Immunogenicity cut point setting & outlier evaluation – part 1

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Tiered Testing Strategy

- Tier 1: Identify "reactive" samples
  - Samples with signal above screening cut-point
- Tier 2: Identify "Ab+" samples by testing reactive samples in the absence and presence of drug
  - Samples with percent inhibition above confirmatory cut-point
- Tier 3: Determine a sample titer value by serial dilution of Ab+ samples in Tier 2
  - Titer is based on the screening cut-point or a higher "titer cut-point". Can be continuous (requires interpolation) or discrete
- Tier 4: Evaluate neutralizing effects of antibodies
  - Usually based on cell-based bioassay using Ab+ samples

Cut point evaluation process

Cut point experimental design

Analyst	Assay Run	Assay Plate	Subject groups with serum samples tested over six runs		
			$S_1 – S_{17}$	$S_{18} – S_{34}$	$S_{35} – S_{51}$
A_1	R_1	P_1	X		
		P_2		X	
		P_3			X
	R_2	P_1		X	
		P_2			X
		P_3	X		
	R_3	P_1			X
		P_2	X		
		P_3		X	
A_2	R_4	P_1	X		
		P_2		X	
		P_3			X
	R_5	P_1		X	
		P_2			X
		P_3	X		
	R_6	P_1			X
		P_2	X		
		P_3		X	

Alternative visual

Plate Order	Analyst 1			Analyst 2		
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
1	Group A Plate 1	Group B Plate 1	Group C Plate 1	Group C Plate 1	Group B Plate 1	Group A Plate 1
2	Group B Plate 2	Group C Plate 2	Group A Plate 2	Group A Plate 2	Group C Plate 2	Group B Plate 2
3	Group C Plate 3	Group A Plate 3	Group B Plate 3	Group B Plate 3	Group A Plate 3	Group C Plate 3

All samples get tested in every run and every plate, by both analysts.

Plate layout for the balanced CP Experimental Design

	1	2	3	4	5	6	7	8	9	10	11	12	
A	NC		S2		S6		S10		S13		S17		A
B	NC + drug		S2 + drug		S6 + drug		S10 + drug		S13 + drug		S17 + drug		B
C	LPC		S3		S7		S11		S14		NC		C
D	LPC + drug		S3 + drug		S7 + drug		S11 + drug		S14 + drug		NC + drug		D
E	MPC		S4		S8		NC		S15		LPC		E
F	HPC		S4 + drug		S8 + drug		NC + drug		S15 + drug		LPC + drug		F
G	S1		S5		S9		S12		S16		MPC		G
H	S1 + drug		S5 + drug		S9 + drug		S12 + drug		S16 + drug		HPC		H
	1	2	3	4	5	6	7	8	9	10	11	12	

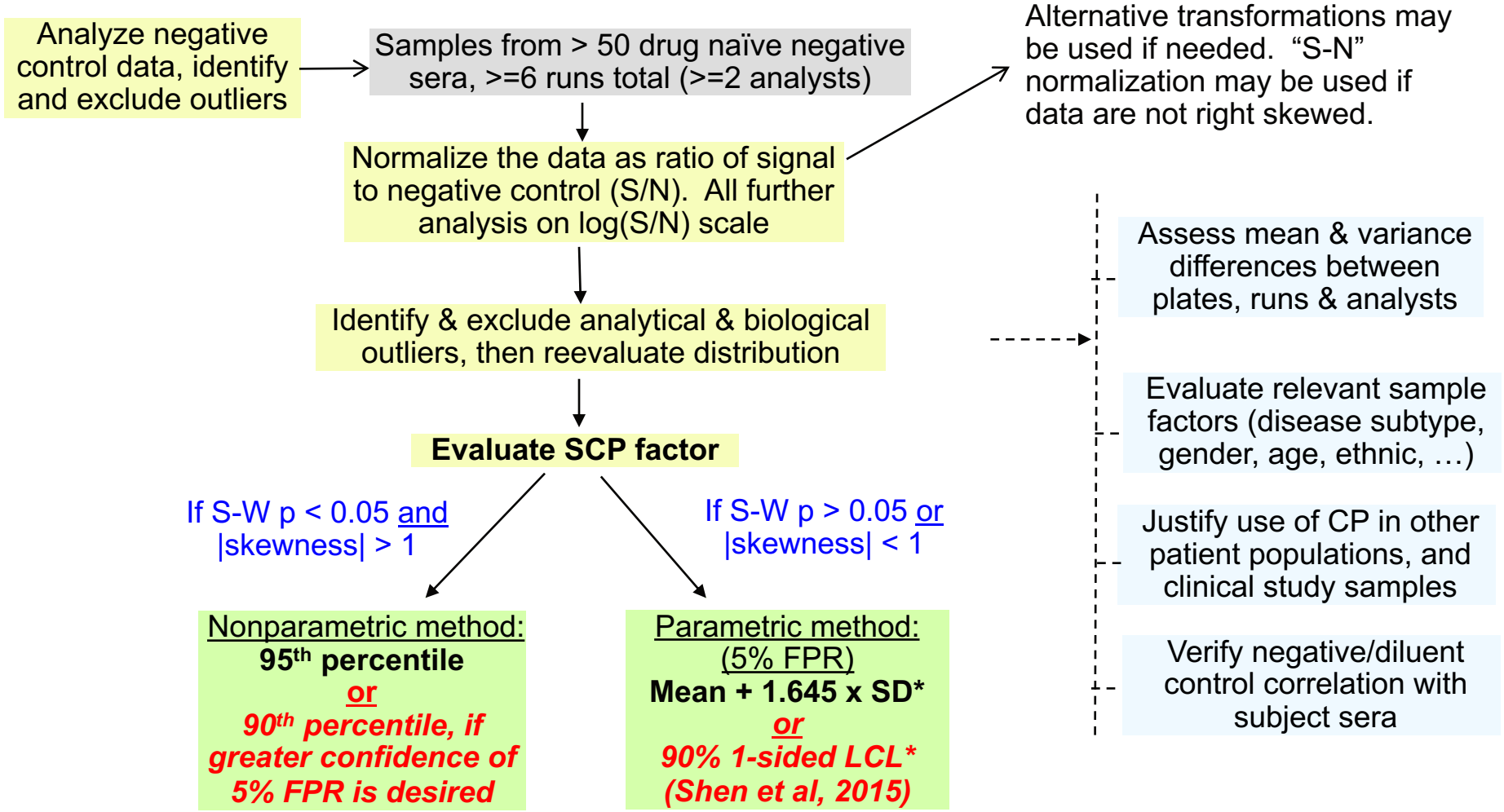
- *NC & LPC with and w/o drug, MPC & HPC*
- *17 subject sera per plate.*

Rationale for this plate layout & controls

- Controls are *spread across the plate* to account for potential non-uniformity issues.
- *Drug-spiked NC*:
  - Potential need to float the CCP.
  - Confirms suitability of CCP during in-study phase.
- *3 reportable results* for NC and drug-spiked NC (6 wells):
  - Due to the additional importance (normalization factor).
- *Drug-spiked LPC*:
  - Demonstrates ability to confirm low ADA level.
- *MPC*: demonstrate performance across full dynamic range.

Screening CP Evaluation (SCP)

Simplified flow-scheme that should work in most cases

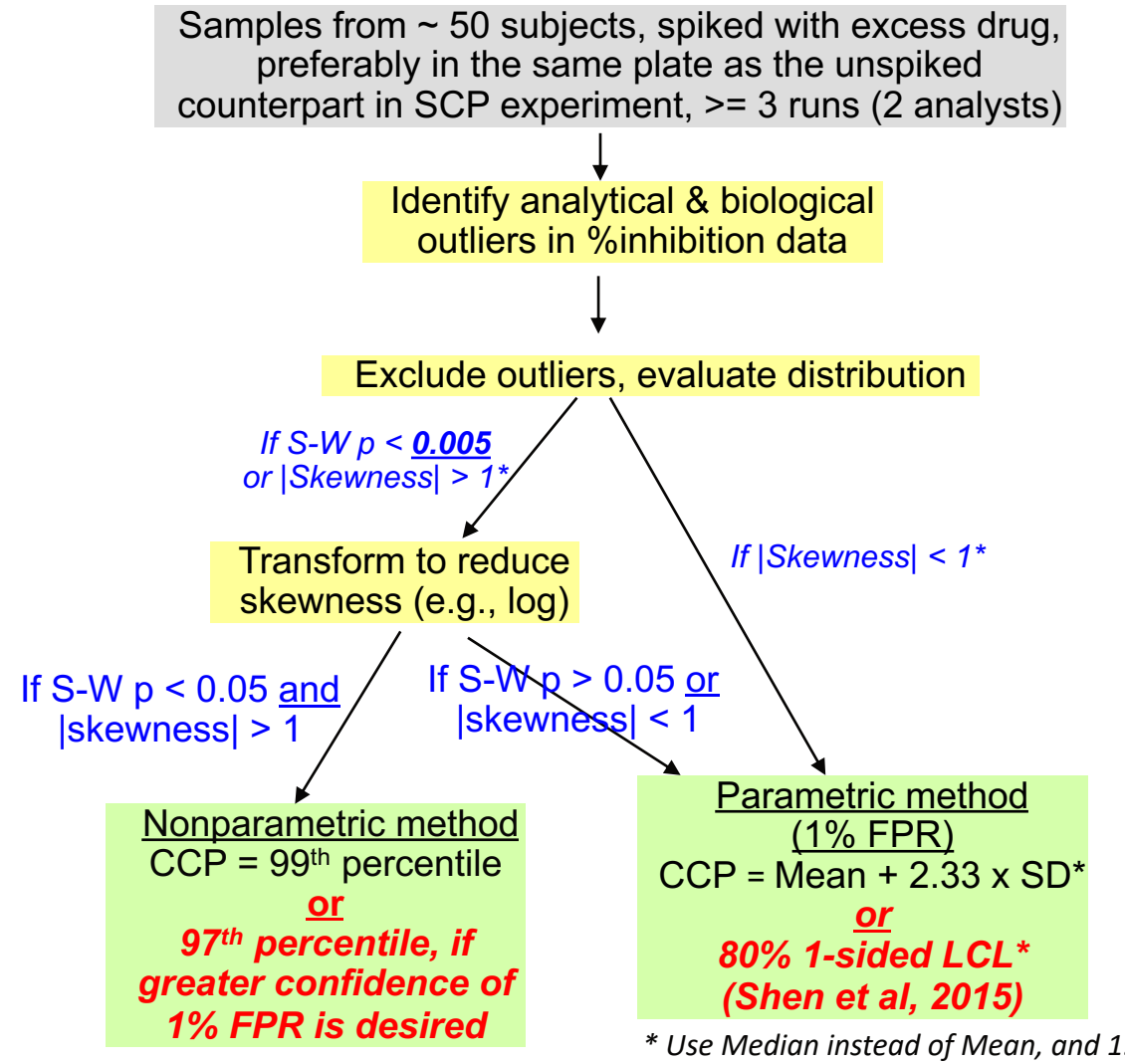


** Use Median instead of Mean, and 1.4826xMAD instead of SD to ensure robustness when there are borderline outliers.*

Devanarayan et al., 2017

Confirmatory Cut Point (CCP) Evaluation

Simplified flow-scheme that should work in most cases



If log transformation is needed, analyze log(s/us) due to negative inhibition

Evaluate inter-plate/run and Inter-analyst mean & variance differences. Also evaluate other sample factors (e.g., demographic, disease subtype, etc.)

If inter-plate/run means are significantly different, and if drug-spiked NC is correlated with subject sera, use Floating CCP

** Use Median instead of Mean, and 1.4826xMAD instead of SD to ensure robustness when there are borderline outliers.*

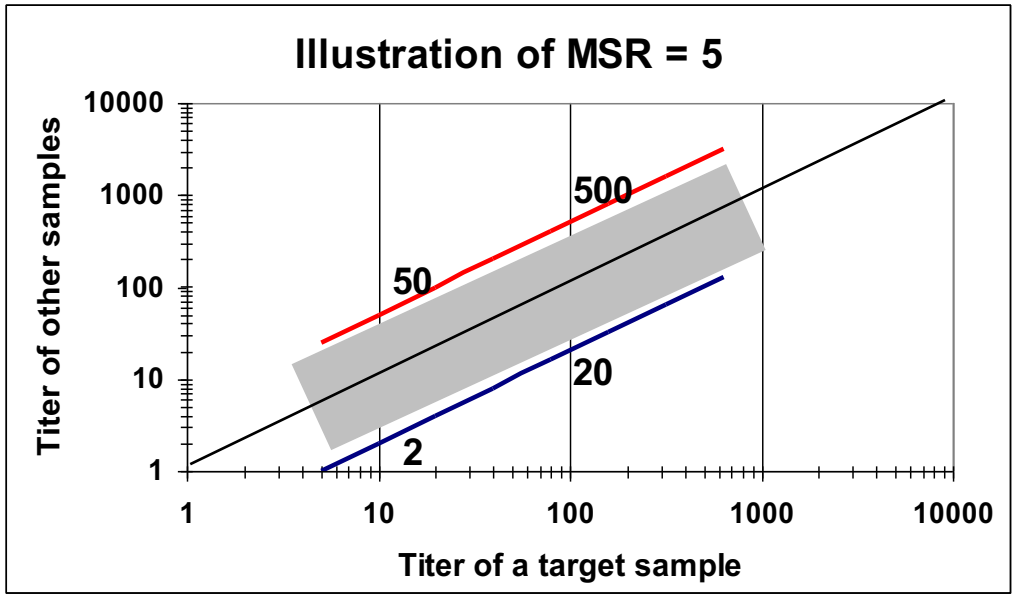
Devanarayan et al., 2017

Titer CP, Titer Precision & Treatment-boosted ADA

Precision of Titers (MSR)

Minimum Significant Ratio (MSR) *Ref: USP chapter <1106>*

- Useful for defining *Titer Precision*, and for setting threshold for *Titer CP*
- Criteria for **Treatment-boosted ADA**



Titer of a sample (x-axis) is not significantly different from samples falling in the grey area.

If MSR of titers = 5, and if pre-dose titer = 10, post-dose titer should be > 50 to be treatment-boosted ADA.

Criteria:

MSR < 3 for most assays from our experience, and is considered desirable.

Evaluation of Titer MSR

Use the data from sensitivity experiment (pre-study validation)

- 2-fold serial dilutions of HPC pools (or MPC), >= 3 runs, >= 2 analysts
- Compute titer by interpolating from each dilution curve
- >= 6 titer values (3 runs x 2 analysts)
- Evaluate *SD of log(titer)* results, for use in the formula below.

$$\text{MSR} = 10^{t_{0.05,n\_1} * \sqrt{2} * SD}$$

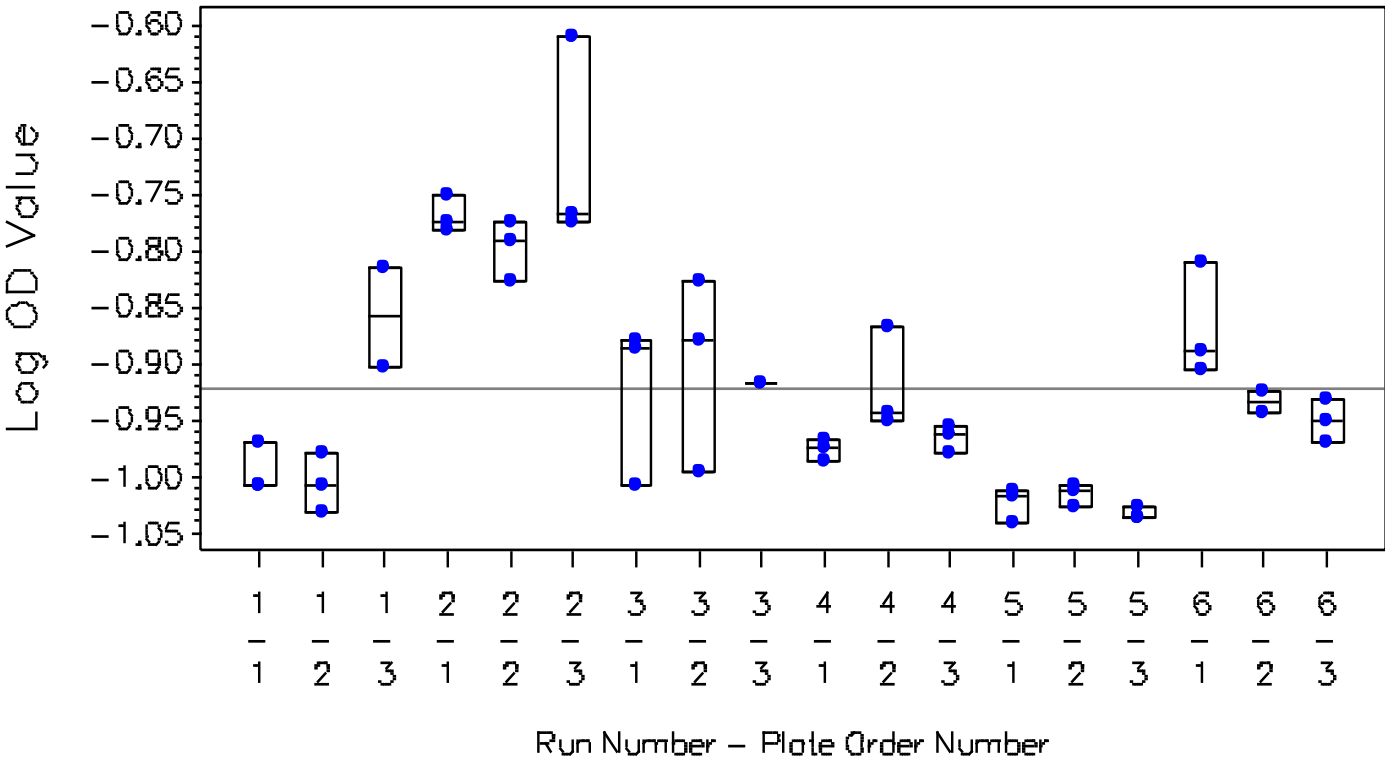
- Derived from 95% one-sided upper confidence limit of the difference of two results.
- $t_{0.05,df}$ is the two-sided t-distribution threshold for 5% error rate
- n = # of titer results
- Anti-log (10^) of the difference of log(titers) = Ratio of Titers.
- Hence this is the Minimum Significant Ratio of two titer results (T-MSR).

Where to set the Titer cut point (TCP)?

- If SCP > lower plateau of PC dilution curve, TCP = SCP
  - More likely to happen when SCP factor is high enough, (e.g., > 1.2).
- If not, samples may not dilute down to the SCP.
  - Titers may not be measurable, and will be highly noisy/variable.
  - Progressively raise TCP threshold to 99%, 99.9% and 6xSD, *until Titers are measurable & precise* (use MSR)
  - ***TCP at 99% or 99.9% upper limit works well most of the time.***
  - Alternative methods based on NC data may be used when appropriate.
- *Titer = MRD for confirmed positives that fall between SCP & TCP.*

Negative Control

Natural analytical variation (negative control pool)



This illustrates the natural analytical variation across assay plates and runs. This is just the practical reality of these data.

Normalizing the patient samples with negative control helps.

Evaluation of NQC as a normalization factor

Floating SCP (or "SCP correction factor") *assumes that NC drifts in the same direction as individual subject samples*.

- i.e., assumes that NC is correlated with subject sera.

This can be formally justified using validation data.

- Plot the NC mean versus mean of subject sera from each run/plate.
- Evaluate Slope & Rank Corr. (Need Slope close to 1, Correlation > 70%).

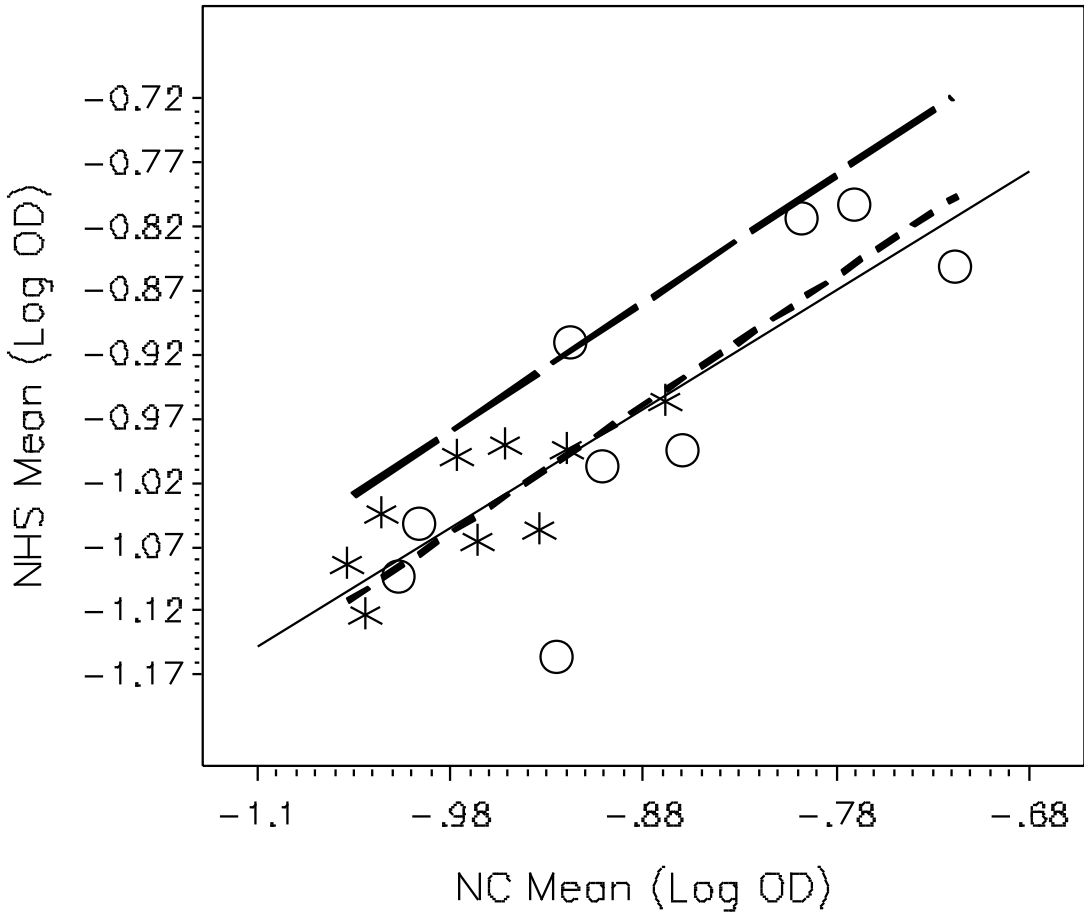
If no correlation, using NQC to normalize may not be helpful.

- More likely when analytical variability exceeds biological variability.

Alternatives:

- New pool, Diluent/buffer control, other controls (same disease/demographic), ….
- *Assess the correlation of these alternative controls with the subject sera.*

Normalizing patient sample results with negative control



Regression Equation:
NHS_Mean = −0.151008 + 0.922165*NC_Mean

Legend: Analyst 1=Circle, Analyst 2=Star

This illustrates how the negative human sera (NHS) drifts in the same direction as the negative QC.

Hence, normalization with negative QC and the use of floating cut-point correction factor is necessary in most scenarios.

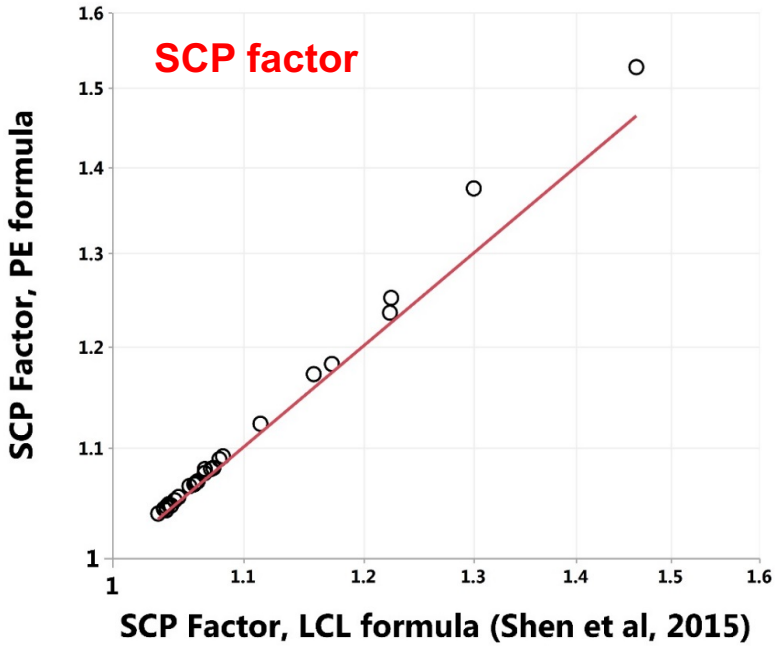
The traditional CP formula (point estimate) versus the Shen et al formula (Lower Confidence Limit)

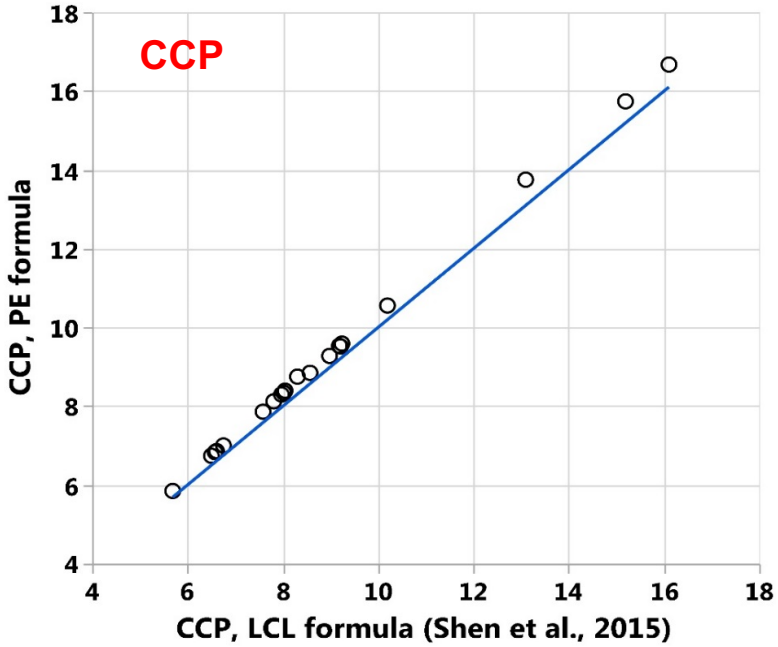
Point estimate (PE) vs. Lower confidence limit (LCL)

- Screening CP was defined to yield *approximately* 5% FPR.
- "Point Estimate" (**PE**) of 95th percentile was proposed in several white papers:
  - ***Mean + 1.645 x SD**, or its robust alternative (Median instead of Mean, 1.4826xMAD instead of SD).*
  - Implies 5% FPR around *half the time* (50% confidence)
- Shen et al (2015) proposed a **Lower Confidence Limit** (**LCL**) for the 95th percentile (SCP) and 99th percentile (CCP).
  - Defined to yield 5% FPR with 90% confidence for SCP.
  - i.e., ***90% one-sided LCL for SCP** and* 80% LCL for CCP.
- We now explore practical difference with several datasets….

SCP & CCP results from PE vs. LCL formulae

Data from ~ **25 assays** (mostly ECLs).





- CP values from the PE & LCL formulae are ***practically similar*** for most assays.
- As expected, LCL values are slightly lower, and thus the FPRs are slightly higher.
- *Differences are more visible for small sample size (e.g., early-phase in-study samples)*

In-study cut points

In-study cut points

Based on simulations, with the sampling design of Shankar et al (2008):

- FPR of SCP factor can vary between 2 to 11% (Amaravadi et al., 2015)
  - After excluding samples with pre-existing Ab.
- FPR < 2% or > 11% can trigger the need for in-study cut point.

This may occur if:

- Pre-study validation samples are not representative of the study samples
  - Differences in demographics, disease or clinical characteristics
- Changes in reagent quality or other analytical factors

Visual and statistical assessment may provide some insights.

- Box plots, comparison of means and variances (ANOVA, w/o outliers), etc.

In-study cut points

Design / data requirements & analysis

Pre-dose samples from > *50 subjects* (phase-II), > 100 subj. (phase-III)

- One reportable result per subject will suffice.
- Samples should be tested across > 3 plates/runs and >=2 analysts.
- Stratification with respect to key baseline characteristics (esp. phase-III).
- *Overall variability from these data will reflect all the relevant sources of variability (analyst, inter-run, intra-run, etc.). Therefore, it is not necessary to test the samples multiple times via a balanced design format.*

For rare disease or pediatric studies where samples are limited:

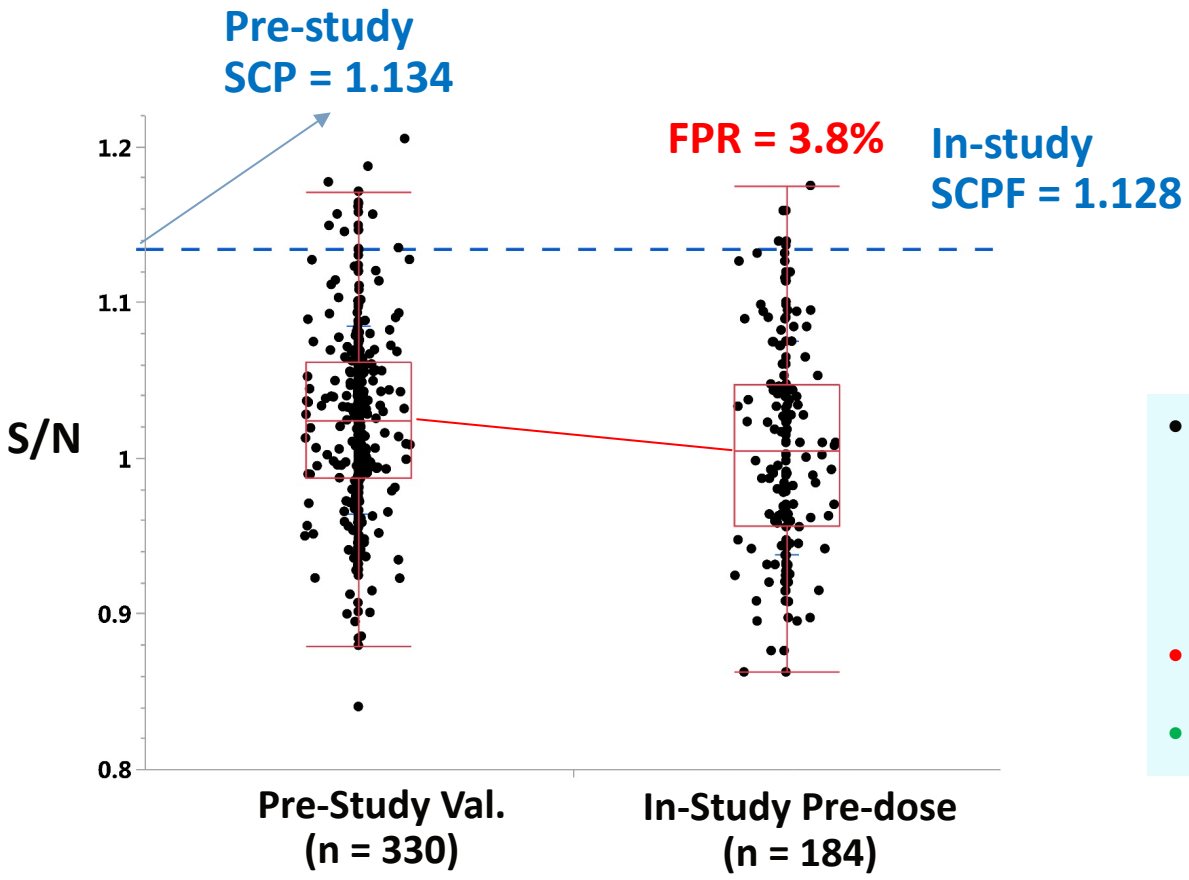
- Use SCPF as the starting point until more pre-dose samples are accrued.
- Alternatively, skip screening, and tests all samples in confirmatory assay.

CP calculation method:

- Similar process as outlined for pre-study validation (assess distribution, exclude outliers, etc.)

In-study cut points

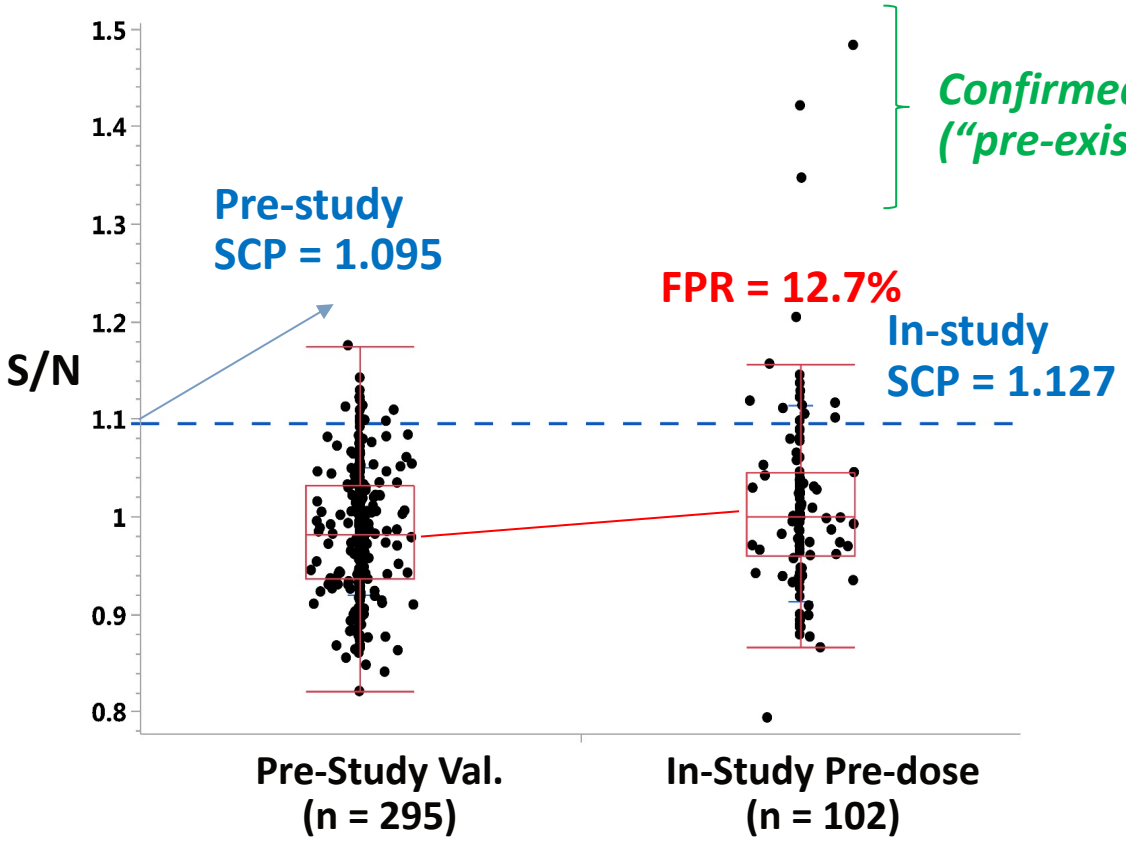
Case Study 1



- *Although distributions look similar, means & variances are significantly different (due to large "n")*
- **FPR = 3.8%**
- ***Don't need in-study CP***

In-study cut points

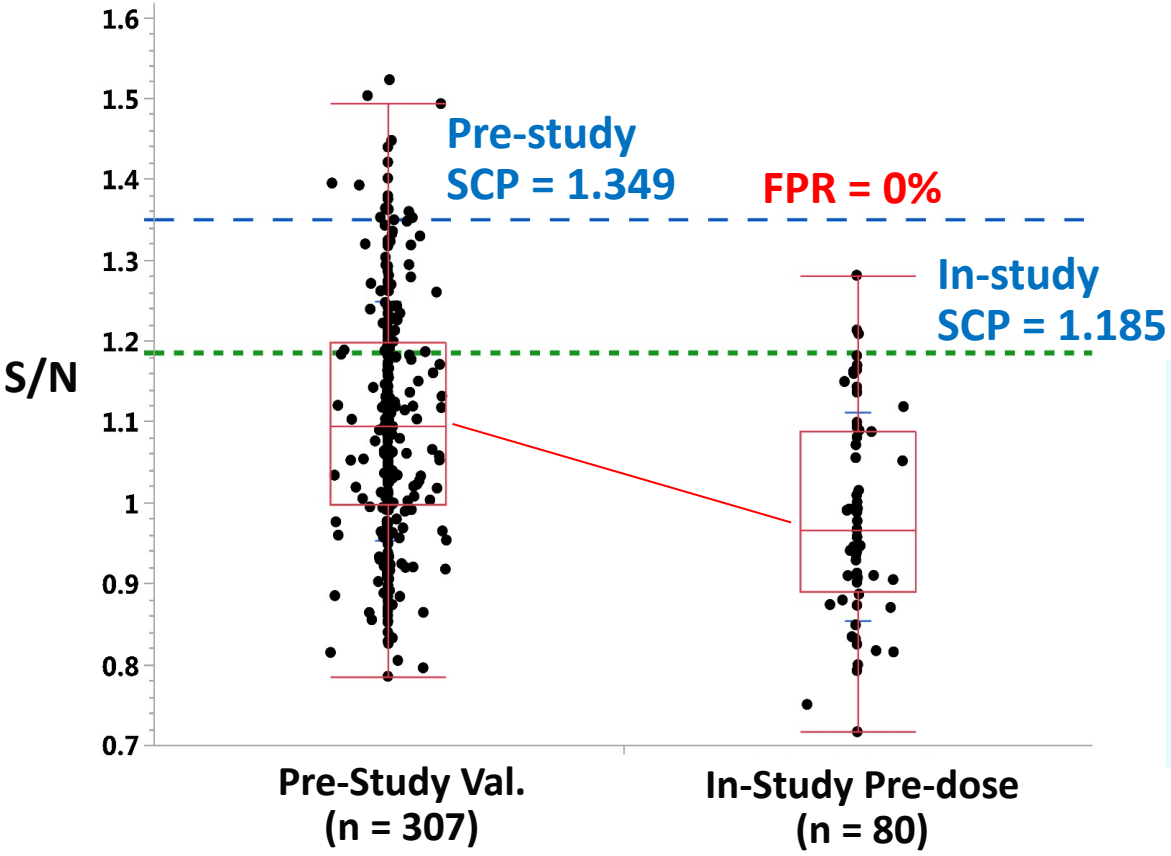
Case Study 2



- *Variances are **not** significantly different.*
- *But means are significantly different.*
- **FPR = 12.7%** *(after excluding 3 confirmed positive samples)*
- ***In-study CP can be used***

In-study cut points

Case Study 3



- *Variances are **not** significantly different.*
- *But means are significantly different.*
- **FPR = 0%**
- ***Need to use in-study CP***

Thank you for your interest & attention!

Questions ?

Break