Free vs. total drug/ligand measurements: A hot topic since 2008

Presenter: Daniela Stoellner, on behalf of EBF

EBF 4th Open Symposium 2011
18-Nov-2011
Barcelona

Free vs. total drug/ligand measurements: a “hot topic” since 2008

1. LBABFG July 2008 Quarterly Newsletter: Why do we care whether a LBA is measuring “total” or “free” protein therapeutic or biomarker? Report from 2008 NBC Hot Topic session.
2. 2009 NBC session on “Free (specific) and Total (Generic) Immunoassay in Biotherapeutic Development”.
3. Kuang B, King L, Wang HF: Therapeutic monoclonal antibody concentration monitoring: free or total? Bioanalysis (2010) 2(6), 1125-1140.
4. AAPS webinar on “Free and total assays of antibody biotherapeutics and target: utilities, challenges, and impact on PK/PD applications”, 2010.
5. White paper on “Bioanalytical Approaches to Quantify “Total” and “Free” Therapeutic Antibodies and Their Targets: Technical Challenges and PK/PD Applications over the Course of Drug Development”. Lee J et al, AAPS Journal (2011).
6. Ahene AB: Application and interpretation of free and total drug measurements in the development of biologics. Bioanalysis (2011) 3(11), 1287-1295.
7. Yang J, Quarmby V: Free versus total ligand-binding assays: points to consider in biotherapeutic drug development. Bioanalysis (2011) 3(11), 1163-1165.
8. 2011 NBC symposium on “Free and total measurements of biotherapeutic and target ligand for PK-PD interpretations”

EBF

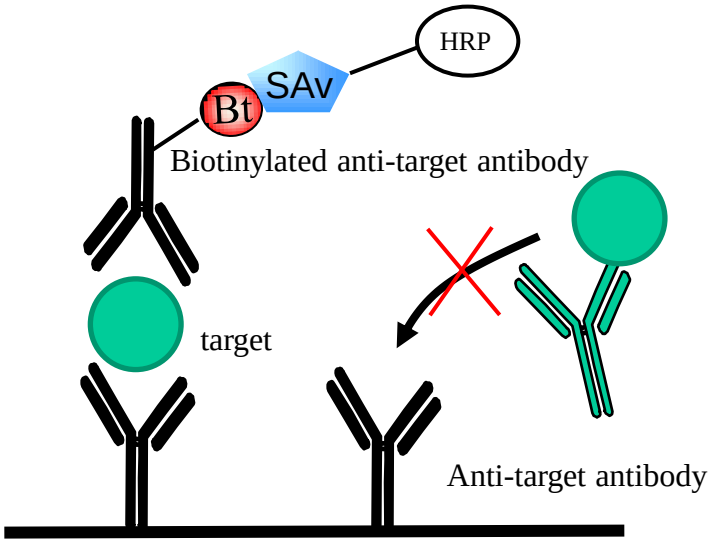
Free vs. total drug/ligand measurements: a “hot topic” since 2008, cont.

9) Topic team “free vs total” formed by EBF-IGM members in 2011

10) 2011 EBF Open Symposium Barcelona: Break-out session on “Challenges of free and total macromolecule quantification”

WHY A HOT TOPIC?

Case study 1: Free target assay



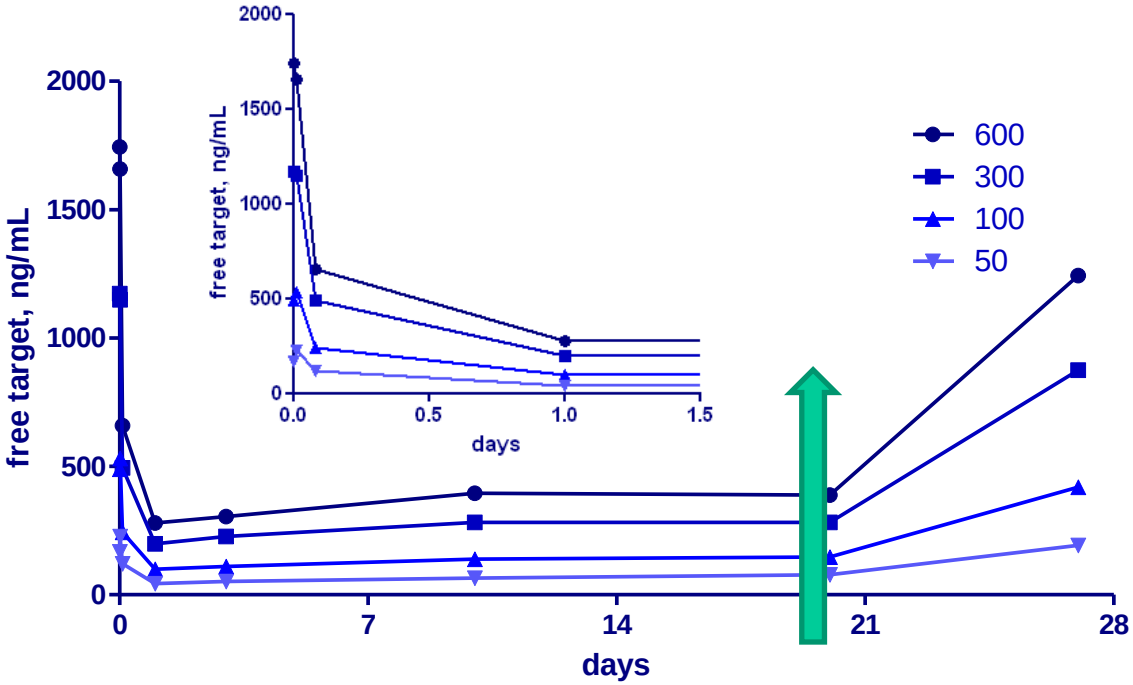
Target bound to therapeutic Mab not able to bind to coated anti-target Ab

Important facts about the assay / target:

- Endogenous level: 2-5 ug/mL
- No parallelism shown up to 1:600 dilution
- MRD: 600

Case study 1: Impact of dilution on sample results

- Samples from preclinical study were analysed diluted 1:50, 1:100, 1:300, 1:600



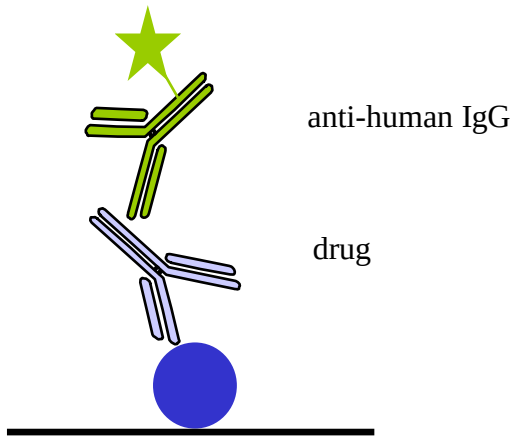
The higher the dilution, the higher the measured concentration of free analyte → matrix effect ? → dissociation of bound forms

Case study 2: Free drug assay

One format – 2 assay conditions

Condition 1:

Coating conc:
100 ng/mL
MRD: 10



Condition 2:

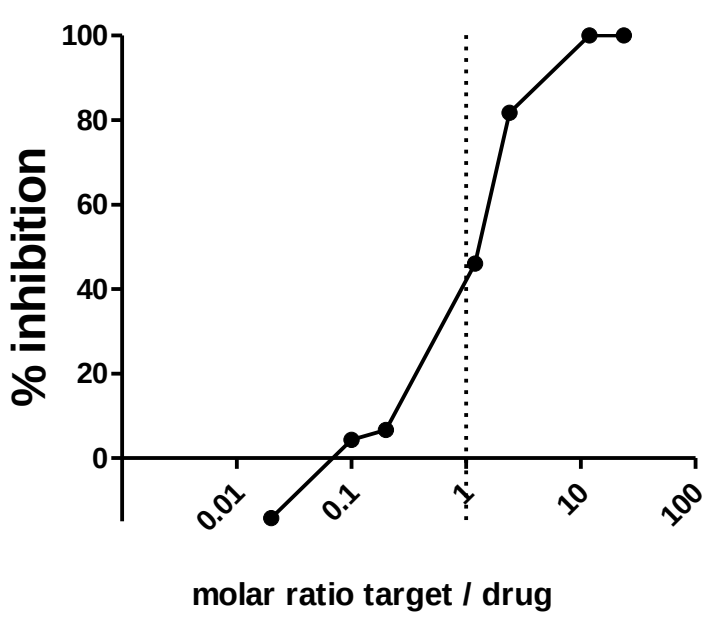
Coating conc:
1 ug/mL
MRD: 20

Drug specific LBA using target as capture reagent

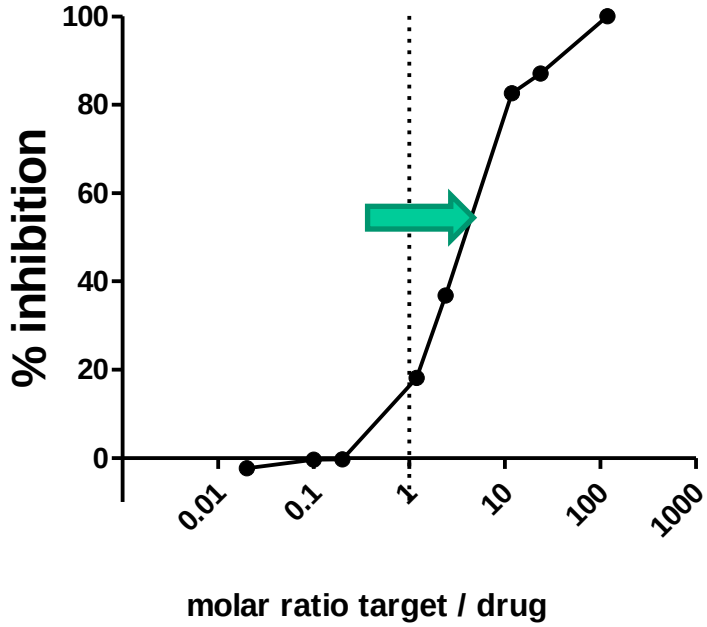
Case study 2: Impact of coating conc and MRD on free drug levels

- **Interference testing**

MRD: 10, coating 100 ng/mL



MRD: 20, coating 1 ug/mL



- For free drug assay → IC_{50} would approach 1

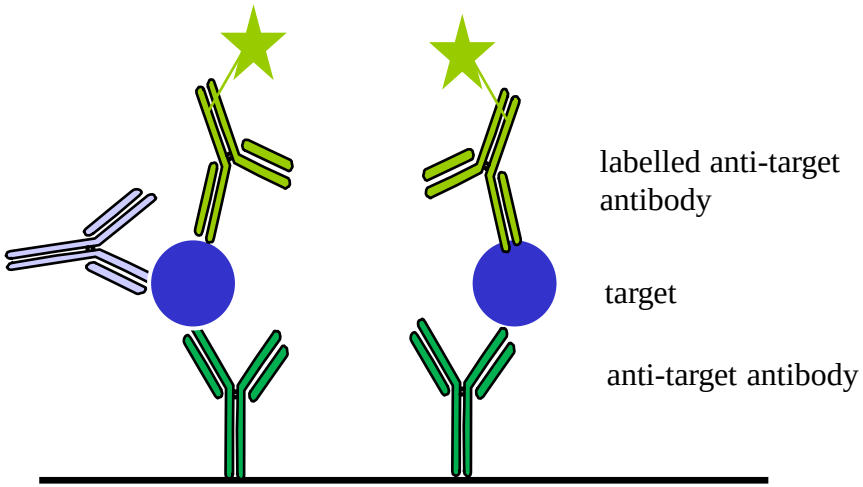
Case study 3: Total target assay

- Need to determine **suppression of free target** levels to validate the ability of the drug to bind its target → drives clinical response
  - ✓ Limitations:
    - Low endogenous levels → high sensitivity required
    - Impact of sample processing and assay conditions on binding equilibrium → over-estimation of free levels

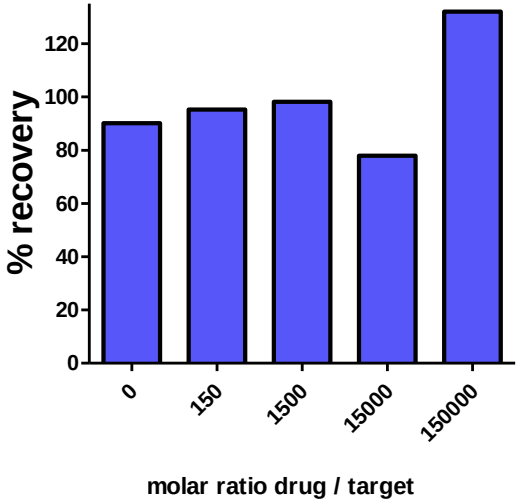
- Therefore: measure **total target** levels
  - ✓ true values
  - ✓ Binding to thMAb usually results in accumulation of total target
- BUT....

Case study 3: Total target assay

....**technical challenge** to find tool antibodies used as capture/detection reagent binding to different epitopes and not competing with the thMab:



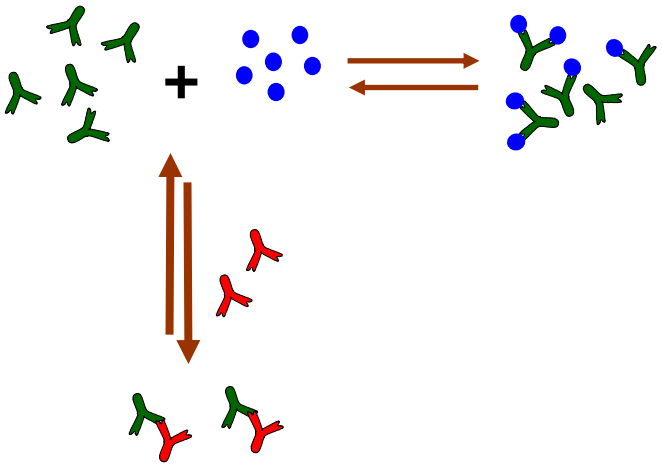
Target bound to therapeutic Mab and unbound target

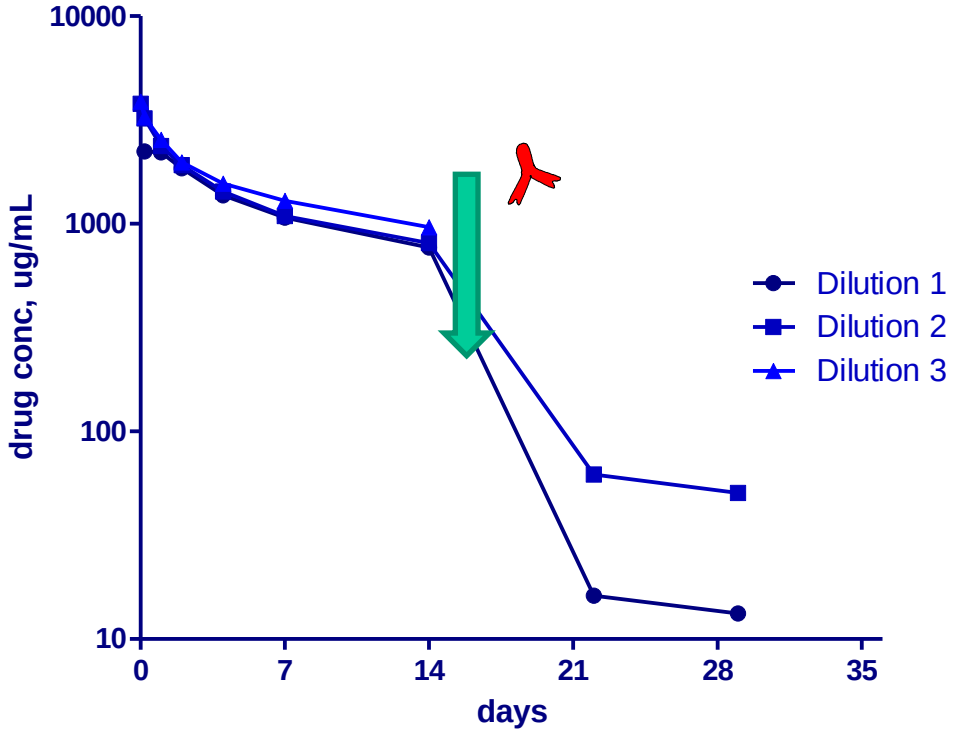


Interference testing confirmed total format:
% target recovery 70 to 130 %

Case study 4: Impact of ADA and dilution on free drug levels

- Anti-drug antibodies: new binding partner





Acknowledgement

Thanks to

- EBF Topic Team 20

  Dietmar Seemann, Sherri Dudal, Michaela Golob, Marianne Scheel Fjording, Margarete Brudny-Kloeppel, Marie-Helene Pascual, Eva Vieser and Daniela Stoellner

Program

1. **Lindsay King (Pfizer)**
   Risk assessment for the measurement of Free and Total drug and target
2. **Roland Staack (Hoffmann-La Roche)**
   Mathematical simulation tools in bioanalytical assay development
3. **Philip Lowe (Novartis)**
   Integration of physiological and biochemical concepts into the development of biopharmaceuticals
4. **Panel Discussion**