RED THREAD®
by Ariadne Software

Anti-Drug Antibody Cut Point, Sensitivity and LPC Determination

ariadnesolutions.com

PROBLEM: With the use of biologics on the rise, unwanted immunogenicity has become a grave concern. However, the nascency and complexity of biologics, the technical challenges of the methodologies used to measure immunogenicity, and the lack of prescriptive guidance by the FDA compound the challenges. Varied approaches for determining cut points brings about subjective interpretation of the available guidance, thus often resulting in confusion. Adding to this uncertainty is the lack of transparency within the bioanalytical group. While the data is generated by the bioanalysts, the analysis to determine the cut point is performed by statisticians, who have little to no knowledge of the experimental design. This may result in "over-scrubbing" of the data through the removal of data points as outliers when in fact these data points should not be excluded. Conversely, you may have non-statisticians performing the data analysis who possibly lack the background experience to perform the analysis consistently and within the appropriate mathematical constraints. Lastly, increased uncertainty is introduced as a result of inconsistent approaches to data analysis and outlier removal within an organization, or even within a bioanalytical group. The above-mentioned challenges are apt to yield unwanted skewness in the resulting data.

RED THREAD SOLUTION: Ariadne's Red Thread® uses bioanalytical expertise, regulatory guidance, and industry best practices to help you address these issues with cut point analysis for immunogenicity / ADA studies. Compliant with the FDA and EMA guidance, Red Thread brings consistency and standardization of data while providing transparency and avoiding excessive or restrained outlier removal.

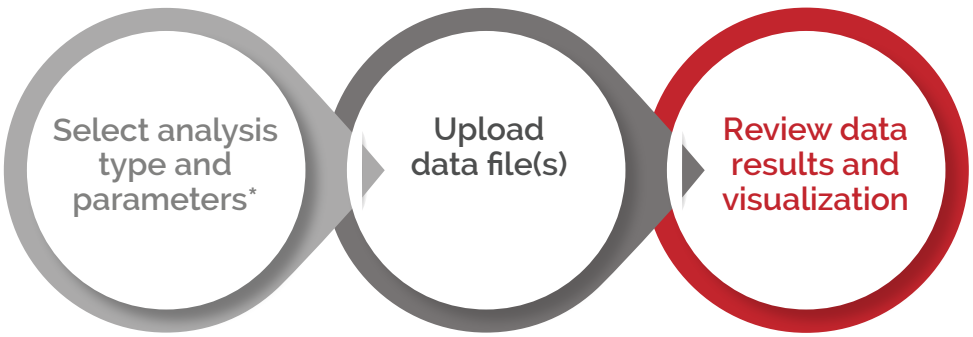
Designed for bioanalysts, statisticians and regulators, our software can help with:

- Screening, confirmatory, and titer cut point determination
- Screening and confirmatory sensitivity analysis
- Screening and confirmatory low positive control (LPC) determination

VALUE:

- **Expertise:** Expertise from multiple subject matter serves as a force multiplier for your team.
- **Efficiency:** Improve workload management and increase efficiencies.
- **Time and cost savings:** With only minutes to run your data sets and calculate the cut point, sensitivity and LPC, save time and resources with no training required.
- **Consistency and Objectivity:** Bring a robust and consistent approach to your group and organization with every report.
- **Transparency:** Bring increased oversight and meticulous removal of outliers while facilitating transparency between bioanalysts and statisticians.
- **Compliance:** Red Thread complies with FDA and EMA guidance as well as industry best practices.

HOW IT WORKS



*Determine your cut point in 3 simple steps. *Prompted user field selections.*

ARIADNE® SOFTWARE

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CUT POINT DETERMINATION

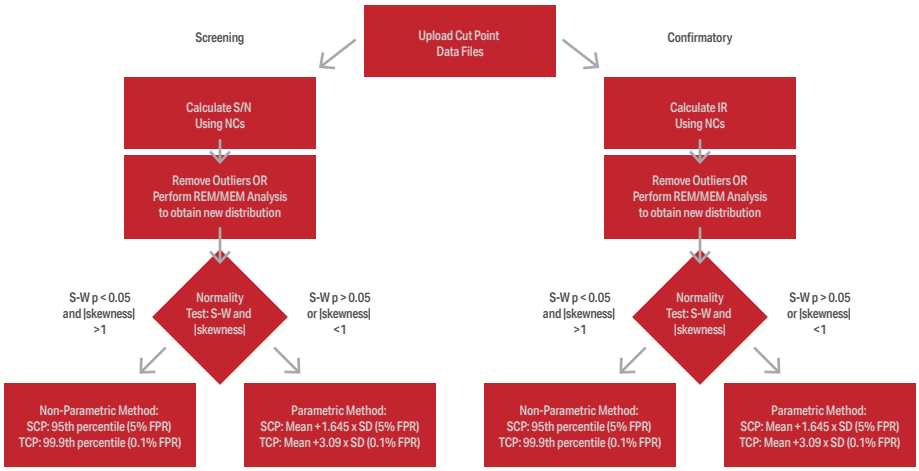
Outlier Removal

Using the Tukey Outlier test, Red Thread determines analytical (intra-subject) and biological (inter-subject) outliers at varying Interquartile Ranges (IQR) from 1.5 to 3. This workflow is the more common industry practice.

Random/Mixed Effects Model

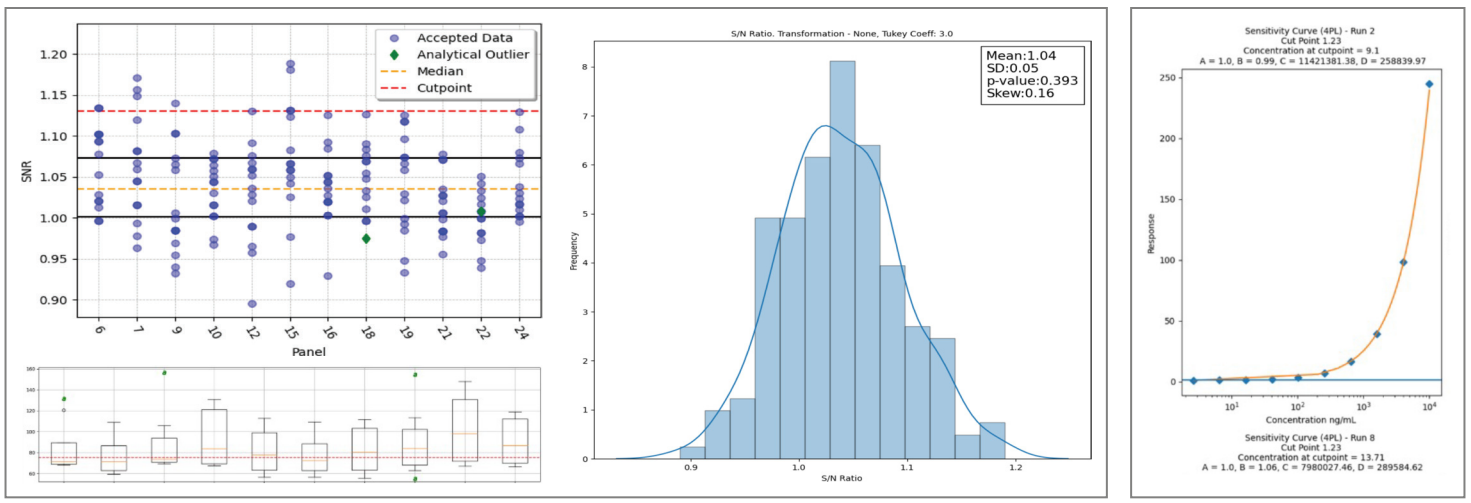
Random/Mixed Effect Model (REM/MEM) accounts for analytical and biological variability with an all-inclusive dataset using statistical modeling. While REM addresses unobserved heterogeneity in the response of each individual subject within the data set, an MEM approach utilizes the combination of REM with assigned Fixed Effects (FE), which accounts for variability induced by sample plates, analysts, collection dates, collection times, and more.

Following outlier removal or the treatment of data using REM/MEM, the normality of the data is assessed using the Shapiro-Wilk test. The results from the normality test, along with a measurement of the skewness of the population data, are then used to determ e level. Under the nonparametric approach, where the population follows a non-normal distribution, the cut point is calculated based on a percentile calculated directly from the data distribution and the level of confidence.



SENSITIVITY AND LOW POSITIVE CONTROL (LPC) DETERMINATION

Prior to completing the remaining validation parameters, CP determination is followed by the confirmation of the sensitivity of the assay as well as the determination of the appropriate LPC concentration in order to meet regulatory requirements and industry best practices. The LPC should be calculated contemporaneously to avoid any additional work that would be required if a new LPC concentration had to be determined after the full validation was completed. Providing the sensitivity for both, the screening and confirmatory tiers, allows for the potential differences in sensitivity of both tiers to be fully visible (i.e. "Should we have separate screening and confirmatory LPC values?"). Sensitivity is typically determined using either a four-parameter logistic (4PL) or five-parameter logistic (5PL) regression and the LPC is calculated at a 99% upper confidence level using a t-distribution function.



Red Thread's data visualization facilitates uncompromised decision making. The histogram, box plot, and scatter plot represent cut point data (left), and line graph represents sensitivity data (right).