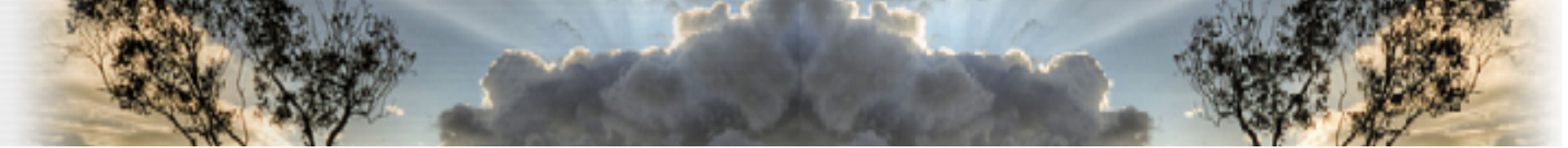


EBF consideration for NAb assay development and design with emphasis on matrix, sensitivity and sample pre-treatment

Anna Laurén – on behalf of the EBF NAb project team

Objective

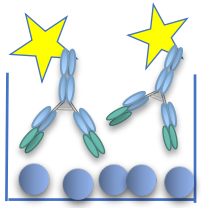
- In the recent year, the EBF team for neutralising antibody assays (NAb) has focused on discussing assay constraints for a successful NAb assay
- Aim has been to create a reality check across industry based on team experiences what makes a sufficiently good NAb assay:
  - Assay formats
  - NAb negative control pool matrix and individual samples
  - Reflection on theoretical assay sensitivity
  - Ways to solve serum interference and drug interference by sample pre-treatment
- Experience built on ~25 cases from the NAb expert team

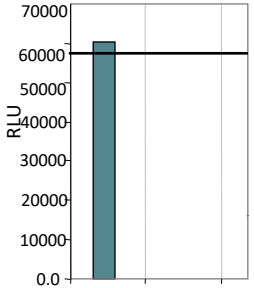
NAb assay competitive ligand binding assay (CLBA)

Example: CLBA for Mab-Drug with soluble target*

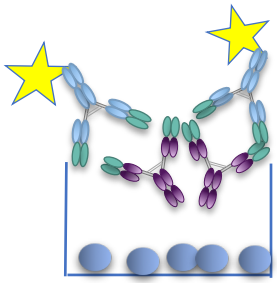
- Target coated on plate

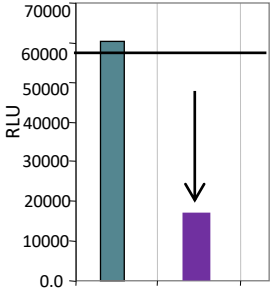
Binding of labeled drug yields high assay signal:



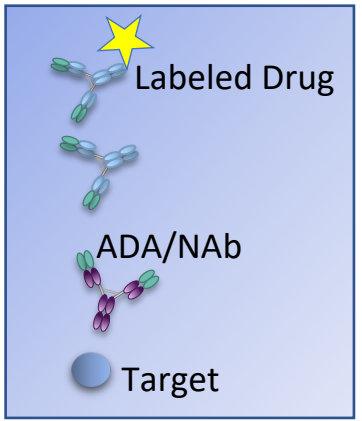


Signal decrease by NAb:



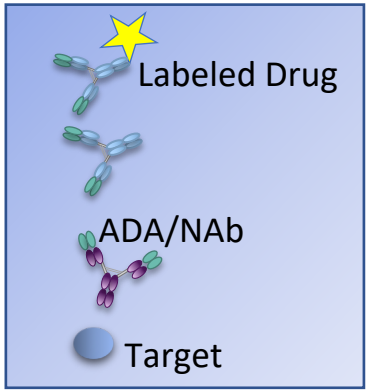


NAb



*opposite set up (drug coated + read out via labeled target)
+ sequential protocol might be beneficial

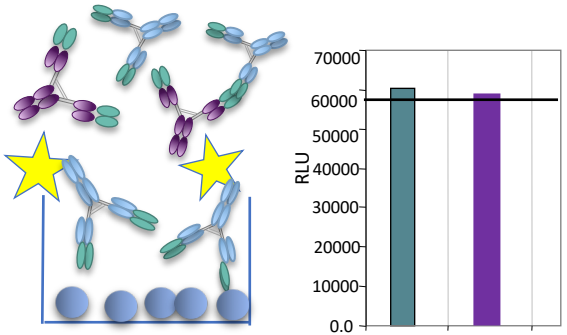
CLBA Drug and Target Interference



Example: CLB for Mab-Drug with soluble target

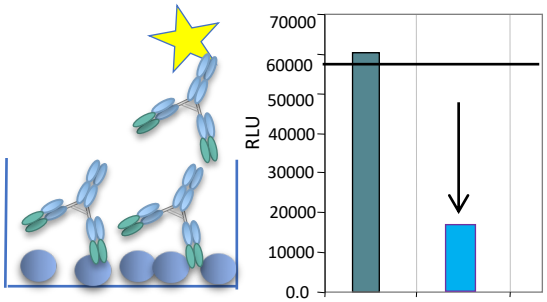
- Target coated on plate
- Consider optimal protocol and coating for each project

Case a):
False negative due to “Drug on Board“:



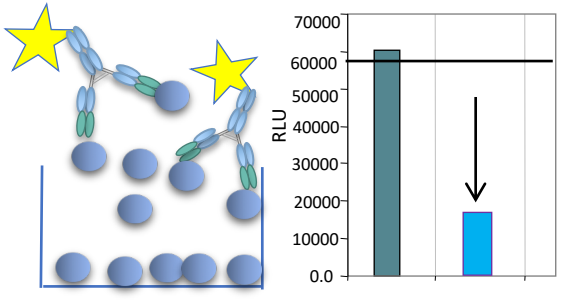
NAb + Drug

Case b):
False positive due to “Drug on Board“:



No NAb; Excess of unlabeled Drug

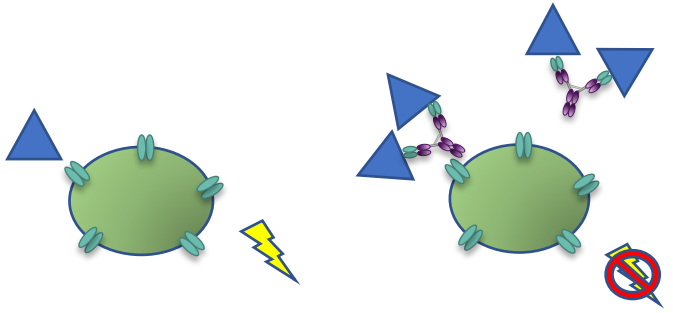
Case c):
False positive due to Target Interference



No NAb; interference of soluble target

Direct Cell based assay (CBA) for agonistic Drug

- Stimulation by drug yields high signal
- Nab againts drug yields low signal

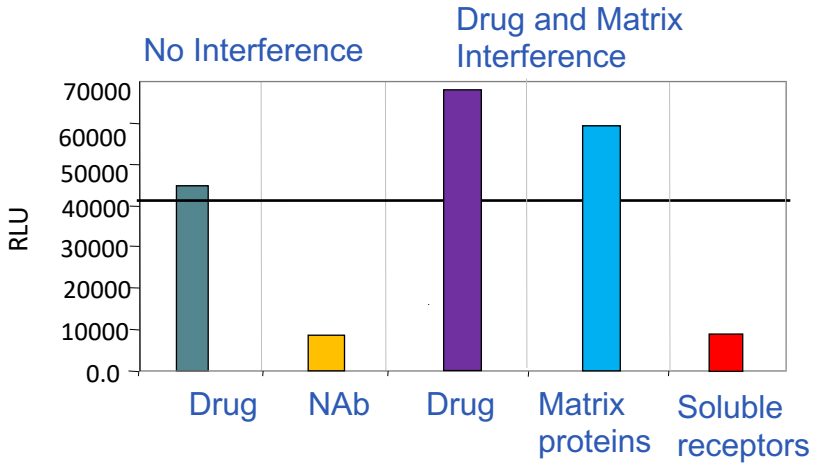


Drug and matrix interference and increase assay signal:

- false negative

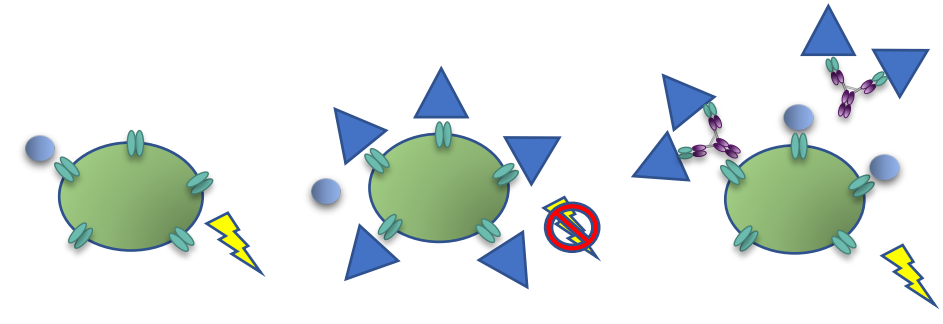
Soluble receptors may decrease assay signal

- false positive

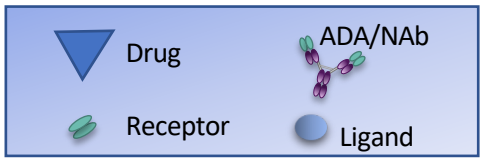


Indirect CBA for antagonistic Drugs

- Stimulation by target yields high assay signal
- Nab againts drug yields high assay signal



Target stimulates cells | Drug inhibits target | NAb inhibits drug

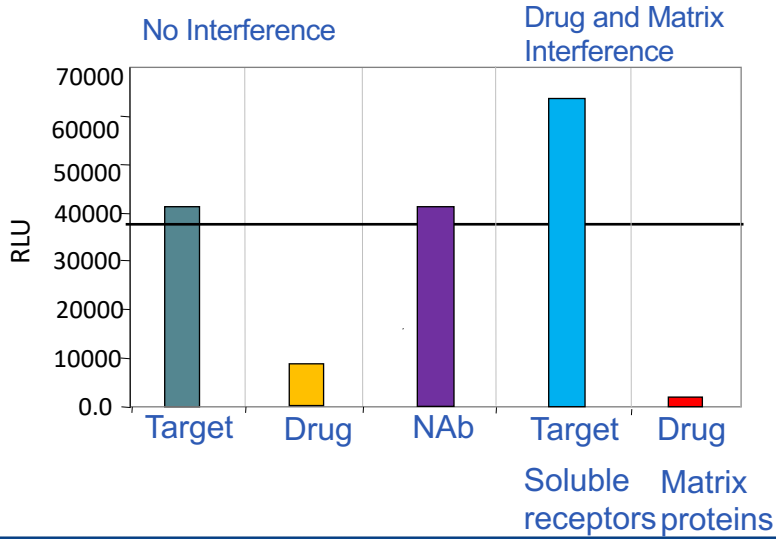


Drug/matrix interference may decrease assay signal:

- false negative

Target interference/soluble receptors increase assay signal:

- false positive



How can the matrix influence your NAb assay?

- Cell based assays (CBA)
  - Endogenous toxicity can kill cells: example Complement system can kill cells – reduced signal

- Cell based assays (CBA) and Competitive ligand binding assay (CLBA)
  - Higher variability of assay signals at low MRDs
  - Circulating target can interfere with drug-target-PC interaction
  - Circulating natural antagonist can inhibit target
  - Concomitant medication can interfere in the assay system

Selection of matrix pool during assay development

Experiences from the NAb team

- Most frequent matrix: Serum
- NC matrix pool based on healthy individuals (majority of cases)
- If endogenous proteins influence assay signal: establish a pool from target population (when available)
- Screen individuals in the NAb assay before pooling and remove outliers
- Include 6-50 individuals in the NC matrix pool
  - Better to use a higher number of samples to minimise variability when bridging with new pool

Establishment of cut-point (CP)

- Validation
  - ✓In majority of cases the individuals representative of study population were used for CP (~30 individuals)
  - ✓Healthy matrix individuals were used when assay was included already in Phase 1 or when the target population was rare
- In Study:
  - ✓Establish communication with clinical teams for early sample analysis for CP
  - ✓In study CP shall be assessed with ADA negative pre-dose samples
  - ✓For high risk project: In study CP should be determined as soon as the first 30 individuals were screened and included in the study

Bridging of new matrix pool

- Experience with matrix pool based on at least 10-20 individuals gives less variance between pools
- Two alternatives exists from the team experience:
- Alternative 1: Bridging is most common
  - Comparing to old acceptance criteria
  - Statistical comparison of old and new pool, adjustment of CP in case of difference
  - Performed by 7 out of 9 companies
- Alternative 2: No Bridging in rare cases
  - Re-validate the CP was done by 2 out of 9 companies
- Scientific justification should be done by company

Theoretical sensitivity in cell based assays

- A theoretical sensitivity can be calculated based on below information
- When selecting the cell line during assay development the following is important to consider:
  - Concentration of drug and ligand in the linear assay range
  - Molecular weight of the drug
  - MRD of the sample
- Another important factor: Selection of PC and how it would compete with drug to drug target
  - Consider epitope binding and affinity for PC to drug and receptor
- Already available potency assays may be un-relevant
  - Concentration of drug used in potency assays may be to high
  - Matrix interference unknown - Potency assays are developed with drug in buffer

How to calculate theoretical sensitivity

- Concentration of drug used for stimulating the cells is converted to a molar concentration
- Concentration of antibody is converted to a molar concentration
- Assumption, 1 antibody can bind 2 drugs and will give 100% Neutralisation
- Example:
  1) Drug X: 20 kDA
  2) MRD 20
  3) Control Ab: 150kDa
  4) 25 ng/ml drug @ 20kDa = **1.25nM** drug in well with cells
  5) 1.25nM/2 = **0.625nM** Ab can neutralise drug fully in well with cells
  6) 0.625nM Ab x MRD = **1875ng/ml Ab in sample** = Theoretical sensitivity

Theoretical sensitivity gives an estimate on how good the assay can be

Examples from cell based assays with no sample pre-treatment

Drug	Drug concentration for stimulation (ng/mL)	Molecular weight (kD)	MRD	Theoretical sensitivity (ng/mL)	Actual sensitivity (ng/mL)	Type of PC
A	25	20	20	1875	330	mAb
B	840	20	4	12600	- unknown	affinity pAb
C	0.25	20	100	94	277	affinity pAb
D	0.04	15	20	4	28	mAb
E	0.035	20	10	0.0013	130	mAb
F	0.9	30	20	45	70	mAb
G	1.5	30	20	75	122	mAb
H	3.5	30	14	126	150	mAb
I	2.5	30	17	115	85	mAb
J	0.4	4	4	30	500	mAb
K	0.13	4	4	5	15	mAb
L	6	4	2	225	3000	affinity pAb

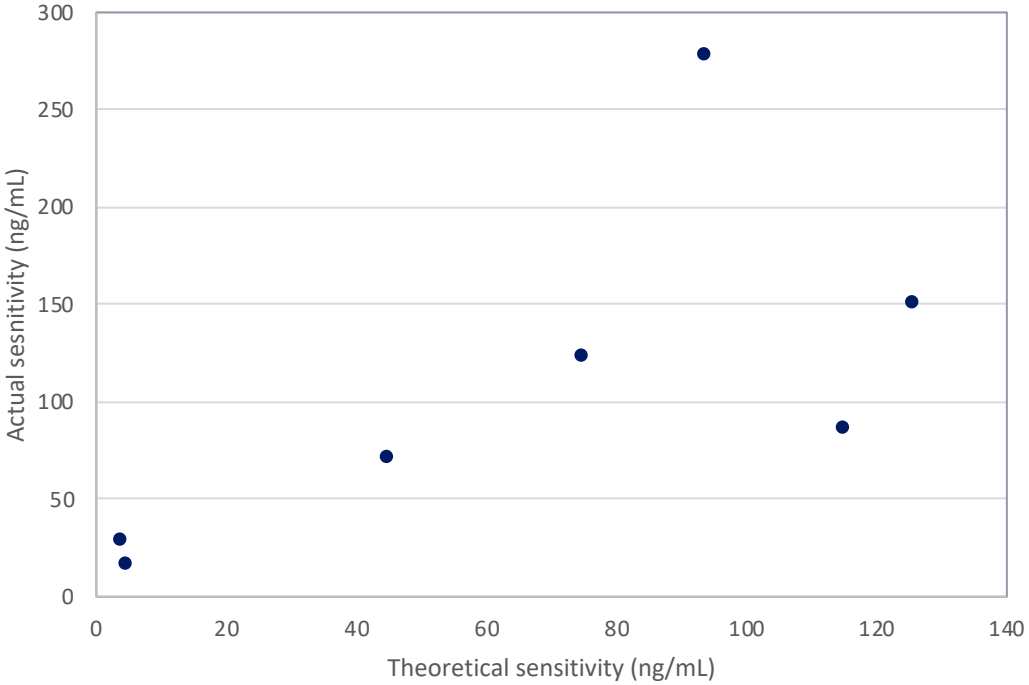
Reasonable correlation between Theoretical and Actual sensitivity in 7 out of 12 assays

Theoretical sensitivity (ng/mL)	Actual sensitivity (ng/mL)	Type of PC
94	277	affinity pAb
4	28	mAb
45	70	mAb
75	122	mAb
126	150	mAb
115	85	mAb
5	15	mAb
12600	- unknown	affinity pAb
1875	330	mAb
0.0013	130	mAb
225	*3000*	affinity pAb

* outlier

Reasonable indication

No indication

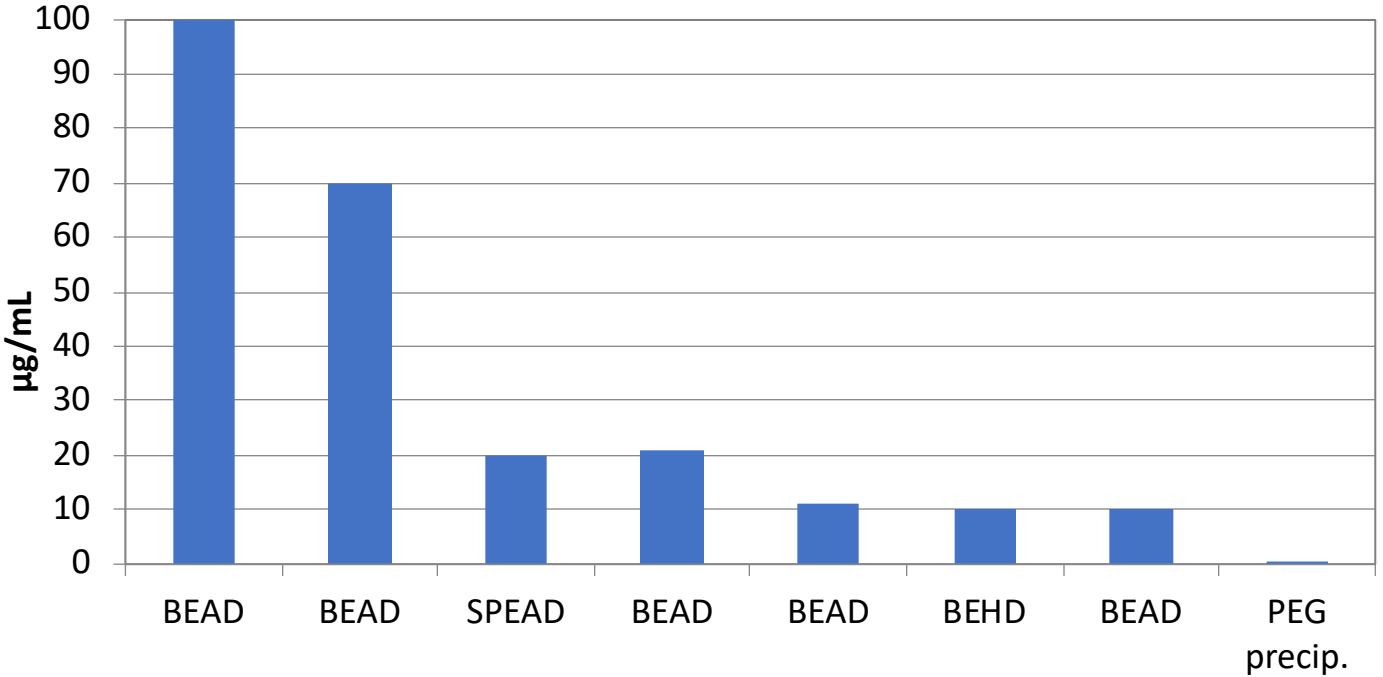


Sample Pre-treatment, Matrix and Drug Tolerance

- Residual drug tolerance issues in NAbs is a common problem
- Can lead to false negatives as well as false positives
- Investigate both NC and PC (at least LPC) spiked with drug at Cmin
  - Add additional drug and PC concentrations if relevant
- If sufficient DT cannot be achieved, then a number of pre-treatments can be tested to address this including;
  - SPEAD, BEAD, acid dissociation, PEG precipitation, ACE etc
  - References given in back-up slides

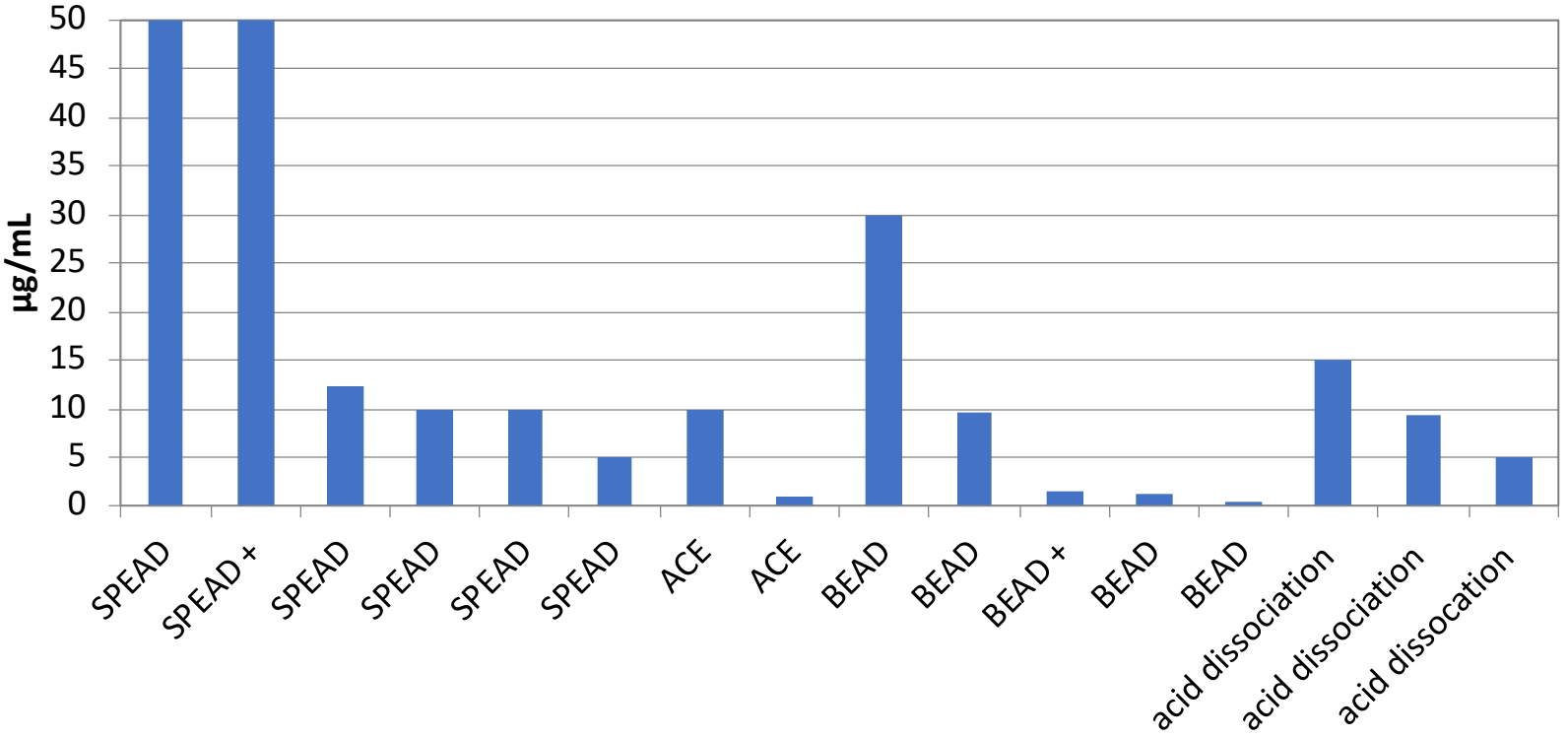
Typical Drug Tolerance levels achieved using Sample Pre-Treatment in CBA Nabs

- 1 outlier removed



Typical Drug Tolerance levels achieved using Sample Pre-Treatment in CLBA Nabs

- 1 outlier removed



Typical Data observed after Sample Pre-Treatment

Cell based NAbs;

- 6 out the 9 examples (67%) have DT of ~ 0.5 – 20 µg / mL
- DT > 30 µg/mL can be reached in a rare cases (3 out of 9)

CLBA based NAbs;

- 13 out the 17 examples (76%) have DT of ~ 0.5 – 20 µg / mL
- DT > 30 µg/mL can be reached in a rare cases (4 out of 17)

Summary

- Matrix can impact outcome of NAb assay performance
  - Screen to select a suitable serum pool
  - New matrix should be bridged (when possible) with no need to re-establish CP
- Estimates of theoretical sensitivity can be used to understand if available cells are feasible to use for a NAb assay
- NAb assays can have problems with interference from matrix and drug in samples
  - Interference can give both false positive and false negative NAb results
  - Assay design and sample pre-treatment can be used to minimise interference
  - Sample pre-treatment have worked successfully in both CBA and CLBA

Acknowledgment

- EBF community
- Lead: Anna Laurén - Svar Life Science
- Team members:
  - Richard Weaver– Covance
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  - Weifeng Xu – Merck and Co / MSD
  - Joanna Grudzinska-Goebel – Bayer
  - Marcel van der Linden – Genmab
- Team Sponsor: Tobias Haslberger - Abbvie

Thank you and time for questions

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Back-up References pre-treatment in ADA/NAb assays

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