An evaluation of methods for determining confirmatory cut-points in enzyme-linked immunosorbent assays (ELISA)

Thomas Jaki[1] John-Philip Lawo[2] Martin J Wolfsegger[3]
Peter Allacher[3] Frank Horling[3]

[1]Lancaster University, Lancaster, United Kingdom
[2]CSL Behring GmbH, Marburg, Germany
[3]Baxter Innovations GmbH, Vienna, Austria

NCS Conference
September 25, 2012

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

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

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)

Confirmatory cut-points:

- Recommendation in Shankar *et al.* (2008)
- Alternative method proposed in Neyer *et al.* (2006)

Methods

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

$$\text{I} = 100 * \left(1 - \frac{\text{competion value}}{\text{screening value}}\right)$$

- Positive sample if I > 50 %
  ($\times$ fix inhib (50%))
- Positive sample if I > $\bar{I} + z_{1-\alpha} * sd(I)$ with $\alpha = 0.001$
  ($\circ$ %inhibition)

Overall cut-point

1. based on the average of the runs per sample
2. based on the average of the per run cut-points
3. based on pooling all data (ie runs are treated as independent samples)

Methods

2. Difference $-$ ($\triangle$ difference)

$$D = \text{screening value} - \text{competition value}$$

- 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 screening vs competition
   - Positive sample if p-value < 0.01

Simulation setting

- 2-stage simulation:
  1. Generate data containing mostly (all) negative samples to find cut-points
  2. Generate data containing 5% true negative, 90% true positive and 5% false positive samples
- Three runs per sample (with and without differences between runs)
- Normal and log-normal data
- $n = 40$, 80 and 160 to find cut-point, $n = 1000$ to evaluate it
- 10,000 Simulation runs

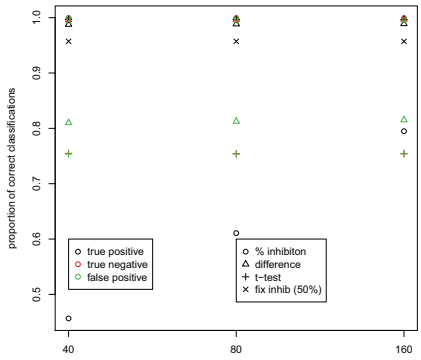
Comparators

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

  samples

Sample size

Figure 1: Classification rates for different sample sizes (Scenario 1).



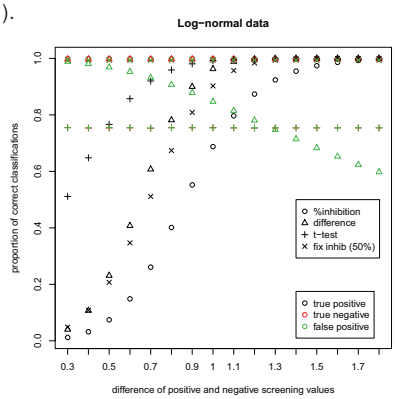
Scenarios

Table 1: Scenarios. Log-normal data.

#	positive vs negative samples	Means different between runs	SD different between runs
1	no positive samples	No	No
2	10% positive samples	Yes	No
3	10% positive and 5% false positive samples	Yes	Yes

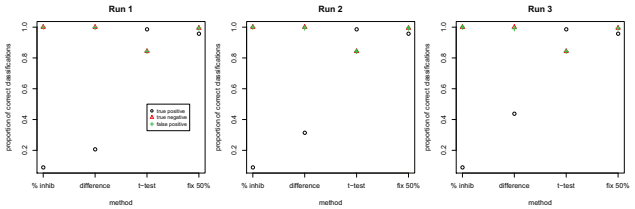
Difference between positive and negative

Figure 2: Classification rates for different effect sizes. SD=0.2 (Scenario 1).



Difference between positive and negative

Figure 3: Classification rates for difference of 2 sd between positive and negative screening values. Mean level in runs varies by 0.5 sd (Scenario 2).

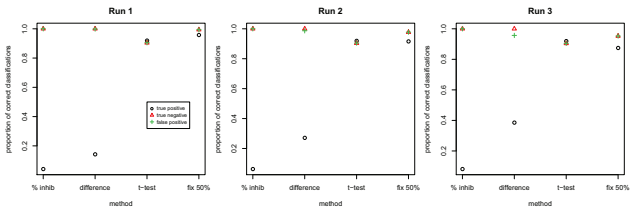


Discussion

- No uniformly superior method available;
- Large differences between positive and negative samples required;
- Variation between runs causes concern.

Difference between positive and negative

Figure 4: Classification rates for difference of 2 sd between positive and negative screening values. Mean level and sd in runs varies (Scenario 3).



References

Hoffman D, Berger M (2011) Statistical considerations for calculation of immunogenicity screening assay cut points. Journal of Immunological Methods 373:200â208.

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. ournal of Pharmaceutical and Biomedical Analysis; 55:1148-1156.

Neyer L, Hiller J, Gish K, Keller S, Caras I (2006) Confirming human antibody responses to a therapeutic monoclonal antibody using a statistical approach. Journal of Immunological 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. Journal of Immunological 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. Journal of Pharmaceutical and Biomedical Analysis, 48, 1267-1281.