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a Paper created with KCAS Bioanalytical & Biomarker Services

Artificial Intelligence increases efficiencies and reduces bias for bioanalytical pharmacokinetic studies and anti-drug antibody statistical determinations

CRO automates bioanalytical data review and statistical data generation to meet sponsor deadlines and provide high quality data

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ABSTRACT:

Artificial Intelligence (AI) and advanced computational techniques are increasingly serving as the primary choice for determining data accuracy and reviewing large sets of bioanalytical data[1]. Expert systems, an AI component that uses a rule-based approach, can provide possible solutions for reviewing and auditing of Pharmacokinetic (PK) bioanalytical data and for statistical analysis of immunogenicity Anti-Drug Antibody (ADA) data by removing subjective decision making from the analysis. Historically, these assay data are reviewed first by the Principal Investigators (PI), then Quality Control (QC) and finally Quality Assurance (QA). Based on this data review model, the amount of time PI, QC and QA spend on generating and reviewing data has increased significantly with each complexity added by the agency in the new FDA guidance's of 2018 and 2019. With this increased complexity and the number of Projects or Studies moving through the laboratory, it is impossible for all reviewing teams to find all issues, missed data tables or inadvertent subjectivity and bias introduced in population statistics. Since these errors are spread over many different projects, identifying the root causes of these errors is burdensome. This paper examines the adoption of an AI-enabled software in a Contract Research Organization (CRO), the operational bioanalysis changes resulting from its adoption, and the return on investment from this AI tool. Focusing on the areas and technical modifications to the bioanalysis process, which has reduced the errors and removed any subjective decision making on Immunogenicity population statistics for ADA analysis.

BACKGROUND:

WHAT IS ARTIFICIAL INTELLIGENCE?

Artificial Intelligence (AI) entails systems or machines that mimic human intelligence to assist humans with tasks that involve immense amounts of data or with everyday tasks that are repetitive and tedious[2]. Some examples of AI tools include chatbots that assist in serving customers, complex AI solutions that predict which drug candidates may perform better for certain disease state targets, and software that analyze financial transactional data to identify fraudulent patterns.

While machine learning (ML) is the most known form of AI, it encompasses many other domains, such as Expert Systems, Computer Vision, Natural Language Processing, and Robotics, to name a few[3]. Additionally, AI is also used to bolster advanced computational techniques, such as image processing, to provide further incremental benefits that image processing cannot provide as a standalone technology. With the broad range of domains, various applications, and its capability to provide more powerful solutions alongside other existing technologies, AI can be transformational in the processing and analysis of data.

SOME COMMON SUB-FIELDS OF ARTIFICIAL INTELLIGENCE

Machine Learning: Machine Learning uses algorithms to find patterns and features within data and use them for making decisions and predictions on new sets of data, using either labeled data (supervised learning) or unlabeled data (unsupervised learning). Deep Learning is a field of Machine Learning that can use labelled or unlabeled data to create a model without human intervention to extract and assign features to different sets of categories of data. These capabilities of machine learning are driving predictive modeling for bioactivity and toxicity in drug screening[3,5].

Expert Systems: These are rule-based systems that mimic decision-making of a human expert. Expert systems acquire knowledge in the form of data files or inputs from experts. It then uses a rule-based engine housed within the expert system to process this information to provide output to the end-user. Expert systems are aiding doctors with patient diagnosis[2,3,4].

Computer vision: A field of AI that enables hardware and software systems to process digital images, videos and other visual inputs to take actions and/or make recommendations. While machine learning and expert systems serve as the mind of AI solutions, computer vision serves as its eyes. For instance, computer vision is used in assisted driving functions to assist a vehicle to remain in their lane and to prevent collisions[2,3].

Natural language processing: Natural Language Processing, a branch of AI, helps AI solutions process text and spoken words in a manner that is similar to how humans process them. It combines computational linguistics—rule-based modeling of human language—with statistical, machine learning, and deep learning models. Its common uses include fake news identification and spam detection[2,3].

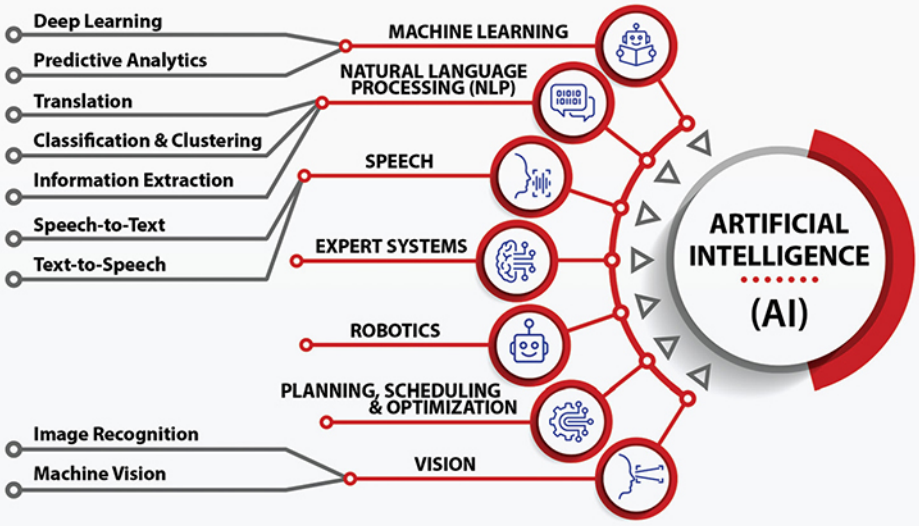

Figure 1: Artificial Intelligence and its subfields (Das, 2018).

INTRODUCTION:

As the amounts and complexity of information available is increasing, AI and advanced computational techniques are playing a critical role in determining data accuracy and reviewing large bioanalytical data sets[1]. AI is assisting in simulating activities associated with human intellect by not only processing and comprehending data, but also by thinking, acquiring new abilities, and adapting to new contexts and challenges[4]. Therefore, the use of AI in the pharmaceutical industry can increase accuracy, reduce the human workload, remove bias and errors, as well as reduce the time taken to evaluate these data sets[2,3,4]. Due to its immense potential, AI solutions have found a place in multiple sectors of pharmaceutical industry, ranging from bioactivity prediction in drug screening and regulation of in-line quality in Quality Control (QC) and Quality Assurance (QA) to assisting in subject selection in clinical trials and market prediction and analysis

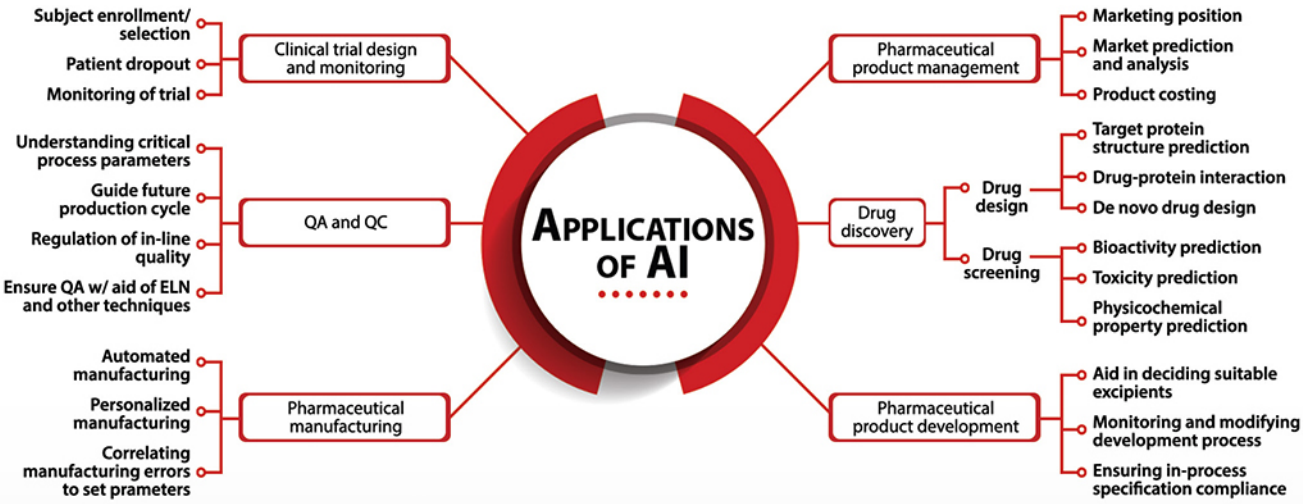

Figure 2. Applications of artificial intelligence in pharmaceutical value chain (Paul, 2021).

in product management lifecycle[5]. The scope of this paper is limited to the application of AI in QA and QC processes of large molecule bioanalytical pharmacokinetic (PK) and immunogenicity Antidrug-antibody (ADA) data.

Currently, the amount of data, tables and number of validation reports for pharmacokinetics (PK) has increased dramatically with the new regulatory guidance of May 2018. The new guidance, increased complexity arising from new technologies, and aggressive timelines are having a negative impact on data quality. On the other hand, due to the regulatory guidance of January of 2019 and numerous white papers on the statistical analysis, the statistical assessment of Anti-Drug Antibodies (ADA) has become significant in the regulatory acceptance of the resulting data from these experiments[6,7]. For accurate and appropriate statistical analysis, it is critical to provide objective and unbiased data. To ensure high quality and thorough review of large molecule pharmacokinetic data and to generate unbiased and objective statistical analysis of ADA data, KCAS Bioanalysis and Biomarker Services (KCAS) will evaluate the use of Red Thread® by Ariadne Software®.

WHAT IS RED THREAD?

Red Thread uses expert systems to create if-then rules that guide the process and analysis of pharmacokinetic and ADA data. When a user uploads a PK report, Red Thread uses expert system rules along with a dictionary of table titles to classify various data tables in the report. The user is then prompted to confirm the classification of these tables with a simple 'Yes/ No' selection. Each data table is then analyzed and audited per the rule-based engine defined for that data set. Data is then flagged green for compliance, red for non-compliance and yellow for requiring additional review within the context of experimental design or other parameters. Red Thread also uses a combination of expert systems and advanced statistical modelling for ADA data. Similar to the PK module, expert systems define the flow and progression of data from step to step. Different statistical models and techniques are used to remove biological and analytical outliers, followed by cut point determination and/or sensitivity analysis.

PK VALIDATION REPORT ANALYSIS:

THE PROBLEM

Drug development is a high cost and high stakes process that is in dire need of time and cost savings. Despite the global pharmaceutical industry going virtual, the increased complexity of new drug modalities and technologies along with the newly proposed guidance of 2018 are adding on to the already inflated cost and time consumption.

While the quality of the PK validation report is extremely important to the sponsor and the regulatory agencies, it can be challenging for a CRO to transfer data to a sponsor for an Investigational New Drug application or clinical assessment with little or no errors. The process of review and auditing of bioanalytical data is designed to ensure high quality data through review of data tables, descriptive information on these data tables and key indicators from the data. However, most CROs are often racing to meet tight deadlines for data and report delivery due to limited resources and overburdened staff.

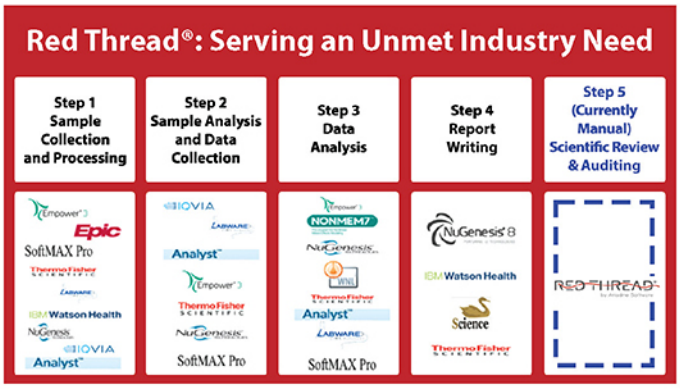

Figure 3. While several automation tools support the flow of PK bioanalytical data from sample collection to report writing, Red Thread meets an unmet need of automating scientific review and auditing of bioanalytical data.

Security and Compliance

- **Red Thread** complies with 21 CFR Part 11, Good Laboratory Practices (GLP), International Organization for Standardization (ISO) 27001, Service Organization Control (SOC) 2, and General Data Protection Regulation (GDPR).
- **Red Thread** deletes the client's data file as soon as it has been processed and does not save client's proprietary information, even in the output report.
- **Red Thread** provides an audit trail.
- **Ariadne** has information security management system (ISMS) policies in place with trained employees. Its ISMS is ISO 27001 certified as of Jan 14, 2022 and is expected to receive its SOC 2 attestation report in June 2022.
- **As an Amazon Web Services (AWS)** customer, Ariadne benefits from AWS data centers and a network architected to protect its information, identities, and applications.

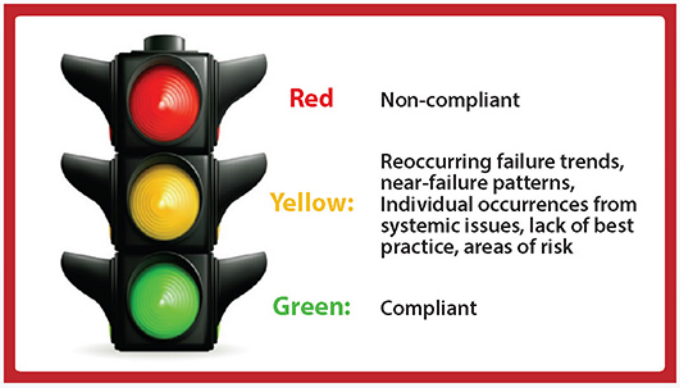

Figure 4. Red Thread Alerting system flags data to draw an end-user's attention to sections that require action or decision-making, thereby allowing for a faster review.

THE SOLUTION

The Red Thread AI software evaluated in this white paper provides a possible solution to these issues. It will increase throughput, transparency, and oversight in operations prior to QC or QA evaluation. The tool is designed by industry experts and provides a remote data review solution. It works by highlighting more findings than the manual process, thereby assuring robust review and auditing of bioanalytical data that ultimately reduces potential rework. By providing faster review of data tables and reports, it decreases time and cost consumption, as well as allows the end user/reviewer to spend more of their time troubleshooting errors, gaps, and potential concerns. Additionally, it enables the identification of problems and potential gaps within the CRO operations systems to correct, improve, and find efficient methods for the generation and review of data tables. The Red Thread AI tool fills the void of a commercially available scientific review and auditing tool for PK bioanalytical data, a manual process that is subject to missed errors. (Figure 3).

Red Thread is built on bioanalytical expertise, using clinical and preclinical reports from various sponsors and CROs. The software uses an alerting system to communicate areas of concern found in the analysis of the PK report to the end-user and prompts them to decide whether to accept the data or take action to address a finding. The alert system is based on regulatory guidelines and on industry best practices, where there is ambiguity or gap in guidance. It flags compliant data in green, non-compliant data in red, and data requiring additional review in yellow. Data flagged as yellow can highlight near-failure patterns, recurring failures, high risk areas due to subjective interpretation of any open-ended guidance, or data that requires additional information/context before a decision can be reached (Figure 4).

The software uses an extensive dictionary mapping of analytically meaningful terms in order to properly classify tables found in a report and translate the data into an internal representation that can be checked against a comprehensive list of checks (as per regulatory guidance and industry best practices). The expert system rules analyze and check the data in the PK validation report for the following bioanalytical parameters: Individual Batch Performance, Sample Concentration, Reanalysis, Stabilities (Reference Material, Storage, etc.), Incurred Sample Reanalysis (ISR), Quality Controls, Calibration/Standard (STD) Curves and Regression Models. As wider variety of data and report formats are used to test the application, the semantic mapping logic used will grow to become more complete, flexible, and context dependent.

Designed to accept PDF, Microsoft Word, and Microsoft Excel files as input, depending on the analysis module, the Red Thread platform maintains data integrity and does not change, modify, or correct data on the submitted report document. Instead, it parses and audits the uploaded document using an alert system that highlights the findings in an output report that is separate from the document submitted (Figure 5). The uploaded document is deleted right after processing, and the output report contains little to no proprietary information to ensure a high level of confidentiality and security.

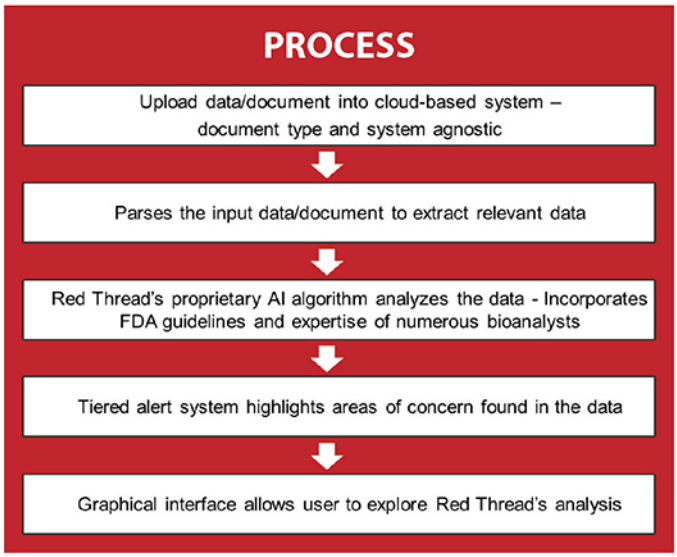

Figure 5. The step-by-step process of submitting a PK validation report for review through Red Thread.

METHOD AND DATA

For PK bioanalytical data, Ariadne Software offers method validation as well as sample analysis modules for both small and large molecules. For the purpose of this evaluation, KCAS used the Red Thread Large Molecule PK Module for its Ligand Binding Assays (LBA) data. For the evaluation, a generic PK validation report was created and small errors were added within the data. This report was then uploaded as a PDF file with analyte name as "Generic." To ensure brevity, only three bioanalytical parameters are being used as examples. Figures 6, 7 and 8 demonstrate the alert results for Batch Summary Run, Selectivity, and Validation Summary Table for this PK validation report.

Table 1. Batch Run Summary for the Method Validation of GENERIC in Human Serum

Green
All batches assayed within established long-term stability

Red
[Batch 16] assayed date after expiry date of 14-Feb-2021
[Batch 17] assayed date after expiry date of 14-Feb-2021
[Batch 18] assayed date after expiry date of 14-Feb-2021
[Batch 19] assayed date after expiry date of 14-Feb-2021
Batch 12 and Batch 13 failed consecutively
Batch 13 and Batch 14 failed consecutively

Yellow
[Batch 7] Missing Instrument ID information in Batch Run Summary Table
Missing batch numbers: 6
No Extraction Stability Provided
No Extraction Date Found
77.8% batch acceptance status confirmed for verifiable batches

Run ID	Run Status	Analyst	Assay Date	Instrument ID	Run Description	Comments
1	Accepted	Jim Doe	05-Feb-2021	001	A&P - Fresh QCs	
2	Accepted	Jane Doe	09-Feb-2021	002	A&P - Frozen QCs	
3	Accepted	Jane Doe	09-Feb-2021	001	A&P - Frozen QCs	
4	Accepted	Jim Doe	10-Feb-2021	001	Qualification and Target Interference	
5	Accepted	Jane Doe	10-Feb-2021	005	A&P - Frozen CQs and Drug Interference	
7	Accepted	Jim Doe	10-Feb-2021		Dilution Linearity	QCs do not meet acceptance criteria
8	Accepted	Jane Doe	11-Feb-2021	002	A&P - Frozen QCs and 1-3x F/T Stability	
9	Accepted	Jane Doe	11-Feb-2021	001	4-5x F/T Stability and H MI	
10	Accepted	Jane Doe	11-Feb-2021	002	A&P - Frozen QCs and Target Interference Repeat	
11	Accepted	Jane Doe	11-Feb-2021	001	4C Stability	
12	Accepted	Jim Doe	11-Feb-2021	001	L MI and Selectivity 1-5	QCs do not meet acceptance criteria
13	Accepted	Jane Doe	11-Feb-2021	005	Selectivity 6-10	QCs do not meet acceptance criteria
14	Accepted	Jim Doe	12-Feb-2021	005	Dilution Linearity Repeat	QCs do not meet acceptance criteria
15	Accepted	Jane Doe	12-Feb-2021	002	MI Repeat and 5x F/T HQC Repeat	
16	Accepted	Jane Doe	16-Feb-2021	001	Dilution Linearity Repeat	
17	Accepted	Jane Doe	16-Feb-2021	002	Selectivity 1-8 Repeat	
18	Accepted	Jane Doe	16-Feb-2021	001	Selectivity 9-10 Repeat and 5x F/T HQC Repeat	
19	Accepted	Jane Doe	18-Feb-2021	001	QC Qualification	

Figure 6. Red Thread alerts for a batch run summary table (top). The findings in the alert system are indicated as "Green", "Red", and "Yellow" flags. The colored boxes in Red Thread output report highlight the corresponding data in the data table (bottom).

Table 3. LLOQ Selectivity Results for GENERIC in Human Serum

Red
Sample: Sample 6 was tested more than once, matrix samples must be from a minimum of 10 individual sources
[Batch 17] Sample: Sample 4 %RE above 25% on Column: LLOQ Selectivity Concentration, ng/mL for concentration 6.12*
[Batch 17] Sample: Sample 5 %RE above 25% on Column: LLOQ Selectivity Concentration, ng/mL for concentration 6.35*

Yellow
[Batch 17] Less than 10 matrix samples were tested for selectivity in the Batch Run, minimum sample requirement was not met
[Batch 17] Sample: Sample 2 %RE above 20% on Column: LLOQ Selectivity Concentration, ng/mL for concentration 7.55
Could not find overall statistics

Table 4. High OC Selectivity Results for GENERIC in Human Serum

Red
[Batch 17] Sample: Sample 3 %RE above 20% on Column: HQC Selectivity Concentration, ng/mL for concentration 1180*
[Batch 17] Sample: Sample 4 %RE above 20% on Column: HQC Selectivity Concentration, ng/mL for concentration 1190*
[Batch 17] Sample: Sample 8 %RE above 20% on Column: HQC Selectivity Concentration, ng/mL for concentration 1240*
[Batch 17] Selectivity at the spiked High QC concentration level did not pass, less than 80% of the matrix samples met the acceptance criteria

Yellow
[Batch 17] Sample: Sample 7 %RE above 15% on Column: HQC Selectivity Concentration, ng/mL for concentration 1120
Could not find overall statistics

Table 3. LLOQ Selectivity Results for GENERIC in Human Serum

Assay Date	Run ID	Matrix Type	Lot No.	Nominal LLOQ Selectivity Concentration, ng/mL	LLOQ Selectivity Concentration, ng/mL	%RE
16-Feb-2021	17	Normal	Sample 1	10	10.1	10
		Normal	Sample 2	10	7.55	-24.5
		Normal	Sample 3	10	10.6	6.0
		Normal	Sample 4	10	6.12*	-38.8
		Normal	Sample 5	10	6.35*	-36.5
		Normal	Sample 6	10	10.3	3.0
		Normal	Sample 6	10	10.9	9.0
		Normal	Sample 7	10	9.67	-3.3
		Normal	Sample 7	10	10.2	2.0

Table 3. LLOQ Selectivity Results for GENERIC in Human Serum

Assay Date	Run ID	Matrix Type	Lot No.	Nominal LLOQ Selectivity Concentration, ng/mL	LLOQ Selectivity Concentration, ng/mL	%RE
16-Feb-2021	17	Normal	Sample 1	960	1050	9.4
		Normal	Sample 2	960	1090	13.5
		Normal	Sample 3	960	1080*	22.9
		Normal	Sample 4	960	1190*	24.0
		Normal	Sample 5	960	1000	4.2
		Normal	Sample 6	960	1030	7.3
		Normal	Sample 7	960	1120	16.7
		Normal	Sample 8	960	1240*	29.2
		Normal	Sample 9	960	984	2.5
		Normal	Sample 10	960	1010	5.2

Figure 7. Red Thread alerts for Lower Limit of Quantitation (LLOQ) and High Quality Control (HQC) Selectivity (top). The findings in the alert system are indicated as "Green", "Red" and "Yellow" flags. The colored boxes in Red Thread output report highlight the corresponding data in the data table (bottom).

Method Validation Report - Summary

VALIDATION SUMMARY TABLE FOR THE DETERMINATION OF GENERIC IN HUMAN SERUM

Red
Number of freeze/thaws validated reported as 5 but specified as 4 by user
QC header value of 100 ng/mL not reported in Validation Summary
The ULOQ Intra A&P accuracy range calculated from the concentration values reported in the data table (-5.73 to 10.68) does not match the ULOQ Intra A&P accuracy range reported in the Validation Summary (-5.47 to 10.94)
The Analytical QC Intra A&P accuracy range calculated from the concentration values reported in the data table (-13.33 to 8.51) does not match the Analytical QC Intra A&P accuracy range reported in the Validation Summary (-13.33 to 8.33)
The ULOQ Inter A&P accuracy value calculated from the concentration values reported in the data table (-0.52) does not match the ULOQ Inter A&P accuracy value reported in the Validation Summary (-0.78)

VALIDATION SUMMARY TABLE FOR THE DETERMINATION OF GENERIC IN HUMAN SERUM

Method description	Method #### is an ECLIA method for the determination of GENERIC in human serum
Sample volume	25 µL
Regression	5PL regression
Weighting factor	1/Y
Dynamic range	10.0 ng/mL - 1280 ng/mL
Calibration standard concentrations	10.0, 20.0, 40.0, 80.0, 160, 320, 640, and 1280 ng/mL
Anchor points	5.00 ng/mL (anchor 1) 5000 ng/mL (anchor 2
QC concentrations	10.0 ng/mL (LLOQ) 30.0 ng/mL (Low QC) 960 ng/mL (High QC) 1280 ng/mL (ULOQ) 500,000 ng/mL (Ultra High QC)
Analyte	GENERIC
Linearity	R^2 > 0.99
Lower limit of quantitation (LLOQ)	10.0 ng/mL
Upper limit of quantitation (ULOQ)	1280 ng/mL
LLOQ Intra-run precision (%CV)	< 12.96
LLOQ Intra-run accuracy (%RE) range	-16.43 to 2.00
LLOQ Inter-run precision (%CV)	8.66
LLOQ Inter-run accuracy (%RE)	-7.55
LLOQ Inter-run total error (%TE)	16.21
Low, Mid, and High QC Intra-run precision (%CV) range	0.43 to 11.19
Low, Mid, and High QC Intra-run accuracy (%RE) range	-13.33 to 8.33
Low, Mid, and High QC Inter-run precision (%CV) range	6.35 to 7.24
Low, Mid, and High QC Inter-run accuracy (%RE) range	-7.31 to 0.01
Low, Mid, and High QC Inter-run total error (%TE) range	6.94 to 14.031
ULOQ Intra-run precision (%CV)	< 4.70
ULOQ Intra-run accuracy (%RE) range	-5.47 to 10.94
ULOQ Inter-run precision (%CV)	6.33
ULOQ Inter-run accuracy (%RE)	-0.78
ULOQ Inter-run total error (%TE)	7.11
QC sample short-term (bench-top) stability	24.5 hours at 2-8°C
QC sample freeze/thaw stability	5 freeze (-80°C)/thaw (2-8°C) cycles
Dilution linearity	Up to 500,000 ng/mL diluted (250,000-fold)
Hook (prozone) effect	Hook effect observed
QC sample long-term storage stability	99 days at -80°C

Figure 8. Red Thread alerts for validation summary table (top). The findings in the alert system are indicated as "Green", "Red" and "Yellow" flags. The colored boxes in Red Thread output report highlight the corresponding data in the data table (bottom).

RESULTS

On a manual evaluation of all green, yellow, and red flags, it was found that the AI-empowered Red Thread flagged all errors that were introduced in a fully validated method report with 100% accuracy.

RETURN ON INVESTMENT FOR PK METHOD VALIDATION AND SAMPLE ANALYSIS MODULES

A return on investment (ROI) was calculated based on the cost of KCAS' QC and QA performing a 100% review (to maximize finding all errors of this type data). The ROI was calculated by assessing hourly costs, time consumed in manual report review, and time taken for corrective actions if findings are not timely addressed, and then compared to Ariadne's licensing costs. Based on this calculation and considering the time/cost of Red Thread to find all these errors, ***the net ROI was approximately 13.6 times the cost of the Red Thread software.***

ADA STATISTICAL DATA GENERATION BY RED THREAD

THE PROBLEM

Although the 2019 FDA guidance of ADA data provides a general direction for cut point analysis, however in order to allow for scientific judgment on a case-by-case basis, the document is not highly prescriptive in the preferred statistical approach. This has raised several questions about outlier determination and which statistical models should be used. ADA data can lend itself to subjective removal of biological and analytical outliers due to lack of expertise, non-standardized best practices, and individual biases. This subjective outlier removal can sometimes lead to over-refining of data, thereby misrepresenting the statistical analysis of the data. Additionally, it is not always easy to determine which statistical model or test best serves a data set, as different statistical approaches are required for data sets with different complexities and variabilities[7].

THE SOLUTION

Red Thread uses an expert system, one of the oldest forms of AI that uses an if-then based rule algorithm to process data and provide an appropriate outcome[3,4]. In Red Thread, expert systems are used in parsing and organizing inputs, determining the correct statistical approach for cut point determination, and then executing the steps to obtain the results. This emulates the decision and reasoning process that would be performed by a subject matter expert conducting this analysis in accordance with regulatory guidelines and industry best practices. An example of a rule that the program follows is testing the distribution of cut point data for normality using the Shapiro-Wilk test and measuring the skew of the distribution in order to obtain a proper threshold for cut point determination.

In addition to calculating the screening, confirmatory and titer cut point values, Red Thread uses the expert systems to guide the determination of the sensitivity of the screening and confirmatory assays as well as provide statistically derived low positive control (LPC) for each of these tiers in accordance with the FDA 2019 guidance and industry best practices.

METHOD AND DATA

Cut Point Determination

The Shapiro-Wilk test, developed by Samuel Sanford Shapiro and Martin Wilk in 1965, is a statistical test for normality in a population. A p-value that is lower than a chosen alpha level indicates rejection of the null hypothesis that the population data is sampled from the normal distribution, while a higher p-value conversely fails to reject this hypothesis (Figure 9). The result from the Shapiro-Wilk test, along with a measure of the skewness of the population data, is used by Red Thread to determine whether to find cut point values using a parametric or nonparametric approach. Under the parametric approach, in which the population is assumed to follow a normal distribution, cut point values are determined using the mean and standard deviation along with a Z score corresponding to the desired confidence level. Under the nonparametric approach, when the data has been categorized as non-normal, the cut point is determined based on a percentile calculated directly from the data distribution and the level of confidence[6,7,8,9] (Figure 10, 13).

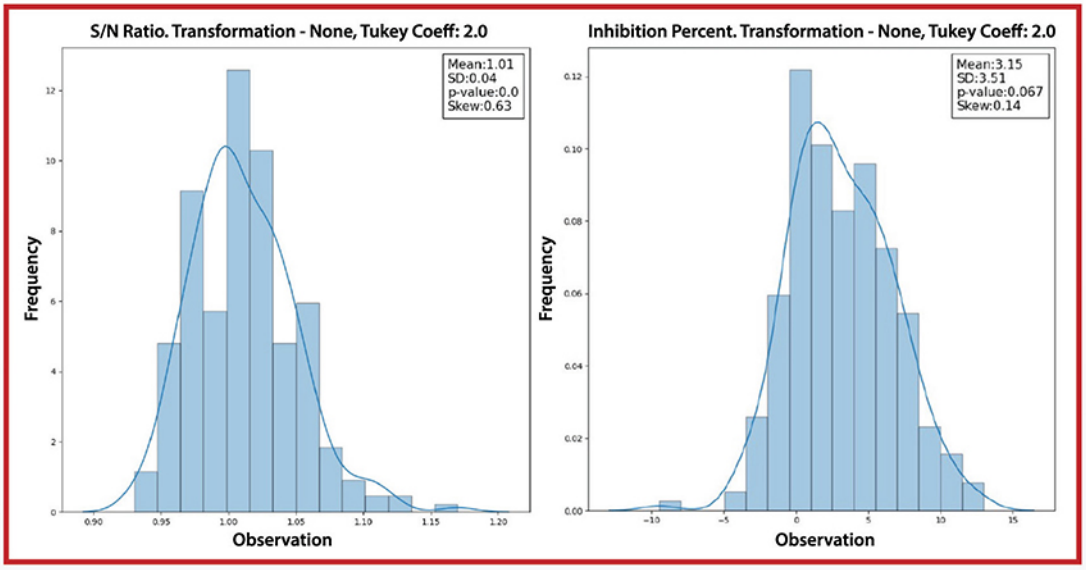

Figure 9. Histograms of the screening (left) and confirmatory (right) cut point data sets – graphic visualization of the normally distributed data.

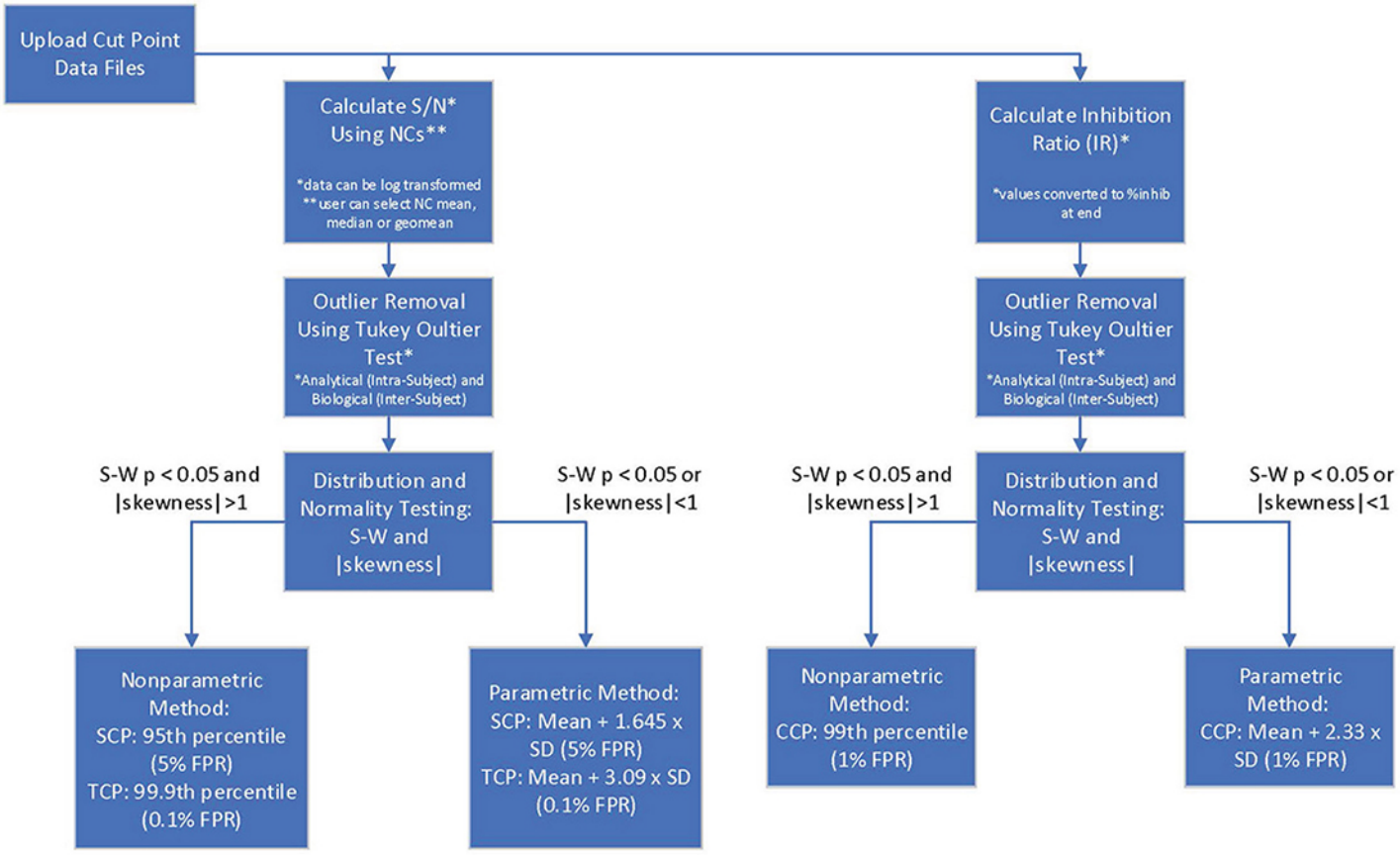

Figure 10. Red Thread's decision making process for screening (left) and confirmatory (right) cut point determination.

Red Thread also uses various other statistical techniques in its ADA module for calculations[6,7]. To determine both analytical and biological outliers, the Tukey Outlier Test can be applied at varying Interquartile Ranges (IQR) from 1.5 to 3 (more restrictive to more inclusive outlier test). To determine analytical, or intra-subject, outliers, each individual subject's observed value (signal-to-noise, or S/N, ratio for screening samples and inhibition ratio for confirmatory samples) is compared to the median of all the subject's values across multiple runs using the Tukey Outlier Test. Once identified, all the analytical outliers are removed from the data set and the median values for all observations across each subject are recalculated. Next, to determine the biological, or inter-subject, outliers, each median subject value is compared to the global mean of the entire data set using the Tukey Outlier Test and removed. (Figures 11, 12, 13).

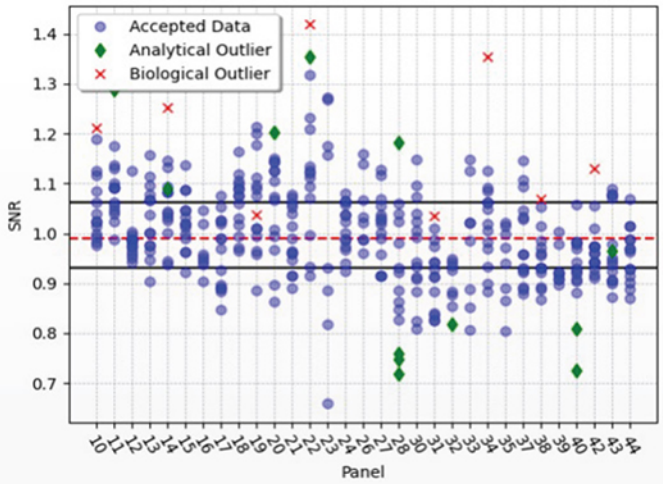

Figure 11: Screening Cut Point Scatter Plot of S/N Values Vs Panel Number.

CP type	Total Observation	Analytical Outliers	Biological Outliers	Accepted Samples	Normality Test	Cut Point	Transformation	IQR	Confidence Level
Screening	391	12	8	371	Pass	1.15	None	2	95
Confirmatory	392	8	16	368	Pass	15.06 %	None	2	99
Titer	391	12	8	371	Pass	1.28	None	2	99.9

Table 1: Red Thread provides a snapshot of statistical analysis of ADA cut point determination data that includes outliers, corresponding IQR, normality test results, cut point values, and confidence level.

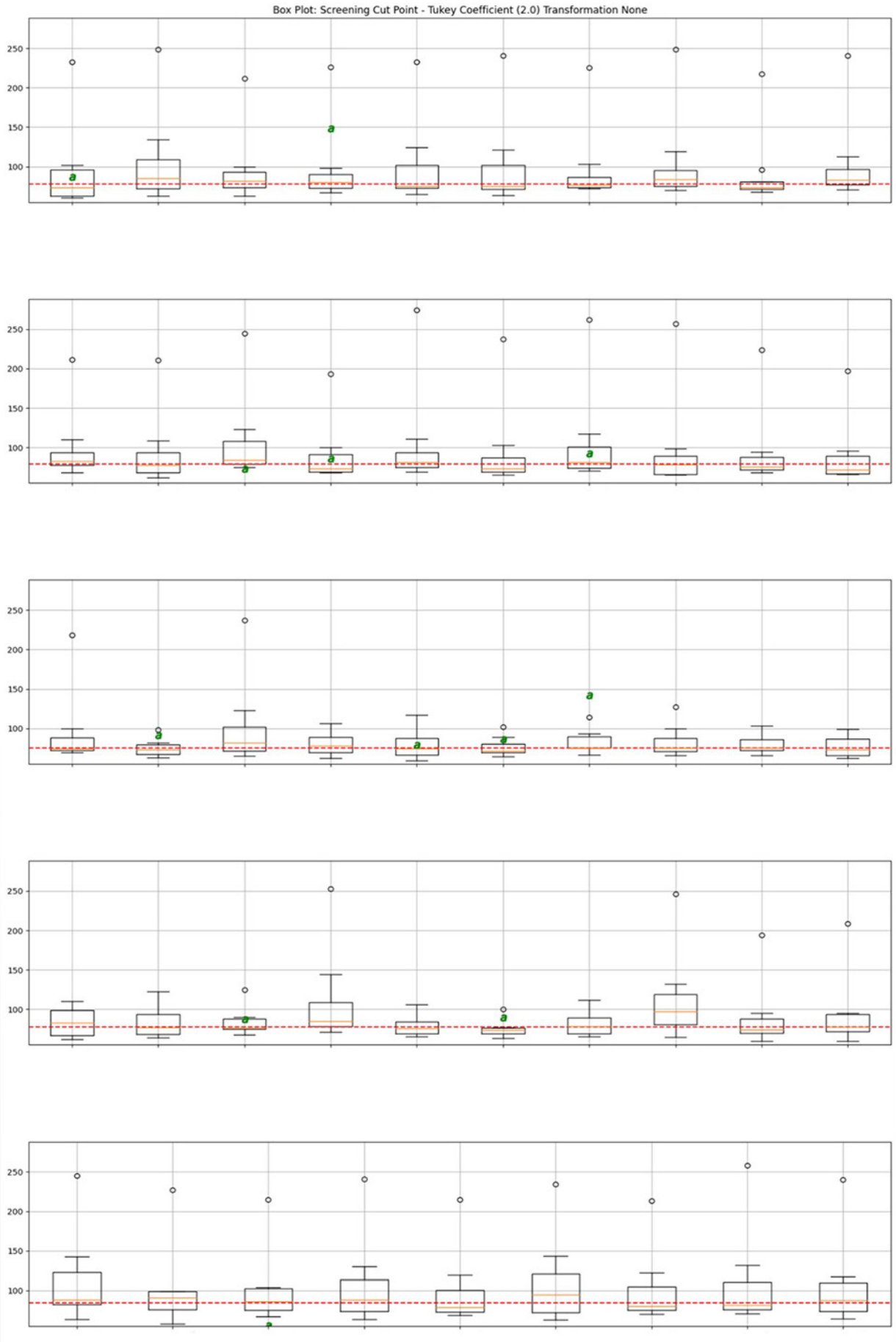

Figure 12: Screening Cut Point box plot without analytical and biological outliers marked.

Sensitivity and Low Positive Control Determination

After determining the various cut point values, Red Thread can also determine the sensitivity of the assay as well as calculate the appropriate concentrations for the low positive control (LPCs) with a 99% confidence level (as per guidance) for both, the screening and confirmatory tiers. Red Thread uses numerical optimization techniques to find the best fit equations using a 4- and 5-parameter logistic regression as well as the power of multiple validated Python libraries to visualize the distribution of the data and the results[8,9]. The resulting regression equations are used to back-calculate the mean concentration of anti-drug antibodies where the cut point intercepts the regression curve. These results are then reported as the Mean Positive Control (PC) Concentration (Figure 14). The sensitivity of the assay is defined as the concentration at which the assay would produce a positive result with 95% of the time (95% Upper Confidence Level), and is calculated as follows:

Sensitivity at 95% Upper Confidence Level = Mean SCP Conc +(1.645*SD)

where the Mean Screening Cut Point (SCP) Conc is the mean of the concentrations found across sensitivity runs, 1.645 is the Z-critical value from the normal distribution with 90 percent of the data centered in the middle with 5 percent of the data in each tail, and the SD is the standard deviation of the means. Using the same sensitivity curve data, Red Thread statistically derives the appropriate LPC concentration for both, screening (sLPC) and confirmatory tiers (cLPC), using the following equation:

sLPC = Mean PC Concentration at SCP + (t0.01,df × SD)

where t0.01,df is the t-critical value at 99 percent with degrees of freedom (df) equal to the sample size (number of sensitivity curves run) minus one, and the SD is the standard deviation of the means.

By using the t-critical value, the data is appropriately modelled at the 99th percentile when in the presence of a small sample size (such as the 6 sensitivity curves typically conducted). This signifies that a t-critical value is better at the 99th percentile than a normal critical value, which was used in the sensitivity assessment, because it enables monitoring the assay at the lower end of the cut point values so that the positive response is captured 99 percent of the time. Using the normal distribution critical value in the presence of a small sample size would result in a greater failure rate when used during sample analysis. This implies that the more sensitivity curves that are run, the greater the confidence we have in the Mean PC Concentration and the SD used in the calculation.

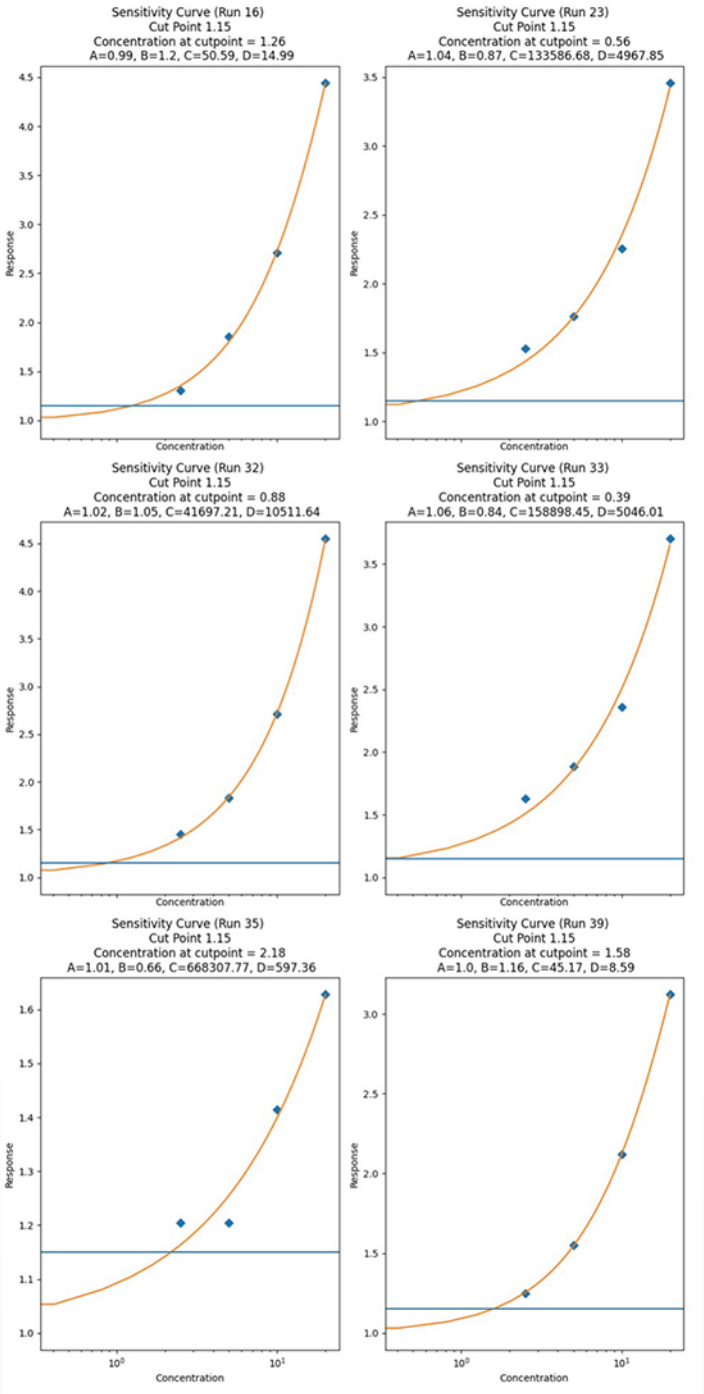

Figure 13. Estimation of the screening Low Positive Control (sLPC) from the sensitivity data run during the screening cut point determination.

Run	Sensitivity (ng/mL)
16	1.26
23	0.56
32	0.88
33	0.39
35	2.18
39	1.58
Mean SCP Conc (ng/mL)	1.14
SD	0.67
CV%	58.77
n	6
Sensitivity, Upper 95% (ng/mL)	2.24
t0.01 (df = 5)	3.36
sLPC (ng/mL)	3.39

RESULTS

KCAS needed these statistical determinations and outlier tests need to be performed in an objective manner[10,11]. Through the use of AI and advanced computational techniques, Red Thread can not only increase throughput but also facilitate objective decision making around outliers. KCAS has completed over 17 ADA statistical reports for clients with increased efficiencies and objectivity as well as with higher consistency in its statistical approach.

The analysis for the cut point, LPCs, analytical outliers as well as biological outliers takes minutes to calculate using Red Thread. KCAS currently avails service provided by Ariadne where KCAS shares data with Ariadne for these analyses with a typical turnaround time of up to 3-5 business days for the statistical analyses, with a same-day turnaround time offered if a study needs to be expedited. The formal statistical report is provided shortly afterwards to be appended to the final validation report. The traditional, non-automated approach usually involves sharing the statistical analysis and the final report all together in a few weeks. This calls for the lab to wait for further validation or repeat the work if the lab continued with its assumptions around the expected CP values or LPC concentrations to be more or less statistically accurate. With Ariadne providing the cut point and LPC statistical analysis within days rather than weeks, KCAS can proceed with the post-cut point analysis validation work more promptly than a traditional, non-automated approach while maintaining regulatory compliance and avoiding costly rework.

ROI FOR SCREENING, CONFIRMATORY AND TITER CUT POINT DETERMINATION

A comparison of manual resources used for outlier removal, cut point determination, and final report generation to the use of Ariadne Software and services yielded ***a net of 20.5 times the cost of using Red Thread***. The ROI was calculated by assessing hourly costs, time spent in manually reviewing the reports and time taken for corrective actions if findings are not timely addressed.

CONCLUSION

For large molecule PK data, Red Thread was able to find non-compliant data and concerning data and/or trends, and flag it accordingly for the bioanalysts to decide on whether the findings need to be accepted or addressed through rework. The software also provided time savings in addition to better resource management in an environment where CROs need to meet aggressive timelines with available resources. In summary, KCAS will continue to use Red Thread for operational review of reports before submitting to QC and QA, thereby providing its clients with a more qualified product for their use in regulatory filings.

For large molecule ADA data, Red Thread provides a compliant and robust method to calculate biological and analytical outliers, followed by cut point determination and sensitivity analysis. It is able to objectively perform these analyses in a fraction of the time with a consistent approach with each data set. Additionally, Ariadne Software's service to create a final report has helped KCAS focus its attention on designing and performing experiments for its clients. In summary, KCAS will continue using Red Thread for generating ADA statistical data and final reports for its clients' submission to the regulatory bodies.

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