Pitfalls in ADA Analysis Workarounds for Clinical Meaningful Immunogenicity Assessment

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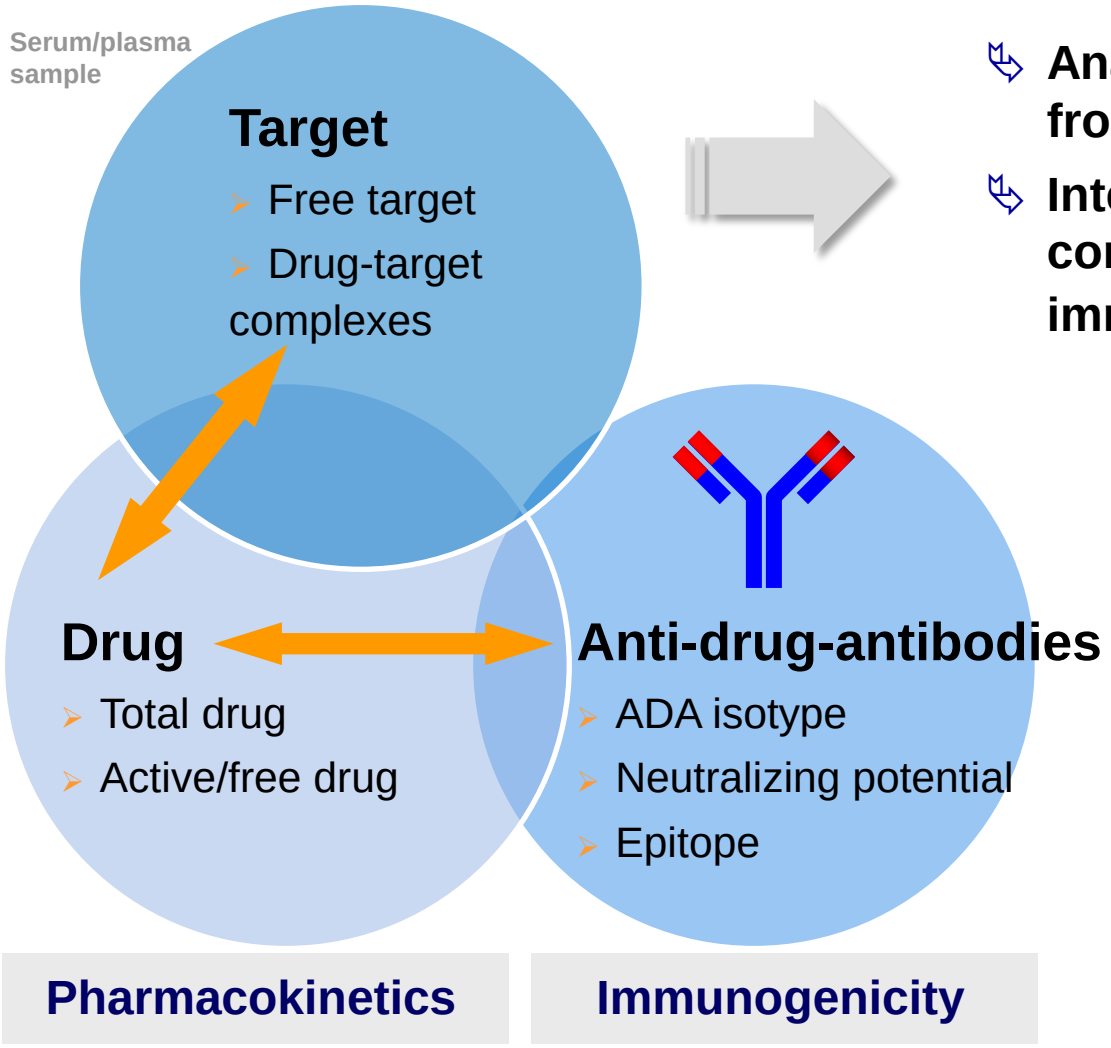
Immunogenicity - Pitfalls in ADA Analysis



- **Analytes are not independent from each other**
- **Interactions have to be considered for immunogenicity assessment**

Clinical consequences

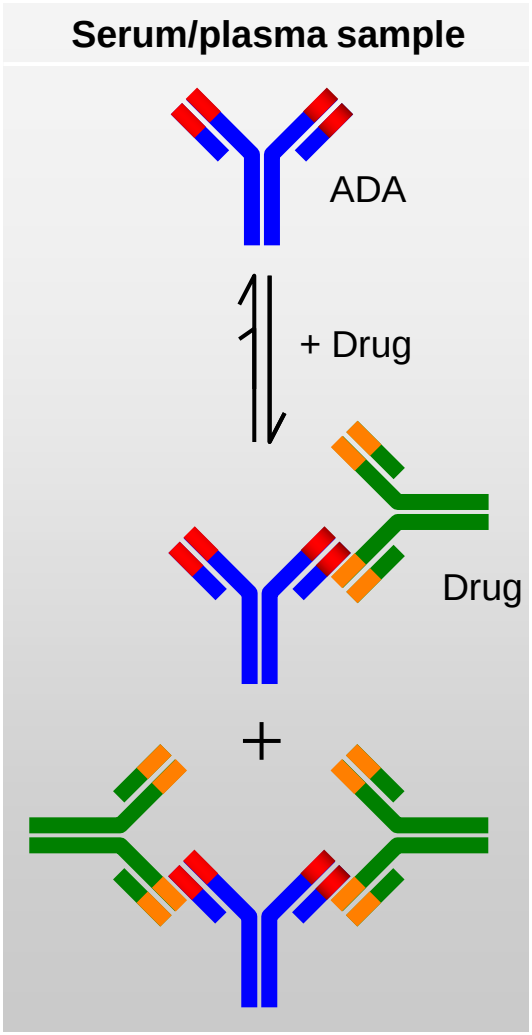
Safety

- **Hypersensitivity / Anaphylaxis**
- **Depletion of endogenous proteins** (e.g. Epo)

Efficacy

- **Reduced/increased exposure**
- **Diminished/loss of efficacy**

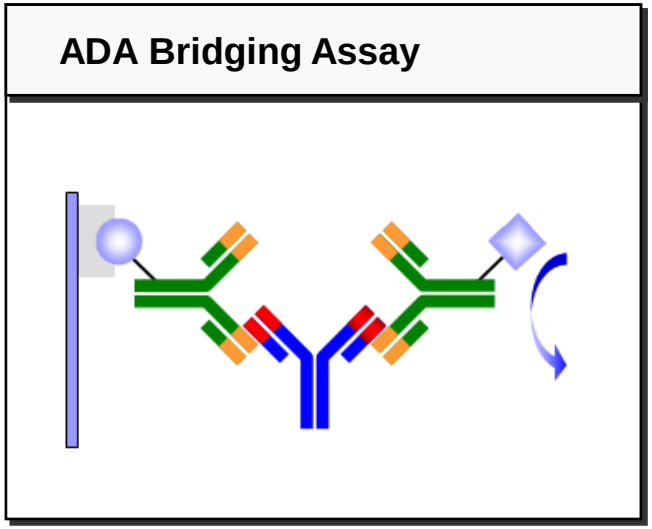
Immunogenicity testing by ligand binding assay
Drug interference



Signal in ADA assay

No signal in ADA assay

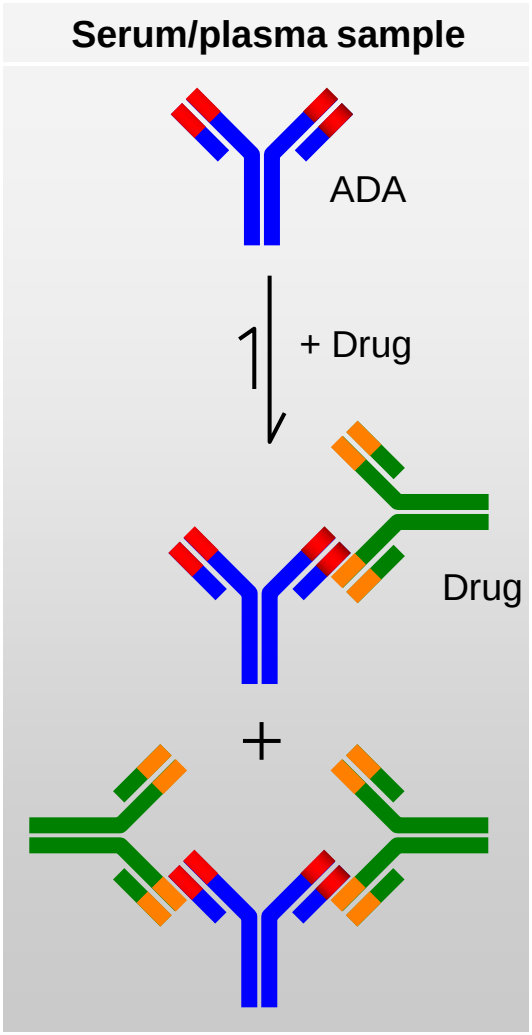
No signal in ADA assay

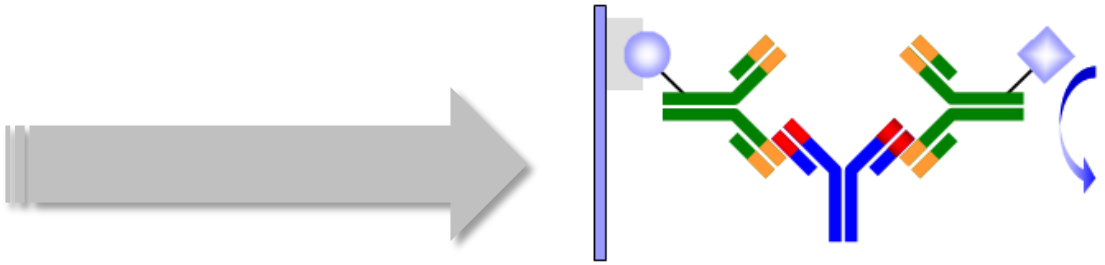


Analytical consequence

- **Drug-ADA-Interaction can result in false-negative ADA testing result**

Immunogenicity testing by ligand binding assay
Drug interference





ADA Bridging Assay

- Colorimetric detection
- Chemiluminescent detection
- Fluorescent detection
- …

1. **Adequate analytical sensitivity for free ADA detection**
2. **Influencing the equilibrium towards free ADA**
3. **Dissociation of ADA-drug complexes by sample pre-treatment**
4. **Detection of ADA-drug complexes**

   Stubenrauch et al., (2012): Analytical Biochemistry 430, 193-199

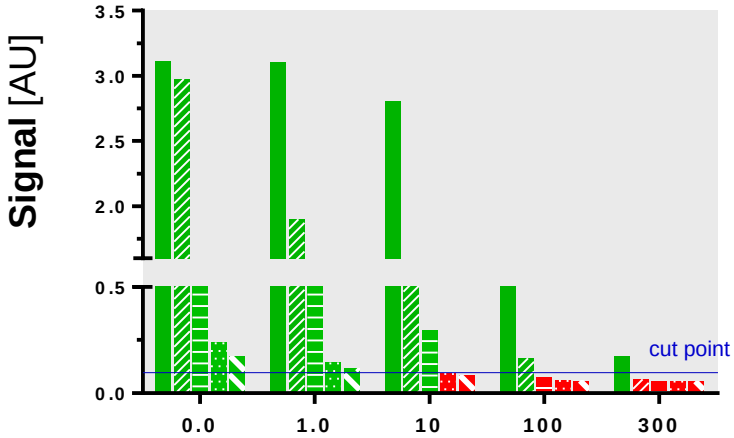
   Wessels et al., (2016); Bioanalysis 8, 2135-2145
5. **ADA enrichment/purification**

Analytical sensitivity of ADA detection

mAb<A> ADA Immunoassay – Assay platforms

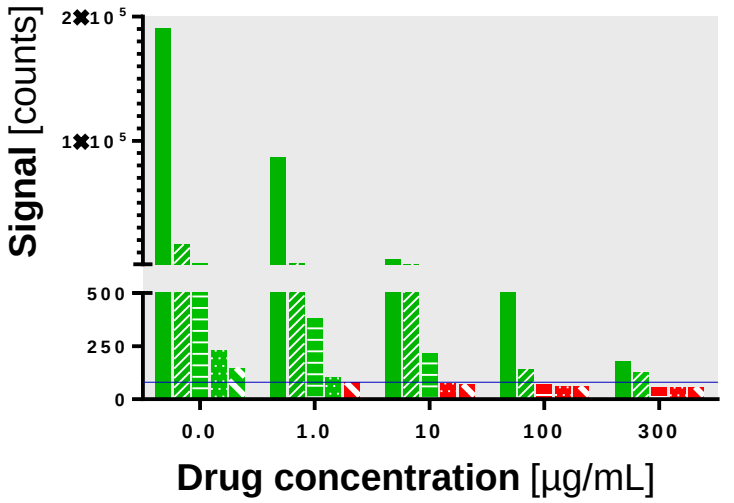
ELISA

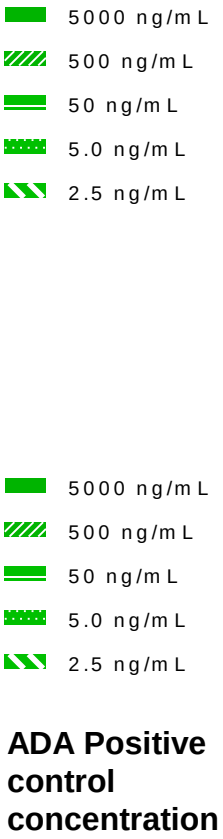
(Biotin/ Digoxigenin)



ECLIA

(Biotin/ Ruthenium)





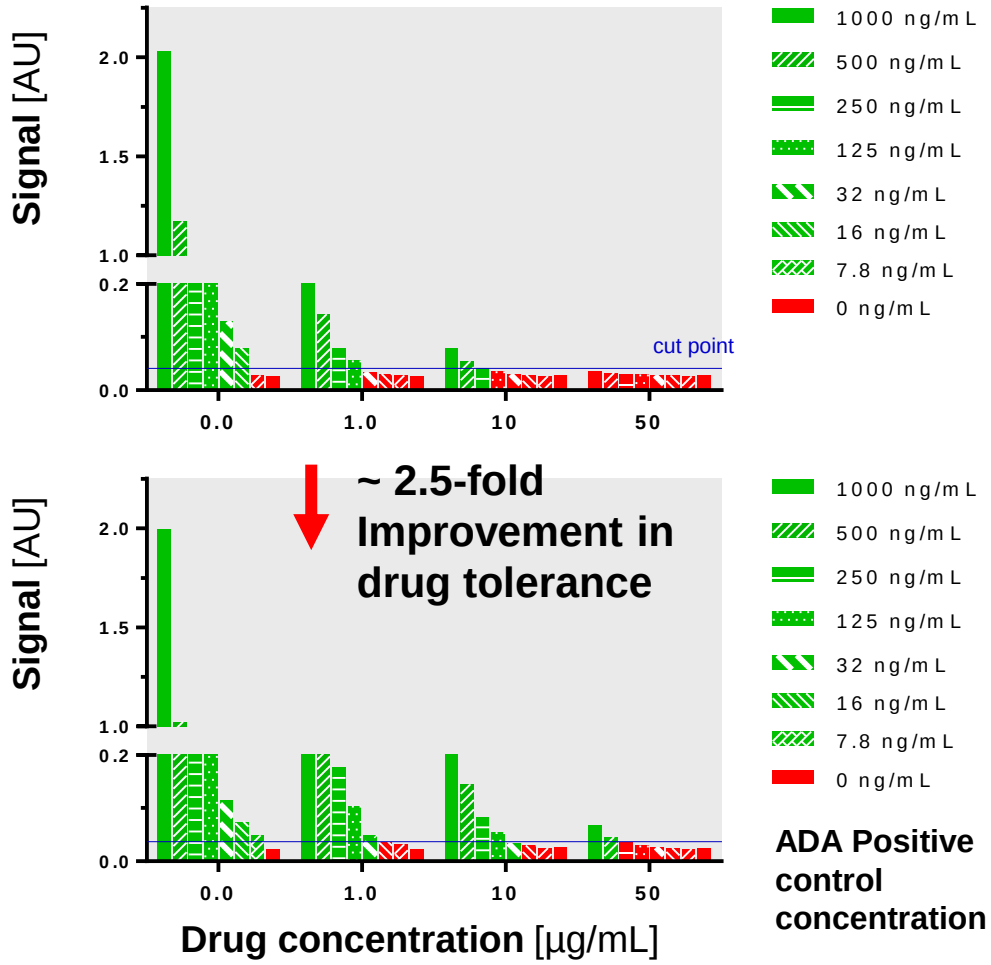
- **Sensitive detection of ADAs with optimized immunoassays**
  - e.g.
  - <2.5 ng/mL ADA in the absence of drug
  - 500 ng/mL ADA in the presence of 100 µg/mL drug
- **Minor impact of platform observed with optimized ADA immunoassays**

Shifting the equilibrium to improve drug tolerance

mAb<B> ADA ELISA – Impact of incubation time

1 h
(Biotin/
Digoxigenin)

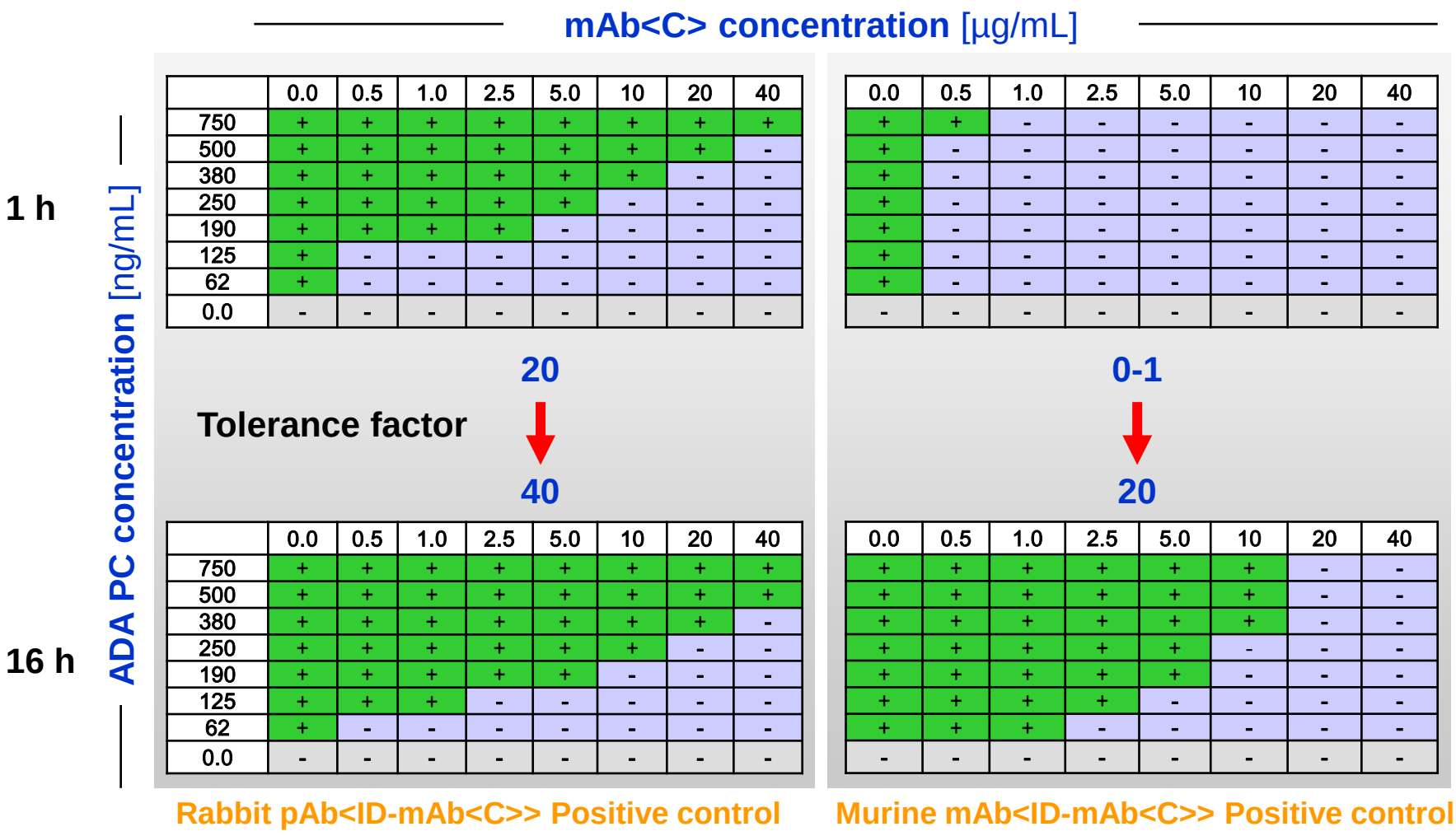
16 h / oN
(Biotin/
Digoxigenin)



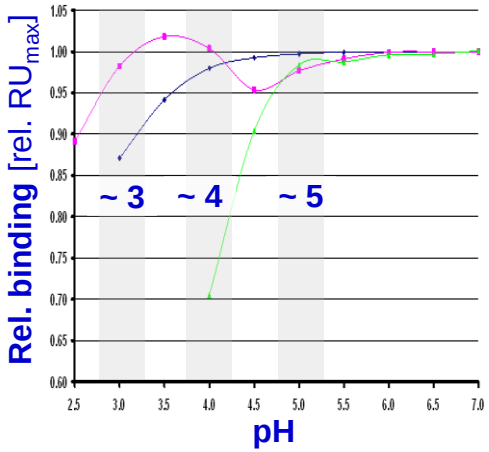
- **Prolonged incubation time improves overall sensitivity <u>and</u> drug tolerance**
  - e.g.
  - 8 vs. 16 ng/mL ADA in the absence of drug
  - 32 vs. 250 ng/mL ADA in the presence of drug

Shifting the equilibrium to improve drug tolerance

ADA characteristics – ADA´s behave differently



Impact of ADA affinity to pre-treatment conditions
pH dependent properties of ADA-drug complexes



- **mAb<mAb<D>> high affinity**
- **mAb<mAb<D>> low affinity**
- **pAb<mAb<D>> / QC**

Set-up

- mAb<D>/anti-<mAb<D> complex pre-bound on SPR chip
- Analysis of pH-dependent dissociation of drug–ADA complexes

- **Reduction of specific ADA binding to mAb<D> strongly depends on characteristics and affinity of ADAs**
- **Dissociation of high-affinity-ADA-drug complexes in general requires harsh pH conditions are required (pH < 3.0)**

- **Reduced recovery observed in acid pre-treated ADA PC samples spiked at QC concentrations**
- **Reduction of binding to drug varies with ADA affinity and time**
- **Potential underestimation (false-negative) of real ADA-positive serum samples**

Acid pre-treatment time **Recovery rel. to untreated samples** [%]

Acid pre-treatment time	ADA Control	pH 3.5	3.0	2.5
1 h	pAb<mAb<D>> / QC	75	75	77
	mAb<mAb<D>> high affinity	91	73	17
	mAb<mAb<D>> low affinity	68	47	43
15 min	pAb<mAb<D>> / QC	70	82	85
	mAb<mAb<D>> high affinity	93	84	58
	mAb<mAb<D>> low affinity	68	49	44

Set-up

- mAb<D> ADA ELISA
- Analysis of acid-treatment conditions on ADA recovery

Impact of acid pre-treatment to ADA integrity

Pro`s and con`s of acid pre-treatment

Pro´s

⇨ Advantages of acid pre-treatment

- Improved drug tolerance
- Easy handling compared to other sample preparation procedures
- Easy to adapt to existing immunoassay platforms

Con´s

⇨ Disadvantages of acid pre-treatment

- Impact on denaturation of human ADAs of study samples cannot be tested
- Impact on different ADA affinities and ADA isotypes (IgG_x, IgM, IgE, etc.) is not known
- Different and maybe species-dependent performance characteristics under pre-treatment conditions (rabbit ADA vs. human ADA)

Goal:

ADA testing strategy that mimimizes the risk of **false-negative results** due to high levels of residual drug (unsufficient analytical sensitivity) or due to ADA denaturation in test samples (ADA inactivation)

Assay optimization towards improved drug tolerance

Example: mAb<B> ADA assay: Evaluation of testing conditions

mAb<C> conc. [µg/mL]

ADA Positive Control conc. [ng/mL]

1 h

[ng/mL]	0.00	1.00	10.0	50.0
1000	2.032	0.296	0.079	0.036
500	1.171	0.143	0.055	0.033
250	0.650	0.079	0.041	0.030
125	0.366	0.056	0.036	0.030
31.3	0.130	0.034	0.030	0.028
15.6	0.080	0.031	0.029	0.028
7.81	0.028	0.028	0.027	0.026
0.00	0.027	0.027	0.028	0.028

Cut-point: 0.041

0.00	1.00	10.0	50.0
13748	1693	442	127
6973	786	282	84
3646	421	165	71
1929	234	107	58
563	97	64	51
312	77	53	53
50	52	52	42
40	50	51	38

Cut-point: 89

16 h

1000	1.996	0.678	0.281	0.069
500	1.023	0.338	0.145	0.046
250	0.584	0.177	0.084	0.036
125	0.319	0.105	0.055	0.030
31.3	0.115	0.049	0.034	0.026
15.6	0.075	0.037	0.030	0.025
7.81	0.049	0.032	0.025	0.023
0.00	0.023	0.024	0.026	0.025

Cut-point: 0.037

14040	3807	2007	513
6655	1822	917	252
3234	926	487	143
1718	509	270	92
532	180	110	60
310	116	78	56
176	81	65	48
46	59	45	46

Cut-point: 97

Native serum sample

+ acid-pre-treatment

1000	2.022	1.613	0.713	0.146
500	1.124	0.878	0.389	0.090
250	0.576	0.442	0.200	0.056
125	0.309	0.238	0.112	0.041
31.3	0.097	0.081	0.049	0.030
15.6	0.061	0.053	0.037	0.028
7.81	0.044	0.039	0.031	0.026
0.00	0.025	0.028	0.027	0.026

Cut-point: 0.039

12935	10579	4906	1047
6156	4787	2323	510
3037	2443	1156	273
1507	1176	596	159
398	327	180	66
212	177	98	56
128	105	75	49
40	42	46	45

Cut-point: 86

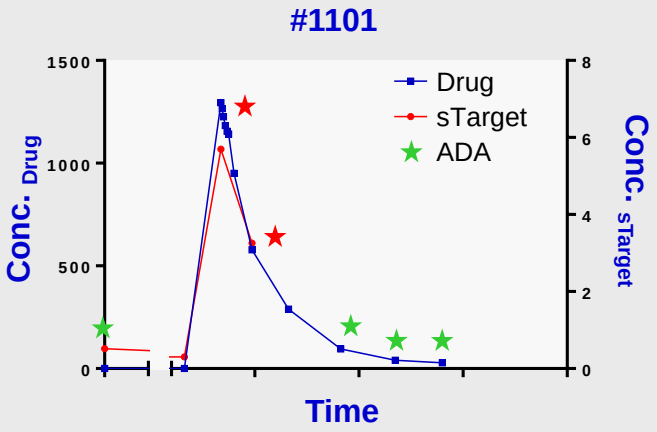
Pre-treated serum sample

ELISA **ECLIA**

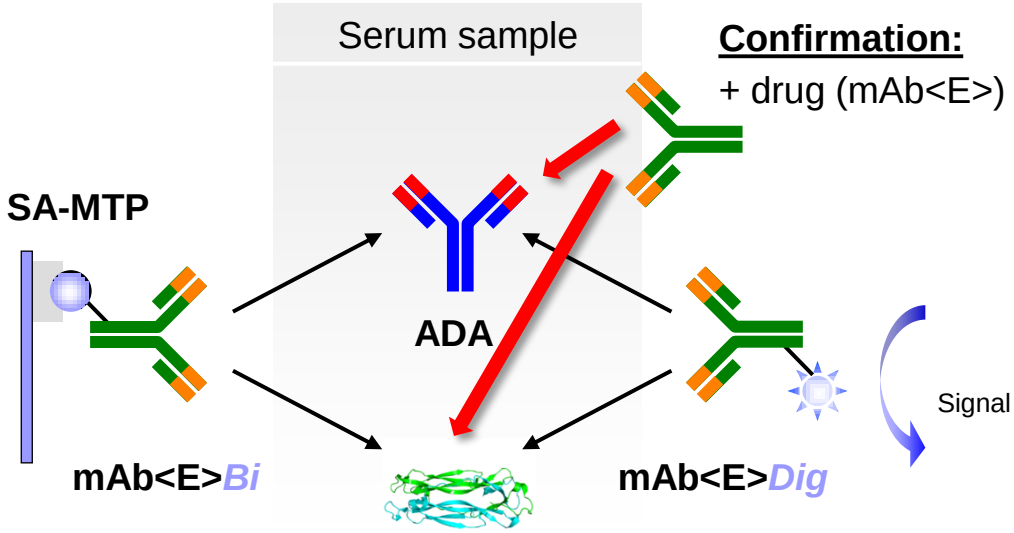
Immunogenicity testing by ligand binding assay
Target interference

Case study: **mAb<E>**

- Drug: rec. humanized mAb
- Target: soluble cytokine
- SAD/MAD study in HV/patients



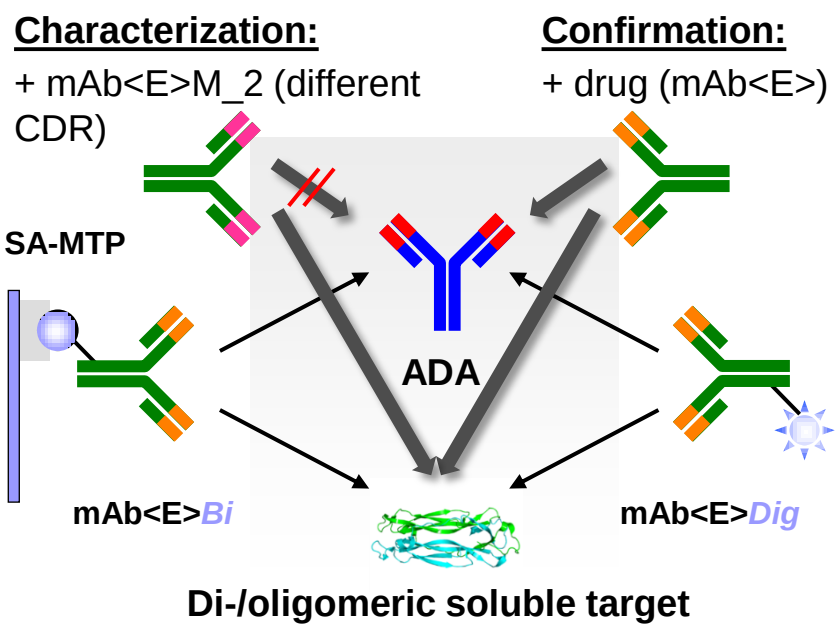
ADA Assay format: Bridging assay

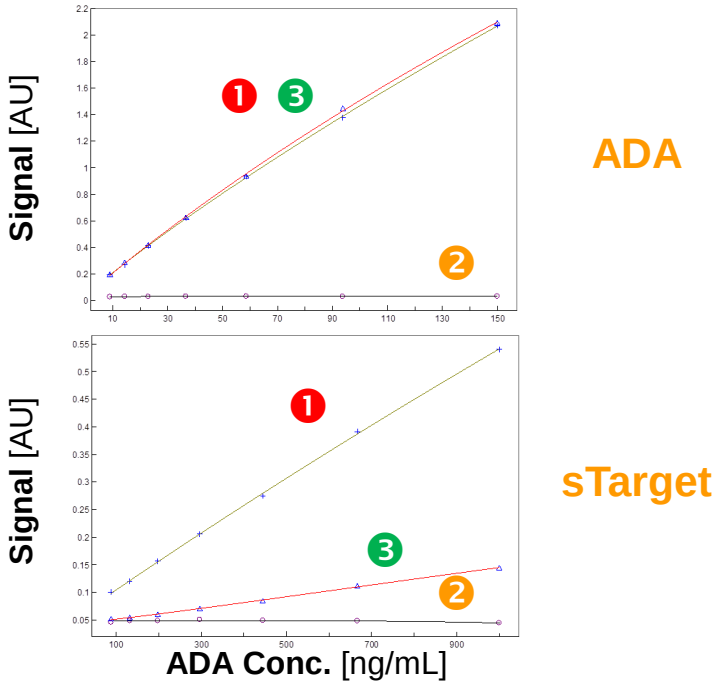


Sample	ADA Screening	ADA Confirmation (+ Drug / mAb<>)
ADA	Positive signal	⇩ Signal quenching
sTarget	Positive signal	⇩ Signal quenching
ADA + sTarget	Positive signal	⇩ Signal quenching

Immunogenicity testing by ligand binding assay

Target interference





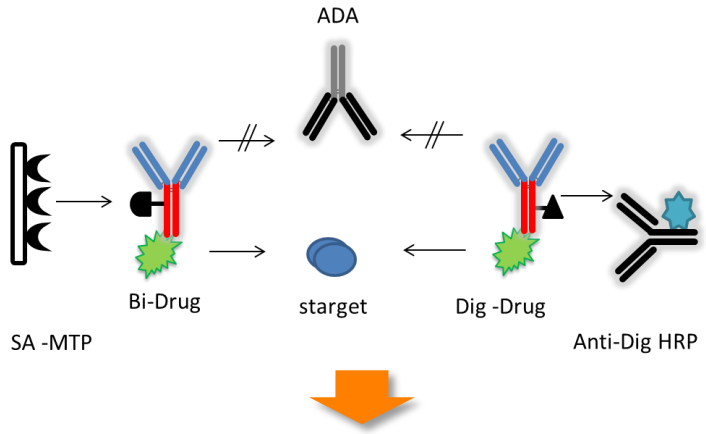
- **Presence of di-/oligomeric soluble target can result in false-positive ADA results in classical ADA bridging assays**
- **Development of a characterization reagent/assay allows discrimination between positive and false-positive ADA results**

	❶	❷	❸
Sample	**ADA Screening**	**ADA Confirmation** (+ Drug/ mAb<E>)	**ADA Characterization** (+ mAb<E>M_2)
ADA	Positive	⇩ Signal	⇔ No quenching
sTarget	Positive	⇩ Signal	⇩ Signal
ADA + sTarget	Positive	⇩ Signal	⇔ No Quenching

Equilibrium shift to eliminate target interference

Reduction of target interference by sample dilution

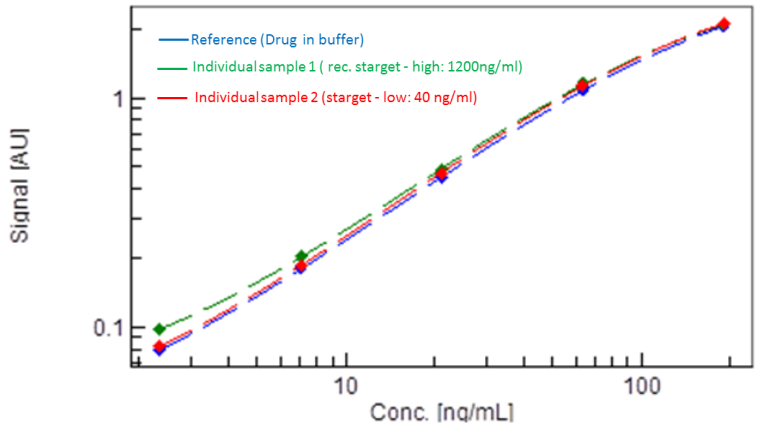
ADA Bridging Assay



- **Reduction to 1% serum matrix content reveals increased specificity**
- **Risk mitigation strategy for false-positive ADA results due to target interference**
- **Balance between reduced sensitivity by sample dilution and benefit from increased drug tolerance/sensitivity due to complex dissociation**

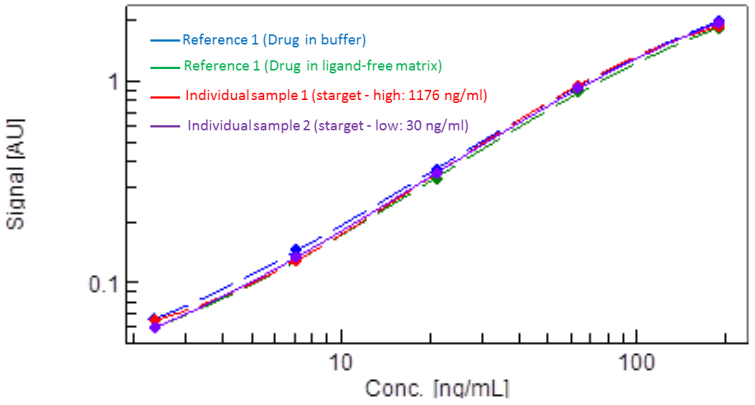
Spiked recombinant target

(1200ng/ml; 40 ng/ml)



Endogenous target level

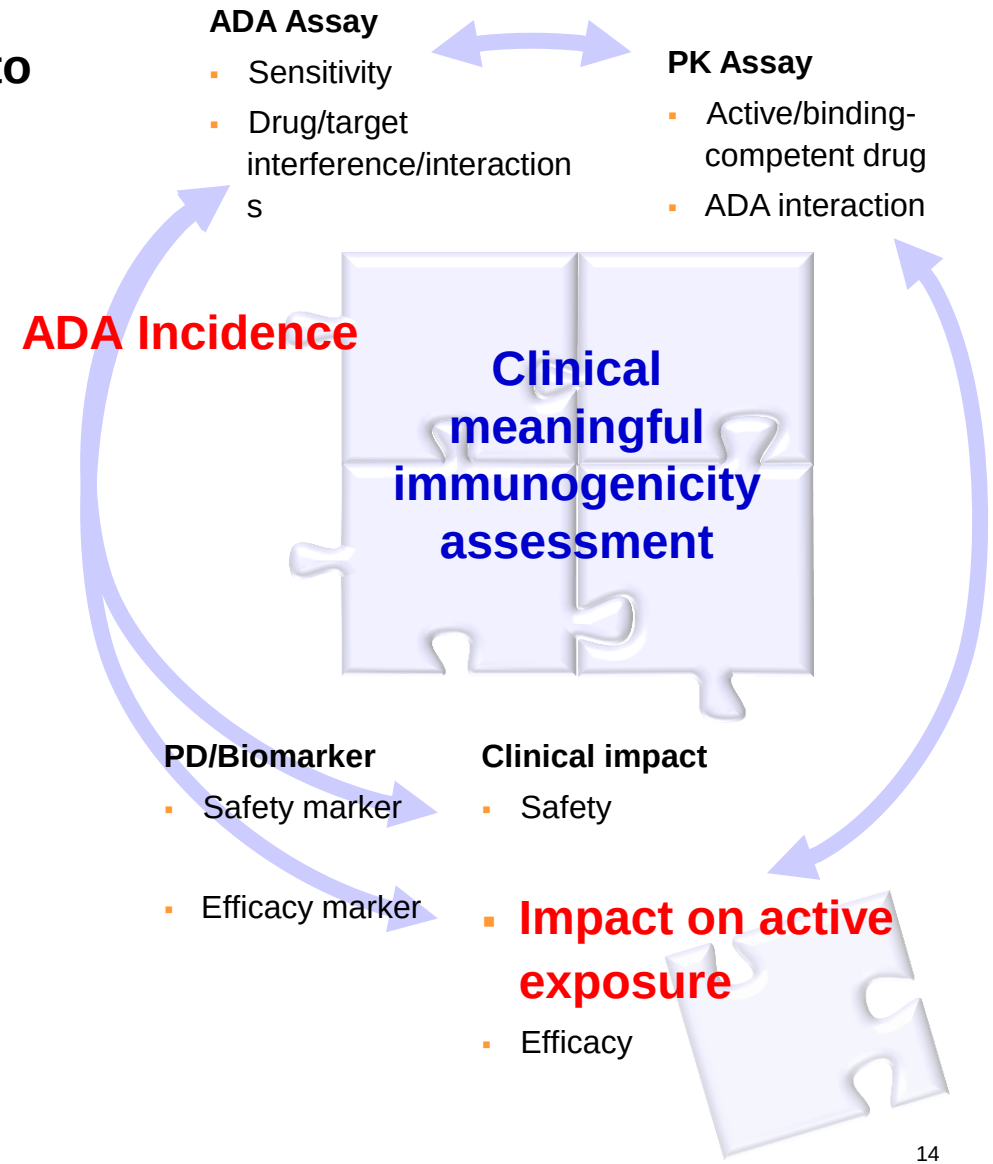
(1176 ng/ml; 30 ng/ml)



See also: Staack et al. (2012): Bioanalysis; 4(4):381-95

Summary and Conclusions

- **Testing for anti-drug antibodies to assess clinical immunogenicity requires deep understanding of interacting proteins of anti-drug antibodies**
  - biologic conditions
  - sample/assay conditions

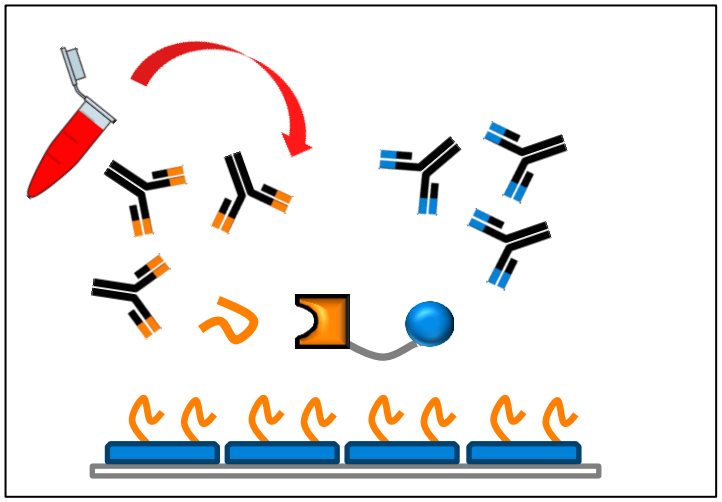


ADA-dependent Neutralization

ADA characterization vs. assessment of ADA effect on efficacy

ADA Characterization

- **Cell-based nAb Assay**



- Full mode of action covered
- Only **qualitative** data
  - Obtained information:
    (At least) Some ADAs of the polyclonal immune response neutralize the drug effect
- „Selectivity“ → sol. Ligand could also cause neutralization
- „Technical challenges“: drug tolerance, sensitivity……

Assessment of ADA Effect on Efficacy

- **Ex-vivo Potency Assay**
  Schäfer, Challand, Schick, Bader, Hainzl, Heinig, Müller, Papadimitriou, Heinrich.
  Bioanalysis; 2015 (24):3063-72

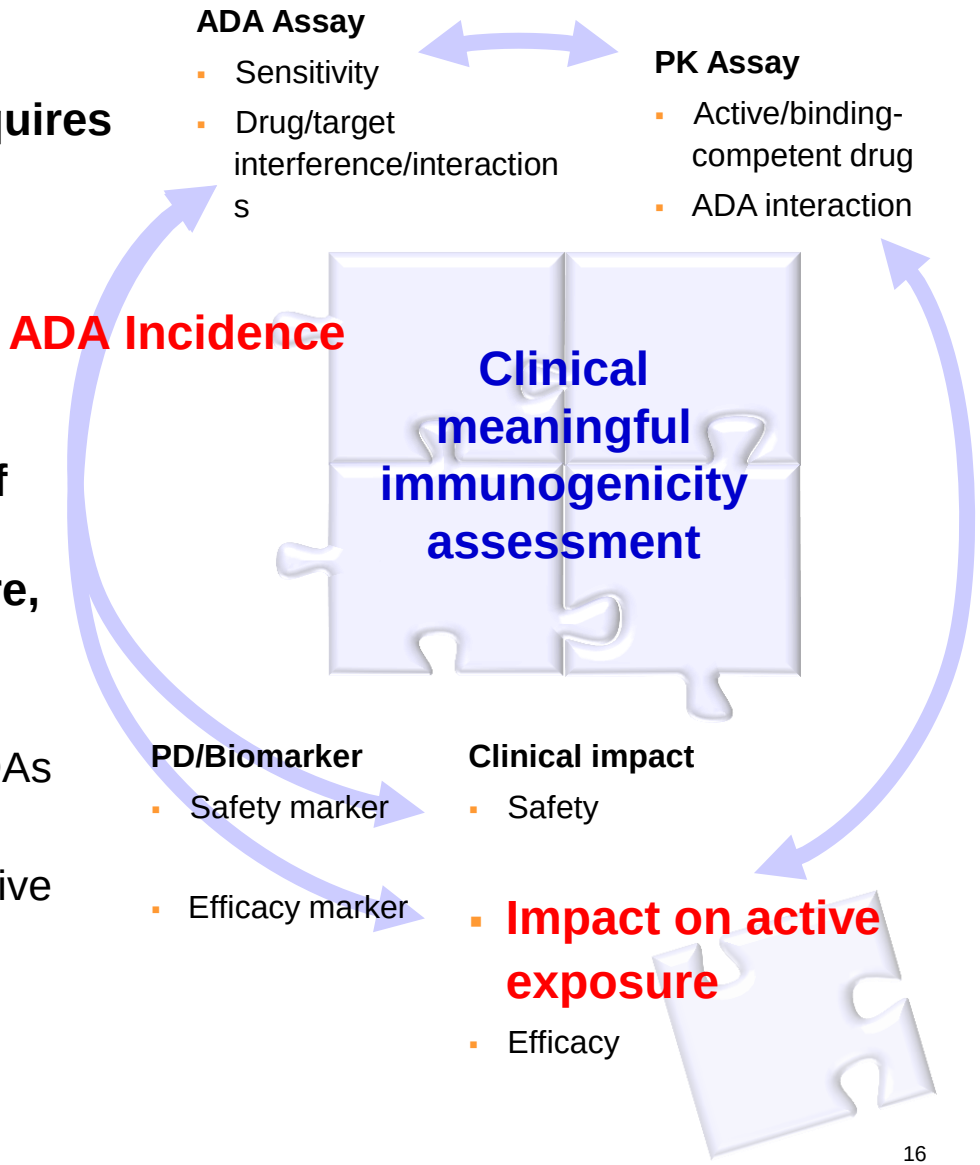
- **Cell-based PK Assay**
  Hu, Gupta, Swanson, Zhuang.
  J. Immunol. Methods 345(1–2), 70–79 (2009).
  Wei, Grill, Heatherington, Swanson, Gupta
  Journal of pharmaceutical and biomedical analysis; 2007 ;43(2):666-76

- **Active LBA PK Assay**
  Staack, Jordan, Viert, Schäfer, Papadimitriou, Heinrich.
  Bioanalysis; 2015 (24):3097-106

Relevant information:
Active drug exposure

Summary and Conclusions

- **Testing for anti-drug antibodies to assess clinical immunogenicity requires deep understanding of interacting proteins of anti-drug antibodies**
  - biologic conditions
  - sample/assay conditions
- **Clinical meaningful investigation of immunogenicity is an integrated analysis of ADA impact on exposure, safety and is always an interplay between**
  - sensitive and specific detection of ADAs under study conditions
  - measurement of pharmacological active drug
  - clinical safety and efficacy markers



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Doing now what patients need next