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

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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Development of New Ph. Eur. “Horizontal Standards” for Monoclonal Antibody Analysis: An Overview from the EDQM

AT Europe 2022

23-25 May 2022

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EDQM, Council of Europe

Presentation Outline

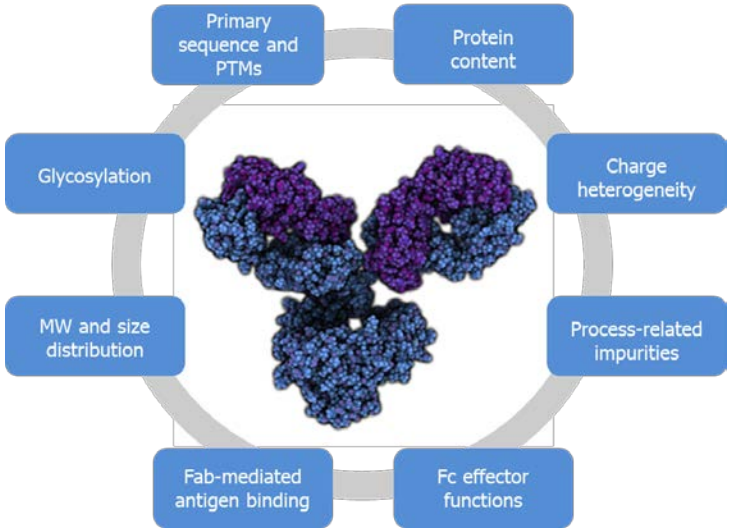
- **Ph. Eur. standards for monoclonal antibodies: development approaches**
- **“Horizontal standards”:**
  - **TNF-alpha product class case study**: bioassay collaborative study and *NEW* draft general chapter 2.7.26.
  - TNF-alpha bioassay package
- **New general texts: recent developments**
- Concluding remarks

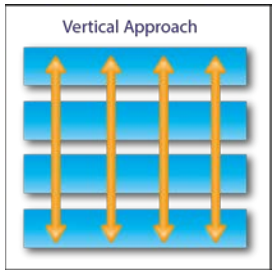
Ph. Eur. Approach to Public Standard-Setting

Monographs

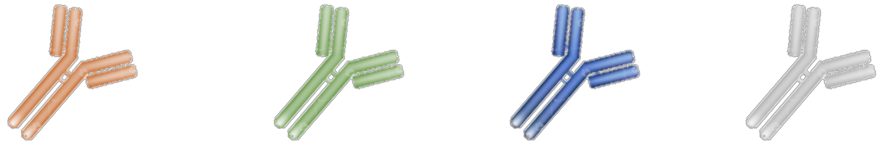
- Ustekinumab (3165)*
- Adalimumab (3147)*
- Golimumab concentrated solution (3103)*
- Infliximab concentrated solution (2928)

Definition
Production
Identification
Tests
Assay/Content
Assay/Potency





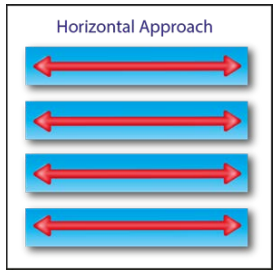
Horizontal standards (general chapters)



Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)**

Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies (2.5.43)*

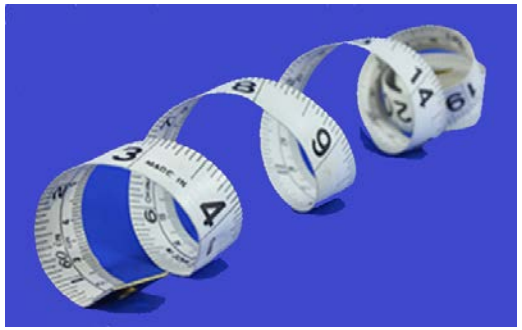
Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies (2.5.44)*



*under elaboration
** to be published in Ph. Eur. Supplement 11.1 (Oct. 2022)

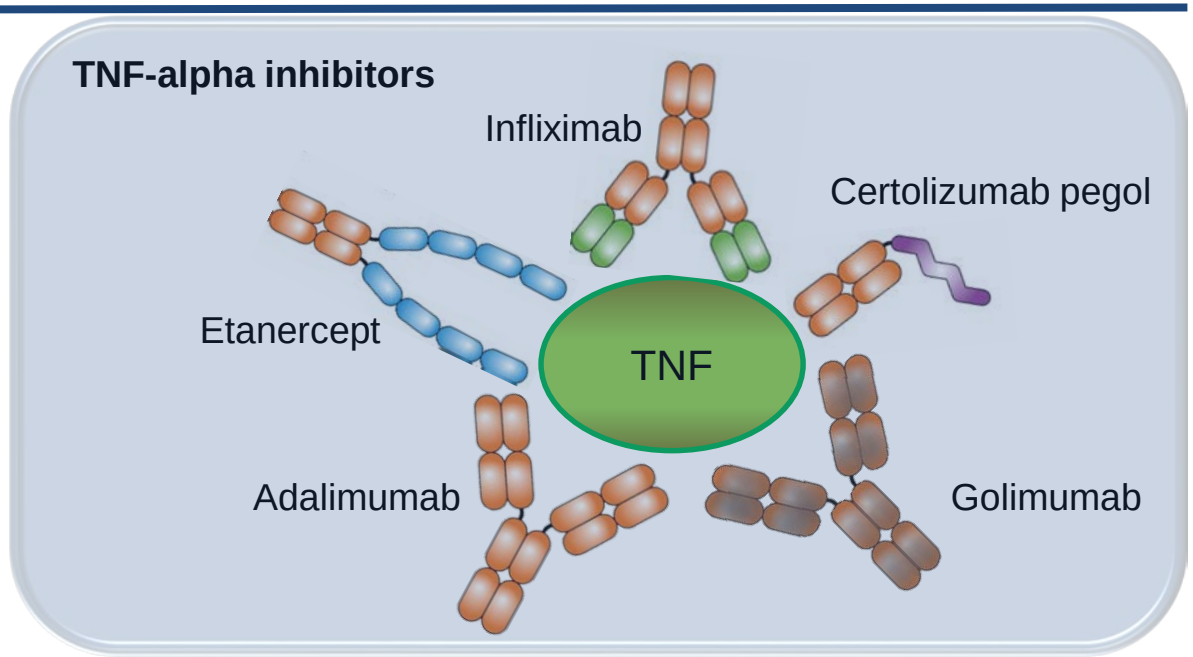
Ph. Eur. Standards for mAbs: Development Approaches

- **Expand** the portfolio of quality standards for mAbs:
  - Target **product classes** and specific drug substance; evaluate new opportunities on a case-by-case basis with support from key stakeholders
  - Develop **general methods of analysis** to support analytical testing → broad applicability, performance characteristics; multi-laboratory collaborative studies
- Explore **flexible concepts** and **new types of standardisation**:
  - Focus on key quality attributes and associated testing strategies
  - Establish suitable common expectations and general methodologies with broad applicability



Standardisation of TNF-alpha Bioassays

- **Rapidly growing number** of TNF-alpha antagonists on the market
- **Increased variety of approaches** to bioassay selection for assessing and comparing potencies
- Questions raised concerning the **appropriate choice of potency assays** for particular products and how they should be designed, conducted, analysed and applied



Elaboration of a **new general chapter** to provide **suitable common expectations** and **general methodologies** for potency determination, widely applicable to the class of **TNF-alpha antagonists (Bioassay "Horizontal" Standard)**

TNF-alpha Bioassay Collaborative Study

AIM:

- to investigate suitability of selected bioassays to be applied as multi-product procedures, suitable to assess the TNF-alpha inhibitory effect
- using a number of TNF-alpha antagonist substances (different sources/ manufacturers) as test samples, as well as corresponding reference standards:
  - to assess the **suitability** of candidate procedures for the aforementioned substances
  - to **evaluate and compare assay performance**
  - to **compare the relative potency results obtained with the different assays** for each of the TNF-alpha antagonist substances.

TNF-alpha Bioassay Collaborative Study: Design

Cell-based assay	Target cell line	Type of assay	Type of readout
Procedure A	U937	Apopotosis	Luminescence
Procedure B	WEHI-164	Cytotoxicity	Absorbance
Procedure C	HEKBlue CD40L	Reporter gene	Absorbance
Procedure D	L929	Cytotoxicity	Absorbance

Procedures A, B, C and D initially validated for specific TNF-alpha antagonist substance:

- Procedure A – etanercept [monograph]
- Procedure B – infliximab [monograph]
- Procedure C – certolizumab pegol
- Procedure D – adalimumab

Common sample panel:

- ✓ 7 different preparations (test samples) and 2 in-house reference standards
- ✓ Ph. Eur. Etanercept BRP batch 1 (10 000 IU/ampoule)
- ✓ Ph. Eur. Infliximab BRP batch 1 (500 IU/ampoule)
- ✓ non-TNF-alpha control antibody

Study participants

Ph. Eur. Experts' laboratories; EDQM Laboratory

EDQM statistical analysis: 4-parameter logistic model; validity based on slope-ratio (parallelism)

TNF-alpha Bioassay Collaborative Study: Results

	WEHI-164 cytotoxicity assay				U937 apoptosis assay				NF-κb-inducible reporter gene assay				L929 cytotoxicity assay			
APPC / SST	ETN	**IFX**	ADA	CERT	**ETN**	IFX	ADA	CERT	ETN	IFX	ADA	**CERT**	ETN	IFX	**ADA**	CERT
Specificity	no detectable activity of non-TNF-α antibody				no detectable activity of non-TNF-α antibody				no detectable activity of non-TNF-α antibody				no detectable activity of non-TNF-α antibody			
Controls	cells only/cells +TNF-α > 3 (*n* = 96)				cells +TNF-α/cells only > 2.5 (*n* = 96)				cells +TNF-α/cells only > 3 (*n* = 96)				cells +TNF-α/cells only > 2 (*n* = 96)			
Correlation	*r* ≥ 98.5% in 90% of plates				*r* ≥ 97.5% in 90% of plates				*r* ≥ 99.5% in 90% of plates				*r* ≥ 99.5% in 90% of plates			
Mean bias (%)[1]	≤2.5	≤2.5	≤5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	n.a.	n.a.	n.a.	n.a.
Repeatability (%)[2]	≤10	≤10	≤5	≤10	≤15	≤5	≤10	≤10	≤10	≤10	≤15	≤10	≤15	≤15	≤10	≤10
Intermediate precision (%)[3]	≤15	≤10	≤10	≤10	≤20	≤ 0	≤10	≤10	≤10	≤10	≤15	≤15	≤15	≤15	≤10	≤10
Reproducibility (%)[4]	≤20	≤20	≤15	≤10	n.a.	≤20	≤15	≤15	≤20	≤20	≤15	≤20	n.a.	n.a.	n.a.	n.a.

ETN: Etanercept; IFX: Infliximab; ADA: Adalimumab; CERT: Certolizumab pegol.

[1] Reference standard.

GCV%: [2] between plates within an assay; [3] between different plates and assays within a lab;
[4] between different plates, assays and laboratories. GCV% are averaged over the results of all labs, assays & plates.

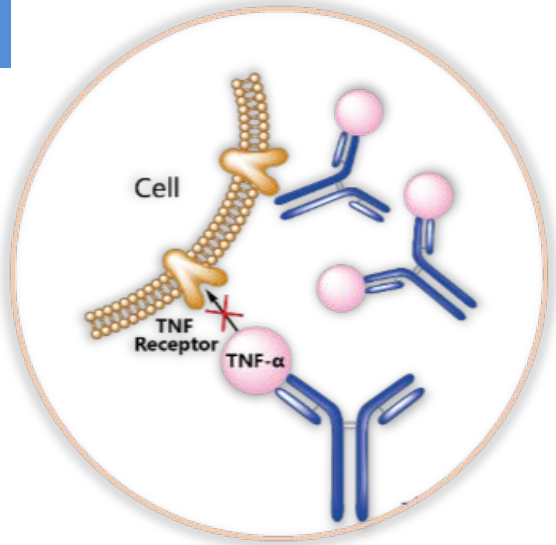
TNF-alpha Bioassay Collaborative Study: Conclusions

- **Proof of concept** demonstrated.
- **Same assay procedure works equally well for all TNF-alpha antagonists tested**:
  - concentration range may need to be modified for different substances;
  - curve fitting for all curves very good;
  - lower asymptote very stable; upper asymptote appears to vary between analysis in different days;
  - assay variability considered acceptable.
- Experimental data generated in the collaborative study **set the basis for defining**:
  - system suitability parameters and criteria included in the general chapter;
  - specific procedures to be described in the general chapter, including sufficiently prescriptive conditions to facilitate successful independent analysis;
  - a common set of analytical expectations and approaches.
- **Critical parameters** and possible sources of variation identified:

⇨ level of details/prescriptive conditions to be suitably reflected in the chapter.

NEW General Chapter 2.7.26: Outline (1/3)

Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)



- **INTRODUCTION AND SCOPE**: **4 procedures considered comparable**, unless otherwise specified in an individual monograph
- **PRINCIPLE** [different assay models]
- **PROCEDURES (A, B, C, D)**
  - detailed description of assay conditions;
  - preparation test/reference solution; TNF-alpha working solutions;
  - cells preparation; plate preparation; addition of staining reagent.
- **DATA ANALYSIS:**
  - general guidance applicable to all assay procedures, with the recommendation to use the 4-PL statistical model (according to the described validated/verified assay procedures)
  - general requirement for acceptable similarity/parallelism for the dose-response curves, with reference to the general chapter *5.3 Statistical analysis*

NEW General Chapter 2.7.26: Outline (2/3)

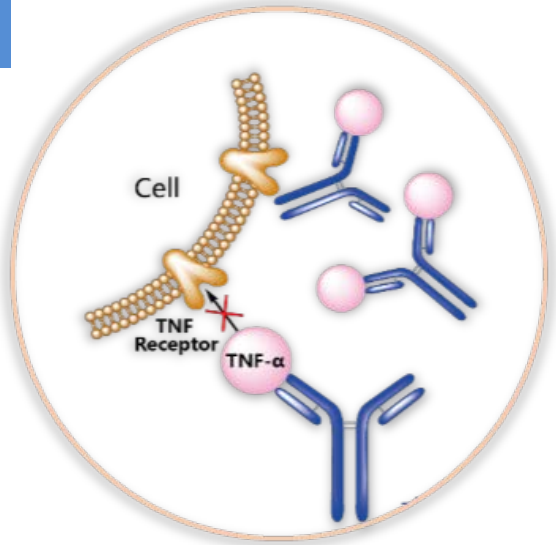
Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)

- **SYSTEM SUITABILITY**: reference standard dose-response curve:
  - sigmoid curve with well-defined upper and lower plateaus and linear part;
  - values of the upper and lower plateaus within pre-defined range established from the minimum and maximum values of the corresponding controls;
  - coefficient of determination calculated (R^2);
  - ratio 'cell+ TNF-alpha control' to 'cells only'.
- **SAMPLE ACCEPTANCE CRITERIA:** test sample dose-response curve:
  - sigmoid curve with well-defined upper and lower plateaus and linear part;
  - coefficient of determination calculated (R^2);
  - sample and reference standard dose-response curves: similarity/parallelism (see general chapter *5.3. Statistical analysis of results of biological assays and tests*).
- **RESULTS**
  - general guidance on how to estimate the relative potency, including condition for assay variability, applicable to all assay procedures

= sets of **universally applicable parameters/criteria**, confirmed by the experimental verification
→ apply to all 4 assay procedures described

NEW General Chapter 2.7.26: Outline (3/3)

Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)



- **GENERAL RECOMMENDATIONS AND ADJUSTMENT OF ASSAY CONDITIONS**
  - discusses aspects (common to all assay procedures covered by the chapter), contributing to the variability of the assay performance;
  - describes the extent to which the various assay conditions may be adjusted to satisfy the system suitability criteria without fundamentally modifying the procedures described:
    - determination of TNF-alpha working concentration;
    - internal controls;
    - determination of analyte working range and dilution series;
    - cell maintenance;
    - assay design/plate layout.

NEW TNF-alpha Bioassay General Chapter 2.7.26

Cell-based assays for potency determination of TNF-alpha antagonists

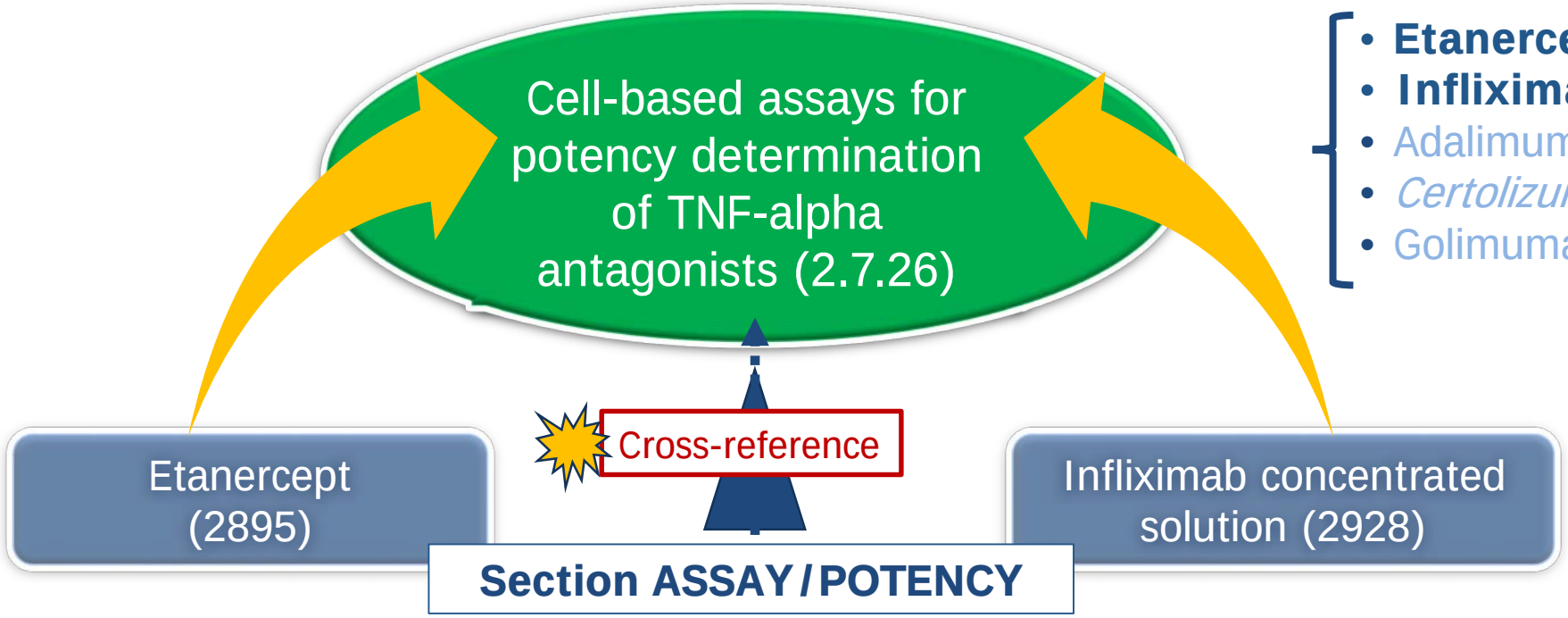
- **NEW type of general chapter** with experimentally verified specific procedures.
- Assays described (procedures A, B, C and D):
  - ➔ validated for potency determination of specific TNF-alpha antagonists;
  - ➔ suitability in terms of specificity and precision demonstrated for each TNF-alpha antagonist substance, during verification experiments;
  - ➔ procedure applied to substances outside the scope of the initial validation or not covered in an individual monograph for a TNF-alpha antagonist, require validation.
- The chapter does not exclude the use of other procedures that are acceptable to the competent authority.

Scope of validation/verification

Anti-TNF-alpha antagonist	U937 apoptosis assay	WEHI-164 cytotoxicity assay	NF-κB-inducible rep. gene assay	L929 cytotoxicity assay
Etanercept*	●	◗	◗	◗
Infliximab*	◗	●	◗	◗
Certolizumab pegol	◗	◗	●	◗
Adalimumab**	◗	◗	◗	●
Golimumab**	○	◗	○	○

● signifies that procedure has been validated
◗ signifies that suitability has been demonstrated during verification experiments
○ signifies that suitability has not been evaluated
* Ph. Eur. monograph
** monograph under elaboration

Link between Chapter and Individual Monographs



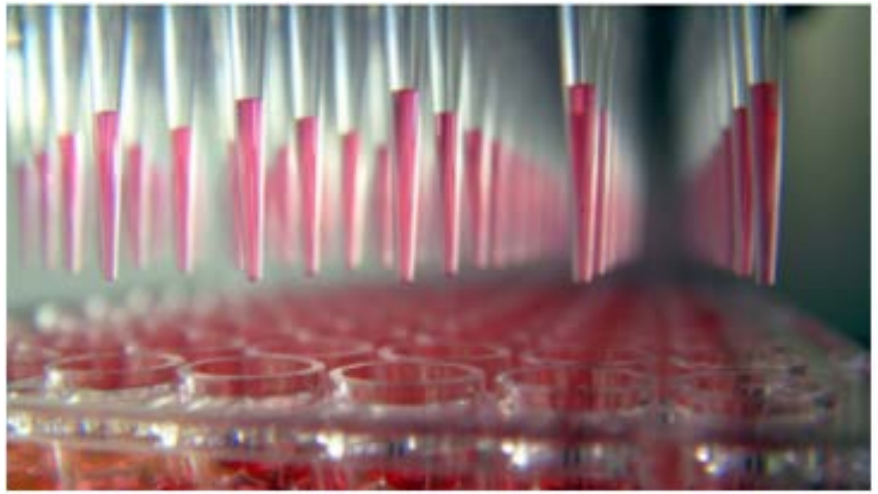
- **Etanercept**
- **Infliximab**
- Adalimumab [under elaboration]
- *Certolizumab pegol*
- Golimumab [under elaboration]

***Suitable* TNF-alpha neutralisation assay – calibration with:**	
Etanercept BRP	Infliximab BRP
Example procedure	
▪ **U937 apoptosis assay (2.7.26, "Procedure A")**	▪ **WEHI-164 cytotoxicity assay (2.7.26, "Procedure B")**
"In addition, the following procedures have been found *suitable*:"	
▪ 2.7.26, B, C and D	▪ 2.7.26, A, C and D

"suitable", "example" defined in Ph. Eur. General Notices

TNF-alpha Bioassay Package

- **New chapter 2.7.26**:
  - provides analytical tools and practical guidance to further build on and support testing.
  - helps establish an accepted and shared analytical language that will help standardise the potency determination of TNF-alpha antagonists, both currently available and in the pipeline.
- **Link created with monographs on TNF-alpha antagonists:**
  - diversifies the choice of suitable bioassays for potency determination
  - reinforces and maintains the flexibility already built into the monographs and the use of Ph. Eur. reference standards.



Ph. Eur. Supplement 11.1

- Publication: October 2022
- Implementation: April 2023

Horizontal Standard Development Beyond Product Class

- *2.5.44 Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies:*
  - (i)cIEF procedures for analysis of charge heterogeneity of mAbs, to monitor identity, quality, production consistency
  - based on data generated in multi-laboratory verification study
  - guidance on the aspects to consider for product-specific application (validation)

- *2.5.43 Size exclusion chromatography for recombinant therapeutic monoclonal antibodies:*
  - widely used methodology for determination of size variants (monomer, HMWS); quantitation of LMWS can be highly variable depending on the mAb analysed
  - SE-HPLC and SE-UPLC procedures, widely applicable to mAbs, given as examples
  - suitability demonstrated by collaborative study

➡ well-defined analytical procedures and **tools to control performance** (including reference materials) and facilitate analytical assessment of key quality attributes of mAbs

New General Texts: Recent Developments

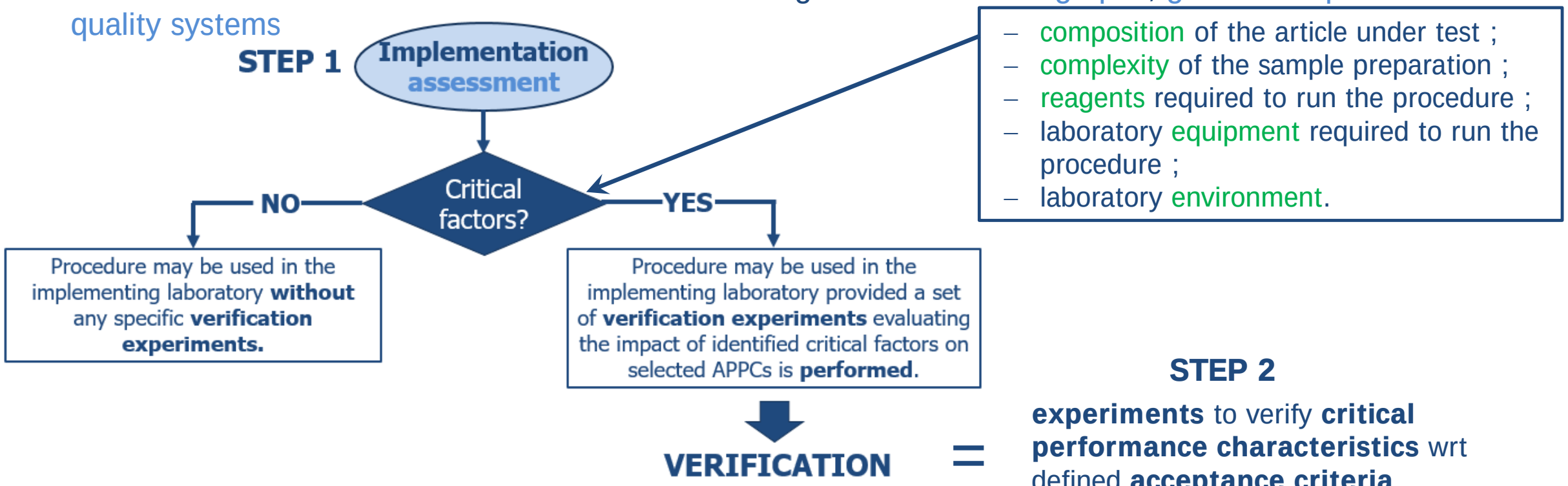
- **Implementation of pharmacopoeial procedures (5.26)**
  - ➔ guidance on setting up an approach for implementation of analytical procedures given in Ph. Eur. monographs;
  - ➔ approach described valid only when used in accordance with the principles laid down in the Ph. Eur. General Notices (including a suitable quality system);
  - ➔ **“for information”** chapter; other approaches may be appropriate.

11th Edition

- Publication: July 2022
- Implementation: January 2023

Implementation of Pharmacopoeial Procedures (5.26)

- **Ph. Eur. General Notices:**
  - the test methods given in monographs have been **validated** in accordance with accepted scientific practice and current recommendations on analytical validation [1.1.2.4 Validation and implementation of Ph. Eur. analytical procedures], unless otherwise indicated;
  - the user must **assess whether and to what extent** the suitability of the method under the actual conditions of use needs to be demonstrated according to relevant monographs, general chapters and quality systems



“Implementation”: Ongoing Activities

- Elaboration of **illustrative examples** on implementation of pharmacopoeial procedures
- Among the selected procedures under consideration:
  - Assay by LC (chemically-defined active substance)
  - Related substances test by LC (medicinal product)
  - **Potency determination by cell-based (monoclonal antibody)**
  - Microbial enumeration tests non-sterile products
  - Identity by IR (excipient)

- Identification of any critical factors and impact on APPC affecting the performance of the procedure
- Develop a verification plan (critical APPCs together with the corresponding acceptance criteria) to assess the procedure according to its intended purpose

New General Texts: Recent Developments

Ph. Eur. General Notices:

"*The tests and assays described are the official analytical procedures upon which the standards of the Ph. Eur. are based. With the agreement of the competent authority,* ***alternative analytical procedures*** *may be used for control purposes, provided that they enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of doubt or dispute, the analytical procedures of the Ph. Eur. are alone authoritative."*

- **Draft general text on Comparability testing of alternative procedures (5.27)**

DRAFT

  - ➔ guidance on possible approaches to assessment of comparability of an alternative procedure that is used instead of a pharmacopoeial procedure
  - ➔ preliminary conditions to the comparability study: validation of alternative procedure, implementation of pharmacopoeial procedure
  - ➔ comparability study: design, acceptance criteria for comparability, approach for data evaluation (equivalence testing)
  - ➔ **"for information"** chapter; other approaches may be appropriate

Comparability of Alternative Procedures (5.27)

https://www.edqm.eu/en/-/new-general-text-on-comparability-of-alternative-analytical-procedures-european-pharmacopoeia-launches-public-consultation

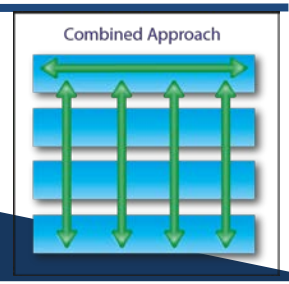
Pharmeuropa 34.2

- Public deadline: 2022-06-30

"Once adopted by the Ph. Eur. Commission, this new general text will represent a major addition to the Ph. Eur. as it provides more detailed information on one of the processes that offers users greater flexibility in their demonstration of compliance with the Ph. Eur. monographs. It is also expected to prove valuable both to users who choose to employ alternative analytical procedures and to assessors during evaluation".

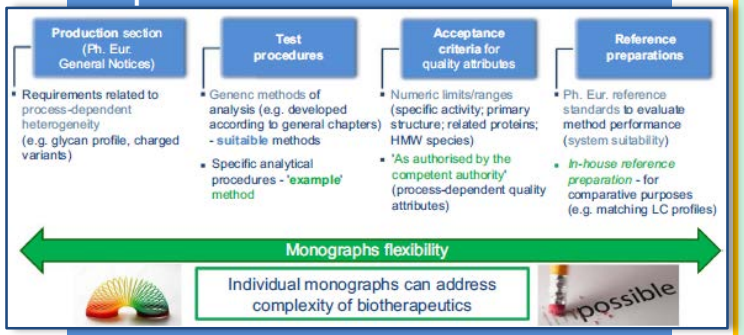
Ph. Eur. Standards for mAbs: Summary

Ph. Eur. Standards for Therapeutic Monoclonal Antibodies: Development Approaches



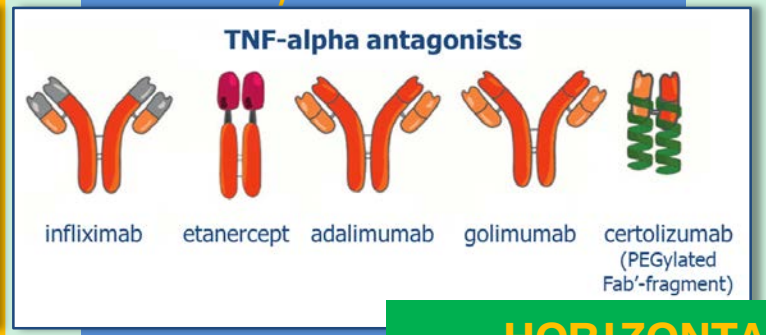
MONOGRAPHS*

- built-in *flexibility*
- *examples* of suitable procedures



PRODUCT CLASS - BASED STANDARDS

- product classes/sub-classes quality attributes
- *TNF-alpha neutralisation*



PERFORMANCE-BASED STANDARDS

- *platform* methodologies
- performance characteristics
- reference standards

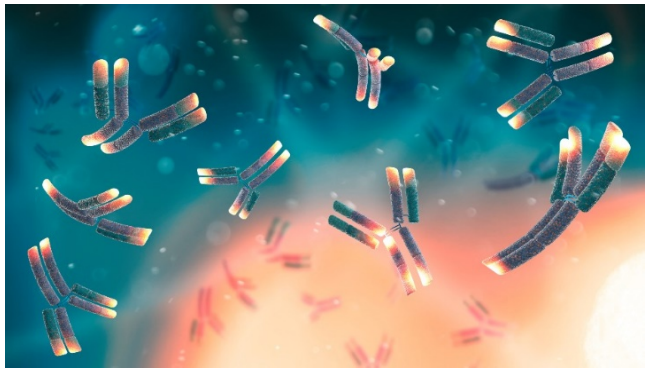
HORIZONTAL STANDARDS

PRODUCT KNOWLEDGE, CASE STUDIES, COLLABORATIVE TESTING

*Buda M., Kolaj-Robin O., Charton E. *Biotherapeutic Products in the European Pharmacopoeia: Have all Challenges Been Tackled?* Generics and Biosimilars Initiative Journal. 2022;11(1)

Horizontal Standard Development: Concluding Remarks

- Explore flexible concepts of standardisation in an increasingly evolving multi-product market
- Reflect key quality attributes and associated testing strategies
- Provide common expectations and general methodologies applicable to wide range/classes of mAbs
- Provide guidance on aspects to consider when an analytical procedure is suitable for its intended purpose
- Contribute to standardisation of therapeutic monoclonal antibodies through rationalisation of methodologies and common functionalities



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