CONSIDERATIONS IN PERCEIVED ASSAY SENSITIVITY & DRUG TOLERANCE – SENSE AND NONSENSE OF POSITIVE CONTROLS

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Synthon

Scope

In ADA analysis both relative sensitivity and relative drug tolerance are key parameters of an assay.

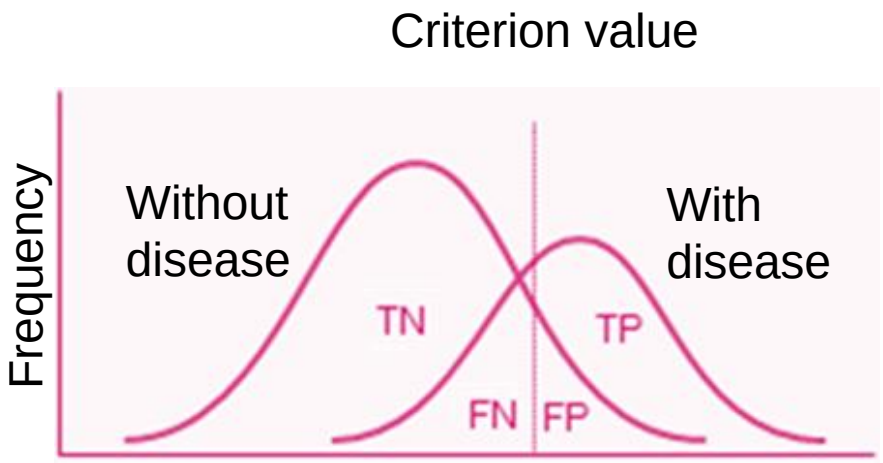
Calculation of sensitivity is somewhat defined*, calculation of drug tolerance varies within the industry (EBF publication in preparation)

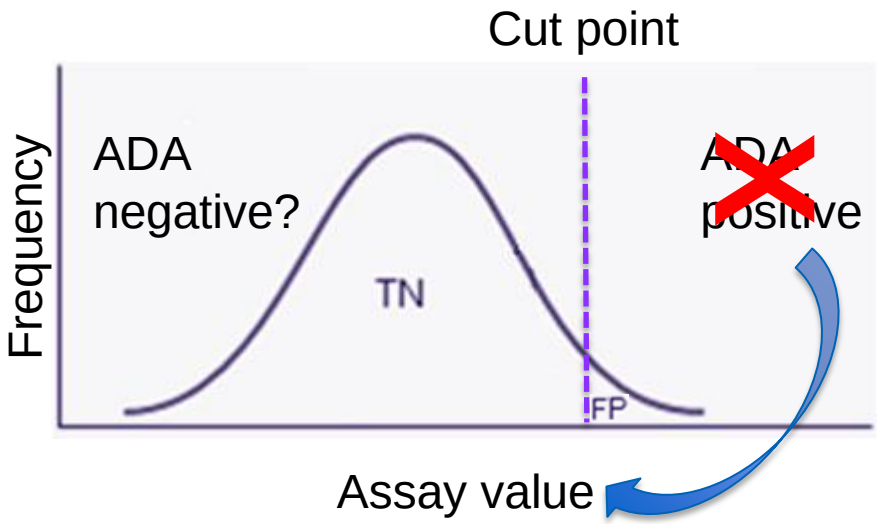
Both concepts are centered around positive controls and assay cut points.

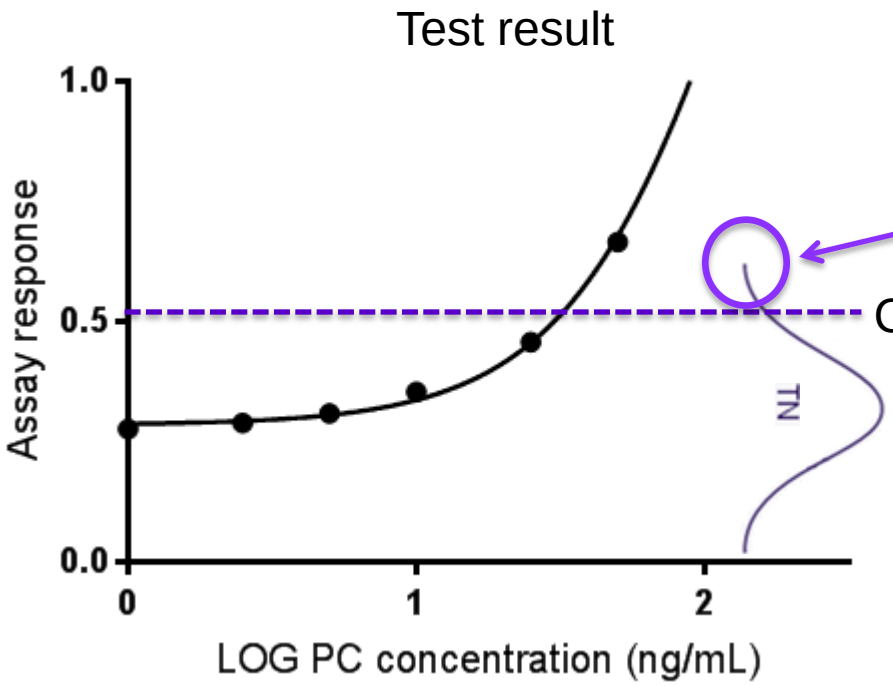
In this presentation we provide some µcase-studies of day-to-day practice to illustrate where harmonization may be beneficial and where it may be difficult.

**E.g. Shankar et al. 2008, FDA (Draft) guidance, USP1106*

Cut point and sensitivity - introduction







*e.g. Shankar et al. 2008, Shen et al. 2015, Zhang et al. 2017, Devanarayan et al. 2017

Cut point and sensitivity

Reported sensitivity in relation to the CP is dependent on:

•The associated confidence limit/intended FPR, *e.g.* 1% for a confirmatory assay, 5% for a screening assay

•The requirement on certainty with regards to the confidence limit/intended FPR, *e.g.* on average 5% or at least 5% with 90% certainty

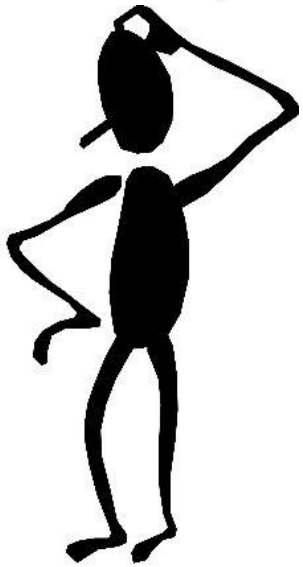
In addition variability may exist in how sensitivity is reported (*e.g.* mean vs 95% confidence limit estimate)

Cut point and sensitivity – µcase study 1

Example of PC A with compound 1 in an Alphalisa bridging assay

Description	CPF	Sens. (mean)	Sens. (95th percentile)	LPC (99th percentile)	FPR validation set
Shankar*[1]	1.15	17	99	318	7.6%
Shen et al*[2] FDA draft	1.11	14	80	264	11.8%

Sensitivity and LPC values in ng/mL



It's all about definitions, the same underlying data may still result in different reported values for sensitivity.

*[1]Shankar et al. 2008, *[2]Shen et al. 2015*

Cut point and sensitivity – µcase study 1

Example of PC A with compound 1 in an Alphalisa bridging assay

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Sensitivity and LPC values in ng/mL

Sensitivity is linked to the false positive rate

Cut point and sensitivity – µcase study 1

Example of PC A with compound 1 in an Alphalisa bridging assay

Description	CPF	Sens. (mean)	Sens. (95th percentile)	LPC (99th percentile)	FPR validation set
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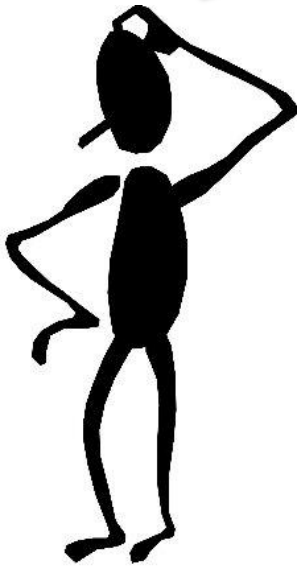
Sensitivity and LPC values in ng/mL



Estimate of relative drug tolerance (mean) at LPC level of PC A which is still found positive:

LPC (99th percentile)	Mean drug-on-board
318 ng/mL	847 ng/mL
264 ng/mL	681 ng/mL

… yet, drug tolerance calculation is not harmonized

Cut point, sensitivity and drug tolerance

The required confidence limit may be more defined for some assays compared to others, *e.g.* screening and confirmatory vs. titer vs. nAb assay. → **opportunity to harmonize?**

(Lack of) current definitions/calculation of reported drug tolerance and to some extent sensitivity may provide an **opportunity for harmonization**

Global (Re-)Harmonization should help to align expectations, yet the biggest impact on sensitivity is often found in selection of a PC…

Positive control, sensitivity and drug tolerance

Positive controls may come in various formats, *e.g.* polyclonal hyper immunized animals, monoclonal antibodies or pooled ADA from treated subjects

PCs are often used to guide platform selection and development…

… but the results of one PC may be misleading in our decision making

Positive control - µcase study 2

Screening assay using MSD platform performed well for mAb A

Compound	PC	MSD homogeneous bridging assay
mAb A	PC 1	19 ng/ml

By default the MSD platform was chosen for ADC B as well

Positive control - µcase study 2

Screening assay using MSD platform performed well for mAb A

Compound	PC	MSD homogeneous bridging assay
mAb A	PC 1	19 ng/ml
ADC B	PC 2	Poor S/N → required extensive optimization

Using the same assay settings as mAb A for ADC B resulted in poor performance

Positive control - µcase study 2

Compound	PC	MSD homogeneous bridging assay	SPR direct assay	Alphalisa homogeneous bridging assay
mAb A	PC 1	19 ng/ml		
ADC B	PC 2	1613 ng/ml	415 ng/ml	3010 ng/ml

Still found poor sensitivity, even in multiple assays

Ranking sensitivity between assays SPR<MSD<Alphalisa

Positive control (PC 2) affinity for ADC B in nM range

Investigated a second positive control

Positive control - µcase study 2

Compound	PC	MSD homogeneous bridging assay	SPR direct assay	Alphalisa homogeneous bridging assay
mAb A	PC 1	19 ng/ml		
ADC B	PC 2	1613 ng/ml	415 ng/ml	3010 ng/ml
ADC B	PC 3	164 ng/ml	456 ng/ml	14 ng/ml

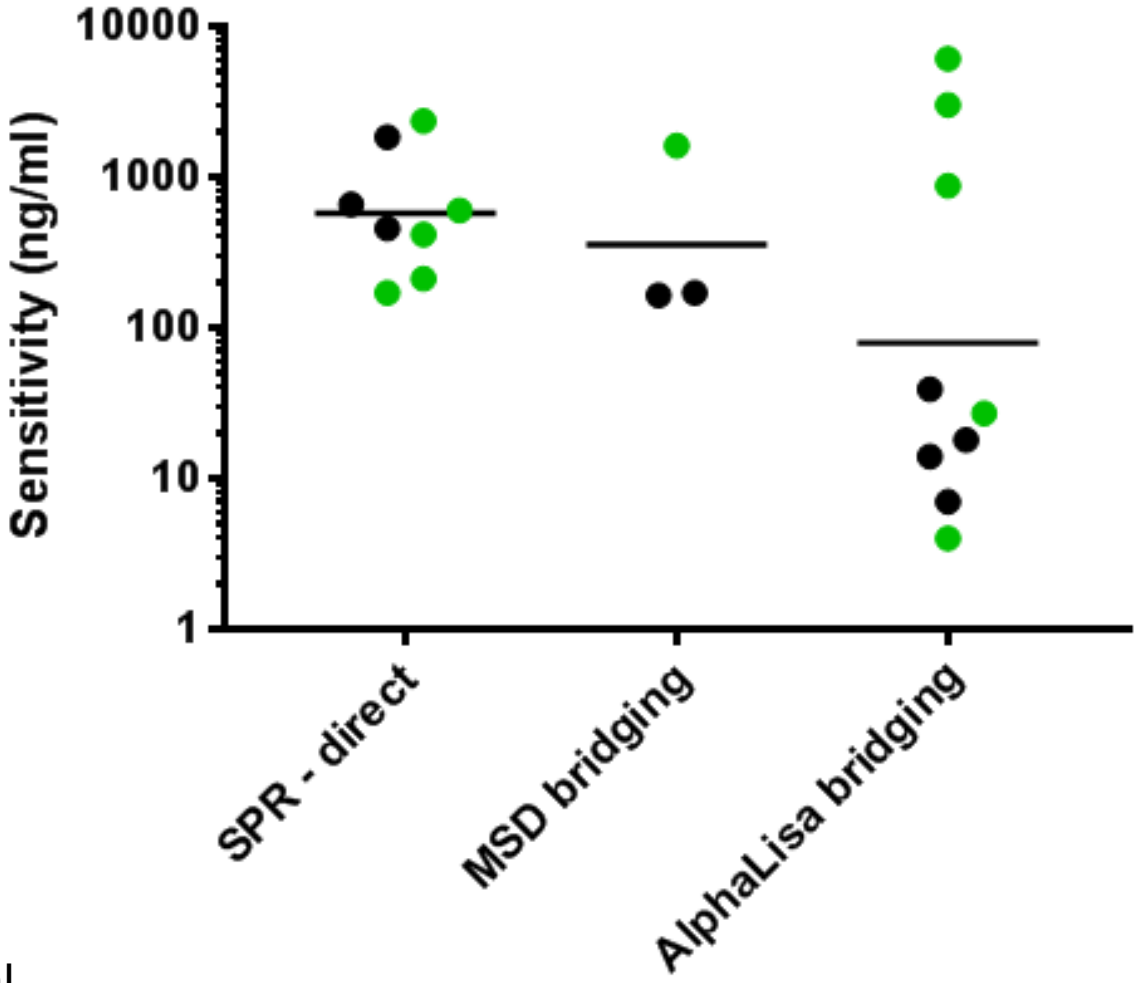
Ranking sensitivity between assays Alphalisa<MSD<SPR

Positive control (PC 3) affinity for ADC B in nM range

Investigated more positive controls…

Positive control - µcase study 2

Results highlight the relative nature of sensitivity in context of PCs

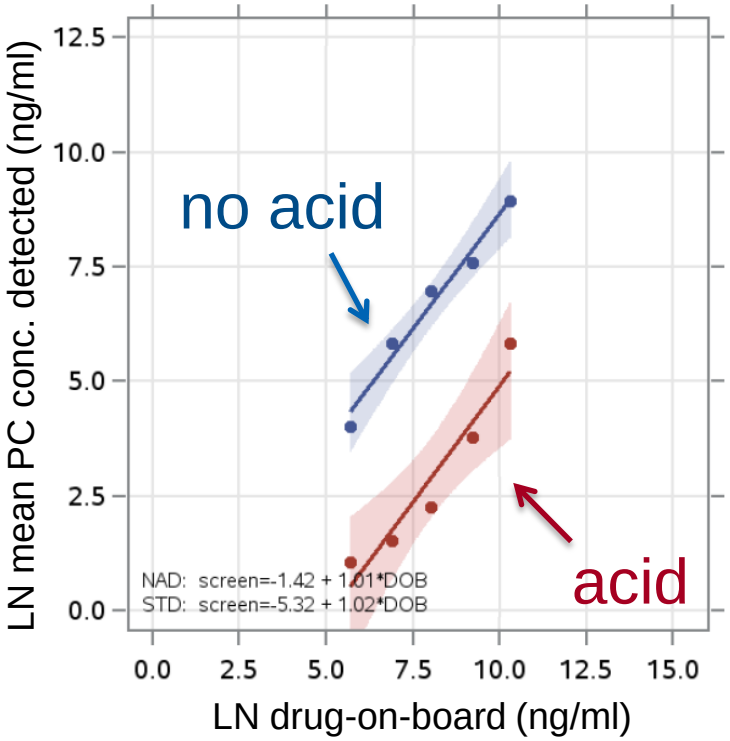


Positive control - method development

Even using the same assay format the behavior of one PC may guide development in a different direction than if another PC we're to be used…

Positive control - method development

Results from a single positive control may be misleading *e.g.*:



Example for an Alphalisa based homogeneous bridging assay:

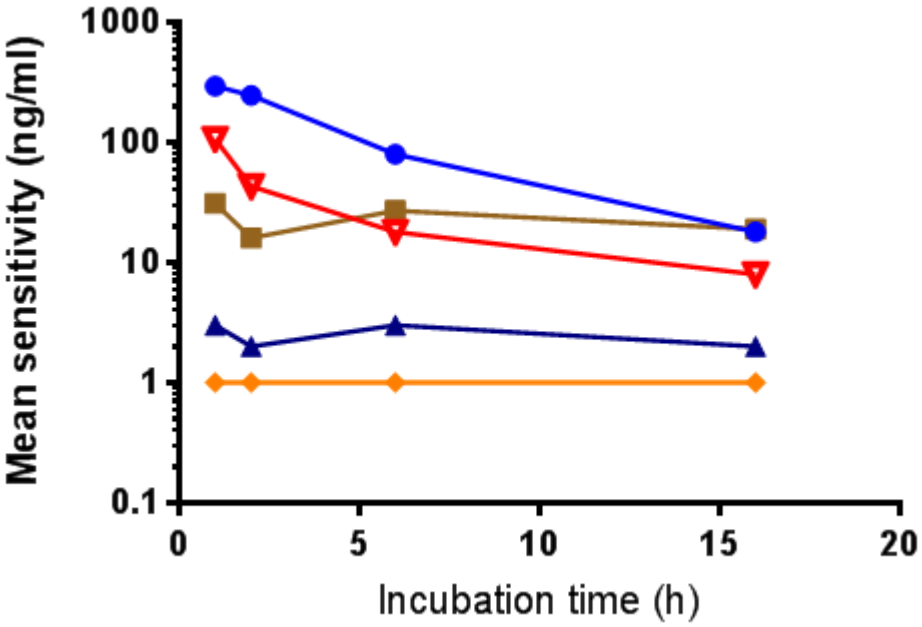
During a development step a set pH (Glycine-HCl) and incubation time showed a moderate improvement in drug tolerance (Sensitivity$_{DOB}$ to DOB)

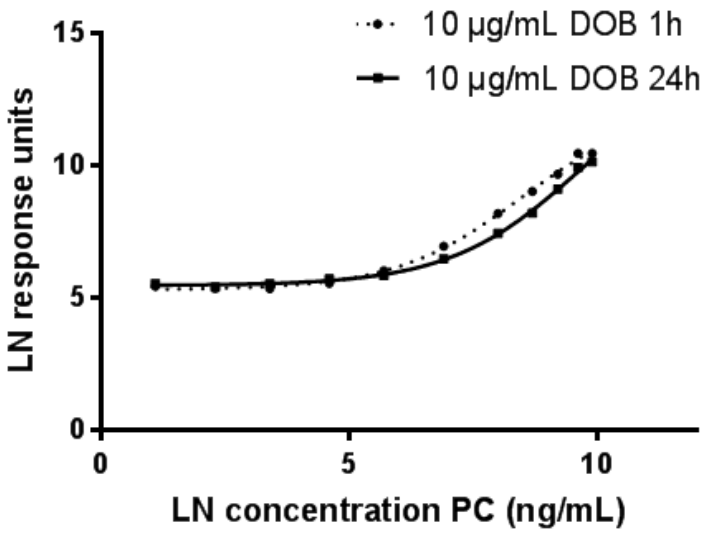
Effect on drug tolerance

Clonality	~	+	-
Polyclonal	3	7	0
Monoclonal	3	3	1

Results from several PC and compounds show that for the investigated experimental settings this did not universally hold true

Positive control - method development





Example of the effect of sample incubation time for an Alphalisa based homogeneous bridging assay.

Overall increasing incubation times had no or a positive effect.

In rare instances an apparent negative effect was found on drug tolerance

Positive control, sensitivity and drug tolerance

PCs may give different results under the same assay settings

Selection of a platform or method development on basis of a single positive control may be misleading

Reported sensitivity and drug tolerance are heavily dependent on the positive control and assay settings → **limited/no opportunity for harmonization?** (except (bio)similars?)

Is characterizing our assays using multiple positive controls the answer?

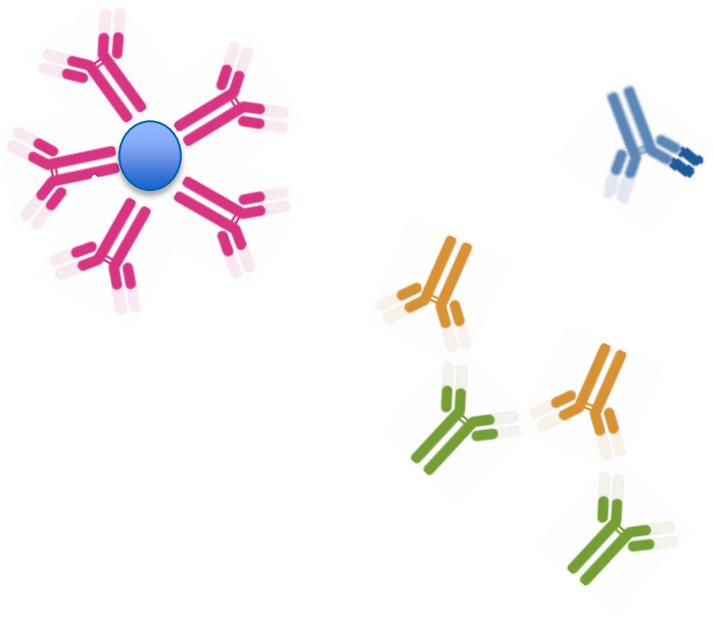
Positive control

Is simply using multiple positive controls the answer? → Should we be asking ourselves the following questions:

1. Do we know for our assays which PC characteristics drive sensitivity and drug tolerance?

2. Do we know the characteristics of the ADA we're trying to detect?

- Affinity?
- Valency - avidity?
- Complex formation?*
- Physicochemical properties?
- …….

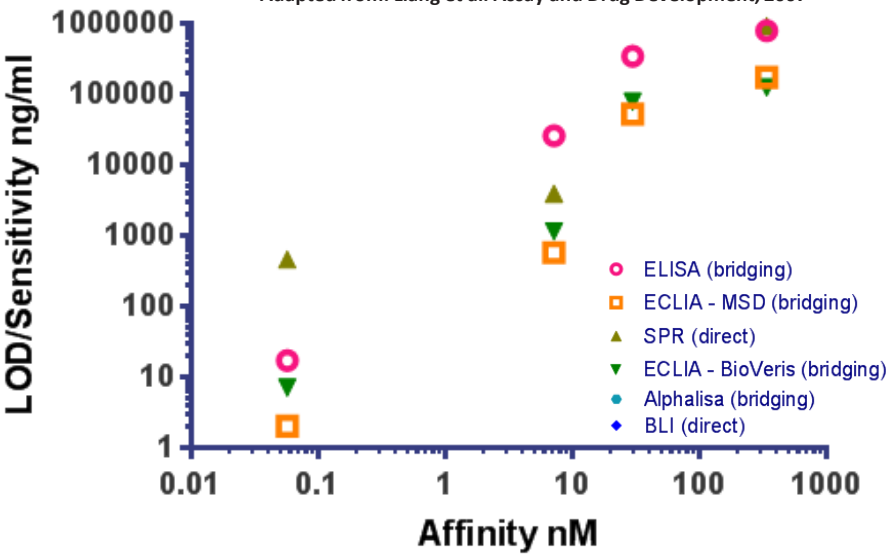


*Examples from literature on complex formation in slide after acknowledgement

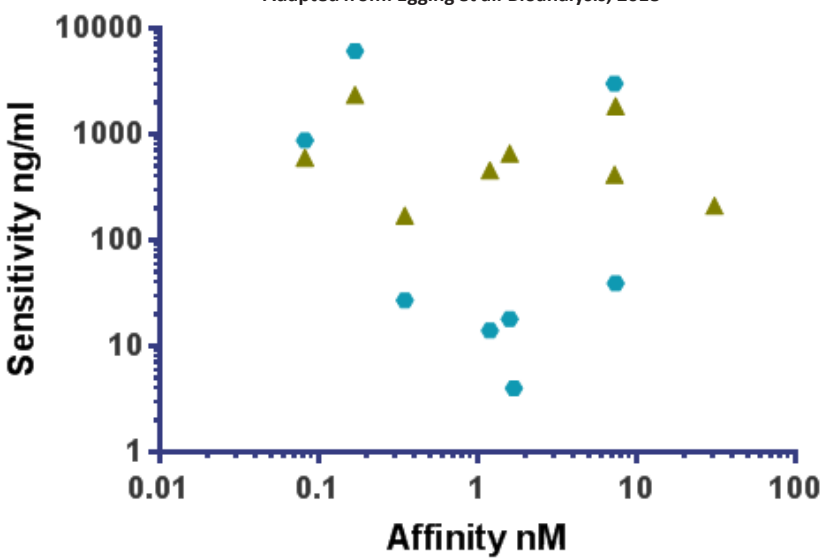
PC characteristics - sensitivity & drug tolerance

Is affinity a good predictor for sensitivity in general?

Adapted from: Liang et al. Assay and Drug Development, 2007



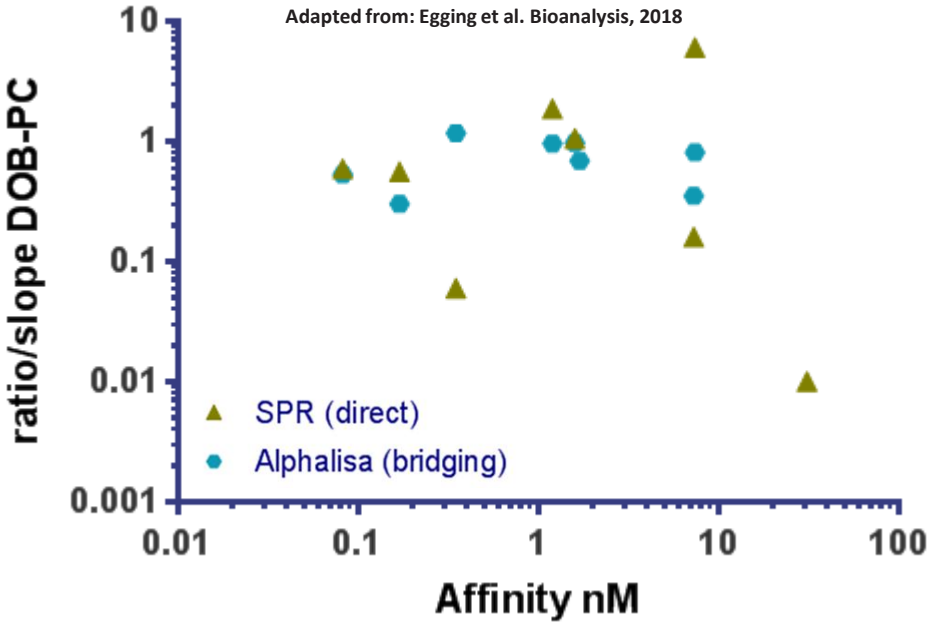
Adapted from: Egging et al. Bioanalysis, 2018



- Li *et al.* JPBA, 2011 reported a trend but no correlation (BLI- direct, ELISA & ECLIA bridging)
- Lofgren *et al.* JIM, 2007 reported a correlation for an ELISA bridging assay, but found no correlation for a direct SPR based method

PC characteristics - sensitivity & drug tolerance

Is affinity a good predictor for drug tolerance?



Lofgren *et al.* JIM, 2007 reported a positive correlation for an ELISA bridging assay and a negative correlation for a direct SPR based method for affinity and drug tolerance.

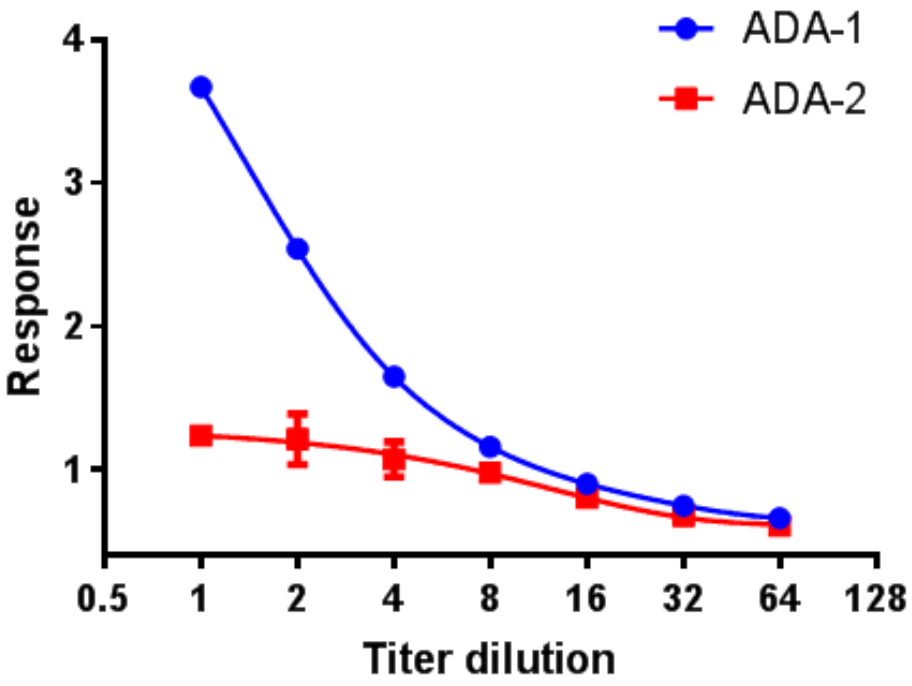
What about other characteristics?

Can we really predict the behavior of our PCs in our assays?

ADA characteristics

Should a large set of PCs be used in sensitivity and drug tolerance estimation? If so, **how should reporting be harmonized?**

ADA1≠ADA2 as exemplified below (ELISA - direct, ADA to compound X), even if you take multiple PC they may behave differently from ADA.

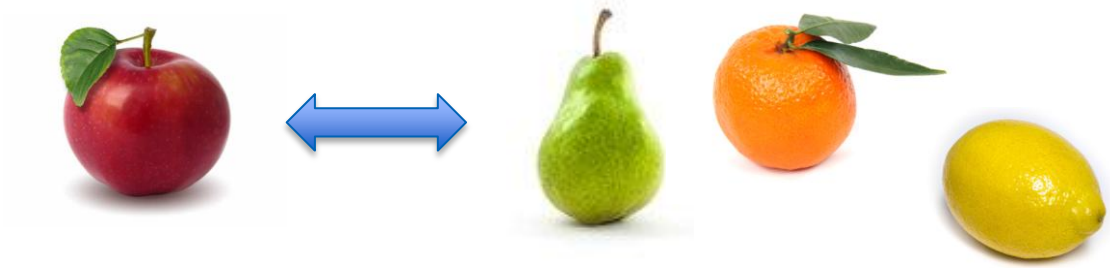


In conclusion

(Re-)**Harmonization of definitions** for sensitivity and drug tolerance should **help align expectations for/from bioanalysts**.

Yet, in all cases **caution** should be applied **in interpretation**, *i.e.*

At least we'll be comparing (assay results)



in the same way

Harmonization should not deter scientists from investigating new (more efficient) ways to work, *e.g.* would a S/N approach be a suitable alternative to titration (Starcevic Manning et al. 2017)?

Acknowledgements

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Examples describing complex formation ADA in vivo

1. Pierog P, Krishna M, Yamniuk A, Chauhan A, Desilva B: Detection of drug specific circulating immune complexes from in vivo cynomolgus monkey serum samples. *J.Immunol.Methods* 416, 124-136 (2015).

2. Rojko JL, Evans MG, Price SA *et al:* Formation, clearance, deposition, pathogenicity, and identification of biopharmaceutical-related immune complexes: review and case studies. *Toxicol.Pathol.* 42(4), 725-764 (2014).

3. Rojas J, Taylor R, Cunningham M *et al:* Formation, distribution, and elimination of infliximab and anti-infliximab immune complexes in cynomolgus monkeys. *J.Pharmacol.Exp.Ther.* 313, 578-585 (2005).

4. Johansson A, Erlandsson A, Eriksson D *et al:* Idiotypic-anti-idiotypic complexes and their in vivo metabolism. *Cancer* 94(4 Suppl), 1306-1313 (2002).

5. van Schouwenburg PA, Kruithof S, Votsmeier C *et al:* Functional analysis of the anti-adalimumab response using patient-derived monoclonal antibodies. *J.Biol.Chem.* 289(50), 34482-34488 (2014).

6. Tung KS, DeHoratius RJ, Williams RC: Study of circulating immune complex size in systemic lupus erythematosus. *Clin.Exp.Immunol.* 43(3), 615-625 (1981).

7. Boysen M, Schlicksupp L, Dreher I, Loebbert R, Richter M: SEC Based Method for Size Determination of Immune Complexes of Therapeutic Antibodies in Animal Matrix. *J.Immunol.Res.* 2016, 9096059- (2016).