High drug tolerant immunogenicity testing: Is there space for improvement?

Gregor Jordan
Pharmaceutical Sciences, Roche Pharma Research and Early Development, Roche Innovation Center Munich

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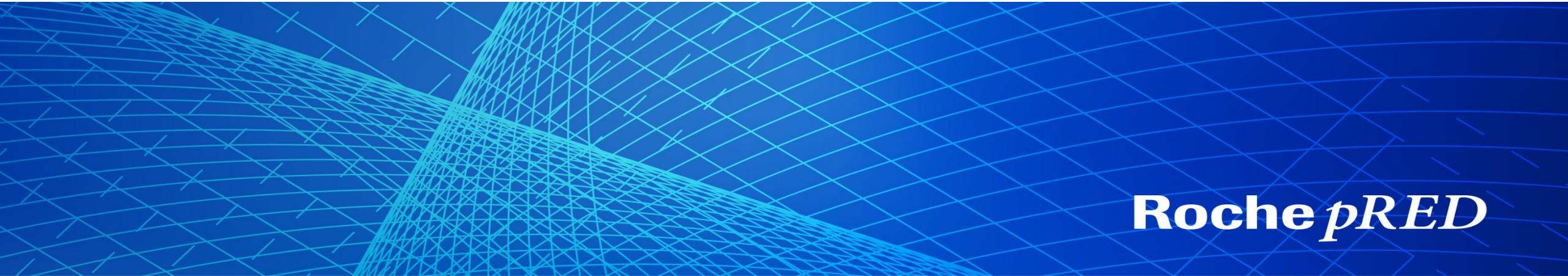


What we often have to deal with...

…"and how to tackle it"

Rapid turnaround time (TAT)

…**easy & robust protocol to reduce handling error and repeats**

Challenge of Soluble Target (sTarget)

… **disrupt complexes followed by e.g. masking or depleting sTarget**

Robust assay protocol with false positive rate in patient samples as pre-defined (5%)

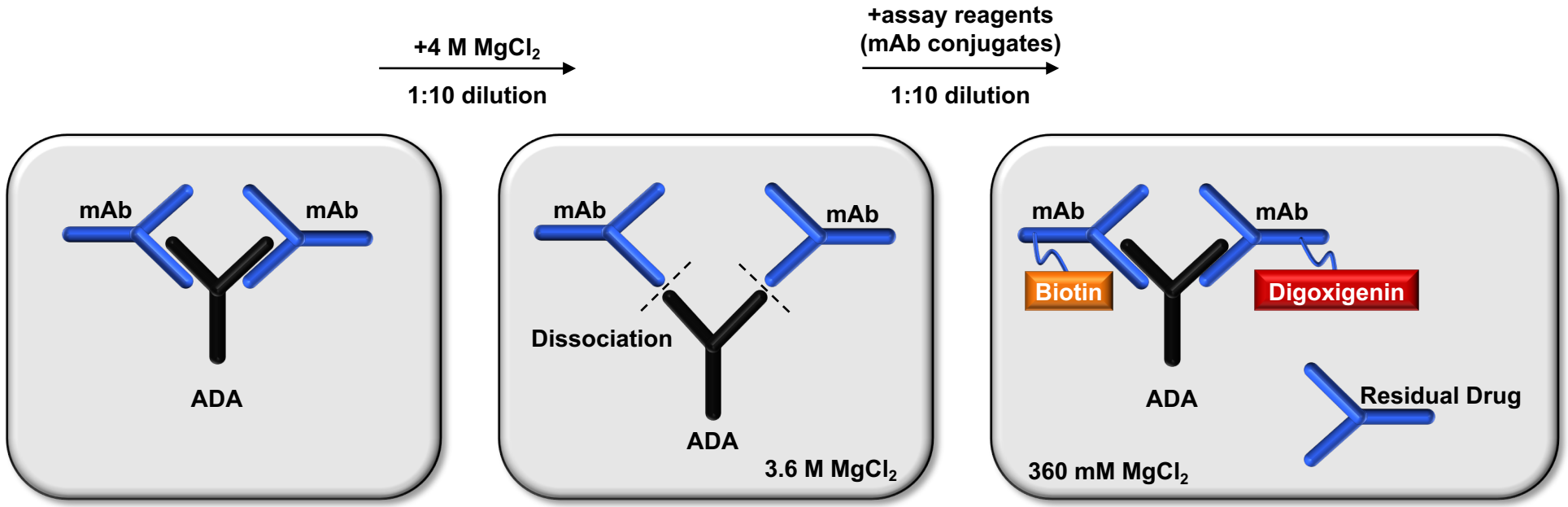
…**outlier exclusion procedure during CP calculation process to hopefully avoid in study CP**..

High drug tolerant assay especially when washout samples are not available

… **disruption of complexes and/or depletion of complexes:** ACE, SPEAD, PandA, BEHD and **HISDA**

HISDA Assay format

…is the predefined MRD=100 a drawback in terms of achieving high drug tolerance?

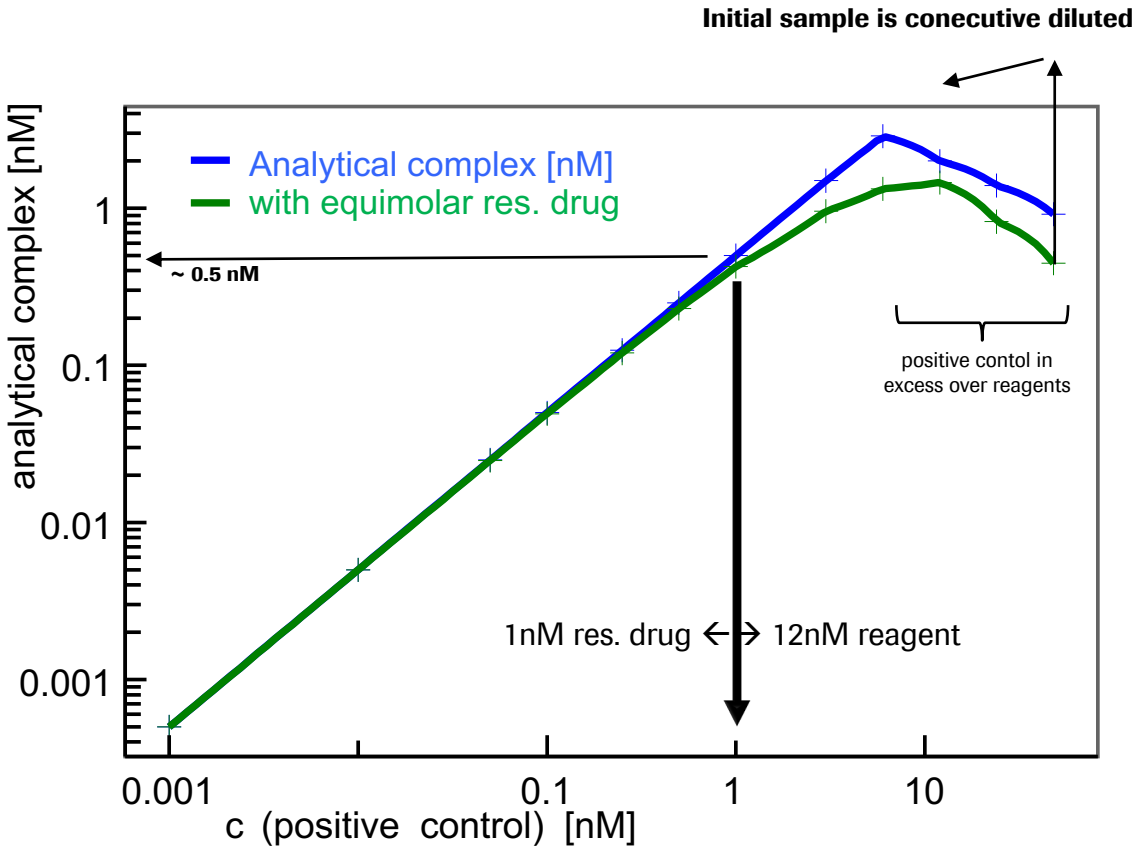


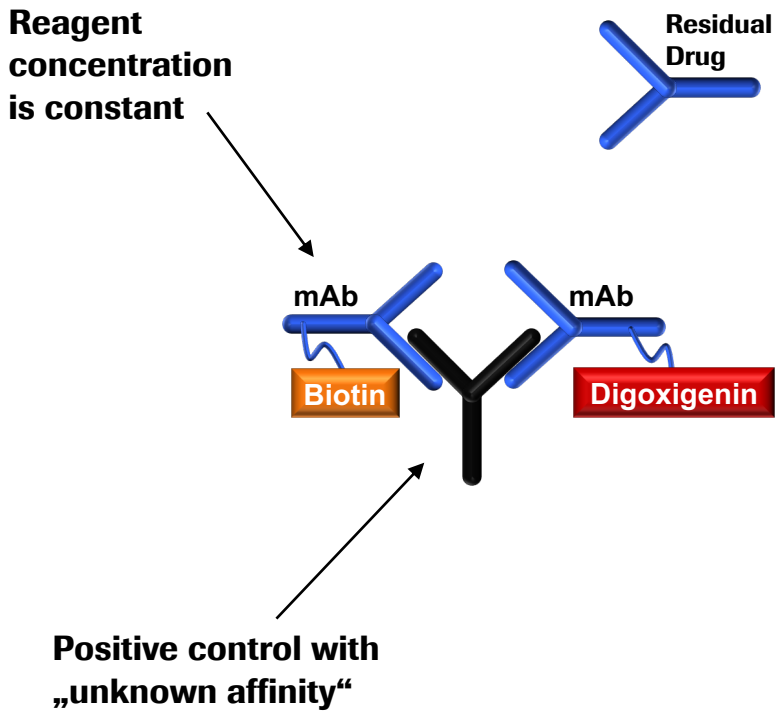
- Two step assay with two consecutive 10 fold dilution with total runtime of ~ 4h
- Disruption of residual drug ADA complexes with mild conditions
- Assay MRD = 100

Jordan, Pöhler, Guilhot; Zaspel, Staack . Bioanalysis. 2020 Jun;12(12):857-866

How does the MRD affect the sensitivity a homogeneous assay

….with an eye on drug tolerance

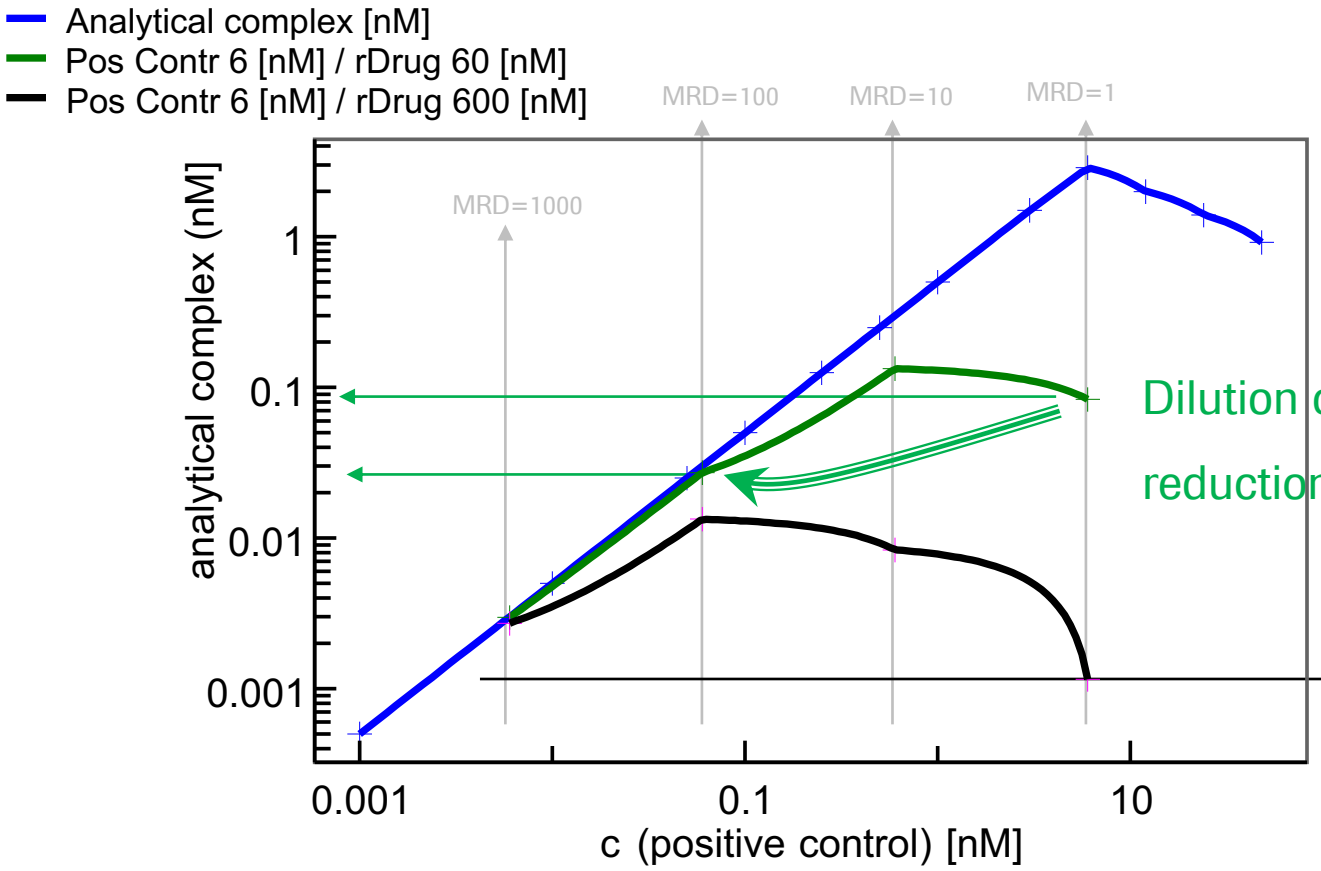




In this example @ < 1 nM ADA the two calibration curves become increasingly similar

How is the dilution affecting the analytical complex?

…in case of high residual drug concentration?



In the presence of high concentrattion of residual drug, the drug toleranece is improved with dilution F ~ 100

Dilution of F=100 results theoretically only in a reduction of signal by a factor of only ~3

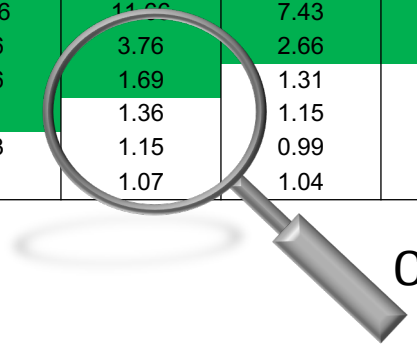
Even a 1000 fold dilution would improve the sensitivity of the sample

Drug Tolerance of the HISDA Assay – Case Example

…with complex disruption and MRD=100

positive control [ng/mL]	Drug Tolerance table residual drug in 100 matrix sample								
	+ 0.5 µg	+ 1.0 µg	+ 5.0 µg	+ 10 µg	+ 50 µg	+ 100 µg	+ 250 µg	+ 500 µg	+ 1000 µg
4000	0.13	0.25	1.25	2.50	12.5	25	63	125	250
2000	0.25	0.50	2.50	5	25	50	125	250	500
500	1	2	10	20	100	200	500	1000	2000
125	4	8	40	80	400	800	2000	4000	8000
62.5	8	16	80	160	800	1600	4000	8000	16000
31.3	16	32	160	320	1600	3200	8000	16000	32000
15.6	32	64	320	640	3200	6400	16000	32000	64000

positive control [ng/mL]	Signal to Blank table residual drug in 100 matrix sample								
	+ 0.5 µg	+ 1.0 µg	+ 5.0 µg	+ 10 µg	+ 50 µg	+ 100 µg	+ 250 µg	+ 500 µg	+ 1000 µg
4000	39.13	38.60	38.55	36.44	31.52	26.32	19.20	12.57	6.97
2000	26.05	26.99	26.23	24.27	20.23	16.46	[illegible]	7.43	4.20
500	10.01	9.98	9.62	8.67	6.91	5.56	3.76	2.66	1.78
125	3.59	3.43	3.35	3.11	2.60	2.06	1.69	1.31	1.14
62.5	2.35	2.28	2.19	2.15	1.84	1.51	1.36	1.15	1.03
31.3	1.66	1.70	1.66	1.60	1.44	1.23	1.15	0.99	0.99
15.6	1.43	1.31	1.35	1.33	1.20	1.11	1.07	1.04	0.98

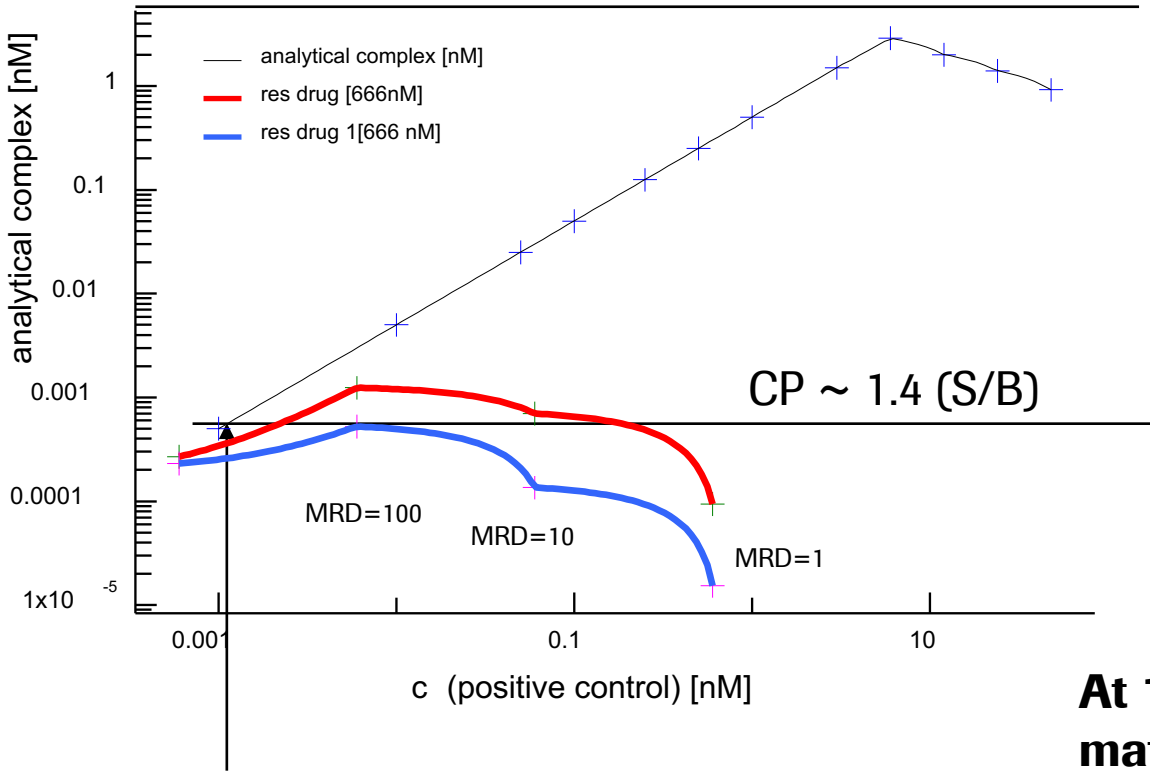


CP ~ 1.4 (S/B)

Drug tolerance factor of >1000 could be achieved

Is the drug tolearance in line with calculations?

…by spotting on 100µg/mL and 250 µg/mL residual drug sample



positive control [ng/mL]	residual drug	
	+ 100 µg	+ 250 µg
4000	26.32	19.20
2000	16.46	11.66
500	5.56	3.76
125	2.06	1.69
62.5	1.51	1.36
31.3	1.23	1.15
15.6	1.11	1.07

Green indicates obove CP

~16 ng/mL in sample

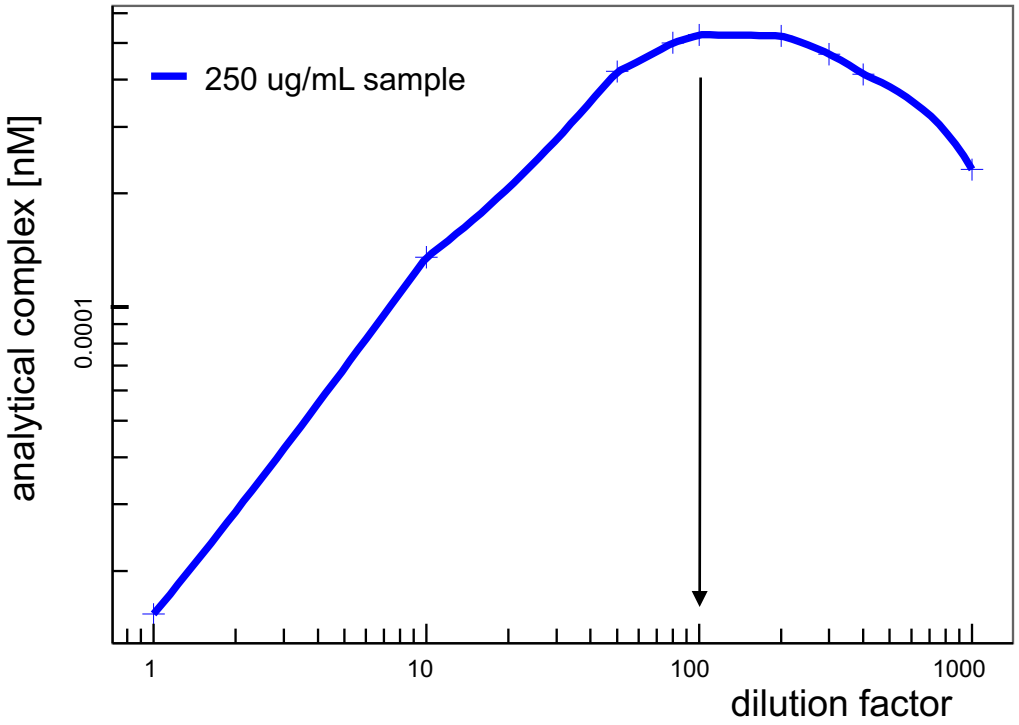
analytical sensitivity of the assay with MRD=100

At 100 and 250 µg/mL the drug tolerance matches the expectations.

The MRD of 100 seems to be optimal in terms of drug tolerance at high residual drug level

Can the drug tolerance be improved further more?

…by adapting the dilution…



By changing the dilution factor the drug tolerance can‘t be improved

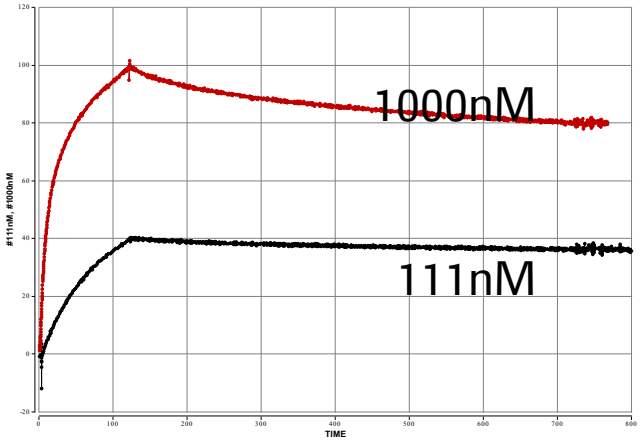
A dilution factor of 100 seems to be optimal for high residual drug samples (based on calculation)

Reaching equilibrium is assumed … but how long does it take to reach the equilibrium in case of over night incubation?

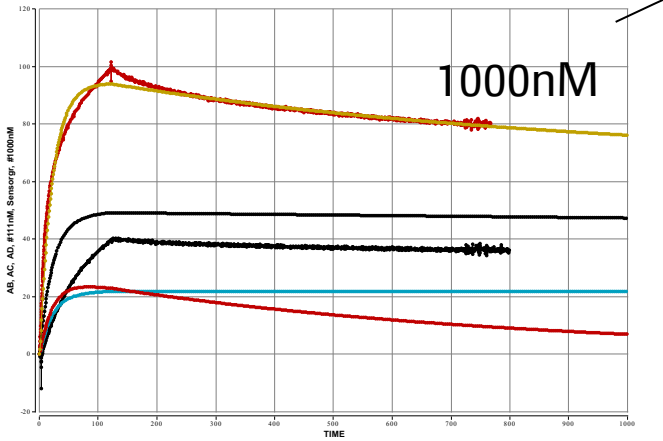
Kinetic characterizatrion of a polyclonal positive control

…how poly is poly and what „koff distribution“ is observed?

Biacore sensorgram

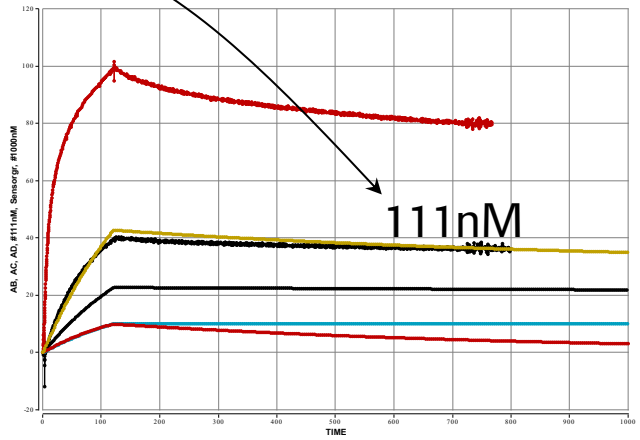


Calculation vs sensorgram



F=9 of sample

All kinetic and Biacore specific parameter left const



It is per default assumed that three IgG are present:

With these pre assumpton the best fit provides:

mAb 1 in mixture = 28% contributing to sensorgram after 120 sec to 24% → koff=1.03e-7

mAb 2 in mixture = 64% contributing to sensorgram after 120 sec to 53% → koff= 4.34e-5

mAb 3 in mixture = 8% contributing to sensorgram after 120 sec to 23% → koff= 1.366e-3

Is a disruption of complexes needed

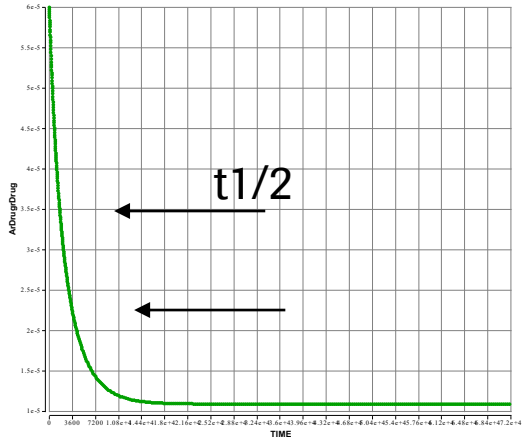
…or is a over night incubation sufficient to form quanitative measurable complexes?

Dilution of 0.6 nM / 60 nM sample F=10000

Dilution with reagent

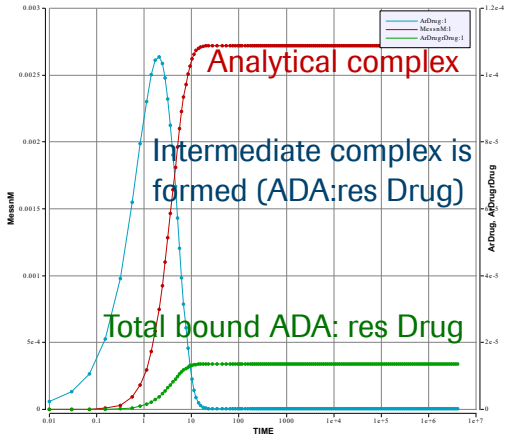
No initial complex dissociation

Koff~4e-4 1/sec → t1/2~30min

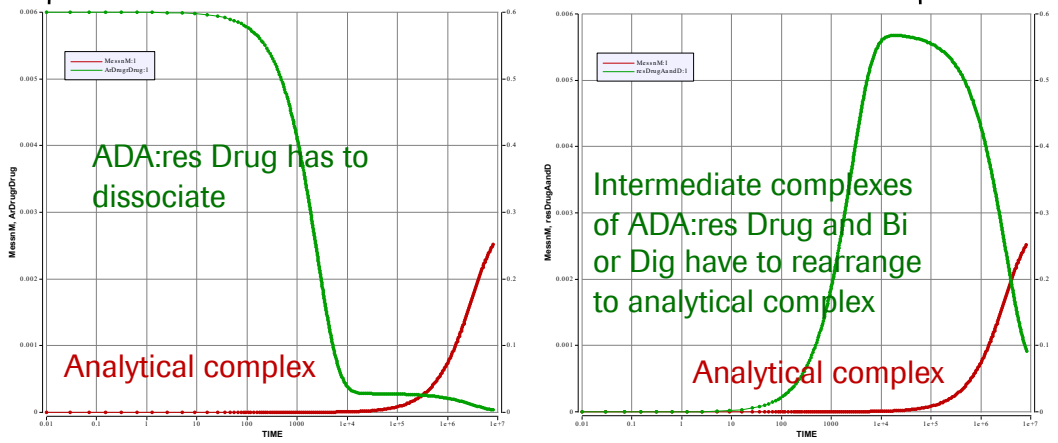


High dilution – complexes dissociate over time

1:100 of with disruption of complexes



Formation of complexes driven by association



Formation of complexes driven by dissociation and intermediate complexes which will not give a signal

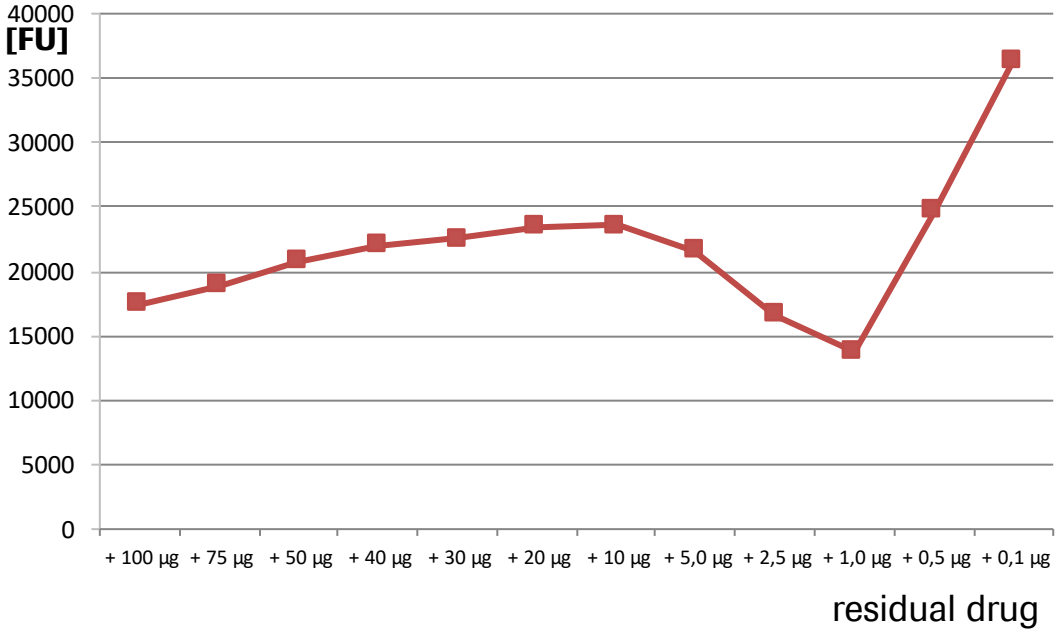
- Dissociation of complexes prior addition of reagent improves time to reach new equlibrium singnificantly
- time limiting is dissociation and formation, while intermediate complexes were formed

Disruption of complexes

…only essential for achieving high drug tolerance? What about soluble target

Extended **drug tolerance** table covering residual drug from **0.1 to 1000 µg/mL** serum concentration for randomly chosen **positive control concentration of 4000 ng/mL**

Over night incubation with no „active“ complex dissociation



spiked drug [µg/mL]	signal [FU]
+ 100 µg	17493
+ 75 µg	18927
+ 50 µg	20743
+ 40 µg	22046
+ 30 µg	22583
+ 20 µg	23422
+ 10 µg	23611
+ 5,0 µg	**21600**
+ 2,5 µg	**16649**
+ 1,0 µg	**13751**
+ 0,5 µg	24719
+ 0,1 µg	36378

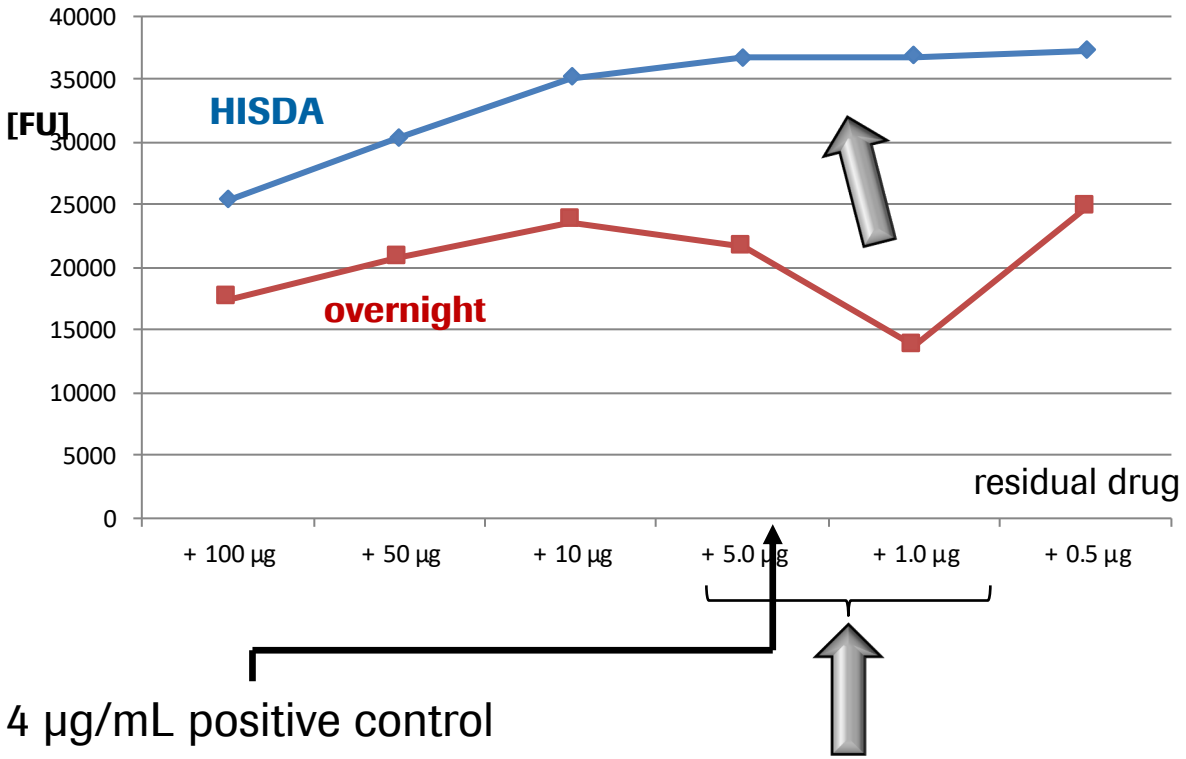
Unexpected behavior of signal in relation to residual drug at medium to low concentration.

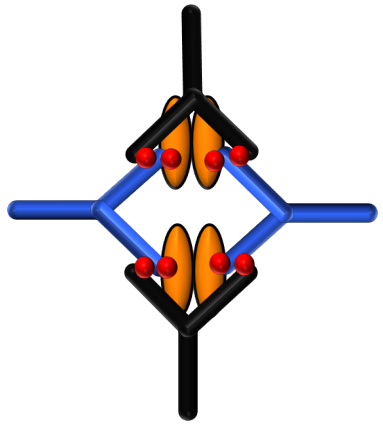
Explanation might be:

At low concentration stable complexes of dimer soluble target with res drug and pos. control are formed which were not detected. Over night incubation does not dissociate complexes

Disruption of complexes bulid up with soluble target

… self contained stable complexes with theoretical 2:2:2 structure

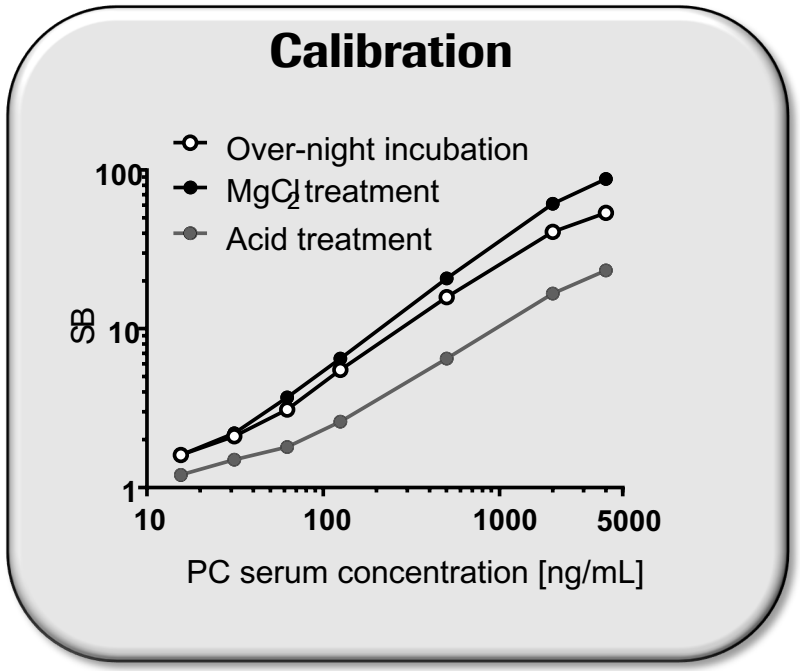


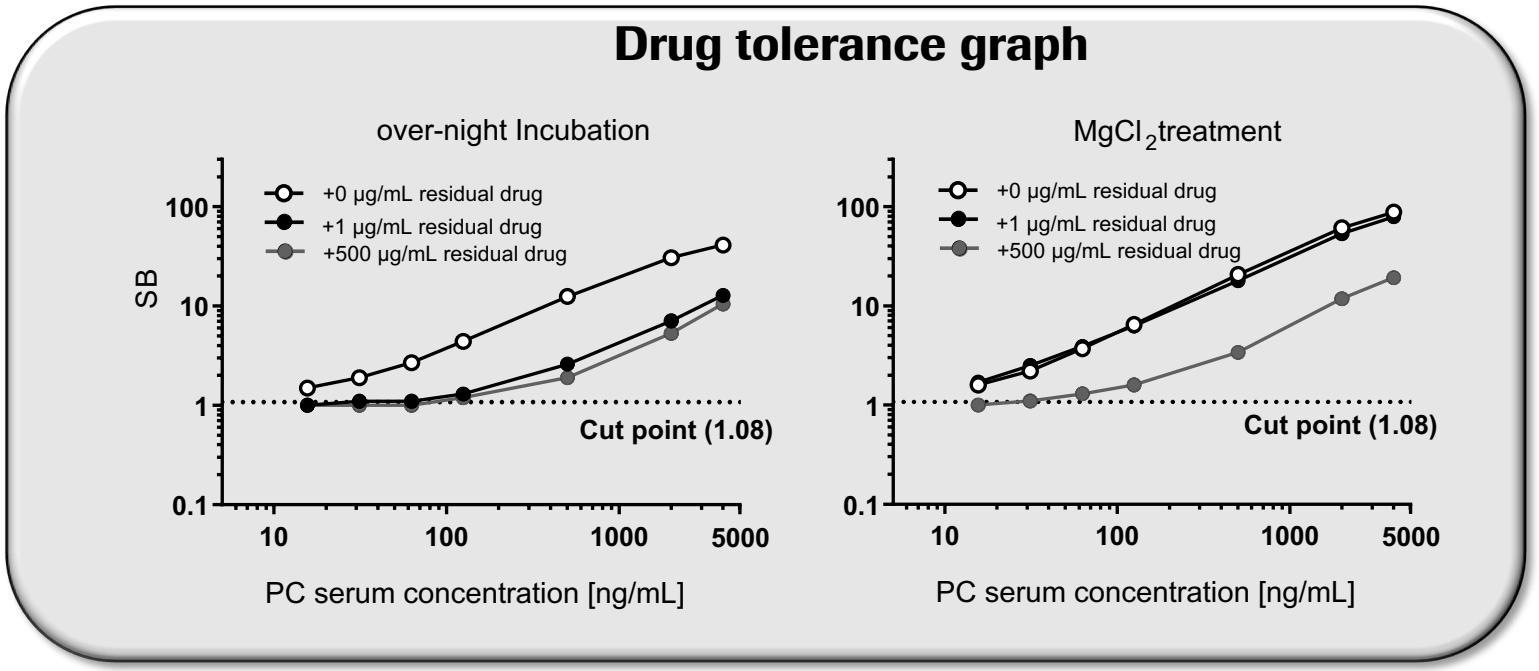


- Soluble target in the low nM range
- Drug in light excess to soluble target
- Stable “hexamer complexes”

Case example 1

…improved drug tolerance with HISDA compared to overnight incubation



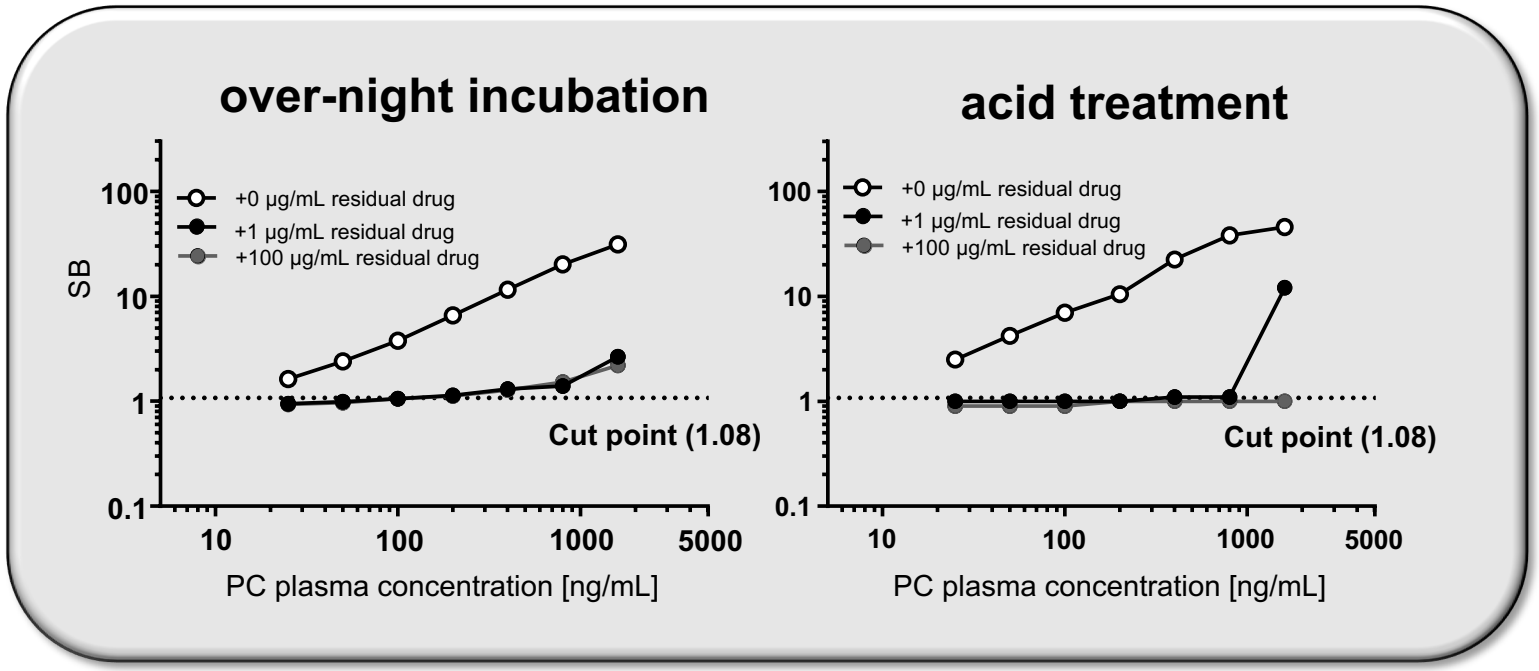


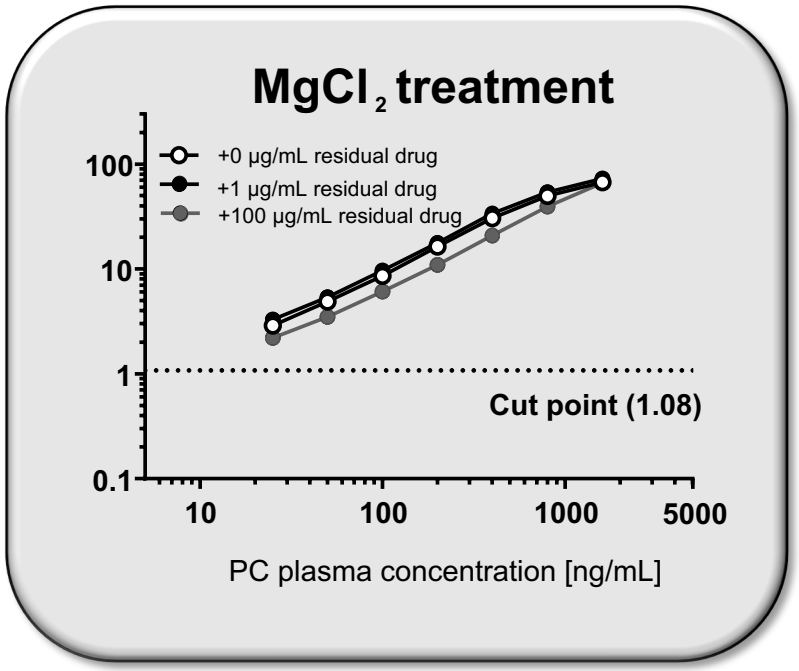
- **HISDA comaprable to over night incubation**
- **Acid treatment seems to negively affect the pos. Control**
- **Improved drug tolerance**

Jordan et al. High ionic strength dissociation assay (HISDA) for high drug tolerant immunogenicity testing, Bioanalysis 2020

Case example 2

…acid pre treatment has a negative impact on the positive control





- **Acid treatment seems to negively affect the pos. control**
- **Improved drug tolerance with HISDA**

Jordan et al. High ionic strength dissociation assay (HISDA) for high drug tolerant immunogenicity testing, Bioanalysis 2020

Summary and conclusion

- High ionic strength dissociation assay
  - Drug tolerance
    - ✓ **Drug tolerance factor of 1000** and more can be achieved
    - ✓ **Potential soluble target interference** (dimer target) by forming even more stable complexes can be prevented
  - **MRD of 100** advantegous for high drug tolerance whereby reducing or rising the dilution shows no improvement
  - Rather simple assay protocol wich makes routine analysis robust
- Kinetic of complexes
  - It might take several hours to reach new equlibrium for maximum signal intesity
- HISDA shows in two case examples improved drug tolerance compared to acid treatment or over night incubation

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Doing now what patients need next