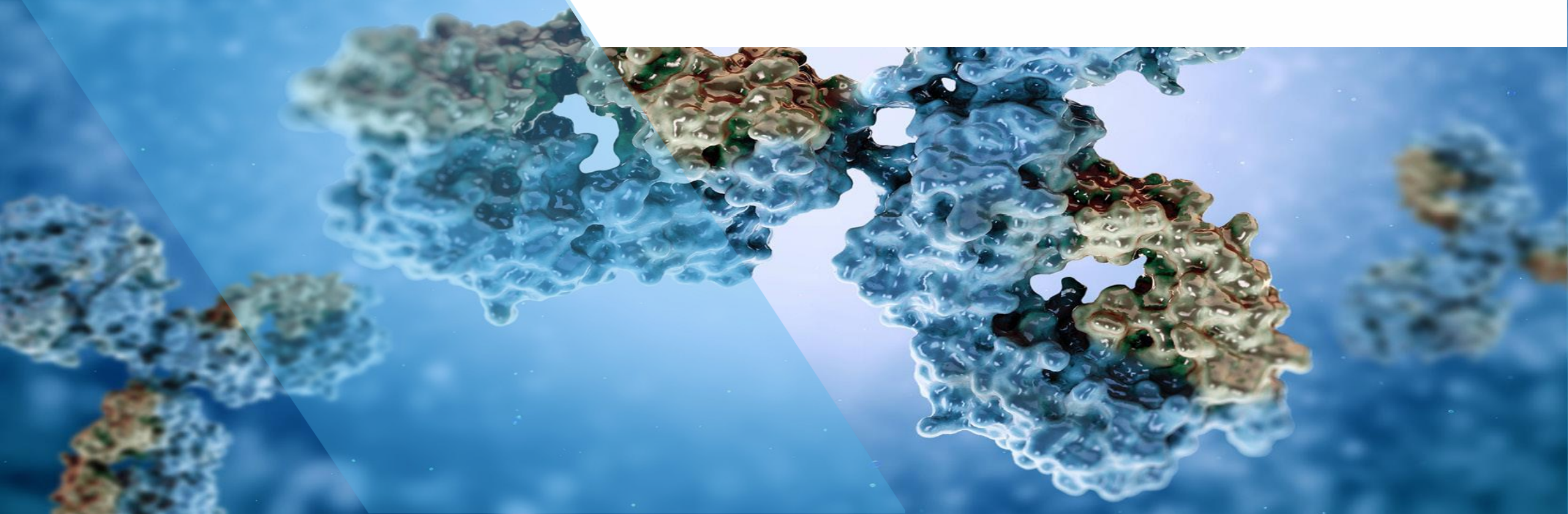
Overview of QC for Biologics



Learning Objectives

Describe the structural and functional complexity of biopharmaceuticals

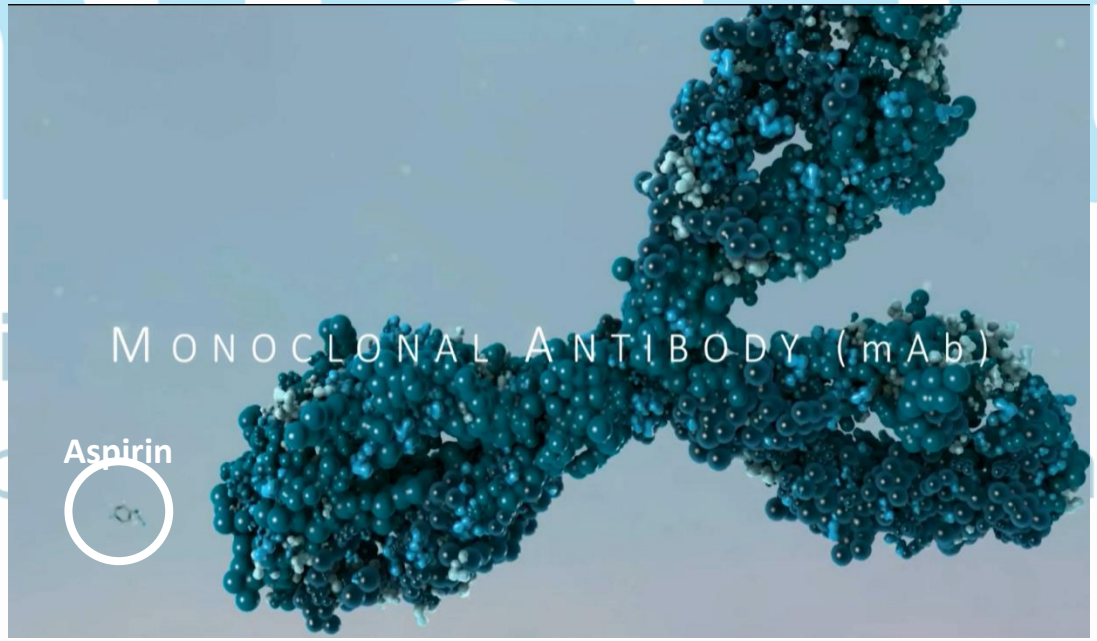
Describe the testing requirements for in process testing, lot release, stability and comparability testing

Topics

- Biopharmaceuticals: A Closer Look
- Analytical Characterisation of Biologics
- Stability Testing
- Comparability Testing

Biopharmaceuticals Are Complex Molecules…

- Biopharmaceuticals are vastly more complex structurally than traditional medicines
- Hierarchical structural organisation
- Mutations, RNA splicing
- PTMs, glycosylation
- Other process effects
- Multiple mechanism of action
- Heterogeneous batches



Walsh, Gary, ed. *Post-translational modification of protein biopharmaceuticals*. Wiley-VCH, 2009.
Image from NIBRT-AbbVie Educational film, https://www.youtube.com/watch?v=fjuF16eQ7iQ

- Process impurities and degradation products

The Structural Features of Biopharmaceuticals Are Not Fixed!

- Many of the **physicochemical properties** of biopharmaceuticals are subject to change during all steps of manufacturing
- Rigorous **control** of the process and sensitive **testing** of drug **substance**, drug **product** and **raw materials** aim to minimise this
- Batches are still a **heterogeneous mix** of structurally related **isoforms** but these must be defined and consistent from batch-to-batch

Where is this heterogeneity coming from?

Amino Acid Substitutions

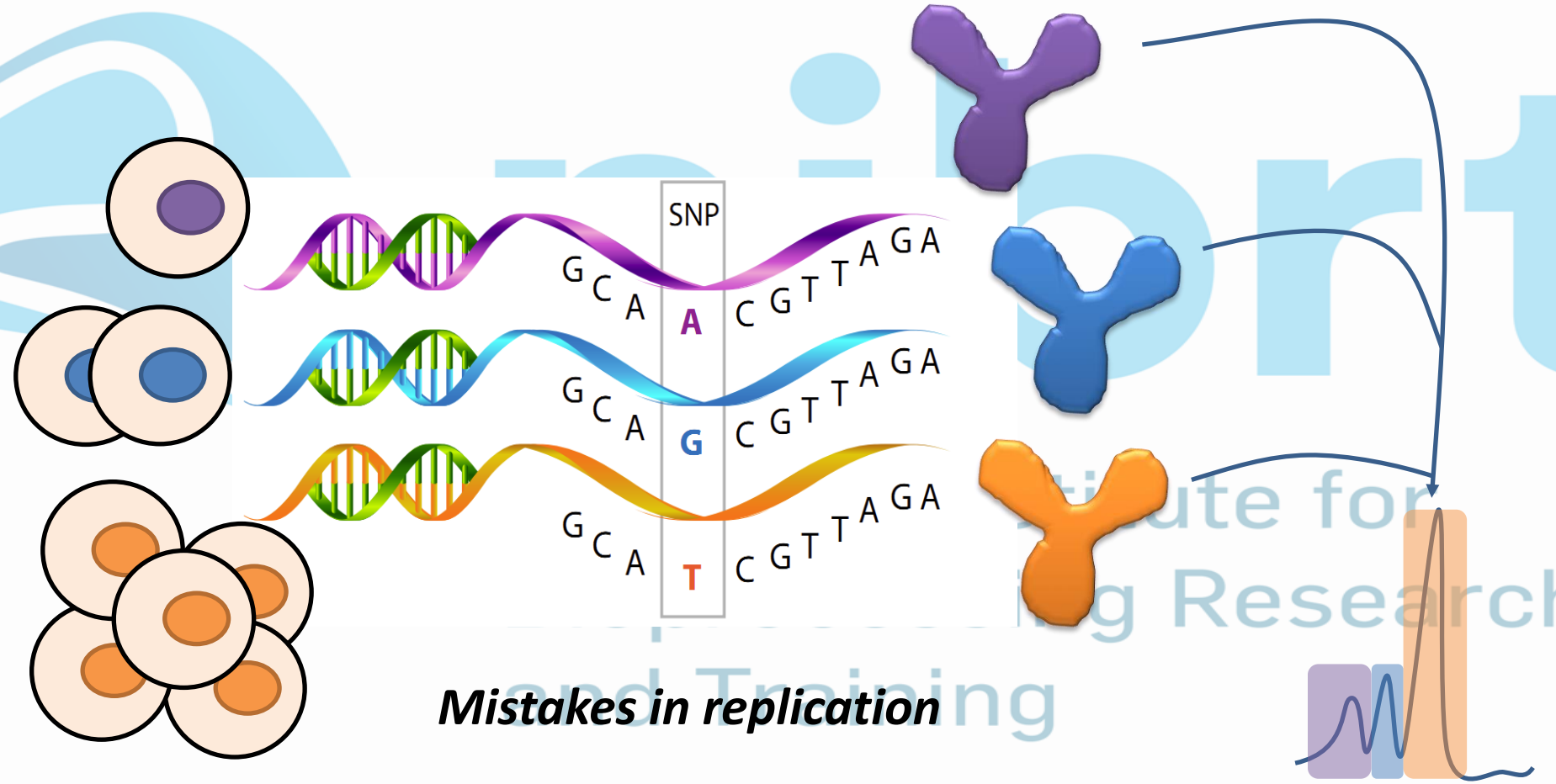
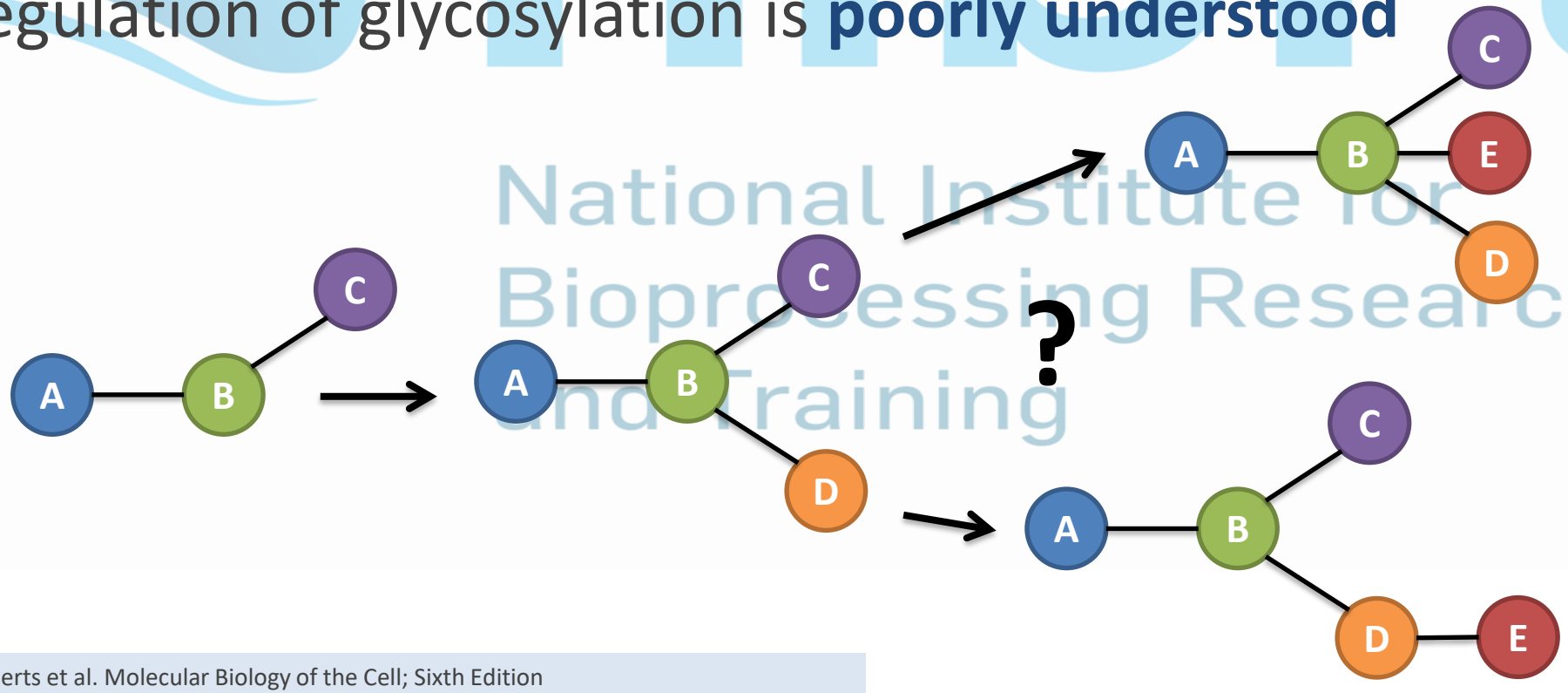


Image modified from https://neuroendoimmune.files.wordpress.com/2014/03/snp.png
Walsh, Gary, ed. *Post-translational modification of protein biopharmaceuticals*. Wiley-VCH, 2009.

Glycoforms: Creating Mixtures

- There is **no DNA template** for glycans
- Their structure depends on **many factors**
- The regulation of glycosylation is **poorly understood**



Alberts et al. Molecular Biology of the Cell; Sixth Edition

Downstream Processing Can Change Protein Structure Too!

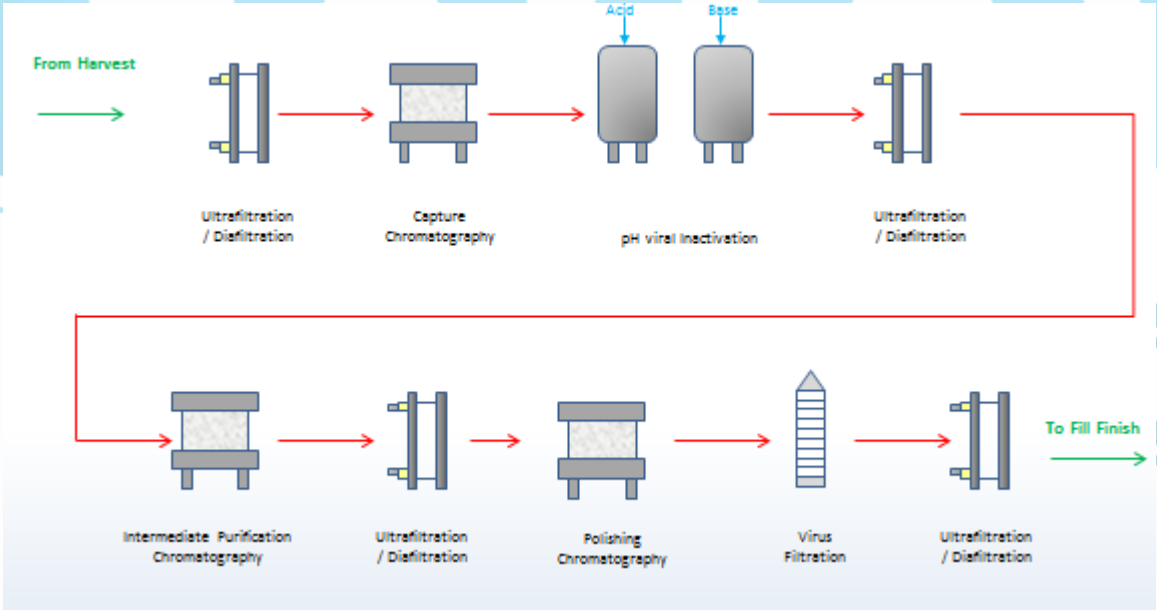
Hydrolysis

Denaturation

Aggregation

Desialylation

Precipitation



Glycation

Deamidation

Pyroglutamate

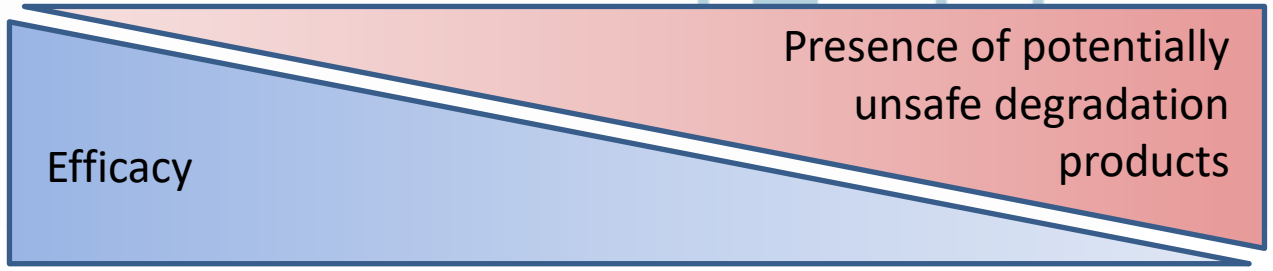
Disulfide scrambling

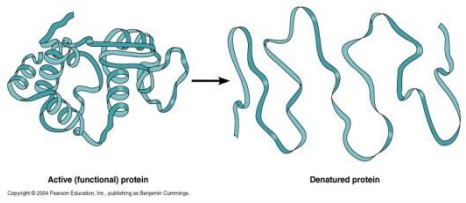
Oxidation

Walsh, Gary. *Pharmaceutical biotechnology: concepts and applications*. John Wiley & Sons, 2007.

Formulation and Stability Issues

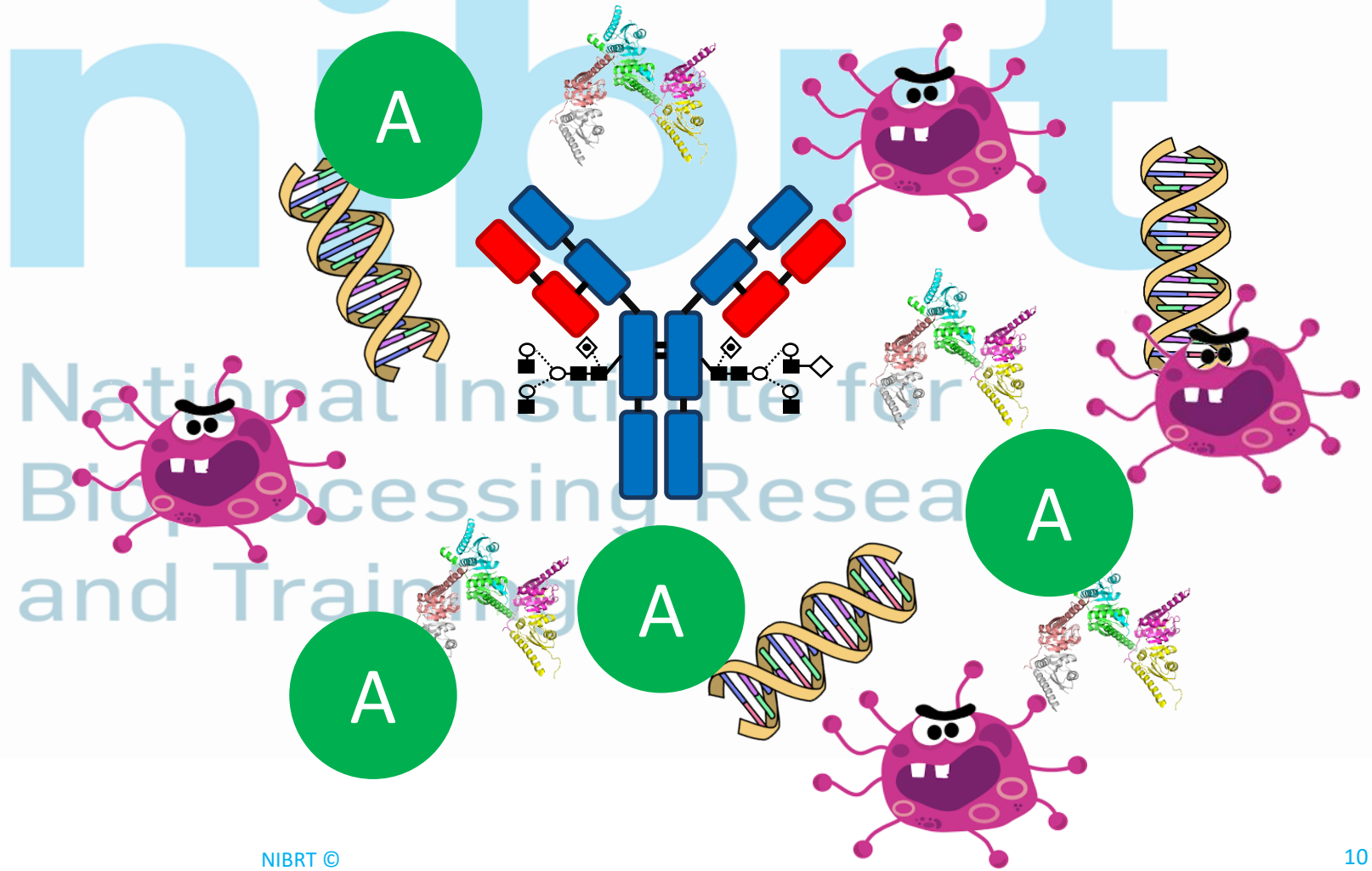
- As traditional medicines degrade, major concern is loss of **efficacy**
- As biopharmaceuticals degrade, there can be loss of **efficacy** *and* potentially increased **safety** issues
  - Aggregation
  - Precipitation
  - Fragmentation
  - Hydrolysis
  - Photolysis
  - Deamidation (ASX,GLX)
  - Oxidation (MET)
  - Disulfide Scrambling
  - Deglycosylation (Glycoproteins)



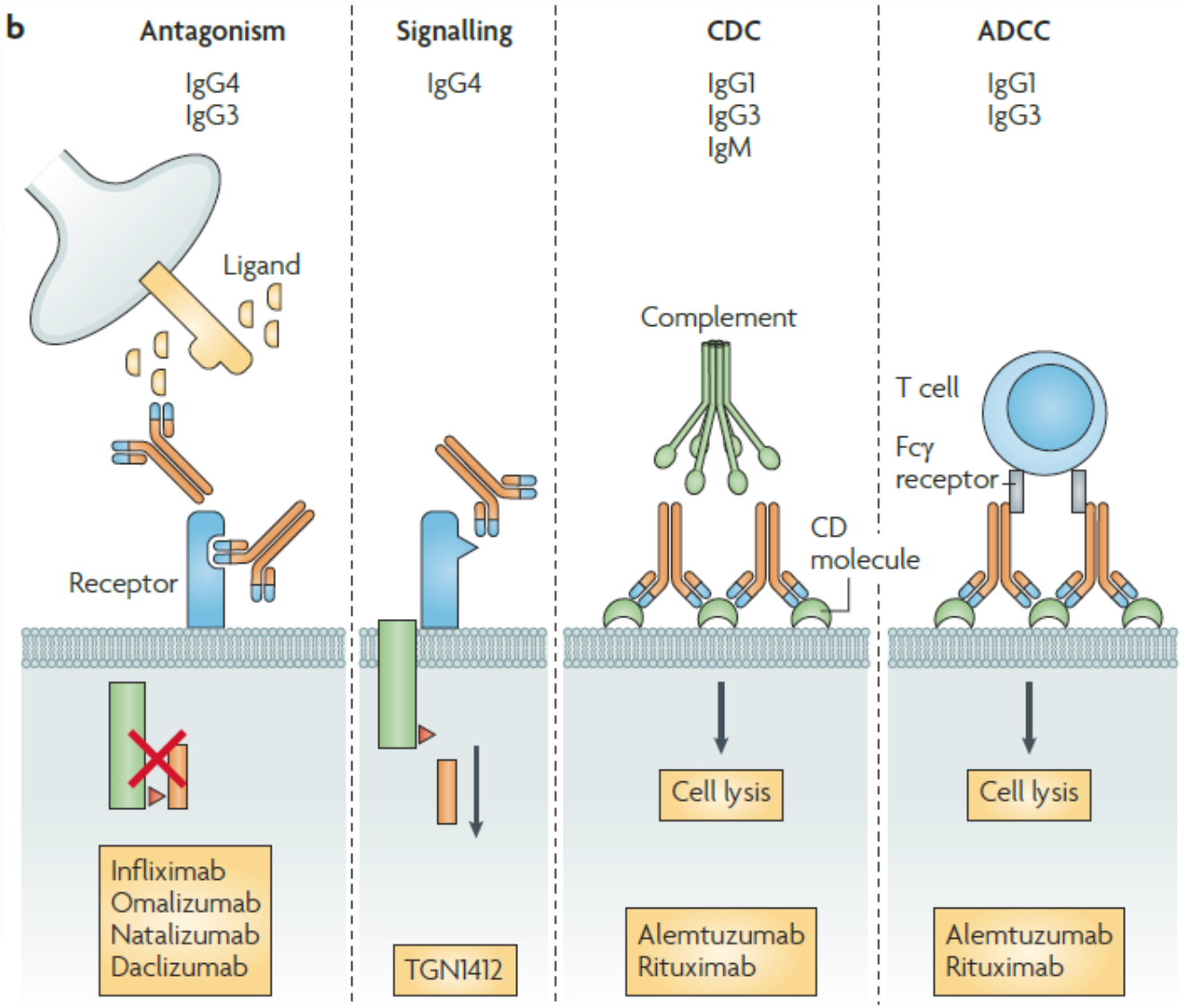


Process-Related Impurities Must Also Be Tackled!

- Detecting and removing **process** related impurities represent another challenge
  - Host Cell Proteins
  - Host Cell DNA
  - Virus
  - Residual Protein A



Mechanisms of Action: More than One Way to Skin a Cat



- **Many mAbs have multiple MoA**
- **Their relative dominance can vary depending on the indication in which they are used!**

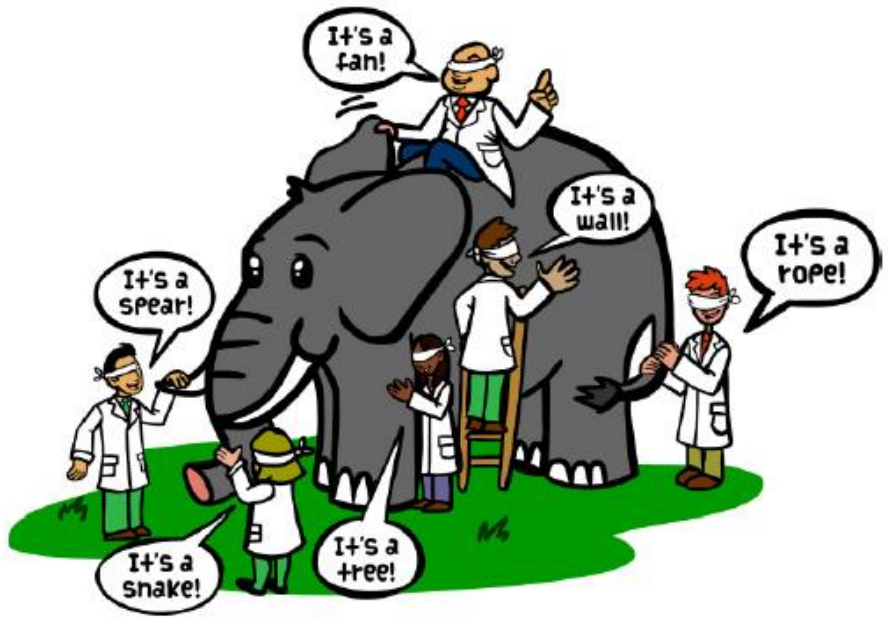
CDC = Complement dependent cytotoxicity

ADCC = Antibody dependent cell mediated cytotoxicity

Hansel, Trevor T., et al. "The safety and side effects of monoclonal antibodies." *Nature reviews Drug discovery* 9.4 (2010): 325-338.

Many Orthogonal Methods Required…

- Due to the **complexity** of protein structure and function, it is impossible to determine the **safety, efficacy** and **quality** with just one test!



- An extensive panel of biochemical and functional analytical tests is required for a complete picture!

http://www.theblindelephant.com/uploads/elephant_pic.jpg

Topics

- Biopharmaceuticals: A Closer Look
- Analytical Characterisation of Biologics
- Stability Testing
- Comparability Testing

The Importance of Analytics

Products are defined by their material and processes of manufacture, which are controlled by sometimes variable and complex analytical methods

$$M^A + P^A = Pr^A$$

- M = Materials
- P = Process
- Pr = Product
- A = Confidence of Analytical Methods

What is Bioanalytics?

Bioanalytics is concerned with testing the **quality** of the product throughout the manufacturing process to ensure its **safety** and **efficacy**

Bioanalytical Methods are used throughout:

- Product characterisation
- In-process testing
- Lot release testing
- Stability testing
- Comparability testing

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, JP. 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

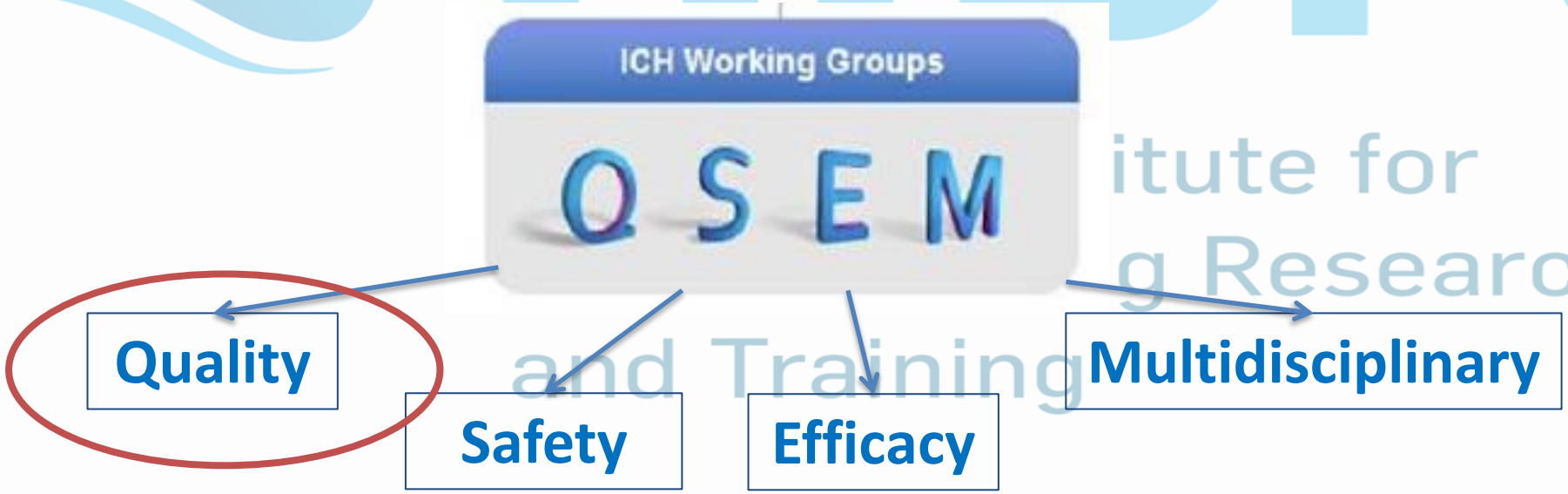
- Once approved, **must continue to use the approved tests for all subsequent batches**

ICH Guidelines for S,E&Q of Pharmaceuticals

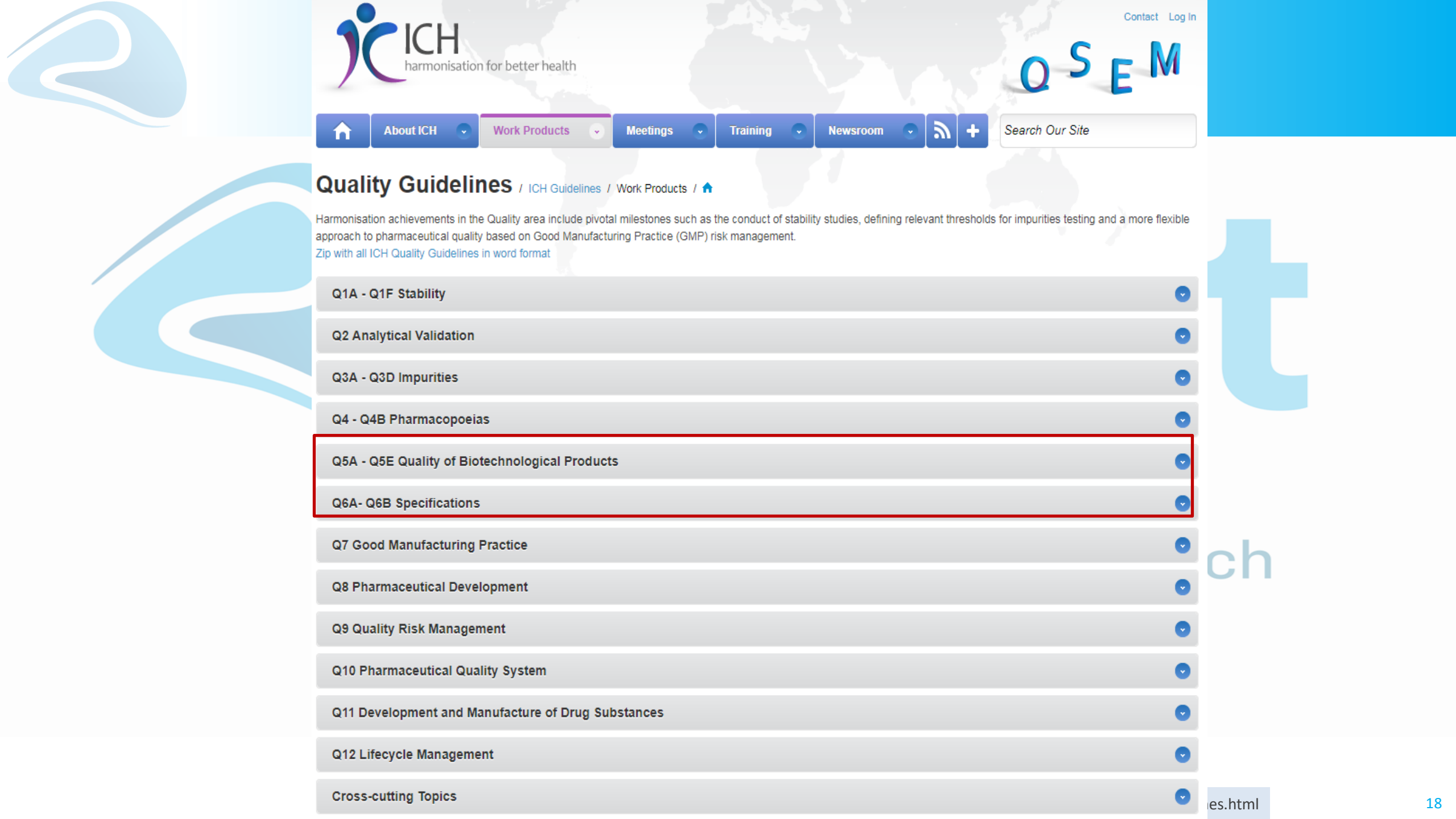
The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

ICH harmonisation for better health

ICH provides **Guidelines on technical requirements under 4 Working Groups**



http://www.ich.org/about/organisation-of-ich/working-groups.html, accessed 01 April 2016


ICH
harmonisation for better health
Contact
Log In
Q S E M
About ICH
Work Products
Meetings
Training
Newsroom
Search Our Site
Quality Guidelines / ICH Guidelines / Work Products /
Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.
Zip with all ICH Quality Guidelines in word format
Q1A - Q1F Stability
Q2 Analytical Validation
Q3A - Q3D Impurities
Q4 - Q4B Pharmacopoeias
Q5A - Q5E Quality of Biotechnological Products
Q6A- Q6B Specifications
Q7 Good Manufacturing Practice
Q8 Pharmaceutical Development
Q9 Quality Risk Management
Q10 Pharmaceutical Quality System
Q11 Development and Manufacture of Drug Substances
Q12 Lifecycle Management
Cross-cutting Topics

Characterisation and Quality Control

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	

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

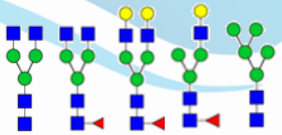
Characterisation

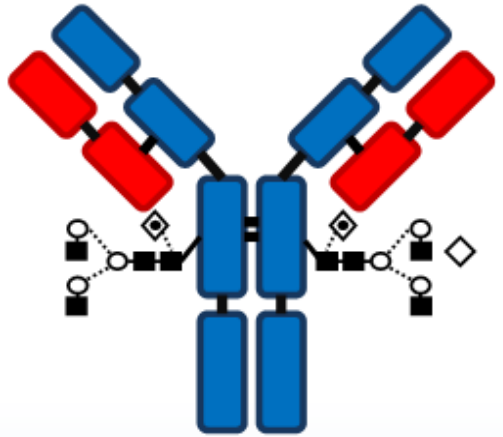
ICH Topic Q 6 B

Characterised products are those whose identity, purity, impurities, potency and quantity have been determined

Structural Characterisation

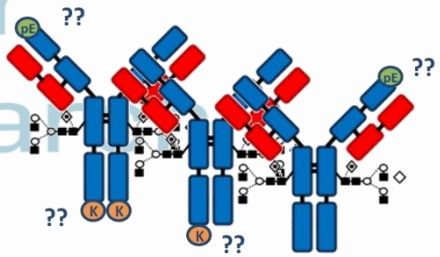
- Amino acid sequence
- Post translational modifications
- Glycan content
- Disulphide bridges





Physiochemical Characterisation

- Size/molecular weight
- Extinction coefficient
- Electrophoretic patterns
- Chromatographic patterns
- Isoforms



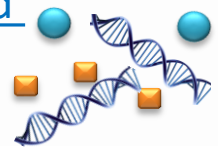
Functional Characterisation

- Potency
- Binding properties

Impurities

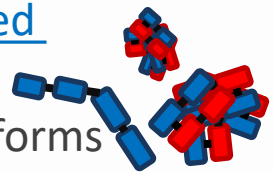
Process Related

- DNA
- Viruses
- Host cell proteins (HCPs)



Product Related

- Aggregates
- Truncated forms
- Chemically altered forms



Protein characterization methods

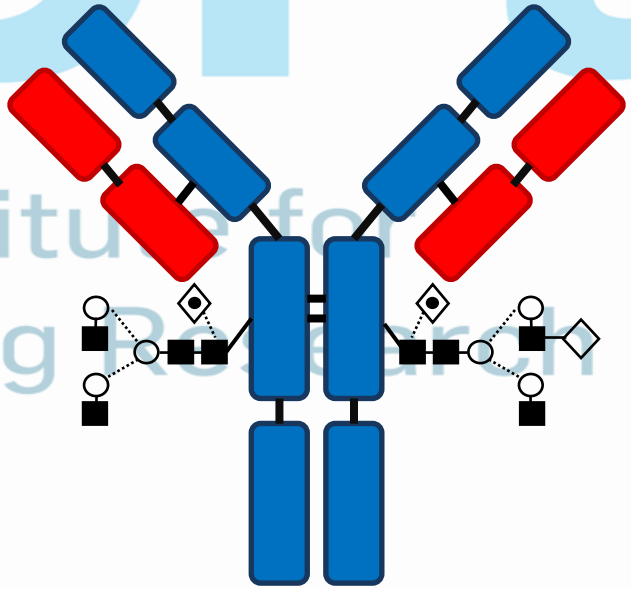
Characteristic	Methods	Application
Concentration	Protein Assays, ELISA, SPR	Protein concentration
Size	1D/2D-PAGE	Identity, purity, sample integrity, separation
	Size Exclusion Chromatography	Identity, purity, separation
Charge	Ion Exchange Chromatography	Identity, purity, separation
	Iso-Electric Focusing	Identity, purity, separation
Hydrophobicity	Hydrophobic Interaction Chromatography	Identity, purity, separation
	Reversed Phase-HPLC	Identity, purity, separation
Biological affinity	Western blotting , ELISA, SPR	Identity, sample integrity
	Affinity Chromatography	Identity, purity, separation
Size:charge	Capillary Electrophoresis	Identity, purity, separation
Peptide sequence	Mass Spectrometry	Identity, sample integrity
Post-translational modifications	e.g. Glycan analysis (by UPLC or UPLC-MS) Phosphorylation, Oxidation, Acetylation, Methylation	Identity, sample integrity

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

Characterisation vs Quality Control

Characterisation

- Refers to elucidation of product biological, chemical and physical characteristics
- Occurs during product and process development
- Informs setting of quality specifications for commercial batches
- Also required following process changes

QC Testing

- Testing of:
  - raw materials,
  - buffers/media,
  - product intermediates (in-process, drug substance)
  - finished drug product
  - ongoing stability
- Confirmation that all of the above meet ***pre-defined*** specifications and/or proven acceptable ranges as set out in Marketing Authorisation

Topics

- Biopharmaceuticals: A Closer Look
- Analytical Characterisation of Biologics
- Stability Testing
- Comparability 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
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	

http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html, accessed 01 April 2016

Stability Testing: Regulations

ICH Topic Q 1 A (R2)
Stability Testing of new Drug Substances and Products

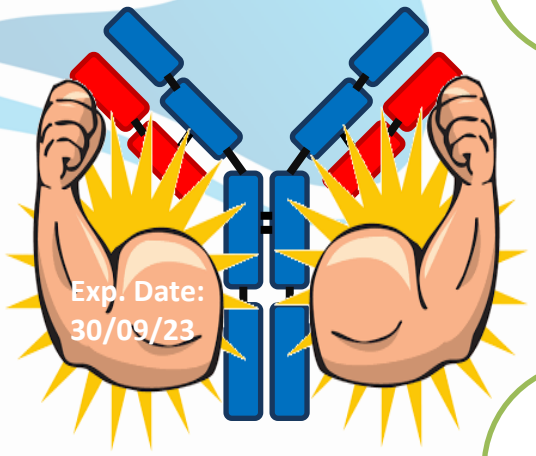
- Parent guideline for stability testing
- Offers guidance for **any** new drug
- Stress testing, batch selection, testing frequency, storage conditions

ICH Topic Q 5 C
Quality of Biotechnological Products:
Stability Testing of Biotechnological/Biological Products

- Offers **extra** guidance for biopharmaceutical products – biopharmaceuticals are very complex, and therefore pose extra challenges

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: Strategies

Will my protein degrade under these conditions??

Real Time (Long Term)

- DS/DP is stored at recommended storage conditions and monitored until it falls out of specification*
- Used to establish storage conditions and/or shelf life – the number of days that the product remains stable
- Continues post approval, up to and beyond the expiry proposed by the manufacturer

Accelerated/Stressed

- DS/DP is stored at elevated **stressful** conditions –atypical conditions
- Indicates which environments that product should **not** be exposed to – determines the point and time when the DS/DP falls out of specification*
- Provides data for future process changes
- Degradation profile
- Risk management

**The characteristics of a biopharmaceutical will change as it ages, but it is considered to be stable as long as these characteristics remain within the manufacturer's specifications*

Drug Product Stability Protocol Example

Testing frequency is based on proposed shelf life; < or > 1 year

	Real-time Testing		Accelerated/Stressed Testing	
Timepoints (number of months)	-20°C	5°C	25°C/60%RH	40°C/75%RH
0	ABCDE	ABCDE	ABCDE	ABCDE
1	ABCDE	ABCDE	ABCDE	ABCDE
2	ABCDE	ABCDE	ABCDE	ABCDE
3	ABCDE	ABCDE	ABCDE	ABCDE
6	ABCDE	ABCDE	ABCDE	
9	ABCDE	ABCDE	ABCDE	
12	ABCDE	ABCDE		
18	ABCDE	ABCDE		

A = appearance
B = electrophoretic method
C = HPLC/UPLC method
D = bioassay/immunoassay
E = bioburden (DS)/sterility (DP)

B, C: purity/identity
D: potency

Forced Degradation Studies

- In order to develop and validate suitable stability-indicating analytical methods, we need to "make ghosts"
- This means forcing our biopharmaceutical to degrade under harsh conditions (e.g. exposure to 5-15 freeze/thaw cycles, agitation for 6-36 hours)
- Most sensitive analytical methods can then be picked to be used as stability-indicating

Will my test methods be able to detect my degraded protein?

Agitation

	A	B	C	D	E	F	G	H
0	-	-	-	-	-	-	-	-
1	-	-	-	+	-	-	-	-
2	-	-	-	+	-	-	-	-
3	-	-	-	+	-	-	-	-
4	-	-	-	+	-	-	-	-
5	+	-	-	+	-	-	-	-

Freeze-Thaw

	A	B	C	D	E	F	G	H
0	-	-	-	-	-	-	-	-
1	-	-	-	-	+	-	-	-
2	-	-	-	-	+	-	-	-
3	-	-	+	-	+	-	-	-
4	-	-	+	-	+	+	-	-
5	+	-	+	+	+	+	-	+

Both Agitation and Freeze-Thaw promote aggregation that was measured using the following methods:

A = Appearance; B = pH; C = SDS-PAGE; D=SEC-HPLC
E = RP-HPLC; F = IEF; G = Peptide Map; H = Potency

Advanced Topics in Analytical CMC Studies for Biotech and Biosimilar Products, N. Ritter, 2017

Yu, J. "Intentionally Degrading Protein Pharmaceuticals to Validate Stability-Indicating Analytical Methods", BioPharm 13:11 (2000), p. 46.

Topics

- Biopharmaceuticals: A Closer Look
- Analytical Characterisation of Biologics
- Stability Testing
- Comparability Testing

Comparability

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	

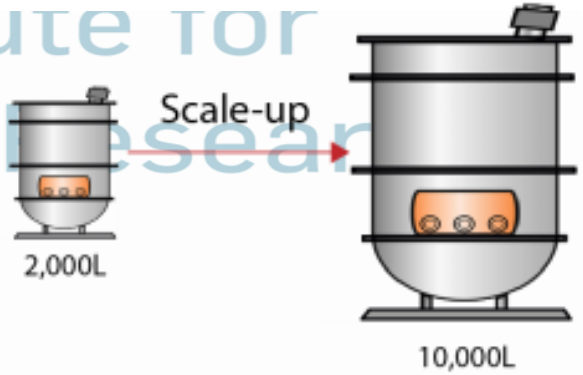
http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html, accessed 01 April 2016

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 harmonisation for better health **Q5E**

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

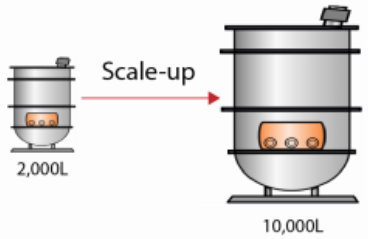
- Which analytical methods are most relevant to detect potential change in corresponding CQA

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

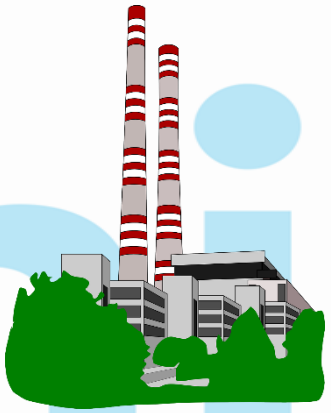
Example of a Comparability Plan

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%

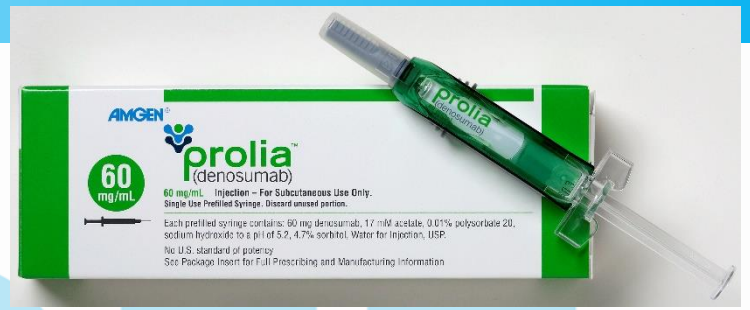
Mélanie Schlegel and Yves Bobinnec, Comparability Protocols for Biotechnological Products; BioProcess International 11(6) June 2013

Comparability Examples

- Manufacturer wanted to have **two sites approved** for manufacture of Cetuximab
- There was a **major difference** observed in human **pharmacokinetics** between products manufactured at the two sites: 30 % difference in mean trough concentration and 50 % difference in mean peak concentration.
- **FDA only approved a single manufacturing site.**



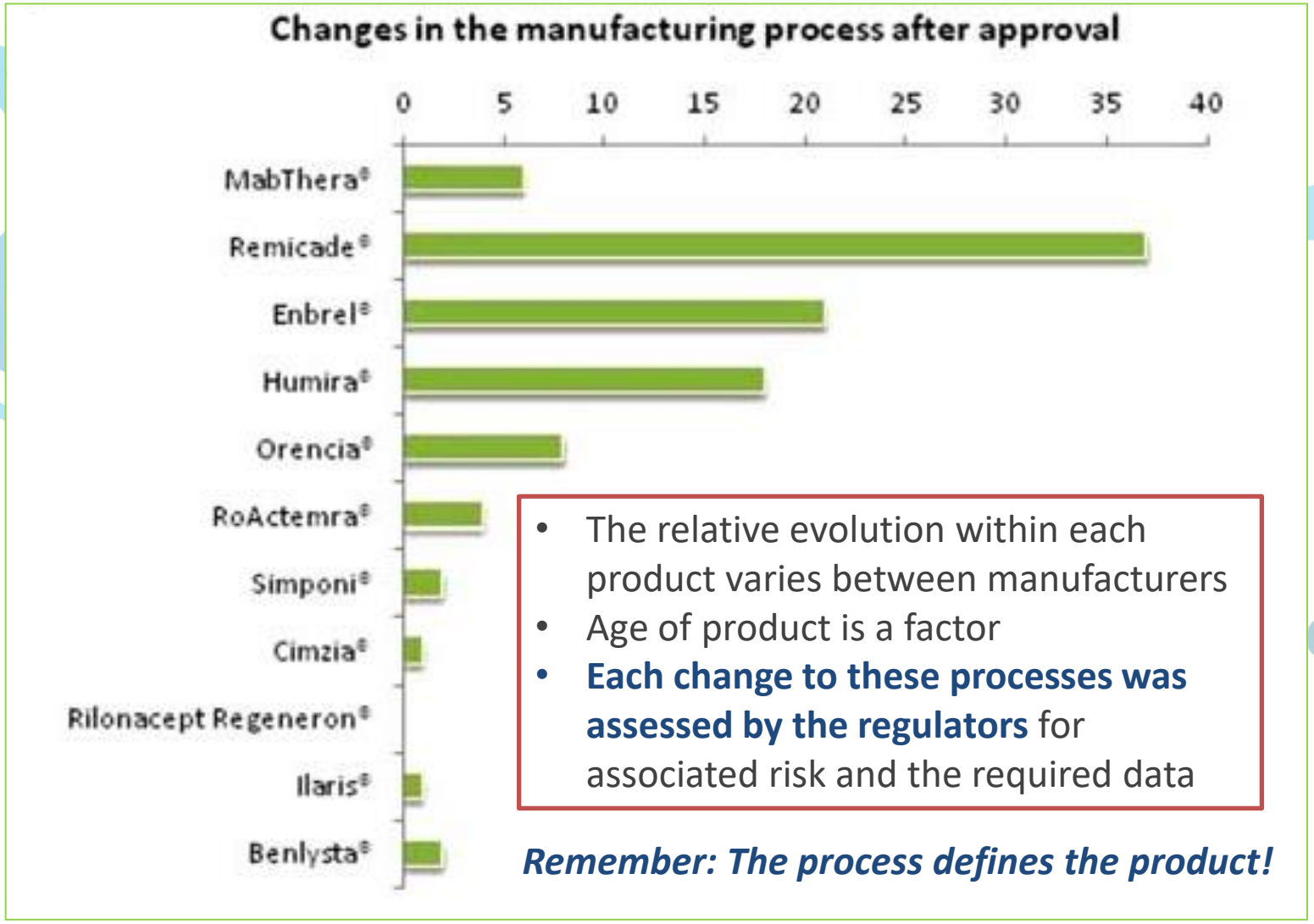
EMA Human Medicine European Public Assessment Report For Prolia (Denosumab) (March 2010); EMA website



- Manufacturer wanted to have **two sites approved** for manufacture of Prolia and **two different dosage forms** (vial and pre-filled syringe).
- **Analytical differences** were observed: minor differences in the glycosylation, size and charge profiles, but with **no impact on the in-vitro potency assay**
- Also **no impact was seen in non-clinical PK/PD study** in cynomolgus monkeys
- **The product received an FDA and EMA approval**

FDA BLA Market Approval of Erbitux (Cetuximab): Approval History, Letters, Reviews and Related Documents – Administrative and Correspondence Documents – Recommendation For Approval Action (February 12, 2004)

Evolution in Biologics



- The relative evolution within each product varies between manufacturers
- Age of product is a factor
- **Each change to these processes was assessed by the regulators** for associated risk and the required data

Remember: The process defines the product!

Schneider, Christian K. "Biosimilars in rheumatology: the wind of change."*Annals of the rheumatic diseases* 72.3 (2013): 315-318.

Topics

- Biopharmaceuticals: A Closer Look
- Analytical Characterisation of Biologics
- Stability Testing
- Comparability Testing

Thank You