

Robust Bayesian model for the determination of the confirmatory cut point during the immunogenicity assessment

October 22-24, 2025



Disclaimer

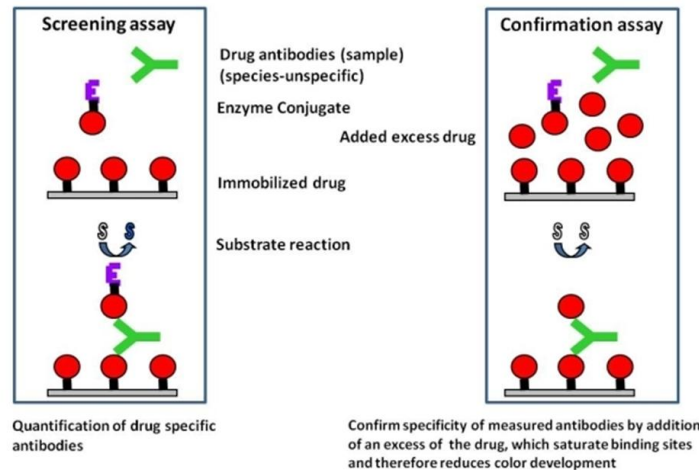
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Immunogenicity introduction

- Unwanted immunogenicity is an immune response by an organism against a therapeutic antigen. This reaction leads to production of **anti-drug-antibodies (ADAs)**, inactivating the therapeutic effects of the treatment and potentially inducing adverse effects.
- Cut point determination is an essential tool for immunogenicity assessment for discriminating samples with ADAs from those without ADAs.
- A confirmatory assay is performed with and without excess drug. Typically, the percent inhibition is modeled to evaluate a confirmatory CP (CP) which separates positive from negative samples.
- Although percent inhibition is a ratio, it is usually assumed to follow a normal distribution...

<http://www.elisa-entwicklung.com/assay/41-ADA.htm>

Principle of a HS sandwich ELISA

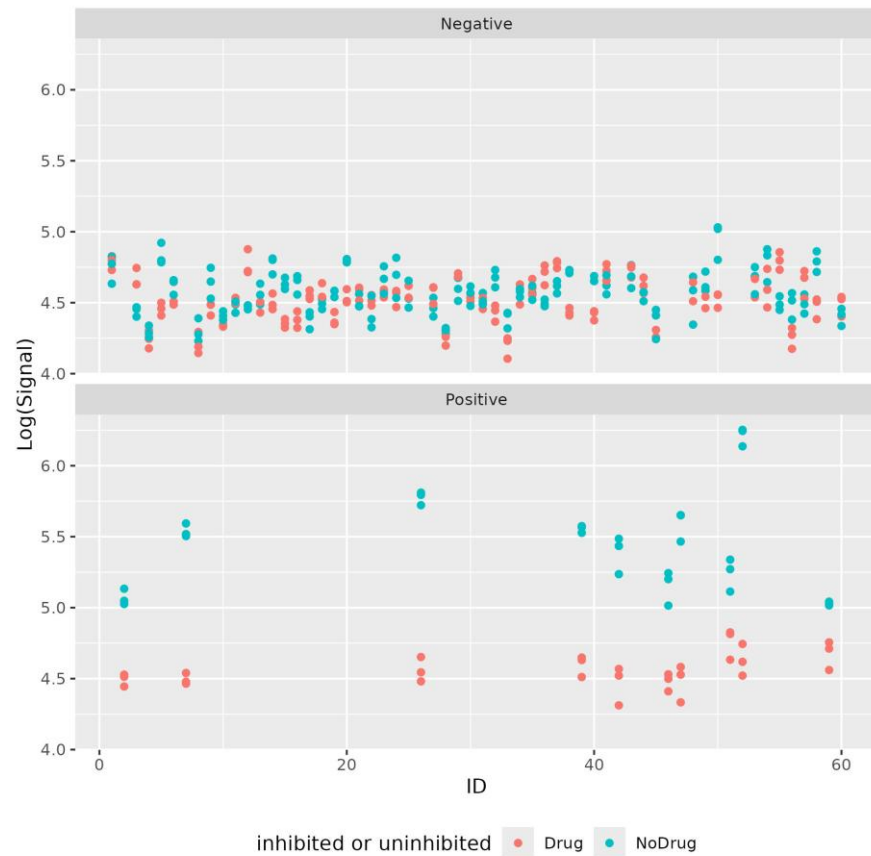


Sensitive

Specific

Uninhibited and inhibited signals

1. Plot represents the log(signals) for positive and negative samples
2. Uninhibited signals (Blue)
 - Correspond to screening assay
 - Positive samples are larger compared to negative samples
3. Inhibited signals (Red)
 - Are similar for both positive and negative samples
4. Naïve population (aka negative samples) is expected when determining cCP
5. If not, usual methodologies includes outlier tests to remove positive samples
6. Practically, this is impossible to avoid some positive samples.



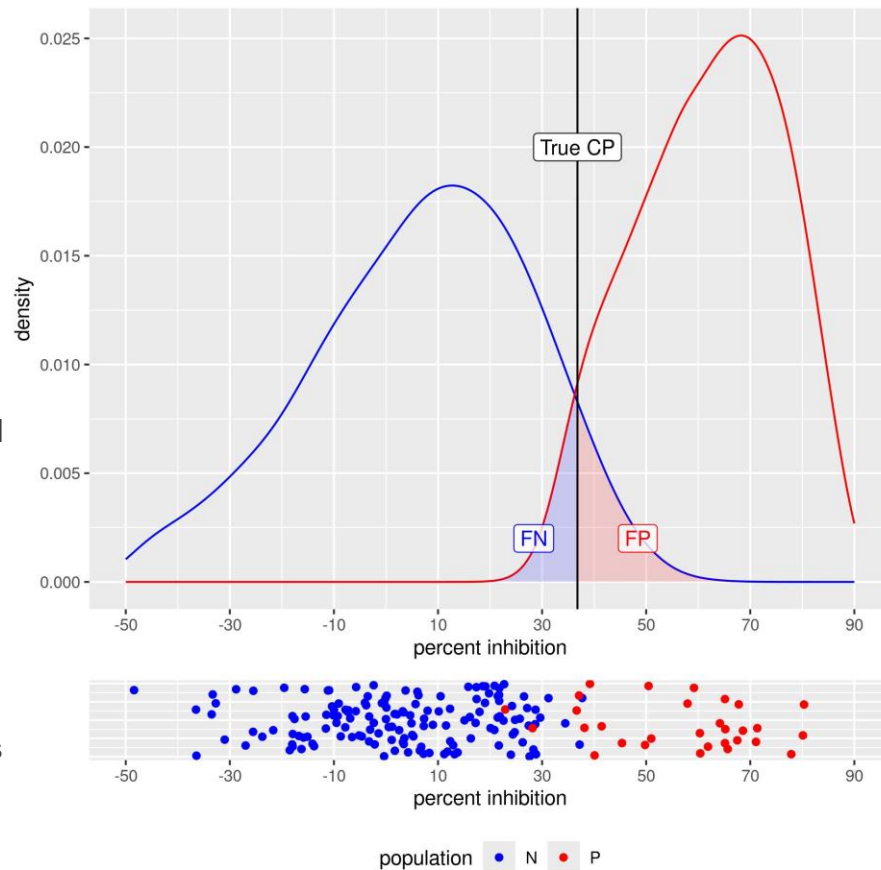
Confirmatory assay - percent inhibition

1. $\% \text{ inhibition} = 100 * \left(1 - \frac{\text{inhibited signal}}{\text{uninhibited signal}}\right)$

2. For negative samples,

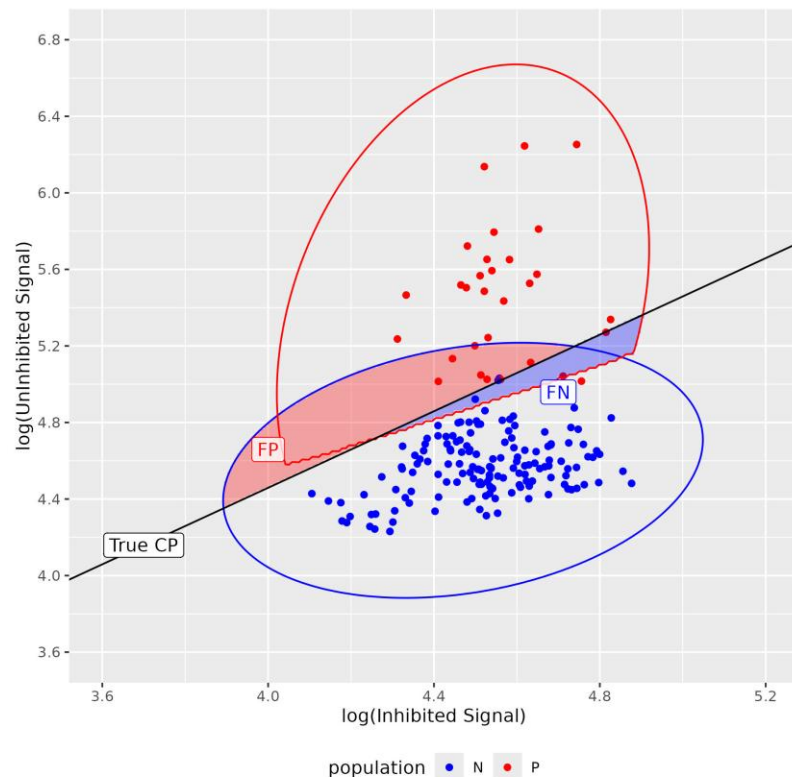
$\frac{\text{inhibited signal}}{\text{uninhibited signal}}$ is a log normal

3. No theoretical distribution for percent inhibition. Bayesian approach makes possible to propagate uncertainty to the percent inhibition distribution from distributions of inhibited and uninhibited signals.
4. Positive population is usually very diversified by nature and often difficult to characterize with a distribution.
5. Objective of the assay is to determine the confirmatory cutpoint (CP) that separate positive from negative population.
6. If distribution parameters used for simulating data are known, the true value of CP is known.
7. On the top plot, the densities of the theoretical distributions are plotted. On the bottom plot, some samples (from positive and negative populations) are drawn.



Confirmatory assay can be analyzed using a bivariate distribution

1. The two signals are correlated at patient and plate levels.
2. On the plot, the densities of the theoretical distributions are plotted
3. As in the previous slide, the distribution of the positive population is difficult to characterize. (here a truncated distribution is used).
4. True CP is obtained using distribution of negative samples



Determination of CP for confirmatory assay **with real data**

Calculation of CP assumes that the samples are negative (naïve patient), but this is rarely the case

Therefore, analyses account for it and generally use 2 steps.

1. Preliminary analysis: Outlier tests are commonly used to remove (extreme) positive samples
 - Depending on the outlier removal methodology, results may vary.
 - Practically, some positive samples usually remain after outlier removal
2. CP determination: A model on the whole population that is evaluated as negative
 - Tolerance intervals (beta-expectation or beta-content gamma-confidence) are used as CP

Alternatively, robust fit can be used as an alternative to outlier tests. Parameter estimates would be less sensitive to outliers.

Therefore, no preliminary preliminary outlier test (single step approach)

Determination of CP for confirmatory assay **with simulated data**

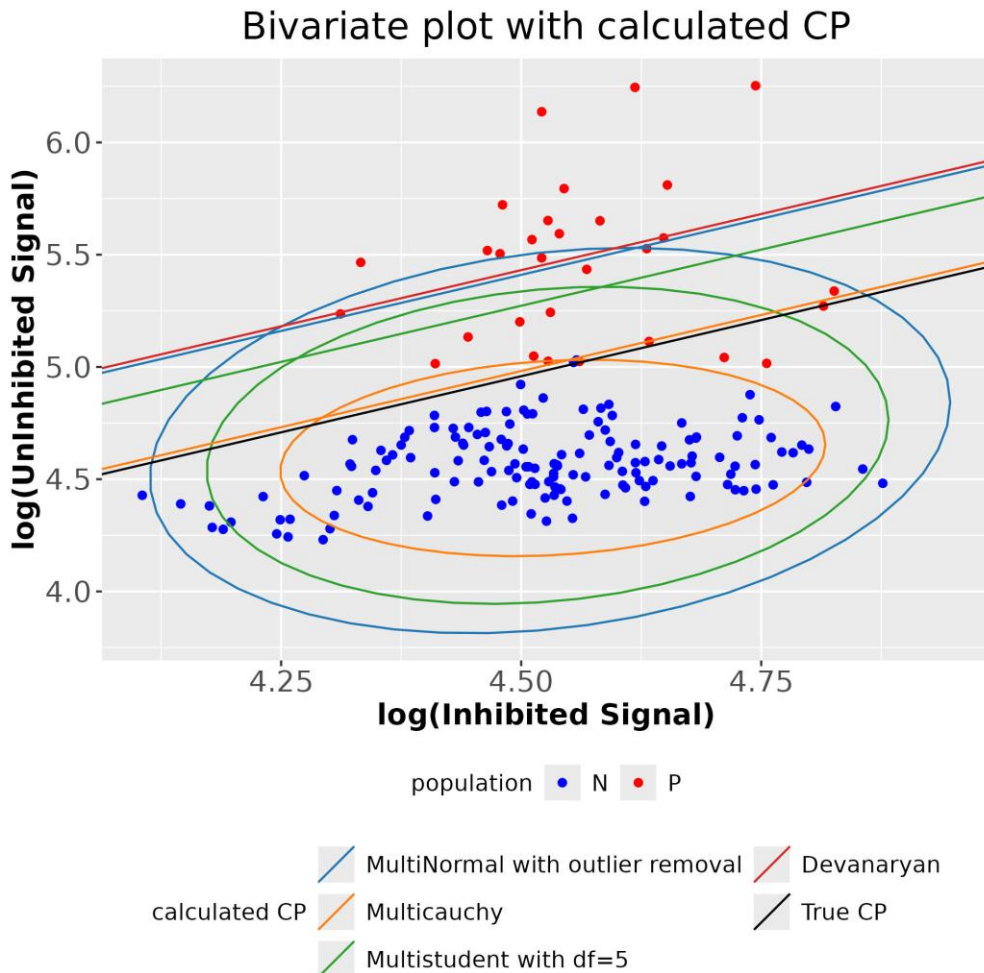
Advantages

- **We can benchmark different outlier tests and CP calculation methods and compare new approach**
- Positive and negative samples are identified. True value of the CP is known.
- FPR and FNR can be calculated.
- Effect of sample size can be evaluated

Results

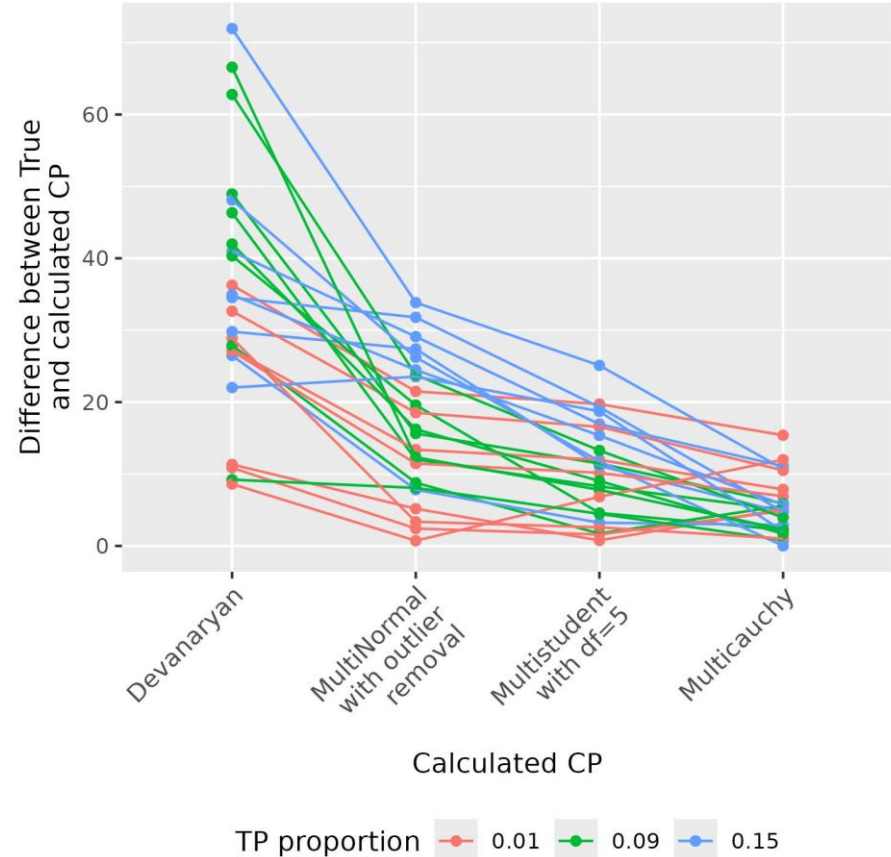
- Data are represented as bivariate plot
- Different CP methods are compared
 - Multistudent (df=5, robust)
 - Multicauchy (df=1, expected to be very robust)
 - Devanaryan is the reference standard method
- Ellipse are plotted to represent densities of the resulting distribution of the fit
- CP are represented as lines

Calculation method	CP
Multinormal with outlier removal	59.8
Multicauchy	38.2
Multistudent with df = 5	53.8
Devanaryan	60.6
true CP	36.8



A few datasets were simulated using a bivariate mixed model

- Although some other covariates are present across the simulated datasets, only the proportion of outliers is represented (in colors).
- Y axis is the difference between true CP and calculated CP
- Lines join individual datasets
- Although a clear impact of outlier proportion is present, robust model is less impacted



Conclusions: Strategies for the determination of CP for confirmatory assay

Analysis using the percent inhibition

- Less- or un-characterized distribution.
- Loss of information (underlying relationship between the inhibited and uninhibited signals).
- Assumes independence between the two signals.

Analysis using multivariate distribution

- Captures the correlation between the inhibited and uninhibited signals.
- Several distributions are available, from which robust fit demonstrate lack of influence of outliers.
- Optionally, hierarchical models (including several layers of random effects) can be used.S