GILEAD
Creating Possible

# Random and Fixed Effects Model for Cut Point Determination

2022 EBF - Points to Consider on Cut Points

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Atiya Taqui

Bioanalytical Chemistry, Gilead Sciences, Inc.

Foster City, CA, USA

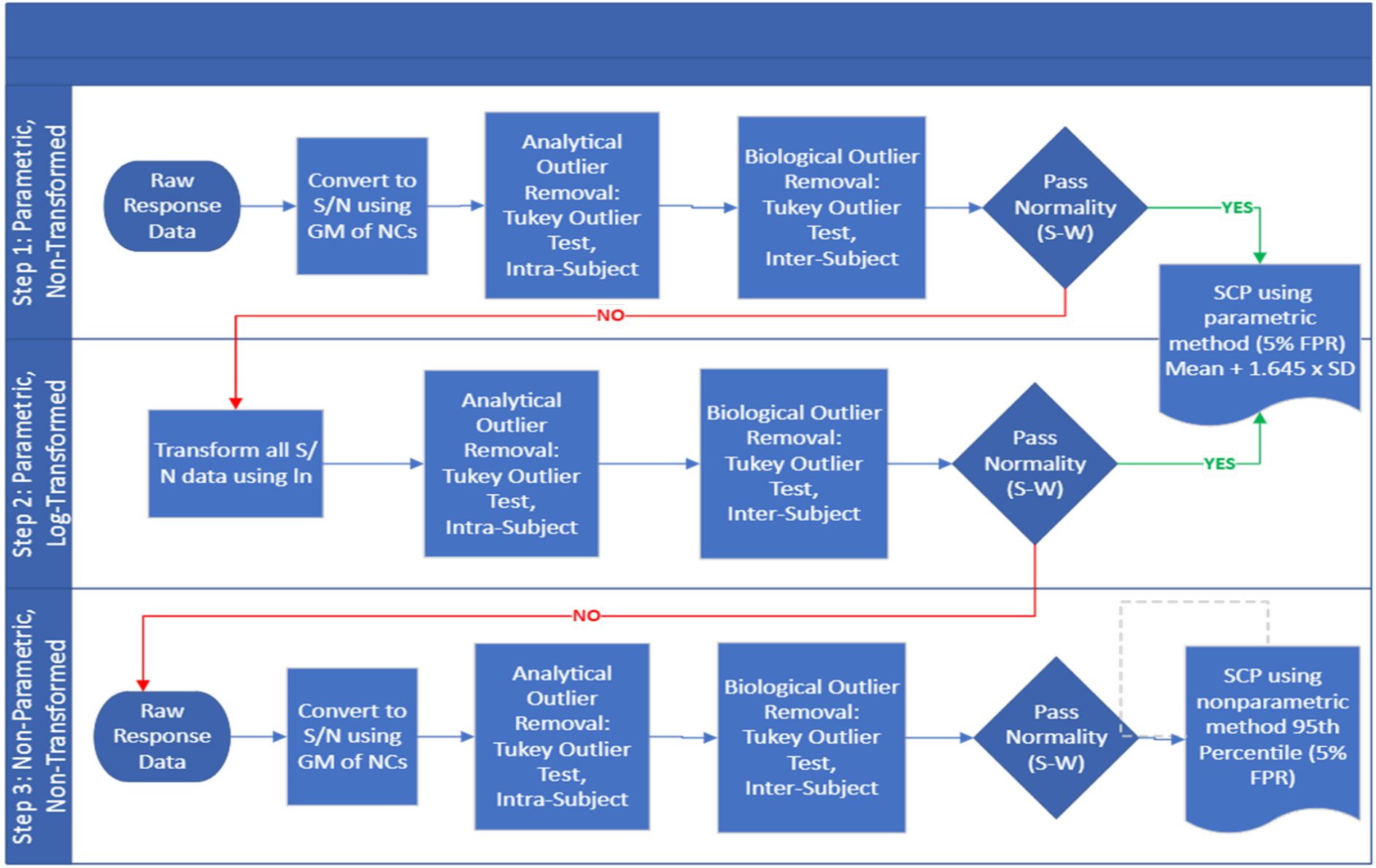
# Common Sources of Variability and Concerns for Appropriate Cut Point (CP) Calculation

- Variations in assay background
- Variability in replicate observations of the same sample
- Poor critical reagents – aggregation, non-specific binding, insufficient purification
- Commercial matrix
- Limited number of replicates for in-study CPs

# The Case Study - Overview

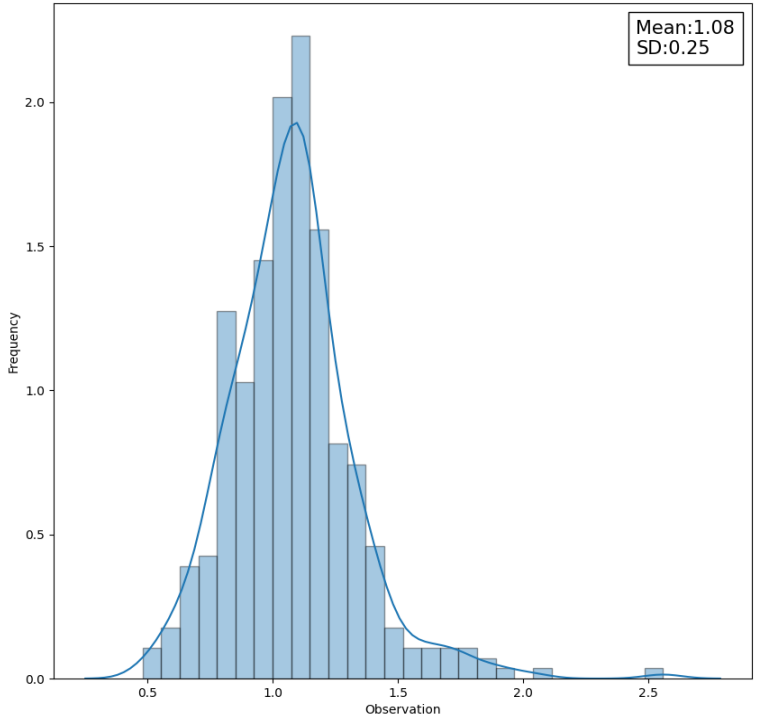
- Electrochemiluminescence (ECL) complex assay procedure with Solid Phase Extraction with Acid Dissociation (SPEAD)
- High background signal in the assay
- Cut points for normal human matrix (serum) and two different cancer type matrices were evaluated during validation
- High variability of sample response within disease populations (from commercial source) as well as normal healthy population
- In-study cut point for three different cancer type matrices was evaluated

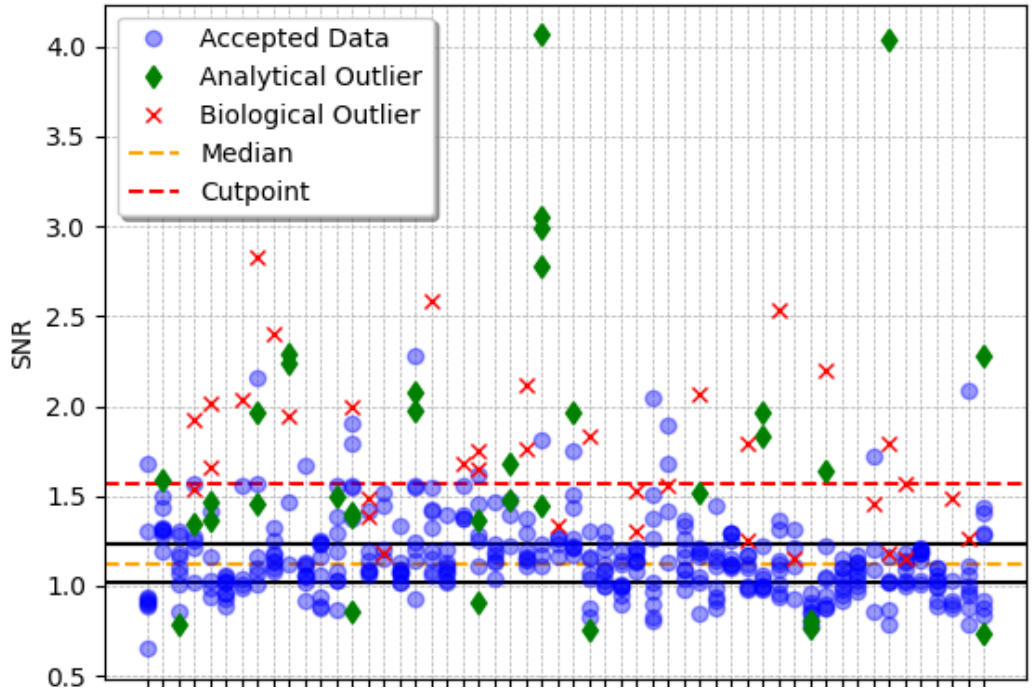
# Non-Model Approach to CP Calculation




- No NCs were removed. NC GeoMean was used (Median can also be used).
- No subjective removal of outliers was performed – all outliers were statistically justified.

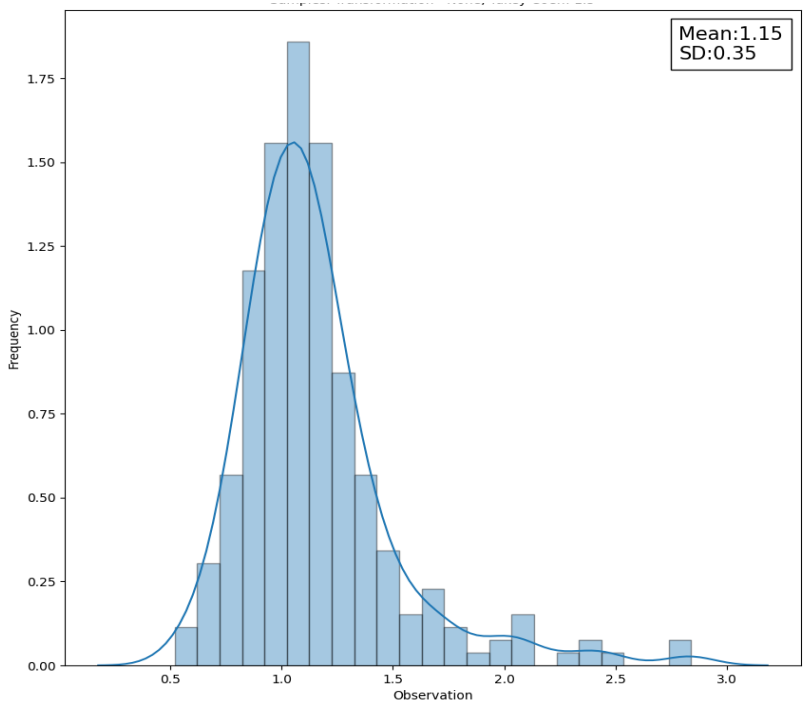
# Normal Human Matrix_CP Analysis in Validation

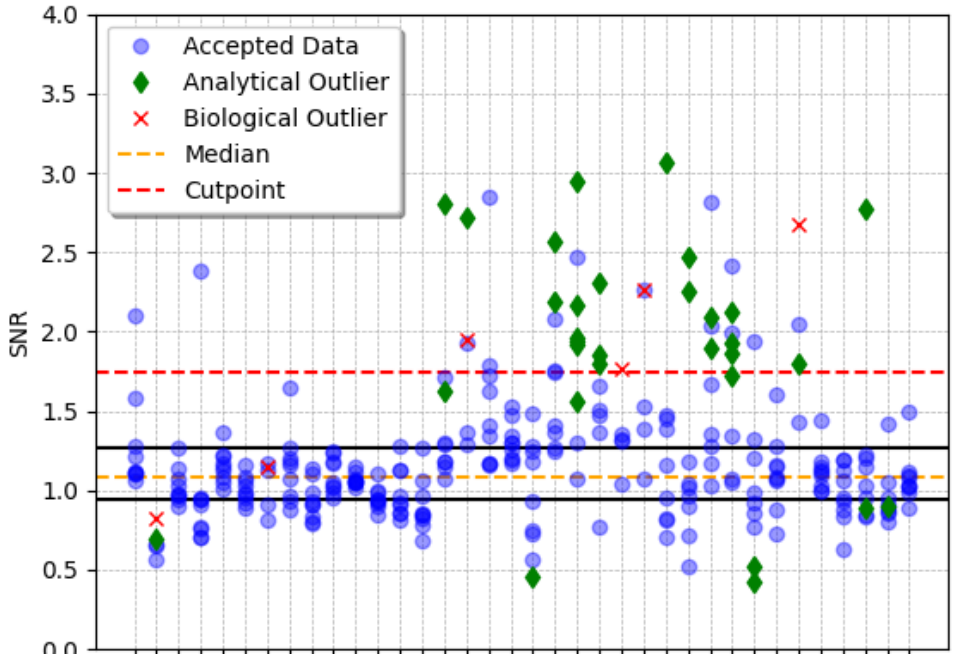



| IQR | CP (SNR) | Biological Outliers (No. of Subjects) | Analytical Outliers (No. of Data Points) | Total Data Points Used for CP Calculation | Normality Test (S-W) |
|---|---|---|---|---|---|
| 1.5 | 1.48 | 4 | 28 | 380 out of 443 (50 subjects) | Failed |
| 2.0 | 1.55 | 3 | 17 | 399 out of 443 | Failed |
| 3.0 | 1.59 | 3 | 10 | 406 out of 443 | Failed |

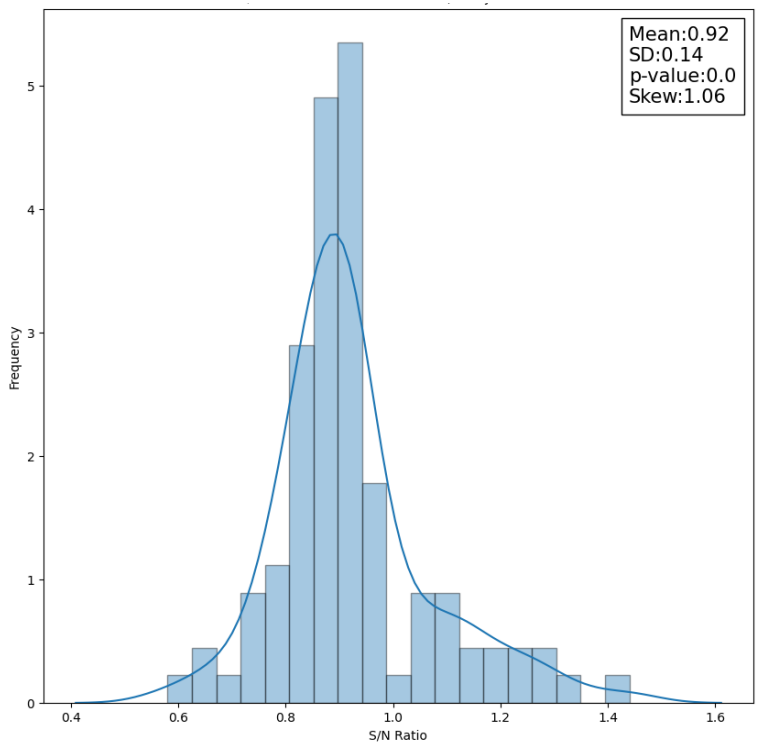
# Cancer Type 1 Matrix_CP Analysis in Validation

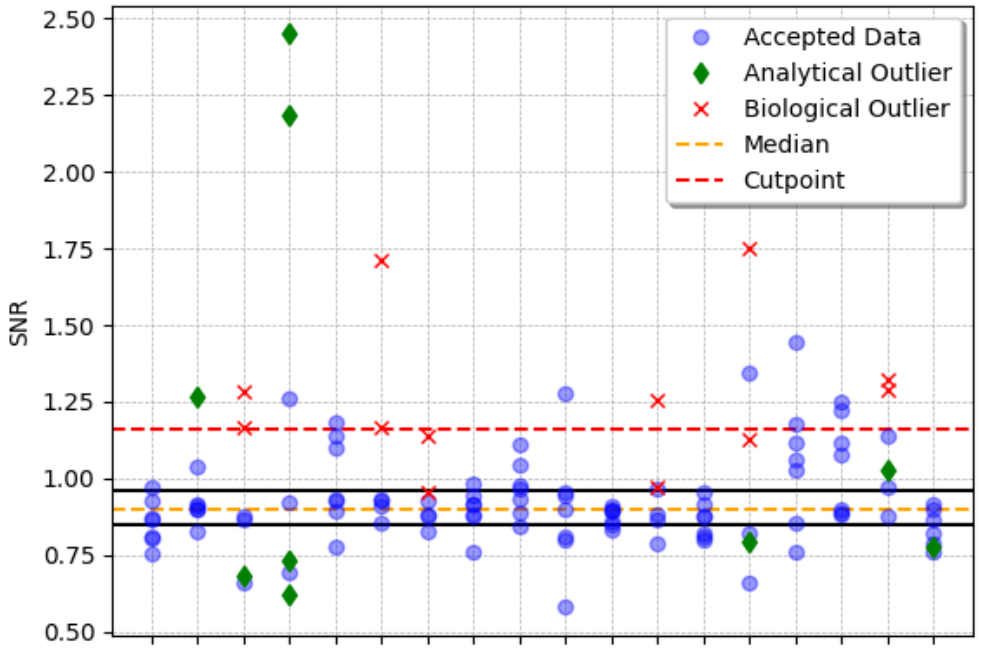



| IQR | CP (SNR) | Biological Outliers (No. of Subjects) | Analytical Outliers (No. of Data Points) | Total Data Points Used for CP Calculation | Normality Test (S-W) |
|---|---|---|---|---|---|
| 1.5 | 1.79 | 1 | 33 | 261 out of 300 (50 subjects) | Failed |
| 2.0 | 1.94 | 1 | 27 | 267 out of 300 | Failed |
| 3.0 | 2.09 | None | 17 | 283 out of 300 | Failed |

# Cancer Type 2 Matrix_CP Analysis in Validation




| IQR | CP (SNR) | Biological Outliers (No. of Subjects) | Analytical Outliers (No. of Data Points) | Total Data Points Used for CP Calculation | Normality Test (S-W) |
|---|---|---|---|---|---|
| 1.5 | 1.16 | 2 | 10 | 98 out of 120 (20 subjects) | Failed |
| 2.0 | 1.28 | 2 | 6 | 102 out of 120 | Failed |
| 3.0 | 1.64 | None | 2 | 118 out of 120 | Failed |

# Summary of CP Analysis in Validation

- Disease state has impact on CP value determined
- High variability between different runs/panels (analytical variability)
- Relatively high percentage of outliers removed
- Analyst-dependent variability was observed and confirmed with ANOVA test
  - Inability to identify which specific analyst data to remove from the data set due to high variability across entire study

*Is there a statistical way to overcome the different sources of variability observed during validation?*

# Thinking Outside of the Box-Plot

General Random Effects Model (REM): $Y_i = \mu + U_i + W_i$

where:
$Y_i$ = S/N values
$\mu$ = average response of entire population
$U_i$ = random effect model on the Gaussian distribution
$W_i$ = individual-specific random error term

REM with Control for Fixed Effect (FE): $Y_i = \mu + \beta_{FE} + U_i + W_i$

where:
$\beta_{FE}$ = fixed-effect terms for assignable parameter (known effect)

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Bioanalysis

Methodology

## Statistical methods of screening cut point determination in immunogenicity studies

Meiyu Shen [1,*] and Tianjiao Dai [2]

"The proposed approach of cut point calculation based on REMs can more appropriately identify the variance components in ADA data from a pre-study experiment design"

"Under current practices, the proposed REM statistical approach, may be helpful in alleviating the issue of pre-study and in-study design differences"

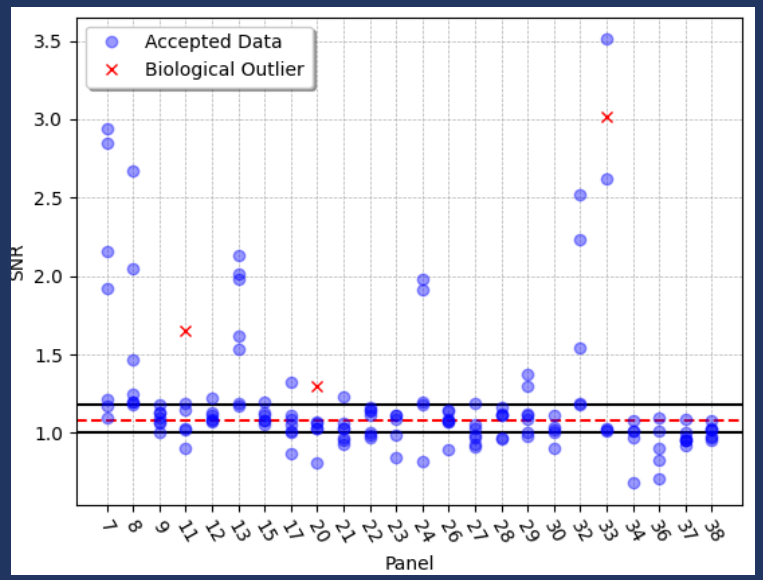
# What is a Random Effects Model (REM)?

- A statistical approach that is able to control for unassignable parameters that lead to variability within the data set, also known as random effects.
- REM can be combined with fixed effects (FE), which are known (assignable) effects that lead to variability in the data set.
- Benefits of REM
  - Avoid unknowingly removing true variability of the population
  - Use modeling to remove impact of random sources of variability that might not have a specific root cause

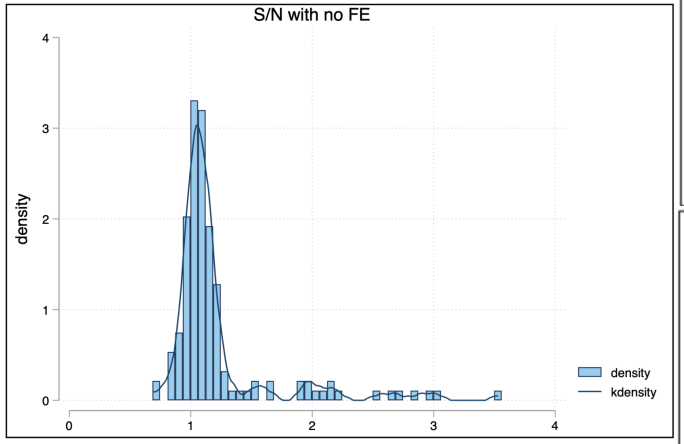
# In-Study Cut Point Run Design

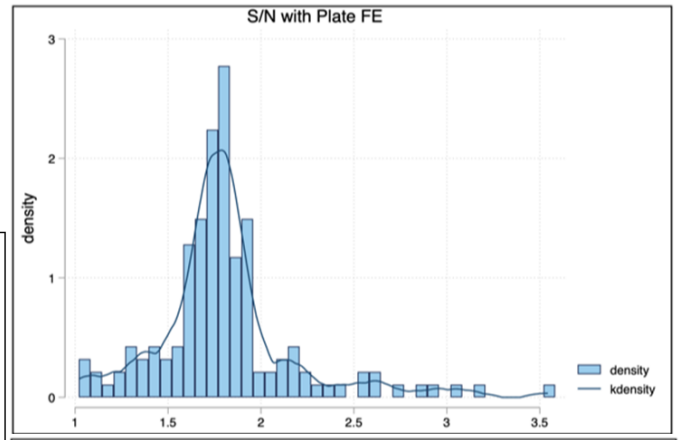
- ≥50 pre-dose study samples
- n=3 replicates (instead of n=6) due to insufficient study sample volume
- ≥ 2 analysts, over ≥ 2 days
- Cut point in matrices from 3 different cancer type populations were evaluated using Random and Fixed Effect Model (R&FEM)

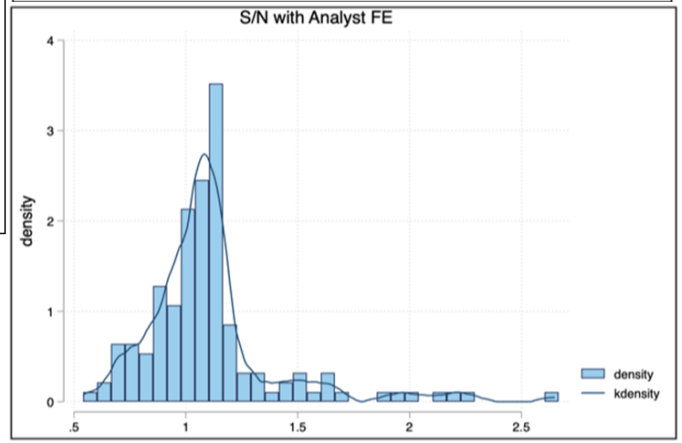
# In-Study CP Analysis for Cancer Type 1 Matrix




Due to high variability, no analytical outlier was statistically determined using Tukey Outlier Test (IQR 1.5 to IQR 3).



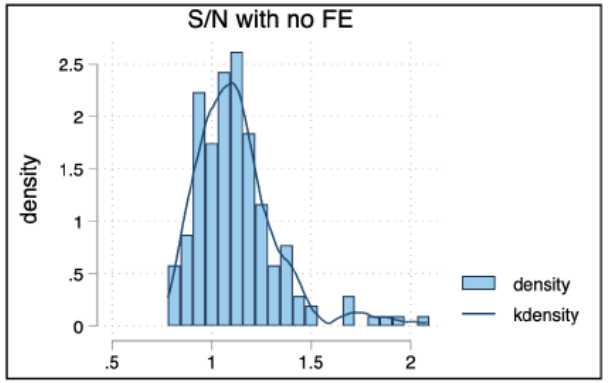

| Statistical Analysis | Data Points | Normality Test | Cut Point (SNR) |
|---|---|---|---|
| Non-model | 147* (50 subjects) | Failed | 2.15 |
| REM with no FE | 150 | Failed | 2.33 |
| REM with Plate FE | 150 | Failed | 2.61 |
| REM with Analyst FE | 150 | Failed | 1.68 ✓ |

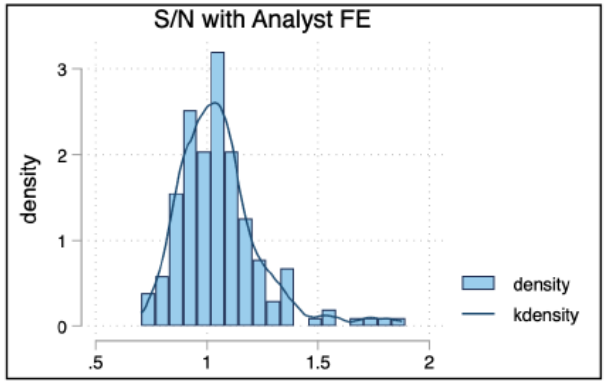
*1 Biological Outlier

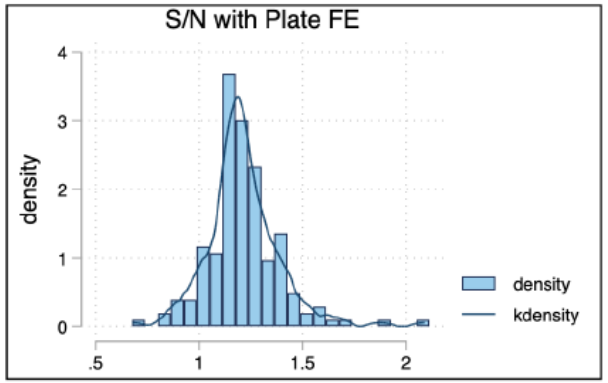
# In-Study CP Analysis for Cancer Type 2 Matrix

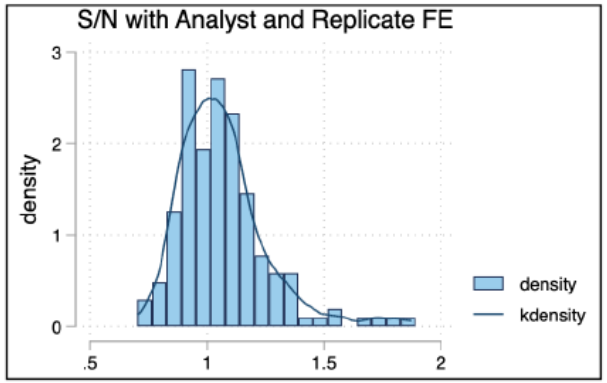
| Replicate | Frequency |
|---|---|
| 1 | 50 |
| 2 | 49 |
| 3 | 45 |

- One individual was depleted after the first replicate was run.
- Four individuals were depleted after the second replicate was run.



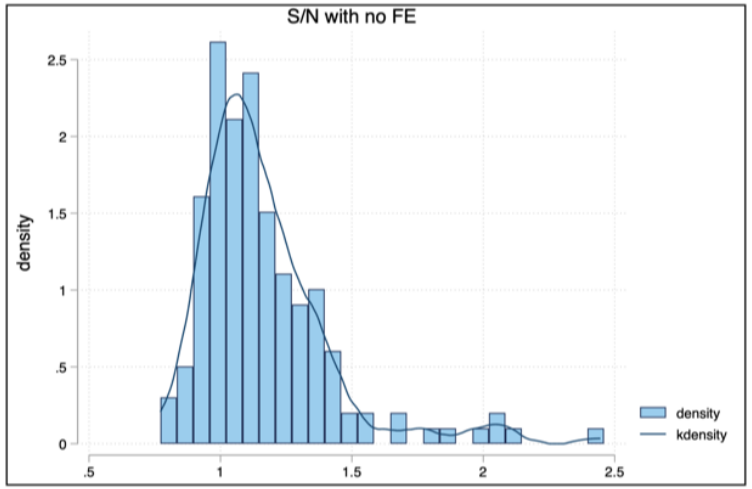



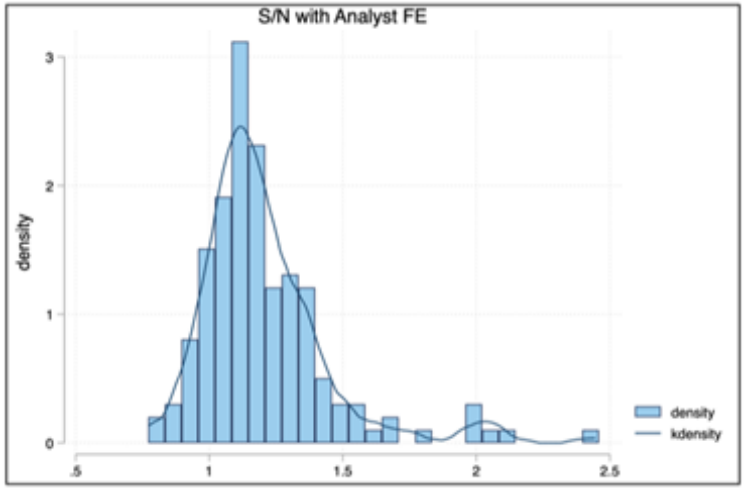



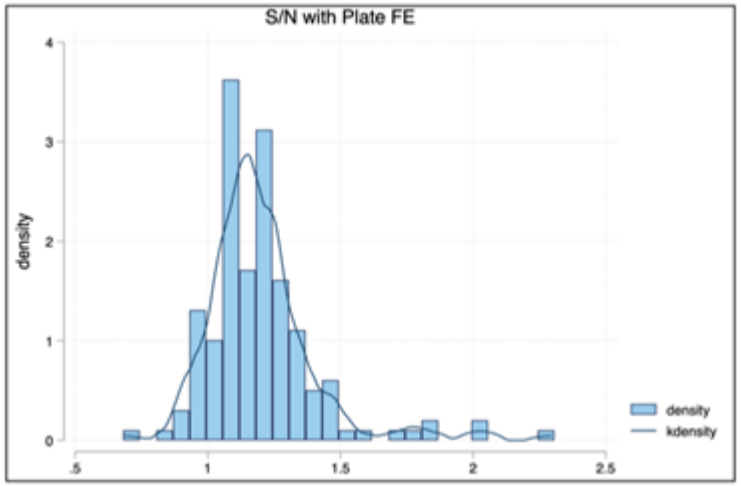




| Statistical Analysis | Data Points | Normality Test | Cut Point (SNR) |
|---|---|---|---|
| REM with no FE | 150 (50 subjects) | Failed | 1.47 |
| REM with Plate FE | 150 | Failed | 1.49 |
| REM with Analyst FE | 150 | Failed | 1.37 |
| REM with Analyst and Replicate FE | 150 | Failed | 1.37 ✓ |

# In-Study CP Analysis for Cancer Type 3 Matrix




| Statistical Analysis | Data Points | Normality Test | Cut Point (SNR) |
|---|---|---|---|
| REM with no FE | 159 (53 subjects) | Failed | 1.70 |
| REM with Plate FE | 159 | Failed | 1.56 |
| REM with Analyst FE | 159 | Failed | 1.70 ✓ |

# Conclusions

- REM with one or more fixed effect was the appropriate statistical approach for cut point calculation for the disease state matrices in this ADA assay.
- With data modeling, outliers do not need to removed from the data set and variability within the data set can be controlled for unassignable and assignable parameters.
- REM with or without fixed effects can be considered and accepted by regulators for data sets with too low or too high variabilities.

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