Evaluation of outlier detection methods for cut-point determination of immunogenicity screening and confirmatory assays

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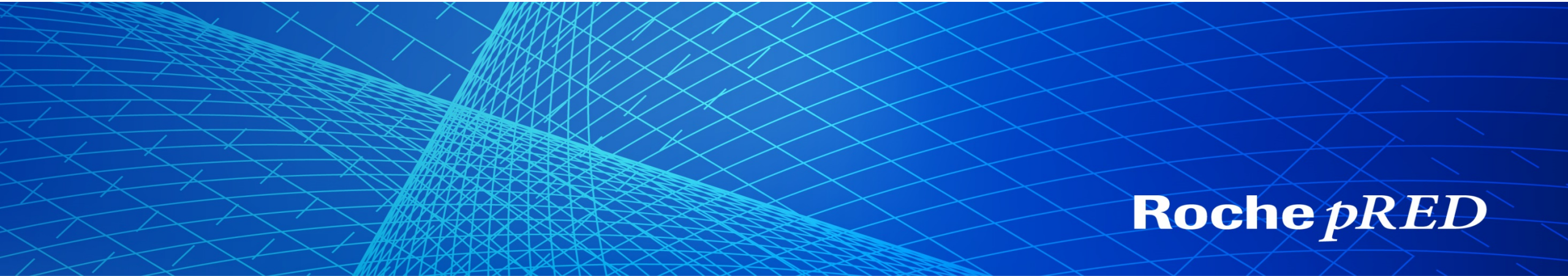
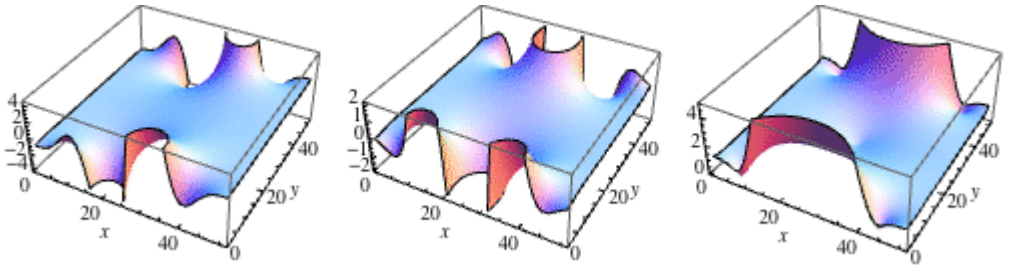


COMPLEX EQUATIONS



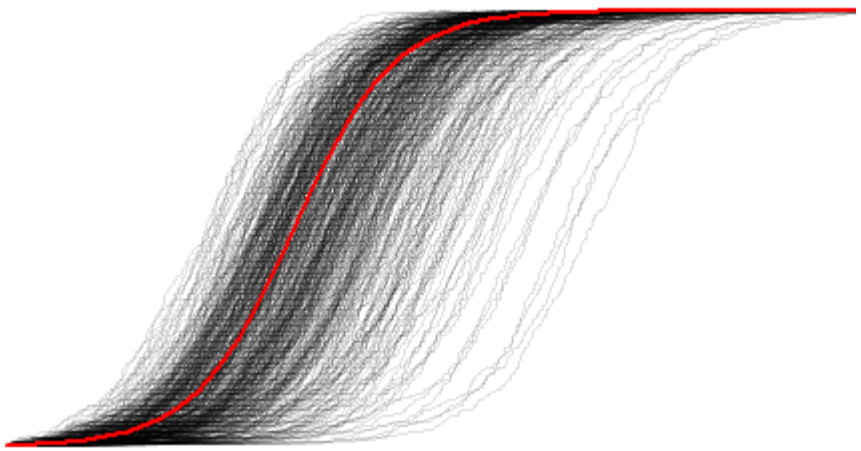
$$\int_0^\infty \frac{e^{-(p+x)y}}{\pi(p+x)} \sin\left(a\sqrt{x}\right) dx = -\sinh\left(a\sqrt{p}\right)$$

$$+\frac{e^{-a\sqrt{p}}}{2}\operatorname{erf}\left(\frac{a}{2\sqrt{y}} - \sqrt{py}\right) + \frac{e^{a\sqrt{p}}}{2}\operatorname{erf}\left(\frac{a}{2\sqrt{y}} + \sqrt{py}\right)$$

$$\int_0^\infty \frac{\sqrt{x}\, e^{-(p+x)y}}{\pi(p+x)} \cos\left(a\sqrt{x}\right) dx = \frac{e^{-\left[py+a^2/(4y)\right]}}{\sqrt{\pi y}} +$$

$$\sqrt{p}\left[-\cosh\left(a\sqrt{p}\right) - \frac{e^{-a\sqrt{p}}}{2}\operatorname{erf}\left(\frac{a}{2\sqrt{y}} - \sqrt{py}\right) + \frac{e^{a\sqrt{p}}}{2}\operatorname{erf}\left(\frac{a}{2\sqrt{y}} + \sqrt{py}\right)\right]$$

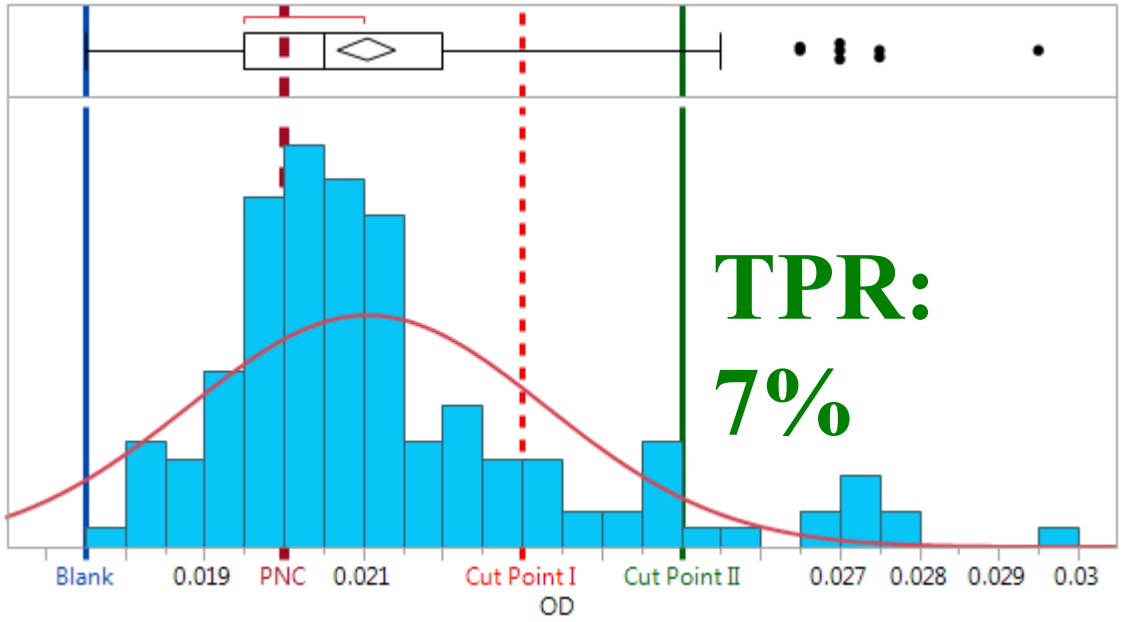
SIMULATIONS



The Challenge

- Good quality reagents and supply
- First-class assay development
- High sensitivity technologies

Sensitivity: 0.96 ng/mL



TPR: 7%

Total Positive Rate: 14%

WHAT IF

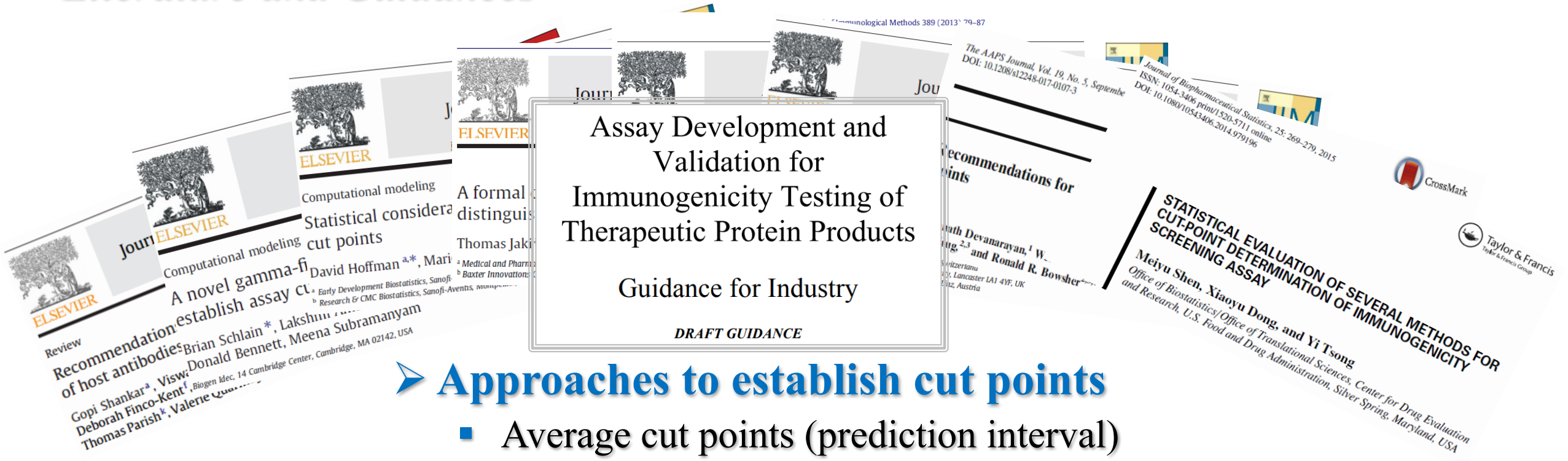
there was a different outlier detection approach?

Outline

- Literature and Guidances
- “Positive Rate” Definitions and Evaluation Scheme
- Outlier Detection Approaches
- Data Assessment Package
- Results & Conclusions

Cut point Determination

Literature and Guidances



➢ Approaches to establish cut points

- Average cut points (prediction interval)
- Confidence-level cut points (quantile lower bound)
- Robust parametric, (non-)parametric
- Mixture models

Cut point Determination

Literature and Guidances

- Significant amount of work on **simulated data sets**
  - frequently with **ideal** (normal) **distribution**
- Less investigation on real data sets
- **Outlier detection rarely considered**
  - focus on analytical vs. biological outliers
  - Shen et al. - *Effect of outliers on the cut-point estimator is not investigated, outlier identification and removal are not discussed either*
  - however, outlier removal can easily further inflate total/false positive rates

Positive Rates

Definitions

Whole vs. **negative population** to calculate rates

- Whole population used in our calculations

TPR

- **Total Positive Rate**: percent of all screened (confirmed) positive samples

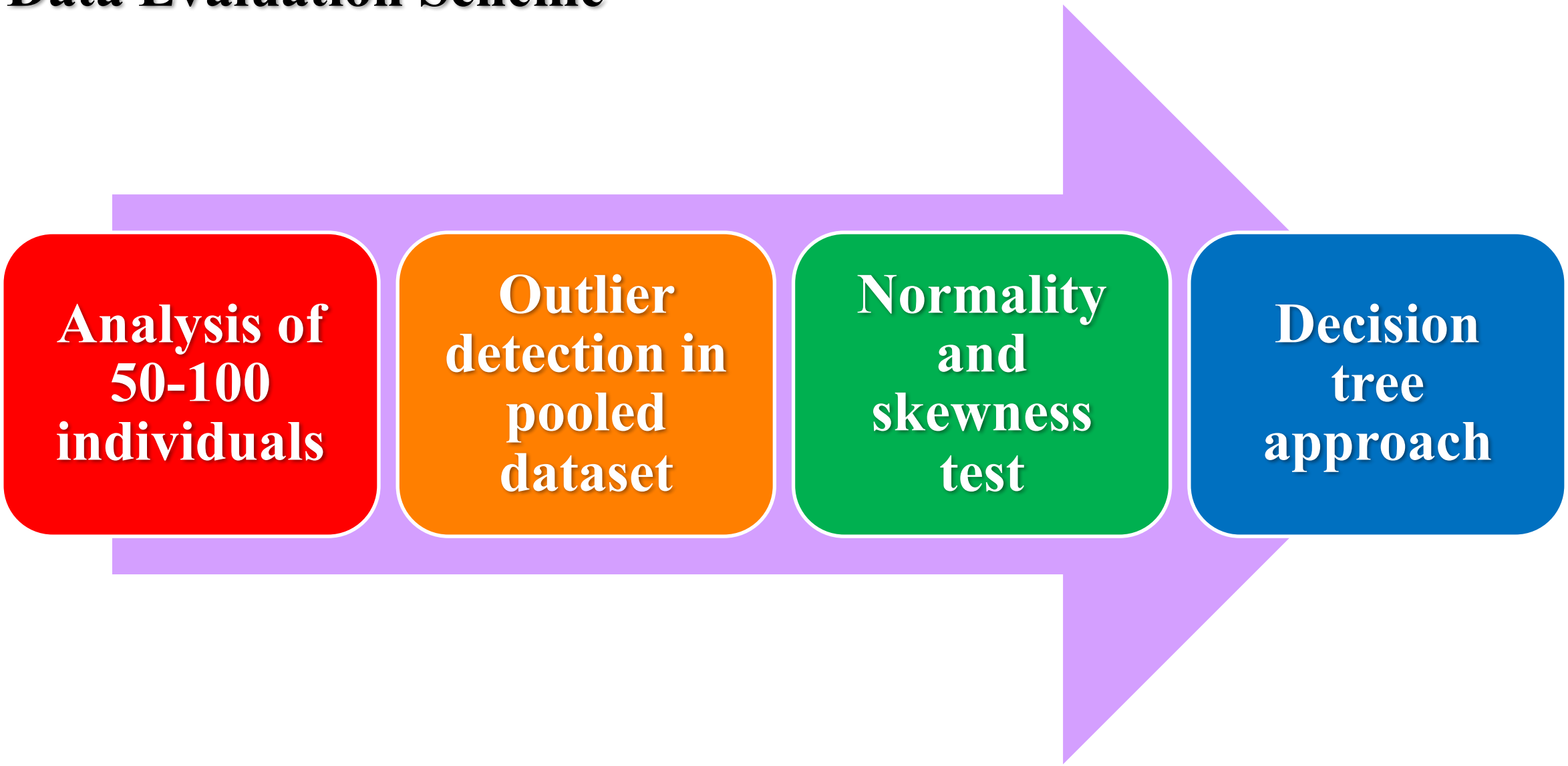
FPR

- **False Positive Rate**: percent of screened but not confirmed positive samples

CPR

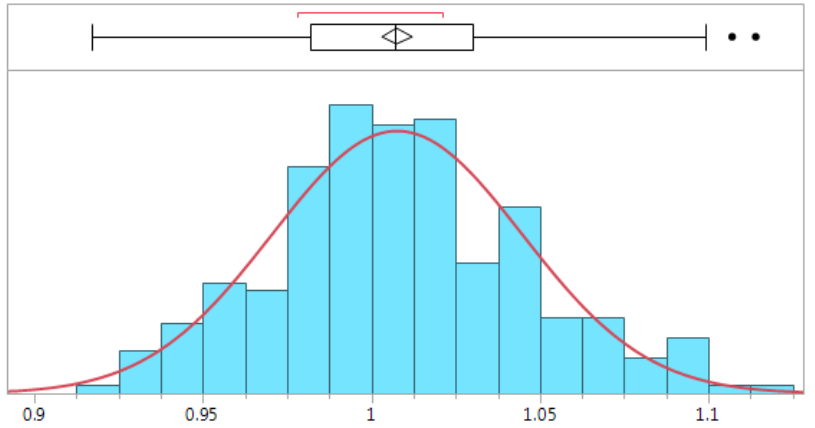
- **Confirmed Positive Rate**: percent of both screened and confirmed positive samples

Data Evaluation Scheme



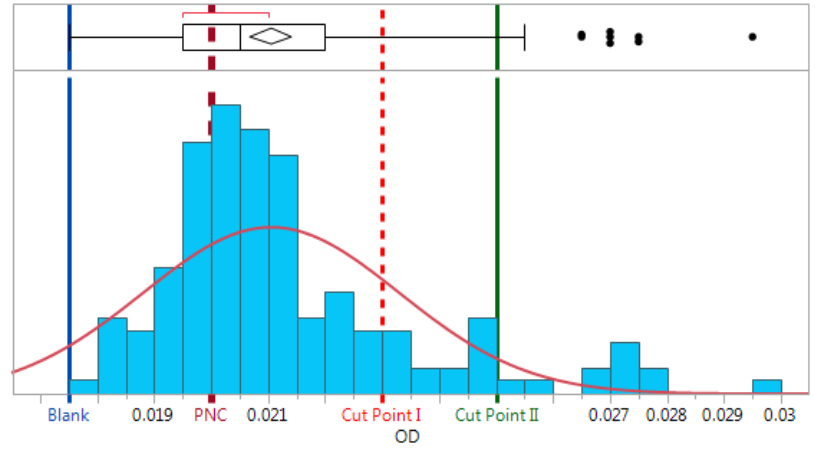
Outlier Detection Approaches

- **Tukey's boxplot (1.5*IQR)**
  - **3*IQR** for extreme values
  - Expected to work nicely when data are symmetric

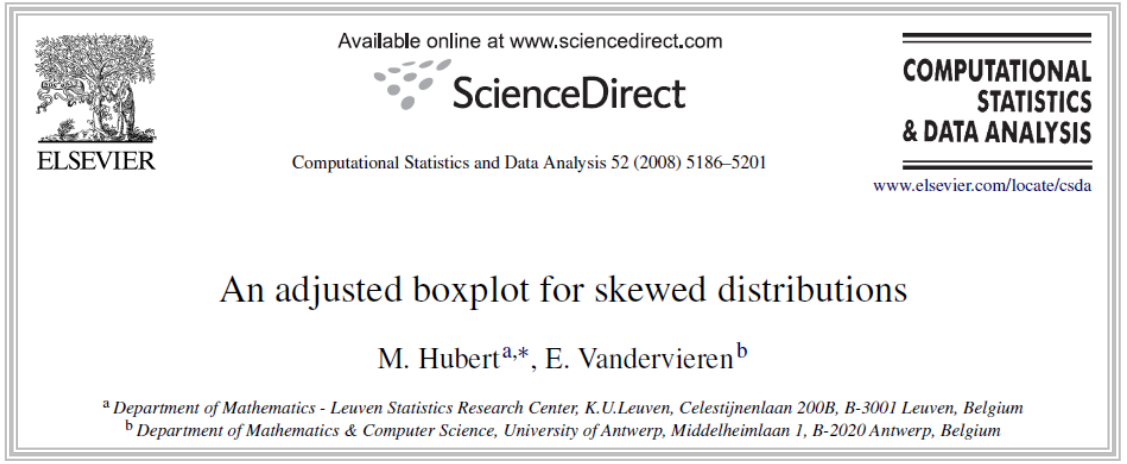


- **3*SD**
  - Estimate of SD significantly biased by the presence of outliers
  - **Robust alternative: 3*1.482*MAD** (MAD: median absolute deviation)
    - less influenced by the presence of outliers

- **ADJUSTED BOXPLOT**
  - Overcomes the problem of skewness / asymmetry
  - Can be applied to non-normal data sets



Adjusted Boxplot



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An adjusted boxplot for skewed distributions

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- Lack of industry experience in immunogenicity validations
- Adjustment of the boxplot that includes a robust measure of skewness in the determination of the whiskers
- Datasets can be processed in "R"
  - *Robustbase* package: *adjboxStats(x, coef = 1.5, a = -4, b = 3)*
- One fits all? Maybe not…
  - generalized boxplot for severely skewed distributions (Bruffaerts et al., 2014)
    - useful when some points are generated from another distribution

Data Assessment Package

Compounds, methods and matrices

- MAb, BsAb, PEG-Prot, Fab, Conj-Ab
- Bridging immunoassays
- Mostly ELISA, ECL
- Healthy and disease validation population

21 validation data sets evaluated

- 3 non-clinical screening
- 10 clinical screening
- 8 clinical confirmatory

+ a few clinical studies

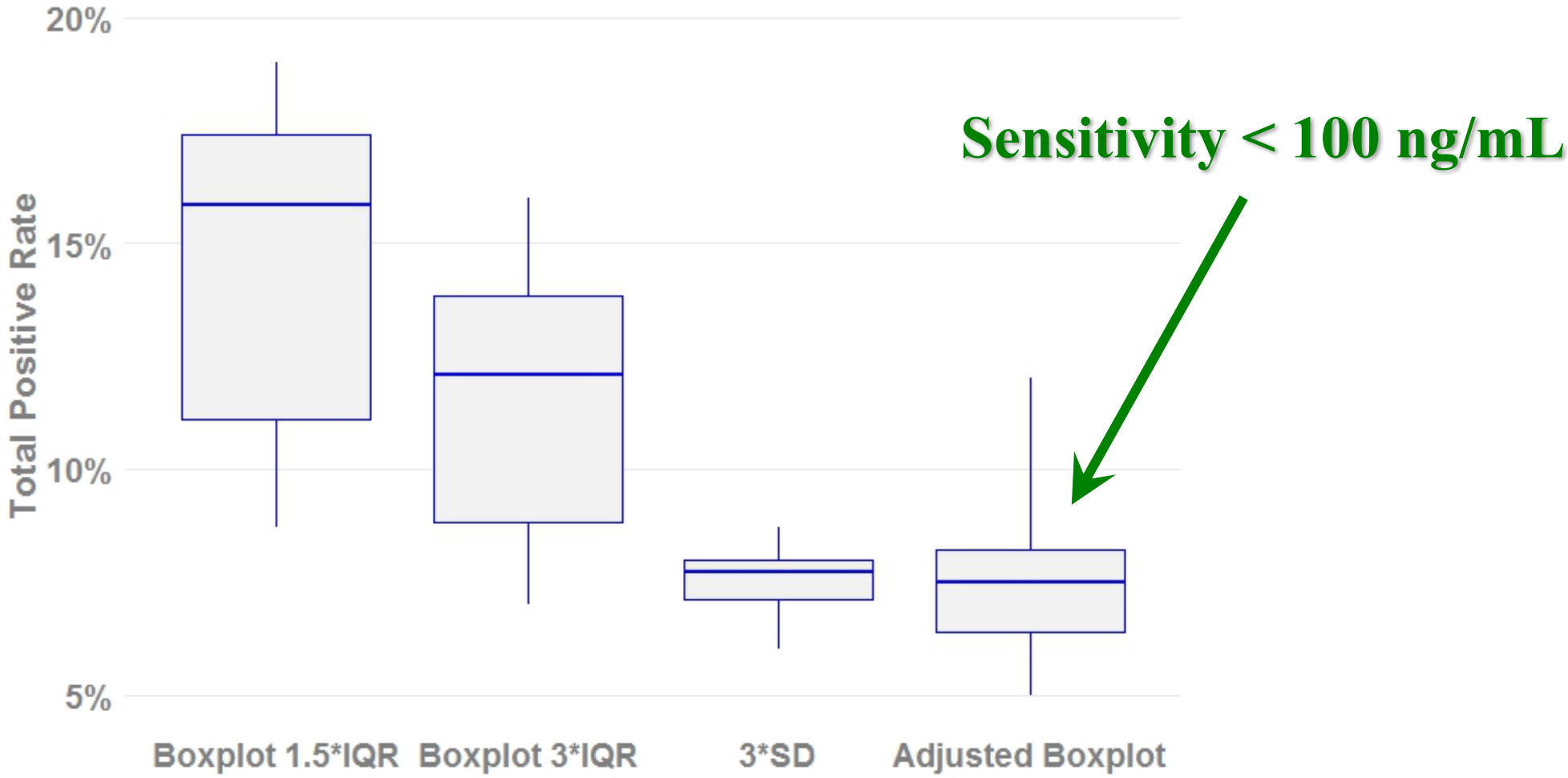
5 outlier detection methods tested

- Boxplot 1.5*IQR
- Boxplot 3*IQR
- 3*SD
- Robust alternative of 3*SD
- Adjusted boxplot

Threshold determination

- Robust parametric
- Parametric
- Parametric after log-transformation
- Non-parametric

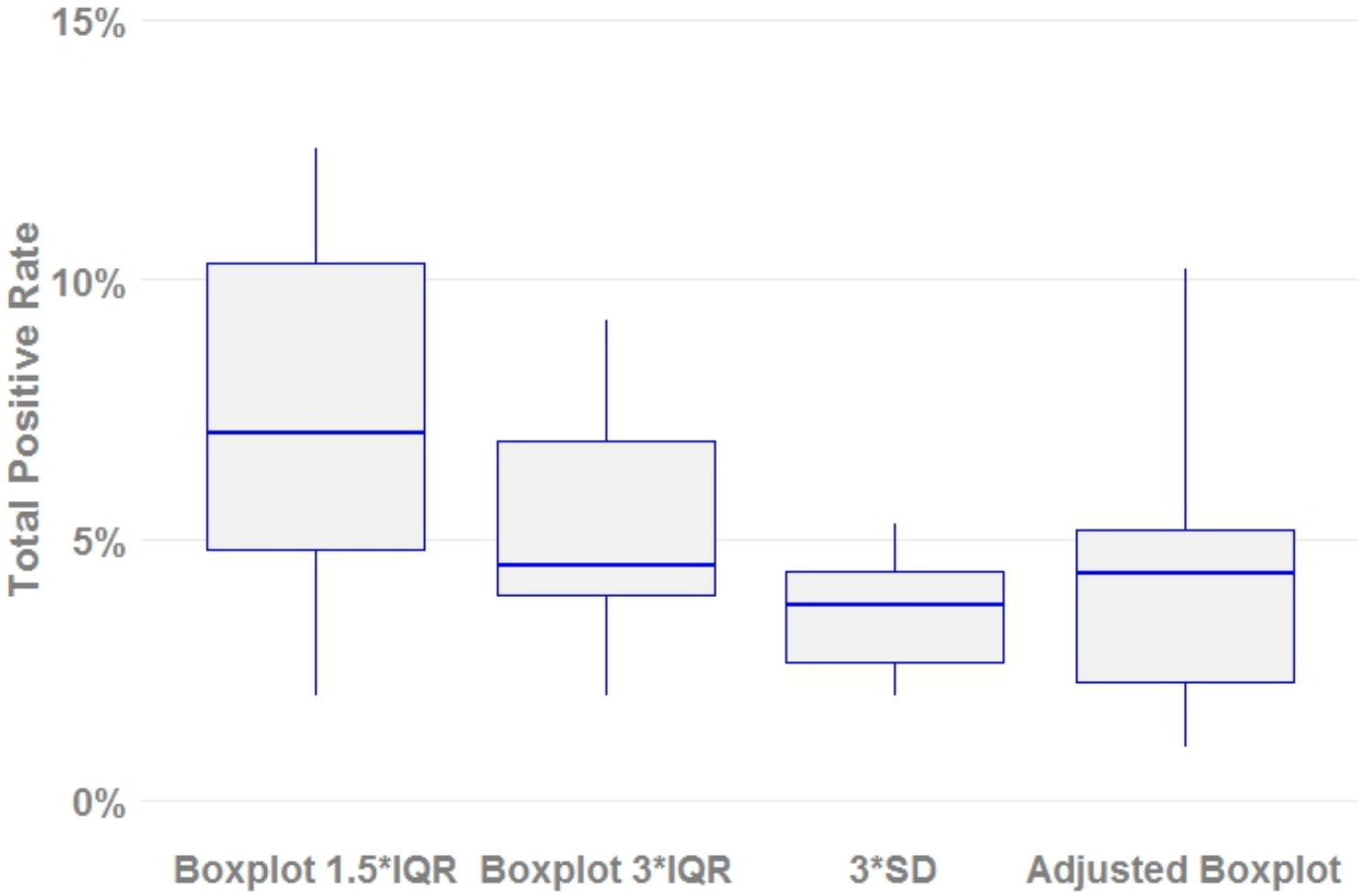
Composite TPR in Clinical Screening Validations



ADJUSTED BOXPLOT: Superior performance with more favourable total positive rate

Composite data of all 10 clinical screening validations

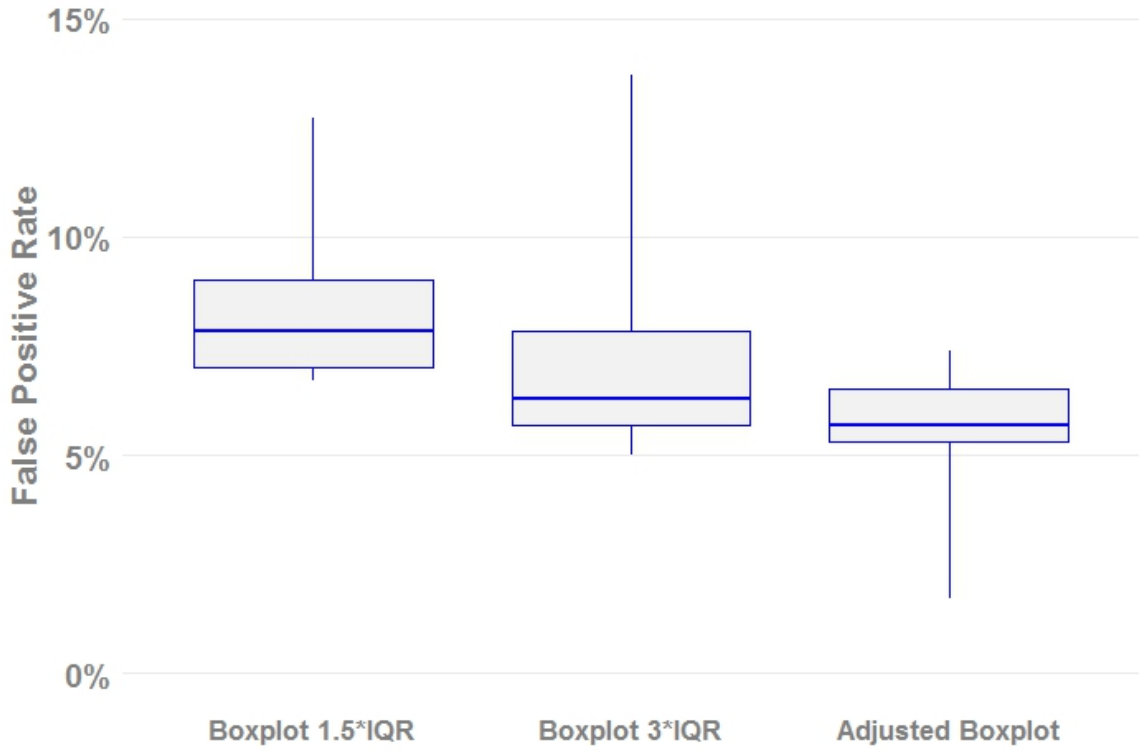
Composite TPR in Clinical Confirmatory Validations

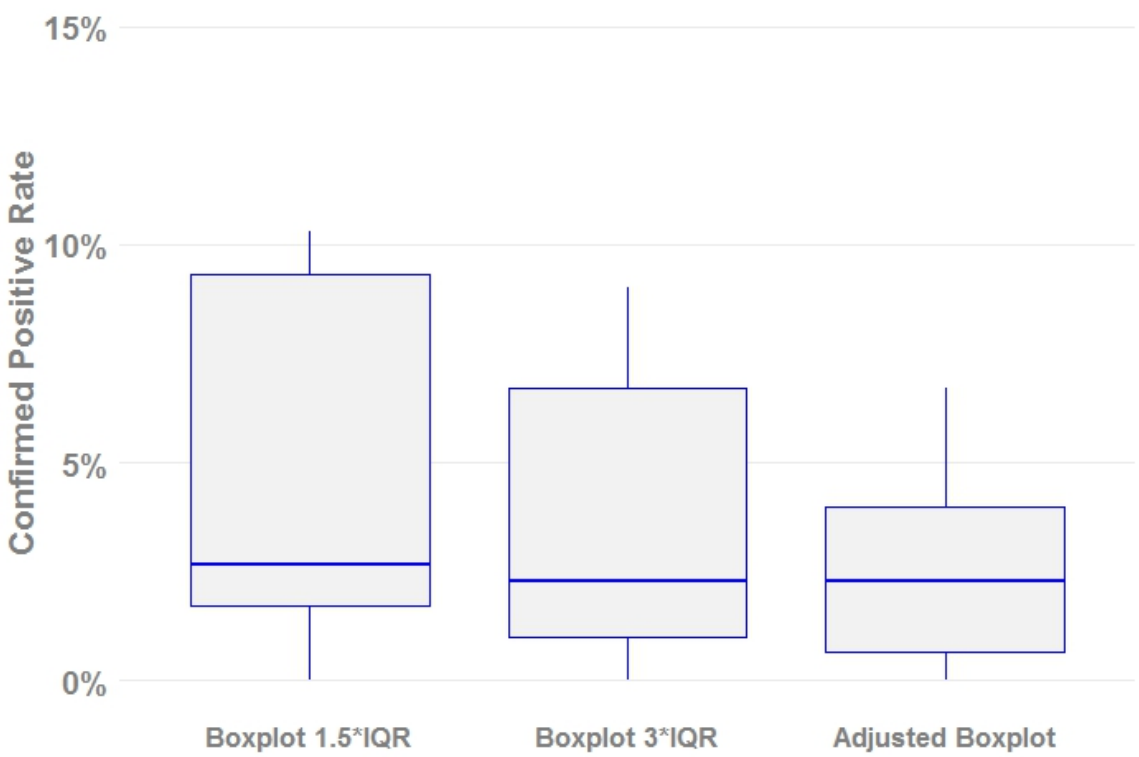


- Distribution of confirmatory ratios tends to be closer to ideal situation
- Less difference among median TPRs
- Adjusted boxplot performs nicely under less extreme conditions

Composite data of all 8 clinical confirmatory validations

Composite FPR and CPR in Clinical Validations





Composite data of 8 clinical screening and confirmatory validations

Non-Inferiority at the Other Extreme

- Human screening method (100 individuals measured three times)
- Bridging ELISA, photometric readout, PEG-protein, sensitivity<10 pg/mL
- Almost normally distributed data set with very low variability
- **Adjusted boxplot shows very similar performance**

Compound RO001	BP 1.5*IQR	BP 3*IQR	ADJUSTED BP
Screening CPF	1.086	1.091	1.091
TPR	8.7%	8.0%	8.0%
Confirmatory CP	9.0%	10.3%	10.1%

Assessment in Clinical Studies I

- Assessment of clinical baseline study samples (n=585)

Compound RO002	BP 1.5*IQR	BP 3*IQR	ADJUSTED BP
TPR in Validation	19.0%	11.2%	5.0%
TPR in Study with Validation CPF	12.0%	7.5%	4.6%

Assessment in Clinical Studies II

Compound RO003 (n=120)		BP 1.5*IQR	BP 3*IQR	ADJUSTED BP
SCREENING ASSAY	TPR in Validation	16.0%	16.0%	12.0%
	TPR in Study with Validation CPF	22.5%	20%	16.7%
	TPR in Study with In-study CPF	16.7%	13.3%	9.2%
CONFIRMATORY ASSAY	TPR in Validation	10.0%	8.7%	6.7%
	TPR in Study with Validation CCP	9.2%	8.3%	5.0%

Conclusions

- There is a need for outlier detection approaches that can deal with skewed data sets
- Adjusted boxplot is a promising outlier detection method exhibiting this feature
- Close-to-ideal screening and confirmatory validation data sets showed non-inferior performance of adjusted boxplot in cases where asymmetry is low
- Superiority of adjusted boxplot was shown in screening data sets (validations and clinical studies) where skewed data sets are more common

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… and many more

Doing now what patients need next