Statistical methods for cut-point determination in enzyme-linked immunosorbent assays

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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 cut-off values that distinguish between positive and negative samples crucial.

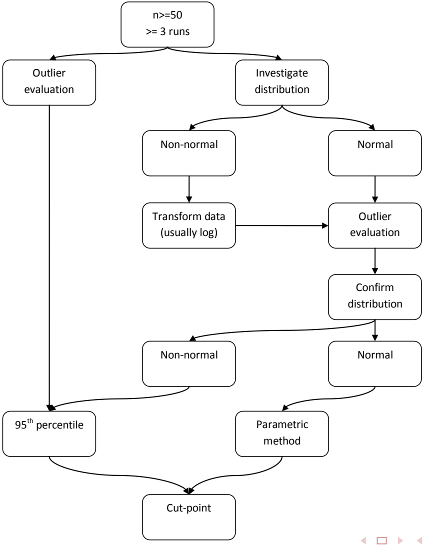
Previous work on cut-point determination

- Several white papers (eg Mire-Sluis *et al.* 2004, Shankar *et al.* 2008);
- Recommendations unspecific;
- Statistical basis for recommendations unclear.

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



Mixture model

Motivation

- Sample could contain positive and negative subjects;
- Positive subjects have higher OD values than negative subjects;
- Covariates (Experimenter, Cage, ...) are not used.

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.

Mixture model
Comments

- Covariates can be included by using regression mixture models;
- Different distributions can be used;
- Predictors for component membership can be included.

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 40, 80 and 160;
- 10 different simulation scenarios;
- 10,000 simulation runs for each combination.

Comparators

- false positive rate
- false negative rate
- proportion of correctly classified
    - true positive
    - true negative
    - false positive

  samples

Scenarios

Table 1: Scenarios investigated

#	positive vs negative samples	distribution	true positive rate	false positive rate
1	no positive samples	log-normal	0.00	0.00
2	small difference	log-normal	0.10	0.10
3	moderate difference	normal	0.05	0.05
4	large difference	log-normal	0.10	0.05

Results

Figure 2: Distribution of cut-points in Scenario 4 over 10,000 simulations. (a) corresponds to 40 samples, (b) to 80 samples and (c) to 160 samples.

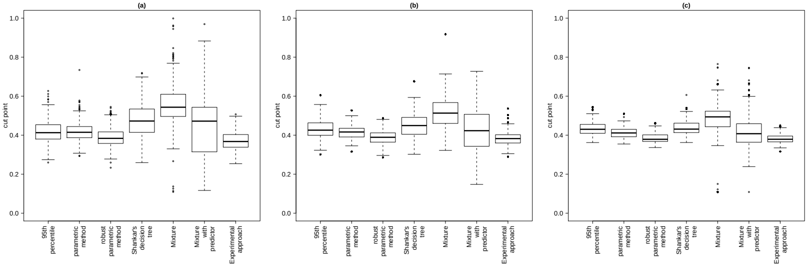


Table 2: Detailed results of classification for Scenario 4 with n=160.

	false positive rate	false negative rate	correct true positive	correct true negative	correct false positive
95th percentile	1.72	6.10	36.54	100.00	62.47
Parametric method	4.64	0.36	96.85	99.99	3.34
Robust parametric method	7.96	0.00	100.00	96.38	0.00
Shankar's decision tree	3.77	3.68	63.96	99.01	35.84
Mixture	7.38	1.92	81.99	95.86	18.00
Mixture (with class predictor)	6.05	0.13	98.81	98.51	1.14
Experimental approach	2.65	4.45	55.59	99.99	44.49

Table 3: Detailed results of classification for Scenario 4 with n=160.

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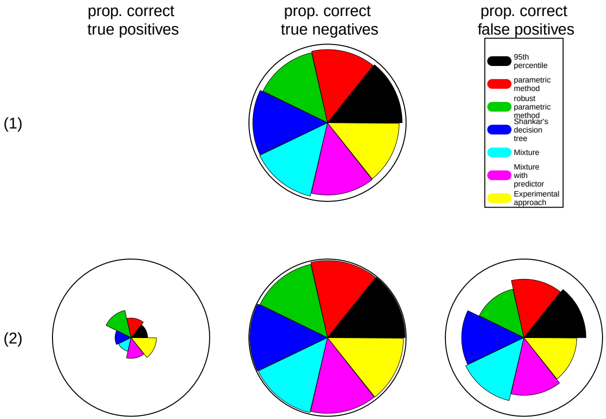
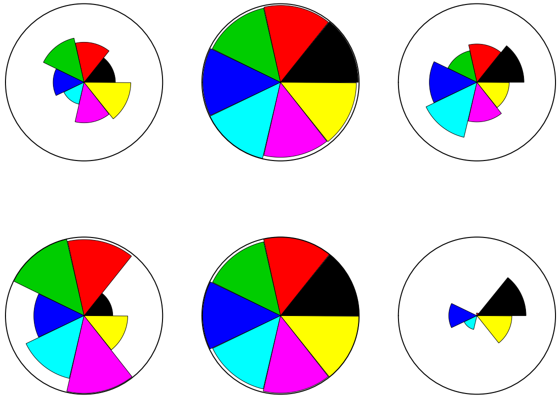

prop. correct true positives
prop. correct true negatives
prop. correct false positives
(1)
(2)
95th percentile
parametric method
robust parametric method
Shankar's decision tree
Mixture
Mixture with predictor
Experimental approach

(3)

(4)



Discussion

- No uniformly superior method available;
- Using screening assays together with confirmatory assays allows elimination of false positives;
- Robust method performs well in the presence of positive values;
- Mixture models provide a flexible tool to tailor cut-point determination.

References

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