Statistical approaches for Anti-Drug-Antibody (ADA) identification

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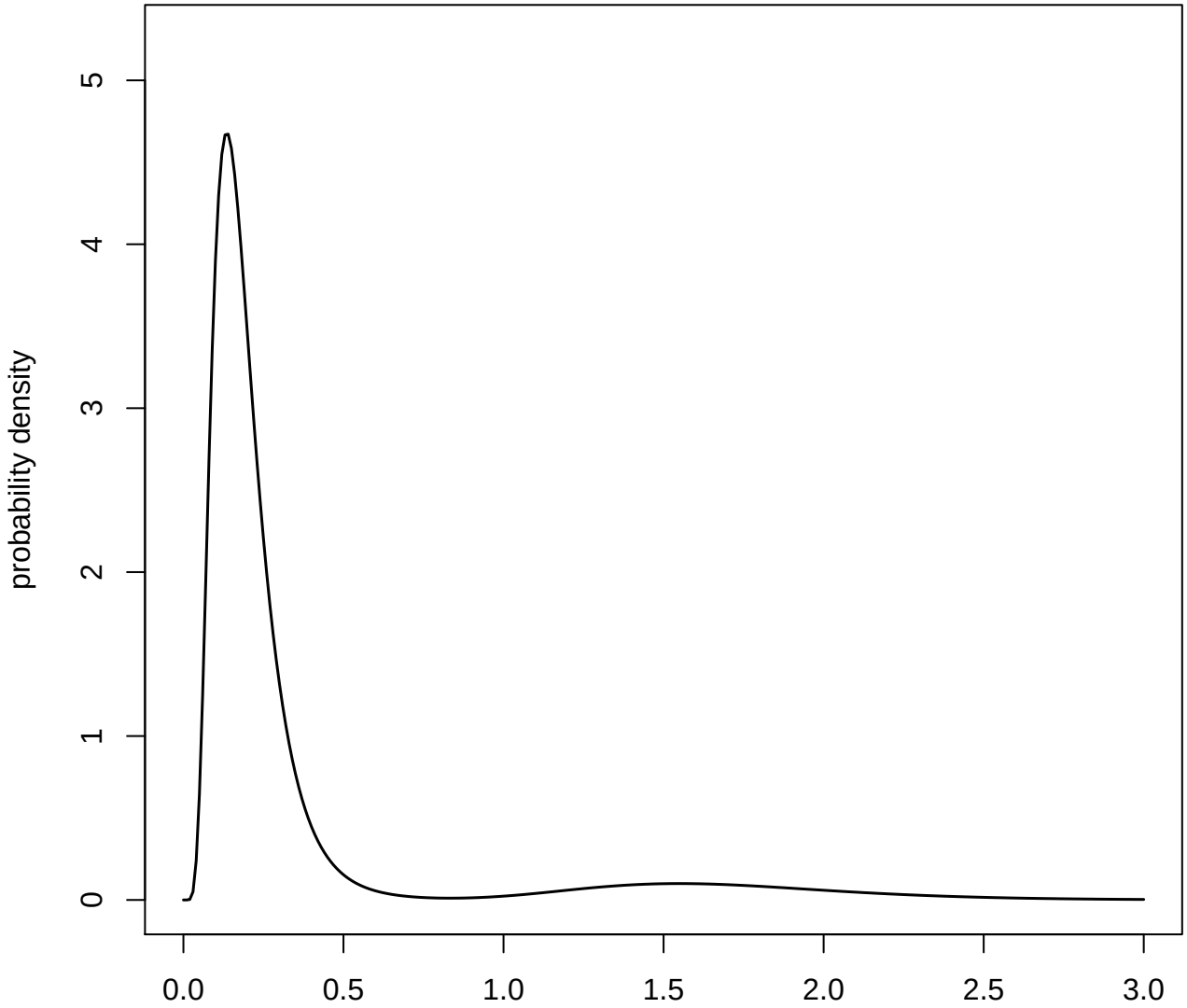
Motivation

- Biotechnology derived therapeutics may induce anti-drug antibodies (ADA);
- ADAs can impair efficacy and safety;
- Assays for the detection of ADAs necessary;
- Appropriate methods for classification (cut-off values) that distinguish between positive and negative samples crucial.

The challenge



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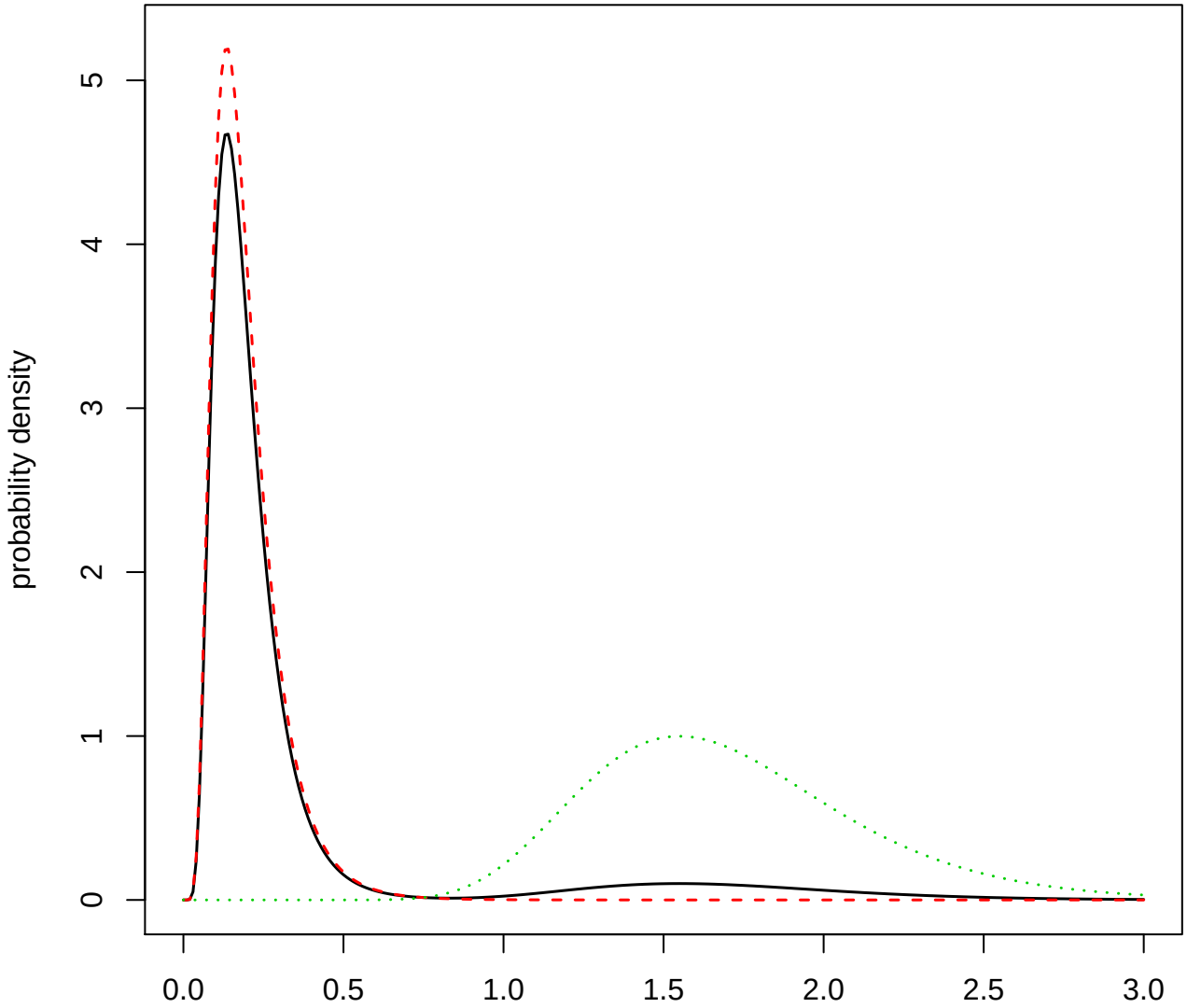
The challenge



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A multi-tier approach

Stage 1: screening assay is used for rapid identification of positive samples

Stage 2: confirmatory assay is used to confirm the results of the screening assay

Stage 3: a functional assay for assessment of the neutralizing capacity of antibodies

Objective of this talk

Objective: Evaluate multi-tier approach

- Simplified setting
  - Several runs undertaken
  - Cut-points based on average of runs
  - No plate/well-effect
  - No true positives when establishing cut-points
- Methods evaluated can be extended to other settings

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Cut-point determination

Screening cut-points

- Several white papers (eg Mire-Sluis *et al.* 2004, Shankar *et al.* 2008);
- Comparison of statistical properties of methods (Jaki *et al.* 2011, Hofman & Berger 2011)

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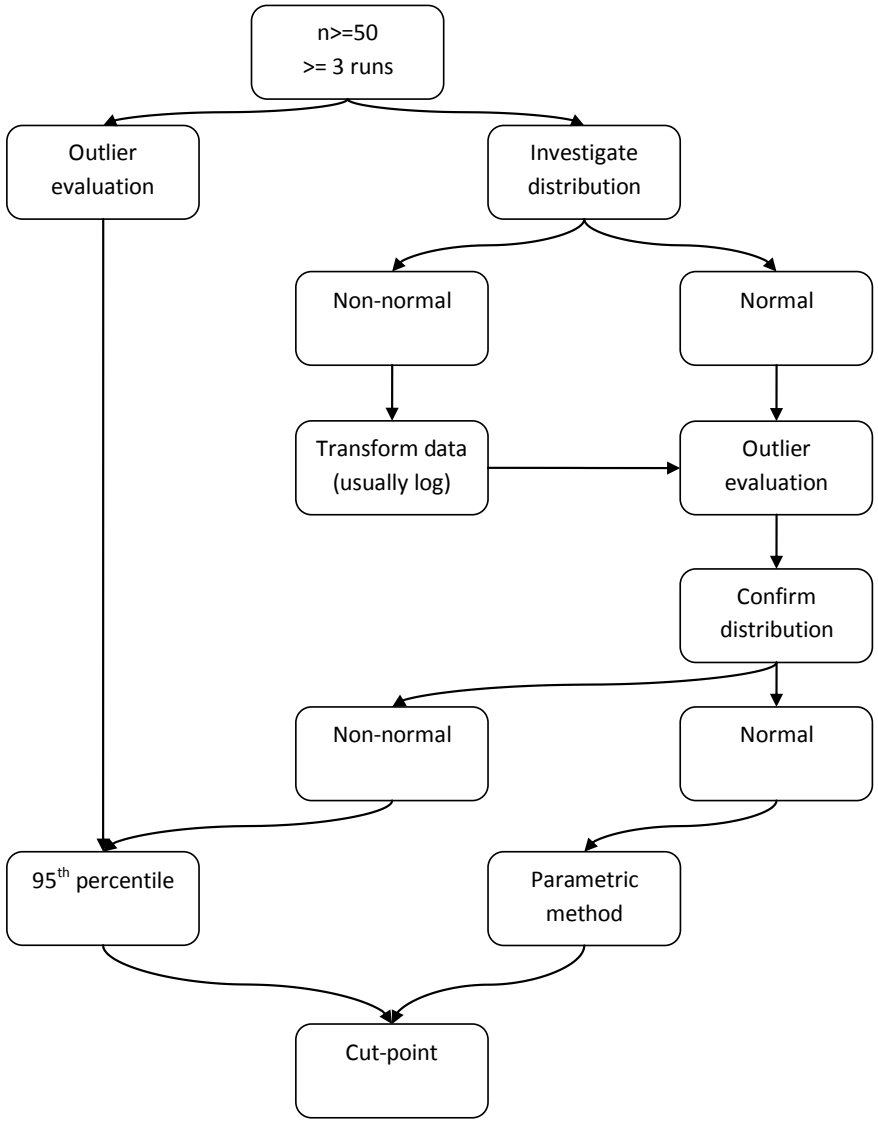
Simple Methods

- 95th percentile;
- Parametric method: $\bar{X} + z_{0.95} * SD(X)$;
- Robust parametric method: $X_{0.5} + z_{0.95} * 1.483 * MAD$, where $MAD = median(|X - X_{0.5}|)$.

Figure : Decision tree according to Shankar et al. 2008.



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Mixture model
Method

1. Fit a 1-component and 2-component mixture model;
2. Select the better fitting model via BIC;
3. Use the 95th percentile of the lower distribution as the cut-point.

Experimental approach

Goal: Eliminate false positives.

- Define the 95th percentile of confirmatory assay data as preliminary cut-point;
- Exclude from the screening dataset observations whose
  - i screening values > preliminary cut-point and;
  - ii confirmatory values > preliminary cut-point;
- Define the cut-point as 95th percentile of new dataset.

Simulation setting

- True positive samples have high OD in screening assays, but low OD in confirmatory assays;
- False positives have high OD in screening assays and confirmatory assays;
- True negative samples have low OD on both assays;
- Samples of size 160;
- 10,000 simulation runs for each combination.

Scenario

- large difference between positive and negative samples
- log-normal observations
- 10% true positives
- 5% false positives

Table : Detailed results of classification

	correct true positive	correct true negative	correct false positive
95th percentile	36.54	100.00	62.47
Parametric method	96.85	99.99	3.34
Robust parametric method	100.00	96.38	0.00
Shankar's decision tree	63.96	99.01	35.84
Mixture	98.81	98.51	1.14
Experimental approach	55.59	99.99	44.49

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Discussion

- No uniformly superior method available;
- Robust method performs well in the presence of positive values;
- Mixture models provide a flexible tool to tailor cut-point determination;
- Decision tree does not work well

Approach - confirmatory

- Test only samples positive according to screening assay
- Competition assay
- Multiple runs per sample with and without pre-incubation

Methods

1. Inhibition (Shankar *et al.*, 2008):

$$\mathrm{I} = 100 * \left(1 - \frac{\text{inhibited value}}{\text{uninhibited value}}\right)$$

- Positive sample if $\mathrm{I}^* > 50$ %
  (fixed 50% inhibition)
- Positive sample if $\mathrm{I}^* > \bar{I} + z_{1-\alpha} * sd(I)$ with $\alpha = 0.001$
  (parametric %inhibition)

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Methods

2. Difference $-$ (parametric difference)

$$D = \text{uninhibited value} - \text{inhibited value}$$

- Positive sample if D* > $\bar{D} + z_{1-\alpha} * sd(D)$ with $\alpha = 0.001$

3. t-test (Neyer *et al.*, 2006) $-$ (t-test)

- perform t-test within sample of uninhibited vs inhibited
- Positive sample if p-value < 0.01

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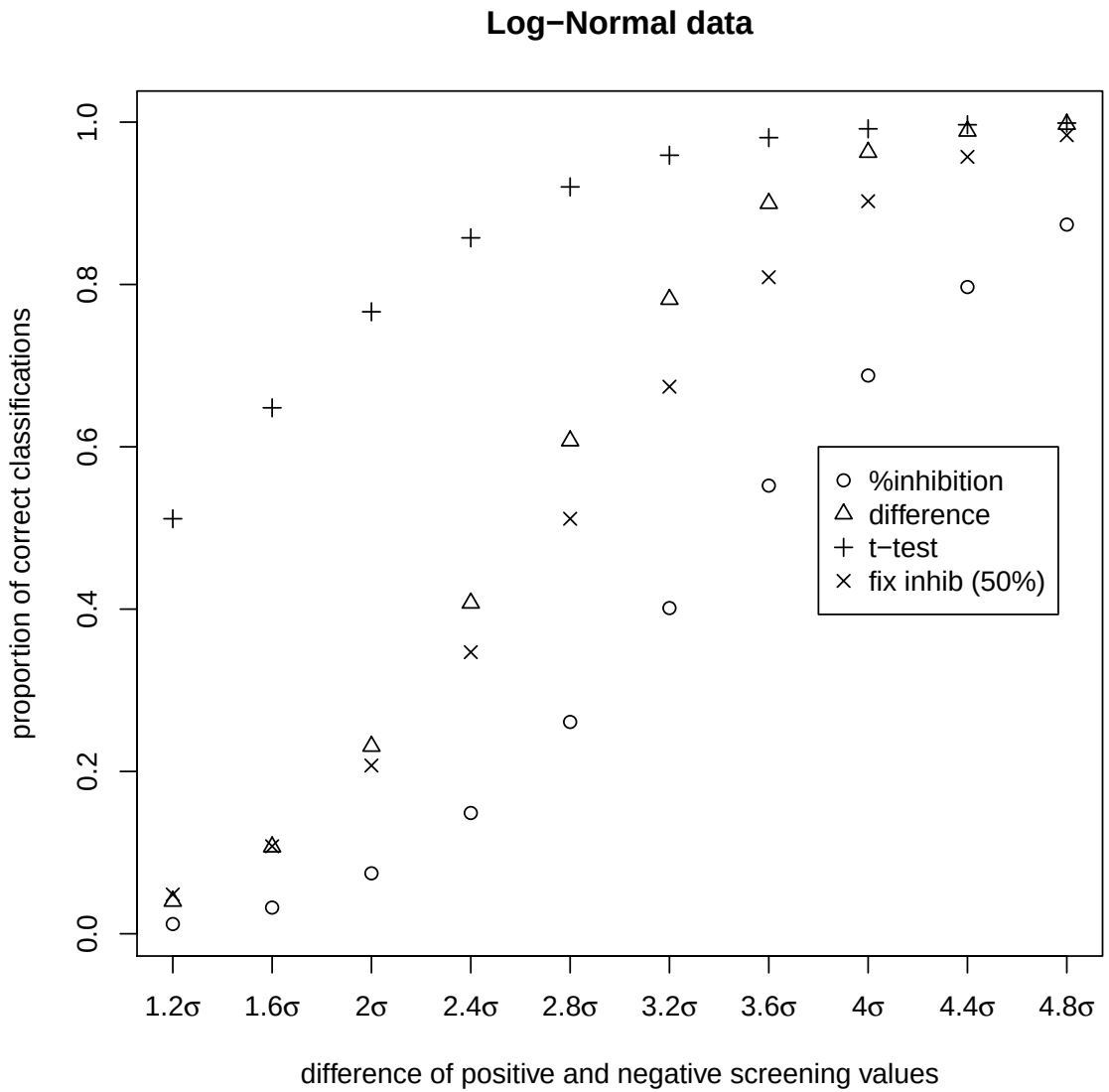
Difference between positive and negative



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Discussion

- Large differences between positive and negative samples required;
- t-test superior to alternative methods;
- Variation between runs causes concern.

Simulation setting

- 2-stage simulation:
  1. Generate data containing all negative samples to find cut-points
  2. Generate data containing 85% true negative, 10% true positive and 5% false positive samples
- Three runs per sample
- Normal and log-normal data
- $n = 160$ to find cut-point, $n = 1000$ to evaluate it
- 10,000 simulation runs

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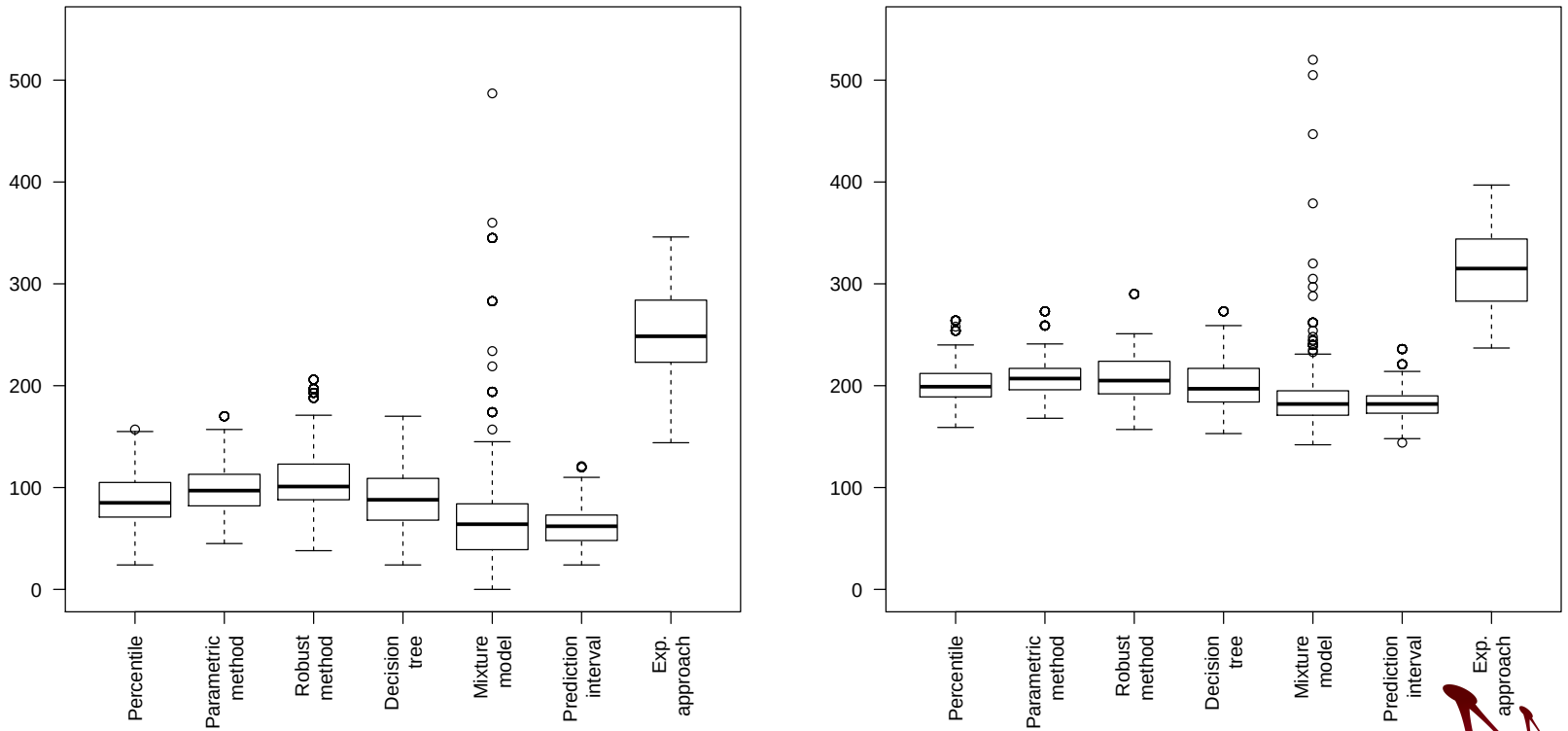
Number of samples passed to confirmation

Normal

Figure : One standard deviation difference between positive and negative samples on left panel and a difference of 5 on the right.



Note: We expect 200 samples to be passed on.

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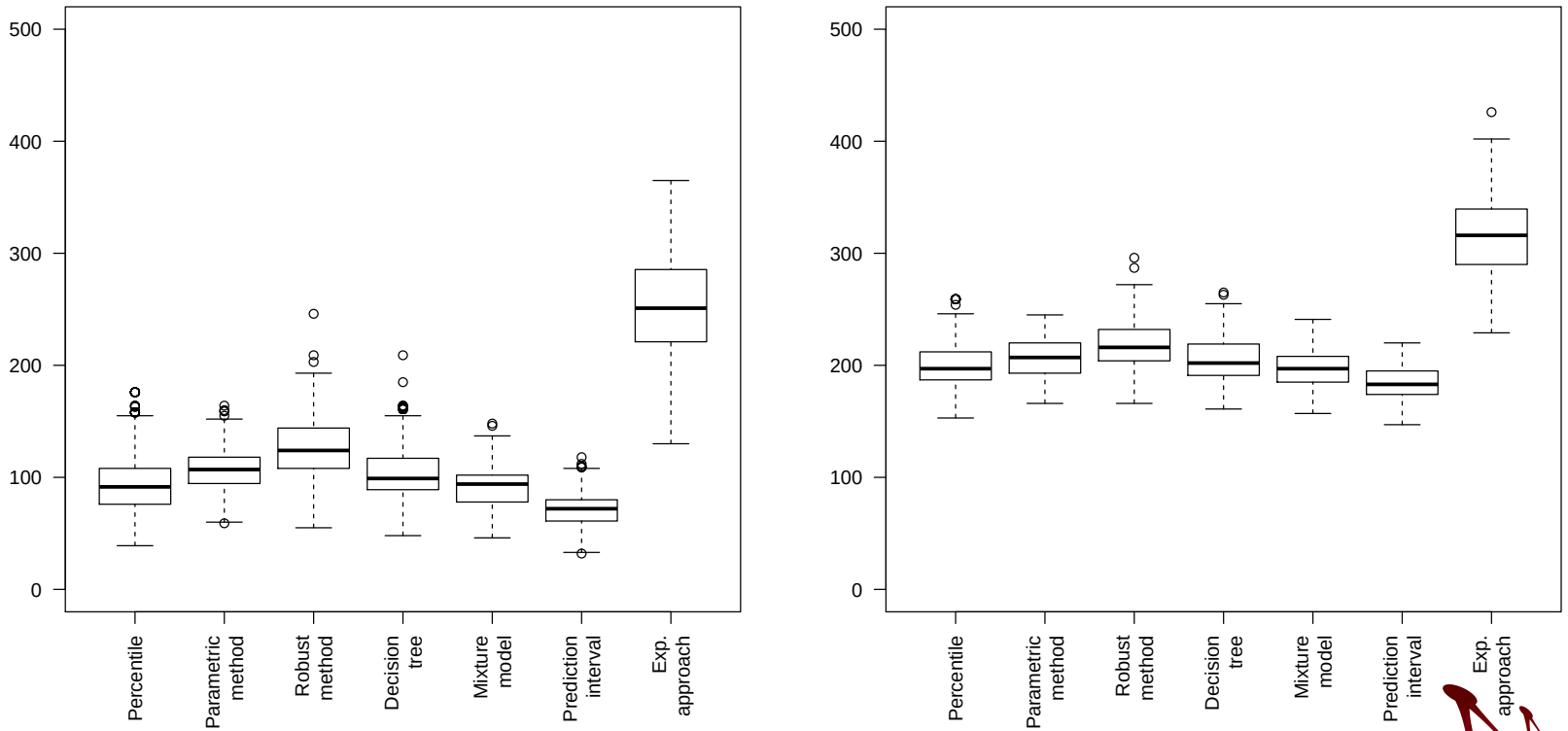
Number of samples passed to confirmation

Log-normal

Figure : One standard deviation difference between positive and negative samples on left panel and a difference of 4 on the right.



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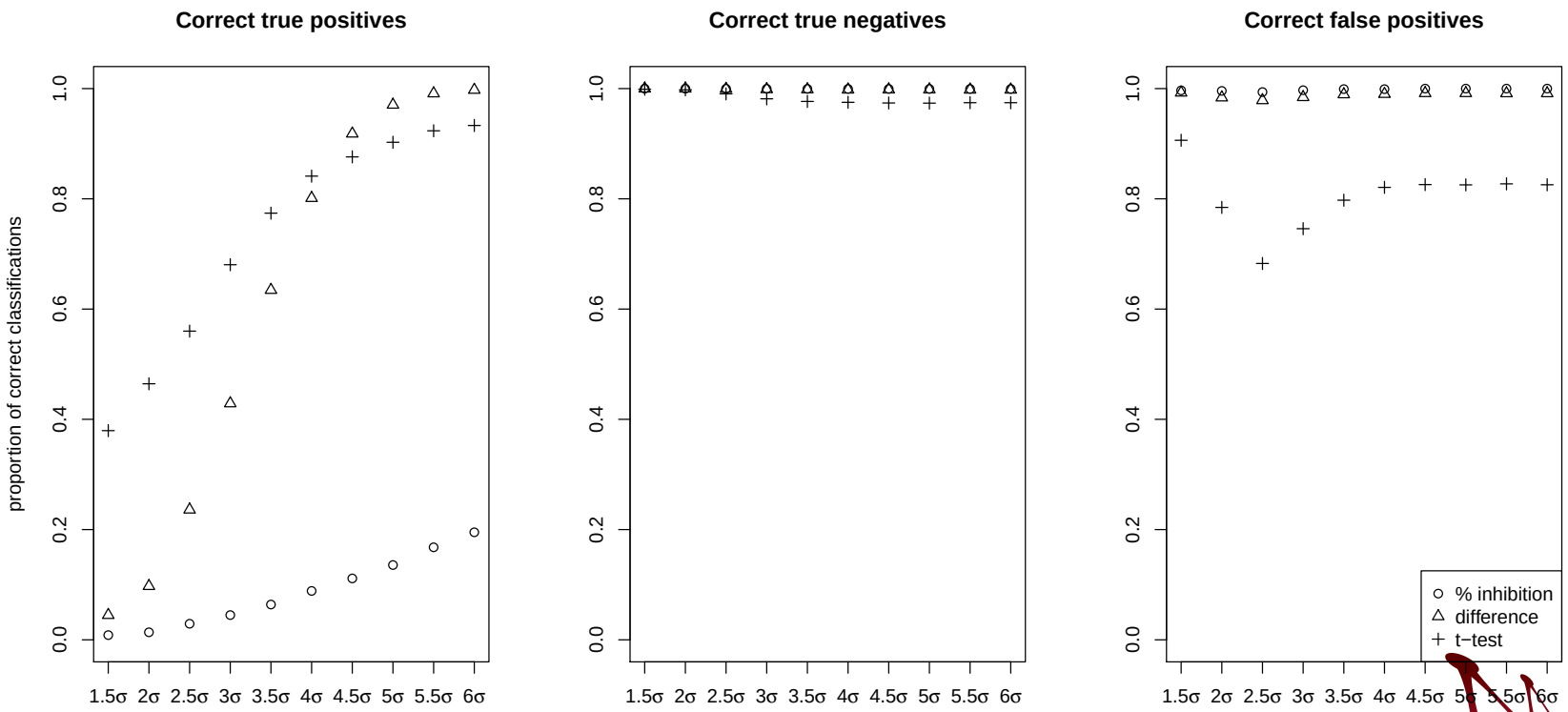
Classification rates: robust method used for screening

Normal

Figure : Classification rates across the two stages when the robust parametrics method is used for the screening assays.



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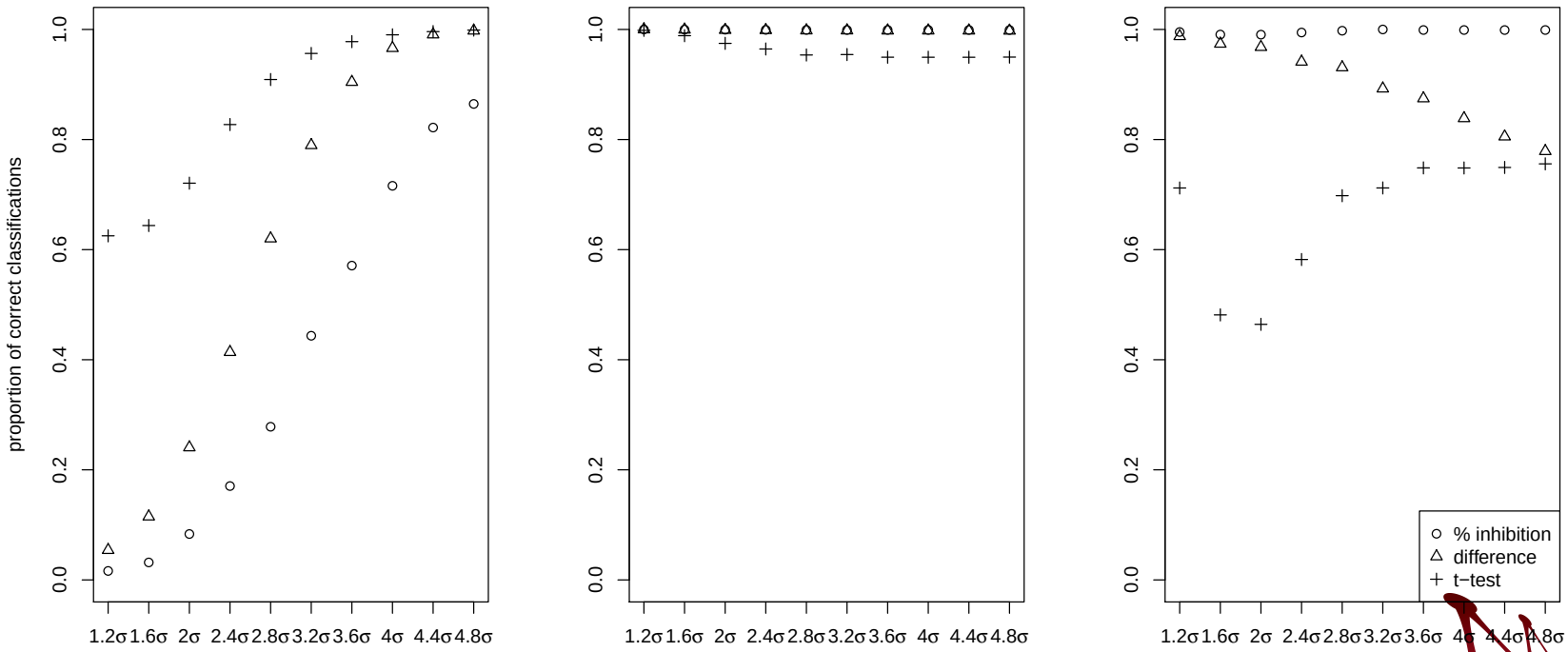
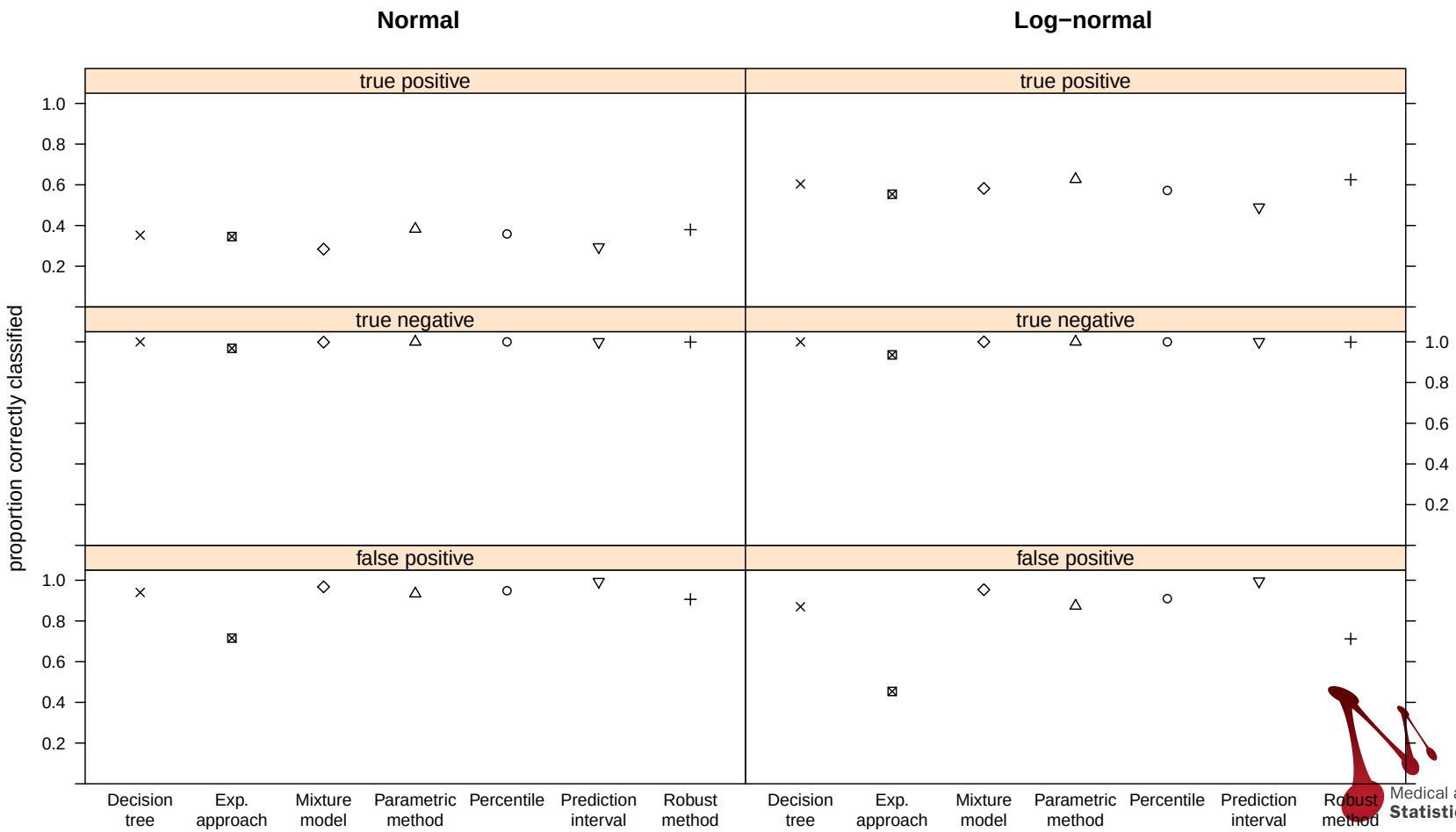


Number of samples passed to confirmation

Figure : Classification rates across the two stages when the t-test is used for the confirmatory assays.



Discussion

- Multi-tier approach works ok IF confirmation assay works
- Method for screening CP of secondary importance
- Results are similar under imperfect scenarios too

Conclusion

Two options:

- Need better methods for confirmatory assays
- Do not even bother with confirmatory assays

References

Hoffman D, Berger M (2011) Statistical considerations for calculation of immunogenicity screening assay cut points. J Immunol Methods. 373:200-208.

Jaki T, Lawo J-P, Wolfsegger MJ, Singer J, Allacher P, & Horling F. (2011) A formal comparison of different methods for establishing cut points to distinguish positive and negative samples in immunoassays. J Pharm Biomed Anal. 55:1148-1156.

Jaki T, Lawo J-P, Wolfsegger MJ, Allacher P, & Horling F. (2014) A comparison of methods for classifying samples as truly specificwith confirmatory immunoassays. J Pharm Biomed Anal. 88:27-35.

Neyer L, Hiller J, Gish K, Keller S, Caras I (2006) Confirming human antibody responses to a therapeutic monoclonal antibody using a statistical approach. J Immunol Methods. 315:80-87.

Mire-Sluis AR, Barrett YC, Devanarayan V, Koren E, et al (2004) Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods. 289:1-16.

Shankar G, Devanarayan V, Amaravadi L, Barrett YC, et al (2008) Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 48:1267-1281.

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