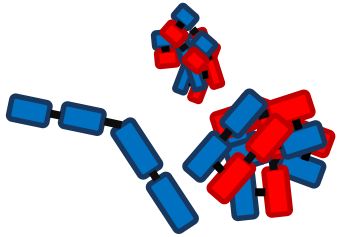
Q6A- Q6B Specifications

Code	Document Title	Previously coded
Q6A	Specifications : Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances	
Q6B	Specifications : Test Procedures and Acceptance Criteria for Biotechnological/Biological Products	

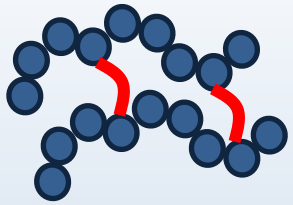
ICH Topic Q 6 B
Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

Process-related impurities

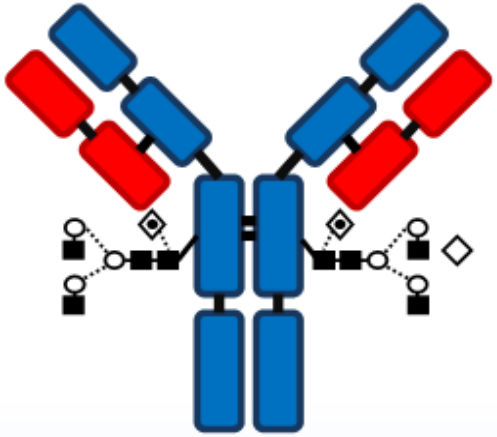
Product-related impurities



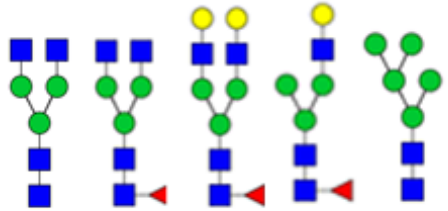
Disulfide bridges



Intact mass
Higher Order Structure
Potency
Binding properties

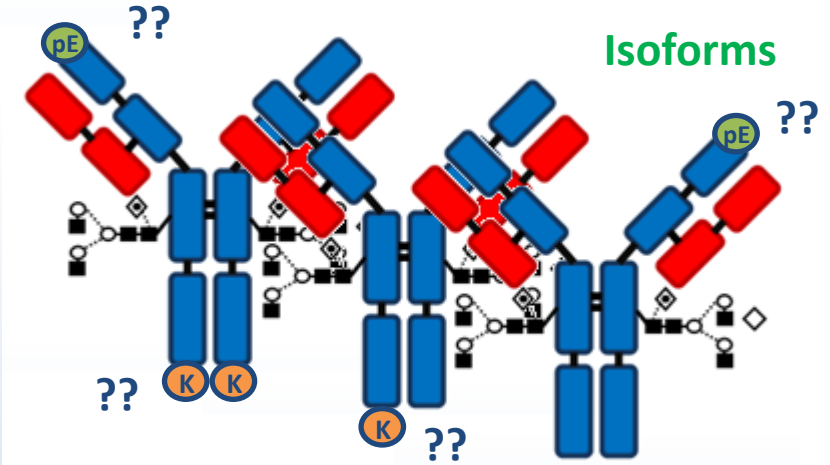


Glycan profile



Amino acid sequence
Mutations
PTM

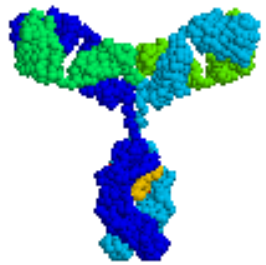
Isoforms



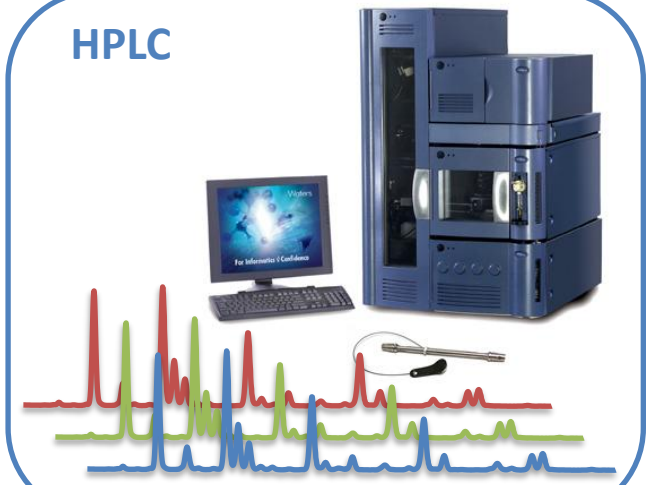
Vial inspection

pH, conductivity, osmolality

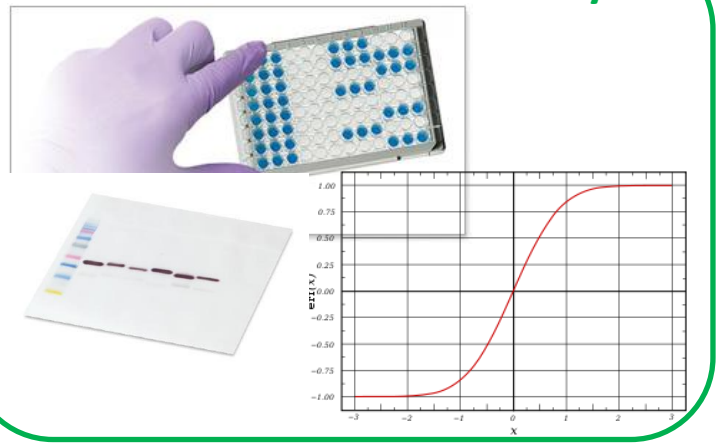
Electrophoresis



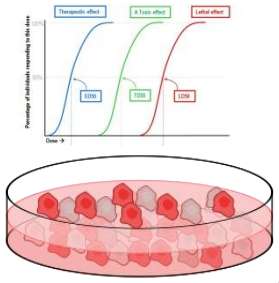
HPLC



Immunoassays



Bioassays



Microbial testing

Stability Testing

Quality Guidelines

Q5A - Q5E Quality of Biotechnological Products

Code	Document Title	Previously coded
Q5A(R1)	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin	Q5A
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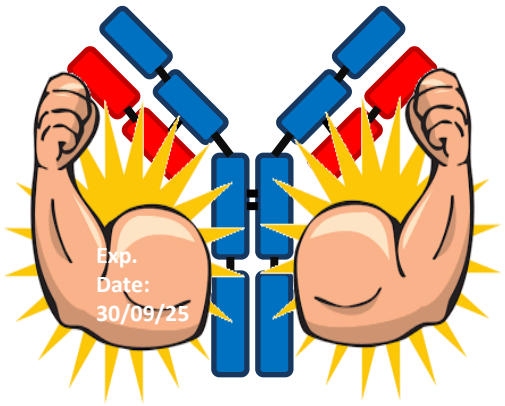
Stability Testing

Key part of QC Program and conducted throughout the product lifecycle



- To understand how the **quality** of a DS/DP **changes over time** under the influence of **different environmental conditions**, and to determine degradation pathways
- Provides information with regard to DS/DP **storage conditions**, **final formulation/packaging** and **shelf life**. This information is necessary for **regulatory approval**
- Ensure continuous **safety** and **efficacy** for the patient

Product must be completely characterised, and CQAs determined before stability studies begin

SGS, "A guide to biologic stability testing" Accessed April 2002 <https://www.sgs.com/en/news/2015/10/a-guide-to-biologic-stability-testing>

Blessy, M., et al. (2014) *"Development of a forced degradation and stability indicating studies of drugs - a review"* Journal of Pharmaceutical Analysis 4(3) pp 159-165

Stability Testing: What conditions do we look at?

Temperature	Humidity	Light	Container/ Closure Systems	Reconstitution
• Storage temperature and above	• Can sometimes be omitted where humidity protecting containers are used (Climate zones I and II)	• Studies to demonstrate the effects of light • Case by base basis	• Inverted, horizontal and upright studies • Effects of closure	• Stability after reconstitution • Storage conditions

The purpose of stability testing is to:

- provide evidence on how the **quality** of a drug substance or product **varies over time**, under the influence of a variety of environmental factors

ICH Q5C: Stability Testing of Biotechnological/Biological Products
ICH Q1A(R2): Stability Testing of New Drug Substances and Products
ICH Q1B: Photostability Testing of New Active Substances and Medicinal Products

Comparability

ICH harmonisation for better health

Quality Guidelines

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Q5B	Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products	
Q5C	Stability Testing of Biotechnological/Biological Products	
Q5D	Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products	
Q5E	Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process	

Q6A- Q6B Specifications

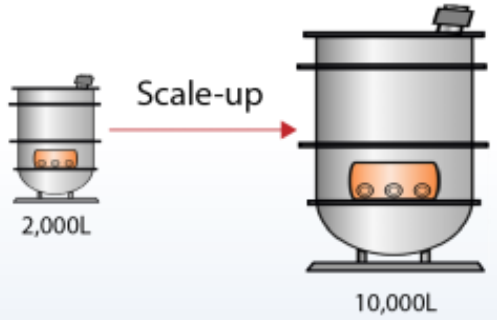
Code	Document Title	Previously coded
Q6A	Specifications : Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances	
Q6B	Specifications : Test Procedures and Acceptance Criteria for Biotechnological/Biological Products	

Changes in CQAs: Drift and Evolution in Biologics

Changes in the biologic can be a result of:

- **Drift:** a result of unknown deviations in the manufacturing process

- **Evolution:** known changes in the manufacturing process (equipment change, scaling, raw materials suppliers, etc.)



ICH Q5E harmonisation for better health

Photo: https://www.wuxibiologics.com/scale-out-vs-scale-up-biomanufacturing/

Ramanan, Sundar, and Gustavo Grampp. "Drift, Evolution, and Divergence in Biologics and Biosimilars Manufacturing." *BioDrugs* (2014): 1-10.

The goal of ICH Q5E

- The goal of this **comparability exercise** is to ensure the quality, safety and efficacy of a drug product produced by a changed manufacturing process

- Demonstration of comparability does not necessarily mean that CQAs of pre-change and post-change product are identical
  - *They are highly similar and that existing knowledge is sufficiently predictive to ensure that any differences in CQAs have no adverse impact*

How do we manage change?

Gather important information

- List of CQAs
- Description of product change and rationale for change
- Historical data and product characterisation data

Identify impact of changes on product quality

- What might be effects on CQAs?
- What might be effects on in-process controls?

Select relevant analytical methods

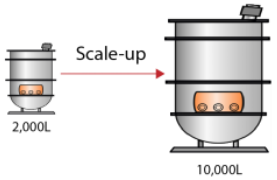
- Which analytical methods are most relevant to detect potential change in corresponding CQA

Determine acceptance criteria

- Based on side by side comparison or statistical comparison

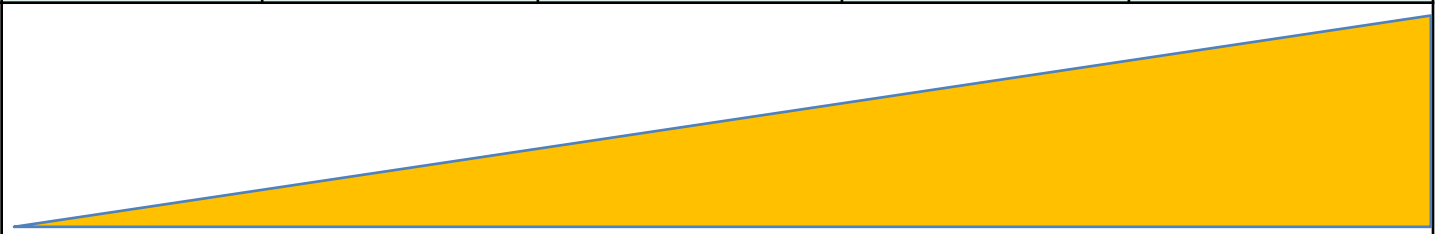
Mélanie Schlegel and Yves Bobinnec, Comparability Protocols for Biotechnological Products; BioProcess International 11(6) June 2013

Example of a Comparability Plan

Process Change	Affected CQA	Impact Assessment	Analytical Method	Acceptance Criteria
Scale-up of cell culture 	Residual HCP	Scale-up is expected to produce more biomass hence more residual HCP	HCP ELISA	≤ 50ppm
	Glycosylation Profile	Scale up might affect cell line growth parameters possibly leading to a change in the glycosylation pattern	Oligosaccharide mapping	Side by side profile comparison
	Isoform profile	Scale up might affect cell line growth parameters possibly generating a different isoform profile	HPLC	Main peak ≥ 90% Pre-peak < 3% Post-peak < 2%

Mélanie Schlegel and Yves Bobinnec, Comparability Protocols for Biotechnological Products; BioProcess International 11(6) June 2013

Change Risk and Data Requirements

Nature of Process Change	Change filter supplier	New equipment in same facility	Move to new production facility	Change cell /culture media	New cell line or major formulation change
Risk Level & Data Requirement					
	Low Risk Analytical data Process data		**Moderate Risk** Analytical/Process data + Stability data		**High Risk** Analytical/Process /Stability data + Non clinical data + Clinical data

The nature of a manufacturing change determines the amount and type of supporting data required to evaluate comparability

Lee JF, et al. Curr Med Res Opin 2012;28:1053–8

Topics

- Complexity of Biologics
- Introduction to QC Testing
- Key ICH Guidelines

Thank You