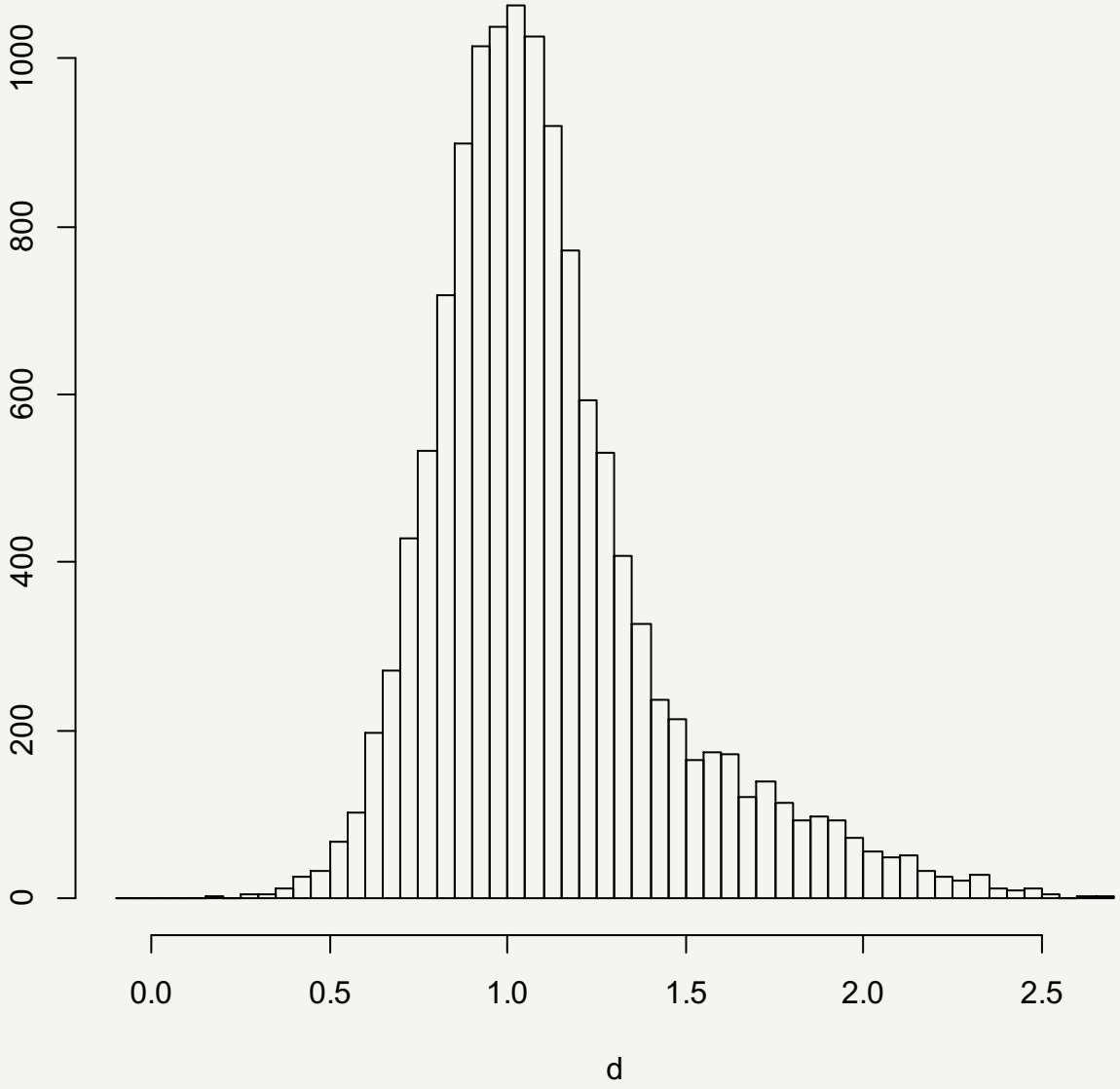


Screening assay data transformation – can a Weibull transformation help to achieve the theoretical FPR?

Gregor Jordan, pRED Pharmaceutical Sciences, Bioanalytical R&D, Roche Innovation Center Munich

Roland Staack, pRED Pharmaceutical Sciences, Bioanalytical R&D, Roche Innovation Center Munich

CP calculation approach

… a look at the literature

G. Shankar et al. / Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281

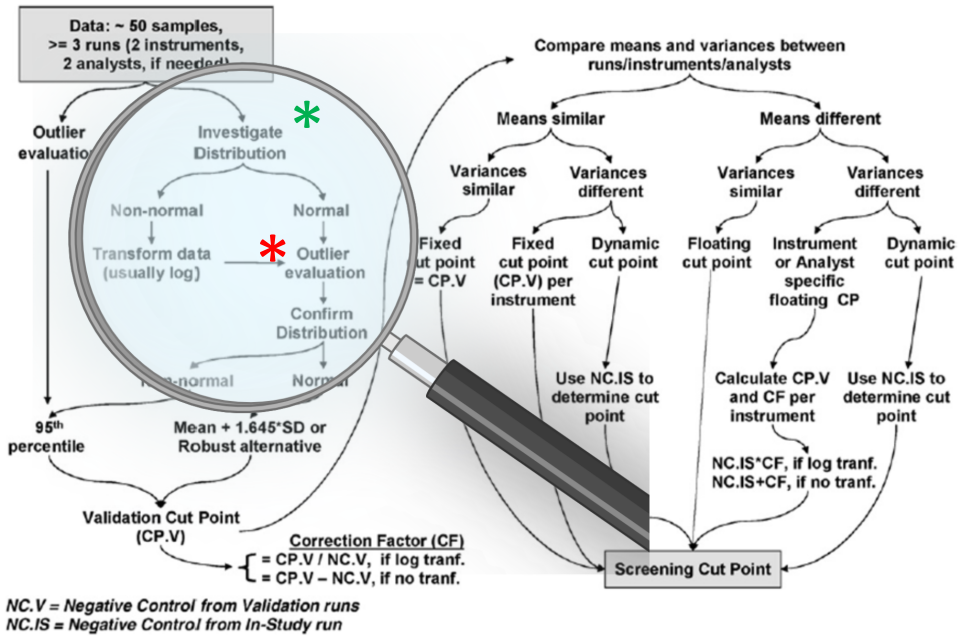


Fig. 1. Scheme for evaluating cut point samples and calculating screening cut point.

Created with BioRender.com

The AAPS Journal, Vol. 19, No. 5, September 2017 (© 2017)
DOI: 10.1208/s12248-017-0107-3

Recommendations for Systematic Statistical Computation of Immunogenicity Cut Points

Viswanath Devanarayan,[1] Wendell C. Smith,[2] Rocco L. Brunelle,[2] Mary E. Seger,[2,3] Kim Krug,[2,3] and Ronald R. Bowsher[2,3,4]

Skewed data

Journal of Immunological Methods 463 (2018) 105–111

Research paper

Excessive outlier removal may result in cut points that are not suitable for immunogenicity assessments

Robert J. Kubiak*,[1], Jianchun Zhang[1], Pin Ren, Harry Yang, Lorin K. Roskos

MedImmune LLC, One MedImmune Way, Gaithersburg, MD 20878, United States

Journal of Immunological Methods 484–485 (2020) 112817

A new method for identification of outliers in immunogenicity assay cut point data

Jianchun Zhang*, Rosalin HGP Arends, Robert J Kubiak, Lorin K Roskos, Meina Liang, Nancy Lee, Cecil Chi-Keung Chen, Harry Yang

Journal of Immunological Methods 389 (2013) 79–87

Computational modelling

Statistical methods and tool for cut point analysis in immunogenicity assays

Lanju Zhang [a,*], Jianchun Jason Zhang [b], Robert J. Kubiak [b], Harry Yang [b]

Potential implication for study sample population

… when we already excluded erroneously to many “outliers”

FDA guideline for Immunogenicity (2019)

The sponsor should consider the impact of statistically determined outlier values and true-positive samples when establishing the cut-point. **The sponsor should provide justification for the removal of any data points, along with the respective method used to determine their status as outliers.** Sponsors should consult with FDA if there is a concern regarding the exclusion of outliers

C. Confirmation of Cut-Point in the Target Population
Samples from different populations can have different background activity in ADA assays. Similarly, the background activity can change when samples used to determine the cut-point during assay validation were not obtained and handled in a manner that represents how samples will be obtained and handled in-study. **Therefore, it is necessary to confirm that the cut-point determined during assay validation is suitable for the population being studied**. A sufficient number of samples from the target population should be used, and justification for the number used should be provided. If sufficient numbers of samples are not available,

In study CP needed? … but when?

The AAPS Journal, Vol. 19, No. 5, September 2017 (© 2017)
DOI: 10.1208/s12248-017-0107-3

Recommendations for Systematic Statistical Computation of Immunogenicity Cut Points

Viswanath Devanarayan,[1] Wendell C. Smith,[2] Rocco L. Brunelle,[2] Mary E. Seger,[2,3] Kim Krug,[2,3] and Ronald R. Bowsher[2,3,4]

In study population FPR not between 2% - 11%

The AAPS Journal (2020) 22: 19
DOI: 10.1208/s12248-019-0400-4

Criteria to Reevaluate Anti-drug Antibody Assay Cut Point Suitability in the Target Population

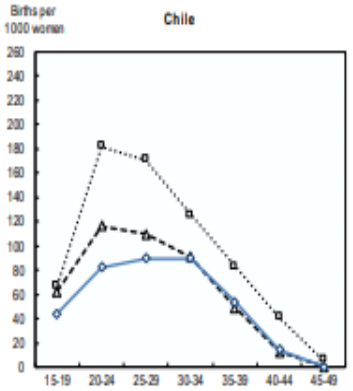
Charles Y. Tan,[1,2] Gregory S Steeno,[1] Zhiping You,[1] Puneet Gaitonde,[1] Chun-Hua Cai,[1] John Kamerud,[1] Boris Gorovits,[1] and Daniel J. Baltrukonis[1]

2% - 11% criterion is sample size dependent

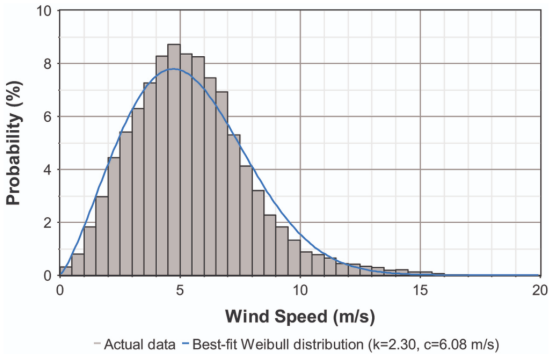
When do we observe skewed distributions 1 of 3

…only biological variability?

…naturally occurring skewed distribution…

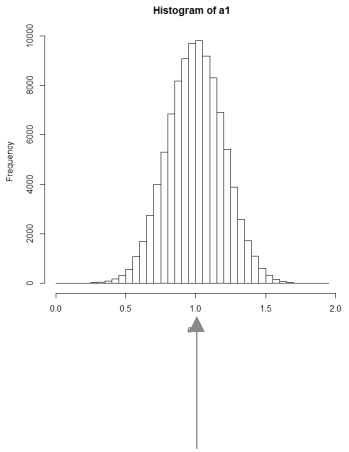


Source: OECD Family Database
http://www.oecd.org/els/family/database.htm

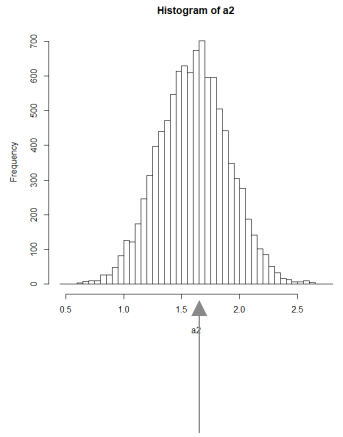


Source: https://doi.org/10.1515/ehs-2017-0003

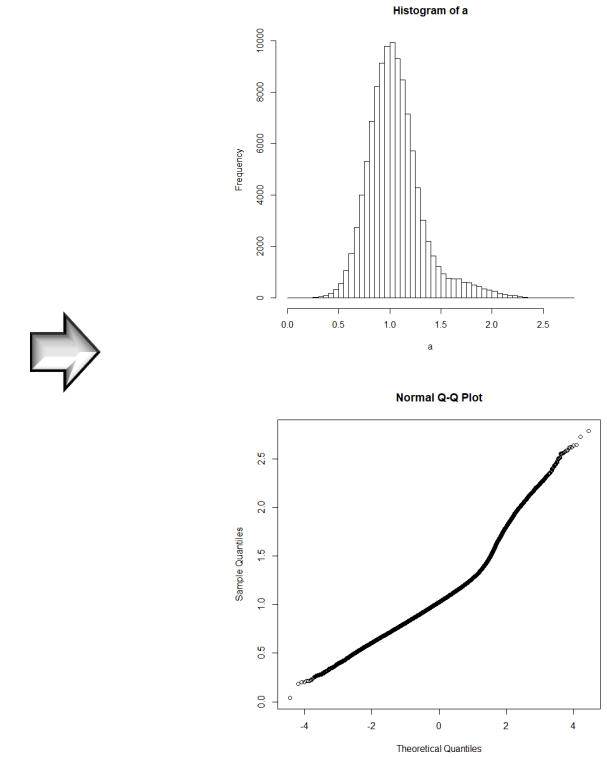
…or e.g. overlay of „two assay distributions“…



Mean=1
sd=0.2



Mean=1.6
sd=0.3

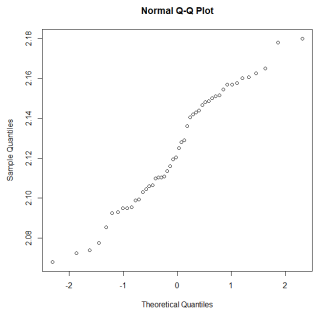


When do we observe skewed distributions 2 of 3

… dilution of normal distributed sample mixture to reader background

TMB is directly converted and stopped…then…stepwise diluted (each well individually) with buffer to a final dilution of 625.
After each dilution step (F=5) the plate is analyzed.

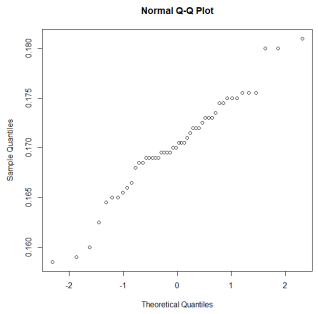
Average signal (n=48) = 2.125



Shapiro.test p-value = **0.121**

Dilution F=5

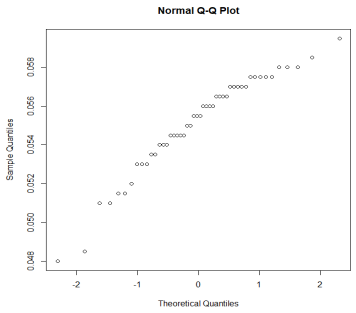
Average signal (n=48) = 0.170



Shapiro.test p-value = **0.2096**

Dilution F=5

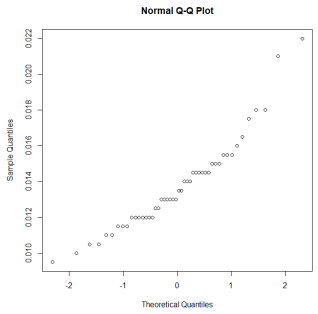
Average signal (n=48) = 0.055



Shapiro.test p-value = **0.01992**

Dilution F=5

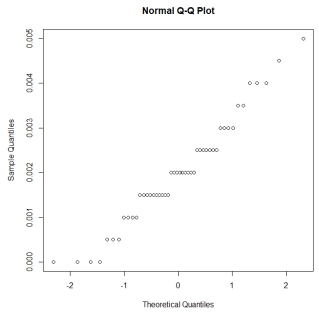
Average signal (n=48) = 0.014



shapiro.test p-value = **0.004579**

Dilution F=5

Average signal (n=48) = 0.002



shapiro.test p-value = **0.1348**

Preparation normal distribution of signals

Intermediate skewed distribution

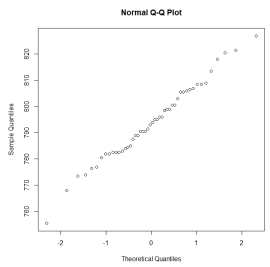
System normal distribution of signals

When do we observe skewed distributions 3 of 3

… and when not?

HPPA* is directly converted and stopped…then…stepwise diluted (each well individually) with buffer to a final dilution of 15625
After each dilution step (F=5) the plate is analyzed.

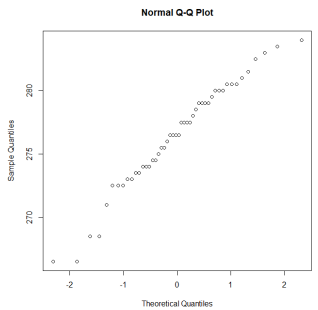
Average signal (n=48) = 793.9



Shapiro.test p-value = **0.9427**

Dilution F=5

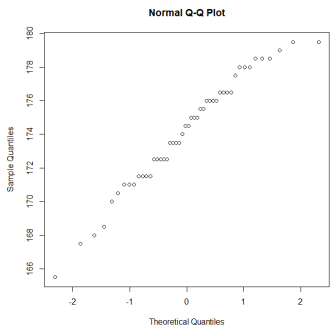
Average signal (n=48) = 276.4



Shapiro.test p-value = **0.2684**

Dilution F=5

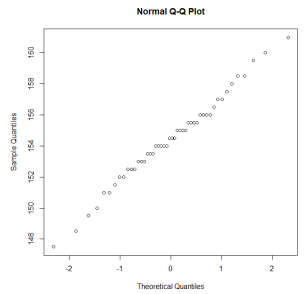
Average signal (n=48) = 174.2



Shapiro.test p-value = **0.2436**

Dilution F=5

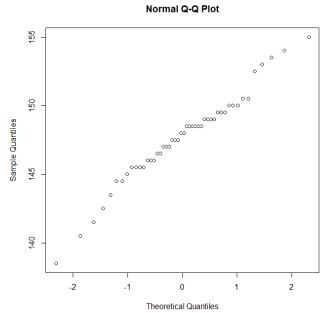
Average signal (n=48) = 154.4



shapiro.test p-value = **0.9538**

Dilution
F=25
& F= 125

Average signal (n=48) = 147.7 (F=25)
Average signal (n=48) = 147.8 (F=125)



shapiro.test p-value = **0.3362 (F=25)**
shapiro.test p-value = **0.8014 (F=125)**

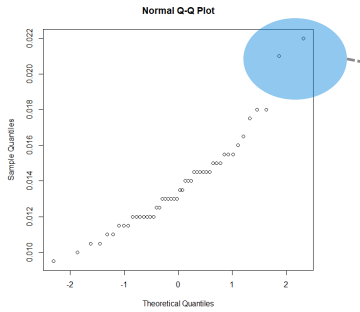
Preparation normal distribution of signals

System normal distribution of signals

* Jordan et a.; Bioanalysis 2017 Feb;9(4):407-418. doi: 10.4155/bio-2016-0225

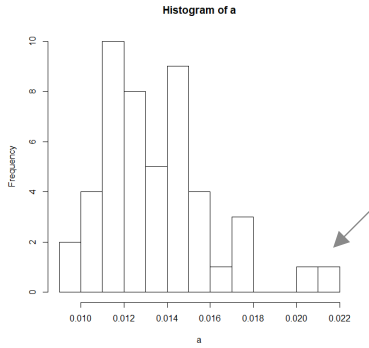
Possible interpretation of the observed skewed distribution

…for the intermediate non normal distribution…



shapiro.test p-value = **0.004579**

Skewness=1.10
R library „moments“



Shapiro.test p-value = **0.3954**

Skewness=0.34
R library „moments“

Outlier when 1.5 IQR rule is applied

No outlier when 3 IQR rule is applied

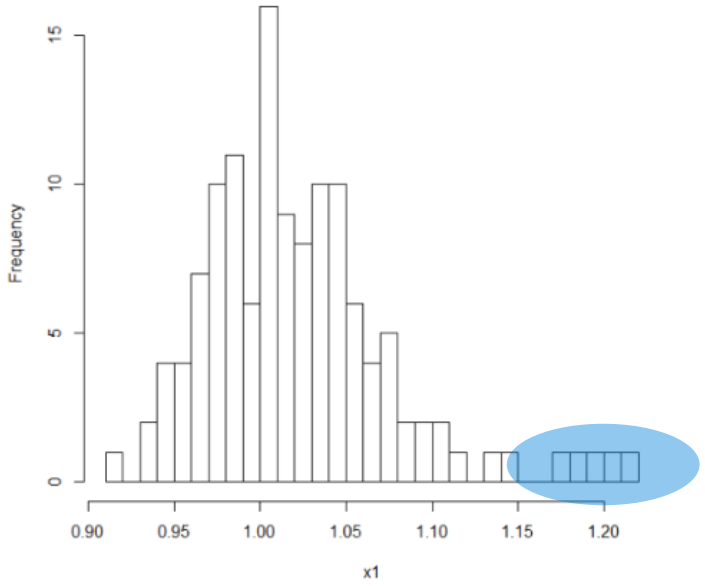
These two data points were initial part of the normal distribution

Assuming these data set would have been “reanalyzed in the clinical setting” the false **positive rate would be ~ 10%** as no outlier could be excluded…

How to bring the two data points, which are part of the distribution, “back to the distribution”?

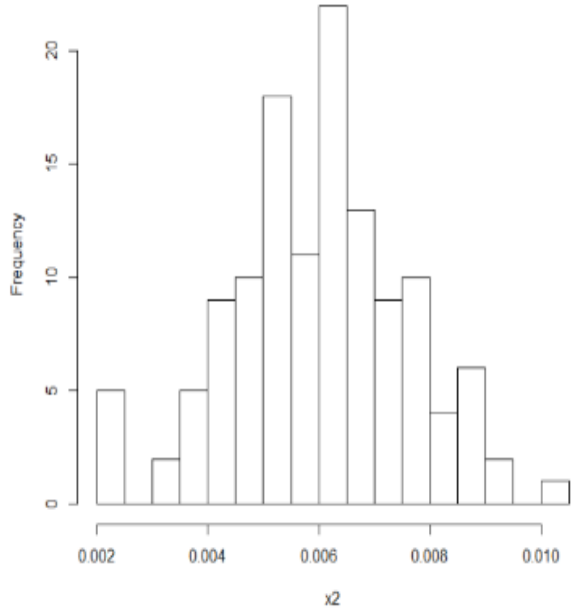
Alternative screening data transformation approach *

… a proposal which might help with skewed data



f(x) = (a/b) (x/b)^(a-1) exp(- (x/b)^a)

a and **b** were determined by driving the skewness of distribution to “zero”



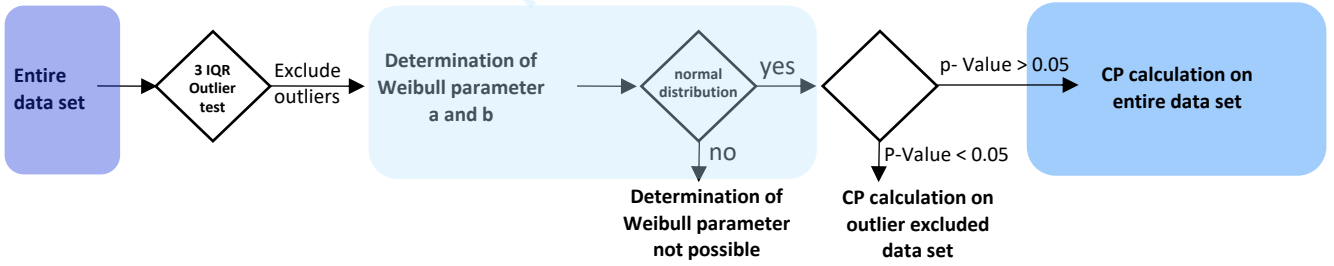
Focus of the presented approach is on the transformation approach and not mainly on the reproducibility of the assay data.
No screening data were excluded based on their reactivity in the confirmatory assay

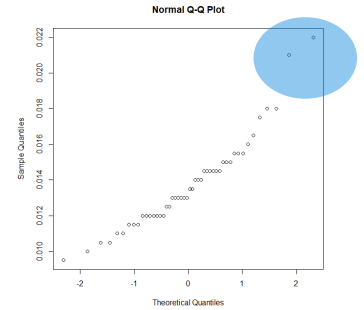
* Jordan, G., Staack, R.F. An Alternative Data Transformation Approach for ADA Cut Point Determination: Why Not Use a Weibull Transformation?. *AAPS J* **23,** 97 (2021).

Weibull based transformation

….based on 10 projects (pre-clinic and clinic)

Data set	Outlier based on 3IQR				CP (based on 3IQR)	All data	CP (on al)
	N outlier	a (shape)	b (scale)	*p*-value		*p*-value	
1	1	0.80	0.09	**0.80**	1.12	0.61	1.13
2	2	0.68	0.09	**0.54**	1.06	< 0.05	
3	4	0.71	0.10	**0.19**	1.45	0.09	1.94
4	1	0.43	15.0	**0.83**	1.99	0.77	2.07
5	1	1.03	0.10	**0.27**	1.16	0.41	1.16
6	10	2.49	0.50	**0.05**	1.11	< 0.05	
7	0	0.66	0.10	**0.95**	1.17		
8	4	0.69	0.10	**0.07**	1.08	< 0.05	
9	8	1.20	0.36	**0.17**	1.36	0.05	1.81
10	3	0.92	0.74	**0.23**	2.17	0.27	2.44



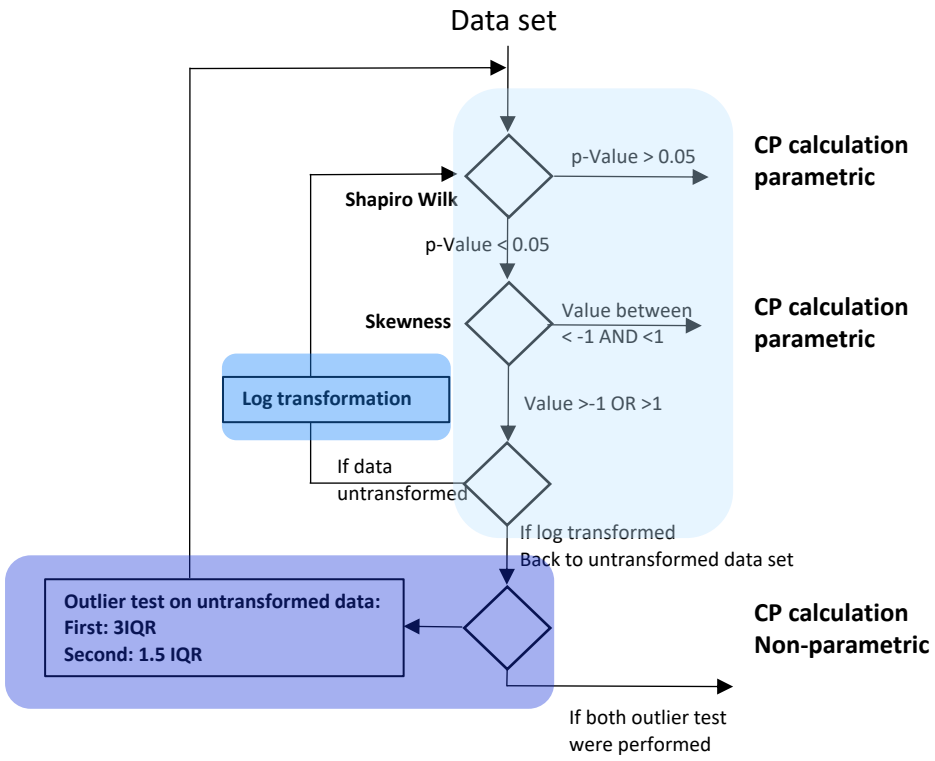


Applicability for 2 clinical data sets for which 3 * 100 data points exist:

(1) both data sets could be transformed to normal distribution by applying the Weibull approach with p-values of 0.40 and 0.24
(2) data set 2: initial (based on N=100) determined values a and b could be kept and the p value dropped from 0.54 (N=100) to 0.40 (N=300)

Decision tree based CP calculation

…on the same data set



applied outlier test:		Original data set				Outlier based on 3IQR					Outlier based on 1.5IQR					CP
trans-formation:		non transformed		Log transformed		non transformed			Log transformed		non transformed			Log transformed		
Data set	N (data)	p-value	skewness	p-value	skewness	N outlier	p-value	skewness	p-value	skewness	N outlier	p-value	skewness	p-value	skewness	
1	128	< 0.05	2.18	< 0.05	1.73	1	< 0.05	1.19	< 0.05	*0.99*						1.11
2	100	< 0.05	8.06	< 0.05	6.41	2	< 0.05	1.06	< 0.05	*0.74*						1.05
3	50	< 0.05	2.30	< 0.05	1.63	4	< 0.05	1.19	< 0.05	*0.93*						1.35
4	60	< 0.05	1.58	*0.12*	*0.62*											2.00
5	64	< 0.05	3.83	< 0.05	3.11	1	< 0.05	1.25	< 0.05	1.08	3	*0.05*	*0.65*			1.10
6	100	< 0.05	8.76	< 0.05	5.62	10	< 0.05	1.71	< 0.05	1.51	15	< 0.05	1.18	< 0.05	1.04	1.06
7	40	< 0.05	1.41	< 0.05	*0.99*											1.15
8	50	< 0.05	4.19	< 0.05	3.26	4	< 0.05	*0.85*								1.07
9	100	< 0.05	4.76	< 0.05	2.62	8	< 0.05	1.11	< 0.05	*0.72*						1.27
10	100	< 0.05	3.58	< 0.05	1.02	3	< 0.05	1.28	*0.09*	*0.44*						2.01

Comparison of calculated CP and FPR

… and the false positive rate when applying the CP on all data…if applicable

	decision-tree based CP calculation	Weibull transformation based CP calculation
Data set	CP	CP
1	1.11	1.13
2	1.05	1.06
3	1.35	1.94
4	2.00	2.07
5	1.10	1.16
6	1.06	1.11
7	1.15	1.17
8	1.07	1.08
9	1.27	1.81
10	2.01	2.44

No outliers Detected in decision tree based approach

	decision-tree based CP calculation	Weibull transformation based CP calculation	
Data set	false positive rate	false positive rate	
1	6%		**6%**
2	8%	7%	
3	18%		**8%**
4	5%		**5%**
5	13%		**5%**
6	19%	16%	
7	8%	8%	
8	16%	16%	
9	17%		**7%**
10	10%		**4%**

CP on „all data“

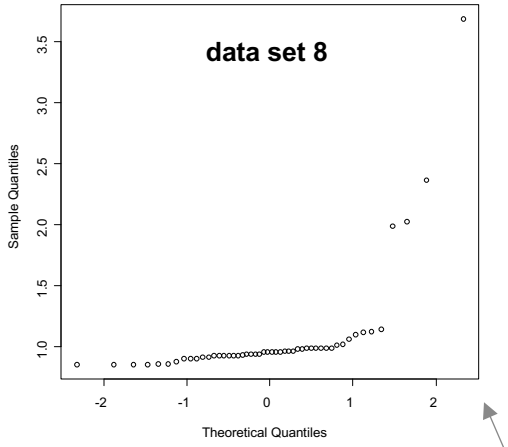
CP on „3 IQR data“

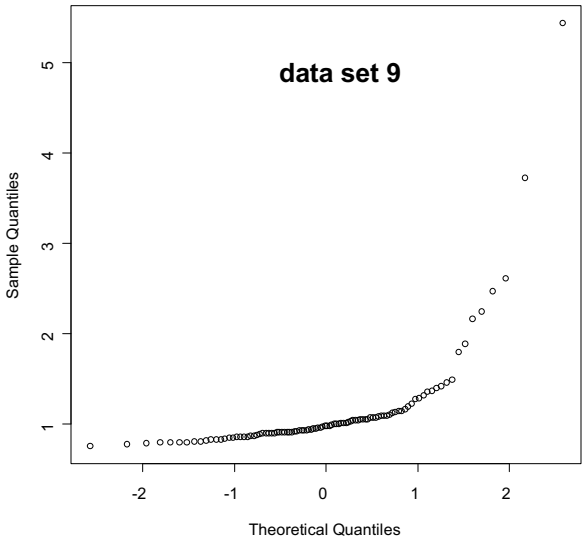
- **Alternative Weibull based transformation brings the false positive rate of the data set close to the target value of 5%**
- **For data sets without excluded data the CP of log transformed and Weibull based transformation are comparable**

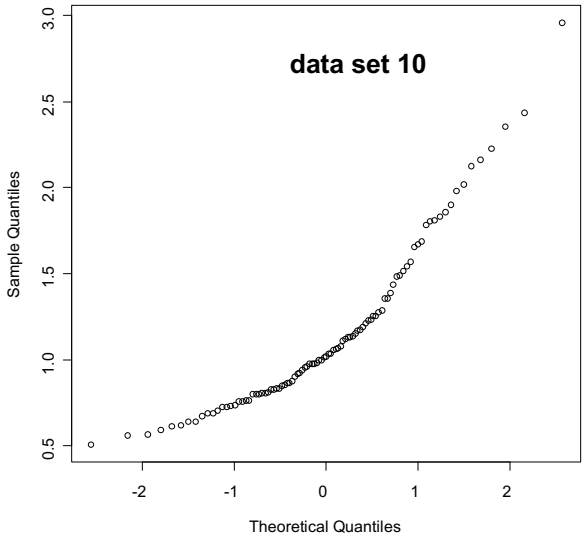
Comparison of calculated CP

… and the false positive rate when applying the CP on all data

Example of the last three data sets
Based on 3IQR
Data set 8: **4** Outlier detected
Data set 9: **8** Outlier detected
Data set 10: **3** Outlier detected







	decision-tree based CP calculation	Weibull transformation based CP calculation
Data set	false positive rate	false positive rate
1	6%	**6%**
2	8%	7%
3	18%	**8%**
4	5%	**5%**
5	13%	**5%**
6	19%	16%
7	8%	8%
8	16%	16%
9	17%	**7%**
10	10%	**4%**

CP on „all data“

Summary and conclusion

- **Weibull transformation** of ADA screening data could be applied **for all investigated data sets**
- **Robustness** of the Weibull based transformation approach could be **demonstrated** based on two data sets for which 3 * 100 data points were available
- **“Detection” of potential erroneous detected outliers** was possible with the Weibull transformation process
- CP and FPR for data sets without any detected outliers were considered to be **comparable for log transformation and Weibull based approach**
- Weibull based transformation brings, over all, the **FPR close to the theoretical value of 5%**

Acknowledgement

Roland Staack

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Tim Marchhauser
Miriam Moheysen-Zadeh

Julia Heinrich

Doing now what patients need next