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

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Development of New Ph. Eur. Bioassay "Horizontal" Standards: An Insight into the Anti-TNF-alpha Product Class Collaborative Study

CMC Forum Strategy Europe 2020

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EDQM, Council of Europe

Presentation Outline

- **Ph. Eur. and bioassays approaches**
- **MAB pilot phase:**
  - 'Bottom-up' approach – **horizontal standards developement**
  - **TNF-alpha product class case study**: bioassay collaborative study
  - **Elaboration of a general chapter on** *Cell-based assays for potency determination of TNF-alpha antagonists*: points to consider
- Concluding remarks and future perspectives

Bioassay Approaches in the Ph. Eur.

General monographs

- classes of substances or products (defined by production method, intended use);
- mandatory requirements for all the products within the scope of definition section.

Individual monographs

- based on approved specification(s) backed up by batch data;
- validated analytical procedures*; acceptance criteria (*unless otherwise stated).

General chapters

- general recommendations for analytical procedures;
- guidance for design of analytical methods and for analysis of their results;
- mandatory when referred to in a monograph.

Chapter 5.12
Reference Standards

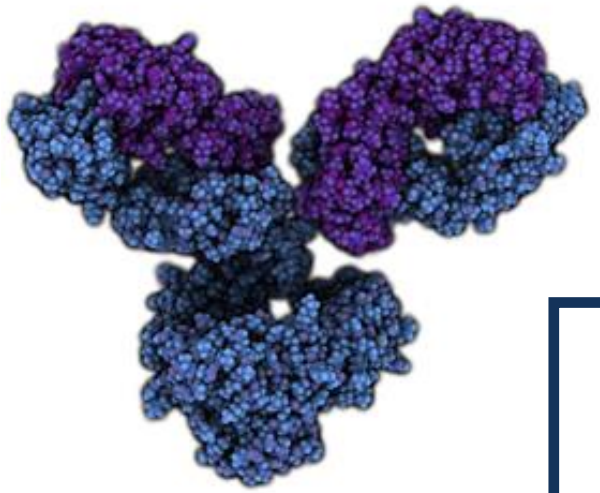
Biological Reference Preparations (BRPs)

Established specifically and exclusively for use in monographs, as prescribed in the procedures given

Bioassay Approaches in the Ph. Eur. – Therapeutic MAbs

Products of recombinant DNA technology (0784)

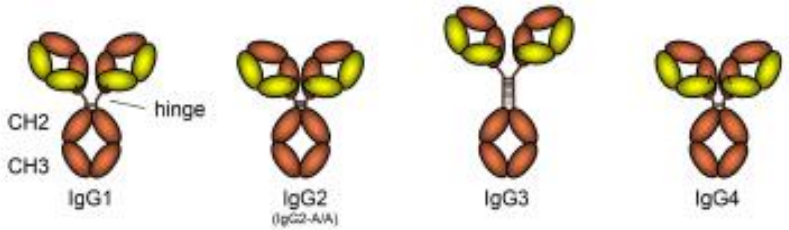
- **PRODUCTION** – **Characterisation:**
  - **Structure**: **biological assays** based on functional activity may serve as additional confirmation of the higher-order structure.
  - **Biological activity**: assessed by **biological**, biochemical **assays**,… as appropriate.
    Mechanism of action: investigated and preferably reflected in the **potency assay**.
- **ASSAY** – **Potency**:
  - **potency assay** established using a suitable reference standard and carried out against this reference standard;
  - general chapter 5.3. may be used to design the assay and calculate the results.



Monoclonal antibodies for human use (2031)

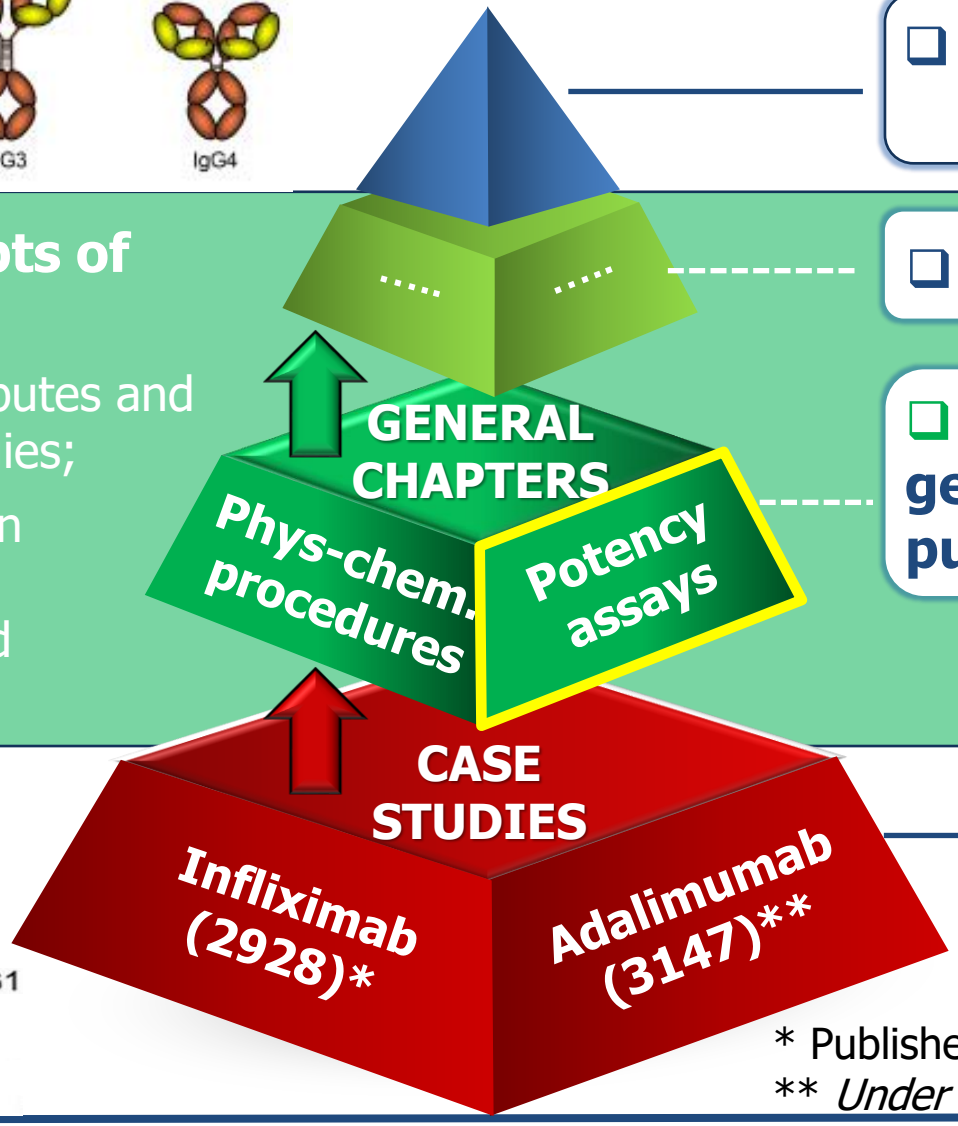
- **PRODUCTION:**
  - **Product characterisation**
  - **Biological assay** chosen in terms of its correlation with the intended mode of action of the monoclonal antibody.
- **IDENTIFICATION:** the assay also contributes to identification.
- **ASSAY:**
  - carry out a suitable **biological assay** compared to the reference preparation;
  - design of the assay and calculation of the results: usual principles (for example, 5.3).

MAB Pilot Phase: A 'Bottom-up' Approach



Explore flexible concepts of standardisation:

- focus on key quality attributes and associated testing strategies;
- establish suitable common expectations and general methodologies with broad applicability.

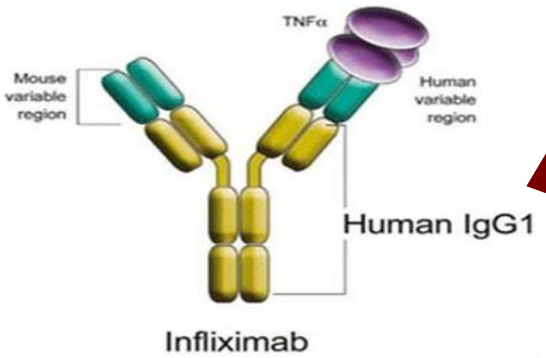


❑ General monograph on *Monoclonal antibodies for human use (2031)*

❑ **Product classes/sub-classes** QAs

❑ **HORIZONTAL STANDARDS: general methods** to be used for **purity** testing, **potency** determination

❑ **Product-specific QAs:** flexibility; method performance criteria; examples of suitable procedures; establishment of Ph. Eur. reference preparations

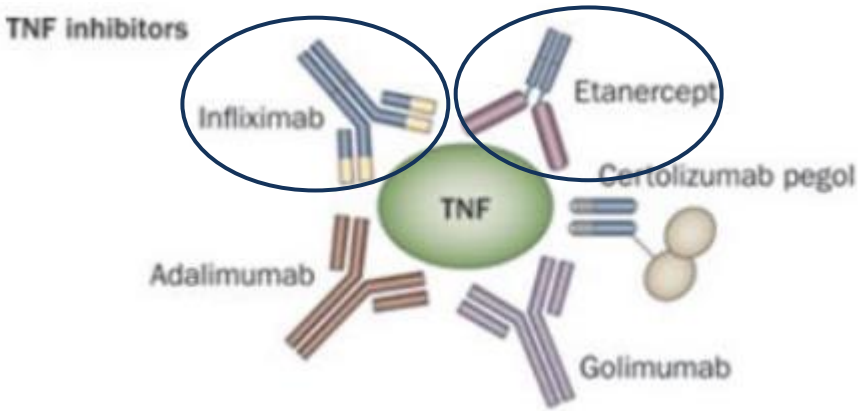


* Published in Ph. Eur. Supplement 9.6 (July 2018)
** *Under elaboration*

Bioassay Standards: the Case of Anti-TNF-alpha Products

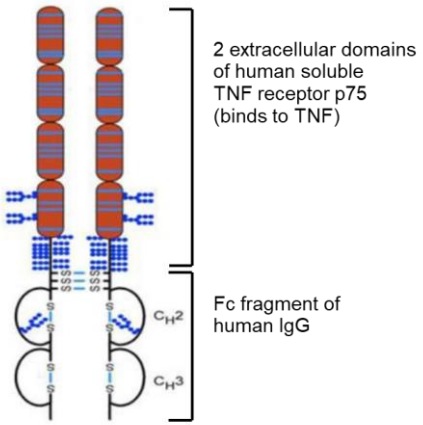
Anti-TNF Biologic Drugs

- prevent TNF-alpha receptor activation by binding to TNF-alpha, thereby neutralising the biological activity of TNF-alpha;
- biological activity evaluated in **cell-based potency assays** using different approaches for **TNF-alpha neutralisation**.



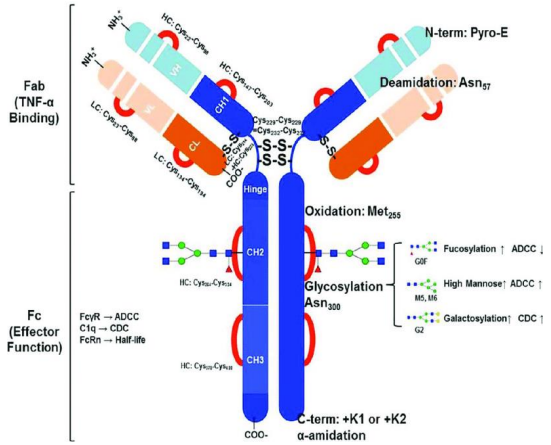
Adapted from Robert S. Woodrick & Eric M. Ruderman
Nature Reviews Rheumatology. vol. 7, 639–652 (2011)

Ph. Eur. monograph for *Etanercept (2895)*



- suitable cell-based assay based on the inhibitory action on the biological activity of TNF-alpha and a suitable readout for assessing this inhibitory effect.
- Ph. Eur. Etanercept BRP
- Example procedure: **U937 cell apoptosis assay**

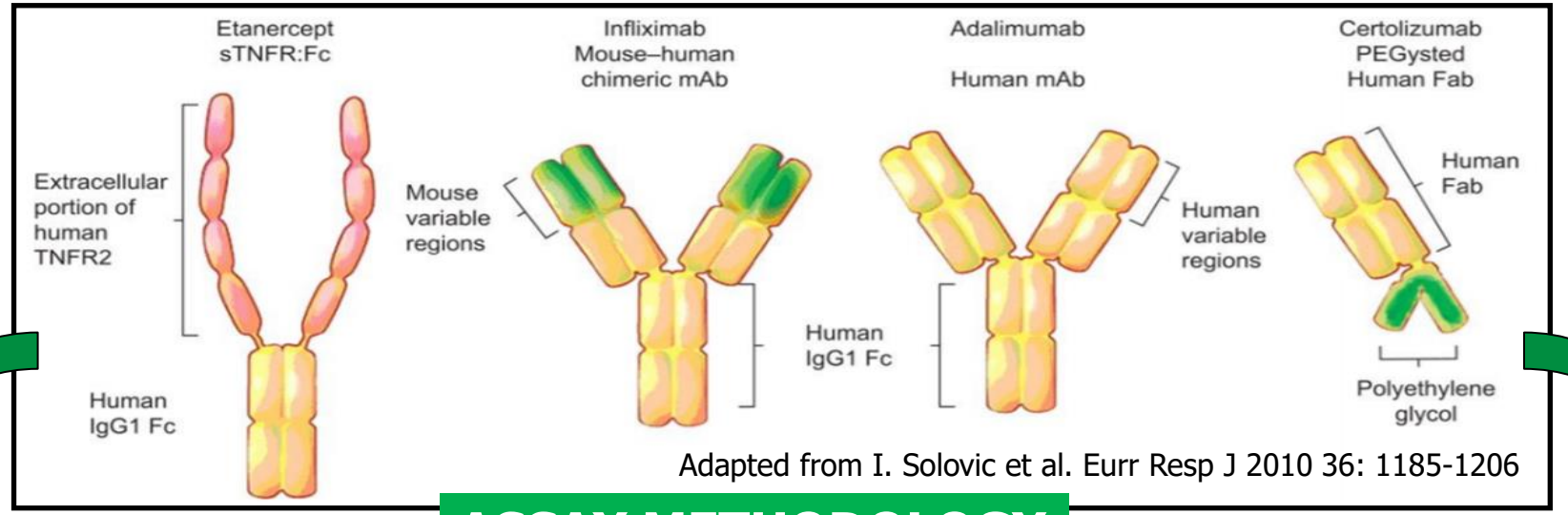
Ph. Eur. monograph for *Infliximab concentrated solution (2928)*



- Ph. Eur. Infliximab BRP
- Example procedure: **WEHI-164 cytotoxicity assay**

Development of Bioassay "Horizontal" Standards

Anti-TNF-alpha Bioassay Collaborative Study



Adapted from I. Solovic et al. Eurr Resp J 2010 36: 1185-1206

ASSAY METHODOLOGY

WEHI-164 cytotoxicity assay	**HEK-Blue CD40L** reporter gene assay	**U937** apoptosis assay

Collaborative study: experimental verification of selected bioassay models

⇨ **AIM: to evaluate suitability** of candidate assays to be applied as universal methods for potency determination of TNF-α antagonists.

Anti-TNF-alpha Bioassay Collaborative Study

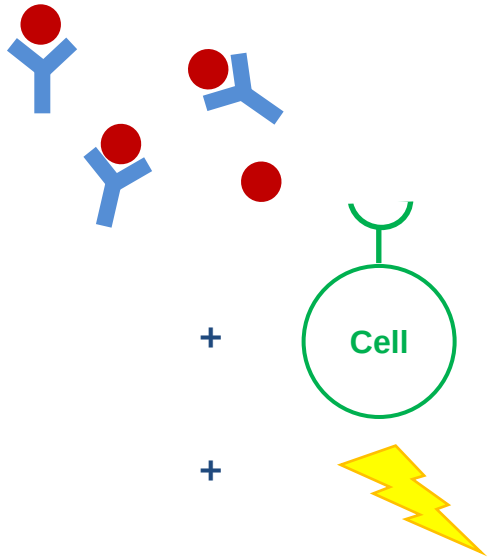
Study design

- **Bioassays** (based on common study protocol):
  - WEHI-164 cytotoxicity assay
  - HEK-Blue CD40L reporter gene assay
  - U-937 apoptosis assay

- **Study participants**: 9 labs, 8 countries (Official Medicines Control Laboratories, National Control Laboratories and EDQM Lab)
- **Sample panel**:
  - Etanercept: samples A, B
  - Infliximab: samples C, D
  - Adalimumab: samples E, F
  - Certolizumab pegol: sample G
- **Reference standards**:
  - Ph. Eur. Etanercept BRP (10000 IU/amp)
  - Ph. Eur. Infliximab BRP (500 IU/amp)
  - Adalimumab in-house RS (I)
  - Certolizumab in-house RS (H)
- **EDQM statistical analysis** (dose-response: 4-parameter logistic model; ratio-of-slopes approach for parallelism)

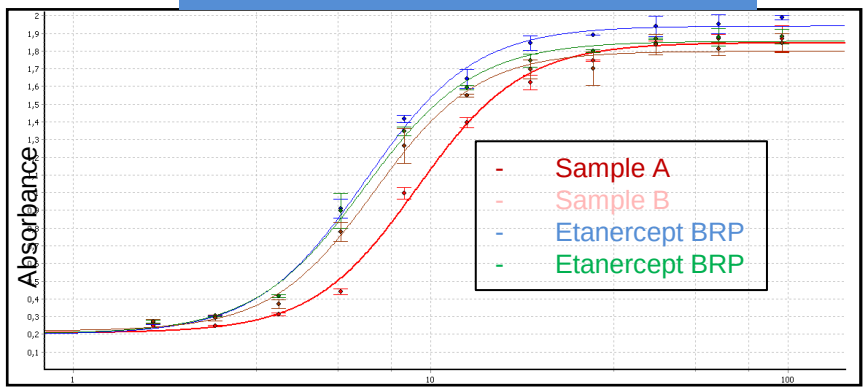
Bioassays Performed in the Collaborative Study

Cell types	rhTNF-alpha	Assay	Readout	No. labs
WEHI-164	60 IU/mL 15 IU/mL 10-40 IU/mL	Cytotoxicity	Absorbance: WST-8	8
U937	40 IU/mL 37.5 IU/mL	Apopotosis	Luminescence: Caspase/Glo 3/7	4
HEKBlue CD40L	40 IU/mL 0.4 ng/mL	Reporter gene	Absorbance: QUANTI-Blue	6



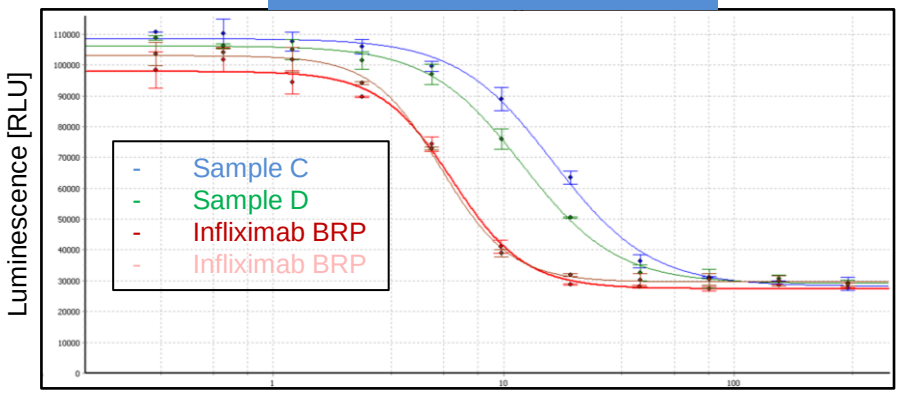
WHO International Standard 3rd International Standard for Tumour Necrosis Factor-alpha (Human, rDNA derived)

WEHI-164 cytotoxicity assay



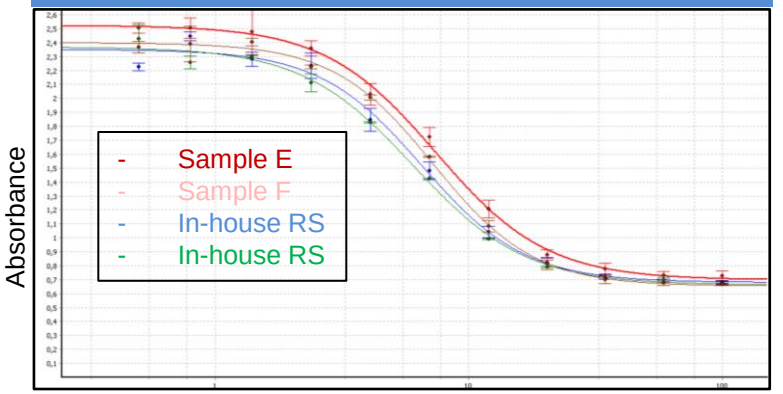
Etanercept [ng/mL]

U937 apoptosis assay



Infliximab [ng/mL]

HEK-Blue CD40L reporter gene assay



Adalimumab [ng/mL]

Study Design

- **Preliminary assay**:
  - TNF-alpha control curve
  - Reference standards:
    - Infliximab BRP (WEHI-164 cytotoxicity assay)
    - Etanercept BRP (U937 apoptosis assay)
    - Certolizumab pegol IHRS (HEKBlue rep. gene assay)
  - Controls:
    - **specificity control (non-TNF-alpha antibody)**
    - 'cells only'; 'cells + TNF-alpha'
- **Final assays:**
  - three assays (each assay spread over 2 days)
  - four plates per day (total of 24 plates/assay type/ lab)
- **Reporting and evaluation** of:
  - specificity
  - precision (plate, assay, lab)
  - recovery (mean vs. target)
  - cells only / cells + TNF-alpha
  - coefficient of correlation

Assay layout (example)

Assay i Day 1 (4 plates)		Assay i Day 2 (4 plates)	
Plate 1 Etanercept BRP Sample A Sample B **Etanercept BRP**	Plate 3 Infliximab BRP Sample C Sample D **Infliximab BRP**	Plate 5 Sample I (IH RS) Sample E Sample F **Sample I (IH RS)**	Plate 7 Sample G Sample H (IH RS) **Sample H (IH RS)** **Infliximab BRP**
Plate 2 Sample A Etanercept BRP **Etanercept BRP** Sample B	Plate 4 Sample C Infliximab BRP **Infliximab BRP** Sample D	Plate 6 Sample I (IH RS) **Sample I (IH RS)** Sample E Sample F	Plate 8 Sample H (IH RS) Sample G **{Infliximab BRP}** **Sample H (IH RS)**

Reference in red = used as standard curve

Reference in black = used as test sample (recovery)

Plate layout (example)

DAY 1 Plate 1

Cell only	Etanercept BRP										
	Sample A										
TNFa CTRL	Sample B										
	Etanercept BRP										

Bioassay Verification Study: Results Summary

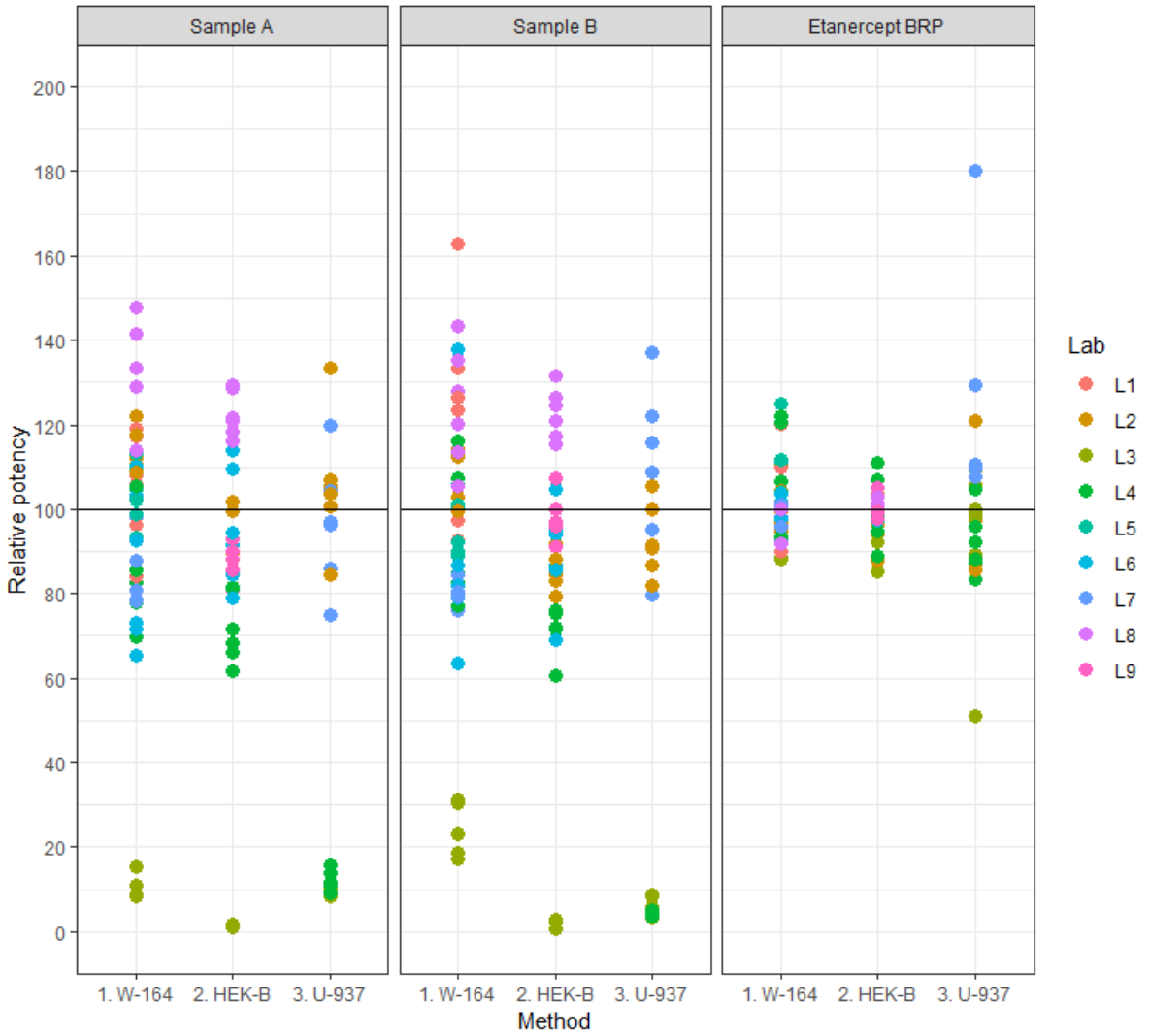
	WEHI-164 cytotoxicity assay				U937 apoptosis assay				HEK-Blue CD40L reporter gene assay			
Parameter	Etaner.	**Inflixim.**	Adalim.	Certoliz. pegol	**Etaner.**	Inflixim.	Adalim.	Certoliz. pegol	Etaner.	Inflixim.	Adalim.	**Certoliz. pegol**
Specificity	no detectable activity of non-TNF-alpha antibody				no detectable activity of non-TNF-alpha antibody				no detectable activity of non-TNF-alpha antibody			
Controls	cells only/cells +TNF-alpha > 3 (n = 96)				cells +TNF-alpha/cells only > 2.5 (n = 96)				cells +TNF-alpha/cells only > 3 (n = 96)			
Correlation	$r \geq$ 98.5% in 90% of plates				$r \geq$ 97.5% in 90% of plates				$r \geq$ 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%**
Repeatability[2]	≤ 10%	**≤ 10%**	≤ 5%	≤ 10%	**≤ 15%**	≤ 5%	≤ 10%	≤ 10%	≤ 10%	≤ 10%	≤ 15%	**≤ 10%**
Intermediate precision[3]	≤ 15%	**≤ 10%**	≤ 10%	≤ 10%	**≤ 20%**	≤ 10%	≤ 10%	≤ 10%	≤ 10%	≤ 10%	≤ 15%	**≤ 15%**
Reproducibility[4]	≤ 20%	**≤ 20%**	≤ 15%	≤ 10%	n.a.	≤ 20%	≤ 15%	≤ 15%	≤ 20%	≤ 20%	≤ 15%	**≤ 20%**

[1] Reference standard.

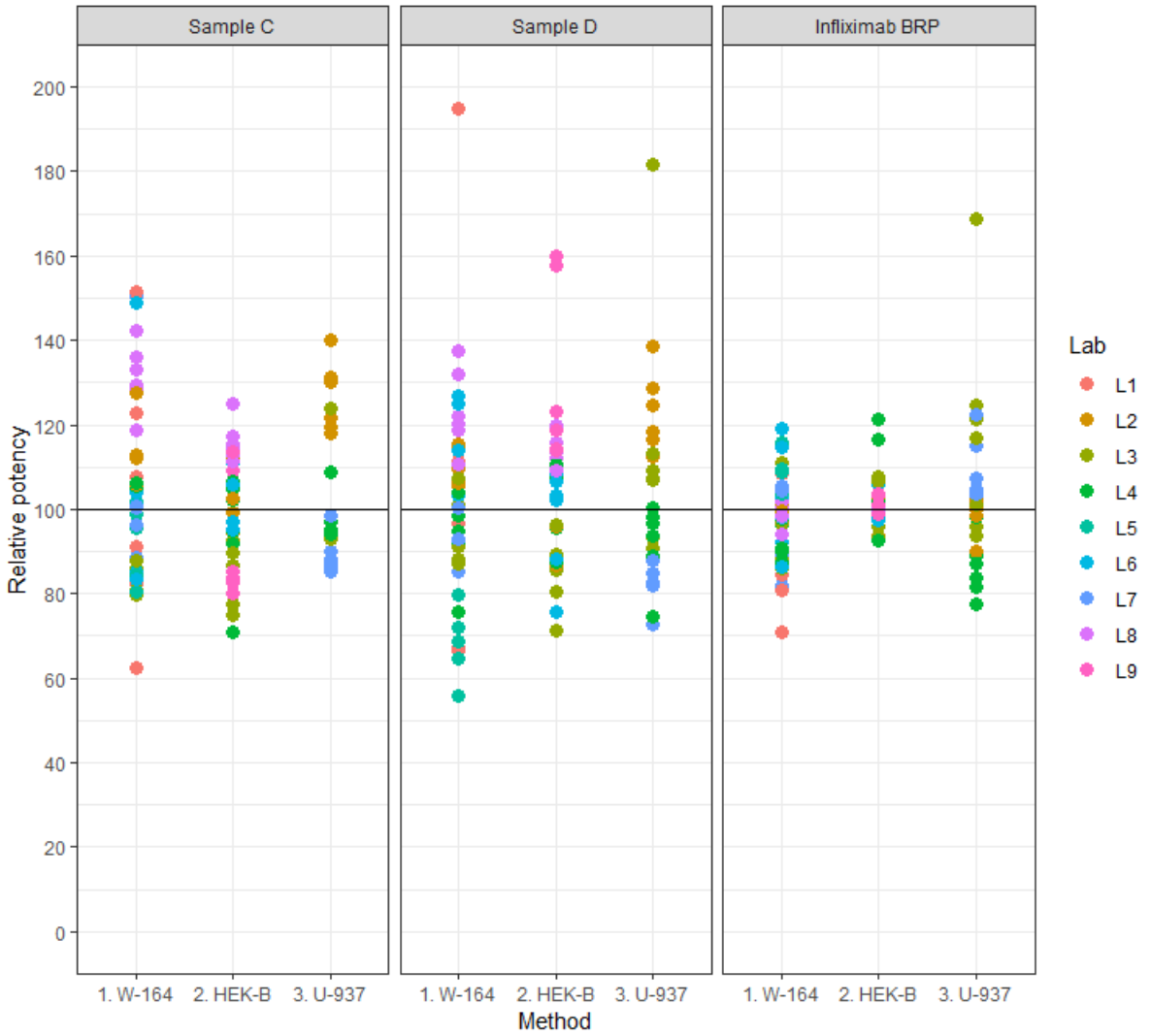
GCV%: [2] between plates within an assay; [3] between different plates and assays within a lab;
[3] between different plates, assays and laboratories. GCV% are averaged over the results of all labs, assays & plates.

Product Cluster/Bioassay Format: Overall Results (1/2)

ETANERCEPT

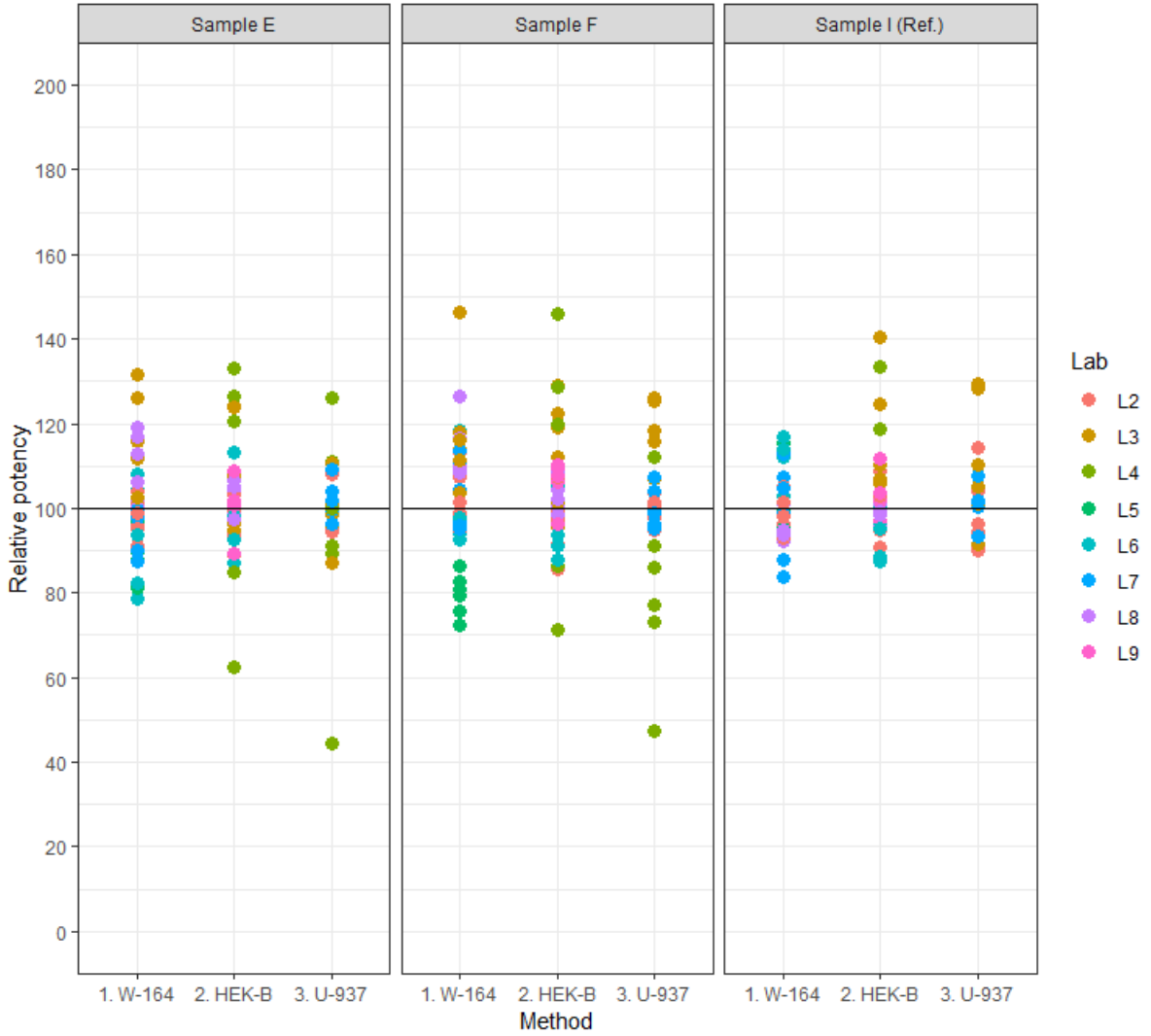


INFLIXIMAB

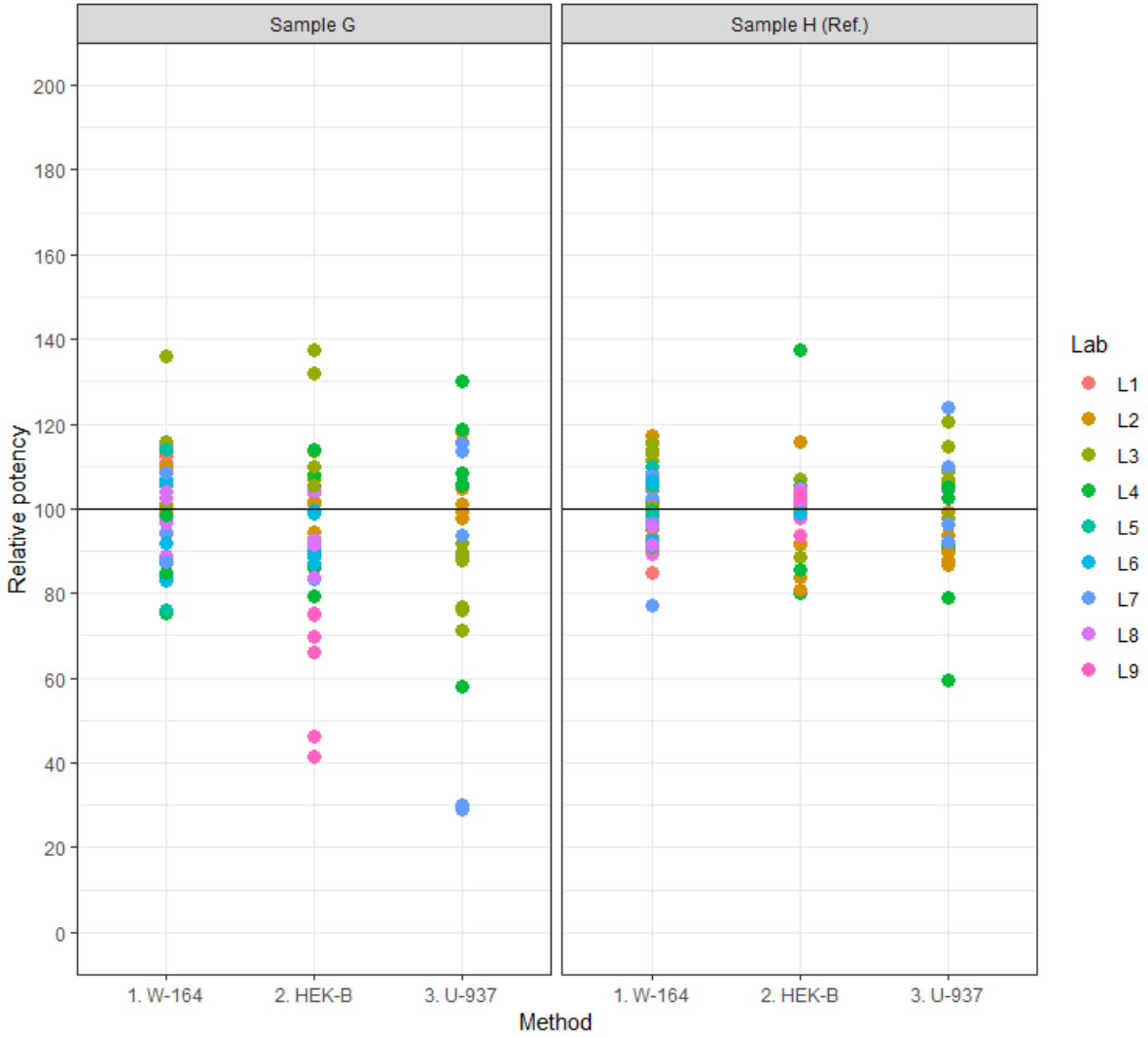


Product Cluster/Bioassay Format: Overall Results (2/2)

ADALIMUMAB



CERTOLIZUMAB PEGOL



Anti-TNF-alpha Bioassay Collaborative Study: Conclusions

- **Proof of concept** demonstrated.
- **Each assay procedure works equally well for all anti-TNF-alpha products tested**:
  - **suitability** in terms of specificity and precision has been demonstrated for all other substances outside the scope covered by the initial validation;
  - concentration range may need to be modified for different products;
  - curve fitting for all curves very good;
  - assay procedure variability considered acceptable.
- Experimental data generated in the collaborative study **set the basis for defining**:
  - system suitability parameters and criteria to be included in the general chapter;
  - specific procedures to be described in the general chapter, including sufficiently descriptive 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 draft chapter.

Draft General Chapter: Outline/Points to Consider

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

- **INTRODUCTION AND SCOPE**
- **PRINCIPLE** [different assay models]
- **GENERAL RECOMMENDATIONS**
  - assay qualification/controls
- **PROCEDURES**
  - preparation test/reference solution; TNF-alpha working solutions;
  - cells preparation; plate preparation;
  - addition of staining reagent
- **ADJUSTEMENT OF ASSAY CONDITIONS**
- **SYSTEM SUITABILITY**
  - test and standard dose-response relationships similar (see general chapter 5.3 for methodologies on assessing similarity/parallelism of four-parameter logistic curves);
  - **standard curve**: sigmoid curve; lower and upper plateaus; number of dilution points
  - **standard curve**: coefficient of determination calculated for the standard curve (r^2);
  - ratio 'cell+ TNF-alpha control' to 'cells only'.

Scope of validation/verification

Anti-TNF-alpha antagonist	U937 apoptosis assay	WEHI-164 cytotoxicity assay	HEK-Blue CD40L rep. gene assay
Etanercept	●	◗	◗
Infliximab	◗	●	◗
Certolizumab pegol	◗	◗	●
Adalimumab	◗	◗	◗

● signifies originally validated procedure
◗ signifies that suitability has been demonstrated during verification experiments

Future Perspectives

Ph. Eur. Bioassay Approaches – Horizontal standards:

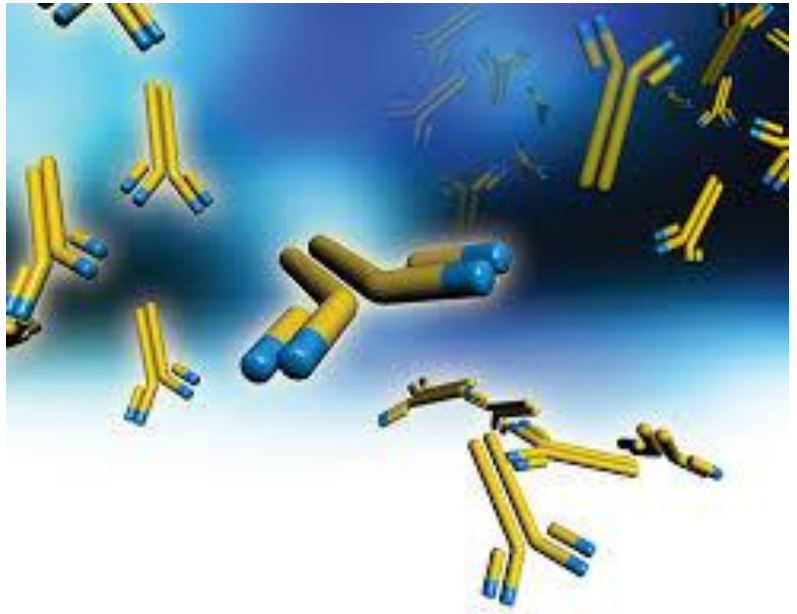
- **Draft chapter *Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)*:** to be published for comments (Pharmeuropa) soon.
- ***New frontiers*** *(different mAbs/shared functionality; types of bioassays; Fc-effector functions….)**?***

Discussion on how to develop these standards needs all stakeholders!

Acknowledgements

- Experts of the Ph. Eur. MAB Working Party
- Special thanks to the participants in the anti-TNF-alpha bioassay collaborative study
- EDQM Colleagues

  David Le Tallec (*Statistics*)
  Emmanuelle Charton



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