Assay Strategies And Technologies To Analyse Soluble Targets Of New Antibody Therapeutics

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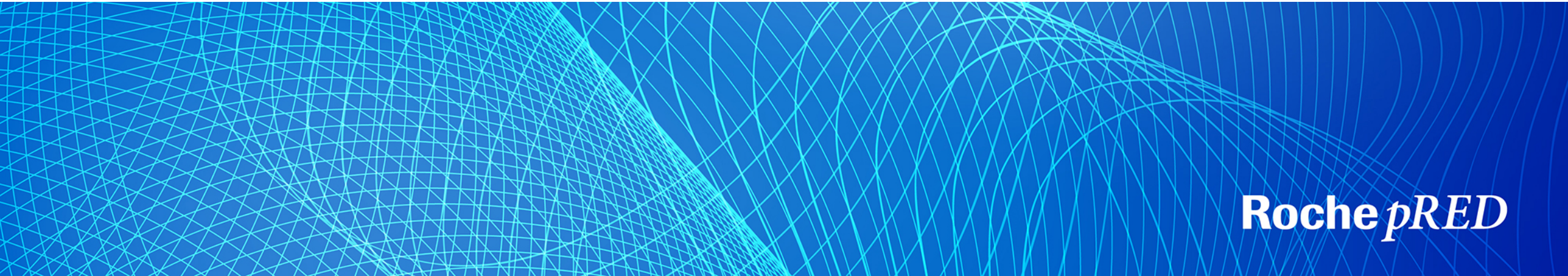
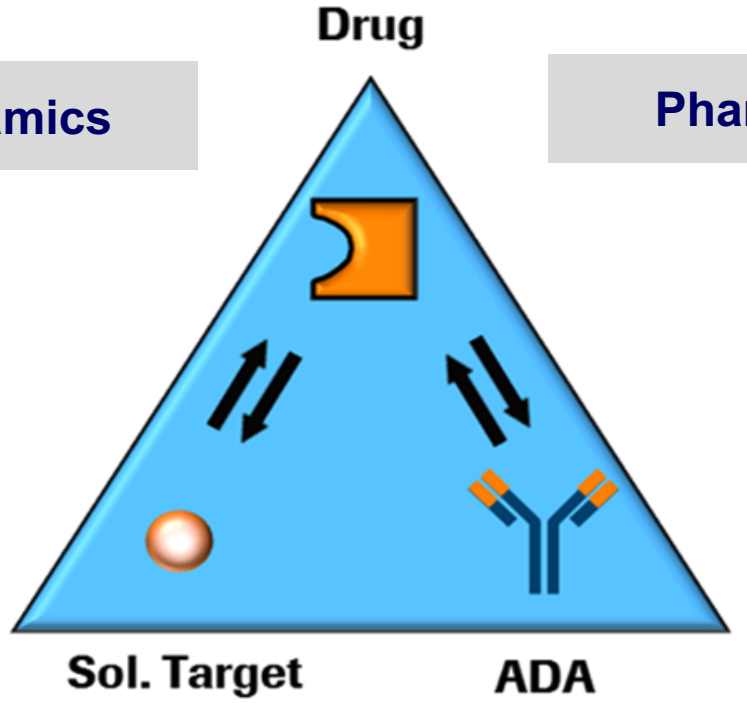


Bioanalytical Assays in Biologics Development

The interplay between drug, target and ADAs

Pharmacodynamics

Pharmacokinetics



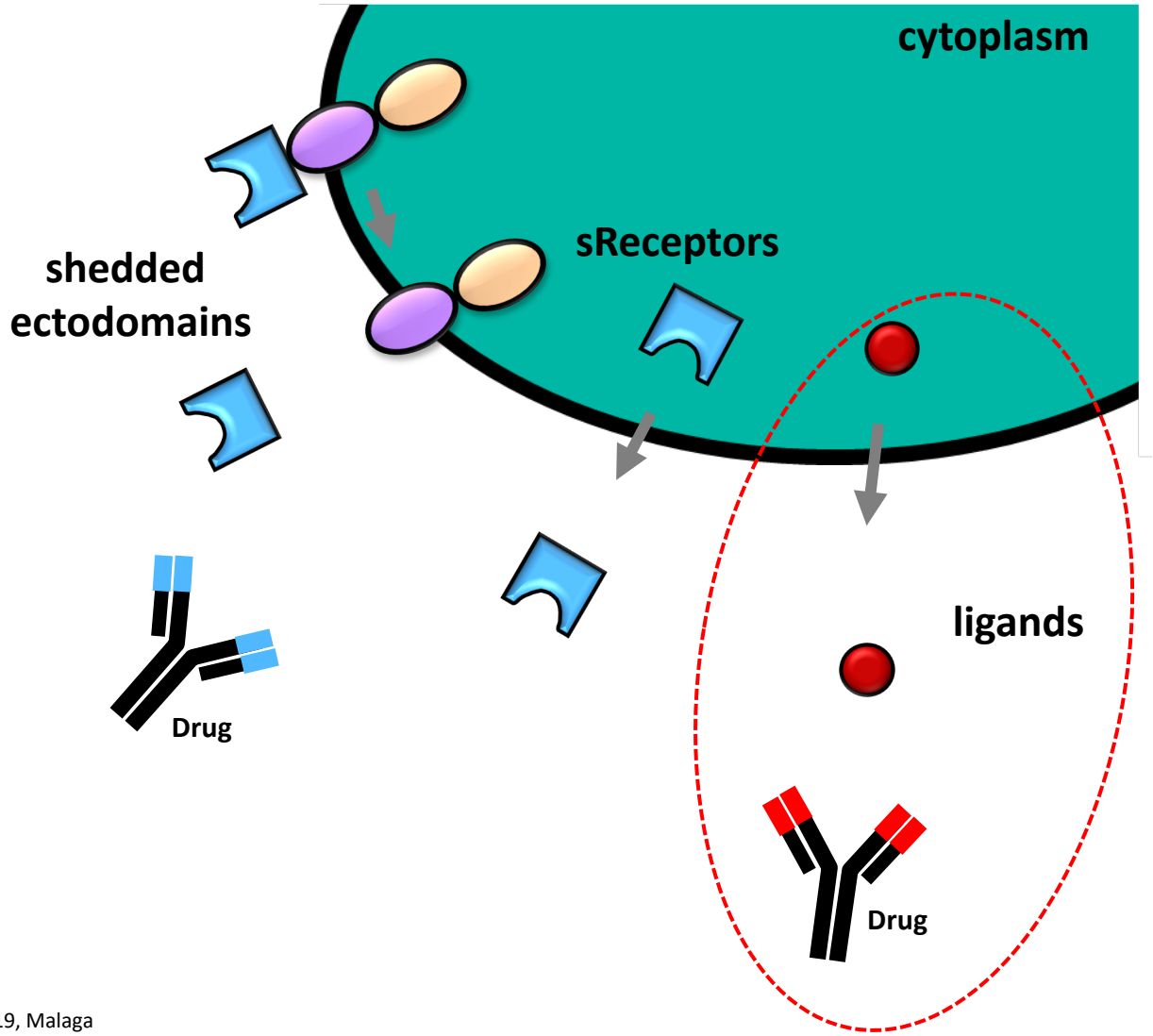
Immunogenicity

- Analytes are not independent from each other
- Interactions have to be considered for
  - Drug exposure assessment
  - Immunogenicity testing
  - Target engagement
  - … many more

Soluble Targets

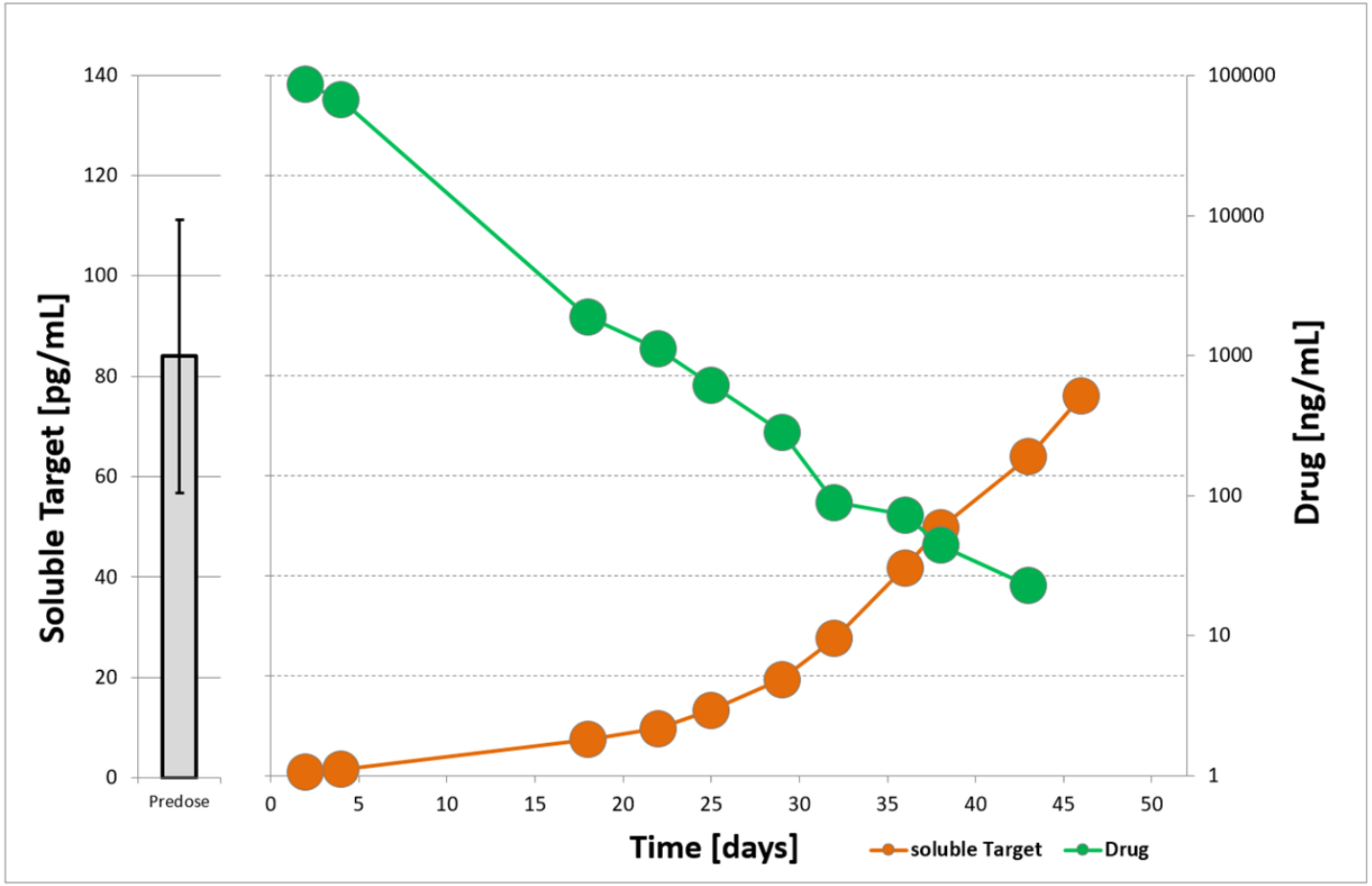
A heterogeneous group of secreted proteins

- Soluble targets comprise
  - Ligands
  - Soluble receptors
  - Shedded receptor ectodomains
- Analysis of soluble targets is challenging because of assay interference by
  - Oligomerisation
  - Isotype variants
  - Ligand-receptor interaction
  - Drug



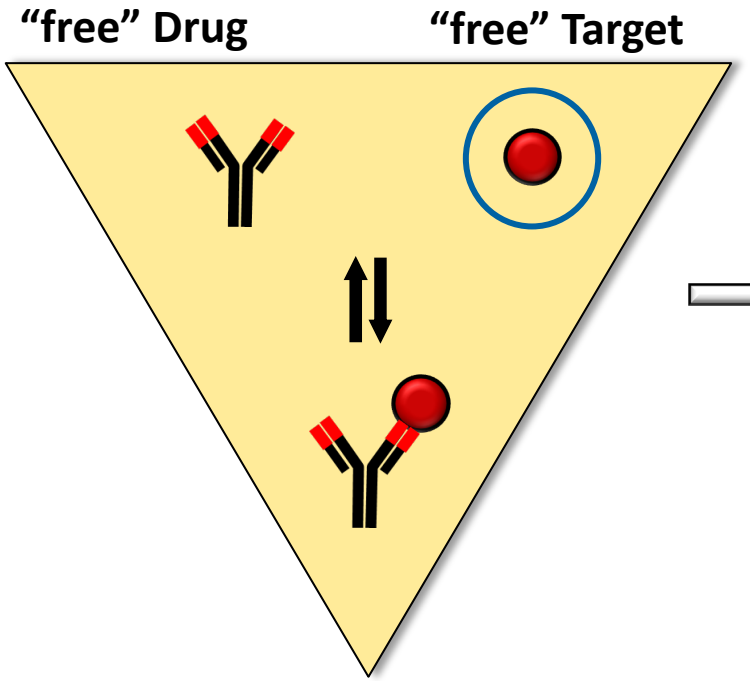
The Interplay between Soluble Target and Drug

PK/PD relationship of an antagonistic antibody and its target

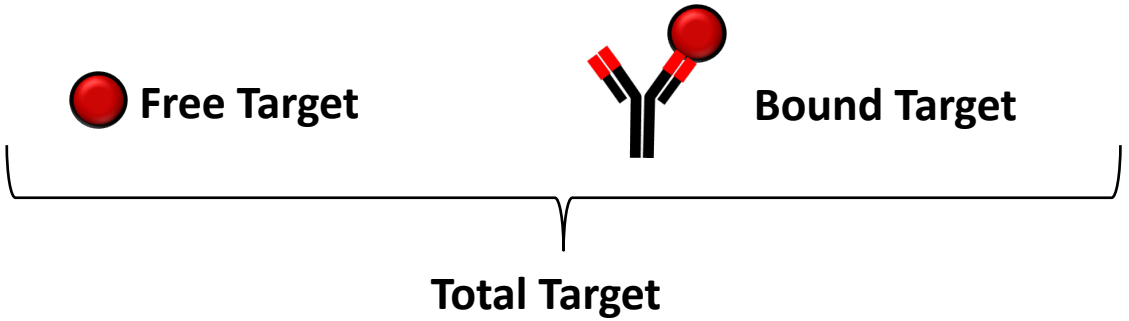


- Combined drug and soluble target analysis provides information on:
  - Drug exposure and clearance
  - Target engagement
  - Definition of dose and dosing frequency & many more
- *In vivo* and assay-related interactions have to be considered

Soluble Target Analysis for Antibody Therapeutics



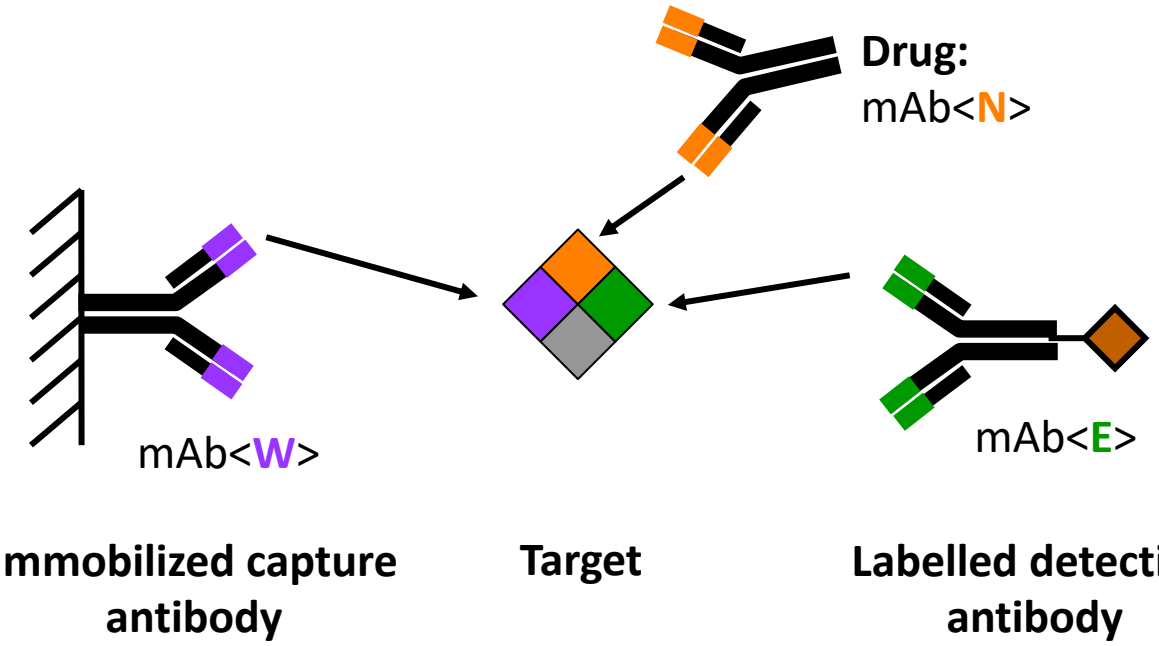
- Proof of *in vivo* binding of mAb to ligand (target engagement)
- Target occupancy and PK/PD relationship
- Efficacious mAb levels
- Dose and dosing schedule selection

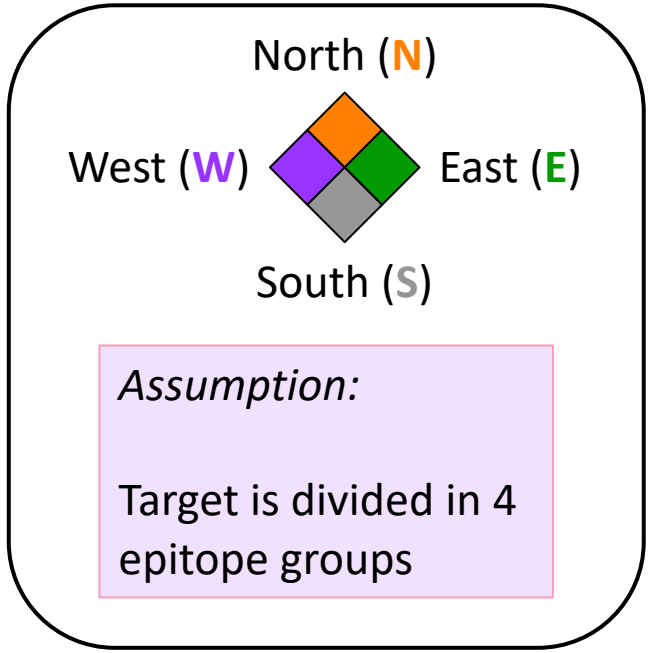


- **Differentiation between total, bound and free target desirable**
- **Challenge: *in vivo* situation (drug-target equilibrium) should kept unchanged by the assay**

Soluble Target Assays for Antibody-Drugs

A) Total target (drug-bound + free target)



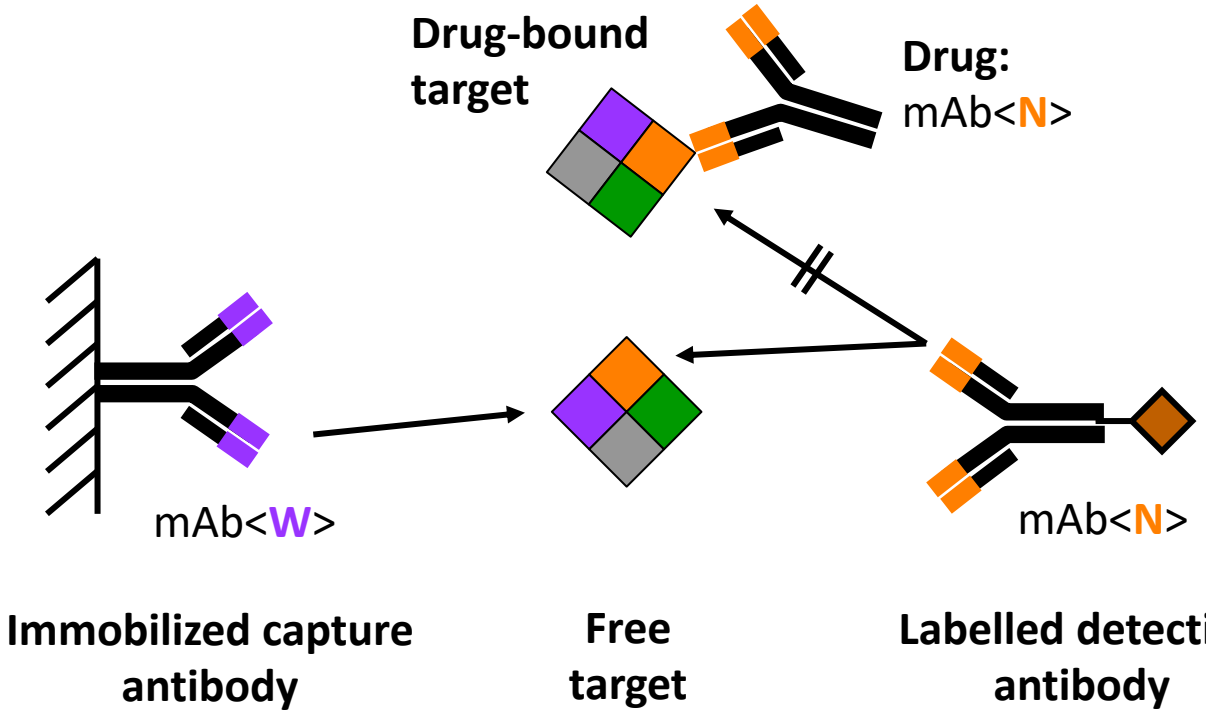


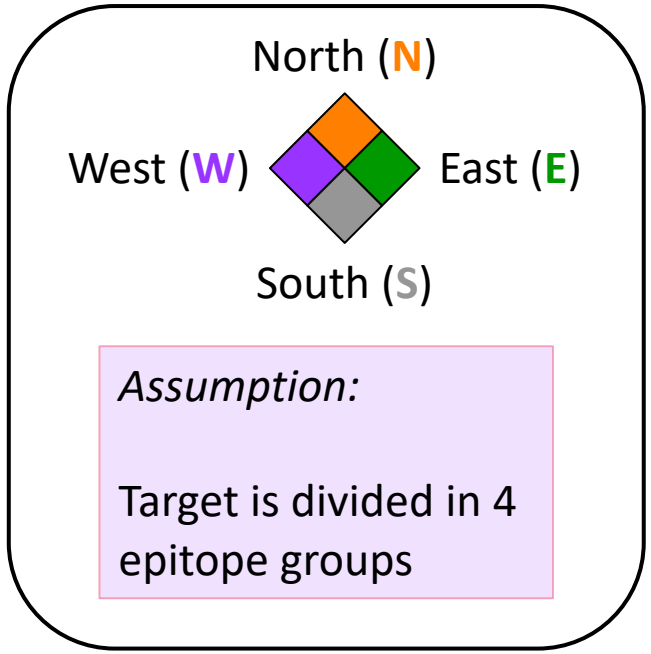
Stubenrauch et al., JPBA.102:459-67. (2015)

- Both, detection (<E>) and capture (<W>) antibody bind to different epitopes than the therapeutic antibody (Drug, <N>)
- Therapeutic antibody (Drug, <N>) does not interfere with target quantification

Soluble Target Assays for Antibody-Drugs

B) Free target





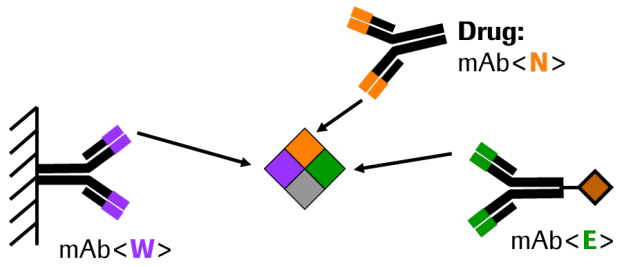
Stubenrauch et al., JPBA.102:459-67. (2015)

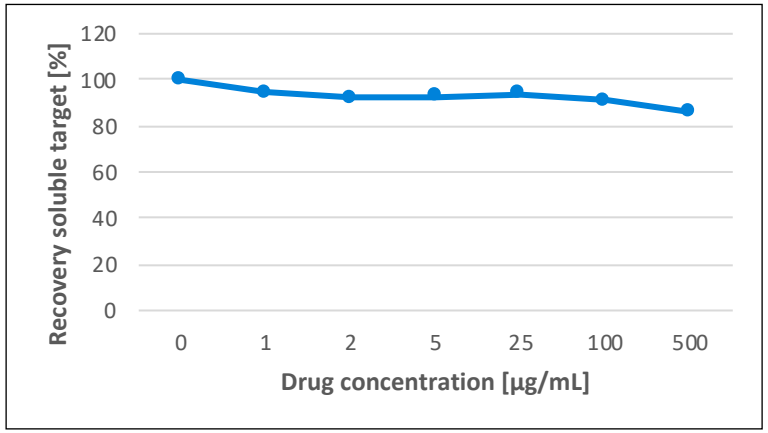
Detection or capture antibody bind to the same epitope as the drug (<N>), but with lower affinity, e.g.: with detection by <N> and capture by <W> as shown

Soluble Target Assays for Antibody-Drugs

Assessment of drug influence is essential to understand what is analyzed

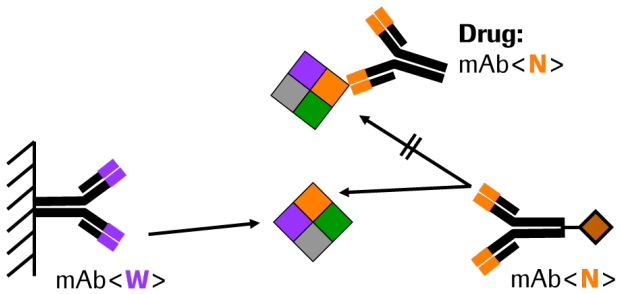
Total Assay

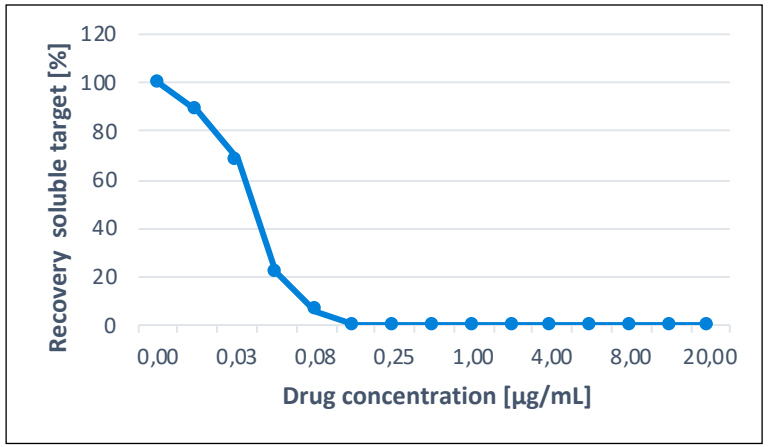




- Increased drug concentration has no influence on soluble target quantification

Free Assay



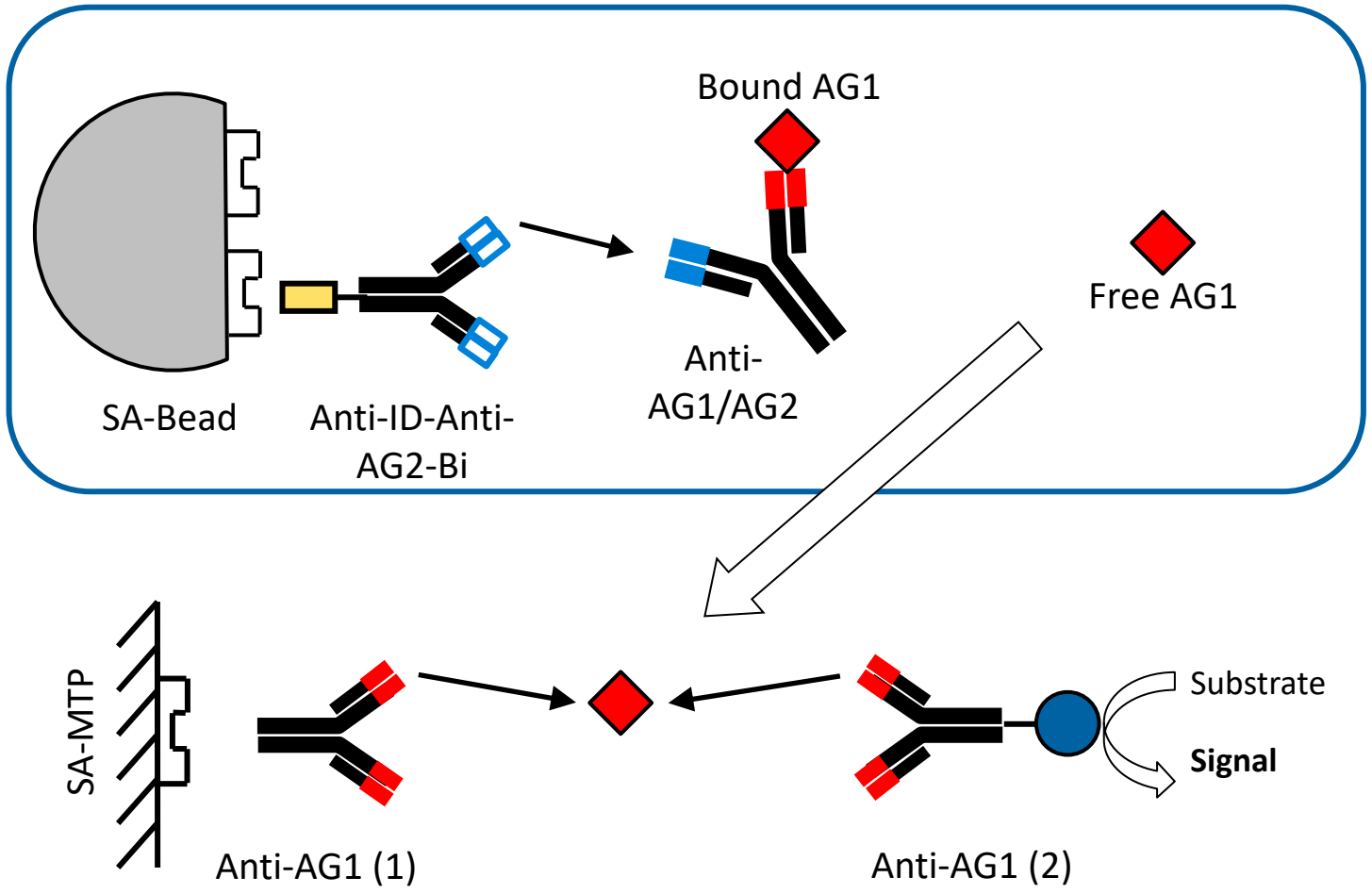


- Free soluble target assay signal is decreased by higher drug concentrations

Assessing of drug influence is essential for understanding your assay and what is analyzed

New Chance for Free Soluble Target Analysis

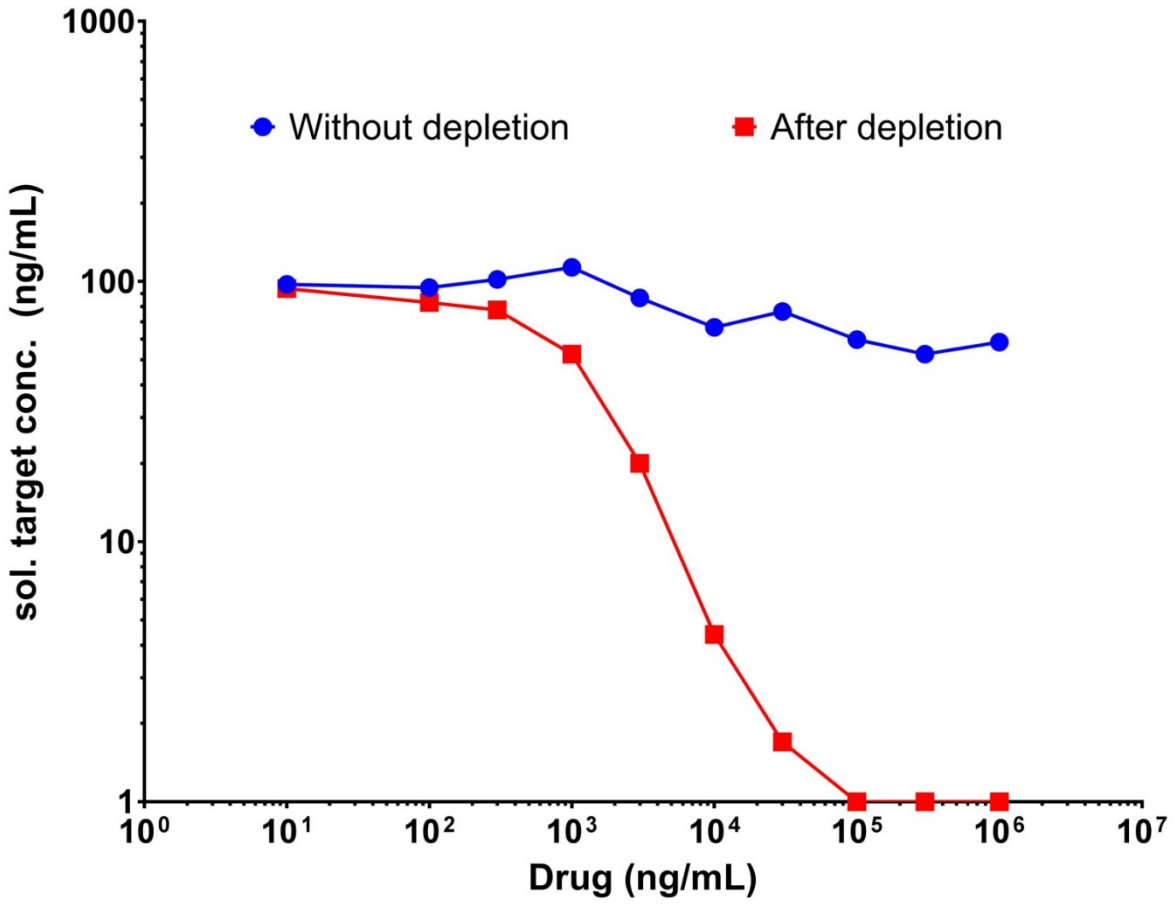
Immunodepletion of drug-bound target



- Removal of drug-bound target advances analysis of free target (free AG1) using AG2 Specificity
- Bispecificity of the drug enables co-depletion as pretreatment w/o impact on the equilibrium between Drug and Target (AG1)

Stubenrauch et al., JPBA.102:459-67. (2015)

New Chance for Free Soluble Target Analysis
Immunodepletion of drug-bound target



- The immunodepletion procedure removes the drug-bound target. Free target remains detectable.
- This results in a free target measurement.

Stubenrauch et al., JPBA.102:459-67. (2015)

Challenges in Soluble Target Assay Development

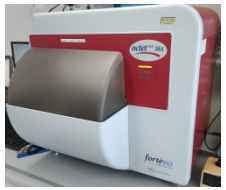
- Physiological concentrations
- Biological variability
- Selecting the most representing reference material
- Assay acceptance criteria to be defined based on scientific judgement
- Clinical needs and the expected picture define the required assay range and LLOQ
- Assay may require to be adapted and re-validated according to the current clinical question
- „Fit-for-purpose“ approach requires constant alignment of the clinical purpose with an in-depth scientific understanding of assay and biomarker biology

➔ **Each Soluble Target requires a unique assay and validation strategy for each clinical purpose**

Technologies - “Purpose-driven” Used in Assay Development

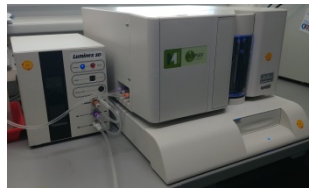
MS, Q Exactive HF-X
(Thermo)

Biacore T200
(GE)



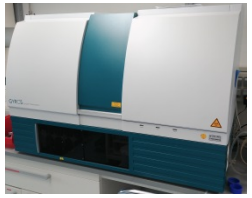
Octet Red96 (Pall)

Cobas e411
(Roche)



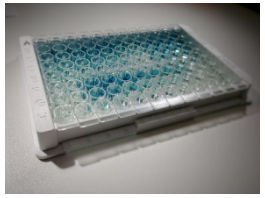
Luminex 200
(Luminex)

Assay Concept



Gyros (Gyrolab)

SP-X
(Quanterix)

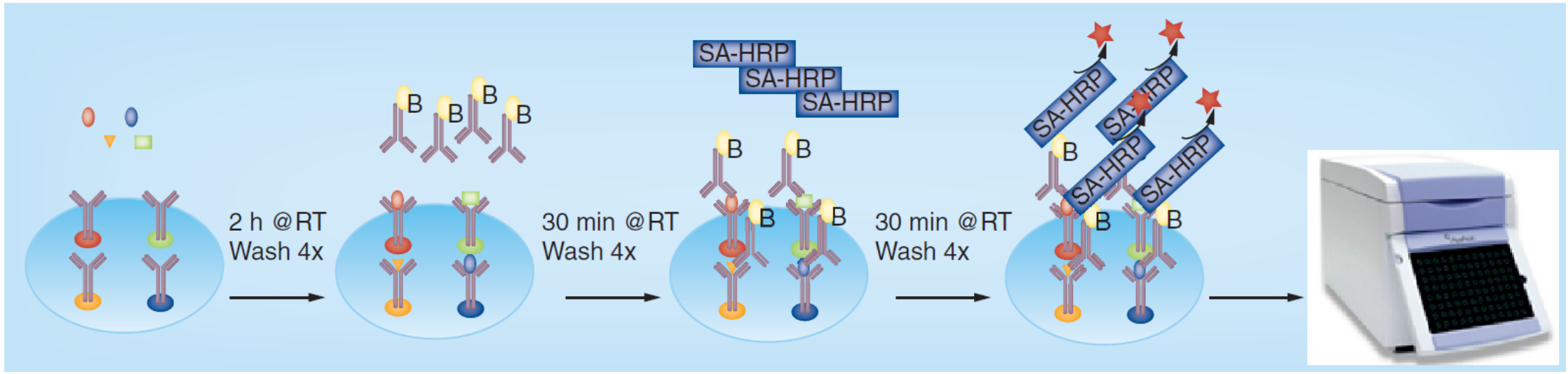


ELISA

Simoa
(Quanterix)

ELLA (Biotechne)

Quanterix SP-X ULTRA Ultrasensitive Assays



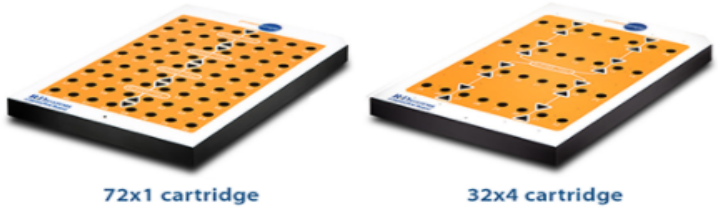
Pro :

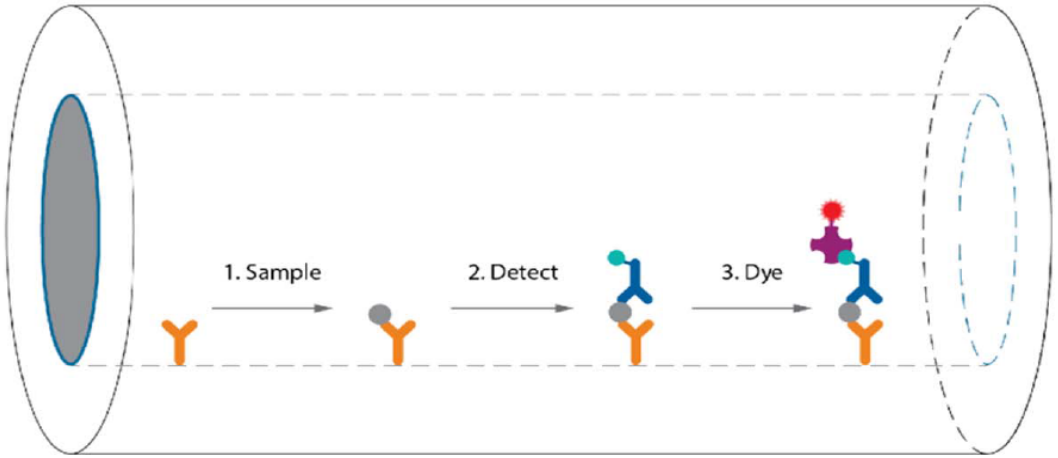
- Similar workflow to ELISA
- Multiplexing
- High sensitivity (Single digit fg/mL range)
- Moderate operator training level required

Con :

- May be susceptible to inaccurate working (Sensitive system – Specific washer needed)
- Vendor-dependent spotting of capture reagents (batch to batch variability to be proven)

ELLA Simple Plex Assays





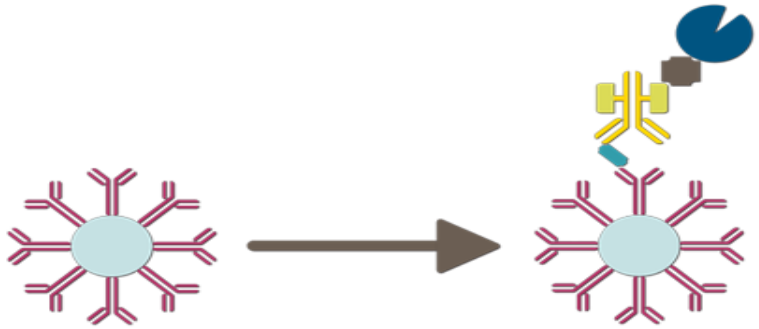
Pro :

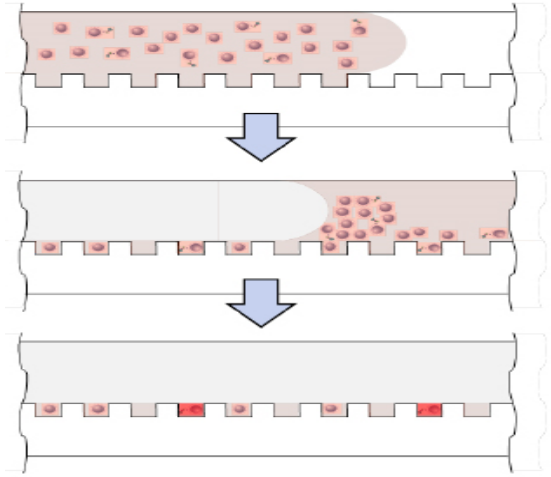
- Relatively low sample consumption (2.5-25µL)
- Low operator training level required
- Low operator hands-on time
- Commercial assays for large number of analytes (>150)
- Good Correlation to Luminex / Simoa data

Con:

- Moderate Sensitivity (LLOQ IL-6 : 0.82pg/ml)
- Limited options for in-house assay development
- Vendor-dependent coating of capture reagents

Simoa™ (Single Molecule Arrays)
HD-1 Analyzer of Quanterix





Pro :

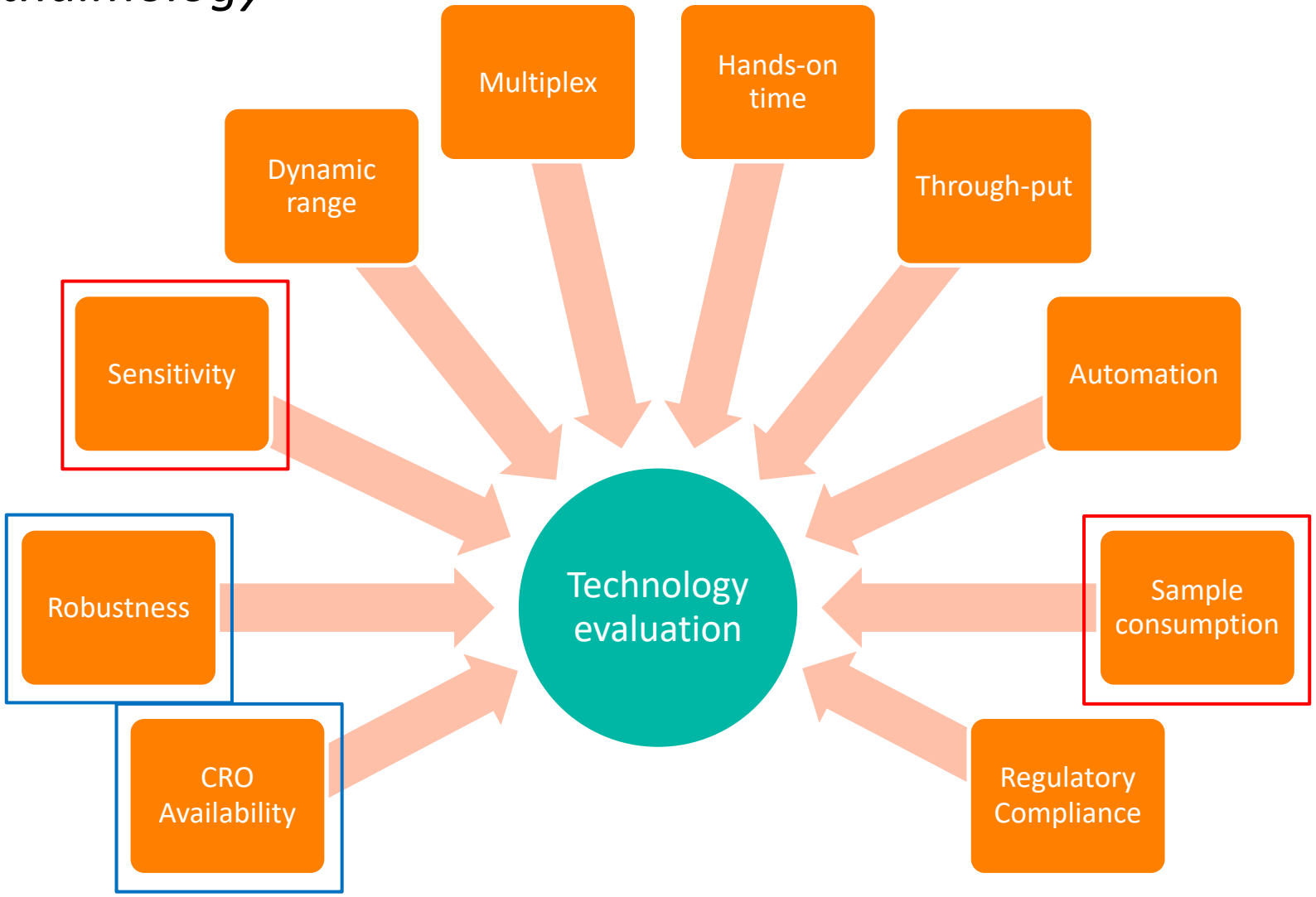
- High sensitivity (two digit fg/mL range)
- Highly automated system
- Robust second generation instruments

Con :

- High instrument costs
- Highly trained operator needed (assay development)

Evaluating Technology Performance Parameter

Example: Ophthalmology



Technology Selection

Case 1 : Quantification of IL-6, based on available Kits

Technology	Sensitivity (pg/mL)	Sample consumptionf or duplicate (Dilution)	Assay Transfer
ELISA	0.78	40µL (1:5)	++
Quanterix SP-X	0.2	25 µL (1:4)	+
ELLA	1.64	25 µL (1:2)	+++
Luminex	19.2	100 µL (1:2)	++
Luminex + Curiox	19.2	10 µL (1:2)	-
Simoa HD-1	0.022	32.5 µL (1:4)	+
Singulex	0.01	200 µL (neat)	-
Pro Quantum	0.064	10 µL (neat)	-*

* - Limited experience

Goal : Quantification of free sol. **IL-6** in Serum samples

Available Volume : 500µL

Required sensitivity : 0.5 pg/mL

Assay Transfer

Decision : SP-X / Simoa HD-1

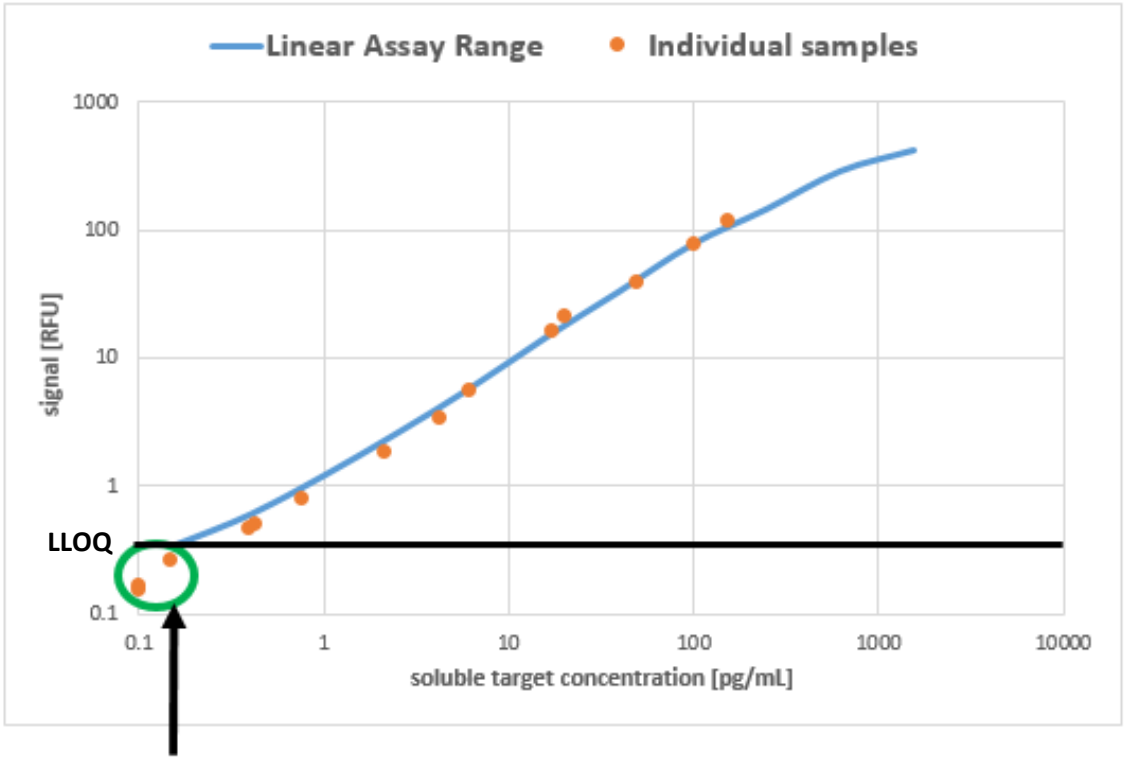
Open question : Free or Total Assay ?

➔ Testing of kits with study samples in presence of drug shows the suitability for measurement of free target.

Technology Selection based on Sensitivity

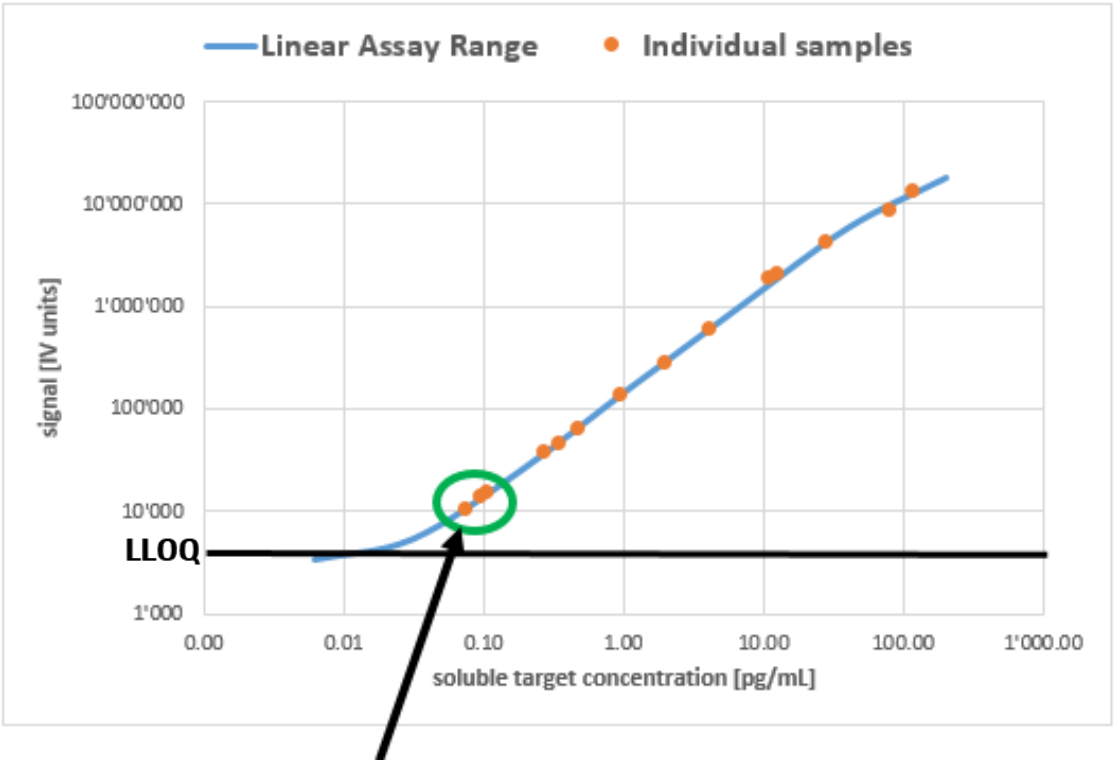
Analysis of individual samples in comparison

Technology 1



below LLOQ ➢ quantification not possible

Technology 2



above LLOQ ➢ reliable quantification

Technology Selection

Case 2 : Quantification of Ang2, based on available Kits

Technology	Sensitivity (pg/mL)	Sample consumption (Dilution)	Assay Transfer
ELISA	1175	40µL (1:5)	++
Quanterix SP-X			+
ELLA	32	25 µL (1:2)	+++
Luminex	360	50 µL (1:2)	++
Luminex + Curiox	360	5 µL (1:2)	-
Simoa HD-1	4.35	15µL (1:8.7)	**+**
Singulex	-		-
Pro Quantum	-		-*

* - Limited experience

Goal : Quantification of free sol. **Ang2** in Aqueous humor samples

Available Volume : 20µL

Required sensitivity : 5 pg/mL

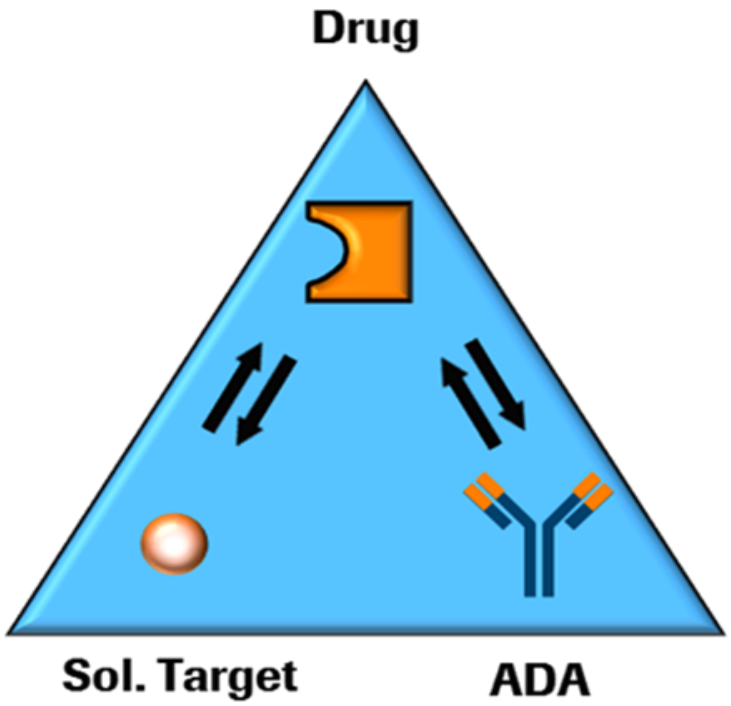
Decision : Simoa HD-1

Open question : Free or Total Assay ?

➔ If no free assay is found, a new homebrew assay must be developed also influencing the choice of technology.

Bioanalysis of Soluble Targets of New Antibody Therapeutics

Conclusions



- Understand your drug and targets to develop assay strategies that result in relevant data
- Individual assay strategies reflecting the biological and clinical context are key for state-of-the-art analysis of Soluble Targets
- Novel antibody therapeutics require purpose-driven technology assessments

Doing now what patients need next