How low is too low

Assessing cut points in anti-drug antibody (ADA) assay validation based on recommendations in the Final FDA guidance

Sebastien Boridy, PhD

Principal Scientist

Charles River Laboratories

Montreal, Qc, Canada

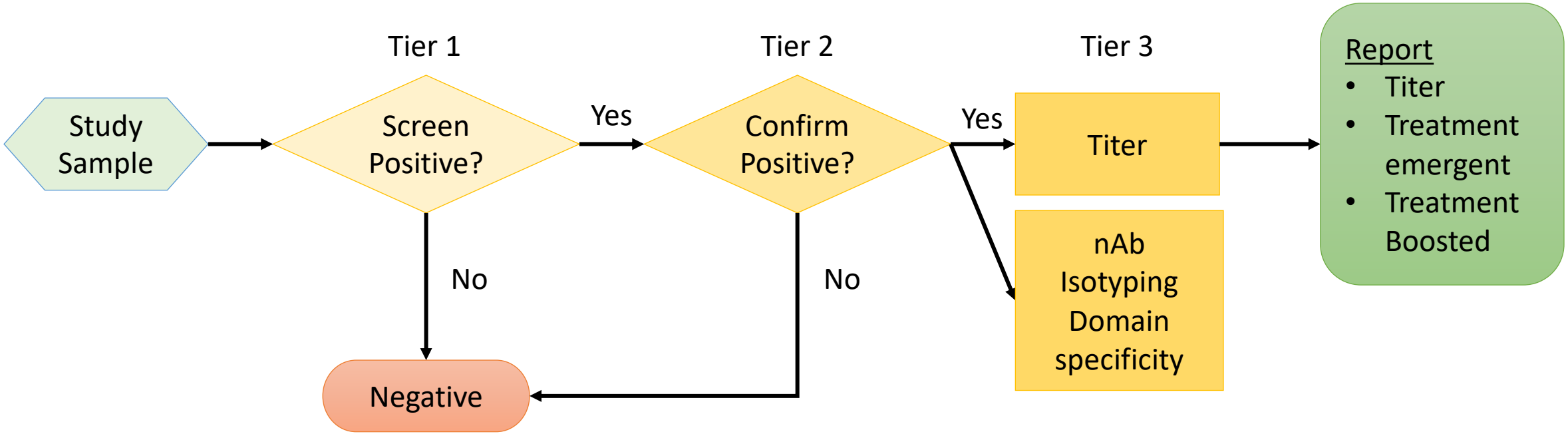
Biography and contact information

- Principal Scientist and Group Head of Immunogenicity at Charles River Laboratories in Montreal, Qc Canada
- Email: Sebastien.Boridy@crl.com

- Close to 6 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

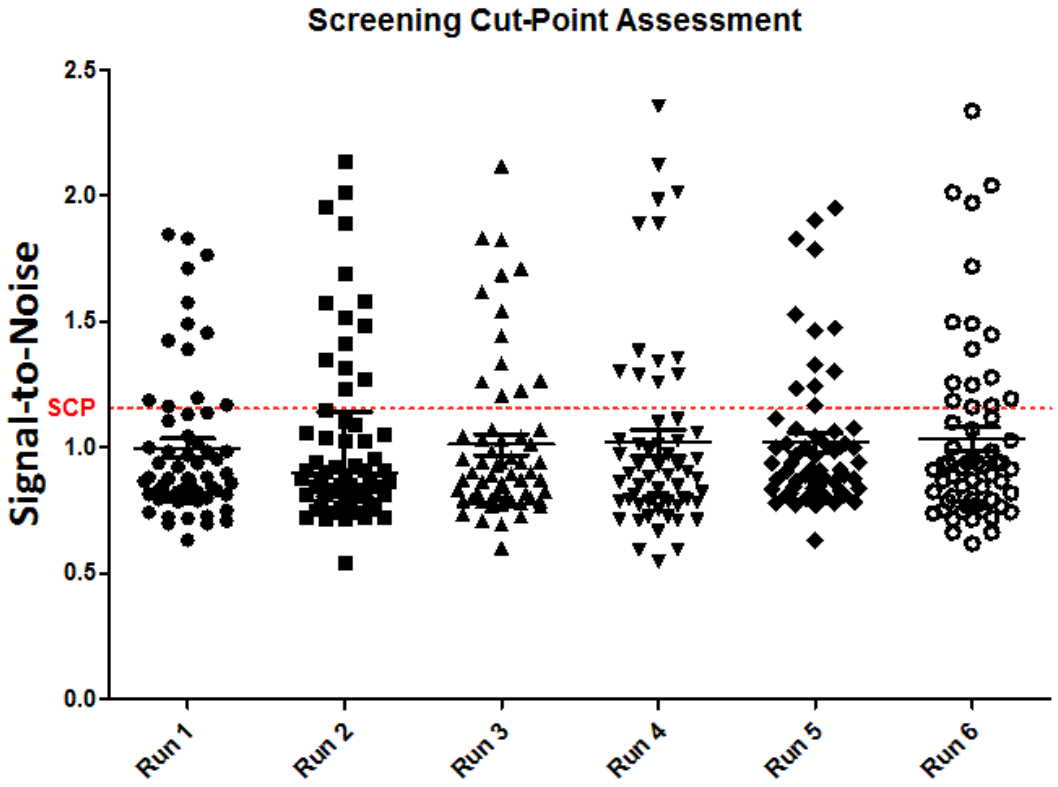
Assumption #1:
Understanding of ADA tiered testing approach using qualitative assay thresholds



Underlying assumptions of this presentation

Assumption #2:
Basic understanding of how assay cut points are derived

- ≥ 50 lots, presumed Negative sera
- Balanced design (≥ 6 runs, ≥ 2 analysts)
- Tier 1 (Screening)
  - 95th percentile of negative population with outliers removed (i.e. 5% FPR)
- Tier 2 (Confirmatory)
  - 99th percentile (1% FPR)



Final FDA Guidance Recommendation

Cut-Point of Screening Assay

The cut-point should be determined statistically with an appropriate number of treatment-naïve samples, generally around 50, from the subject population. Each sample should be tested by at least two analysts on at least three different days for a total of at least six individual measurements.

One approach that allows for high assurance of a 5% false-positive rate is to apply a 90% one-sided lower confidence interval for the 95th percentile of the negative control population (Shen et al. 2016). This will assure at least a 5% false-positive rate with a 90% confidence level. This approach improves the probability of the assay identifying all subjects who may develop antibodies.

Final FDA Guidance Recommendation

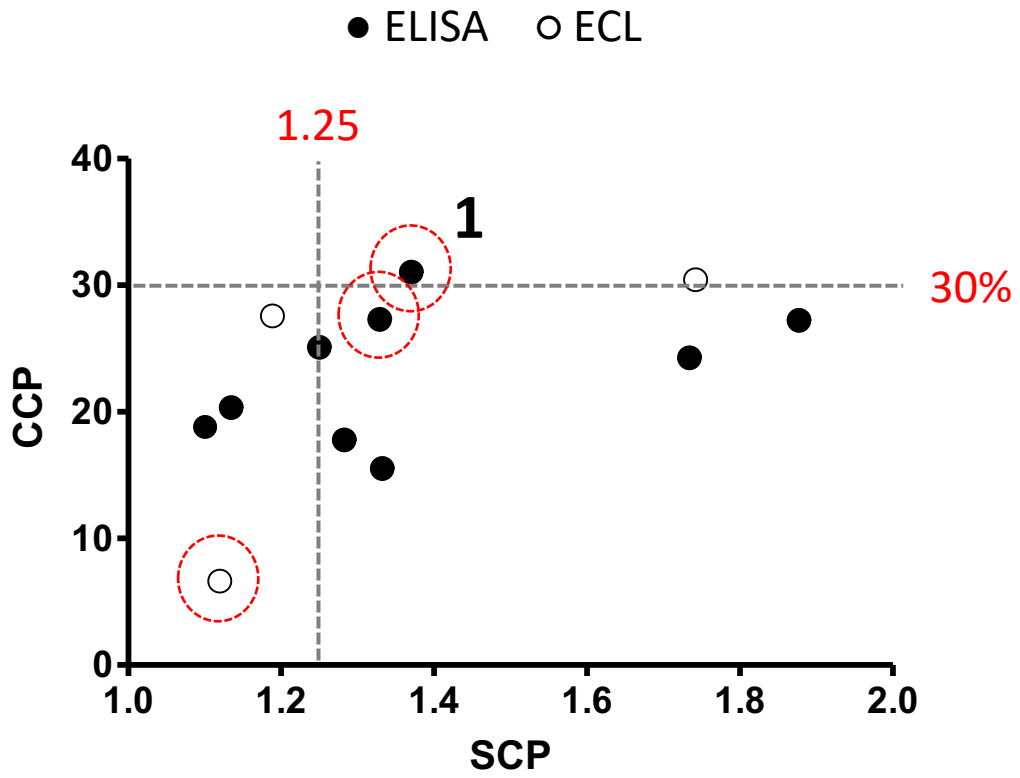
Cut-Point of Confirmatory Assay

Experimental design is similar to screening if the assay is based on signal depletion due to competition for antigen binding (i.e. % inhibition data)

One approach for the estimation of the confirmatory assay cut-point is to use an 80% to 90% one-sided lower confidence interval for the 99th percentile. Because the purpose of this assay is to eliminate false-positive samples arising as a result of non-specific binding, it is adequate to use a 1% false-positive rate for the calculation of the confirmatory cut-point. The use of tighter false-positive rates such as 0.1% is not recommended, but may be acceptable for larger studies.

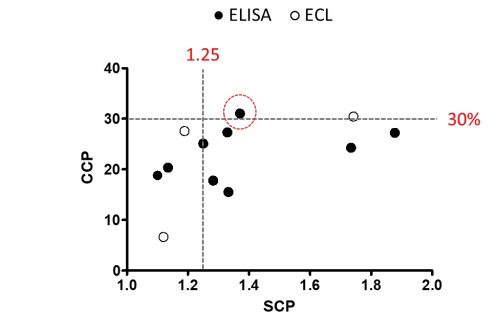
Calculating cut-points using the lower confidence limit (LCL)

- Regulatory:
  - Screening CP (SCP) <2-3x
  - Confirmatory CP (CCP) <70%
- Industry acceptance:
  - 1.25x < SCP < 2x
  - CCP > 30%
- *Three case studies to show that not all SCP/CCP are created equal*



ELISA = Enzyme-linked immunosorbent assay
ECL = Electrochemiluminescence

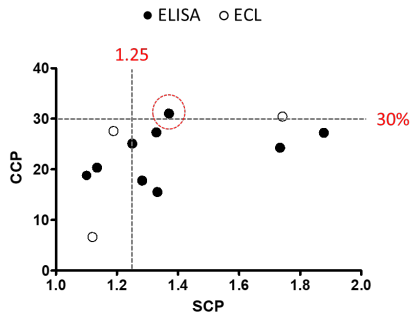
Case Study 1
A case for method re-validation



- Initially validated in 2016
- Phase 1 study 2016-17
  - ~500 subjects enrolled
  - ~6000 samples analyzed
  - ~50 samples screened positive
  - 2 samples confirmed positive (<1% FPR)
- In-study population signal varied little, near instrument noise
- *Challenge*: How to enhance the detection of biological variability to reach targeted 5% FPR?

Drug	Not disclosed
Assay Format	Direct
Readout	ELISA
MRD	1/50
SCP	1.3x
CCP	45%
Sensitivity	~250ng/mL

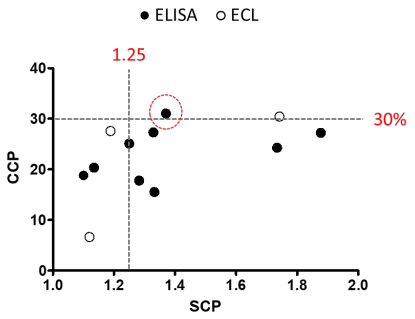
Case Study 1
A case for method re-validation



- Method re-optimized (blocker, assay buffer)
- Method re-validated in 2019
- Phase 3 studies
  - ~1,800 subjects enrolled
  - ~9,000 samples analyzed
  - ~1,600 samples screened positive
  - ~200 samples confirmed positive (~**15% FPR**)
- Difficulty reconciling the clinical relevance of low titer positives (50-400)
- Similar prevalence pre-treatment vs incidence in-study (~2% of subjects)

	Initial	Re-validated
Assay Format	Direct	Direct
Readout	ELISA	ELISA
MRD	1/50	1/50
SCP	1.3x	1.3x
CCP	45%	31%
Sensitivity	~250 ng/mL	400 ng/mL (Scr) 100 ng/mL (Conf)

Case Study 1
Conclusions



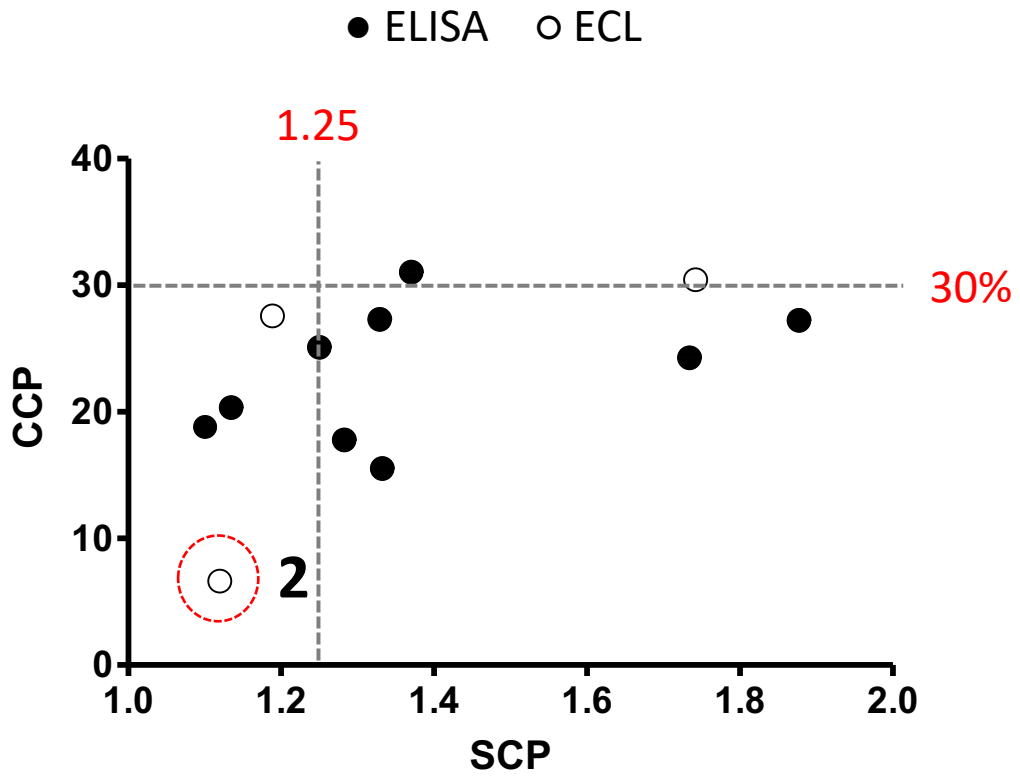
- Method re-validation boosted biological variability
- Combined with LCL approach, higher FPR resulted (<1% to >15%)
- Clinical relevance of the results difficult to interpret
  - No correlation with AEs or safety endpoints
  - No correlation with PK/PD
- 'Improved' method provides greater confidence that positive samples were not missed (i.e. low false negative rate), but questionable whether it is more meaningful than original

Calculating cut-points using the lower confidence limit (LCL)

- *Three case studies to show that not all SCP/CCP are created equal*

Case Study #2

When there is no noise…..



ELISA = Enzyme-linked immunosorbent assay
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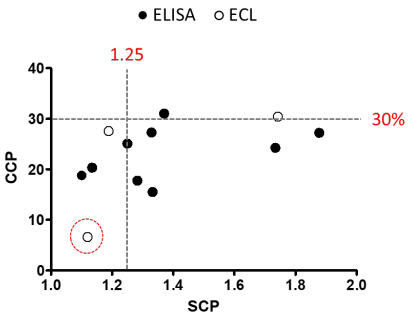
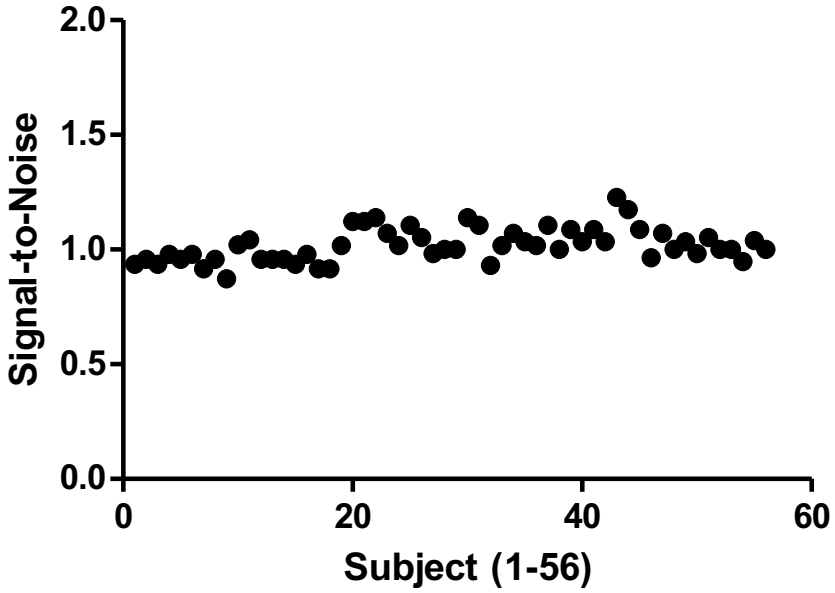
Case Study 2
When there is no noise

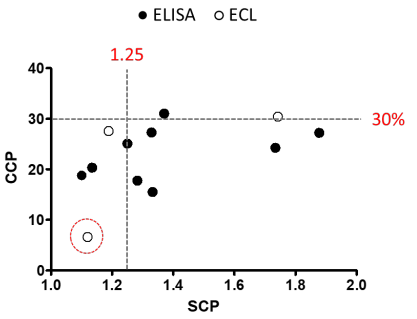


- Homogeneous bridging assay format
- Low variability in signal response for negative population



Drug	Peptide
Assay Format	Bridging
Readout	ECL
MRD	1/4

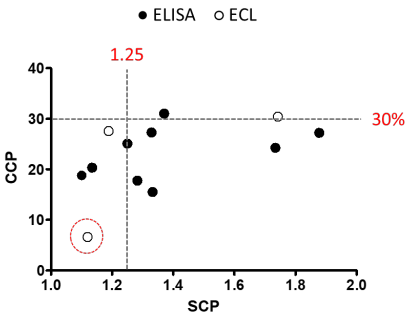
Case Study 2
When there is no noise



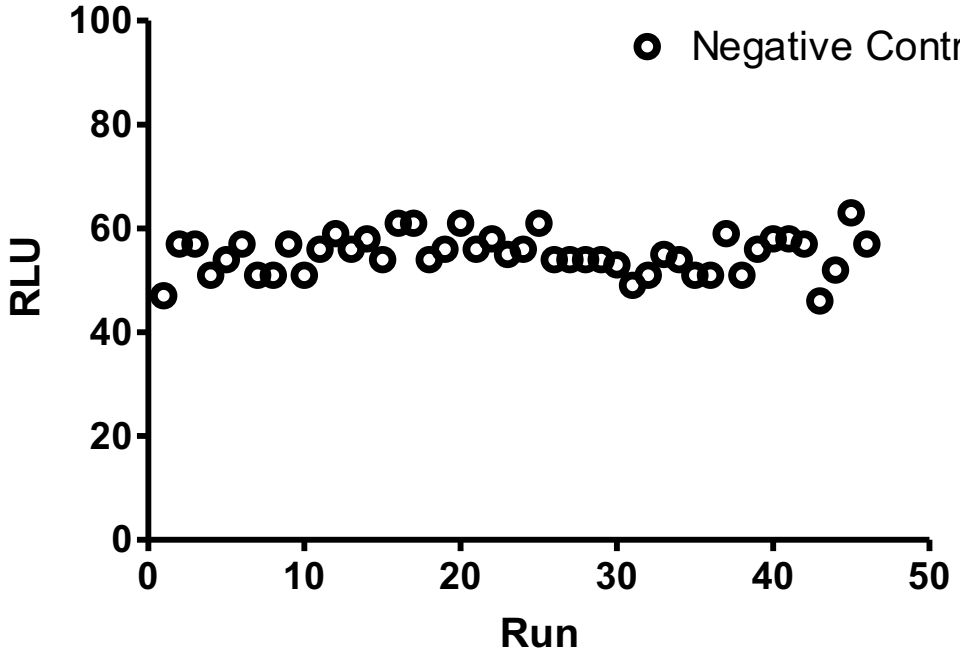
- Assay optimization efforts
  - Blocking/assay buffer optimized
  - Washes minimized
  - Labeled drug concentrations optimized to improve sensitivity
  - Dilution minimized (MRD of 1/2 before the addition of labelled drug)
- Confirmatory CP determined using LCL approach is lower than the negative control variability across validation runs

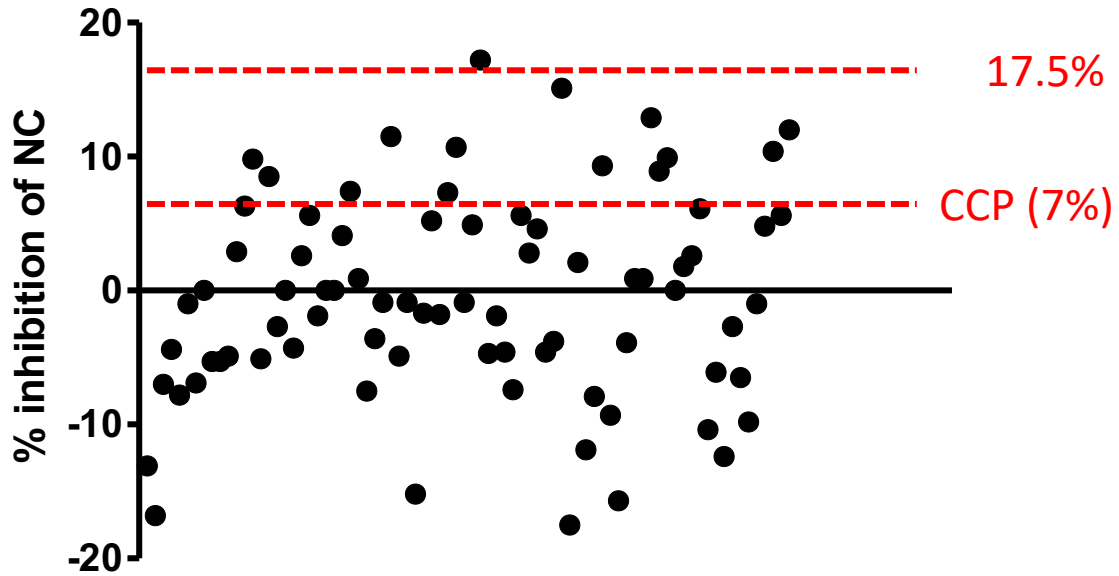
Drug	Peptide
Assay Format	Bridging
Readout	ECL
MRD	1/4
SCP	**1.1x**
CCP	**7%**
Sensitivity	15 ng/mL

Case Study 2
When there is no noise



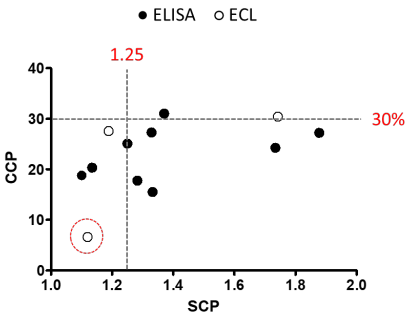
Challenge: How to set the confirmatory assay acceptance criteria for negative control?





Select a 99th upper limit (1% failure)

Case Study 2
Conclusions



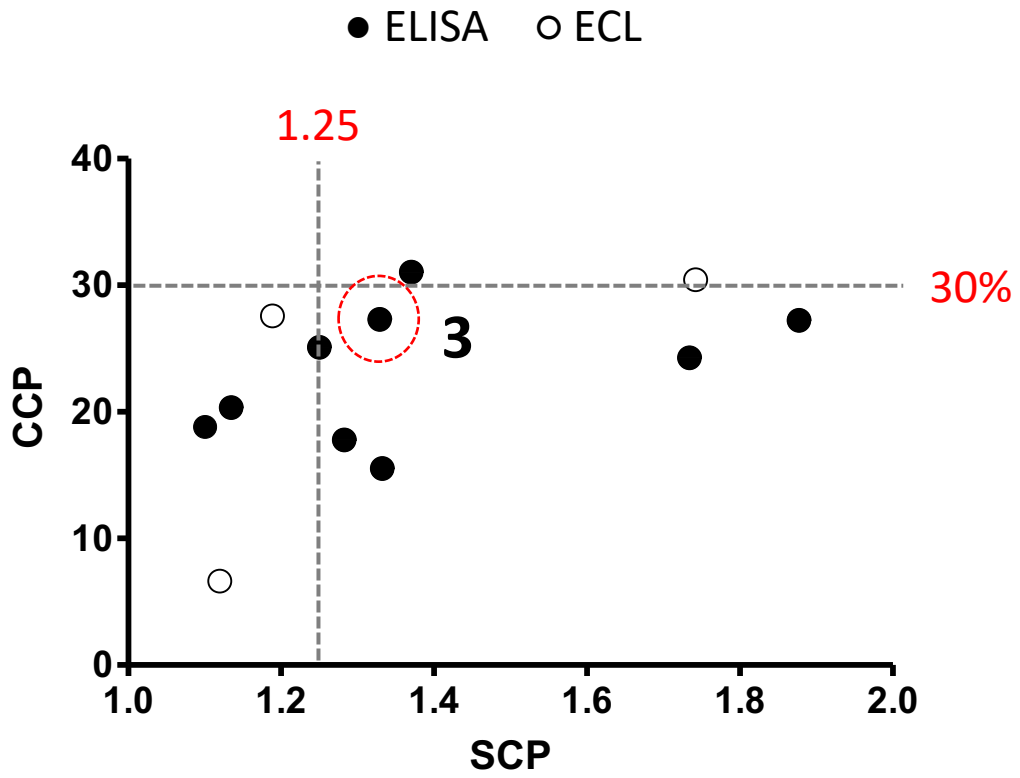
- Low SCP/CCP is appropriate for samples
  - HOWEVER, <1% FPR observed for predose samples due to low screen positive rate – in-study SCP <1x
- Applying CCP threshold to the confirmatory NC increased failure rate of the confirmatory assay
  - Upper limit for NC potentially increases risk of false positives in confirmatory assay, but more representative of NC variability

Calculating cut-points using the lower confidence limit (LCL)

- *Three case studies to show that not all SCP/CCP are created equal*

Case Study #3

A case for an in-study screening cut-point



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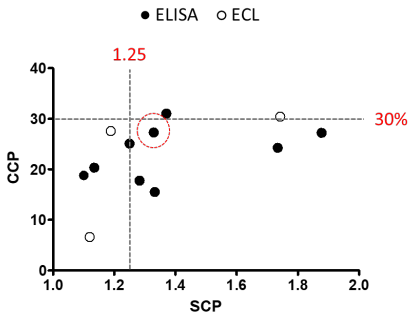
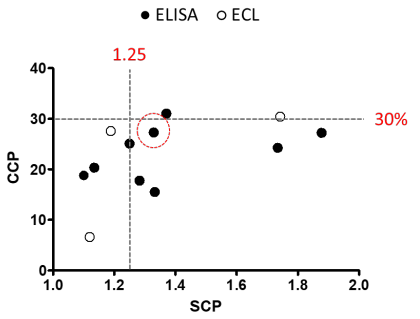
Case Study 3
A case for an in-study CP

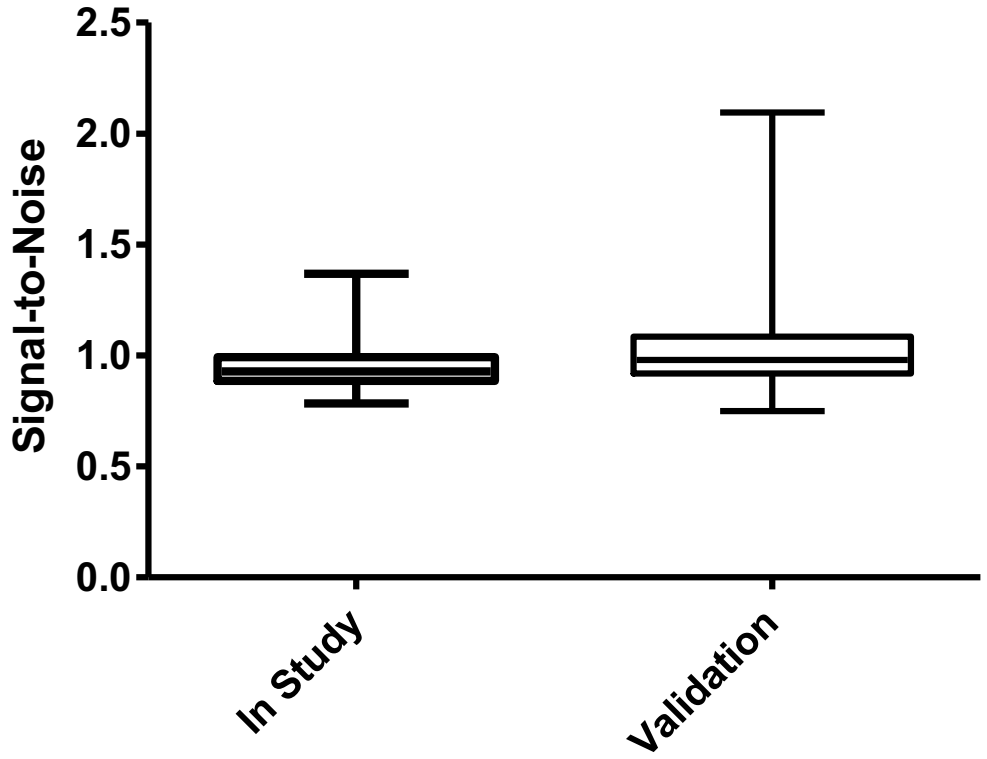


- SCP and CCP were within the “acceptable” range
- In-study:
  - ~1860 samples analyzed from 286 subjects
  - Screen positive rate of 21%
  - Confirm positive rate of 20%
  - FPR <2% (1.4%)
- *Challenge*: How to determine the in-study SCP? What about 1% failure for LPC?

Drug	Not disclosed
Assay Format	Direct
Readout	ELISA
MRD	1/50
SCP	1.3x
CCP	27%
Sensitivity	~45ng/mL

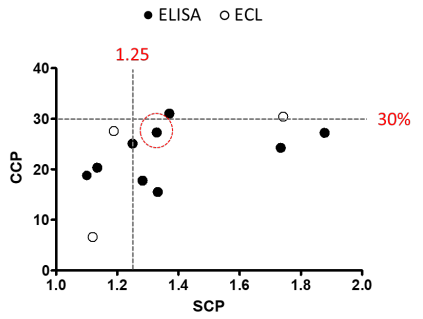
Case Study 3
A case for an in-study CP





	Validation	In-Study
Unique Samples	60	264
Measurements	N=6	N=1
Runs	24	26
Days	6	7
Analysts	2	6
Method	Point Estimate	LCL
SCP	1.3	1.06
Sensitivity	45 ng/mL	10-15 ng/mL

Case Study 3
Conclusions



- Seemingly acceptable SCP not low enough
- Application of in-study CP increased confirm positive rate (…verdict is still out on clinical relevance)
- Lower and upper limit established for LPC based on 0.5 and 99.5 percentiles of the distribution observed, not based on SCP

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

Conclusions

- Reasonable or acceptable SCP/CCP thresholds based on recommendation will vary widely based on the assay format and drug
  - Targeting 5% FPR will increase confidence that false positives are not missed, but clinical relevance of the assay results is lost in certain cases
- Assay robustness should be represented in the SCP/CCP runs to ensure they capture the method's true variability
  - Multiple buffer preparations
  - Operator, equipment, non-disposable material (e.g. plates)
  - *Limitation – accounting for variability with time and critical reagents*
- Alternative approaches to using the SCP and CCP thresholds for system suitability control monitoring are justifiable

Acknowledgements

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- Marie-Soleil Piché
- Sponsors

Questions?