Lääkealan turvallisuus- ja kehittämiskeskus | Säkerhets- och utvecklingscentret för läkemedelsområdet | Finnish Medicines Agency

Performing bioassays - an OMCL point-of-view

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Finnish Medicines Agency - Fimea

Mission: to ensure that the medicines marketed and used in Finland meet the applicable requirements for the efficacy, safety and quality. Fimea operates as a part of the European Medicines Regulatory Network.

- ***Fimea is the national competent authority for medicines***, both veterinary and human
- The regulation and supervision covers the entire life cycle of medicinal products from clinical studies and manufacturing to distribution and postauthorisation measures
- Personnel ~250

The Laboratory

- Is in charge of the quality surveillance of medicinal products marketed in Finland. The Laboratory actively participates in developing the European Pharmacopoeia and regulatory norms.
- Personnel: 17

Finnish Medicines Agency Laboratory

- The Official Medicines Control Laboratory (OMCL) of Finland, belongs to the European OMCL Network
- The network is coordinated by the European Directorate for Quality of Medicines and Health (EDQM) / the Council of Europe
- The laboratory functions according to ISO/IEC 17025:2017. The Laboratory has accreditation by the national accreditation body (FINAS) and approval by EDQM auditors (MJA status)

- We perform chemical, micro-biological and *biological testing*
- *Microbiology:* endotoxins (kinetic and clotting), sterility testing, identification of strains
- *Biology:* binding / immunological assays by ELISA and Western, enzymatic assays, *cell-based assays*

Typical features of work in an OMCL lab

Principles of working

- Independent, impartial testing of a medicinal product / DS / API
  → *Impartiality is a central principle,* important in cases of dispute, investigations on suspected counterfeits and when supporting the Ministry of Social Affairs and Health

- Use of validated methods, compendial or *transferred from MAH*
- Method performance is verified using a limited number of parameters eg. repeatability (RSD, n=6), assay range, system suitability criteria and verification of equipment performance
- Often the method transfer is performed for a single set of analysis
  → *We do not do routine testing*

- We have a wide selection of different methods, maintenance of proficiency is heavily controlled

Analysing the quality of medicines in an OMCL

Different sources of samples

- Different surveillance programs depending on type of marketing authorisation
- Sample selection based on risk analysis
  - Products with National / MRP / DCP authorisation
  - Centrally authorised: *CAP testing program* selection by EMA/EDQM
- Samples from Inspectorate (routine / suspected quality defects / counterfeits)
- Samples from other government organisations
- Preauthorisation testing → support for assessment

Methods

- *Method transfer from MAH* (testing against specifications)
- Ph Eur methods
- In house –methods → eg. Screening methods to identify counterfeits

→ Evaluation of the *quality of the DP* and *suitability of the method*

→ *frequent issues with biological methods*

Results are reported to the MAH and if nonconformities are found, for the competent authority responsible for evaluation of the product. For CAP testing scheme, reporting is to EDQM who reports to EMA

Examples of potency assays

- Enzymatic assays
- Binding assays - ELISA (allergens, different biologicals)
- Cell assays
  - ✓Cytotoxicity / apoptosis / proliferation / reporter gene
  - ✓eg. etanercept, adalimumab, trastuzumab, bevacizumab, abatacept, belatacept, pertuzumab, infliximab, anakinra, basiliximab, alemtutsumab, filgrastim
- Read-out varies: absorbance / fluorescence / luminescence
- Modelling of dose response curves varies: 4 -parameter fit / parallel line / slope comparison
- Obstacles of successful method transfer are often simple
  - ✓ lack of details in the SOP (’tribal knowledge’)
  - ✓ detailed description of handling the cells, eg. the desired density
  - ✓ complex sample preparation
  - ✓ complex dilution series

Developing regulatory norms

EDQM

- Pharmacopoeia work in Ph. Eur. expert groups: 10B (chemical substances), *MAB WP*
- OMCL network: CAP-Advisory group; OMCL MAB group

→ development of surveillance programs for biosimilars and generics and strategies on testing suspected counterfeits

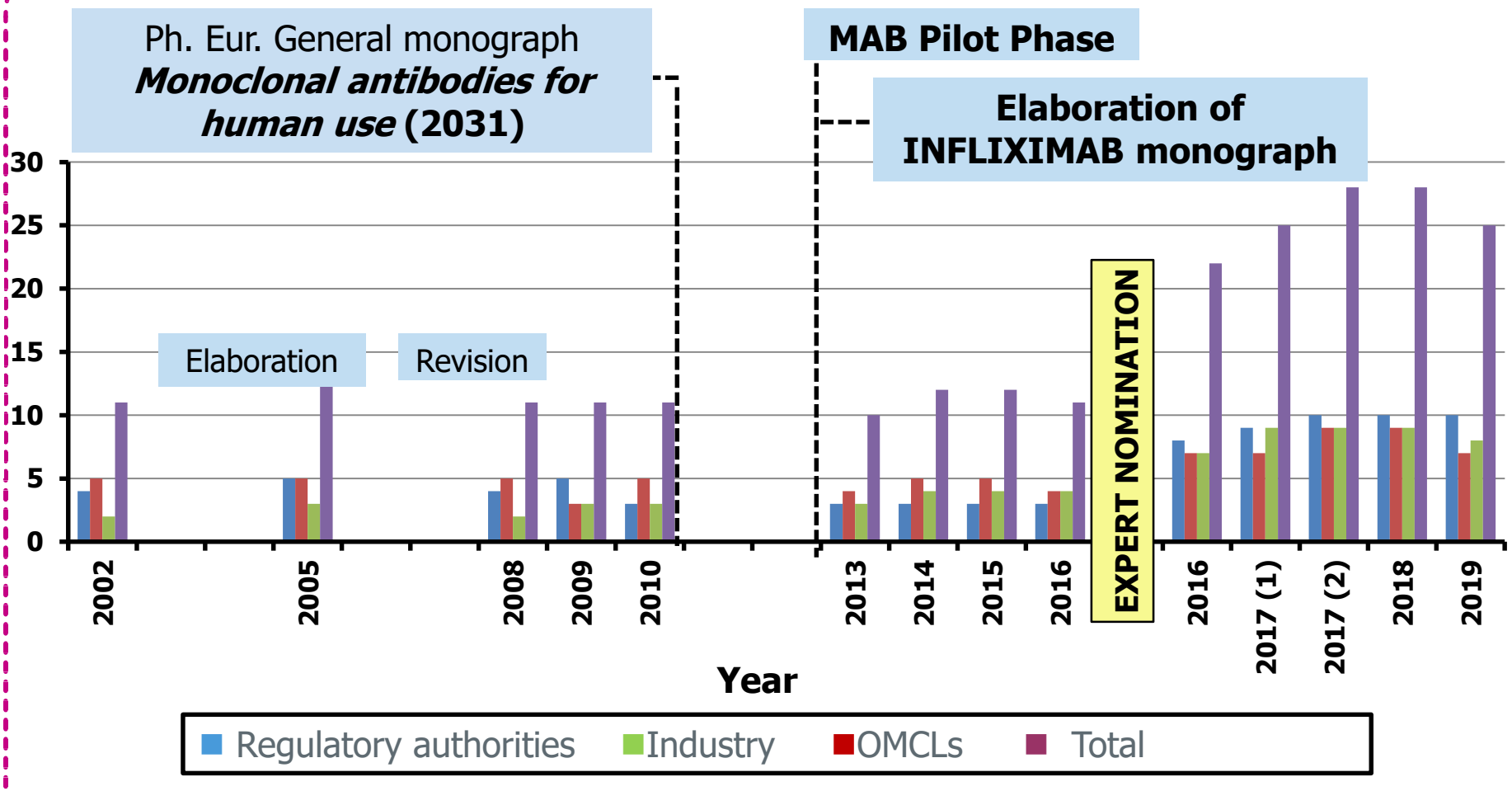
EMA

- Biological working party
- Assessment of biologicals

WHO

- Participation on collaborative studies to support making of international standards

The Ph. Eur. MAB Working Party



2019: MAB WP – Experts and Ad-hoc Specialists:

10 Regulatory authorities / 7 OMCLs / 8 Industry members

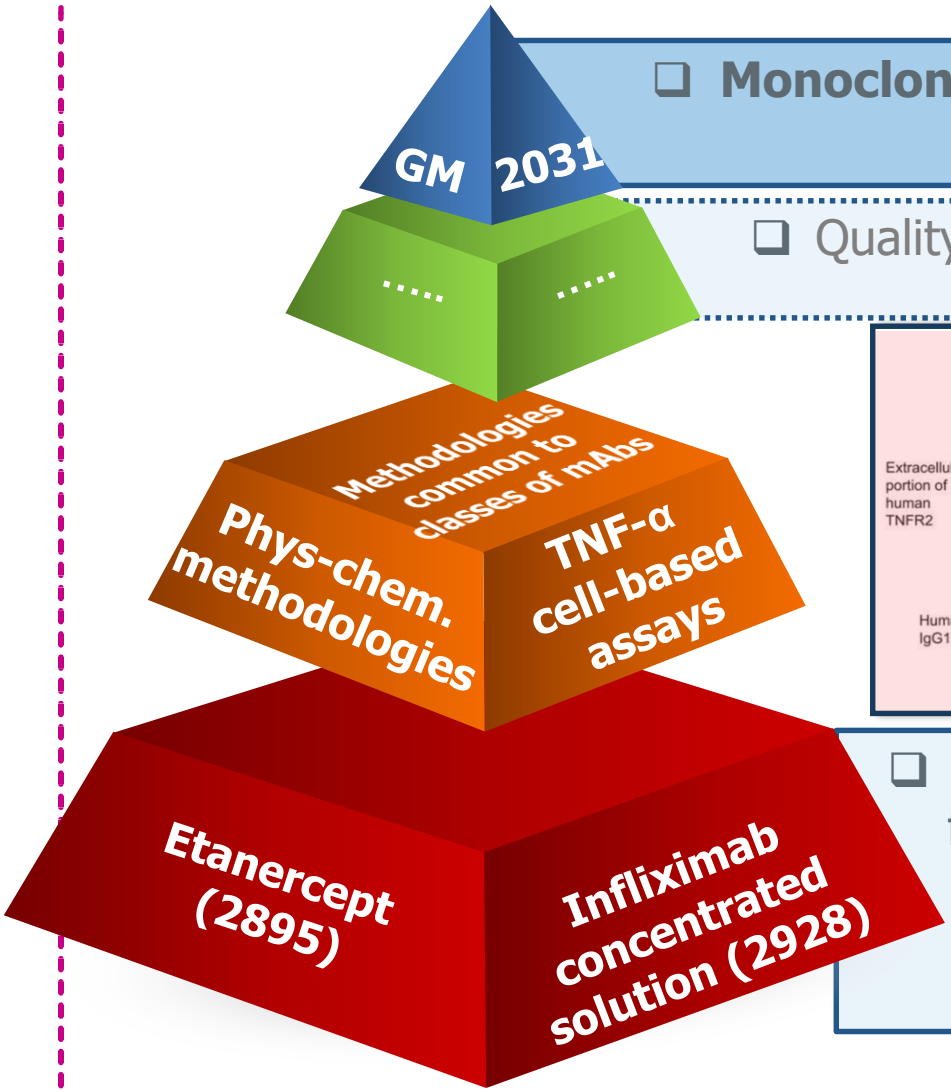
Bioassay Approaches in Ph. Eur.

General monographs	Individual monographs	General chapters
▪ classes of substances or products (defined by production method, intended use) ▪ mandatory requirements for all the products within the scope of definition section	▪ based on approved specification(s) backed up by batch data ▪ validated analytical procedures*; acceptance criteria (*unless otherwise stated) ▪ Eg. Etanercept, Infliximab	▪ recommendations for analytical procedures ▪ guidance for design of analytical methods and analysis of their results ▪ Mandatory <u>when referred to</u> in a monograph

Reference Standards
Biological Reference Preparations (BRPs)

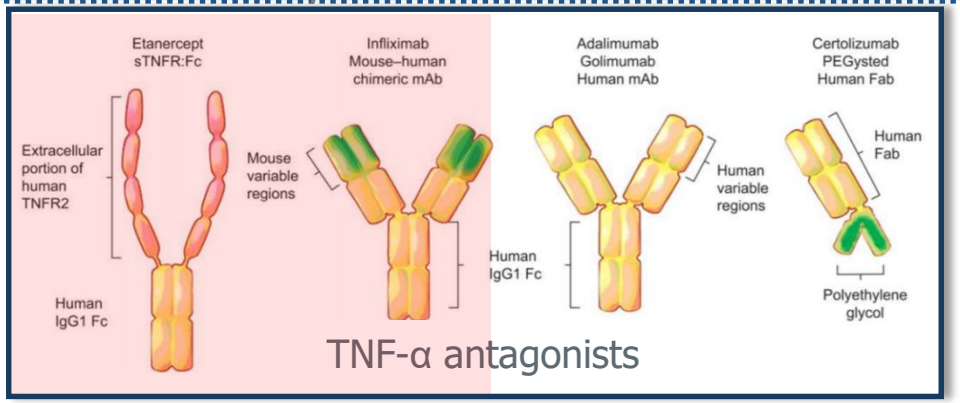
Established specifically and exclusively for use in monographs, as prescribed in the methods given.

PhEur MAB group: developing regulatory norms



❑ **Monoclonal antibodies for human use (2031)**
Overarching requirements

❑ Quality attributes common to (other?) classes/sub-classes of mAbs



❑ **Substance-specific quality attributes**
flexibility allowed; criteria to verify method performance; examples of suitable methods; *establishment of biological reference products*

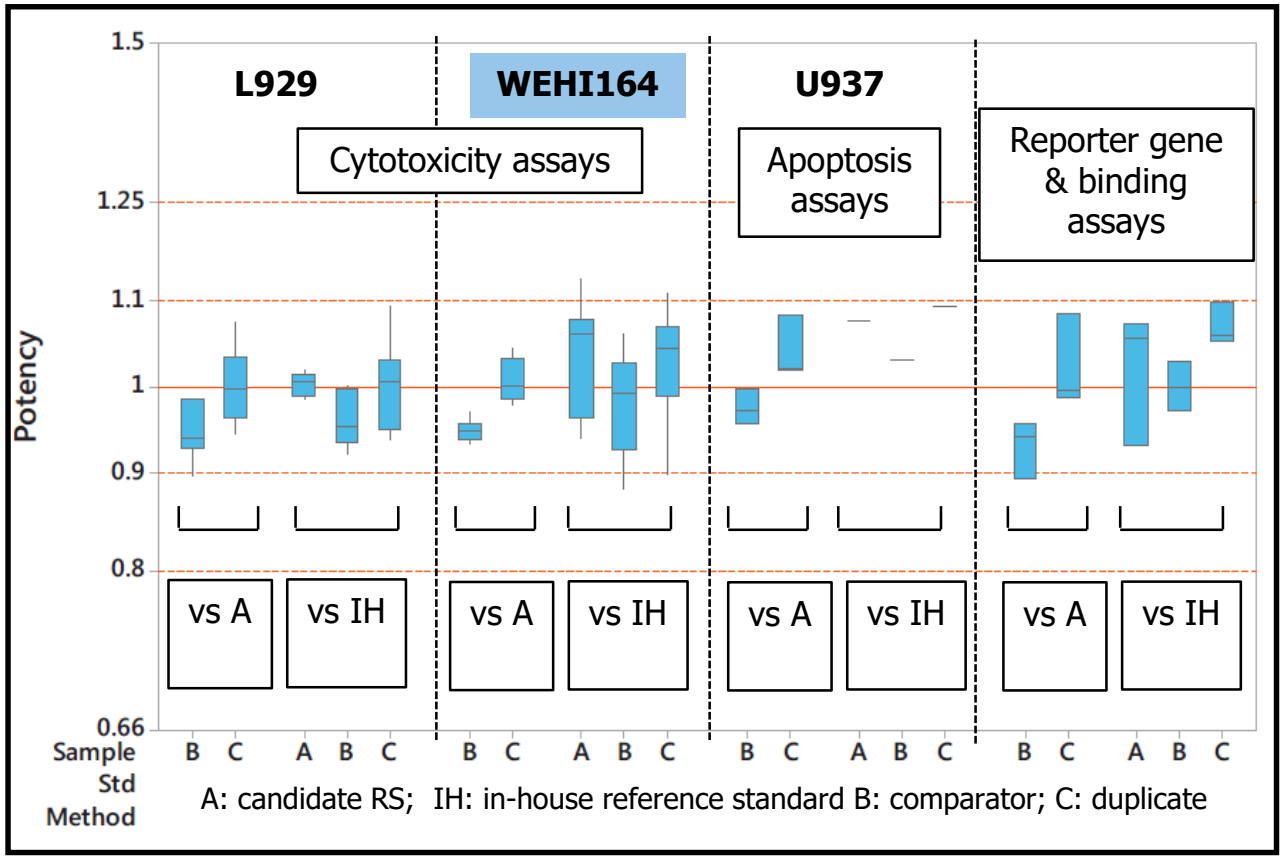
Ph. Eur. BRP for Etanercept – WHO/EDQM Study Results –

Summary of potency estimates relative to candidate RS

Assay	Sample	GM	95% Confidence Limits		Between-lab GCV (%)	n
U937 apoptosis	B	0.93	0.90	0.97	5.5	12
	C	0.93	0.91	0.96	4.7	12
	D	1.00	0.96	1.04	6.8	12
L929 cytotoxicity	B	0.93	0.88	0.97	8.0	12
	C	0.95	0.89	1.01	10.4	12
	D	1.01	0.95	1.08	10.4	12
Other cytotoxicity	B	0.93	0.78	1.10	14.5	5
	C	0.95	0.87	1.03	7.4	5
	D	0.99	0.89	1.09	8.7	5
Reporter Gene	B	0.93	.	.	.	2
	C	0.94	.	.	.	2
	D	1.03	.	.	.	2
Potency (all cell-based assays)	B	0.93	0.90	0.96	7.9	31
	C	0.94	0.92	0.97	7.4	31
	D	1.00	0.98	1.03	8.2	31

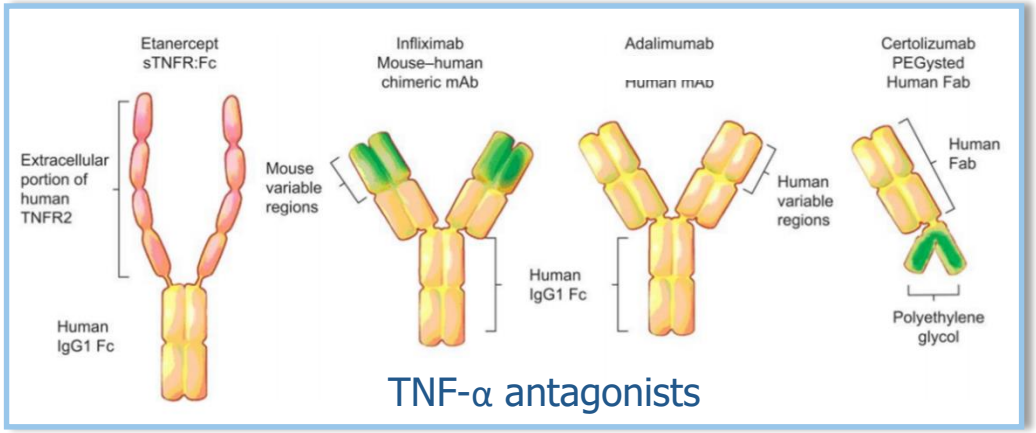
Adapted from M. Wadhwa et al, *Report on a Collaborative Study for Proposed 1st International Standard for TNF receptor II Fc fusion protein (Etanercept)* (WHO/BS/2015.2257)

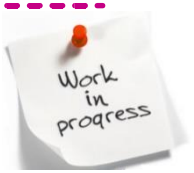
Ph. Eur. BRP for Infliximab
– WHO/EDQM Study Results –



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

MAB Pilot Phase: TNF-α Bioassays





ASSAY METHODOLOGY

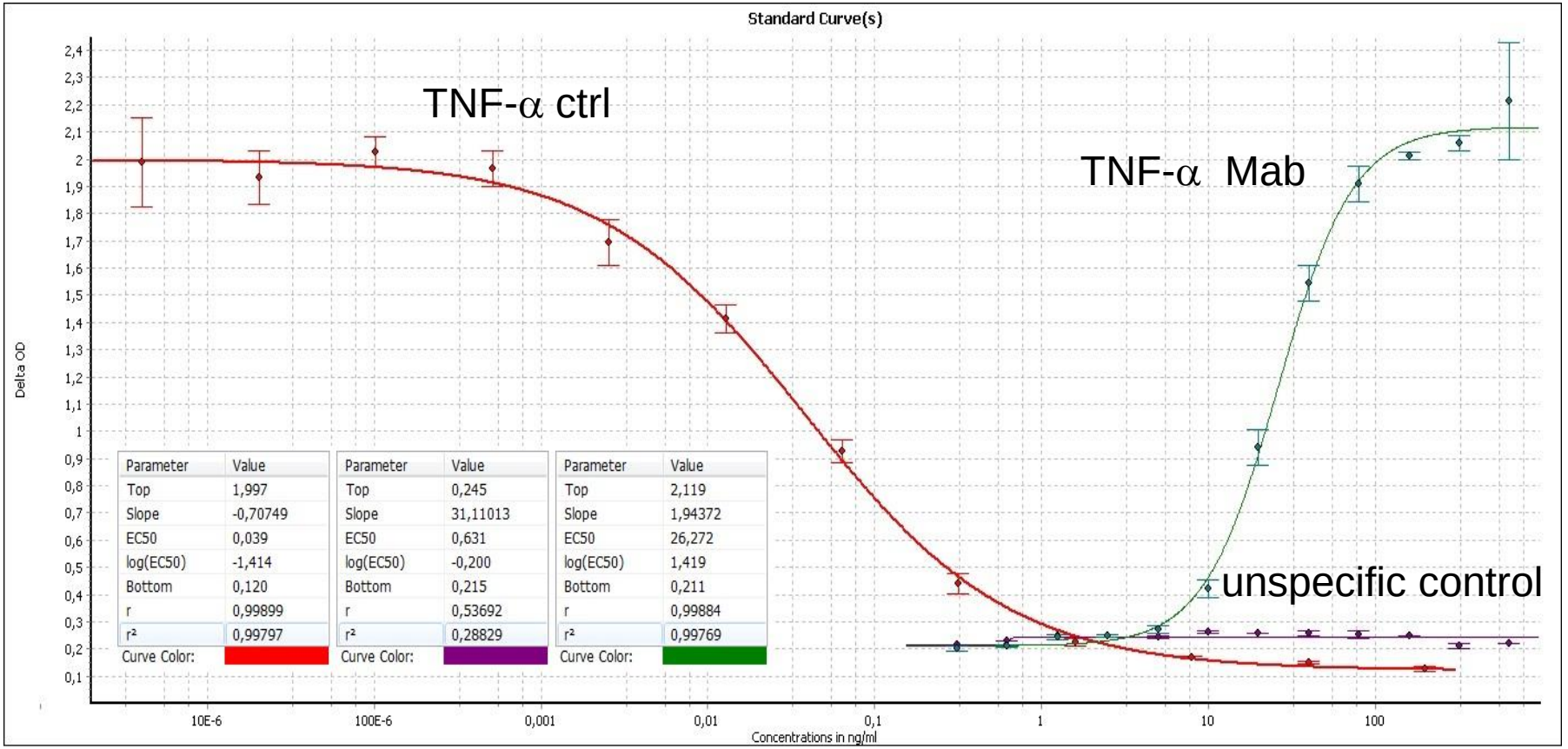
WEHI-164	U937	HEK-Blue CD40L	L929
cytotoxicity assay	apoptosis assay	reporter gene assay	cytotoxicity assay

Collaborative study: experimental verification of cell-based assays based on common laboratory protocol. Performed by OMCL laboratories

⇨ Sample panel: 7 TNF-α antagonists, Etanercept and Infliximab BRP & in house std

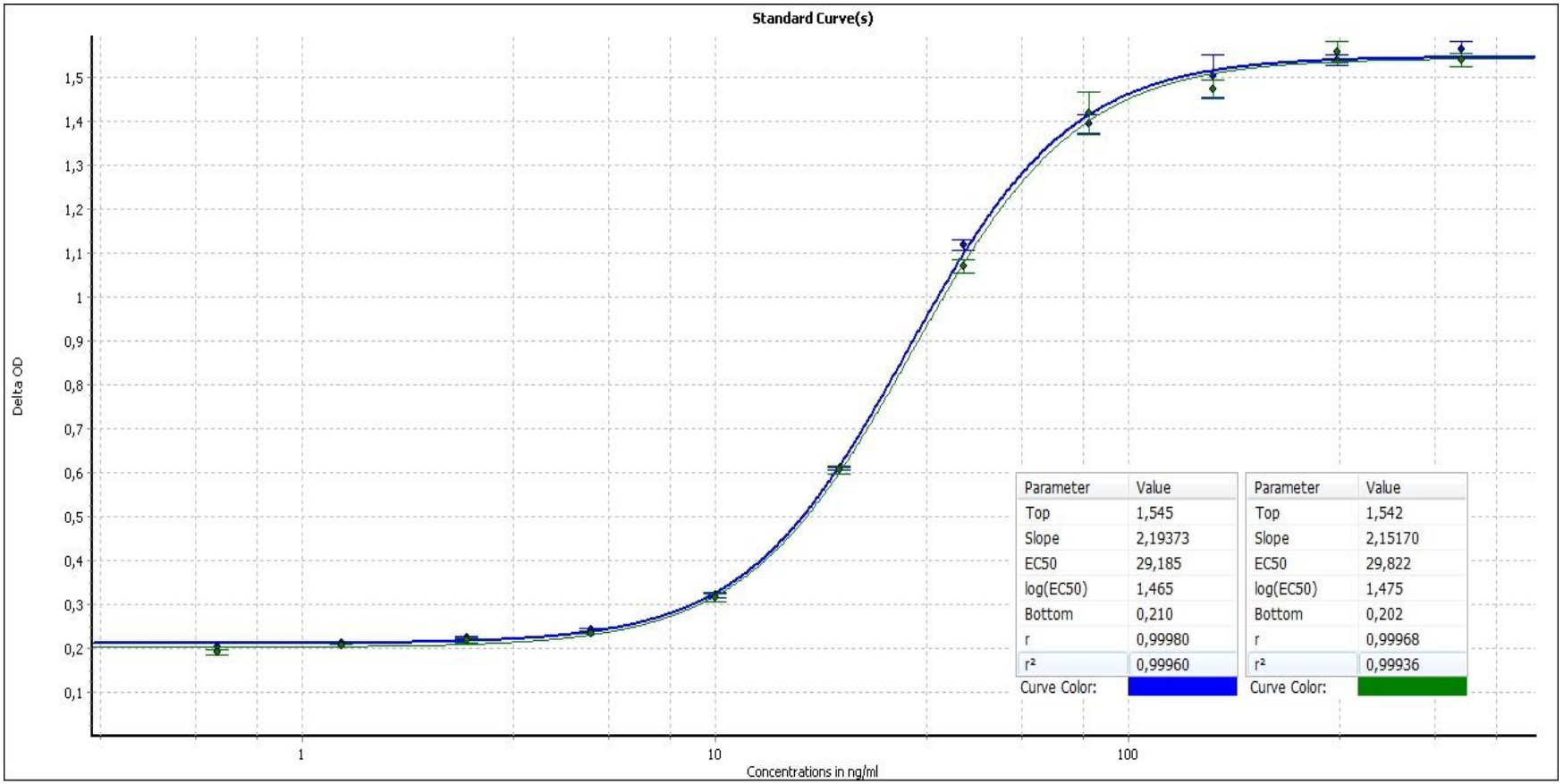
A single assay includes 24 multiwell plates and takes about 7-8 weeks, with adequate no of repetitions. Thanks to Kristiina Järvinen, Pia Lahti!

WEHI-164 Assay



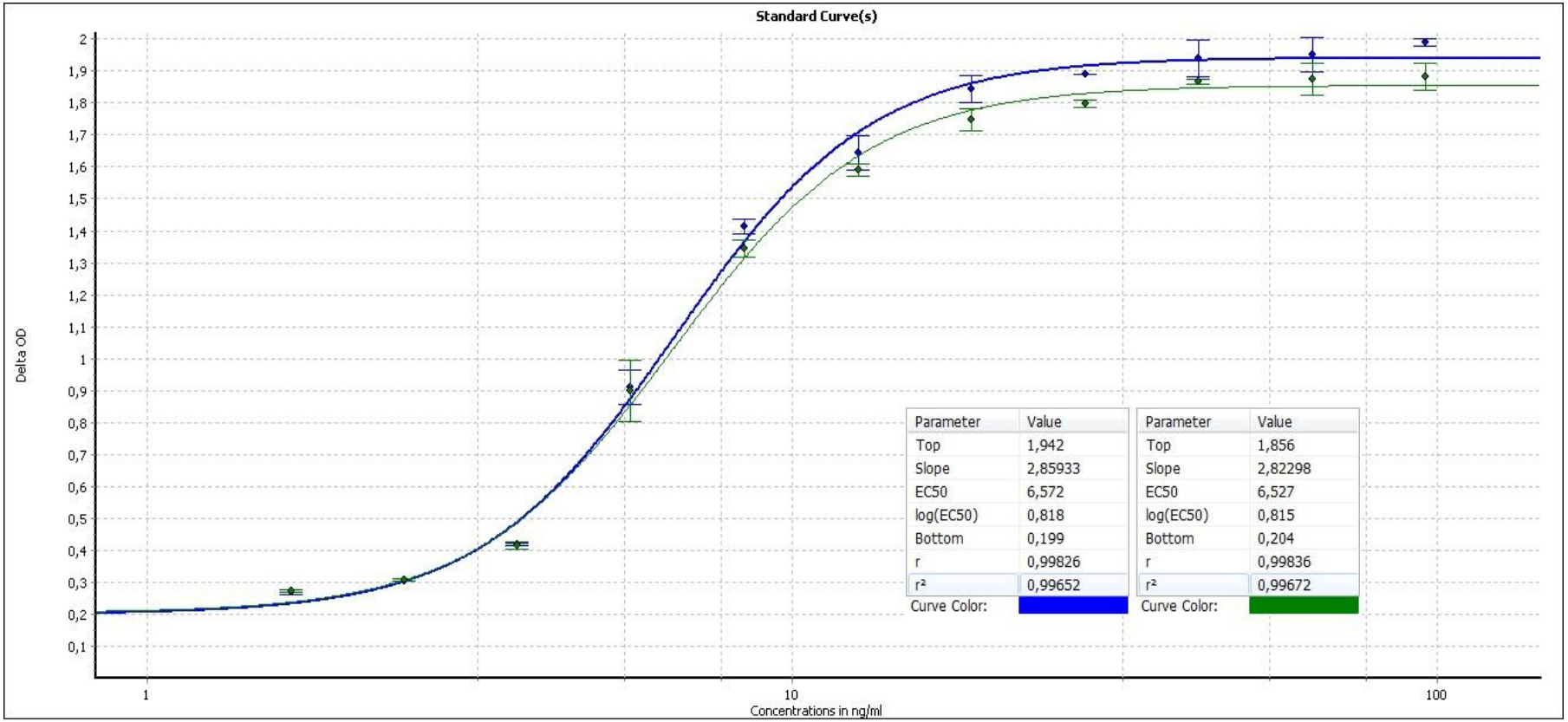
Infliximab BRP

A450-A650 nm: 0.2 – 1.5 AU



Etanercept BRP

A450-A650 nm: 0.2 – 1.9 AU



WEHI-164 Summary

- *The same generic method using WEHI-164 cells works well for all TNF-alpha inhibitors tested*

- RELATIVE potency assay
- Easy to perform, low-cost assay
- Specificity verified with a non-TNF-alpha inhibitor mAb
- Concentration range may need to be modified for different products (Etanercept vs Infliximab)
- Experiments 'self-against-self' look very similar → precision/ accuracy looks promising
- Curve fitting for all curves very good, $r^2 > 0.99$
- Lower asymptote very stable
- Upper asymptote appears to vary between analysis in different days, likely to be caused by cell passage no (increasing read-outs with increasing passage 5-13)

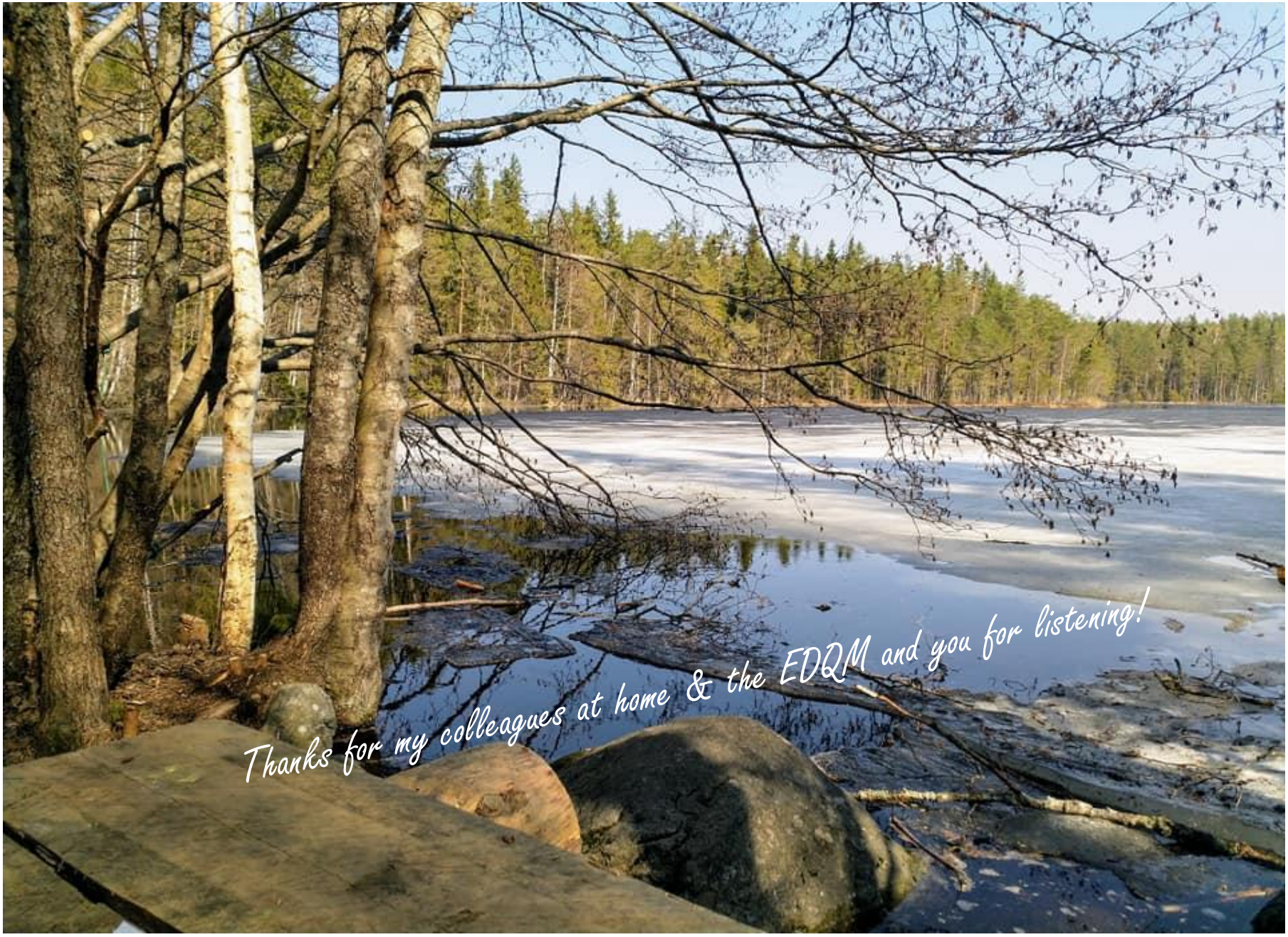
Summary of OMCL activities

- ✓ OMCL laboratory is a neutral, impartial national authority with supervisory role
- ✓ EDQM coordinates the network
- ✓ Quality surveillance programs are based on risk analysis
- ✓ Typically a lot of different methods are within the scope
- ✓ Typically a heavy quality system
- ✓ Participate developing regulatory norms

- ✓ Perform multiple different types of *Bioassays*
  - → accumulating the knowledge of various methods
  - → accumulating the understanding of various products
  - → accumulating the understanding of regulatory possibilities

- ✓ *Working for safety, efficacy and good quality of medicines*

fimea


Thanks for my colleagues at home & the EDQM and you for listening!