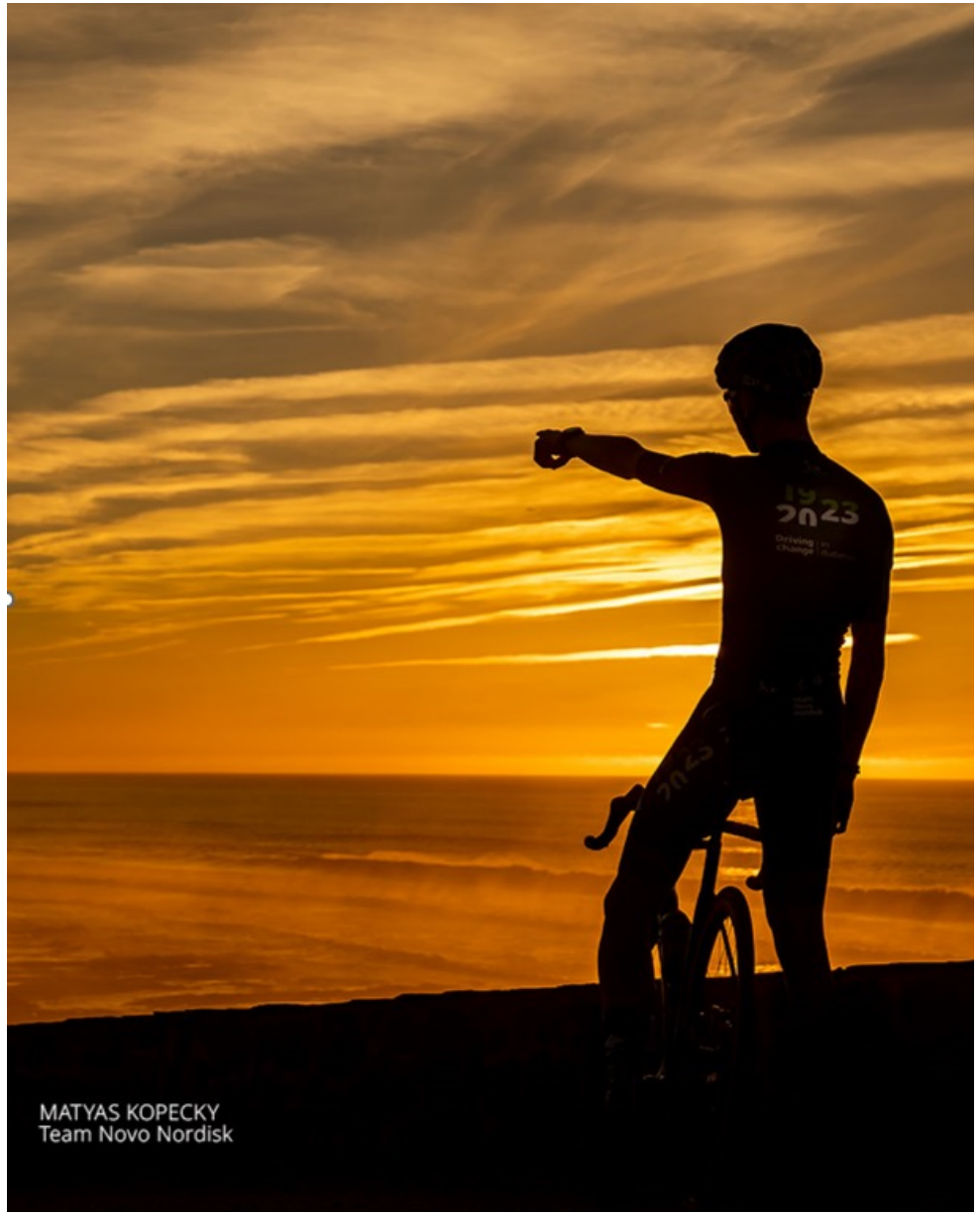


Re-thinking Radioimmunoassay for the detection of anti-drug antibodies against peptide drugs

EBF Autumn Workshop: Challenging Current ADA Analysis Paradigm
Malaga, 21 Sep 2023

Luis C Perez Tosar, Novo Nordisk

1. **Radio-immunoassay (RIA) as ADA platform**

2. **Case studies (validation & sample analysis campaigns)**:

- *Duplicate vs Singlicate*
- *Is Confirmatory Tier needed?*
- *S/N vs Titer as indicator of ADA magnitude*
- *Time & cost saving estimates (sample analysis)*

3. **Reflection points / concluding remarks**

Inspiration from: *Bioanalysis (2021): 13(14), 1123 – 1134 & The AAPS Journal (2022): 24 (81)*

ADA Analysis – Tiered Assay Approach

1st Test: Screening (5% FPR)

≥cut point

< cut point

Binding antibody negative

2nd Test: Confirmation (1% FPR)

< cut point

≥cut point

Binding antibody positive

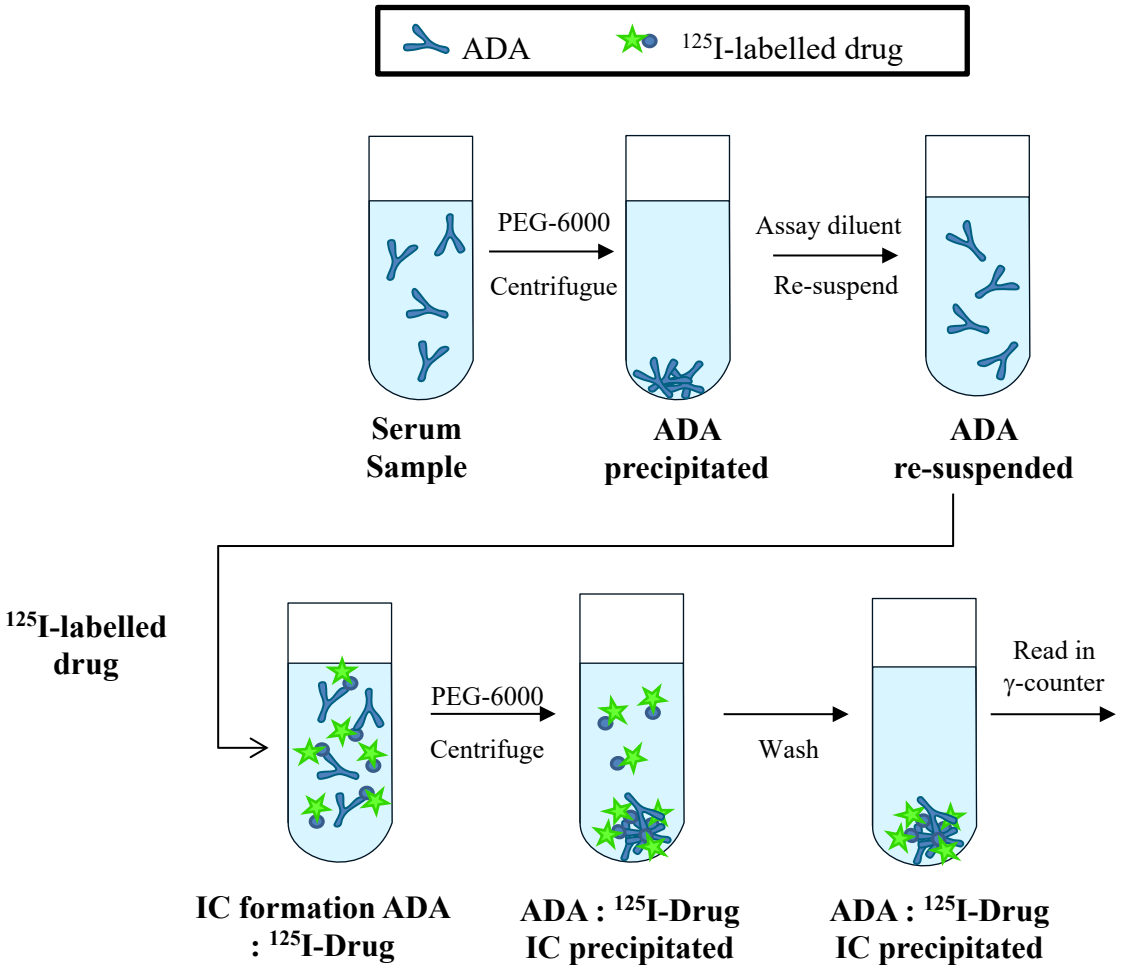
Characterisation
- Titre

Can we simplify this?

Singlicate *vs* duplicate analysis

"FPR": False-positivity Rate

ADA by RIA using PEG-Precipitation



Results expressed as %B/T

"ADA": anti-Drrug Antibody *"PEG": Poly-ethylene Glycol* *"IC": Immune complexes* *"%B/T": Percent bound over total radioactivity*

Singlicate *vs* Duplicate Analysis

Singlicate *vs* Duplicate (Validation)

Retrospective data re-analysis

- Cut points
- Validation parameters
  - Sensitivity and LoQC
  - QC samples Precision & Precision of titers
  - Selectivity

Brief Snapshot

- **2392 experimental points from 37 runs** (RIAs are run in tubes)
- 44 - 116 experimental points per run (88 -232 singlicates)
- **Only 8 experimental points (0,33%) had >20.0% CV**
- **Only 83 experimental points (3.5%) had %CV>10.0%**
- **3.4%CV on average** between duplicates **(all data)**

"QC": Control samples *"LoQC": Low control sample* *"CV": Coefficient of Variation*

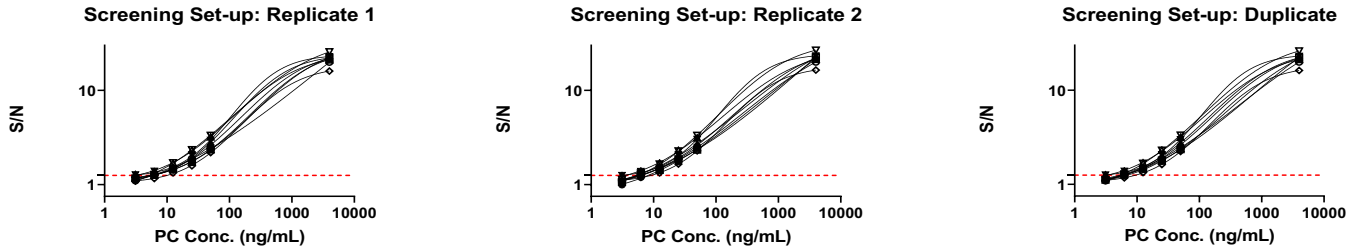
Singlicate *vs* Duplicate: Cut-Points' Determination

	Duplicate	Singlicate 1	Singlicate 2
Screening Set-up			
SCPF (5.0% FPR)	**1.095**	**1.108**	**1.112**
SCPF (1.0% FPR)	**1.147**	**1.172**	**1.173**
BOL	2	2	3
AOL	42	39	44
Confirmatory Set-up			
CCP (1.0% FPR)	**21.2**	**23.7**	**29.9**
BOL	3	3	2
AOL	58	52	38

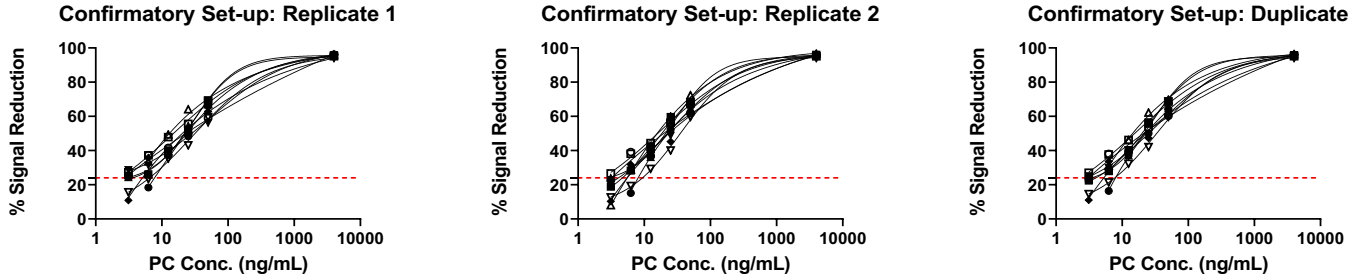
Would these differences in Cut-points have any relevance when analysing samples?

"SCPF": Screening Cut-Point Factor; *"FPR": False positivity rate;* *"BOL": Biological Outliers;* *"AOL": Analytical Outliers*
"CCP": Confirmatory Cut-Point

Singlicate *vs* Duplicate: Sensitivity, LoPC (9 PC curves)



4PL (1/y² weighting) curve fitting



Screening: Interpolated conc. (ng/mL); arbit. SCPF 1.25			
	Singlicate 1	Singlicate 2	Duplicate
Min.	2.48	3.03	2.85
Max.	9.99	9.02	9.50
AVG	**5.50**	**5.90**	**5.75**
StDEv	2.108	1.921	1.952
LoPC (99%CI)	**11.6**	**11.5**	**11.4**

Confirmation: Interpolated conc. (ng/mL); CCP 24.0%			
	Singlicate 1	Singlicate 2	Duplicate
Min.	1.45	2.76	2.84
Max.	6.50	9.38	7.93
AVG	**4.08**	**5.06**	**4.60**
StDEv	2.054	2.385	2.055
LoPC (99%CI)	**10.0**	**12.0**	**10.6**

To facilitate curve fitting, HiQC from the run included as point of the curve and 1.25 (S/N) Arbit. SCPF used to interpolate sensitivity from each run

Comparable sensitivity and LoPC estimation using singlicate(s) and duplicate analysis

"PC": Positive Control *"LoPC": Low positive control* *"arbit. SCPF": arbitrary Screening Cut-Point Factor* *"HiQC": High control sample*

Singlicate *vs* Duplicate: Titer (9 PC curves)

Titration: Interpolated Titer (dilution); arbit. SCPF 1.25			
	Singlicate 1	Singlicate 2	Duplicate
Min.	401	443	421
Max.	1612	1322	1405
AVG	**838**	**757**	**783**
StDEv	368.70	288.10	313.20
%CV	**44.0**	**38.1**	**40.0**

Titer and its precision similar w/ singlicate and duplicate analysis

"PC": Positive Control

"arbit. SCPF": arbitrary Screening Cut-Point Factor

Singlicate *vs* Duplicate: QC Precision

	Screening Set-up (Calculated on S/N)					
	Intra-Assay Precision (%CV)			Inter-Assay Precision (%CV)		
	LoQC	MeQC	HiQC	LoQC	MeQC	HiQC
Singlicate 1	1.7	2.2	2.6	3.3	6.7	5.1
Singlicate 2	1.5	2.0	2.2	3.5	6.0	4.7
Duplicate	1.2	1.7	1.9	2.4	4.6	3.7

	Confirmatory Set-up (Calculated on %Inhibition)					
	Intra-Assay Precision (%CV)			Inter-Assay Precision (%CV)		
	LoQC	MeQC	HiQC	LoQC	MeQC	HiQC
Singlicate 1	0.3	0.5	2.7	0.6	0.9	4.4
Singlicate 2	0.2	0.6	4.1	0.5	1.1	5.7
Duplicate	0.3	0.5	3.0	0.5	0.9	4.5

Comparable QC Precision Results observed using singlicate or duplicate analysis

”LoQC”, ”MeQC”, ”HiQC”: Low, Medium and High control samples.

Singlicate *vs* Duplicate: Selectivity

- **(N=10 individual matrixes)**

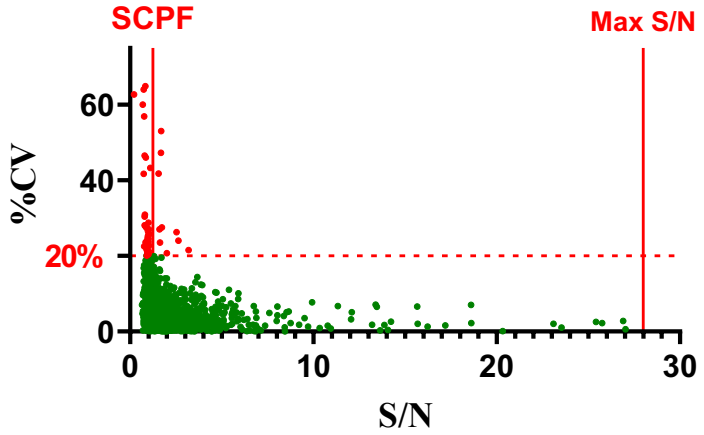
	Negative (Un-spiked)		Spiked w/ LoQC		Spiked w/ HiQC	
	Scr.	**Conf.**	**Scr.**	**Conf.**	**Scr.**	**Conf.**
Singlicate 1	10/10 Neg	10/10 Neg	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**
Precision accross individuals =>			**2.22%**	**5.46%**	**1.34%**	**0.36%**
Singlicate 2	8/10 Neg	10/10 Neg	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**
Precision accross individuals =>			**2.18%**	**4.60%**	**1.30%**	**0.40%**
Duplicate	9/10 Neg	10/10 Neg	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**	**10/10 Pos**
Precision accross individuals =>			**2.20%**	**4.39%**	**1.19%**	**0.33%**

”LoQC”, ”HiQC”: Low and High control samples. *”Scr.”: Screening* *”Conf.”: Confirmation* *”Pos”: Positive*

Singlicate *vs* Duplicate (Sample Analysis)

Phase II ADA sample analysis campaign

- **7975 experimental points from 142 runs**
- On average, 56 experimental points (112 singligates) per run
- **Only 63 experimental points (0.79%) had >20.0%CV**
- **831 experimental points (10.4%) had %CV>10.0%**
- **4.9%CV on average** between duplicates **(all data)**



%CV>20 were at around, or below SCPF

"SCPF": Screening Cut-Point Factor

Can ADA RIA be run in Singlicate analysis?

- Parameters obtained from a validation data set (2400 datapoints) showed equivalent results with singlicate and duplicate analysis

- Decriptive %CV results from a sample analysis data set showed very favourable performance in terms of variability (approx. 8000 datapoints)

SINGLICATE ANALYSIS IS A VIABLE OPTION FOR ADA BY RADIOIMMUNOASSAY

- Continue to build a retrospective knowledge-base on legacy assays
  - Re-interrogation of datasets to compare Singlicate *vs* Duplicate performance
- Approach development of new assays using singlicate analysis
  - No reason why it would perform differently

Value of Confirmatory Set-Up

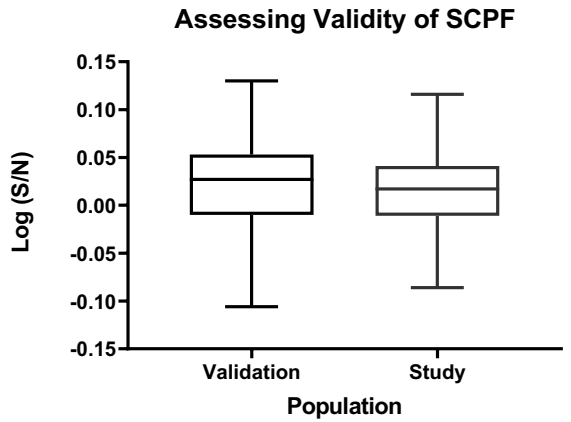
Titer *vs* S/N as expression of ADA magnitude

Case Study: ADA Analysis Campaign; Dose-finding study.

- **Data Set re-analysed:**
  - Value of Confirmatory Set-up
    - Verification of FPR in pre-treatment samples
    - Incidence of TE-ADA
  - Is S/N equivalent to Titer as a surrogate of ADA magnitude?
    - Correlation between S/N and Titer
    - Correlation between PK and ADA magnitude
    - ADA kinetics and pre-existing ADA

Is the confirmatory tier needed? Pre-treatment samples

Pre-treatment Positives using different Cut-Points (Validation)			
	SCPF: 1.245 (5% FPR)	**CCP: 20.6% (1% FPR)**	**SCPF 1.338 (1% FPR)**
Pre-Treatment samples (N = 495)	25	**7**	**12**
FPR observed	**3.7%**		**1.03%**



"SCPF": Screening Cut-Point Factor *"CCP": Confirmatory Cut-Point* *"FPR": False Positivity Rate*

Is the confirmatory tier needed? Treatment-Emergent ADA

TE-positive samples using different Cut-Points			
	SCPF: 1.245 (5% FPR)	CCP: 20.6% (1% FPR)	SCPF: 1.338 (1% FPR)
Post-treatment (N = 2365)	988 "Potentially +ve"	843 Confirmed +ve	868 Positive
% positive	41.8%	35.6%	36.7%

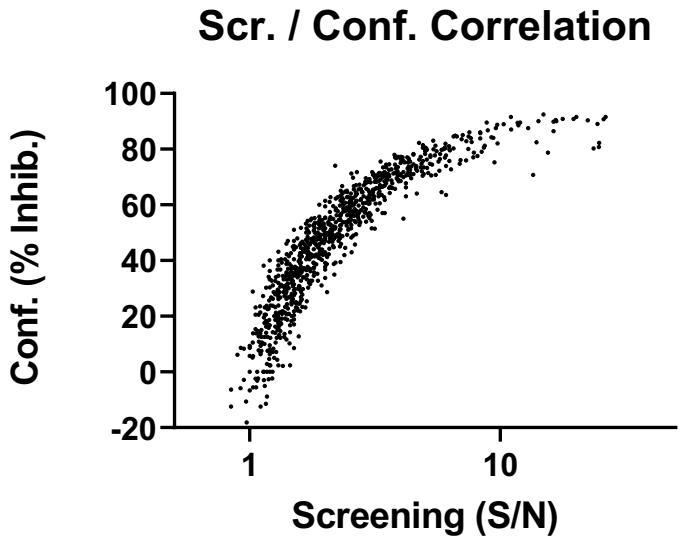
Negligible and, most likely, irrelevant differences in positivity outcome

"TE": Treatment-emergent *"SCPF": Screening Cut-Point Factor* *"CCP": Confirmatory Cut-Point* *"FPR": False Positivity Rate*

Is the confirmatory tier needed?

Very often the <u>Confirmatory Tier is redundant</u>:

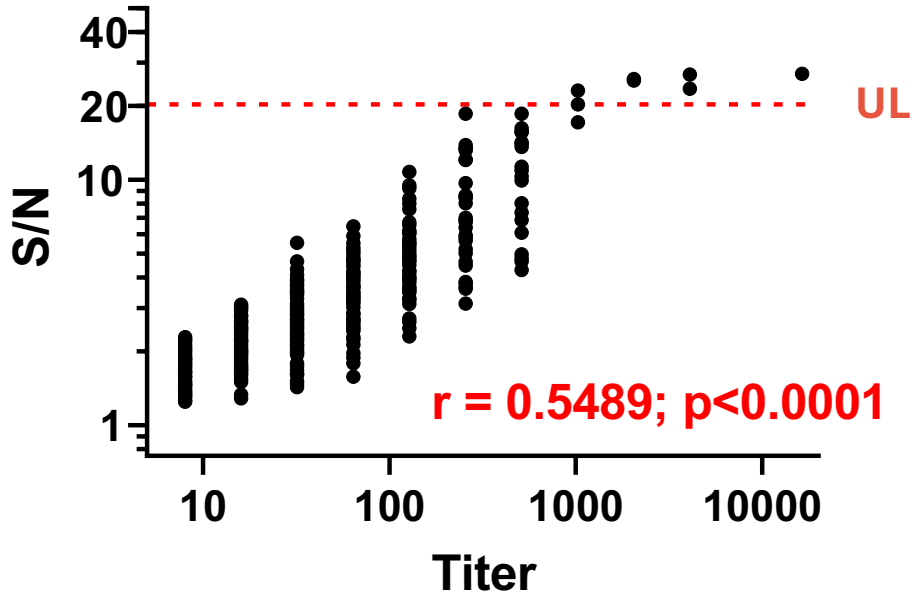
- if correlation between Screening and Confirmation is good



"S/N": Signal to Noise *"%Inhib.": Percentage Signal Inhibition*

S/N *vs* Titer to express ADA magnitude

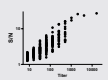
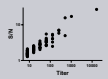
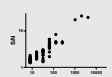
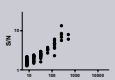
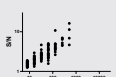
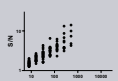
Correlation between S/N & Titer
All ADA-positive samples (N=843)



- moderate, yet highly significant correlation between S/N and Titer
- Only 6 samples at the S/N plateau

"S/N": Signal to Noise

Correlation PK (dose-normalised) *vs* ADA magnitude. ADA +ve samples w/ non-0 PK value (N=574)

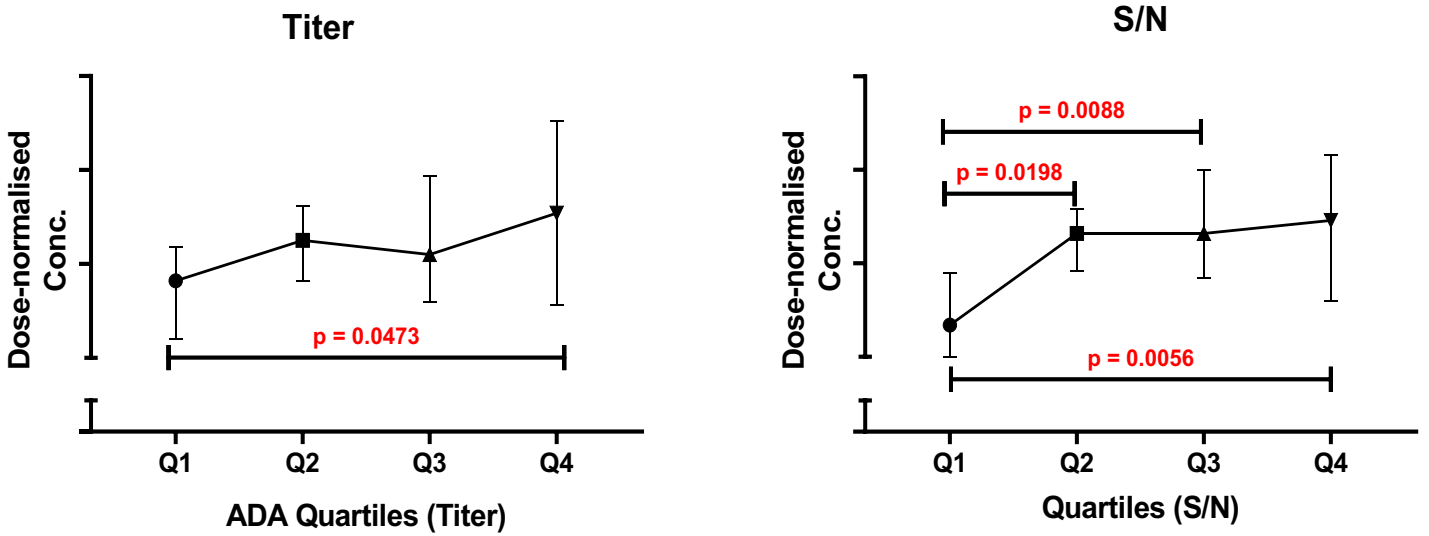
ADA +ve Data set	N	Pearson coefficient (r). Correlation between:		
		S/N *vs* Titer	PK *vs* Titer	PK *vs* S/N
All samples	574	*0.5830 (p<0.0001)* 	-0.03061 (ns)	-0.00995 (ns)
Dose 1	93	*0.7682 (p<0.0001)* 	-0.06567 (ns)	-0.03720 (ns)
Dose 2	96	*0.8617 (p<0.0001)* 	-0.06186 (ns)	-0.08509 (ns)
Dose 3	92	*0.8203 (p<0.0001)* 	*0.4674 (p<0.0001)*	*0.2732 (p=0.0084)*
Dose 4	144	*0.8187 (p<0.0001)* 	-0.03683 (ns)	-0.09342 (ns)
Dose 5	149	*0.7683 (p<0.0001)* 	*0.2553 (p=0.0017)*	0.1525 (ns)

Similar pattern observed with S/N & Titer

- **Titer showed significant correlation in one more group compared to S/N**

"PK": Pharmacokinetics (exposure) *"+ve": Positive* *"S/N": Signal to Noise*

Differences of PK among ADA Quartiles (Titer or S/N)

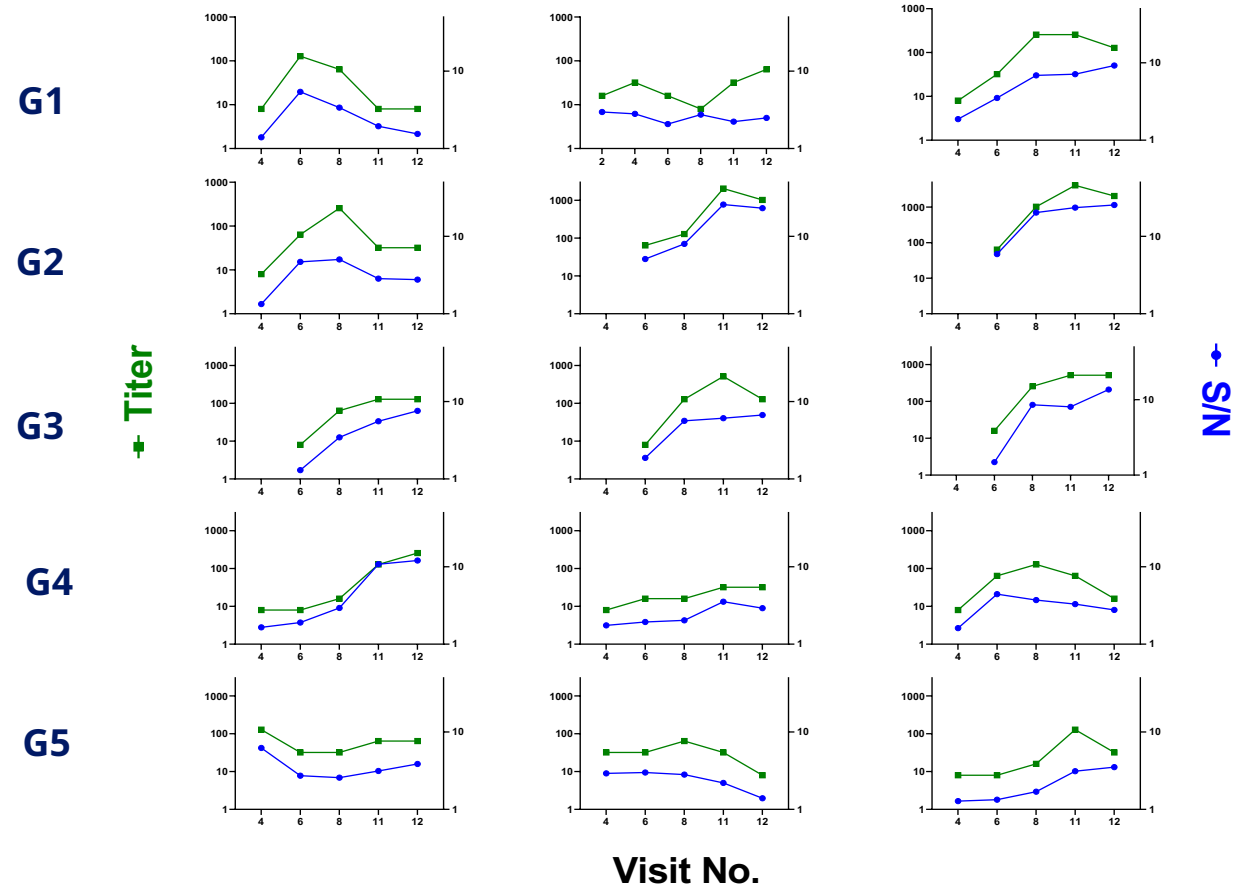


Median +/- 95%CI. Data processed by ANOVA with Kruskal-Wallis test for multiple comparisons

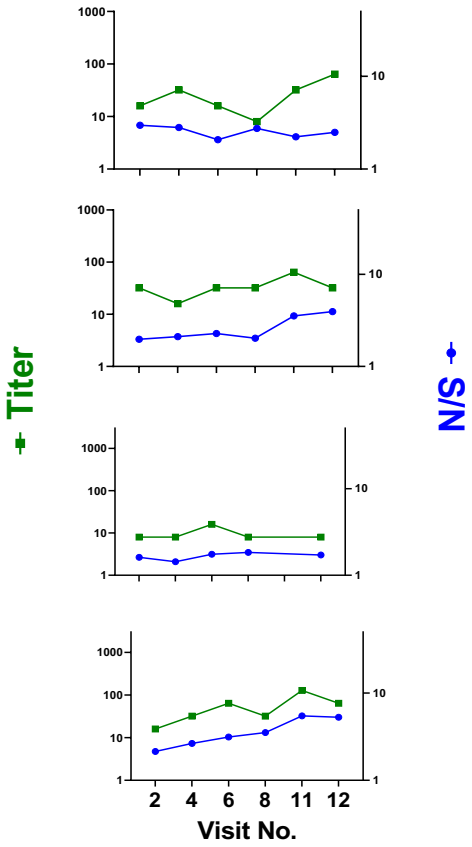
- Similar trend of increasing drug through conc. as ADA magnitude increased over quartiles
  - Significant increase between 1st ad 4th quartiles using both (Titer and S/N)
    - S/N also showed differences between 1st agd 2nd / 3rd quartiles

"PK": Pharmacokinetics (exposure) *"CI": Confidence Interval* *"ANOVA": Analysis of Variance* *"S/N": Signal to Noise*

ADA Kinetics and pre-existing ADA (S/N *vs* Titer)



Subjects with at least 4 time points with a +ve sample



Subjects with pre-existing ADA

Similar overall trend in ADA kinetics using both expressions of ADA magnitude

Can S/N replace Titer as expression of ADA magnitude?

- Case by case considerations are needed
- In our case:
  - Highly significant correlation between S/N and Titer
  - Very low number of samples in the S/N plateau
  - Overall, similar correlation trends between PK and S/N or Titer; however, subttle differences observed, if further analysis performed on data sub-sets
  - Overall, both expressions of ADA magnitud showed similar pattern of ADA kinetics

Time & Cost Saving Simulations

Considerations / assumptions

- Singlicate Analysis
- Runs remain the same size in terms of tubes per run (analysis of 2X more samples)
- Same price per sample
- Titration uses, on average, 5-point dilution series per sample

Only 134 runs used for this simulation (non-hybrid runs)

	Runs / (Time saving)	Cost saving estimates
All Tiers	67 / (50.0%)	Only on reagents
No Conf. (Scr. / Cross-react. / Titer)	53 / (60.4%)	22.0%
No Titer (Scr. / Conf. / Cross-react.)	47 / (65.0%)	38.7%
No Conf. / No Titer (Scr. / Cross-react.)	33 / (75.4%)	56.6%

"Scr": Screening *"Cross-react.": Cross reactivity*

Concluding Remarks & Reflection Points

- Can ADA by RIA be run in singlicate?
- Can we ommit the Confirmatory Set-up?
- Is S/N a viable surrogate of ADA magnitude to replace Titer?

YES

Considerations

- **Regulatory Authorities**: Engage them early and have convincing packages
- **Company Stakeholders (Medical, Regulatory, etc)**: Initiate an educational journey

- **Bio-Analytical & Immunogenicity Scientists**
  - You are on charge and you know well your assay(s)
  - Take balanced risks and fear not
  - Start early. Full implementation in Ph. 3 (maximum benefits)
  - Implications for Software packages / Scripts / Systems / Data processing sheets

Acknowledgements

All my colleagues in IA at NN NCAS

Thank you for your Attention

Questions?


novo nordisk®