

# Cross-Validation of Statistical Workflow for Cut Point Calculation using Red Thread (Python) Vs R

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## INTRODUCTION:

In the traditional approach to cut point determination (here referred to as the outlier approach), analytical (intra-subject) and biological (inter-subject) outliers are removed from the dataset based on the Tukey Outlier test using the interquartile range. The new resulting distribution is then used to determine a cut point, using parametric method for normal/log-normal distributed data and nonparametric method for other types of distributions.

Red Thread's ADA module implements this approach in Python, an open-source programming language, using open-source Python packages. While Red Thread has been thoroughly tested to ensure its results are accurate, an additional level of validation can be achieved by implementing the same approach in another language such as R, a programming language used for statistical analysis. In addition to comparing the final cut point values, it is necessary to compare which outliers are removed and the results of the normality test to confirm equivalence of the entire statistical workflow and decision tree.

## PURPOSE:

The goal of this study is to validate the cut point values of Red Thread's ADA module through the duplication of the entire decision tree and workflow in a separate programming language, R, that is commonly used for statistical analysis. This comparison study used six anonymized datasets that cover a wide range of cases to ensure that the software is validated for all anticipated cases.

DATASET	TEST CASE
Dataset 1	Low SCP (< 1.10, S/N); High CCP (> 50% inhibition); Outliers > 15% (both SCP and CCP)
Dataset 2	Neutralizing antibody (NAB) data
Dataset 3	1.1 < SCP < 2; 10% < CCP < 50%; 5% < Outliers < 15%
Dataset 4	High SCP (> 2); High CCP; Outliers > 15% for both
Dataset 5	Low CCP (< 10% inhibition)
Dataset 6	Outliers < 5%

Table 1: Dataset Summary. SCP – Screening Cut Point; CCP – Confirmatory Cut Point

## APPROACH:

The Python implementation (as used in Red Thread) leverages the modules *numpy*, *scipy*, and *pandas* in calculating cut points: *numpy* and *scipy* provide mathematical and statistical utilities for performing calculations on matrices and distributions; and the *pandas* module provides various data science functions including manipulating for efficient organization and computation on structured data. The R cut point module primarily leverages the R packages *readxl*, *dplyr*, and *stringr*: *readxl* provides functionality of reading data from Excel files; *dplyr* is designed for data manipulation and transformation; and *stringr* provides functionality of string manipulation and pattern matching.

The Python and R programs have been built by different internal teams to implement the following approach to cut point determination (Figure 1):

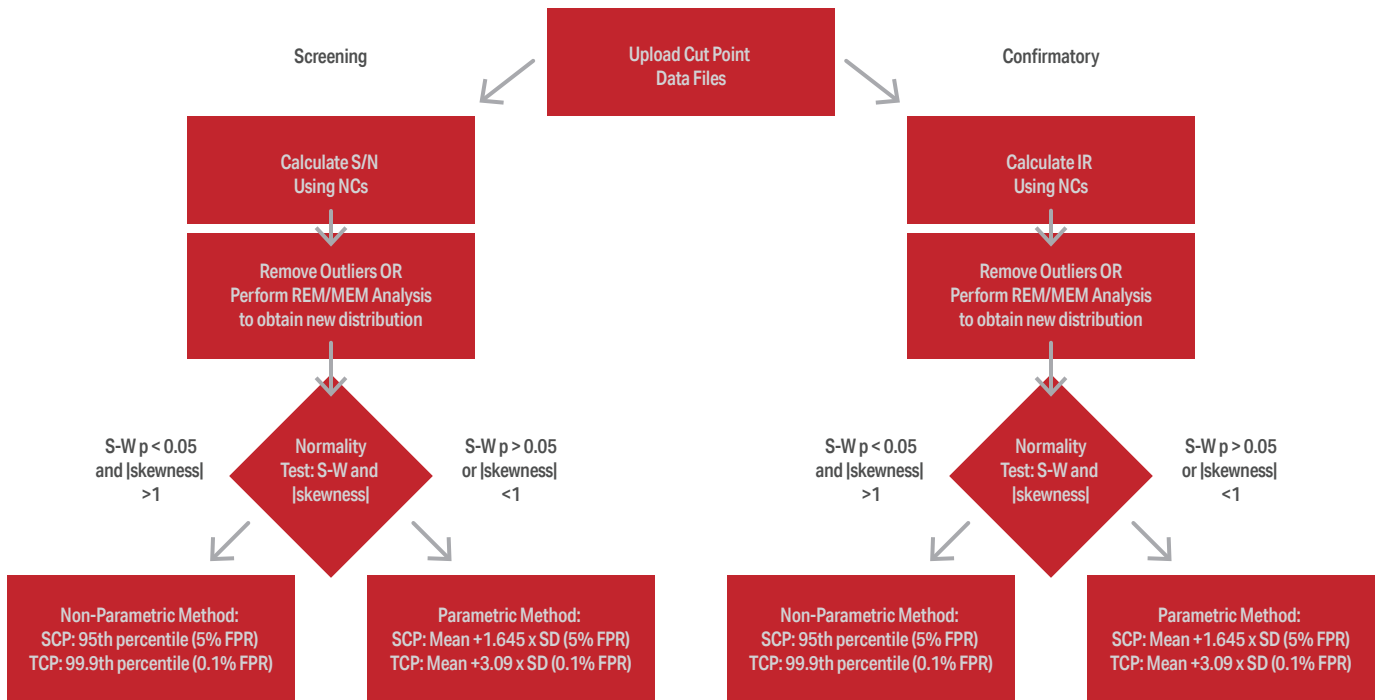


Figure 1: Red Thread ADA module workflow/decision tree

In summary, first analytical (intra-subject) outliers are removed based on the Tukey Outlier Test. The median values for all observations across each subject are then recalculated, and biological (inter-subject) outliers are subsequently removed from the dataset. The distribution/skewness assumption is evaluated subsequently, and if the assumption fails, a log-transformation is performed on the original data, and the outlier removal process is re-evaluated. Finally, depending on the distribution/skewness of the data with outliers removed, a parametric/nonparametric cutpoint is calculated.

**RESULTS:**

The results from Red Thread’s ADA module can be replicated by implementing the outlier approach for cut point determination in R across six datasets. All screening, confirmatory, and titer cut point values are equivalent across all six datasets and out to five decimal places, as limited by Red Thread’s maximum output. Additionally, the outliers removed by both implementations are the same across two IQR levels each (IQR of 1.5 and 3), and the normality test results (parametric vs. non-parametric) are equivalent.

File Name	CP type	Total Observation	Analytical Outliers	Biological Outliers	Accepted Samples	p-value	Skew	Normality Test	CP Value	Confidence Level
Dataset_1 IQR 1.5	Screening -Python	159	14	16	129	$p < 0.05$	<1	Pass	1.09500	95
	Screening -R	159	14	16	129	$p < 0.05$	<1	Pass	1.09500	95
	Titer -Python	159	14	16	129	$p < 0.05$	<1	Pass	1.28540	99.9
	Titer -R	159	14	16	129	$p < 0.05$	<1	Pass	1.28540	99.9
	Confirmatory -Python	156	22	15	119	$p > 0.05$	<1	Pass	53.44733%	99
	Confirmatory -R	156	22	15	119	$p > 0.05$	<1	Pass	53.44733%	99
Dataset 1 IQR 3	Screening -Python	159	7	16	136	$p < 0.05$	<1	Pass	1.09272	95
	Screening -R	159	7	16	136	$p < 0.05$	<1	Pass	1.09272	95
	Titer -Python	159	7	16	136	$p < 0.05$	<1	Pass	1.28584	99.9
	Titer -R	159	7	16	136	$p < 0.05$	<1	Pass	1.28584	99.9
	Confirmatory -Python	156	13	10	133	$p < 0.05$	>1	Fail	66.45868%	99
	Confirmatory -R	156	13	10	133	$p < 0.05$	>1	Fail	66.45868%	99
Dataset_2 IQR 1.5	Screening -Python	220	14	22	184	$p < 0.05$	<1	Pass	0.88263	95
	Screening -R	220	14	22	184	$p < 0.05$	<1	Pass	0.88263	95
	Screening -Python	220	14	22	184	$p < 0.05$	<1	Pass	0.84795	99
	Screening -R	220	14	22	184	$p < 0.05$	<1	Pass	0.84795	99
	Screening -Python	220	14	22	184	$p < 0.05$	<1	Pass	0.80909	99.9
	Screening -R	220	14	22	184	$p < 0.05$	<1	Pass	0.80909	99.9
Dataset_2 IQR 3	Screening -Python	220	3	22	195	$p > 0.05$	<1	Pass	0.87802	95
	Screening -R	220	3	22	195	$p > 0.05$	<1	Pass	0.87802	95
	Screening -Python	220	3	22	195	$p > 0.05$	<1	Pass	0.84110	99
	Screening -R	220	3	22	195	$p > 0.05$	<1	Pass	0.84110	99
	Screening -Python	220	3	22	195	$p > 0.05$	<1	Pass	0.79972	99.9
	Screening -R	220	3	22	195	$p > 0.05$	<1	Pass	0.79972	99.9
Dataset_3 IQR 1.5	Screening -Python	297	41	6	250	$p < 0.05$	<1	Pass	1.03434	95
	Screening -R	297	41	6	250	$p < 0.05$	<1	Pass	1.03434	95
	Screening -Python	297	41	6	250	$p < 0.05$	<1	Pass	1.08903	99.9
	Screening -R	297	41	6	250	$p < 0.05$	<1	Pass	1.08903	99.9
	Confirmatory -Python	294	15	6	273	$p > 0.05$	<1	Pass	14.84036%	99
	Confirmatory -R	294	15	6	273	$p > 0.05$	<1	Pass	14.84036%	99
Dataset_3 IQR 3	Screening -Python	297	18	6	273	$p < 0.05$	>1	Failed	1.03456	95
	Screening -R	297	18	6	273	$p < 0.05$	>1	Fail	1.03456	95
	Screening -Python	297	18	6	273	$p < 0.05$	>1	Failed	1.14247	99.9
	Screening -R	297	18	6	273	$p < 0.05$	>1	Fail	1.14247	99.9
	Confirmatory -Python	294	9	6	279	$p < 0.05$	<1	Pass	15.45937%	99
	Confirmatory -R	294	9	6	279	$p < 0.05$	<1	Pass	15.45937%	99
Dataset_4 IQR 1.5	Screening -Python	597	71	112	414	$p < 0.05$	>1	Fail	4.01757	95
	Screening -R	597	71	112	414	$p < 0.05$	>1	Fail	4.01757	95
	Titer -Python	597	71	112	414	$p < 0.05$	>1	Fail	6.58263	99.9
	Titer -R	597	71	112	414	$p < 0.05$	>1	Fail	6.58263	99.9
	Confirmatory -Python	594	41	0	553	$p < 0.05$	<1	Pass	88.59740%	99
	Confirmatory -R	594	41	0	553	$p < 0.05$	<1	Pass	88.59740%	99
Dataset_4 IQR 3	Screening -Python	597	38	108	451	$p < 0.05$	>1	Fail	5.12445	95
	Screening -R	597	38	108	451	$p < 0.05$	>1	Fail	5.12445	95
	Titer -Python	597	38	108	451	$p < 0.05$	>1	Fail	12.36959	99.9
	Titer -R	597	38	108	451	$p < 0.05$	>1	Fail	12.36959	99.9
	Confirmatory -Python	594	22	0	572	$p < 0.05$	>1	Fail	86.41012%	99
	Confirmatory -R	594	22	0	572	$p < 0.05$	>1	Fail	86.41012%	99
Dataset_5 IQR 1.5	Confirmatory -Python	282	30	22	230	$p < 0.05$	<1	Pass	8.15490%	99
	Confirmatory -R	282	30	22	230	$p < 0.05$	<1	Pass	8.15490%	99
Dataset_5 IQR 3	Confirmatory -Python	282	10	24	248	$p < 0.05$	<1	Pass	8.87391%	99
	Confirmatory -R	282	10	24	248	$p < 0.05$	<1	Pass	8.87391%	99
Dataset_6 IQR 1.5	Screening -Python	884	0	23	861	$p < 0.05$	<1	Pass	1.12877	95
	Screening -R	884	0	23	861	$p < 0.05$	<1	Pass	1.12877	95
	Titer -Python	884	0	23	861	$p < 0.05$	<1	Pass	1.45038	99.9
	Titer -R	884	0	23	861	$p < 0.05$	<1	Pass	1.45038	99.9
Dataset_6 IQR 3	Screening -Python	884	0	6	878	$p < 0.05$	<1	Pass	1.18048	95
	Screening -R	884	0	6	878	$p < 0.05$	<1	Pass	1.18048	95
	Titer -Python	884	0	6	878	$p < 0.05$	<1	Pass	1.53449	99.9
	Titer -R	884	0	6	878	$p < 0.05$	<1	Pass	1.53449	99.9

Table 2: Comparison of Statistical Workflow: Python vs R

**CONCLUSION:**

The Red Thread workflow, implemented in Python, was cross validated in the identical workflow written in the statistical language R as demonstrated by the equivalency of outlier selection, normality results and calculated cut point values for all three tiers across the six tested datasets.

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