EDQM & European Pharmacopoeia: State-of-the-art Science for Tomorrow's Medicines

International Conference organised by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe

19-20 June 2019, Strasbourg, France

Workshop on Biotherapeutics

Moderator

Mr Peter MJM Jongen, Chair of the European Pharmacopoeia Group of Experts on P4 Biologicals

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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Biotherapeutic Products in the Ph. Eur.: Have all the challenges been tackled?

Dr Mihaela Buda
European Pharmacopoeia Department
EDQM, Council of Europe

… EDQM Conference in Tallinn 2016

- Discussion focused on the **role of public standards in the field of biosimilars.**
- Opposition to individual monographs is often caused by an **unfortunate misuse and misinterpretation of monographs** (*e.g.* monographs being used to assess biosimilarity outside Europe) → enhance **communication**, promote trainings, scientific publications……
- Unanimous recognition of **importance of establishment of continuity of biological activity** → achieved by the choice of **robust pharmacopoeial bioassays and appropriate reference standards**.
- **Flexibility** built into individual monographs for biotherapeutic products allows for biosimilars not to be excluded from the market; however, too much flexibility can lead to meaningless standards.
- Further **feedback on the current approach** to address the elaboration of **monographs for complex molecules** is awaited following publication of **Infliximab monograph**.

Presentation Outline

- ❑ **Introduction**: some achievements since the Tallinn Conference
- ❑ **Ph. Eur. and flexibility**: the case of biotherapeutic product monographs
- ❑ **Monograph elaboration/revision process:**
  - participation and role of stakeholders
- ❑ **Monograph implementation** – impact on already approved products:
  - **Infliximab case study**
- ❑ Summary and concluding remarks

… SINCE Tallinn Conference

GaBI Journal
Generics and Biosimilars Initiative

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Home » GaBI Journal » Volume 5 / Year 2016 / Issue 4 » Special Report » The role of European Pharmacopoeia monographs in setting quality standards for

The role of European Pharmacopoeia monographs in setting quality standards for biotherapeutic products

Generics and Biosimilars Initiative Journal (GaBI Journal). 2016;5(4):174-9.
DOI: 10.5639/gabij.2016.0504.045

Published in: Volume 5 / Year 2016 / Issue 4
Category: Special Report
Page: 174-9
Author(s): Emmanuelle Charton, PhD
Visits: 8111 total, 8 today

Keywords: biotherapeutic products, complexity of biologicals, monographs, pharmacopoeia, public standards

Abstract:
European Pharmacopoeia (Ph. Eur.) monographs for biotherapeutic products have existed since the 1990s and remain the publicly available standard defining the quality of these medicines. Continued development of such monographs however faces considerable challenges in the current environment. This manuscript addresses what the main challenges are (complexity of biologicals, setting of specifications, relations with biosimilars) and how they are overcome.

PHARMEUROPA BIO & SCIENTIFIC NOTES

Elaborating Ph. Eur. monographs for biotherapeutic proteins using substances from a single source 129

Elaborating European Pharmacopoeia monographs for biotherapeutic proteins using substances from a single source

M. Buda, S. Wicks, E. Charton[1]

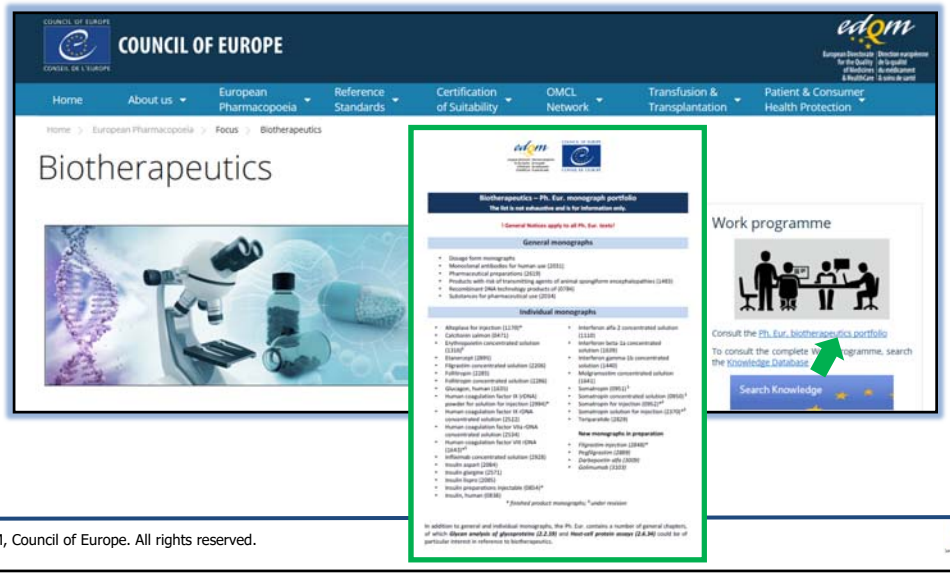
ABSTRACT

For more than twenty years, the European Pharmacopoeia (Ph. Eur.) monographs for biotherapeutic proteins have been elaborated using the multisource approach (Procedure 1), which has led to robust quality standards for many of the first-generation biotherapeutics. In 2008, the Ph. Eur. opened up the way towards an alternative mechanism for the elaboration of monographs (Procedure 4-BIO pilot phase), which is applied to substances still under patent protection, based on a close collaboration with the innovator company, to ensure a harmonised global standard and strengthen the quality of the upcoming products. This article describes the lessons learned during the P4-BIO pilot phase and addresses the current thinking on monograph elaboration in the field of biotherapeutics. Case studies are described to illustrate the standardisation challenges associated with the complexity of biotherapeutics and of analytical procedures, as well as the approaches that help ensure expectations are met when setting monograph specifications and allow for compatibility with the development of biosimilars. Emphasis is put on monograph flexibility, notably by including tests that measure process-dependent microheterogeneity (e.g. glycosylation) in the Production section of the monograph. The European Pharmacopoeia successfully concluded the pilot phase of the P4-BIO during its 159th session on 22-23 November 2016.

Scientific articles on pharmacopoeial matters

EDQM December 2016

New Section Dedicated to Biotherapeutics on EQDM Website



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Home > European Pharmacopoeia > Focus > Biotherapeutics

Biotherapeutics

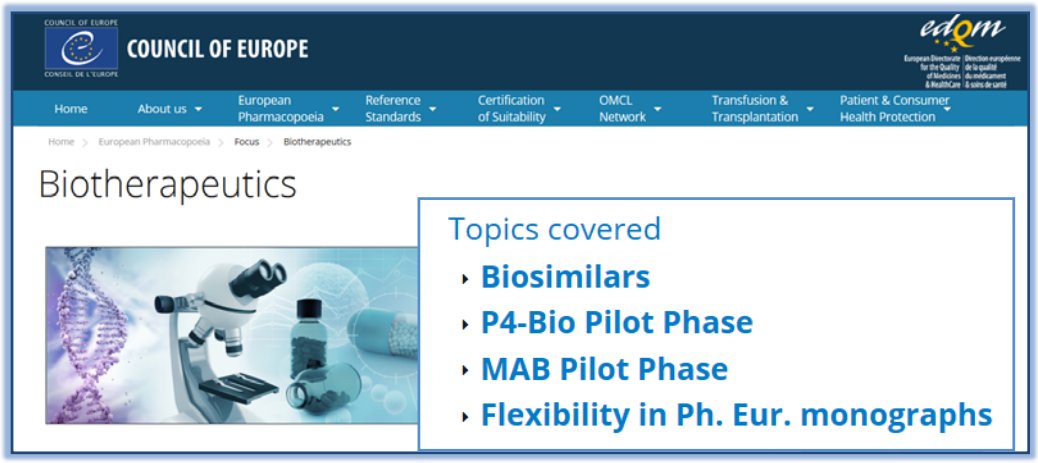
Work programme

Consult the Ph. Eur. biotherapeutics portfolio

To consult the complete Work programme, search the Knowledge Database

Search Knowledge

New Section Dedicated to Biotherapeutics on EQDM Website

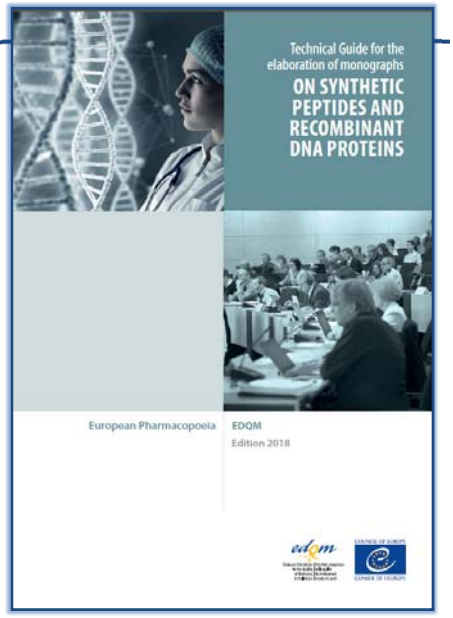


Related **news**; Related **articles**; Related **events** (e.g. trainings, webinars); Additional information (e.g. **Technical guide**)…

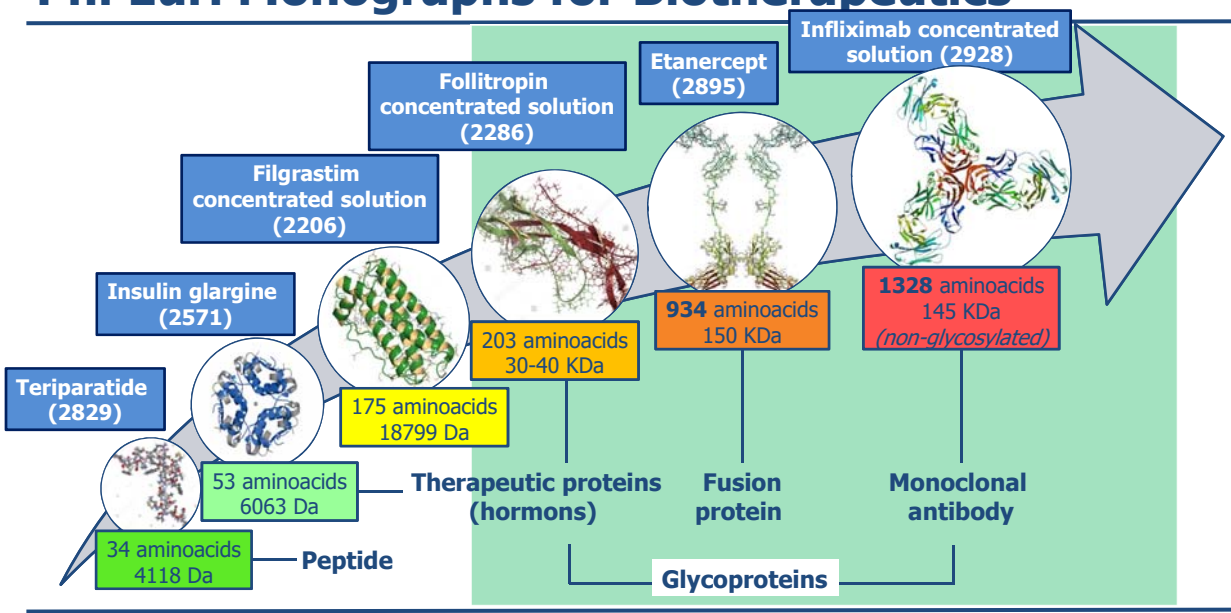
Technical guide for the elaboration of monographs on recombinant DNA proteins and synthetic peptides (Edition June 2018):

- general update to take into account recent experiences on elaboration of monographs for complex proteins;
- new section '**Flexibility**'.

https://www.edqm.eu/en/biotherapeutics



Ph. Eur. Monographs for Biotherapeutics



Ph. Eur. Monographs for Complex Biotherapeutics

Monograph specifications

- Flexibility of expectations, so that they apply to a large variety of products:
  - **Ph. Eur. General Notices** (alternative methods; waiving of tests; enhanced approaches);
  - **"Additional" flexibility**.
- Prescriptive requirements so that the respective test procedures can be applied successfully in a control laboratory/allow independent testing:
  - **method performance (system suitability) criteria**; qualification of analytical methods using Ph. Eur. standards;
  - acceptance criteria; standardisation of potency/functionality.

Ph. Eur. Monographs for Biotherapeutics: Flexibility

Production section (Ph. Eur. General Notices)

- Requirements related to process-dependent heterogeneity (*e.g.* glycan profile, charged variants)

Test procedures

- Generic methods of analysis (*e.g.* developed according to general chapters) – **suitable** methods
- Specific analytical procedures – '**example**' method

Acceptance criteria for quality attributes

- Numeric limits/ ranges (specific activity; primary structure; related proteins; HMW species)
- 'As authorised by the competent authority' (process-dependent quality attributes)

Reference preparations

- Ph. Eur. reference standards to evaluate method performance (system suitability)
- *In-house reference preparation* – for comparative purpose (*e.g.* matching LC profiles)

Monograph flexibility

Individual monographs can address complexity of biotherapeutics

Monograph for Biotherapeutics: Additional Flexibility

SUITABLE METHOD

- **general indications** on the test procedure (main steps to be carried out, type of method, readout, cells, reagents…)
- the term "suitable" is a **conventional term:** '*In certain monographs […], the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.' (General Notices)*

EXAMPLE METHOD

- **specific instructions**, quantities, concentrations, compositions of reagents/buffers, chromatographic conditions etc. together with **system suitability criteria**; method may be used as such but any other suitable validated procedure may be used without demonstrating its equivalence to the 'example' method (subject to approval by the competent authority);
- ***"The following procedure is given as an example."***

Ph. Eur. Monograph Elaboration/Revision: the Process

Monographs are based on quality described for **registered products**

Call for interest

Participation of interested parties

Responding to Pharmeuropa enquiry is a must

OMCLs, assessors, companies

1. Request for monograph elaboration/ revision
2. Endorsement by the Ph. Eur. Commission
3. Assignment to a Group of Experts
4. Creation/ revision of the text by the Group
5. Public enquiry in Pharmeuropa
6. Text adopted by the Ph. Eur. Commission
7. Publication in the Ph. Eur.

survey among NPAs

- **Data package** (current specifications, analytical procedures; validation data; batch and stability data)
- **Material** for testing
- *Candidate material for RS establishment*

- **Review of data package**
- **Draft monograph development**
- **Laboratory study/ collaborative testing – <u>all</u> preparations** (protocol preparation; method verification; data analysis)

- **Draft published for comments** (testing of draft monograph) – 3 months commenting period
- **Evaluation of stakeholder feedback** (technical comments, data)

Once a monograph is published and implemented, MAH's of registered products have to assure their product meets the requirements of the monograph

Impact of Monographs on Already Approved Products

If a monograph is revised/published, what is the **impact** on the **already approved product(s)**?

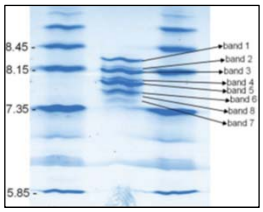
- ❑ Compliance with the Ph. Eur. monograph is mandatory, manufacturers have to meet the requirements of the (revised) pharmacopoeial text at the date of its implementation (6 months after publication of the new/revised text company).
  → Company evaluates and secures compliance with the monograph within 6 months.
- ❑ This is why it is important that key stakeholders get involved in the monograph elaboration (revision) process <u>as early as possible</u>.
- ❑ This is why monographs are published for consultation.

Scenario 1: Charged Variants

Ph. Eur. Monograph

A. Isoelectric focusing – gel electrophoresis (Ph. Eur. 2.2.54)

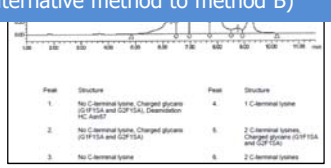
- **test procedure**: example method
  - system suitability: pI markers; infliximab CRS
- **acceptance criteria (isoforms)**:
  - comparison with in-house RS profile



Alternatively, use a **suitable** capillary isoelectric focusing **method** developed according to general chapt

B. CEX-HPLC (Ph. Eur. 2.2.29)

- **test procedure**: prescriptive require
  - system suitability: infliximab CRS
- **acceptance criteria (isoforms ●■◆)**:
  - in-house reference preparation
  - limits: 'as authorised by the competent authority'

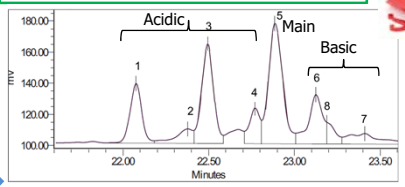


Ph. Eur. General Notices:

- **demonstration of compliance** with the Ph. Eur.
- **alternative methods** (*e.g.* demonstrate equivalence of alternative method to method B)

"Company A" (registered product)

Capillary isoelectric focusing



Limits for main peak, acidic peaks and

Scenario 2: Potency/Specific Activity

Ph. Eur. Monograph

Potency – TNF-alpha neutralisation

- suitable cell-based assay and a suitable readout for assessing the inhibitory effect of infliximab on the biological activity of TNF-alpha; infliximab BRP (assigned potency in IU)
- example method: WEHI-164 cytotoxicity assay; WST-8 colorimetric readout
  - **method performance**/system suitability: infliximab BRP
  - **estimated potency**: 80-120% relative to infliximab BRP (numeric range)

Specific activity*

- 8×10^3 to 12×10^3 IU per milligram of protein

"Company B" (registered product)

- **U937 apoptosis assay**;
  - **in-house RS**
  - estimated potency: x-y% (relative to in-house RS)

- In-house RS (working standard) to be established by **comparison with infliximab BRP** to which it is traceable. (Ph. Eur. *Reference standards (5.12)*)

Protein content + **Potency (IU)**

- **Specific activity*:** fulfills pharmacopeia requirements

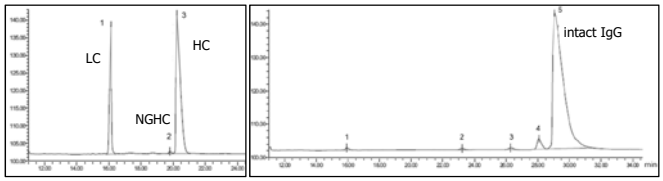
** As indicated under section "Definition"*

Scenario 3: Related Proteins by CE-SDS

Ph. Eur. Monograph

Capillary electrophoresis (2.2.47) under both reducing and non-reducing conditions

- **test procedure**: prescriptive requirements
  - system suitability: infliximab CRS
- **limits**:
  - reducing conditions: ∑ peaks other than HC and LC: ≤ 2%
  - non-reducing conditions: ∑ peaks other than IgG peak: ≤ 8%



LC: light chain; HC: heavy chain;
NGHC: non-glycosylated heavy chain

"Company C" (registered product)

~~**Different CE-SDS method**~~

~~**Ph. Eur. General Notices:**~~
- ~~**demonstration of compliance** with the Ph. Eur.~~
- ~~**alternative methods** (equivalence testing)~~

Higher limits

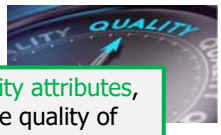
Ph. Eur. Monograph Elaboration/Revision:

Do not miss **Pharmeuropa!**

Ph. Eur. Monographs for Biotherapeutics: Other Cases...

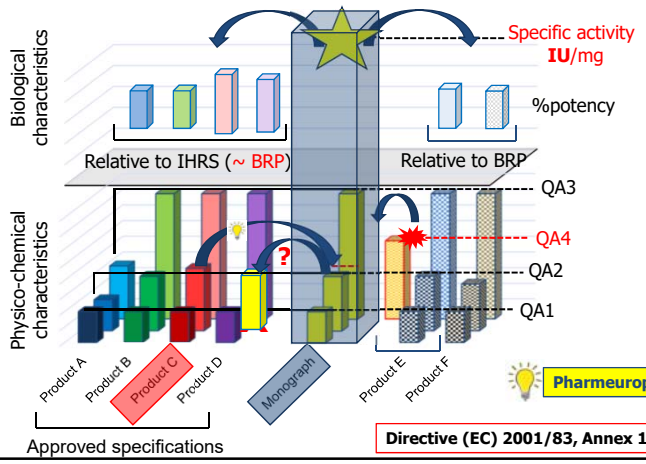
One part of a total control strategy designed to ensure product quality and consistency

Public standard



A set of relevant quality attributes, in order to confirm the quality of the active substance in question

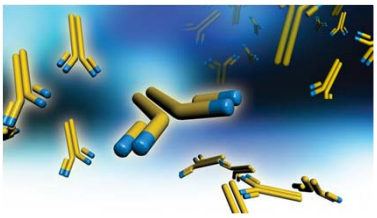
Simulation of impact of a monograph – hypothetical situation



The Pharmacopoeia monograph ensures continuity of product quality

Concluding Remarks

- Individual monographs play a major role in ensuring a standardised level of quality for medicinal products, thus contributing to patient safety.
- Ph. Eur. has over 30 years experience in the elaboration of texts on biotherapeutic products.



- Elaboration of monographs for biotherapeutic products present a number of challenges due to their complexity (meaningful standard *vs* flexibility).
- The latter challenge has proven to be more difficult to overcome; still, with the recent adoption of Infliximab monograph, Ph. Eur. has paved the way for an important class of new generation biotherapeutics.

Discussion on how to continue develop these standards and ensure their effective implementation needs all stakeholders!

Towards Ph. Eur. 10th Edition: Major Milestones

Finished Product Monographs

P4Bio pilot phase

MAB pilot phase

Infliximab (2928)

Etanercept (2895)

Filgrastim injection (2848)

- **General methodologies ('horizontal standards')**
  - Bioassay
  - CE-SDS
  - cIEF
- **Golimumab** concentrated solution (3103)

UNDER ELABORATION

- Teriparatide injection (3130)
- Insuline glargine injection (3129)
- Human coagulation factor VIII (rDNA) powder for injection (3106)
- Human coagulation factor VIII (rDNA), B-domain-deleted, powder for injection (3108)

.....

Acknowledgements

Many thanks to:

Olga Kolaj-Robin
Gwenaël Ciréfice
Emmanuelle Charton

Thank you for your attention

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Ph. Eur. Reference Standards for Biotherapeutic Products

Dr. Marie-Emmanuelle Behr-Gross
Scientific Officer
DBO Department

EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow's Medicines
19-20 June 2019, Strasbourg, France

07/2018:51200

5.12. REFERENCE STANDARDS

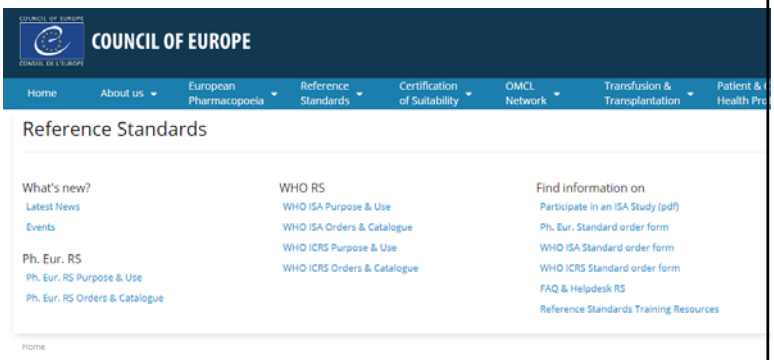
This chapter is published for information.

1. INTRODUCTION

'Reference standard' is used in this chapter as a general term covering reference substances, reference preparations and reference spectra.

Reference standards are frequently necessary to achieve adequate quality control of medicinal products and their components.

Reference standards are established using suitable procedures and their continued suitability for use is monitored according to a predefined programme. Where a reference standard is needed, it is an integral part of the pharmacopoeial monograph or the manufacturer's specification. Where a European Pharmacopoeia reference standard is referred to in a monograph or general chapter, it represents the official standard that is alone authoritative in case of doubt or dispute.



https://www.edqm.eu/

Biological Reference Preparations for Biotherapeutics

European Pharmacopoeia biological reference preparation (BRP). A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. BRPs are either secondary standards calibrated in International Units or primary standards, which may be used to define a European Pharmacopoeia Unit (Ph. Eur. U.). Other assigned contents may also be used, for example, virus titre or number of bacteria.

63 BRPs are currently distributed by the EDQM see list

Example: Infliximab BRP batch 1

Infliximab Biological Reference Preparation (BRP) Batch 1

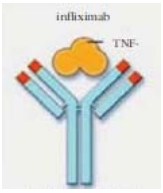
Use in Infliximab potency bioassay in monograph 2928

Principles

Suitable cell-based assay allowing to determine the **inhibitory action of infliximab on the biological activity of TNF-α**

Suitable read out for assessing this effect

Standard Infliximab BRP

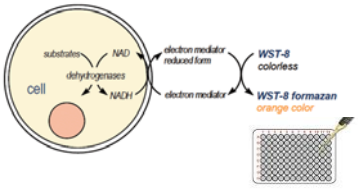


Adapted source: Nestle F. et al. 2009

Example procedure

Cytotoxicity assay, measuring the cytotoxic effect of TNF-α on **WEHI-164 cells.** Cell growth assessed through a **colorimetric assay**

Ability of Infliximab to block inhibition of proliferation of WEHI-164 assessed against BRP1



Infliximab BRP batch 1 – Establishment Frame and Study

Frame: Biological Standardisation Programme **(BSP**) an applied research programme for the establishment of **Biological Reference Preparations (BRP)** carried out with funding by the European Union and by the Council of Europe

Establishement study for Infliximab BRP1: BSP146 performed jointly with 1st WHO International Standard (IS) establishment study through a collaborative study involving 26 laboratories (public and private sector) using different in vitro cell-based bioassays and binding assays

WHO/BS/2017.2323, https://doi.org/10.1080/19420862.2018.1532766

Infliximab BRP batch 1 – Establishment - Some Data

Materials tested

Candidate freeze dried reference preparation (16/170) prepared from a single batch of bulk recombinant „biosimilar" Infliximab samples A and C

Comparator freeze dried product (16/160) prepared from a commercial pharmaceutical preparation sample B

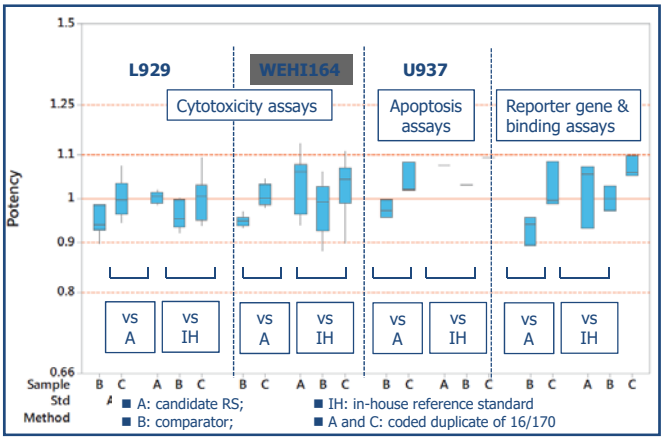
Where available in-house standards IH

Final outcome

Candidate freeze dried reference preparation 16/170 was adopted as the 1st WHO IS and as Ph. Eur. BRP1 with an assigned potency of 500 IU/ampoule

*

Infliximab BRP batch 1 – Establishment – Outcome example



Adapted from C. Metcalfe et al, *The first World Health Organization International Standard for infliximab products: A step towards maintaining harmonized biological activity*, MABS, 2018

Infliximab BRP batch 1 – Establishment - Some Data

Method	Sample	Potencies relative to Candidate A					Potencies relative to IH reference				
		GM	LCL	UCL	GCV	n	GM	LCL	UCL	GCV	n
WEHI-164 cytotoxicity assay	A						1.04	0.97	1.10	7.3%	7
	B	0.95	0.94	0.96	1.3%	9	0.98	0.92	1.04	6.7%	7
	C	1.01	0.99	1.03	2.6%	9	1.03	0.96	1.10	7.5%	7
Overall cell-based neutralisation assays*	A						1.02	0.99	1.06	5.9%	16
	B	0.95	0.94	0.96	2.7%	22	0.98	0.96	1.01	5.1%	16
	C	1.01	1.00	1.03	3.9%	21	1.03	1.00	1.07	6.5%	17

* Cytotoxicity using L929, WEHI-13 cell-lines; U937 apoptosis assay and reporter gene assays used in the WHO study.

WEHI-164 cytotoxicity assay – individual laboratory results:

- potency estimates within labs are very similar for preparations relative to A with GCVs < 11.6%.

(EDQM article under preparation for publication in Pharmeuropa Bio & Scientific Notes)

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Usefullness of BRPs for biotherapeutic testing

The Ph. Eur. reference **BRPs are qualified for the intended use(s)** described in Ph. Eur. monographs and the publication of the results of the collaborative studies run for their establishment allows to grant access to important information on

- **Methods**: critical reagents, SOPs, intra and inter-lab variations, stats…
- **References**: IH or commercial standard, BRP (candidates, former batch), IS
- **Products**: test panel (products of different origins; princeps & biosimilar(s),…)

Infliximab: https://doi.org/10.1080/19420862.2018.1532766

Etanercept: https://doi.org/10.1016/j.jim.2017.03.007

10

Usefullness of BRPs for biotherapeutic testing

Their intended use is for the determination of **pharmaceutical quality** in line with compendial standards and to serve that purpose they are available for

- European Regulatory authorities e.g. EMA for Centrally Authorised Products (CAP) post marketing studies (feasibility study on the model of "generics studies" for Filgrastim completed, Etanercept planned; third to come)
- Official Medicines control laboratories (OMCLs) for batch release, national market surveillance studies, for internal and external QA purposes, …
- Manufacturers for development and control, for batch release and stability studies…

As such they are **not to be used for other purposes and notably in vivo assays, clinical comparability studies nor for defining biosimilarity**, and BRPs should not be inferred as serving this purpose

11

Ph. Eur. Reference Standards for Recombinant Glycosylated Biotherapeutics

Dr. Sylvie Jorajuria
Head of the Biology Section
Laboratory Department

EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow's Medicines
19-20 June 2019, Strasbourg, France

Why is glycosylation important for biotherapeutics?

1) Glycosylation impacts properties of the protein:
   - Bioactivity
   - Pharmacokinetics
   - Stability
   - Immunogenicity

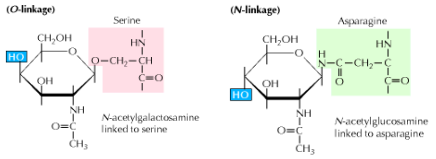
2) Glycosylation:
   - Non-template-driven enzymatic modification
     -> glycan heterogeneity

3) Heterogeneity impacts product quality:
   - Batch to batch variability
     - inconsistency of production
     - risk of out of specification

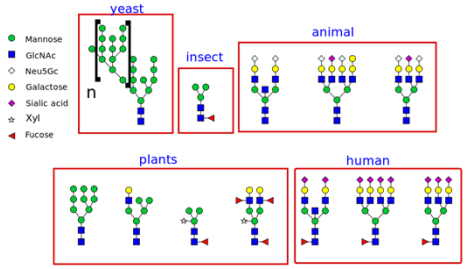
➡ Glycosylation must be: qualitatively and quantitatively controlled at all stages of the lifecycle and therefore must be controlled by the monograph

Heterogeneity of the protein glycosylation

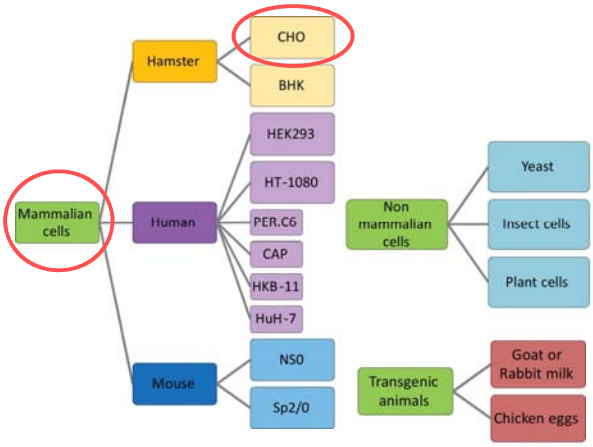
1) Two major types of glycosylation: O- and N-linked



2) Microheterogeneity in N-linked glycans



3) Expression system for rDNA proteins



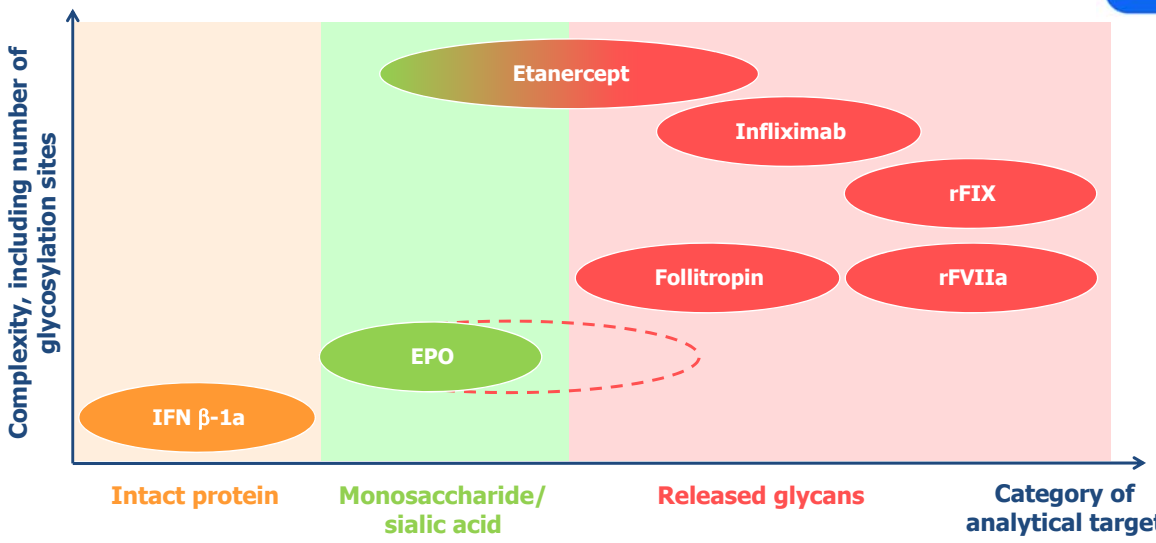
Glycan analysis procedures in the Ph. Eur.

General chapter: 2.2.59. Glycan analysis of glycoproteins

Heterogeneity in glycosylation is assessed by 4 distinct and complementary approaches:

Analytical target	Structure	Resulting information
Intact glycoprotein		overall pattern of glycosylation of the glycoprotein, limited information when the molecule is large and contains multiple glycosylation sites
Glycopeptides		site-specific glycosylation properties, degree of occupancy, oligosaccharide structures
Released glycans:		populations of glycans present on the protein (bi-, tri-, and tetra-antennary profile), degree of sialylation
labelled		
unlabelled		
Monosaccharide:		monosaccharide composition of a glycoprotein
labelled		
unlabelled		

Glycan analysis in Ph. Eur. individual monographs



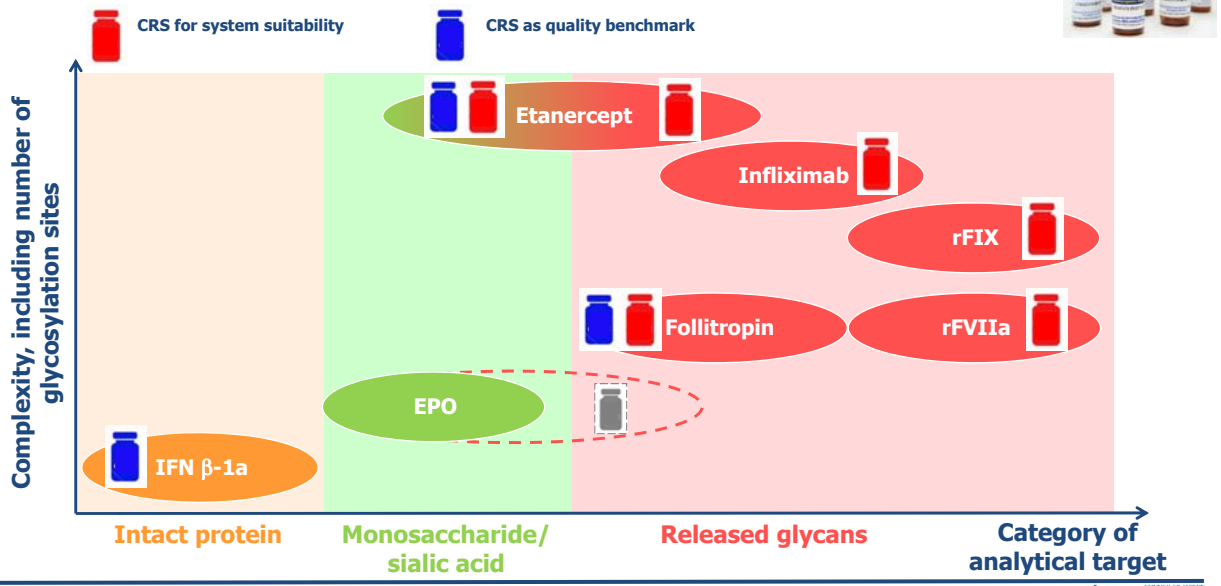
Intended purpose of Ph. Eur. RS in glycan analysis

- Reference standards for glycan analysis may serve 2 functions:
  - to control the performance of the method, including glycan cleavage, recovery and analysis
    -> system suitability

"The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system" Ph. Eur. 2.2.46.

  - to assess acceptance criteria (qualitative, quantitative)
    -> quality benchmark

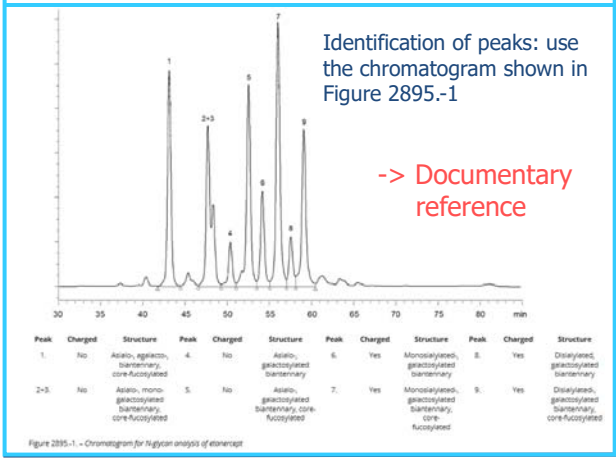
Intended purpose of Ph. Eur. RS in glycan analysis



Confirmation of identity of the analytical target

Means: chromatogram included in the monograph

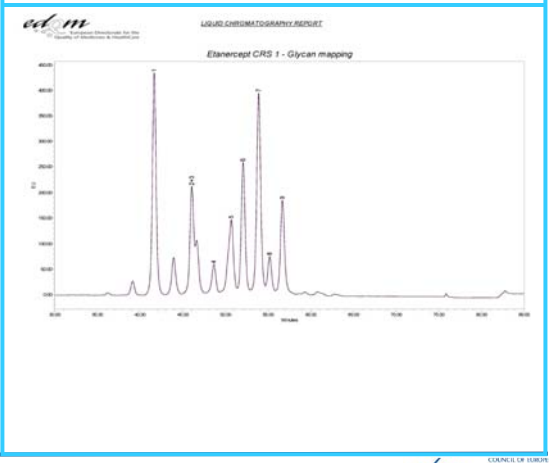
Etanercept monograph – Glycan mapping



Identification of peaks: use the chromatogram shown in Figure 2895.-1

-> Documentary reference

Etanercept CRS



Confirmation of compliance with qualitative requirements

Means: CRS for system suitability and in-house reference preparation

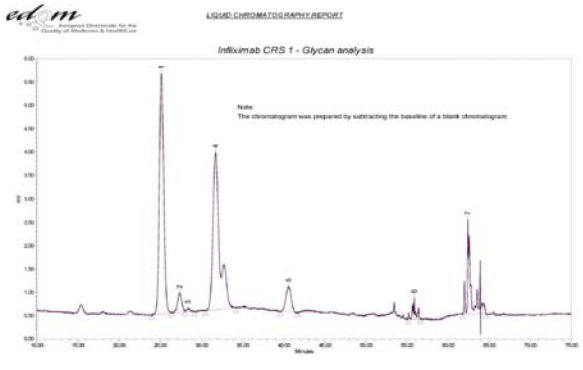
Infliximab monograph – Glycan mapping

- System suitability:
- the chromatogram **obtained** with infliximab CRS is qualitatively similar to the chromatogram **supplied** with infliximab CRS and peaks 1 to 7 are clearly visible

- Results:
- the profile of the chromatogram and the retention times of the peaks obtained with the test solution corresponds to that obtained with the chromatogram obtained with a suitable infliximab in-house reference preparation

-> Consistency of production using a production process specific reference standard

Infliximab CRS

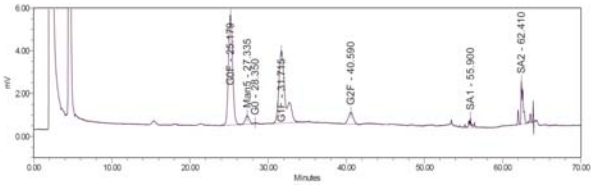


Confirmation of compliance with qualitative requirements

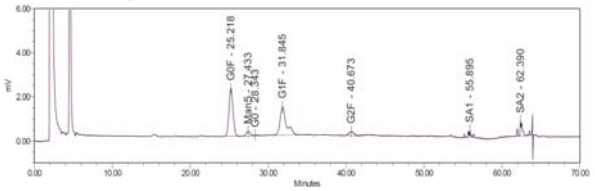
Monograph is applicable to Infliximab produced in mammalian cell expression system

Sp2/0 cell line

Infliximab, manufacturer 1

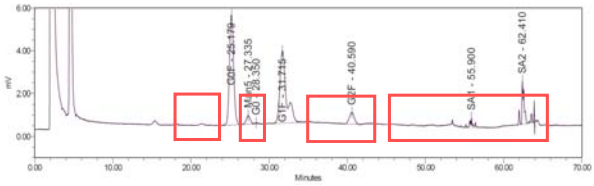


Infliximab, manufacturer 2



CHO cell line

Infliximab, manufacturer 3 *(simulation, expected differences)*



- **Infliximab CRS** is used for system suitability
- For consistency of production purpose, an in-house reference preparation is required

Confirmation of compliance with quantitative requirements

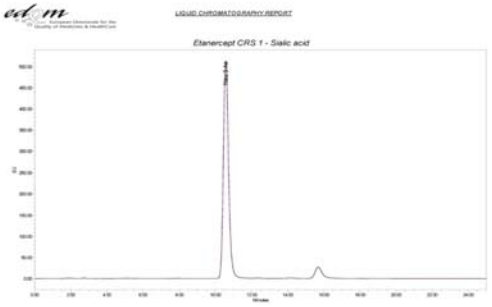
- Quantitative measurement of analyte levels

Means: CRS for system suitability and quality benchmark

Etanercept monograph – Test for Sialic acid

- Reference standard for sialic acid (e.g. N-acetylneuraminic acid, NANA) to prepare a calibration curve
- System suitability: the peak due to sialic acid in the chromatogram **obtained** with etanercept CRS is visible and is similar to the corresponding peak in the chromatogram **supplied** with etanercept CRS
- Results: the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with etanercept CRS

Etanercept CRS



Confirmation of compliance with quantitative requirements

- Quantitative expression of separation profile

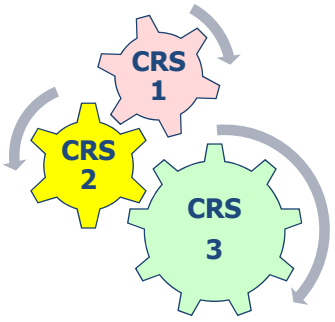
Means: no quantitative criterion of the CRS

Infliximab monograph

Calculate the percentage contents of fucosylated, afucosylated and sialylated glycans, by normalisation

-> only for test solution

-> Sustainable CRS strategy



Opportunities/lessons from the past

- Extend the CRS role to control the performance of the methods
- Acceptance criteria in the monograph must be specific (i.e. measurable attributes) and not embedded in a particular batch of candidate CRS

  Otherwise when the Ph. Eur. RS is replaced, the new batch may not be able to satisfy the same need without impacting the acceptance criteria
- Caution when using Ph. Eur. RS as benchmarking tool because its quality attributes may be mistakenly considered as the monograph specification

Key messages

- Ph. Eur. RS is just "a" material:
  - not necessarily related to the reference product
  - not necessarily related to the monograph specifications
  - is fit for the intended purpose
- Flexibility is built in and monograph provides means for transferability of the analytical procedure and independent testing

Perspective: trends in glyco-engineering

Optimization and control of N-glycan profiles through:

- Cell culture engineering (media, parameters)
- Genetic modification of glycosylation mediators

  Ex: to enhance the desired glycoforms (afucosylated glycoforms: ⬆ mAb ADCC)
- Mathematical models: to predict glycosylation

-> Recombinant glycosylated biotherapeutic monographs and corresponding CRS will have to keep pace with development

Thank you for your attention

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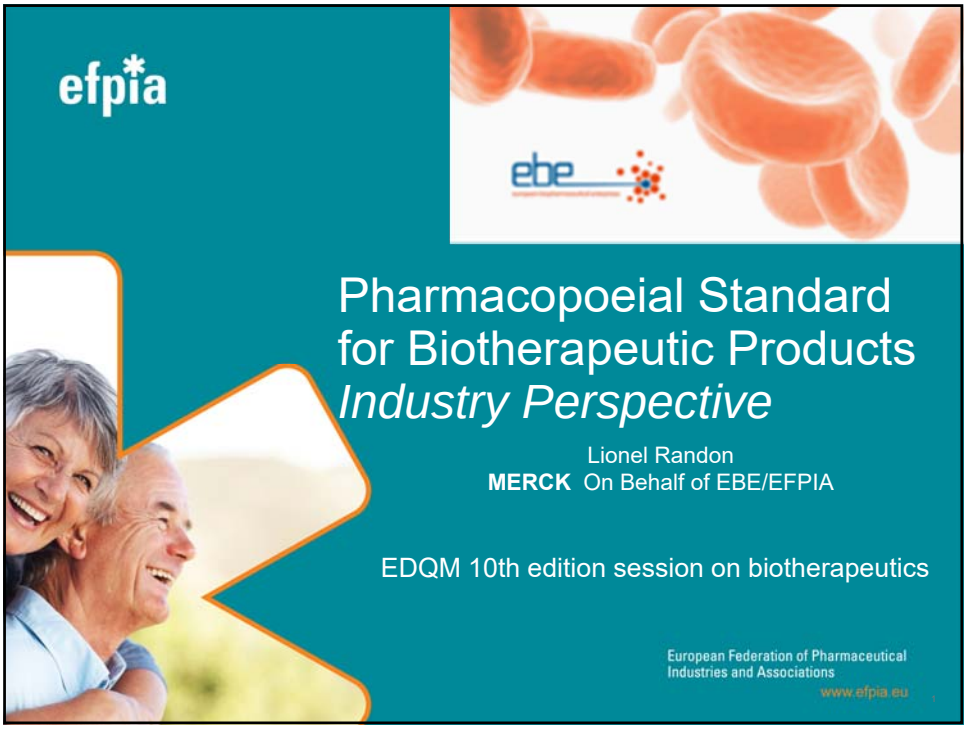

Complying with Public Standards for Complex Biological Molecules

- What must a manufacturer do to ensure compliance with the PhEur. ?
  - Adopt current PhEur. monograph
  - Request for revision of PhEur. monograph
  - Justification of equivalence and/or superiority of a testing in Divergence with PhEur. Monograph
- What does this mean for a biopharmaceutical manufacturer?
  - Substantial and challenging workload to adopt new methods and revise specifications or demonstrate equivalence of Alternative Methods combining with a risk of rejection
  - Regulatory submissions to request approval from HAs for changes

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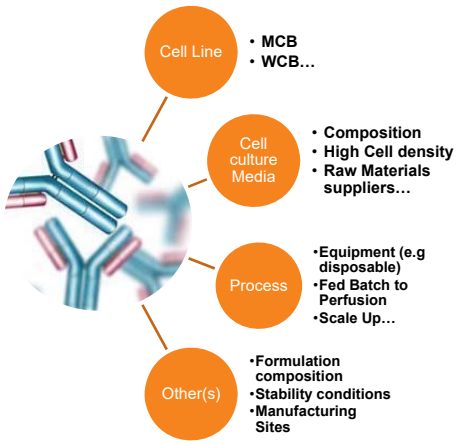
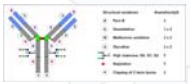
Typical Post CMC Changes having Impact on Quality Product Attributes / Characteristics

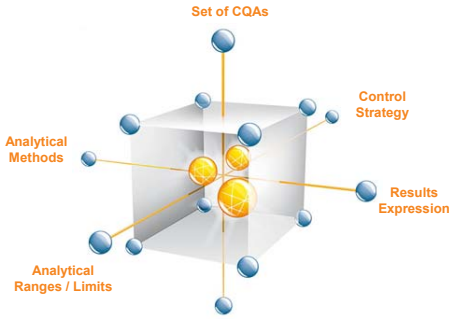
* Process changes are inevitable during LCM looking for optimization due to market demand, patient-centric approach and competitive environment.



Case Study: Introduction

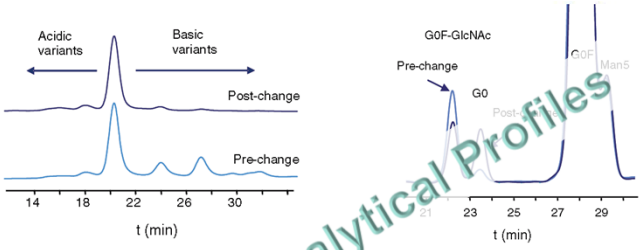


* Substantial disparities between product quality profiles/characteristics and/or analytical results doesn't prevent demonstration of Comparability / Biosimilarity
* Different analytical strategy and methods are used between manufacturers
* The assessment of criticality depends on the demonstration of impact (or non-impact) on the biological activity and PK of the variant

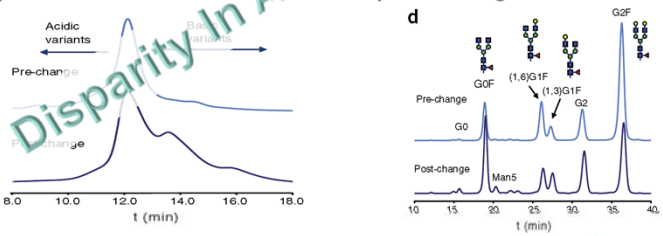


Case Study: Comparability Results on Licensed Products

- Comparison of the different pre- and post-change batches of Rituxan



- Comparison of the different pre- and post-change batches of Enbrel

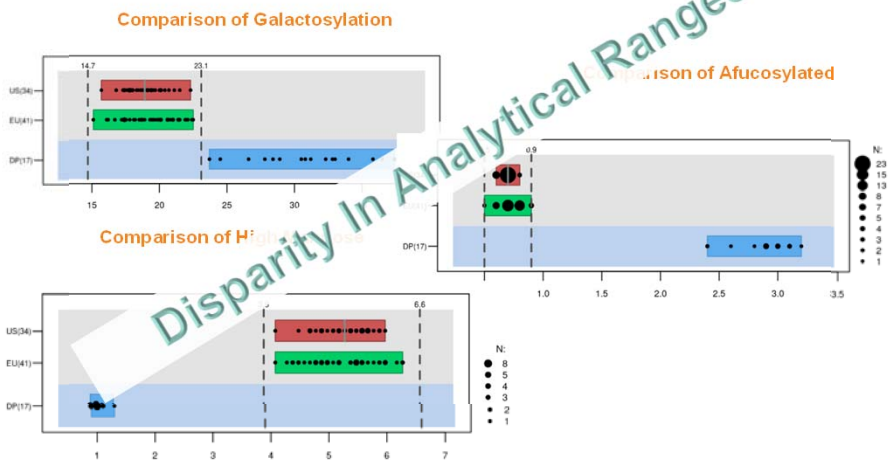


Disparity In Analytical Profiles

(1) Acceptable changes in quality attributes of glycosylated biopharmaceuticals 2011 Nature America, Inc. Martin Schiestl, Thomas Stangler

Case Study: Comparability Results on Licensed IgG1

- Heterogeneity due to glycosylation



Disparity In Analytical Ranges

Case Study: Comparability Results on Licensed IgG1

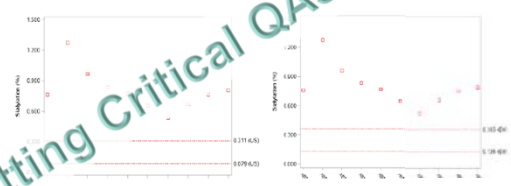
* Heterogeneity due to glycosylation

Manufacturer 1

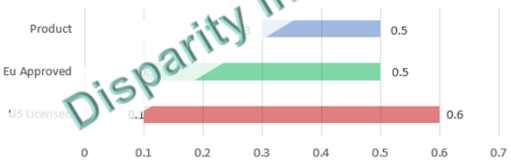
Manufacturer 2

Comparison of Sialylation

Sialylation
Not specifically reported



Comparison of Glycation



Glycation
Not reported

Disparity In setting Critical QAs

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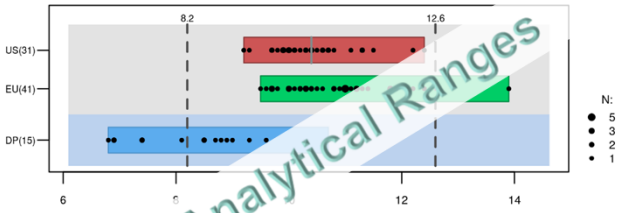
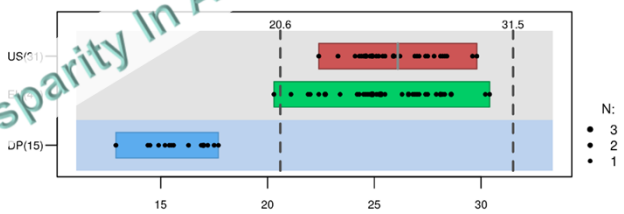
Case Study: Comparability Results on Licensed IgG1

* Product related Impurities_Charge Variants (covering Deamidation, C & N Terminal Variant, Oxidation)

Comparison of Acidic Variants CEX-HPLC



Comparison of Basic Variants CEX-HPLC



Disparity In Analytical Ranges

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Case Study: Comparability Results on Licensed IgG1

Manufacturer 1

Manufacturer 2

Comparison of Purity (aggregates and Product related impurities) Testing Strategy

Quality Attribute	Test Method
Monomer	SEC
HMW variants	SEC & AUC
Dimers	AUC
Antibody fragments (LC & HHL)	SEC & nrCE-SDS
Intact IgG	nrCE-SDS
Acidic/ Basic Variants	CEX-HPLC

Quality Attribute	Test Method
Monomer	SEC & AUC
HMW variants	SEC & AUC
Antibody fragments (HC, LC, HHL, LMW, MMW)	SEC & nrCE-SDS
Intact IgG + Pre peaks	nrCE-SDS
Acidic/Main/Basic Variants	CEX-HPLC

Disparity in Analytical Methods & Expression Results

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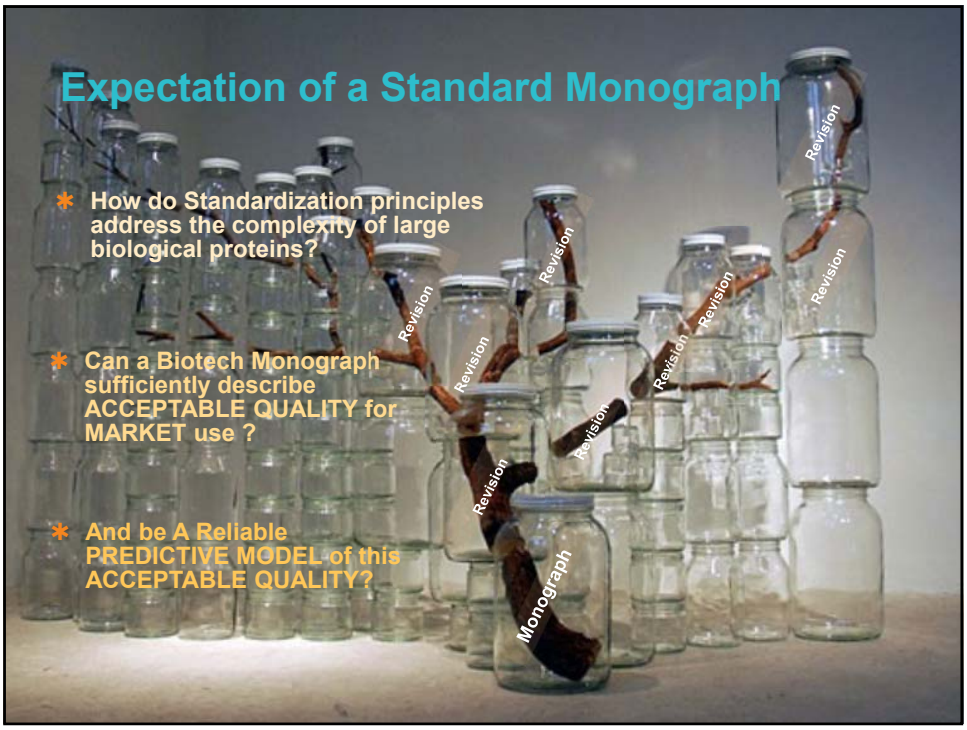
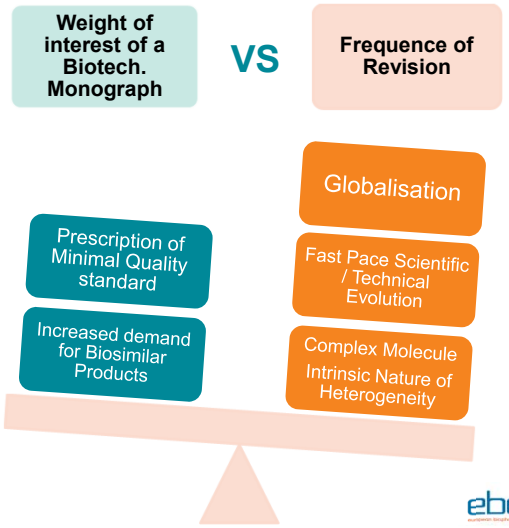
Expectation of a Standard Monograph



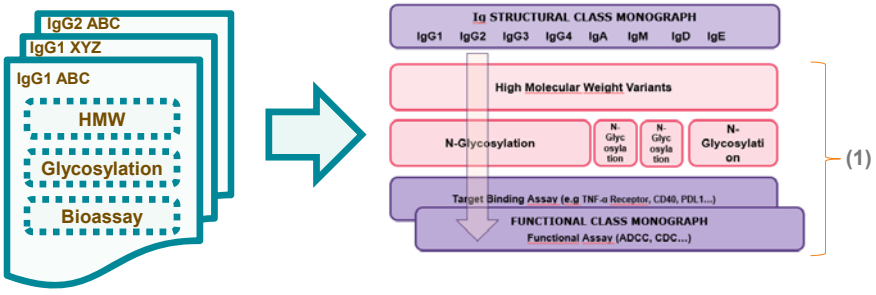
- How do Standardization principles address the complexity of large biological proteins?
- Can a Biotech Monograph sufficiently describe ACCEPTABLE QUALITY for MARKET use ?
- And be A Reliable PREDICTIVE MODEL of this ACCEPTABLE QUALITY?

Expectation of Standard Monograph_Benefice for the Patient ?



Expectation of a Standard Monograph

* A proposed forward-looking Strategy to fulfil Ph.Eur. Core Mission
  * Moving from “Product Specific” toward a “Modular Adaptative” approach involving Class & Performance based Monographs & General Texts



(1) Similarity with Ph.Eur transversale Approach

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We encourage the European Pharmacopoeia to adapt the standardisation for complex biologicals in 21st Century

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The Need for Monographs on Biotherapeutics: A Biosimilar Perspective

Dr. Emmanuel Rossy
Scientific Fellow, Analytical Characterization
EDQM and Ph.Eur.: State of the art Science for Tomorrow's Medicines
19th June 2019, Strasbourg, France

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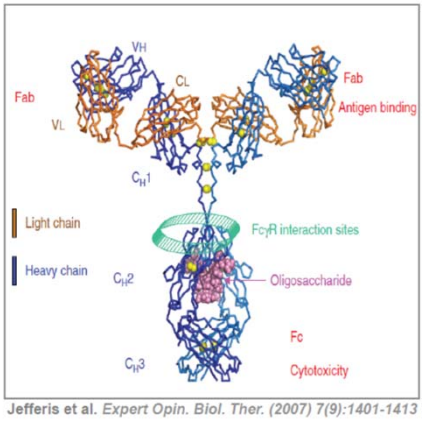
Agenda

1. Introduction
2. Infliximab case study
   - Complexity to define molecule specific attributes due to proteins natural heterogeneity
   - Need for flexible monographs
   - Need for a rapid monograph amendment process
3. Conclusion

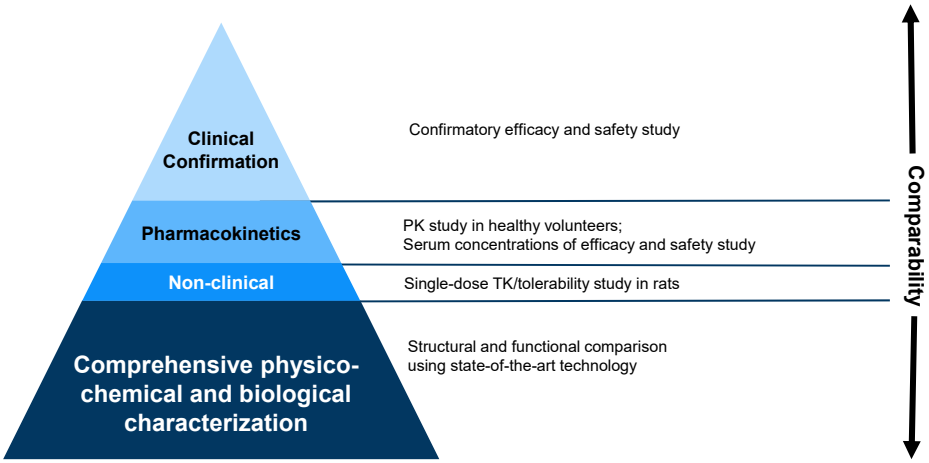
Infliximab - a well characterized monoclonal anti-TNF antibody



Jefferis et al. *Expert Opin. Biol. Ther. (2007) 7(9):1401-1413*

- Infliximab is a recombinant chimeric monoclonal IgG1 kappa antibody composed of a human constant region sequence and murine anti human Tumor Necrosis Factor alpha (TNF-α) variable sequence
- First infliximab approved by EMA in 1999 (Remicade®, Janssen Biologics)
- Monograph for Infliximab concentrated solution (2928) of Ph. Eur. is the first monograph on a monoclonal antibody (effective Jan. 2019)

Totality of the Data from comparative evaluation to the reference medicine



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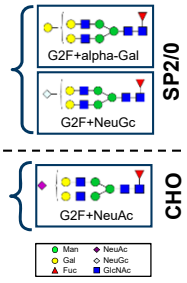
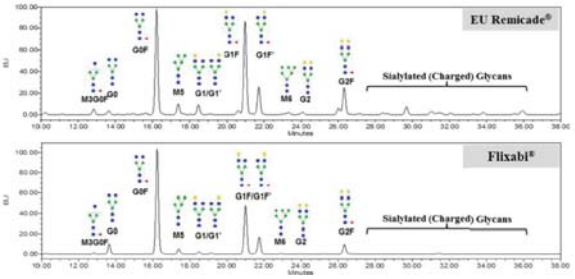
Natural Heterogeneity of Proteins is Complex to Define Adequately

- Infliximab can be produced using a CHO cell line (e.g. Flixabi®), the reference medicine with a SP2/0 cell line
- Some differences are expected e.g.
  - SP2/0 cell lines produce glycans with the alpha-gal motif and N-glycolylneuraminic acid (NeuGc)
  - CHO cells produce typically low levels of glycans with alpha-gal motif or NeuGc (they produce N-Acetylneuraminic Acid - NeuAc-





Glycosylation profiling by 2-AB labeling and hydrophilic interaction liquid chromatography coupled with fluorescence detection

Changsoo Lee, et al. (2017), mAbs, 9:6, 968-977

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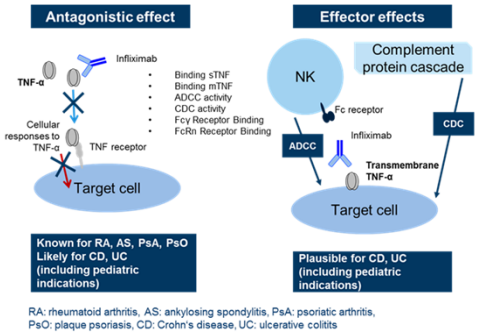
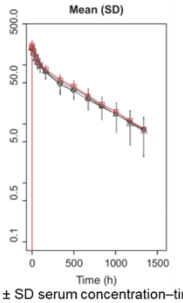
Totality of Data Demonstrated Absence of 'Clinically Meaningful Differences'



RA: rheumatoid arthritis, AS: ankylosing spondylitis, PsA: psoriatic arthritis, PsO: plaque psoriasis, CD: Crohn's disease, UC: ulcerative colitis



Mean ± SD serum concentration–time profiles following a 10-mg/kg single intravenous dose in healthy subjects

Palaparthy et al. Expert. Rev. Clin. Immu. (2018) 14(4), 329-336

- A combination of in-depth *in vitro* biological characterization and comparative PK study showed that total of glycoforms act synergistically and confirmed absence of 'Clinically Meaningful Differences'.
- Knowledge gained from structure-functions studies supports implementation of innovative approaches and latest technologies (e.g. expression system)

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Monograph Flexibility for Class-, Molecule- and Product-Specific CQAs

- Product Specific CQA understanding is gained through development and is reflected into e.g. specifications

Attributes	Limits sets from EP9.6	Potential Alternative Limits for a CHO product
N- linked Glycans	• Percentage of fucosylated glycans • Percentage of afucosylated glycans • Percentage of sialylated glycans	• G0F (Major Species) • G0 (Surrogate for total afucosylation[1]) • G1F (Surrogate for terminal galactosylation[2])
Impurities by capillary electrophoresis under reducing condition	• Sum of all peaks other than heavy chain and light chain: maximum 2 per cent	• EMA approved limits broader

[1] relevant to ADCC
[2] relevant to CDC

- Individual N-linked glycans and their levels are a product-specific CQA
- Need for monograph capable to cover both infliximab specifications

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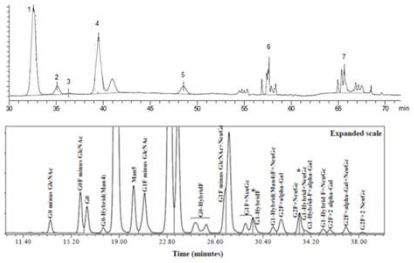
Rapid Monograph Amendment to Maintain Scientific and Regulatory Standards

- Technology, Scientific knowledge and Regulatory expectations are continuously evolving



Example glycan analysis method from monograph 2928 based on strongly basic anion-exchange chromatography couple with pulse amperometry detection (example selected from monograph 2928)

Reference medicine analysis using hydrophilic interaction liquid chromatography coupled with fluorescence detection

- Example glycan analysis method from EP 9.6 is likely not capable to address current expectations in regards to critical glycan species
- Need for a rapid and simple amendment process to adapt to standards and include all infliximabs currently approved

Conclusion

- Current perspective on product specific monographs for biologics is aligned with IFPMA and Medicines for Europe
- Infliximab monograph provides flexibility but illustrates the shortfalls of molecule specific monographs, based on a small molecule format, for complex and large biologics
- Biologics have increased complexity compared to chemicals, and this is reflected in the regulatory pathways
  - Regulatory agencies determine approval of biosimilars as they review the totality of data (Process, Methods, Limits, Analytical, functional and clinical comparability to reference product)
- The single sponsor model has its limitation as limits and methods are based on the sponsor experience with its own process
  - Limits and methods are not necessarily aligned with other scientific and regulatory viable solutions, and limits not set based on clinical impact only
- Monographs should champion high quality products and methods but provide sufficient flexibility to facilitate innovation
  - Leveraging latest technologies and methods or enhancing manufacturability for processes contribute to develop high quality and cost effective biosimilars

Infliximab

Infliximab

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Thank you

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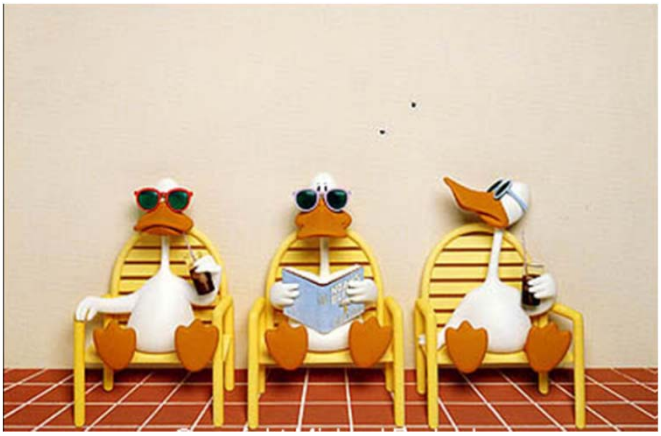
Any questions?



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Pharmacopoeial monographs for biologicals – a regulatory view

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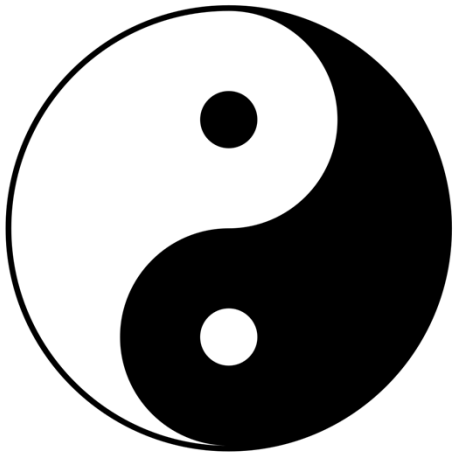
19 June 2019

EDQM and EMA



A philosophical question: Half full or half empty?

Half full or half empty?

- Interesting philosophical question, but limited practical meaning.
- *'Can I drink the contents?'*
- The same applies to monographs!
  - *'Can I use the contents?'*

Reminder: Biologicals (EU) definition

- Definition from 2001/83
  - A biological [active] substance is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physico-chemical-biological testing, together with the production process and its control.
- A biological is complex
  - Complete characterisation difficult
  - Variable
    - Between batches and between manufacturers
  - Control through testing only, not feasible
  - Case by case approach

'Minimum requirements'

- A 'complete' or 'perfect' monograph is an elusive goal
- Consistent EMA-BWP position that 'compendial monographs are minimum requirements'.
- This actually should not hamper monograph development;
  - No need to aim for a 'complete' monograph covering all aspects
  - Instead, aim for a monograph , comprising a number of parameters which are both critical and amenable to standardisation

Biological monographs

- Monographs for biologicals (active substance and finished product) have been around for decades
- Examples
  - Most (common) childhood vaccines and influenza
  - Most (common) plasma derived products, and analogues
  - Several 'classical ' biologicals
  - Several rDNA products
- In addition, many chapters on biological methods available
  - Biological tests 2.6
  - Biological; assays 2.7

Reminder: Meaningful standard vs flexibility

- Need for appropriate equilibrium
- Flexible > applicable to more products/situations
- Sufficiently detailed to be useful for laboratories
- A standard which is too flexible is meaningless

Meaningful standard - concerns

- Commonly voiced concerns are related to flexibilility and maturity

- 'Monographs hamper innovation'
- 'Monographs hamper development of biosimilars'

Monographs do not hamper innovation

- This concern mainly relevant for very new (classes) of substances
  - CQAs not fully understood; or ranges insufficiently qualified
  - Methods not fully optimised
  - Standardisation needs a certain maturity

- Many biological monographs have been around for decades and have demonstrated their usefulness.
  - Maturity achieved
  - May rely on available flexibility (see e.g. General Notices) for implementation of innovative *methods* – CQAs are fixed/'minimum requirement'

Monographs do not hamper biosimilar development

- Concern valid but manageable
  - Need for rapid monograph amendments for new monographs
  - Will diminish with experience gained (better insights in CQAs, both general and product-specific).
- New monograph often based on data from one or few manufacturers
  - Are these data representative for all future manufacturers?
  - Is this a 'true' and reasonable quality profile?
- Example:
  - Reduced CE-SDS in Infliximab (currently 2% impurities allowed; seems unnecessarily tight)
  - Not all new manufactureres can comply

Successes

- EU regulators do apply the monographs!
- Some examples of meaningful standards:
  - Infliximab , potency (test and range)
  - Human Coagulation Factor VIII
    - Both plasma (<275>) and recombinant (<1643>) source
    - Associated method 2.7.4 'Assay of human coagulation factor VIII': Standardisation of biological activity measurement (methods *and* standards = calibration)
    - Associated monographs , e.g. <853> 'Human plasma for fractionation '.
  - Influenza
  - Somatropin, insulin
    - Fixed conversion IU/mg
- Method chapters utilised to full extent?
  - Host Cell Proteins (extremely difficult assay to validate/standardise, esp. Accuracy/Trueness)

Challenges

- Maturity
  - Is a substance/subject sufficiently well developed for standardisation?
- Flexibility
  - Example, glycans (link to potency?);
  - Example, Mab-particles
- Identification of Critical Quality Attributes
  - Example, sialic acids in Infliximab
- Effort/resources
- Main users, different needs/requirements
  - OMCLs, assessors, companies (cave: minimum requirements)

Challenge – finished product monographs

- Finished products monographs for biologicals have been around for decades!
  - See e.g. Influenza vaccine, whole virion, inactivated <159, >Human coagulation factor VIII <275>, Human normal IgG for IM use <338>
  - Monoclonal antibodies for human use <2031>
- Long experience with such monographs >> elaboration not a fundamental challenge
  - May be limited by practical considerations
  - May benefit from similar properties between DS and DP

Some points for future attention

- Assay standardisation/method chapters
  - ADCC and its (glycoform) determinants – mature?
  - Pro coagulant activity in human plasma immunoglobulins
- Protein concentration
  - Easy to determine in relative sense -compared to (paper) standard
  - Difficult to measure in absolute sense
  - Standardised measurement of paramount importance: labelling

Conclusion

- Monographs for biologicals have been around for decades
- Recently adopted monographs (etanercept, infliximab) demonstrate that new monographs can be successfully elaborated
- Balance between flexible and meaningful
  - 'Case by case'
- Focus on what can be standardised
  - Mature aspects (methods, CQAs, etc) based on experience
  - Pragmatic approach

Half full or half empty? Celebrate!