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EIP 16th Open Symposium Workshop Day

CHALLENGING THE CURRENT PARADIGMS FOR CLINICAL IMMUNOGENICITY TESTING — THE PROS AND CONS

History

- The clinical immunogenicity testing strategy as described in current guidelines was established almost two decades ago
- In recent years, several components of this immunogenicity testing paradigm have been heavily challenged:
  - Signal to Noise (S/N) instead of titer to quasi-quantify ADA responses
  - Singlicate instead of duplicate analysis
  - Omitting the confirmatory assay (2-tiered approach)
  - Need for a drug tolerant assay
- Although questioning habits generally is a driver of innovation, some of these proposals seem to be rather workload reduction- than science driven

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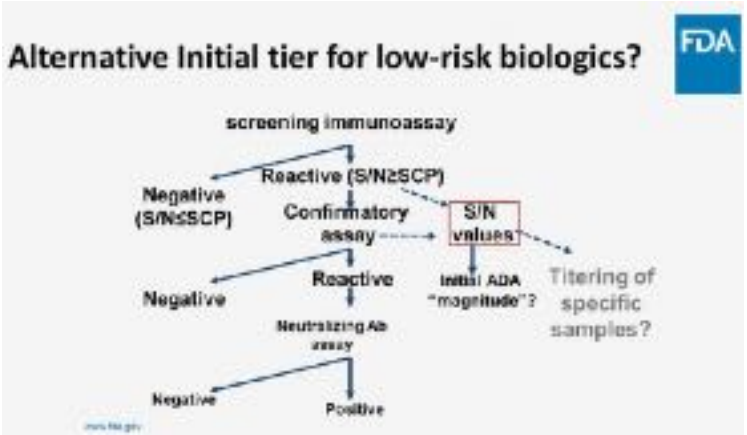
S/N VS TITER

S/N vs Titer – Regulatory Perspective

- Alternative methods of ADA quantitation besides titer may be used:
- 2019 Immunogenicity Guidance:
  - "Several approaches may be used to report positive antibody responses, and the appropriateness of the approach used should be evaluated on a **case-by-case basis**."
- Early-stage Development:
  - Useful for sample semi-quantitation in early-stage development, prior to development of titer assays
- Late-stage Development:
  - Alternative initial tier approach for low immunogenicity risk products
  - Sponsors should provide a justification for choice of S/N in the dossier
    - S/N and titer development data
    - Early clinical study data correlating the effect of ADA on PK using both S/N and titer
    - Establish S/N criteria for assigning study samples as treatment-boosted ADAs



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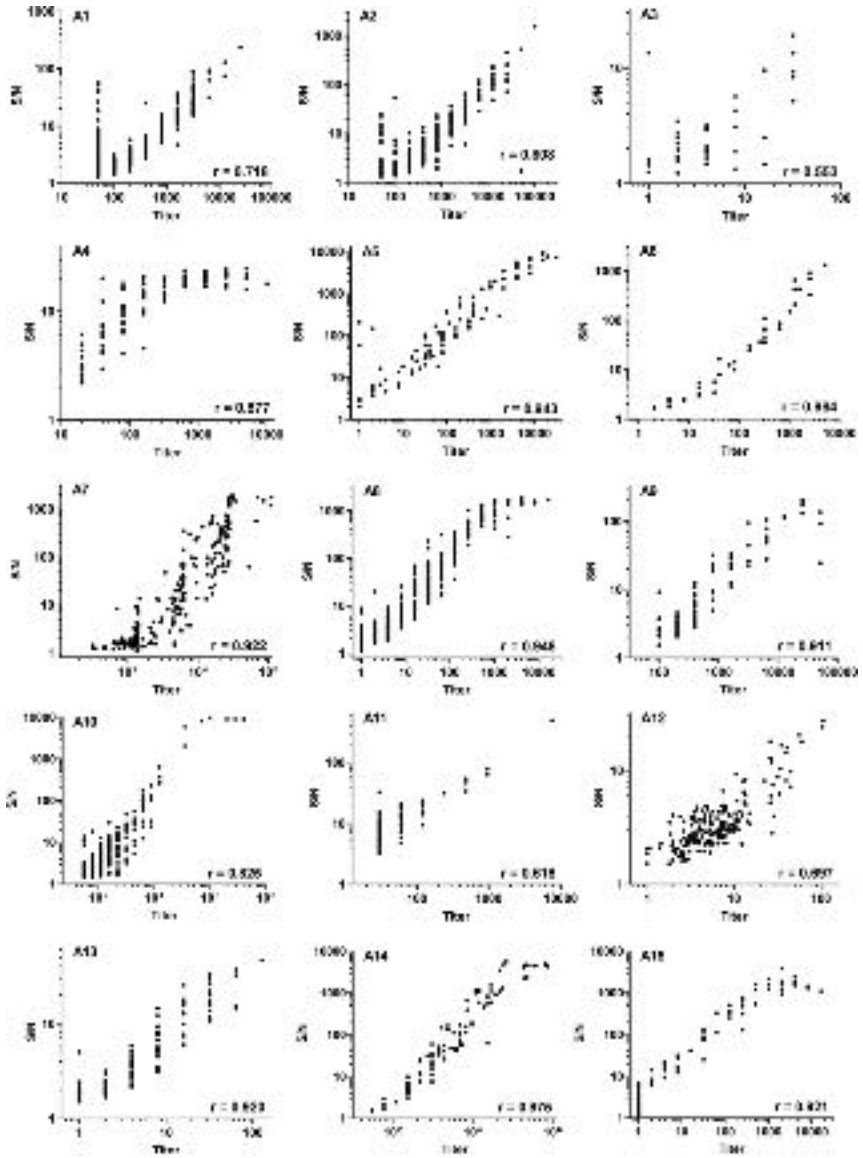
S/N vs Titer Overall Correlation – Published Data



Starcevic Manning M et al, APS J. 2022 Jul 12;24(4):81

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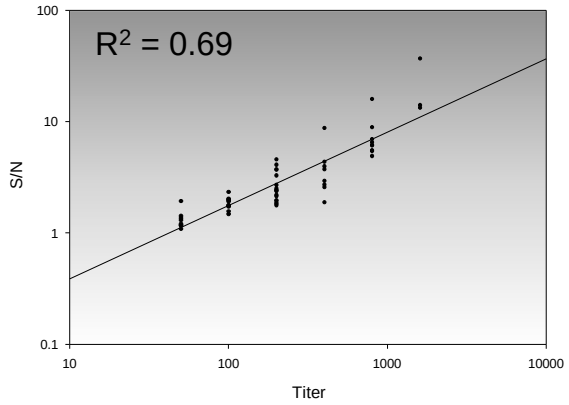
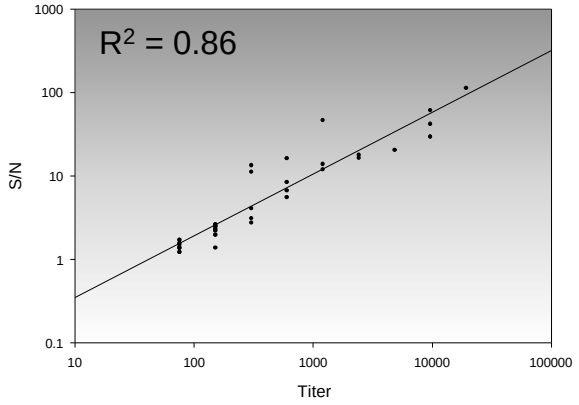
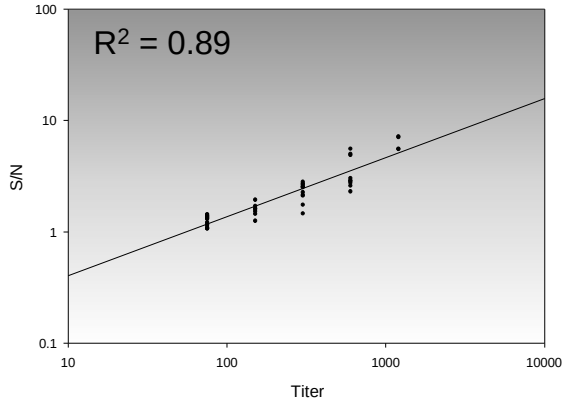
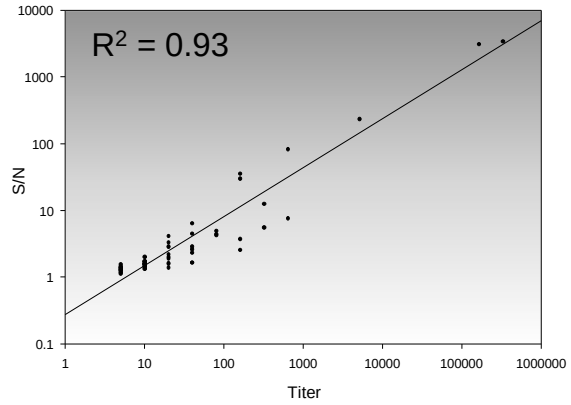
S/N vs Titer Overall Correlation – Internal Data

- Good overall correlation of S/N to titer





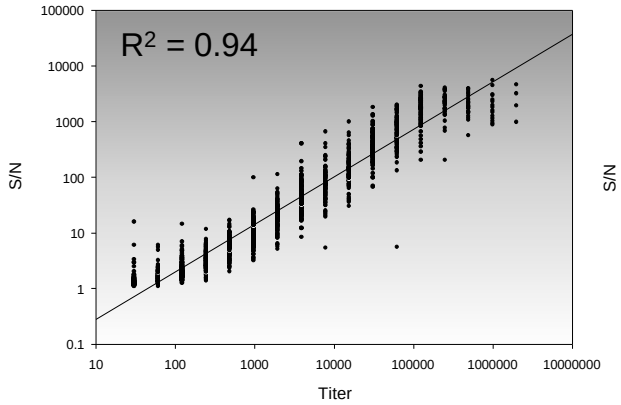


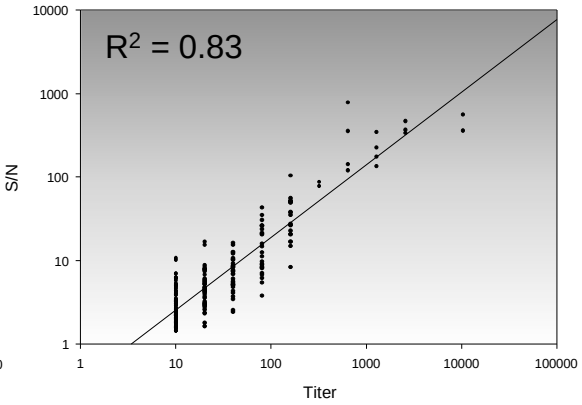


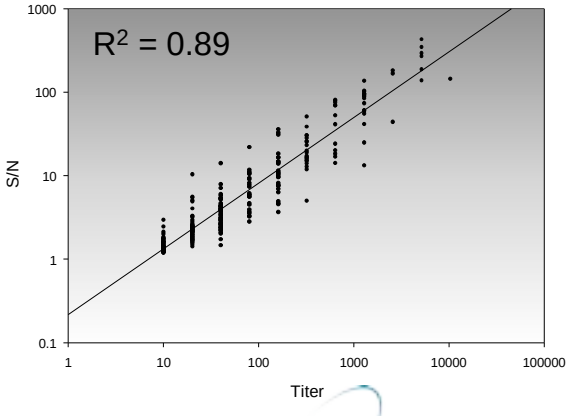
Modalities:

- Polypeptide
- Multi-specific Nanobodies®
- Monoclonal antibody
- NK-cell engager
- Fusion protein

Phase 1 – Phase 3







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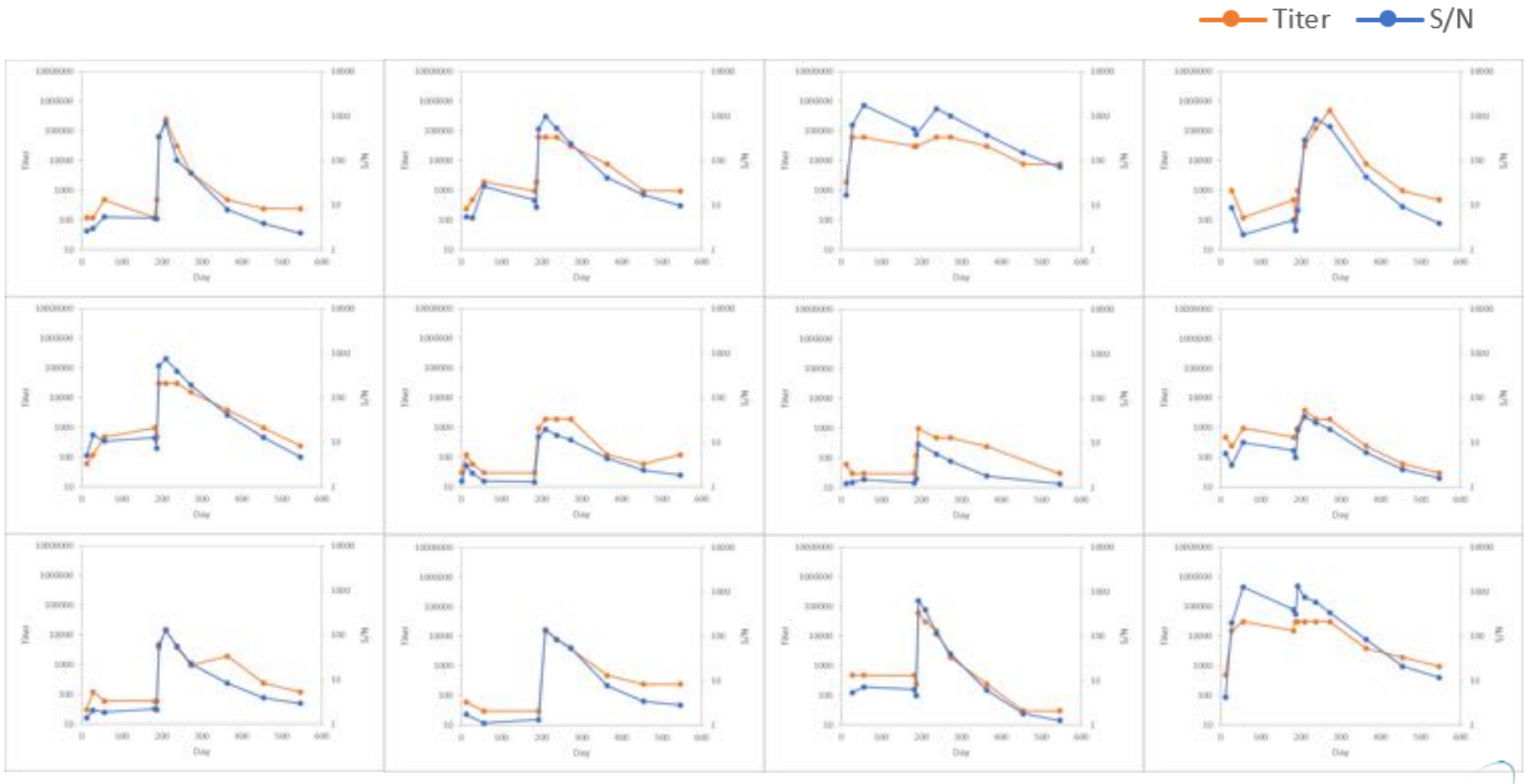
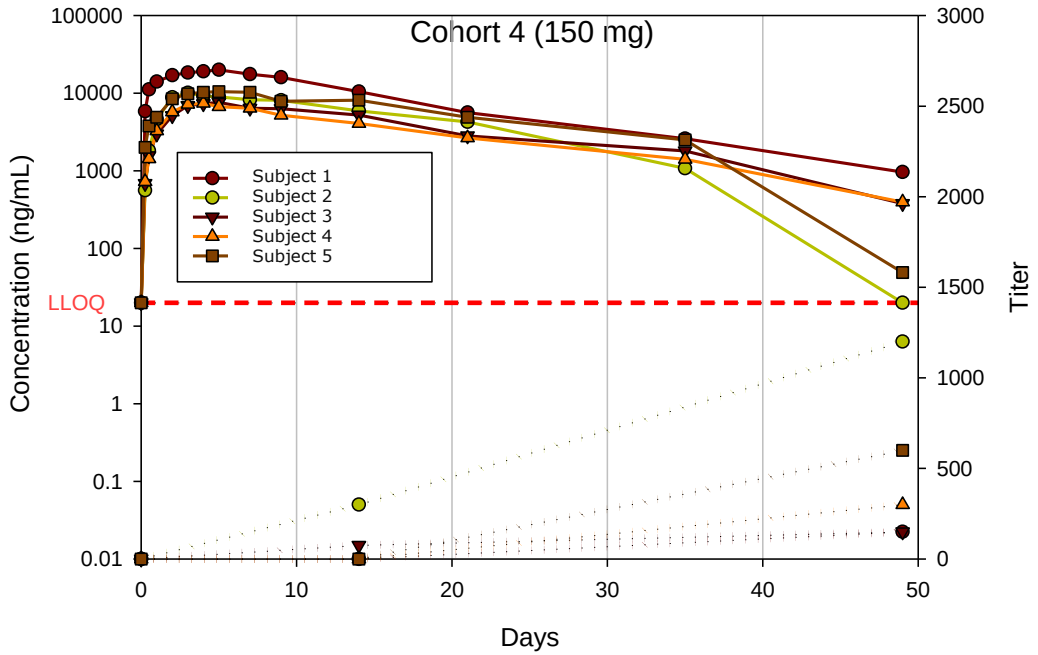
S/N vs Titer Individual Profiles – Internal Data

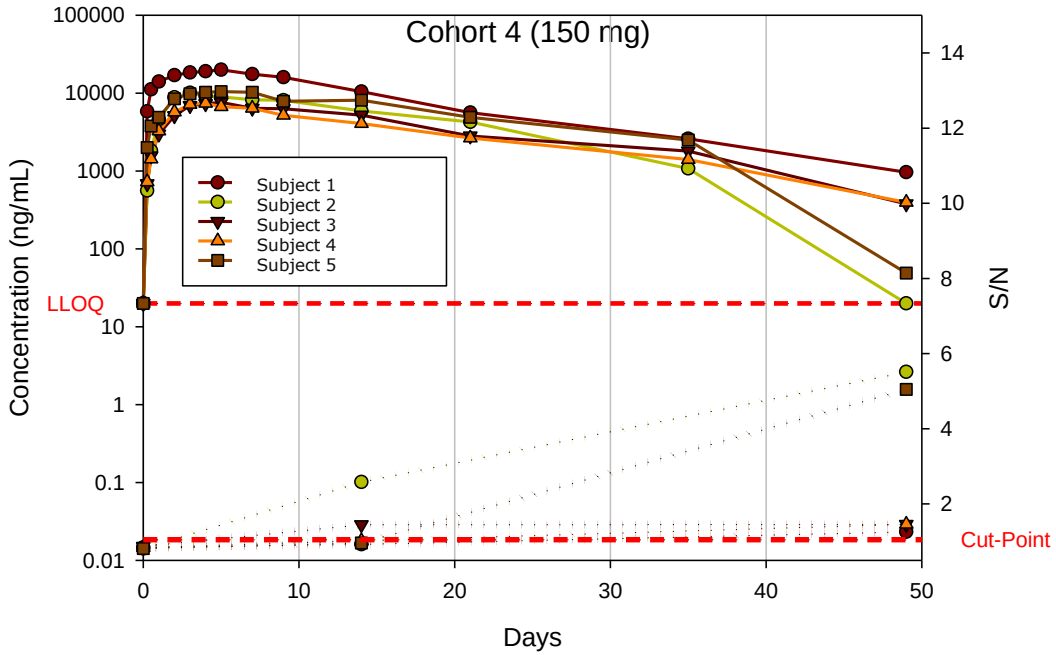


S/N vs Titer – Impact on PK

Titer



S/N



Solid line: PK
Dotted line: ADA

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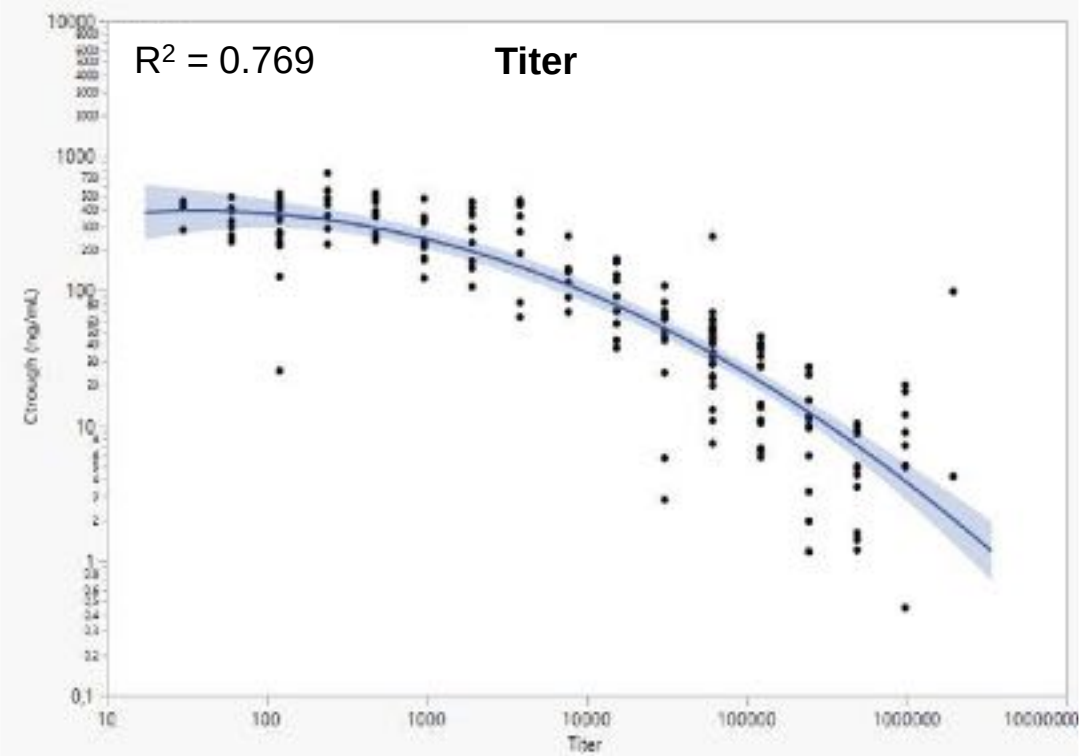
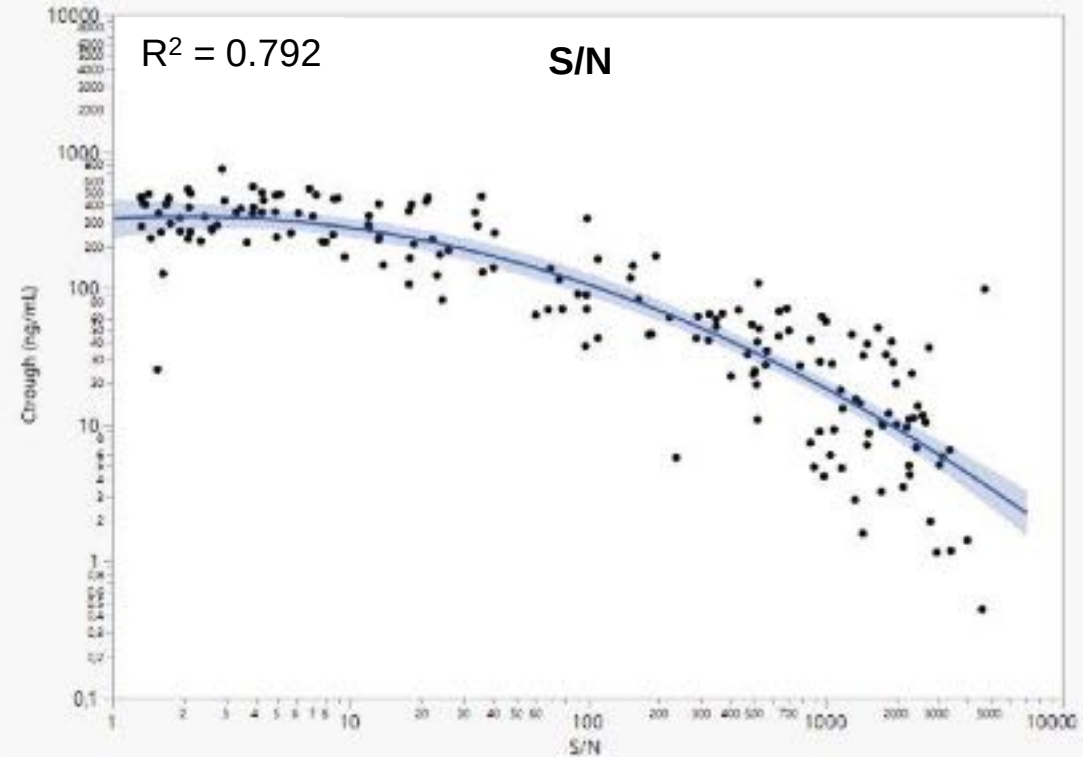
S/N vs Titer – Impact on PK





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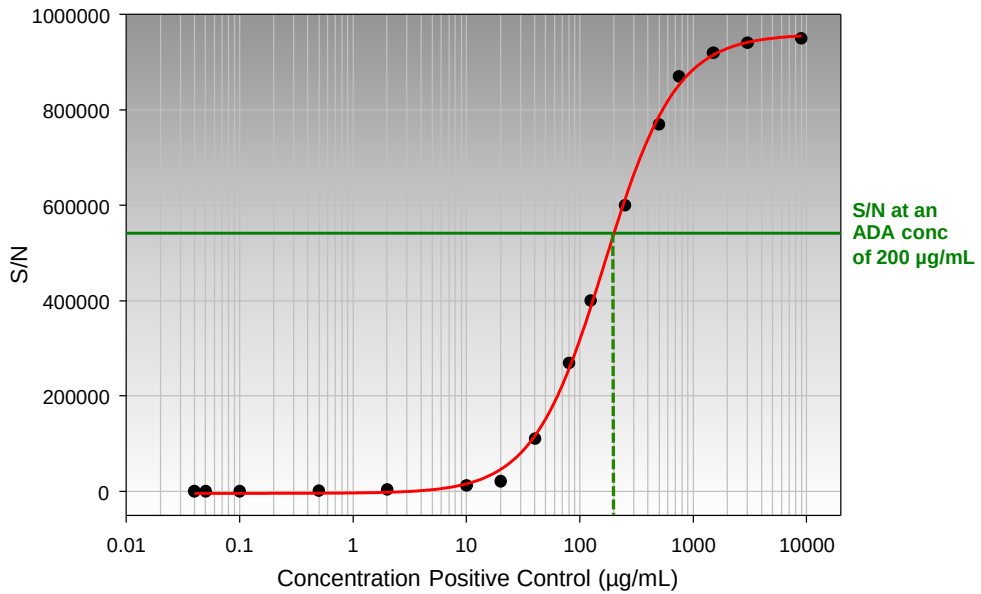
S/N vs Titer – Limitations: Dynamic Range

- S/N data (in contrast to titer values) have the intrinsic disadvantage to plateau at high signals
  - This is frequently observed with colorimetric read-outs (such as ELISAs) due to their limited dynamic range, but could also be seen with ECL assays (if reagents become limited)
  - This can be evaluated during assay validation using high concentrations of the positive control
    - Based on internal and literature data, it is extremely unlikely that ADA responses will show concentrations > 200 µg/mL
    - S/N is deemed feasible if the ADA assay is still in its dynamic range (not plateauing) at an antibody concentration of 200 µg/mL



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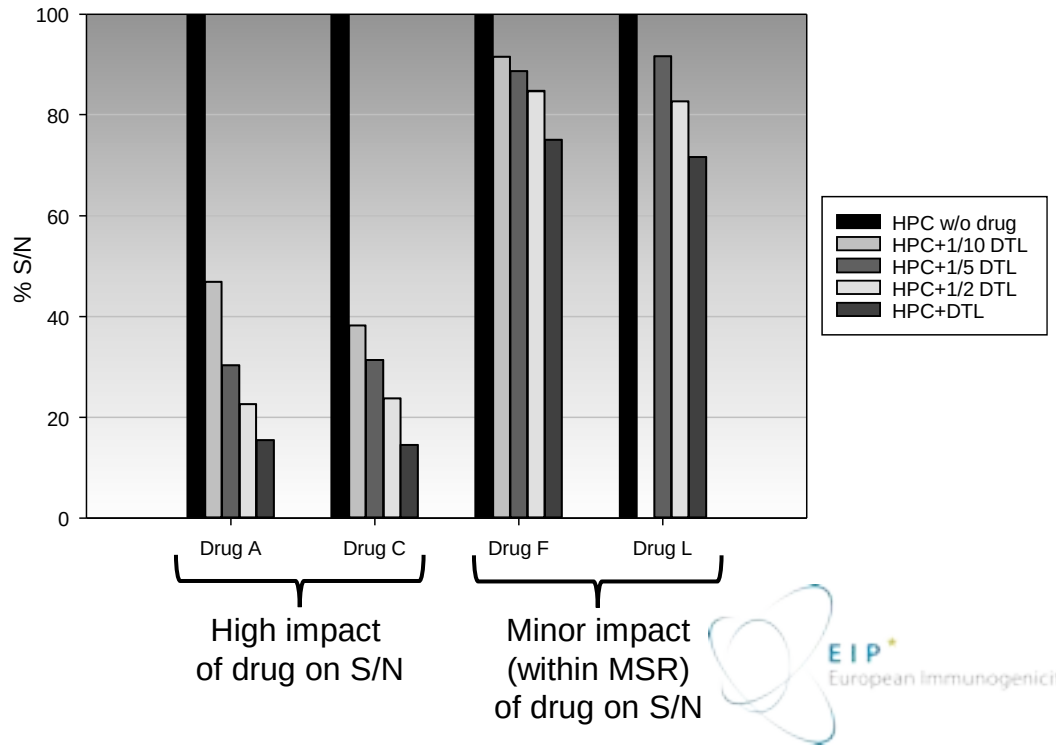
S/N vs Titer – Limitations: Drug Interference

- A more pronounced impact of residual drug on S/N compared to titer was observed for several Sanofi projects
  - Seems to differ from assay to assay
  - Reliable quasi-quantification of ADA results will be impaired
- This can be tested during assay validation by spiking the high positive control (HPC) with increasing amounts of drug up to the drug tolerance limit (DTL) of the ADA assay
  - S/N can be used for quasi-quantification if the S/N of the HPC spiked with the DTL is still within its minimum significant ratio (MSR)

HPC	Titer	S/N
w/o drug	960	20.1
+ 50 µg/mL drug	960	6.2
+ 150 µg/mL drug	1920	2.4



$$\mathbf{MSR} = 10^{\mathbf{t(0.05,n-1)}*\sqrt{2}*\mathbf{SD}}$$

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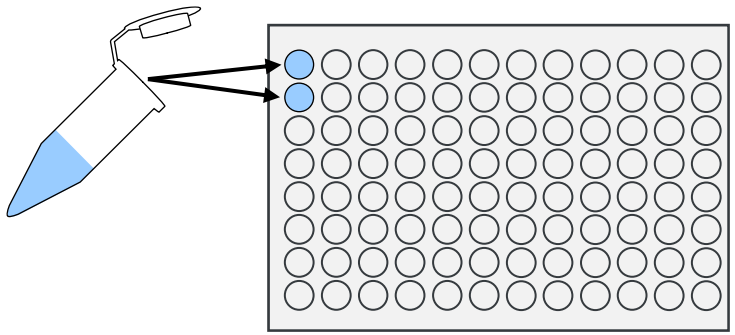
S/N vs Titer - Summary

- Regulatory acceptance of S/N in early (non pivotal) clinical trials
  - Alternative initial tier approach for low immunogenicity risk products in late-stage development
- Good correlation between S/N and titer
- Impact of ADAs on PK can be retrieved with both S/N and titer
- Significant workload reduction
- Liabilities (can be tested/mitigated during assay validation):
  - Influence of an excess of drug
  - Assay saturation range

SINGLICATE ANALYSIS

Singlicate Analysis

- Regulatory perspective:
  - There is no immunogenicity guideline forcing companies to analyze samples and controls in duplicates
- Scientific perspective:
  - It is common practice not to use “real duplicates” (independent dilutions) but applying one dilution into two wells (technical replicate)
    - Limited added scientific value



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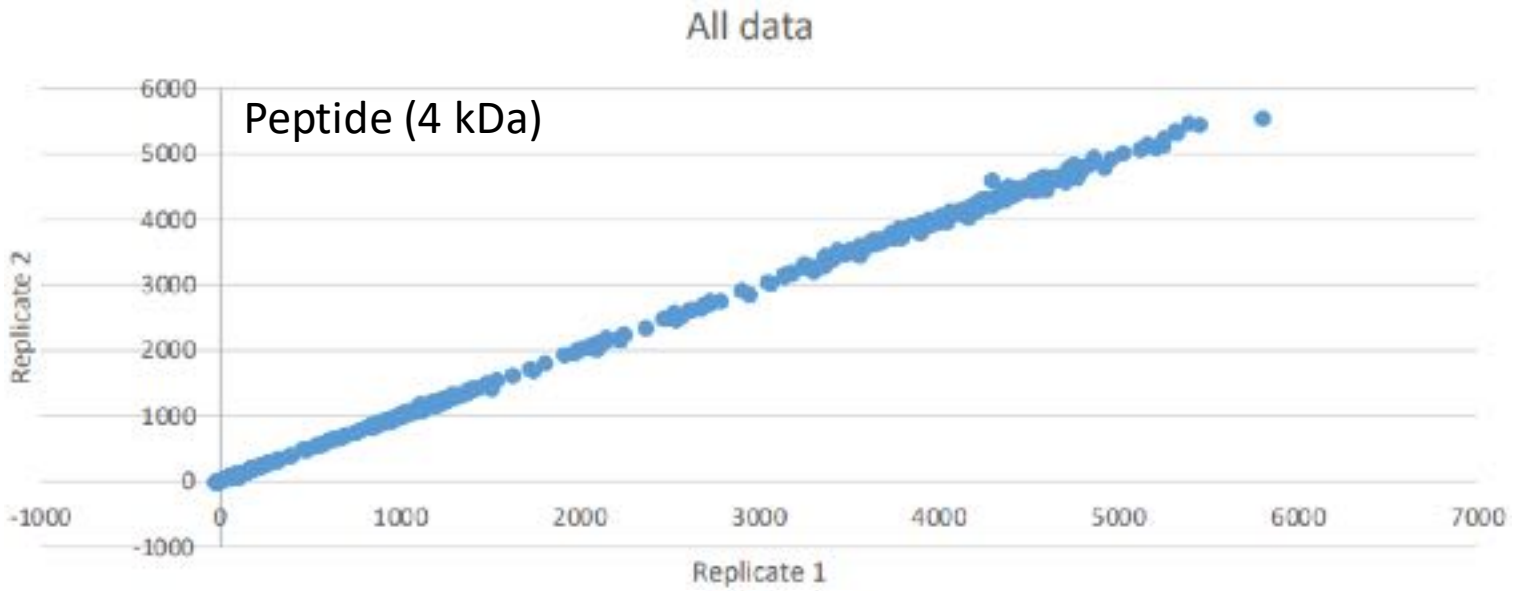
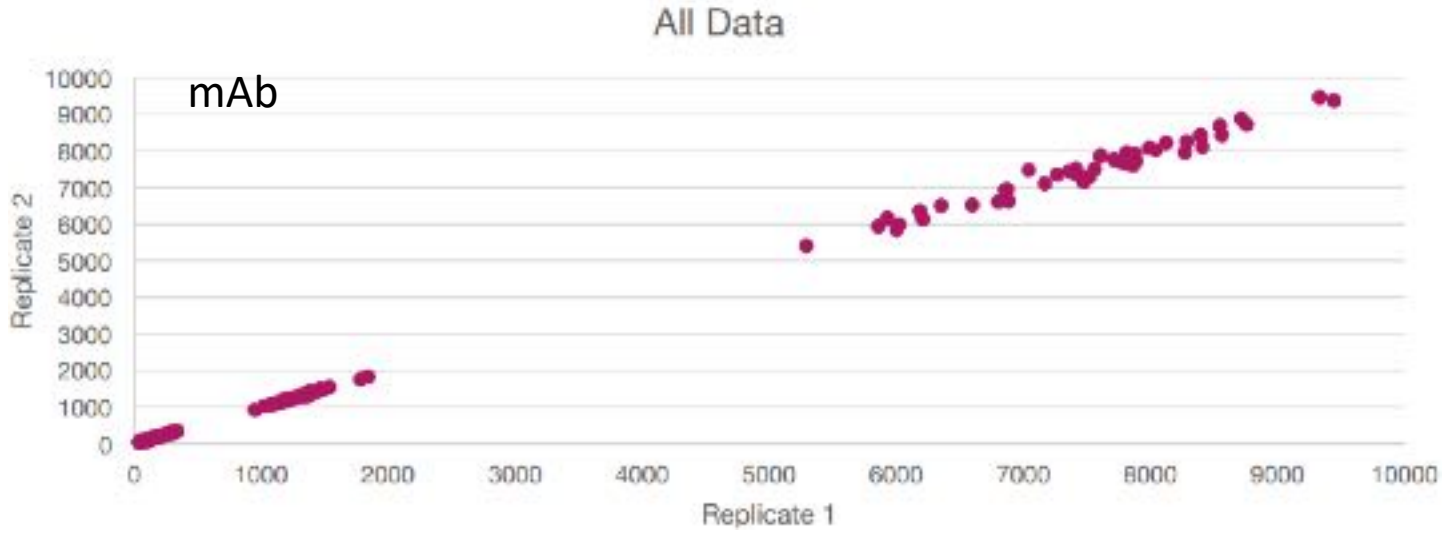
Singlicate Analysis – Examples (1)





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Singlicate Analysis – Examples (2)

- We internally evaluated validation data as those should be most sensitive to differences using singlicates vs duplicates
  - Cut-point statistics was performed individually for both singlicates

Example 1:

	Duplicates	Singlicate 1	Singlicate 2
SCP	**1.15**	**1.16**	**1.17**
CCP (% Inh.)	**23.5**	**29.3**	**25.1**
NC Upper Limit (counts)	**62.6**	**64.4**	**63.7**
Inter-assay Precision (CV %)	**3.4 – 8.6**	**4.3 – 8.9**	**4.9 – 8.7**
Free drug tolerance (ng/mL)	**< 50000**	**< 50000**	**< 50000**

Example 2:

	Duplicates	Singlicate 1	Singlicate 2
SCP	**1.34**	**1.36**	**1.39**
CCP (% Inh.)	**49.0**	**49.1**	**49. 7**
NC Upper Limit (counts)	**134**	**135**	**134**
Inter-assay Precision (CV %)	**3.6 – 10.9**	**3.9 – 11.4**	**3.4 – 10.6**
Free drug tolerance (ng/mL)	**1000**	**1000**	**1000**

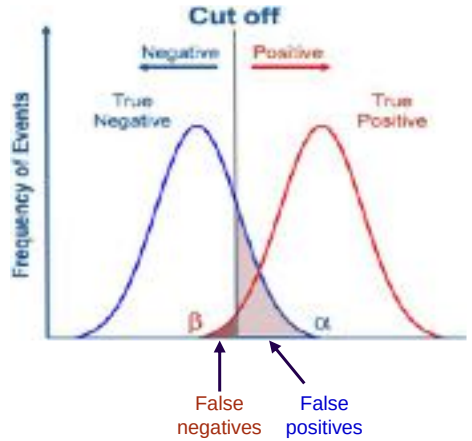
Singlicates - Summary

- No regulatory requirement to employ duplicates
- Similar results were obtained for singlicate 1 vs singlicate 2 in study and validation examples
- No liabilities were observed
- Significantly increased throughput

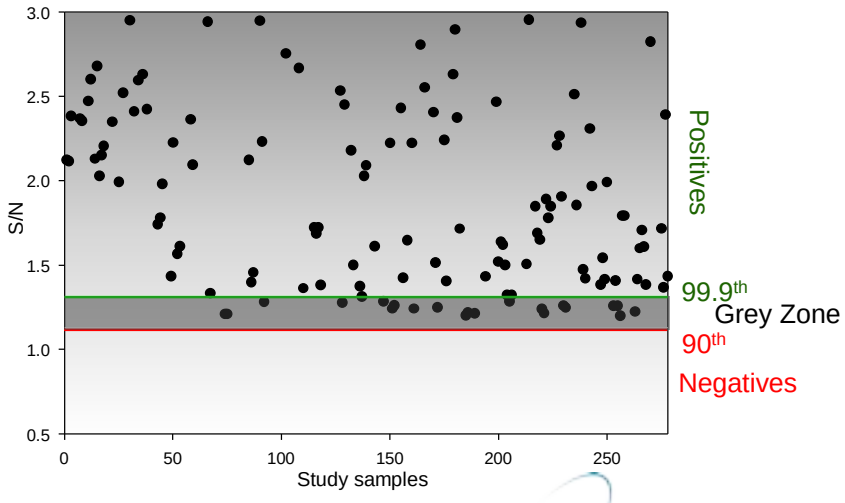
OMITTING THE CONFIRMATORY ASSAY

Omitting the Confirmatory Assay – Expected Impact

- In the standard three-tiered approach the screening cut-point is set at the 95 % prediction interval
  - It was deemed more appropriate to have false positives to avoid/reduce false-negatives during screening
  - This approach is expected to lead to 5 % false positives on average which will get eliminated by the confirmatory assay
- The new proposal is to omit the confirmatory assay and to use a cut-point at the 99 % prediction interval
  - This is expected to decrease the false positives (to 1 % on average) but to increase the false negatives
- An alternative approach could be a “two-tier grey-zone approach” (Devan – personal communication)
  - Using a lower cut point (e.g. 10% false positive rate) to first identify the negatives, and a higher cut point (e.g. 0.1% false positive rate) to identify the positives
  - All samples in-between these two cut points (the “grey zone”) will be subjected to the confirmatory assay



Zhang et al., J. Immunol. Methods, 484-485 (2020), 112817



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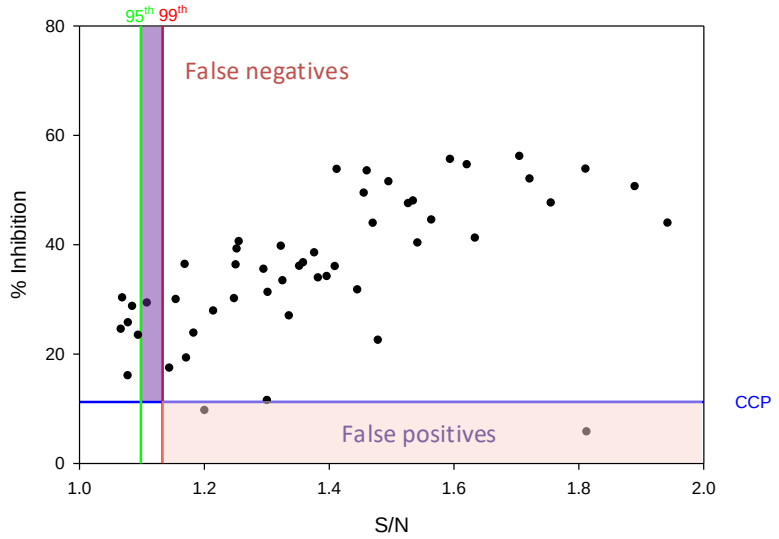
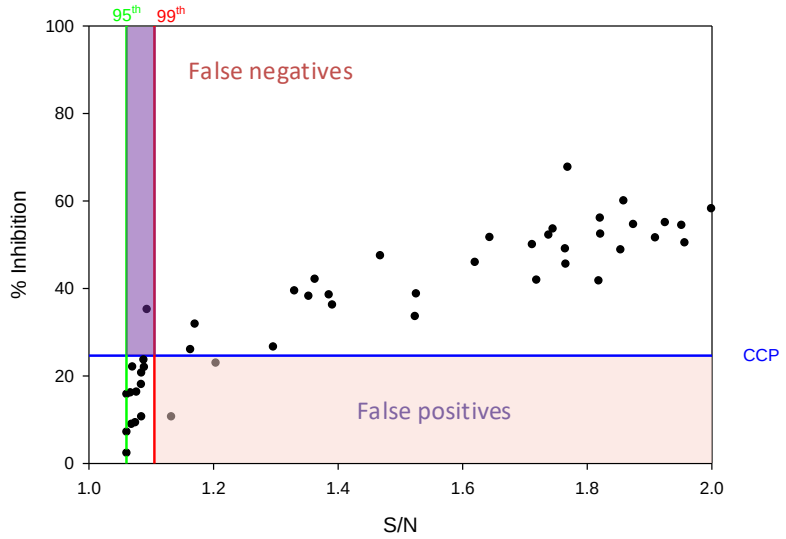
Omitting the Confirmatory Assay – Examples (1)

- A low number of false negatives and false positives was observed
  - This is in line with the statistical expectations
- Approach seems to be feasible assuming that the false negatives don´t have significant clinical impact
  - The “two-tier grey-zone approach” might be used to eliminate false negatives





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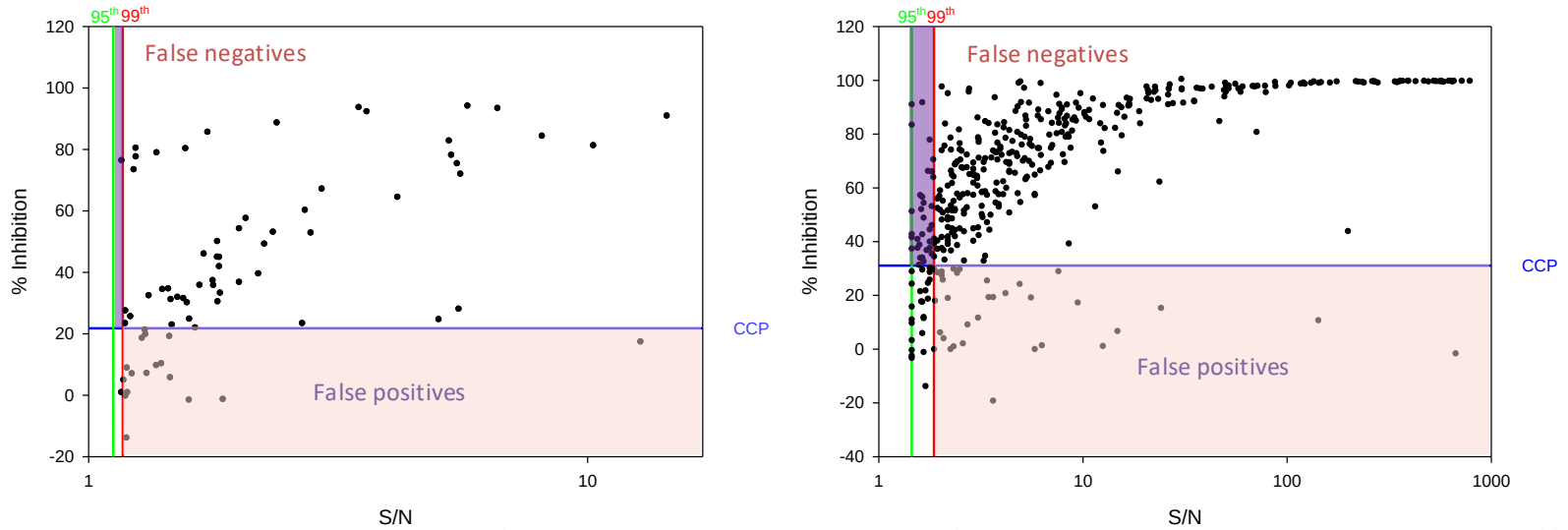
Omitting the Confirmatory Assay – Examples (2)

- Some false negatives but a significant number of false positives
  - False positives are likely due to unspecific binding to matrix components (not due to statistics)
    - False positives cannot be anticipated and tested during assay validation (all assays passed selectivity assessment during validation)
    - False positives will dilute the impact of ADAs (on PK/PD, efficacy & safety)
    - False positives will end up under "incidence" in the product label (without being clinically relevant)
  - The "two-tier grey-zone approach" would help to eliminate false negatives but not false positives



Confirmatory assay not just eliminates statistical false positives but also false positives due to matrix effects

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Omitting the Confirmatory Assay - Summary

- Regulatory acceptance unknown
- Workload reduction depends on the ADA positive rate
- Liabilities:
  - Increased number of false negatives
  - High number of false positives (depending on the assay) impairing correlation with PK/PD, efficacy and safety and leading to a higher ADA incidence in product labels (without clinical impact)
    - Confirmatory assay is not just eliminating statistical false positives but also unspecific binding to matrix components
  - These liabilities cannot be anticipated/mitigated during assay validation

NEED FOR A DRUG TOLERANT ASSAY

Need for a Drug Tolerant ADA Assay

- People recently started challenging the need for a drug tolerant ADA assay
- Proposal:
  - The drug tolerance needed should be based on the immunogenicity risk assessment
- Downsides:
  - The immunogenicity risk assessment is a theoretical exercise and can simply be “wrong”
    - A potential impact of ADAs on PK/PD cannot be anticipated by the risk assessment
    - It is extremely difficult to anticipate hypersensitivity reactions doing a risk assessment
  - Even for a low immunogenicity risk, one would like to know if an impaired PK/PD or safety findings are due to ADAs
    - An assay with insufficient drug tolerance would not allow this (many samples will be false negative)

THANK YOU!!!!!

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