Overcoming challenges in experimental design for in-study cut-point determination

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Biography and contact information

- Principal Scientist and Group Head of Immunogenicity at Charles River Laboratories in Montreal, Qc Canada
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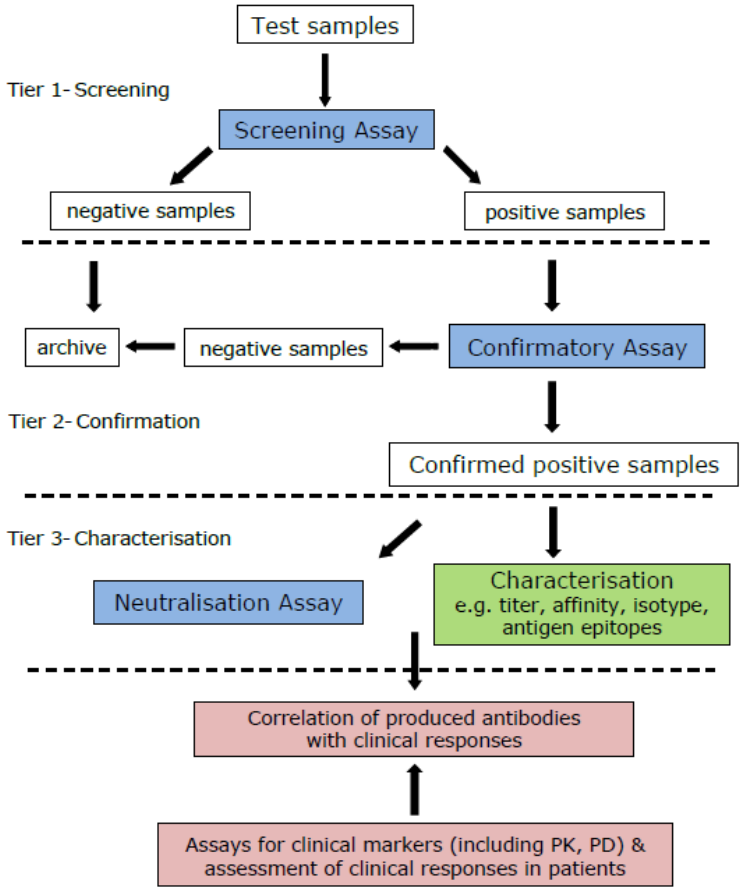
- 8 years developing and validating ADA methods (preclinical and clinical studies)
- Immunology/Pharmacology background, studying inflammatory response to nanomaterials and drug delivery systems (McGill University)
- **Disclaimer: Not a statistician**

Underlying assumptions of this presentation

1) Understanding of ADA tiered testing approach
2) Aware of the balanced experimental design applied during pre-study validation
3) Basic knowledge of how cut points are statistically derived
4) Common understanding of the method used to calculate the false-positive rate



EMA Guidance 2017

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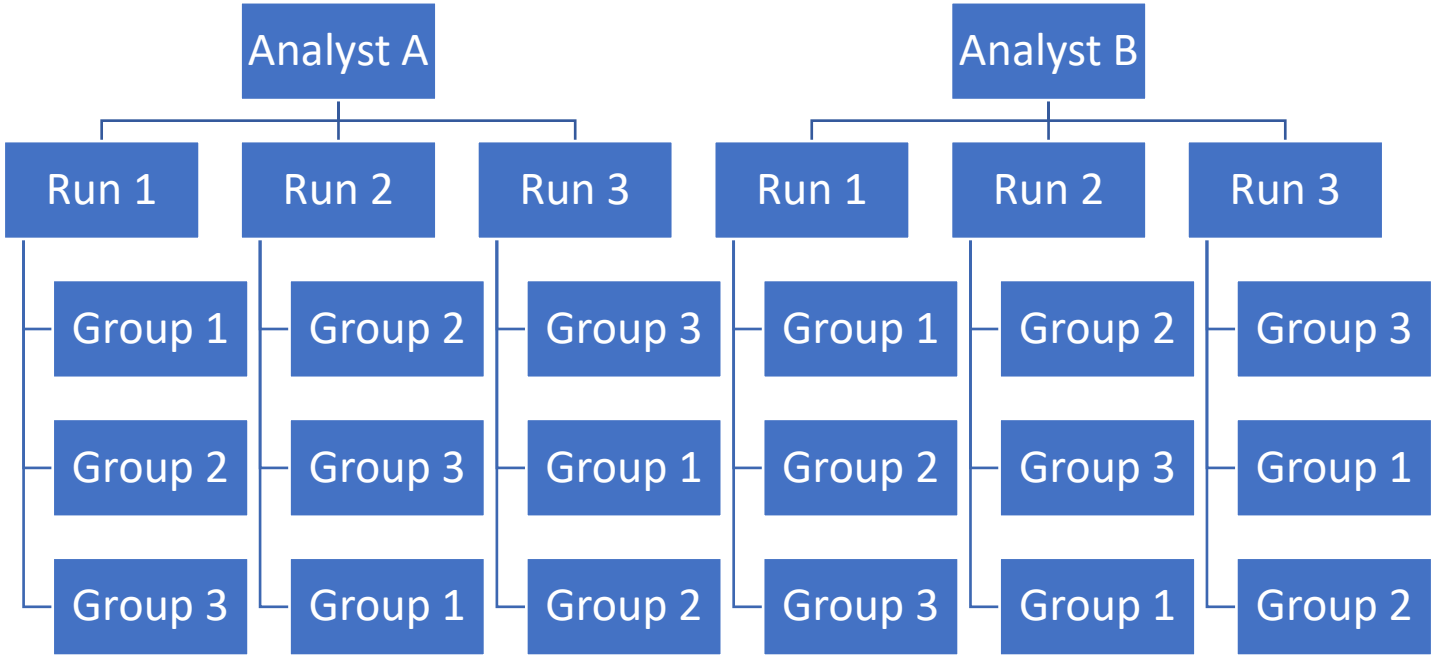

Devanarayan et al., 2017

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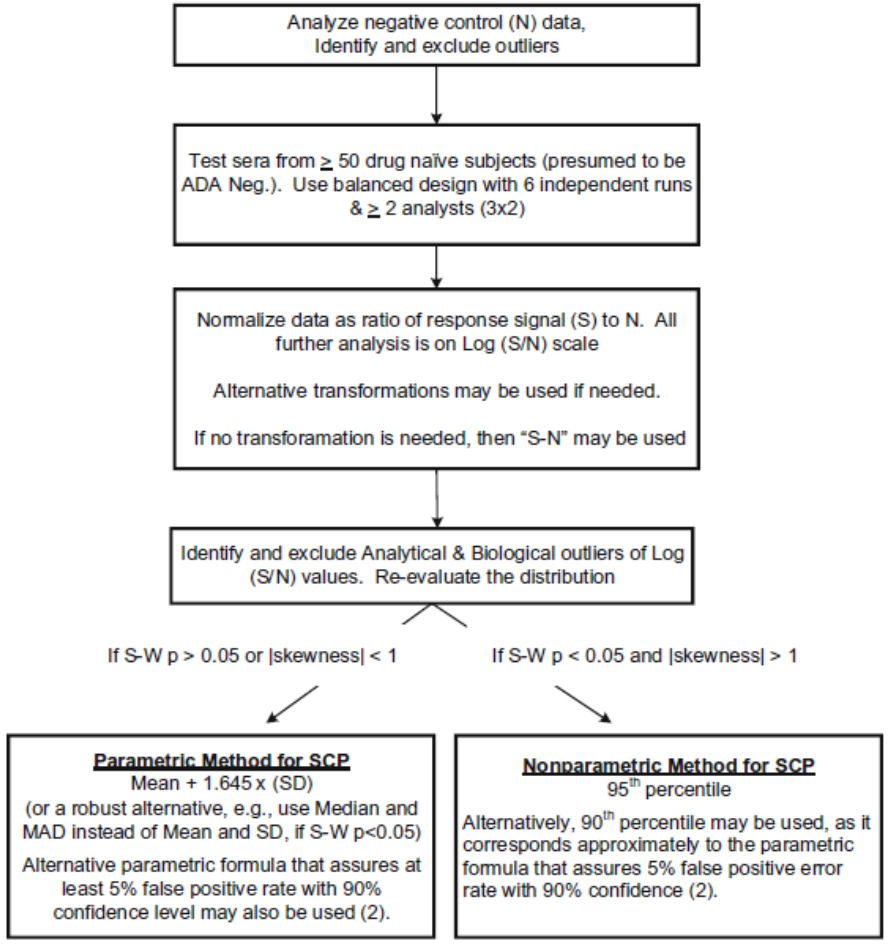

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$$\frac{\textbf{\# of samples confirmed negative}}{\textbf{Total \# of samples – \# of samples confirmed positive}} \textbf{ X 100}$$

Devanarayan et al., 2017

Guidance Recommendations

[…], it is necessary to confirm that the cut-point determined during assay validation is suitable for the population being studied. A sufficient number of samples from the target population should be used, and justification for the number used should be provided. If sufficient numbers of samples are not available, agreement with the Agency should be sought for the number of samples to be used.

– FDA Section VII. C) Confirmation of Cut-Point in the Target Population

*The number of samples required and other design considerations for the evaluation of in-study SCP and CCP depends on several factors, including the **number of baseline samples** available and **adequacy of sample volume for retesting**. When possible, a minimum of 50 baseline subject samples representing the diversity of the population is recommended. […] While each subject sample is only tested once, these samples should be distributed and tested across multiple plates and days by at least two analysts. The variability estimate calculated from all sample data will capture both the analytical and biological variation […]*

– Myler et al., ADA Validation Testing and Reporting Harmonization, AAPS J 2022

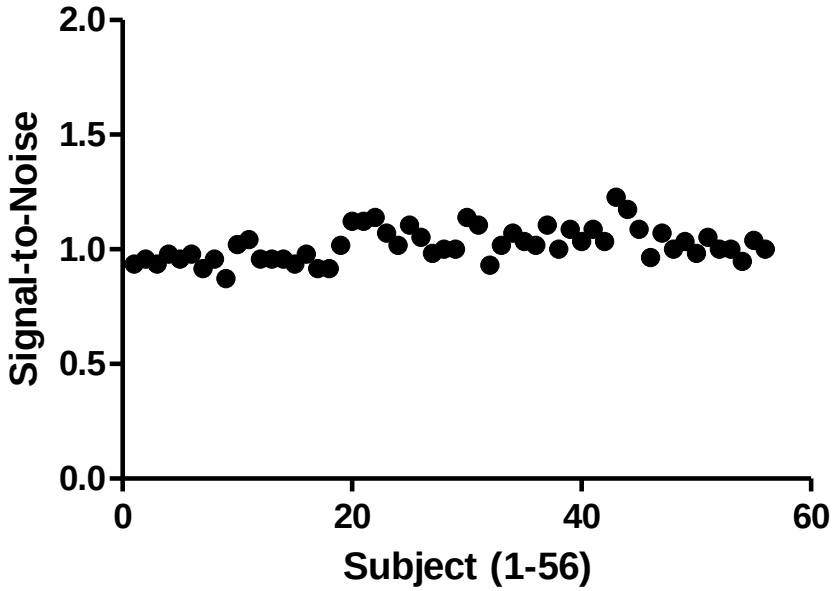
Experimental design challenges for in-study CPs

- Case Study 1: Retrospective interim analyses in early phase
- Case Study 2: Prospective experimental design in late phase to mitigate risk
- Case Study 3: Impact of disease state on CP and sensitivity

Focus of the case studies in on the major regulatory concern of FPR < 2%

Case Study 1
Retrospective interim analyses

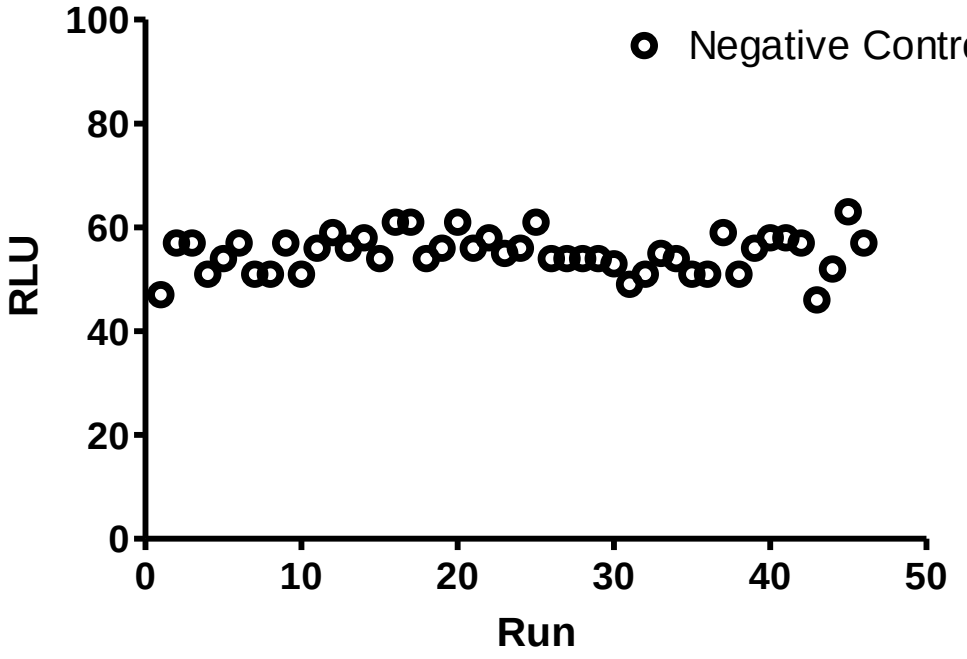
- Low variability in signal response for healthy (negative) population in method validation

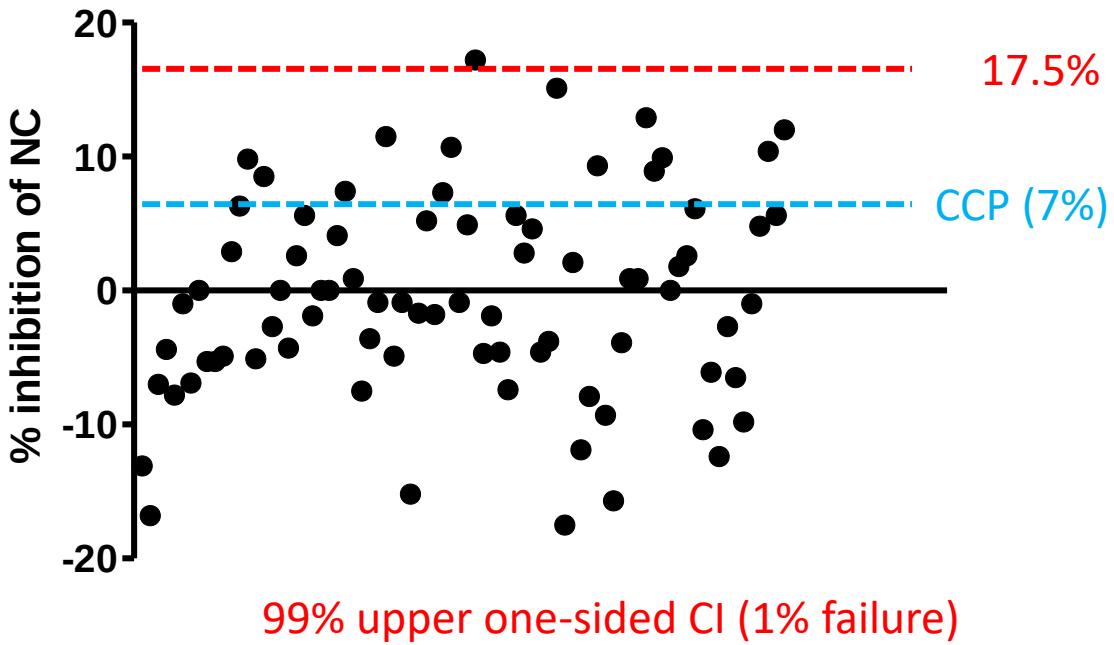


Drug	Peptide
Assay Format	Bridging
Readout	ECL
MRD	1/4
vSCPF	1.1x
vCCP	6.7%
Sensitivity	15 ng/mL
FPR (n=80)	<2%

Case Study 1
Retrospective interim analyses

Initial hint: variability of the NC in confirmatory assay was >CCP

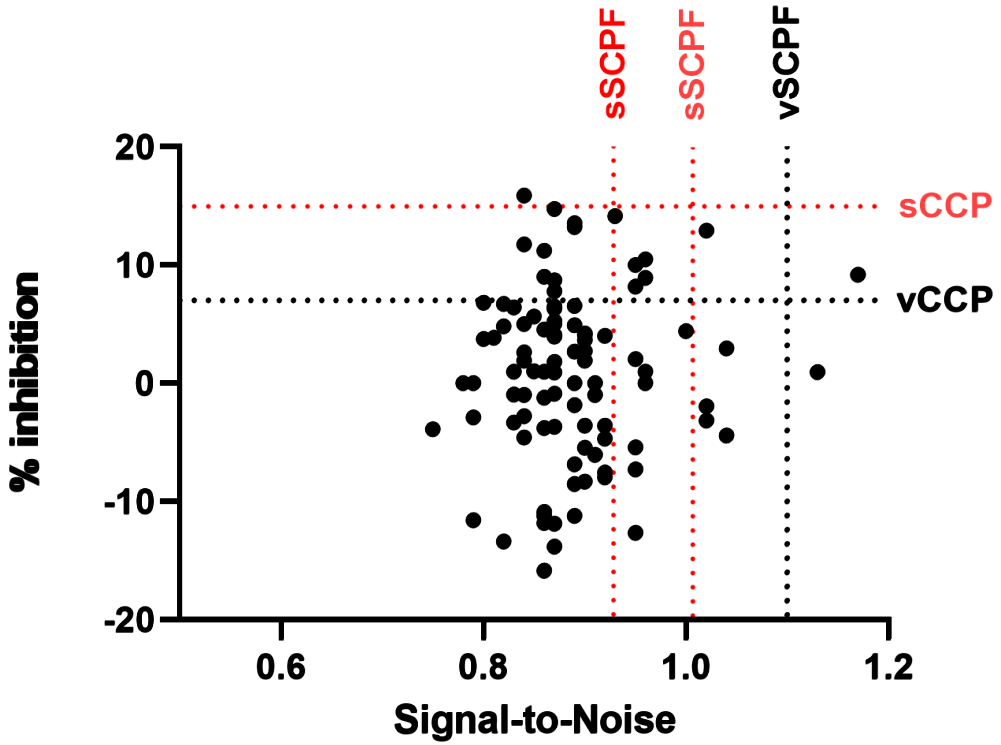




Case Study 1
Retrospective interim analyses

	Validation	In-Study#1
Samples	56	80
Indication	Healthy	Healthy
Measurements	6x	1x
Runs	18	3
Days	6	1
Analysts	2	1
Design	Balanced	unbalanced
SCPF	1.1	0.928
CCP	6.7%	-
FPR	<2%	>11%

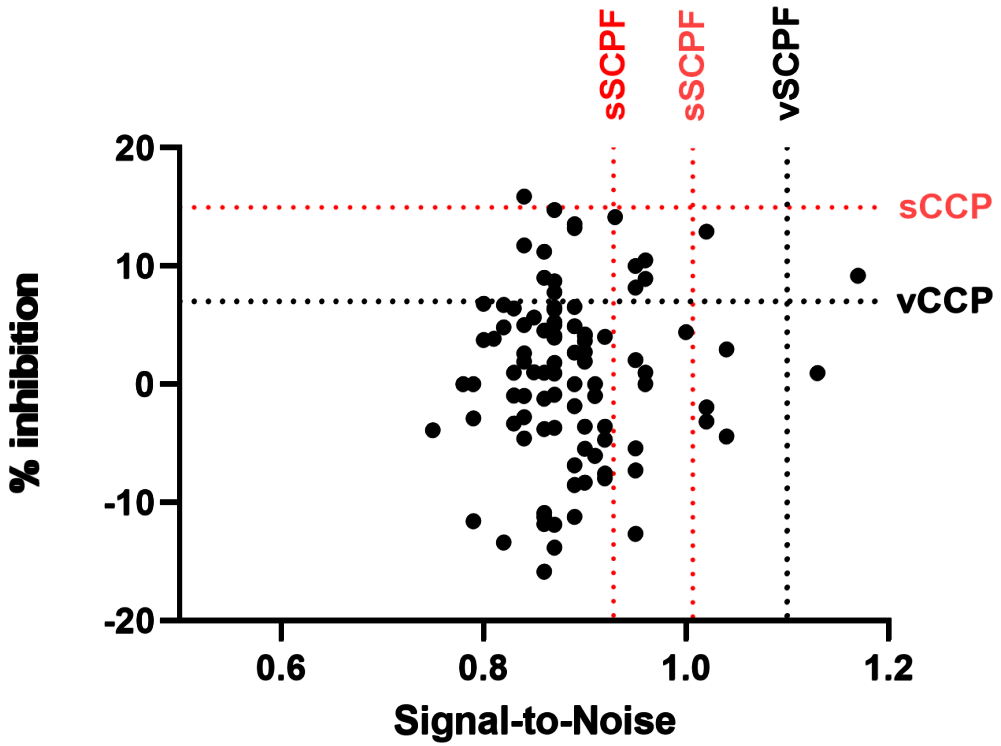
Distribution in in-study data
(baseline, healthy)



Case Study 1
Retrospective interim analyses

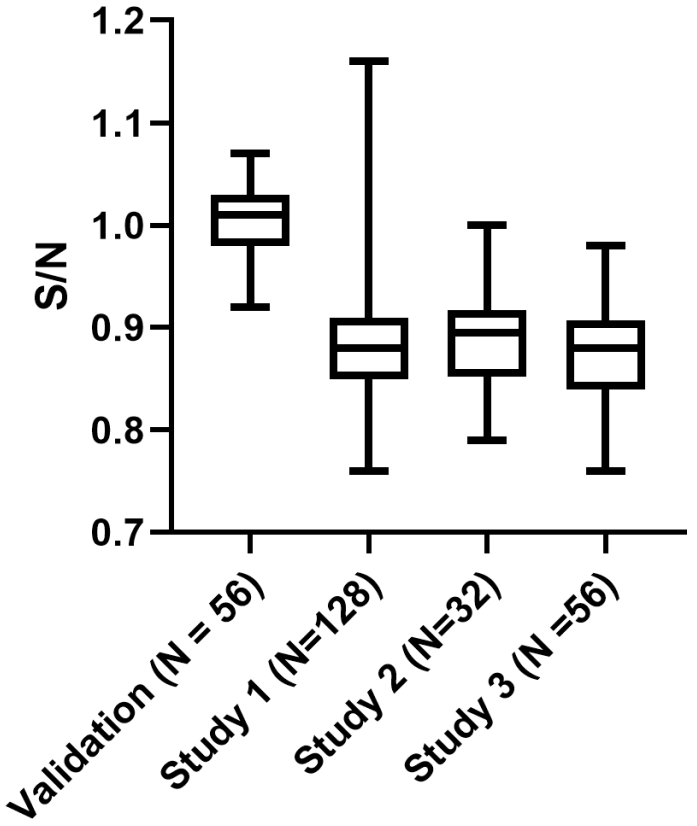
	Validation	In-Study#2
Samples	56	104
Indication	Healthy	Healthy
Measurements	6x	1x
Runs	18	12
Days	6	5
Analysts	2	3
Design	Balanced	unbalanced
SCPF	1.1	1.007
CCP	6.7%	15
FPR	<2%	9.4%

Distribution in in-study data
(baseline, healthy)



Case Study 1
Conclusions and lessons learned

- Consider a prospective design for baseline sample analysis, outlined *a priori*, even when pre-study validation and study populations are similar
- Define bioanalytical strategy for in-study CP
  - Threshold baseline sample number
  - One or multiple in-study CP
  - Define decision tree to trigger in-study CP
- Anticipate the need for in-study CP early on when accounting for deliverable turnaround

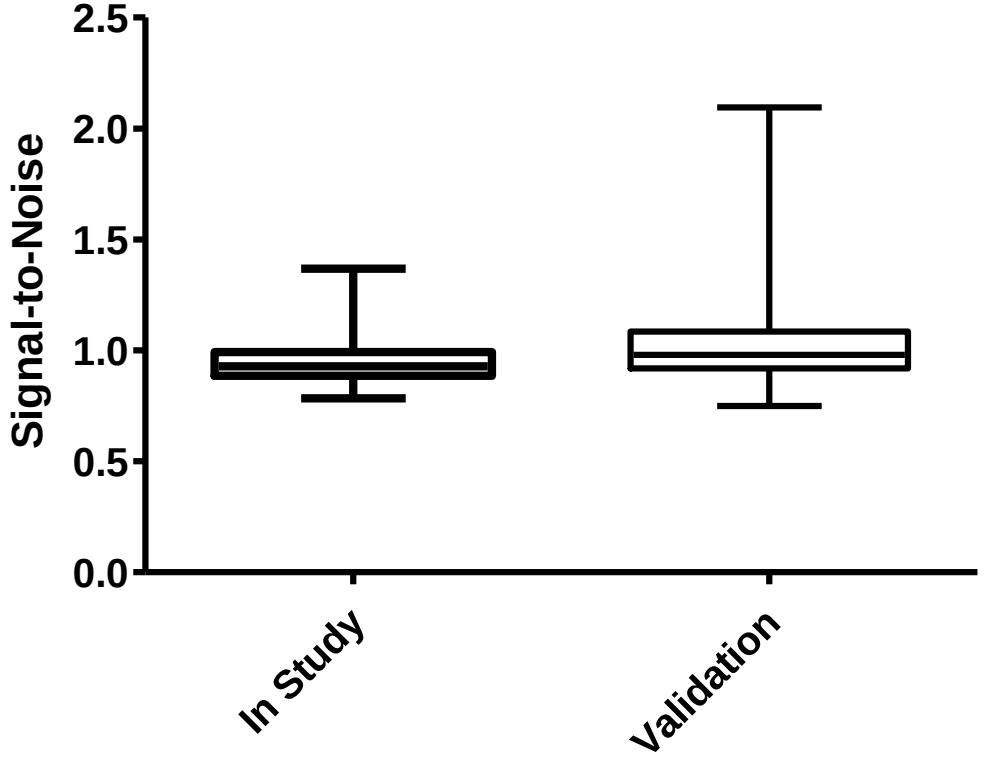


Case Study 2
Prospective in-study CP

- SCP and CCP were within the “acceptable” range
- In-study:
  - ~1860 samples analyzed from 286 subjects
  - Screen positive rate at baseline of 21%
  - Confirm positive rate of 20%
  - FPR <2% (1.4%)

Drug	Oligo
Assay Format	Direct
Readout	ELISA
MRD	1/50
vSCP	1.3x
vCCP	27%
Sensitivity	~45ng/mL

Case Study 2
Prospective in-study CP



	Validation	In-Study
Samples	60	264
Indication	Healthy	Disease
Measurements	6x	1x
Runs	24	26
Days	6	7
Analysts	2	6
Design	Balanced	unbalanced
SCP	1.3	1.06
vCCP	27%	27%
Sensitivity	45 ng/mL	10-15 ng/mL

Case Study 2
Prospective in-study CP

- Seemingly acceptable SCP not low enough
- Application of in-study CP increased confirm positive rate
- Challenge: How to maintain the 1% failure LPC?
  - Lower and upper limit established for LPC based on 0.5 and 99.5 percentiles of the distribution observed, not based on SCP
  - LPC limit is representative of the NC bkgrd

	Before	After
Samples analyzed	1860	1860
Screen positive	21%	29%
Confirm positive	20%	22%
FPR	1.4%	9.3%

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Prospective in-study CP

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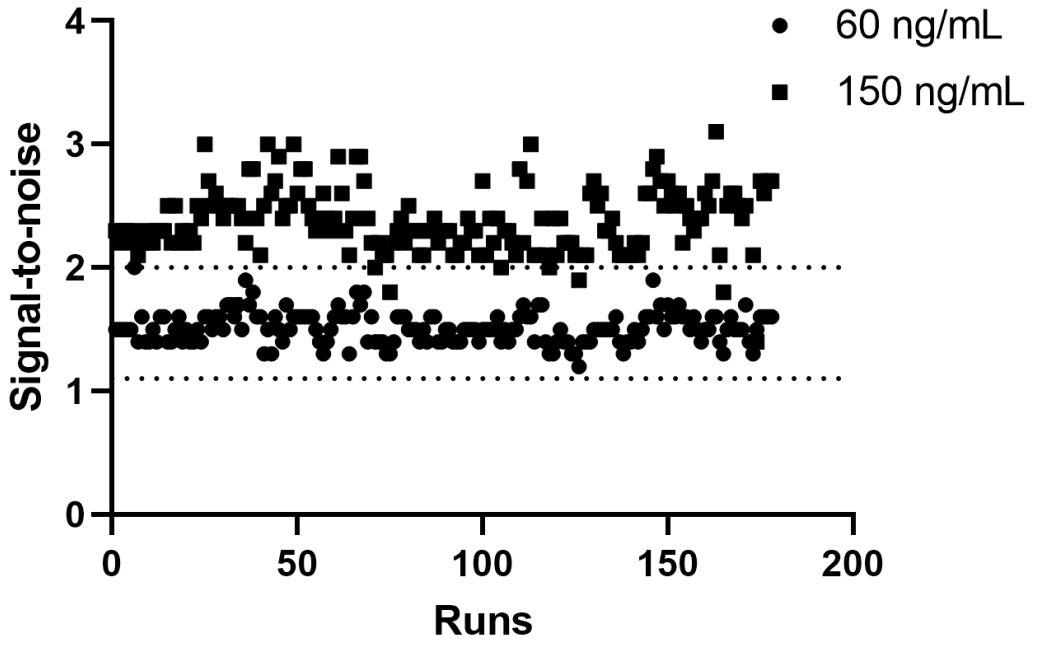


Case Study 3
Impact of disease state on CP and sensitivity

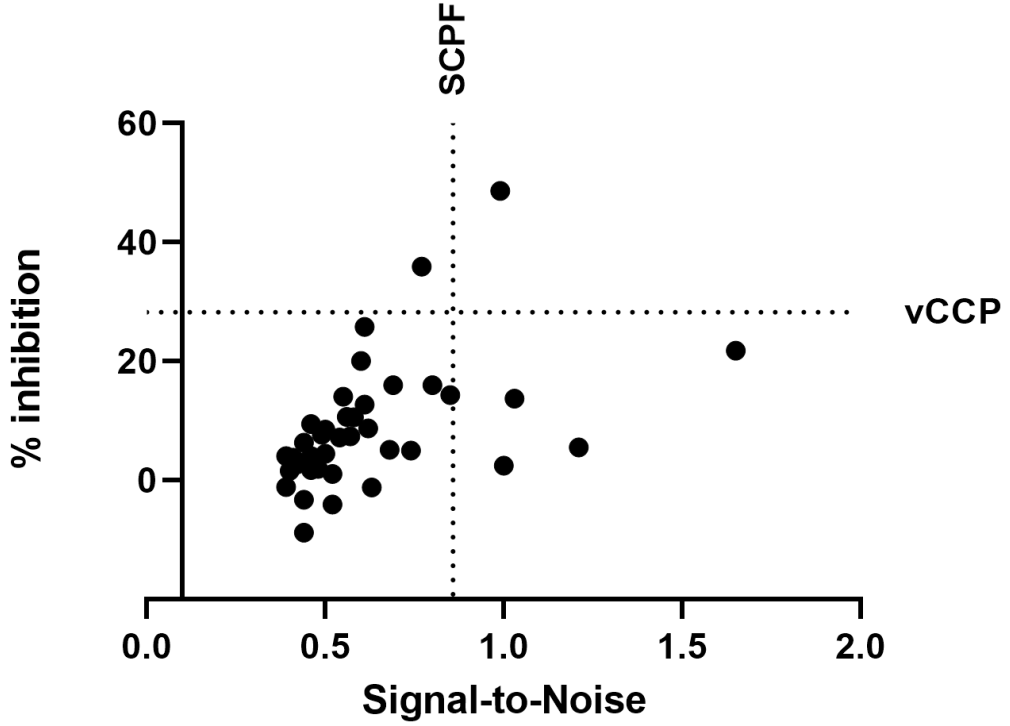
- Sample signal was < NC median signal using an in-house pool from healthy donors

Drug	Oligo
Assay Format	Direct
Readout	ECL
MRD	1/10
NC signal	150-250 RLU

	Validation
Samples	41
Indication	Rare disease
Measurements	4x
Runs	12
Days	3
Analysts	2
Design	Balanced
SCPF	0.858
CCP	28.2%

Case Study 3
Impact of disease state on CP and sensitivity

- Sample signal was < NC median signal using an in-house pool from healthy donors



	Selectivity
Samples	20 pooled
Indication	Rare disease
Measurements	1x
Runs	1
Days	1
Analysts	1
PC unspiked	< PSCP, CCP
LPC-spiked (200 ng/mL)	>PSCP > CCP

Conclusions and Design considerations

- Define the clinical bioanalytical strategy early on as part of *living* ISI
- Consider prospective design for baseline sample analysis and outline *a priori* in analytical plans
  - Critical even when pre-study validation and study populations are similar
- Capture assay variability *expected in lab* when assessing in-study CP
  - Multiple buffer preparations
  - Operator, equipment, non-disposable material (e.g. plates)
  - *Limitation – accounting for variability with time and critical reagents*
- Monitor system suitability controls using assay limits determined in validation based on SSC performance

Acknowledgements

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