PRA Health Sciences

Efficient set-up of a cell-based neutralizing antibody assay using a flow cytometry-based Receptor Occupancy assay format

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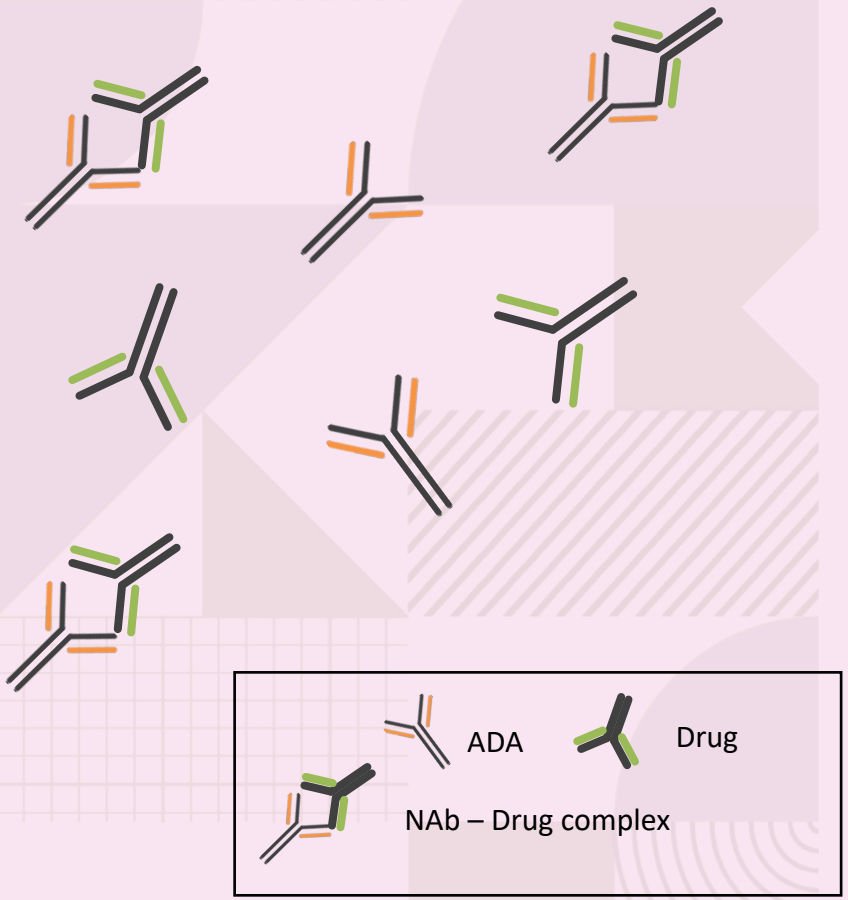
21 November 2019

Need for Neutralizing Antibody assays



- In case of Anti-Drug Antibody (ADA) positivity ➔ Need for Neutralizing Antibody (NAb) assay

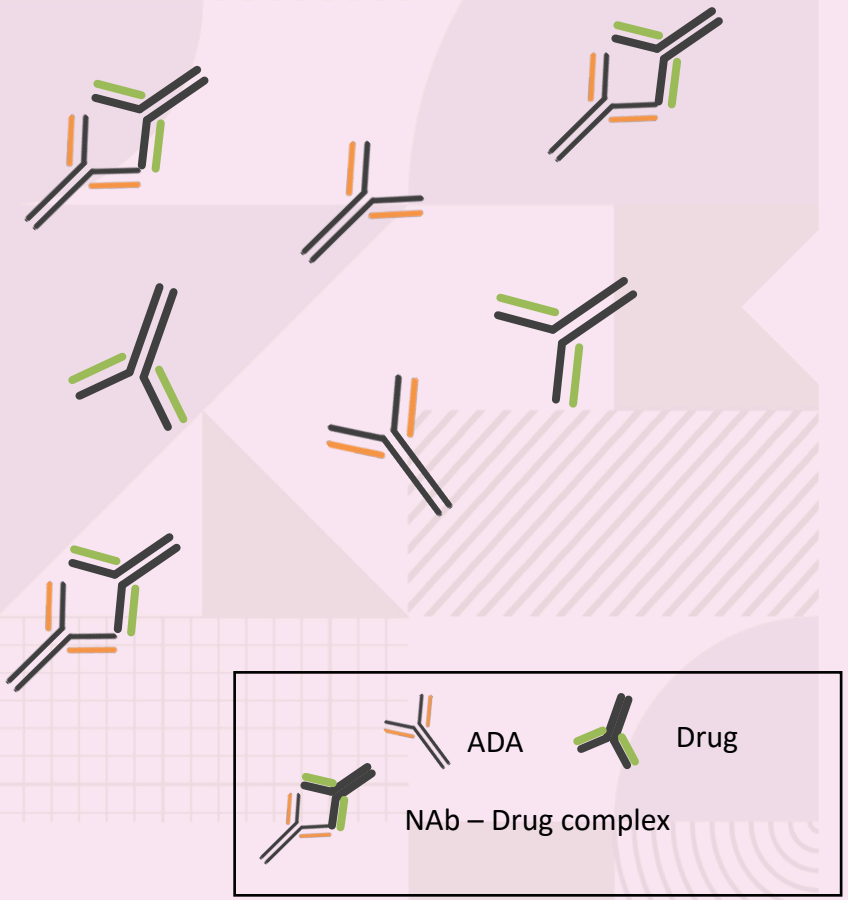
  Determination of the potential of the ADA to inhibit the biological activity of the drug

- Preference for cell-based assay format
- Both cell-based and non cell-based LBA assays are viable options for NAb assessment

  Reflective of the therapeutic mechanism of action (MoA)

Wu B, Chung S, Jiang XR et al. Strategies to determine assay format for the assessment of neutralizing antibody responses to biotherapeutics. AAPS J. 18(6), 1335–1350 (2016).

Need for Neutralizing Antibody assays



- Cell-based NAb assays are often variable and have a limited dynamic range
- Tendency to replace dedicated NAb assays with an integrated assessment of ADAs and PK/PD

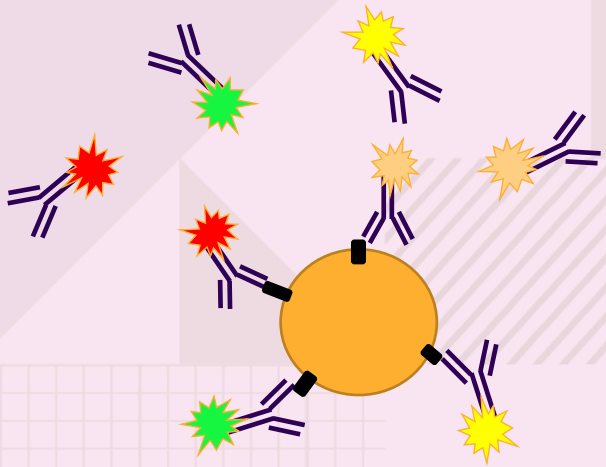
Does this meet the regulatory expectations?

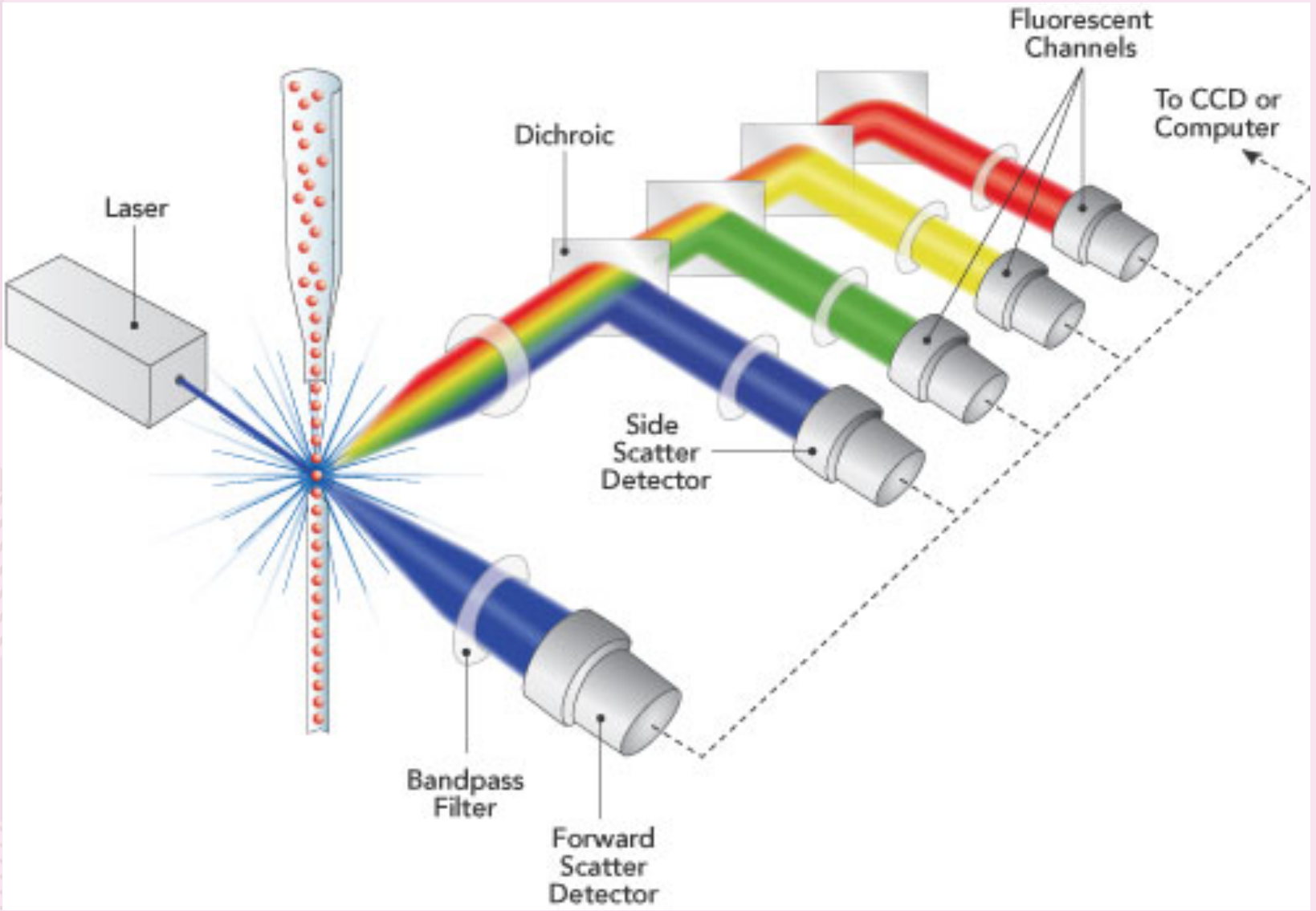
- Alternative approach for setting-up NAb assays

➔ Transformation of a flow cytometry-based Receptor Occupancy (RO) assay into a cell-based NAb assay.

Wu B, Chung S, Jiang XR et al. Strategies to determine assay format for the assessment of neutralizing antibody responses to biotherapeutics. AAPS J. 18(6), 1335–1350 (2016).

Flow cytometry-based Receptor Occupancy (RO) assay

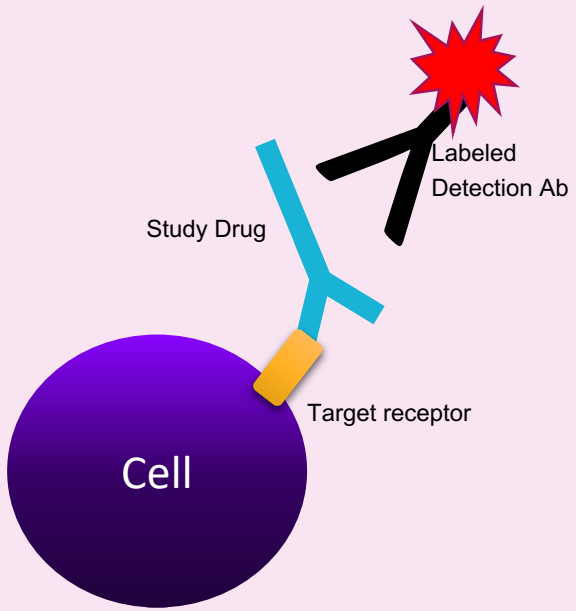




Flow cytometry-based Receptor Occupancy (RO) assay

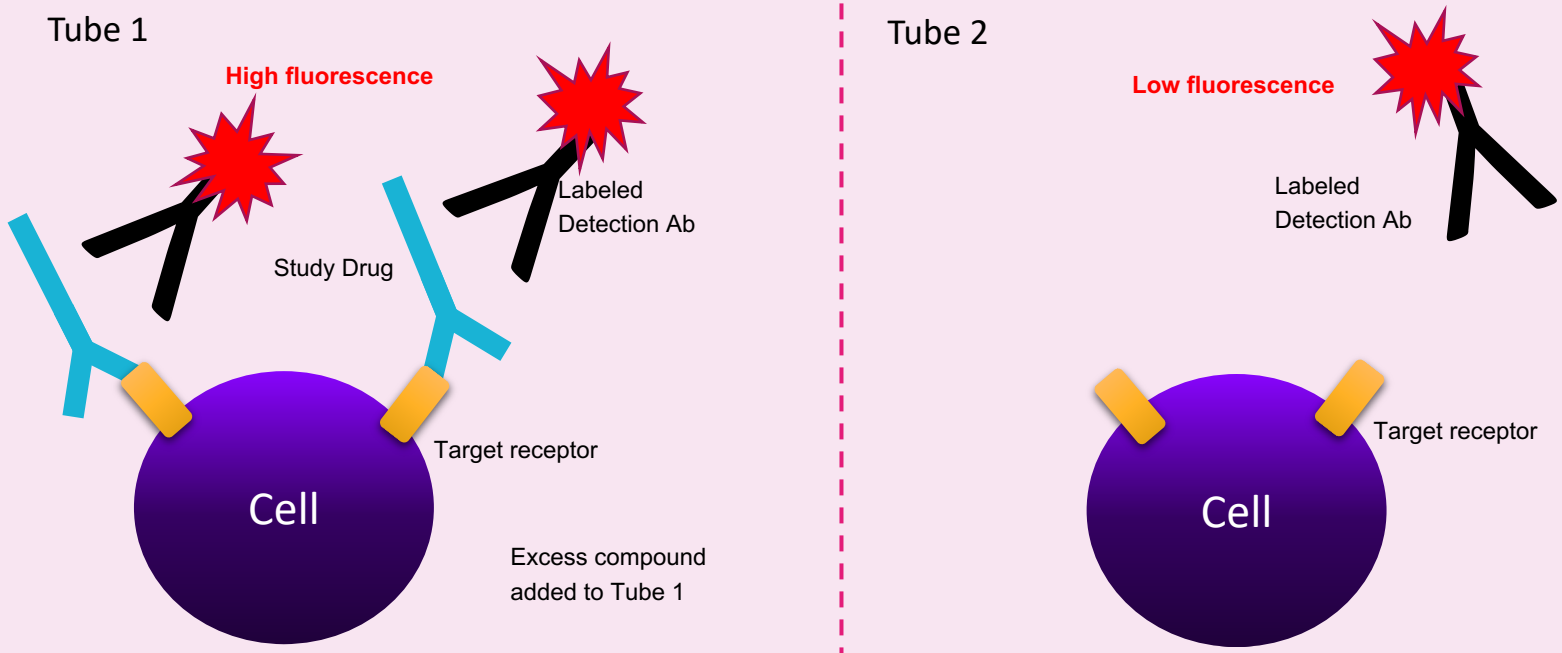
- "Functional" PK assay for compounds that target cell receptors

  Showing Drug Efficacy by target binding



Flow cytometry-based Receptor Occupancy (RO) assay

Assay Format Pre-dose



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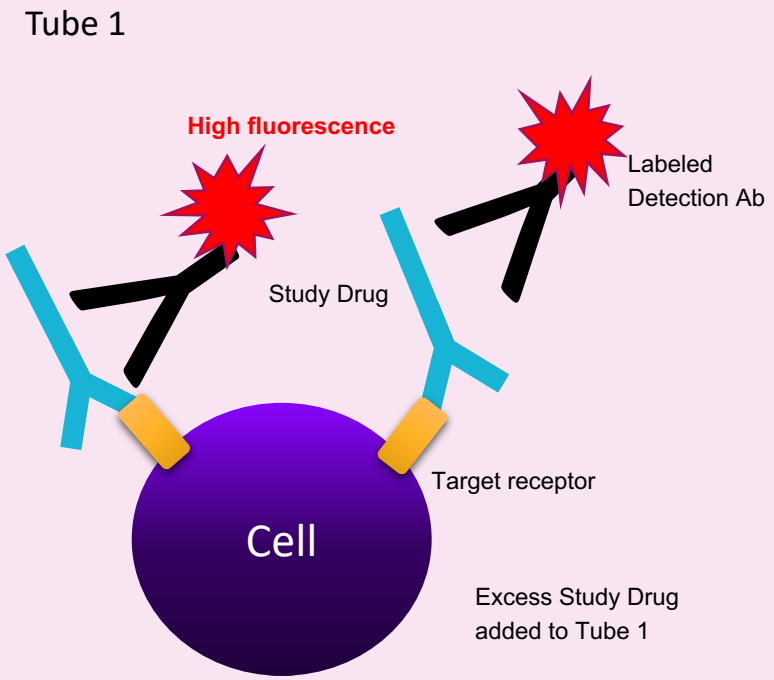
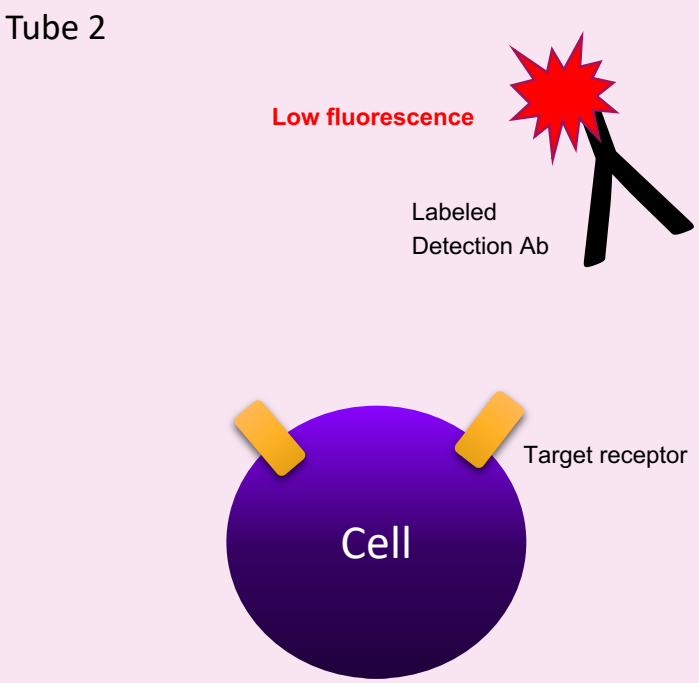
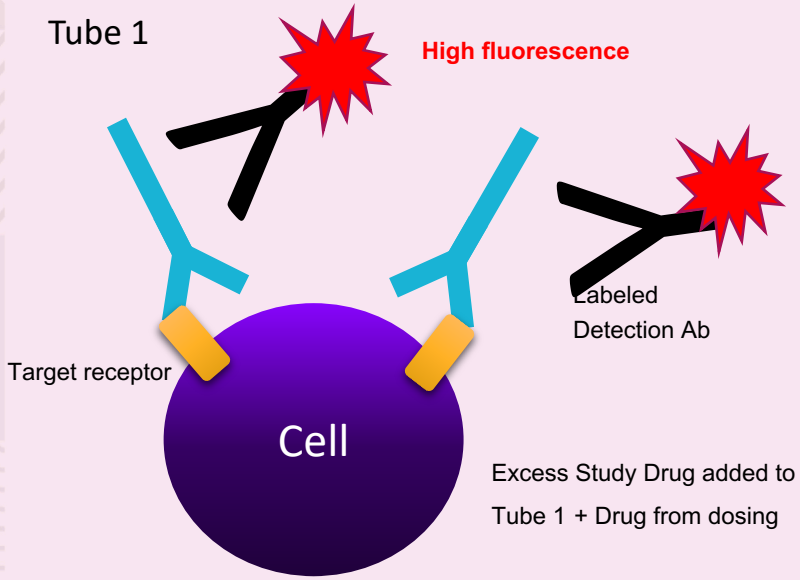
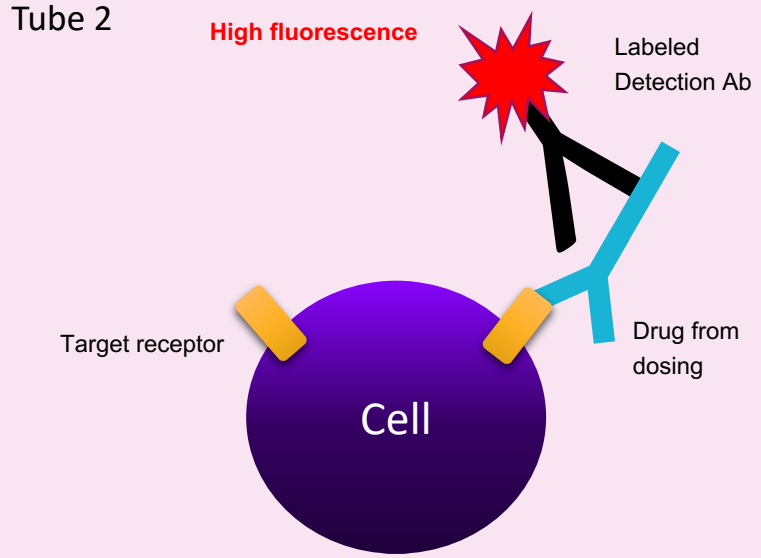




Assay Format Post-dose





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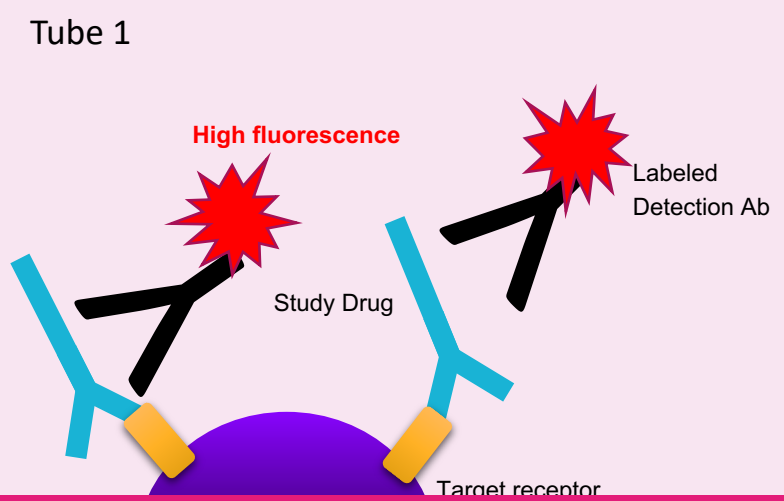
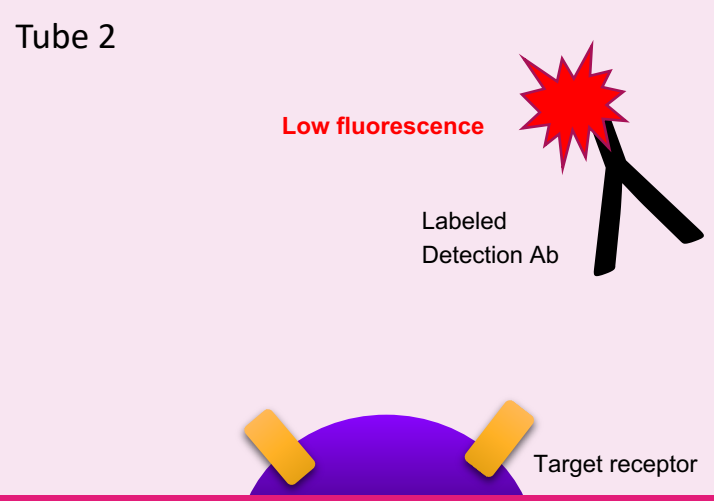
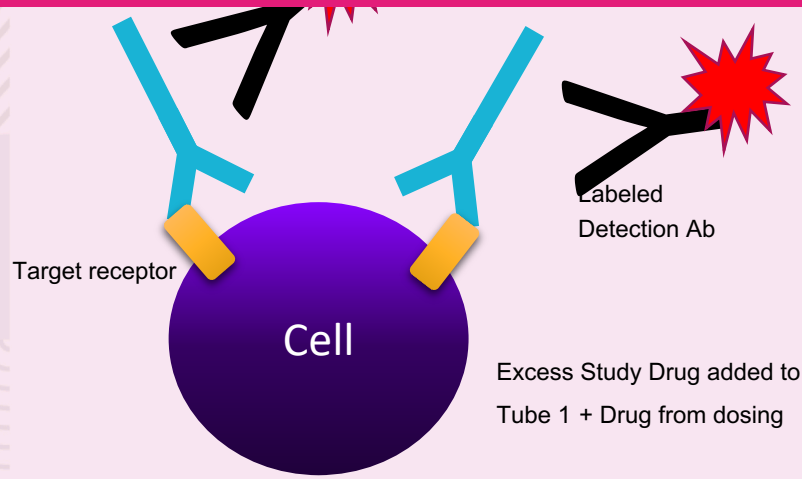


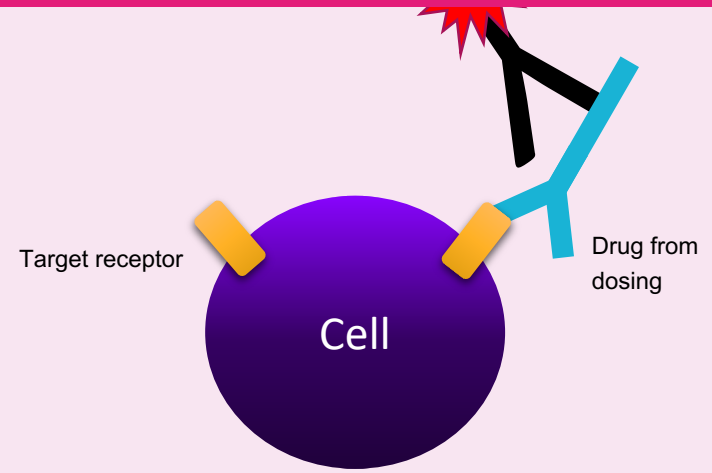


% Receptor occupancy (RO) =

(Tube 2 / Tube 1) * 100%

Assay Format Post-dose





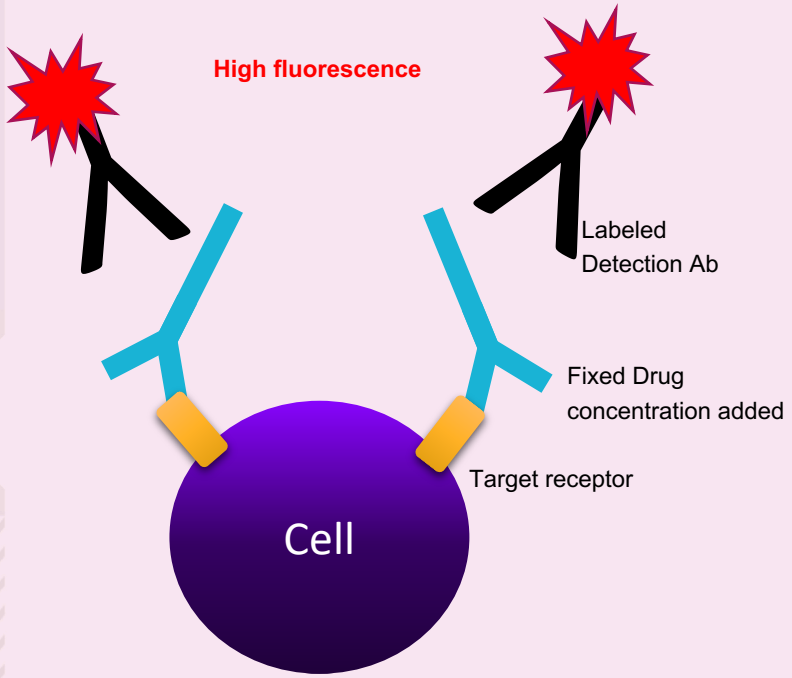
Transformation to a Neutralizing Antibody assay

RO assay → NAb assay

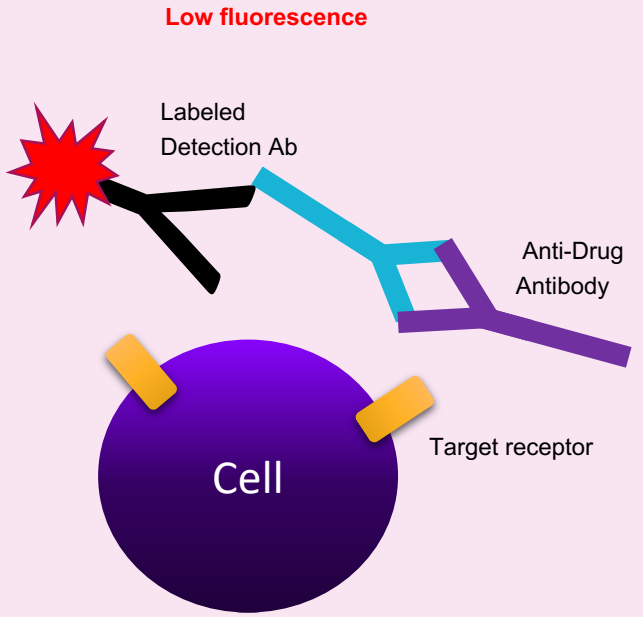
Cell from the study sample which expresses the target receptor, e.g. leukocyte, bone marrow cell etc.

Cell from cell-line that represents the in-vivo situation by expressing the target receptor.

Without presence of NAb



In the presence of NAb



Neutralizing anti-drug Antibodies prevent binding to target receptor

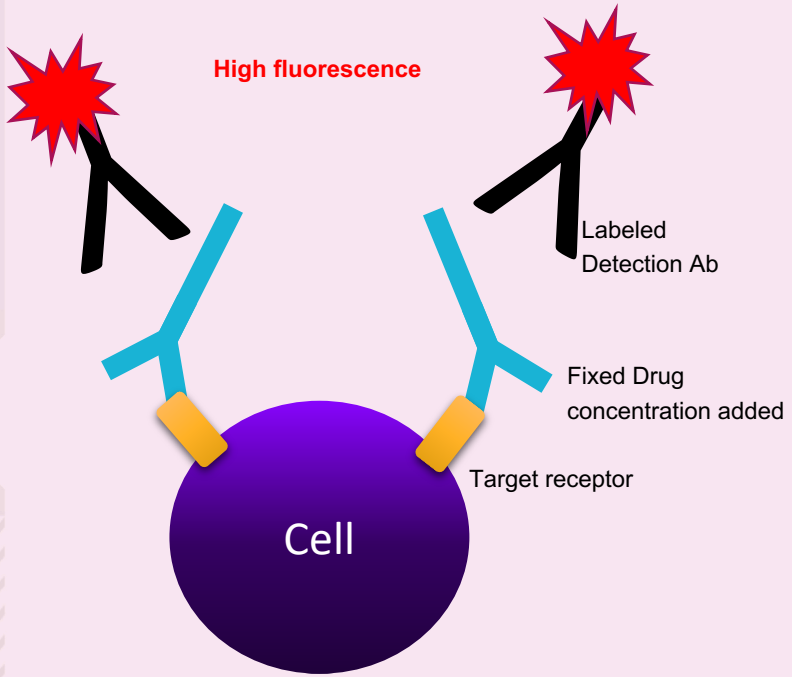
Transformation to a Neutralizing Antibody assay

RO assay → NAb assay

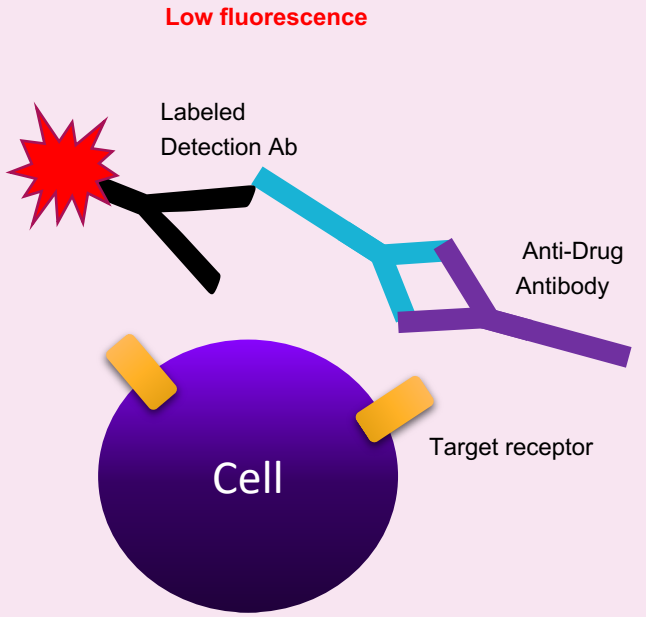
Cell from the study sample which expresses the target receptor, e.g.

Cell from cell-line that represents the in-vivo situation by expressing

Without presence of NAb



In the presence of NAb



Neutralizing anti-drug Antibodies prevent binding to target receptor

Disclaimer:

This assay format shows blocking of the target receptor and not the (possible) subsequent biological consequences

Set-up of the NAb assay

Requirements

Cell: find a cell that (constantly) expresses the target receptor

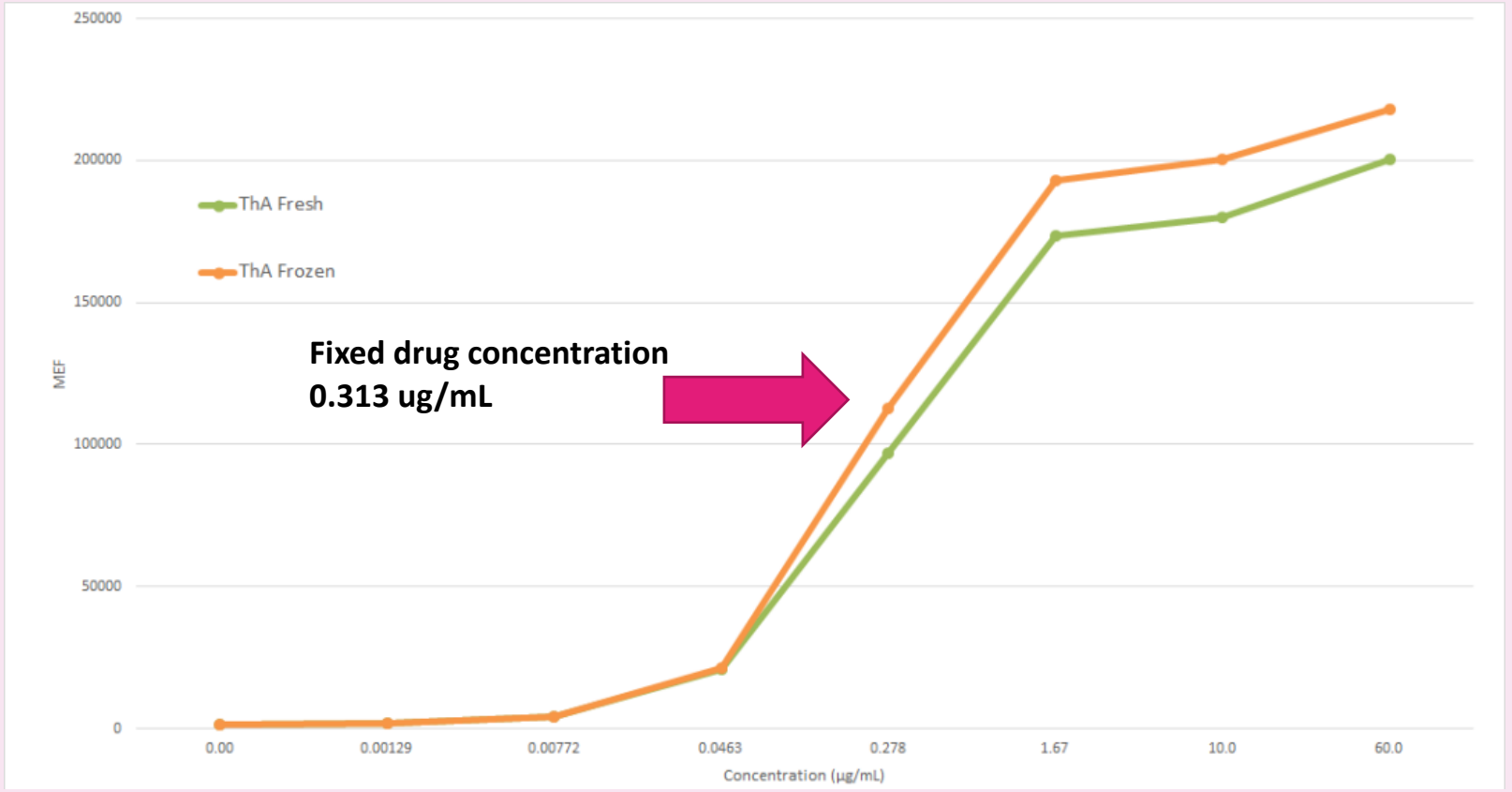
Drug: make sure the target is bound by the study drug

Positive Control Antibody: make sure target binding can be inhibited by the positive control antibody

- Does not have to be the same as in ADA assay
- Can be monoclonal or polyclonal antibodies

Set-up of the NAb assay

The **optimal fixed Drug concentration** was assessed at the linear part of the curve

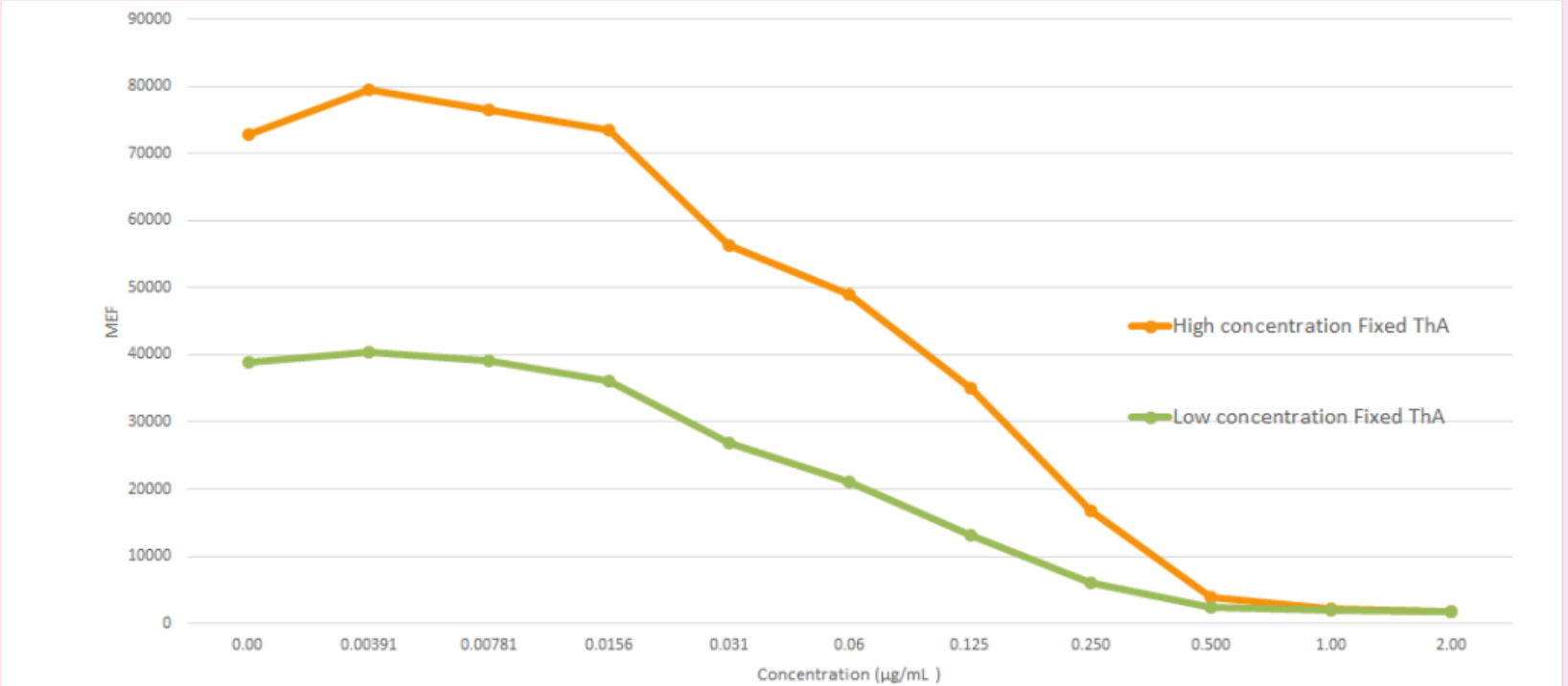


Dose-response curve of the Drug

Set-up of the NAb assay

Titration curve of increasing positive control concentrations at 2 different drug concentrations

Showing neutralizing effects of positive control and dynamic range of the assay

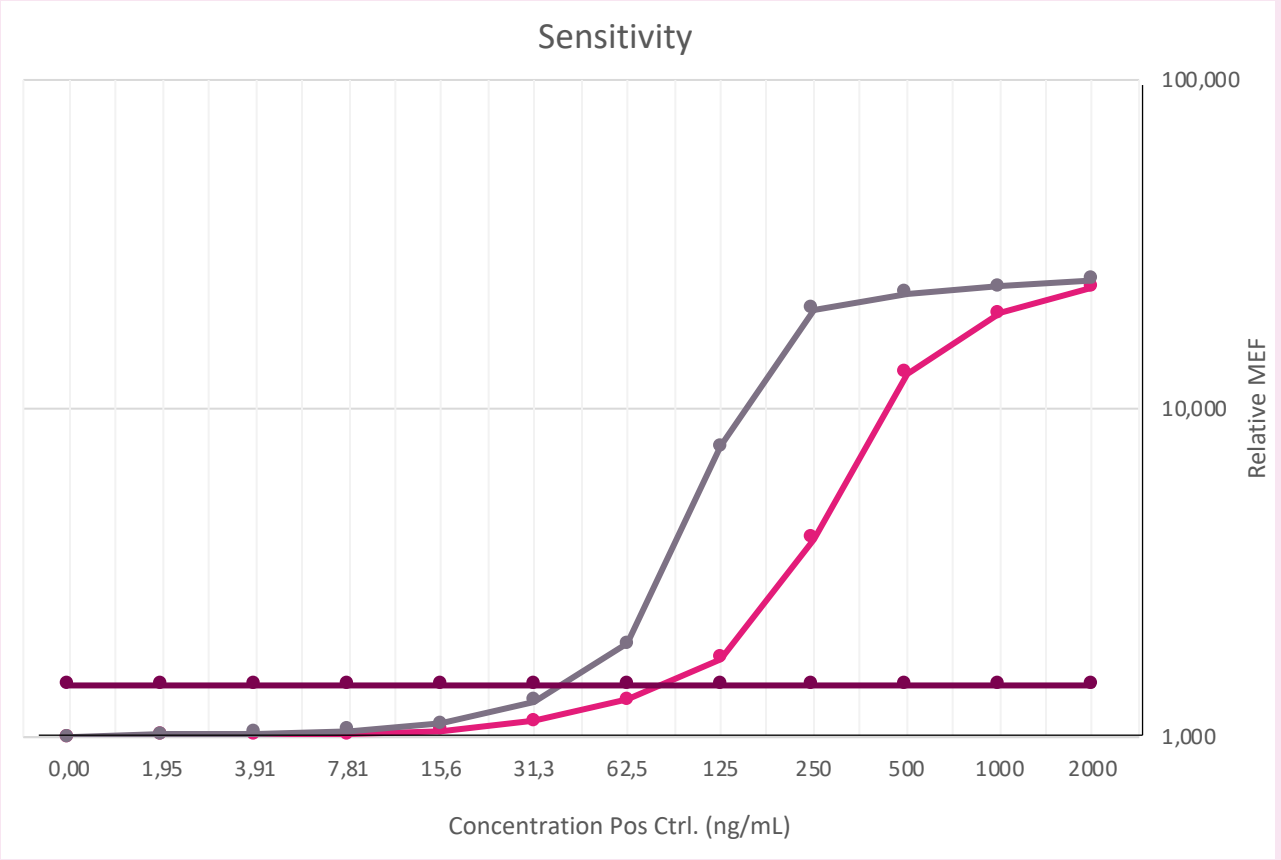


Concentration of NAbs

Set-up of the NAb assay

Sensitivity

Based on sensitivity and dynamic range, QC levels can be determined



Validation of the NAb assay

Validation Parameter	Result
Type of Cut point	
Precision of the (Relative) Response	< 20.0%
Precision of Scoring	< 20.0%
Sensitivity (Limit of Detection)	< 100 ng/mL
Precision of Titers	No deviations of more than 1 titer step
Drug Tolerance	< 100 ng/mL - > 1 µg/mL drug
Matrix Variability	Positive scoring of spiked samples
Stained cell stability	24h at +4°C
Serum stability	> 100 days at -70°C
Freeze/Thaw stability	6 cycles at -70°C

Bioanalysis of the NAb assay

Bioanalysis has been performed

70% of the ADA positive samples was positive for NAb

All NAb positive samples had measurable titers

Conclusions

Feasible and cost-efficient option for NAb assessment in case an activity NAb assay is not needed or possible

However, take a possible lack of sufficient Drug tolerance into account!

- avoid: Integrated assessment of NAb, ADA and PK to misinterpretation
- from: Improve drug tolerance by removing high free drug the samples by pre-treatment

Thank you for your attention!