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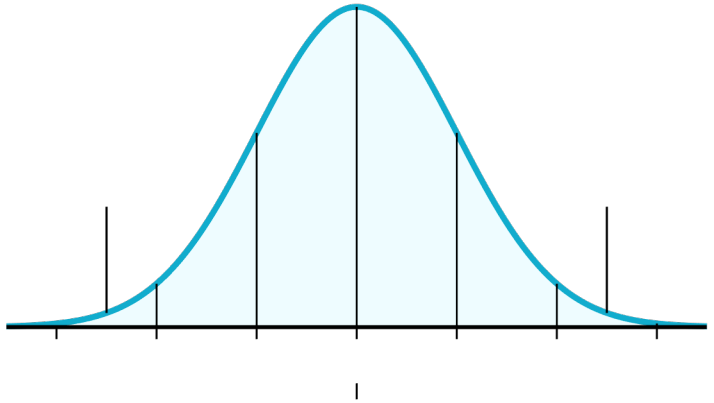
Key publications for cut point calculations

CRO perspective: choosing between multiple implemented experimental and statistical approaches - Ardena case study

EBF Autumn Workshop - 21 September 2023 - Malaga, Spain - Foka Venema
Challenging the Current Testing Paradigm for ADA Testing

Introduction

- Appropriate statistical methods for appropriate data interpretation
- Pre-study method validation recommendations
- Different calculation methods, case studies
- Flexibility of CRO required
- Reduce assay and drug development costs



Immunogenicity study

- ADA evaluation for patient safety and drug effectiveness
- Establishing of the assay cut points is critical
- 1. No prior exposure to the drug
  2. No preexisting antibody is present against the drug (true negatives)
  3. Represents the disease population
- Pre-study method validation Recommendations of EMA (2015) and FDA (2019) - “true” CP

- Different interpretation recommendations Mid-QC, no QC can fail, 4/6 runs analyzed, Inter-assay precision after final SCP and CCP, one/6 runs with final QC
- Appropriate statistical methods for appropriate data interpretation
- Contract Research Organization (CRO) = partnering with client
- Variety of clients for small and large molecule projects, Immunogenicity evaluated

Survey CROs and biotech companies: Fine-tuning the partnership
Mc Kinsey & Company June 09, 2022

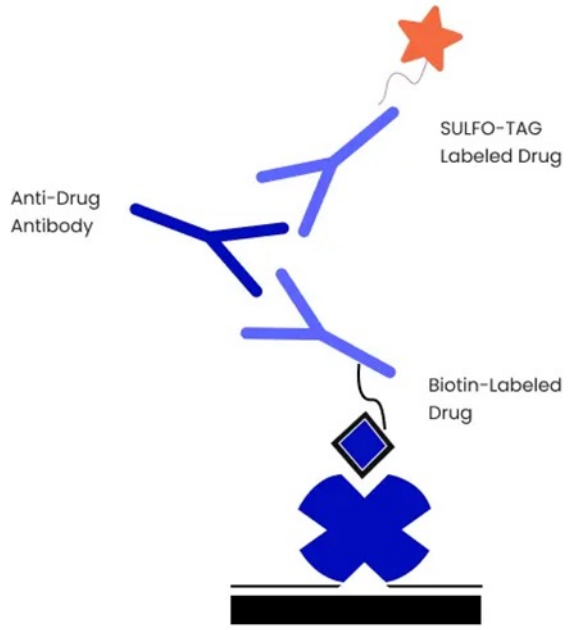
CRO Tailored service - Dedicated - Flexible

- Case Study I
- Case Study II
- Case Study III

Different Sponsors → Human samples → Fit-for-purpose

Different approaches → Takes time and effort to have multiple statistical methods available

- Case Study I : Shankar et al, 2008 - CP calculations in Excel and LIMS
  Case Study II: Ratio pre vs post-dose - CP calculations in Excel
  Case Study III: Devanarayan et al, 2017 - CP calculations in Excel and in LIMS
- A multi-step approach
  Tier 1: Identify “reactive” samples
  - Samples with signal above screening cut-point
  Tier 2: Identify “Ab+” samples absence and presence of drug
  - Samples with percent inhibition above confirmatory cut-point
  Tier 3: Determine sample titer by serial dilution of Ab+ samples in Tier 2
  - Titer is based on the screening cut-point or a higher titer cut-point
- Unspiked matrix pool (NC)
  ADA Positive control spiked (LPC, HPC)



Shankar et al, 2008

Determine effects of analyst, run and plate on the response

- Replicate observations same sample analyzed by different analysts/runs
- Run means and variances compared for Signal

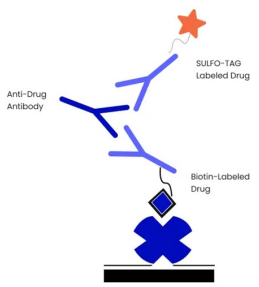
- 51 samples in 3 groups
- 3 different days
- 2 analysts
- 3 plates in each run

- Balanced design
- 6*51 datapoints

- For CCP excess drug added

- Outlier removal Box Plot analysis
- Gaussian Distribution Curve
- Shapiro Wilks Test
- Levene test

- FPR 2 to 11%
- In-study CP using study samples



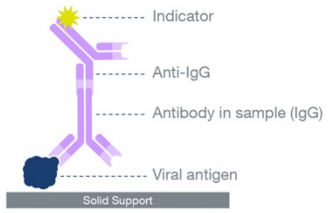
Equal means and variances, a fixed cut point
Unequal means and equal variances, a dynamic cut point
Unequal means and equal variances, a floating cut point
Calculated SCP, CCP, TCP

Ratio pre-postdose

- Straightforward approach for CP calculation
- 15 subjects
- Same plate (time points: pre-dose, day 7, 14, 21, 28)

- Different coating buffers, blocking buffers, sample dilution, different detection antibodies, detection antibody dilution
- Precision of the assay for naïve serum anti-drug IgG responses determined
- Option: study drug added
- Option: ratio NC

- SCP = NC * 1.645
- CCP = 50%
- MRD 50-/100-/250-/500-/1000-fold dilution



Negative baseline + positive post-dose anti-drug IgG antibody response for all subjects
Sample dilution of 1:250 showed the best S/N
No labour-intensive titer determination

Devanarayan et al, 2017

Determine effects of analyst, run and plate on the S/N

Analytical vs biological variation

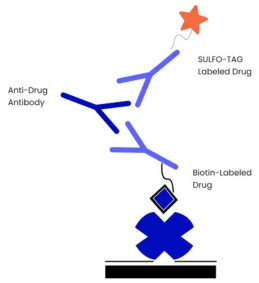
- Replicate observations for the same sample analyzed by different analysts/runs
- Run means and variances are compared for S/N

- 51 samples
- 3 different days
- 2 analysts
- 3 plates in each run

- 306 datapoints

- For CCP excess drug added

- Analytical outlier removal Box Plot analysis
- Biological outlier removal Box Plot analysis
- Gaussian Distribution Curve
- Shapiro Wilks Test
- Levene's test



Calculated SCP, CCP, TCP
Devanarayan recommendation more interpretation as compared to Shankar

Evaluation of 16 CP datasets

- J Garlits · The AAPS Journal 2023 (25-37)

  Systematically investigated 16 validation CP datasets

  Comparison of results obtained from different CP calculations

  Appropriate CP based on:

  - Representative population
  - Sample size (major source of variability in assay signal is biological)
  - Excessive outlier removal

- Different calculation methods have minimal impact on screening and confirmation CP

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The AAPS Journal (2023) 25:37
https://doi.org/10.1208/s12248-023-00806-5

RESEARCH ARTICLE

Statistical Approaches for Establishing Appropriate Immunogenicity Assay Cut Points: Impact of Sample Distribution, Sample Size, and Outlier Removal

John Garlits[1] · Sean McAfee[1] · Jessica-Ann Taylor[1] · Enoch Shum[1] · Qi Yang[1,2] · Emily Nunez[1] · Kristina Kameron[1] · Keilah Fenech[1] · Jacqueline Rodriguez[1] · Albert Torri[1] · Jihua Chen[1] · Giane Sumner[1] · Michael A. Partridge[1]

Received: 14 December 2022 / Accepted: 23 March 2023

Abstract
The statistical assessments needed to establish anti-drug antibody (ADA) assay cut points (CPs) can be challenging for bioanalytical scientists. Poorly established CPs that are too high could potentially miss treatment emergent ADA or, when set too low, result in detection of responses that may have no clinical relevance. We evaluated 16 validation CP datasets generated with ADA assays at Regeneron's bioanalytical laboratory and compared results obtained from different CP calculation tools. We systematically evaluated the impact of various factors on CP determination including biological and analytical variability, number of samples for capturing biological variability, outlier removal methods, and the use of parametric *vs.* non-parametric CP determination. In every study, biological factors were the major component of assay response variability, far outweighing the contribution from analytical variability. Non-parametric CP estimations resulted in screening positivity in drug-naïve samples closer to the targeted rate (5%) and were less impacted by skewness. Outlier removal using the boxplot method with an interquartile range (IQR) factor of 3.0 resulted in screening positivity close to the 5% targeted rate when applied to entire drug-naïve dataset. In silico analysis of CPs calculated using different sample sizes showed that using larger numbers of individuals resulted in CP estimates closer to the CP of the entire population, indicating a larger sample size (~150) for CP determination better represents the diversity of the study population. Finally, simpler CP calculations, such as the boxplot method performed in Excel, resulted in CPs similar to those determined using complex methods, such as random-effects ANOVA.

Keywords anti-drug antibody (ADA) assay · cut point determination · immunogenicity · outlier removal · therapeutic protein products

Introduction

In the past several decades, protein therapeutics have been developed to treat a broad array of diseases, including arthritis, eye diseases, and cancer (1–3). However, administration of biotherapeutics may elicit an unwanted immune response that can result in the generation of anti-drug antibodies (ADA). These ADA responses have the potential to impact the efficacy of the drug or result in serious adverse safety events (4–6).

The assays for detecting antibody responses are typically non-quantitative, titer-based methods that involve potentially three evaluations of a sample: a screening assay to detect antibodies that bind to the drug, a confirmation assay to identify samples with a reduction of assay signal in the presence of excess unlabeled drug, and a titer assay to assess the magnitude of the response.

To determine if an ADA sample is positive, a threshold is established by testing multiple drug-naïve individuals in the assay. These data are examined statistically to set cut points (CPs) with a defined false positivity rate for each tier in the ADA assessment (7, 8). This approach is intended to be

John Garlits, Sean McAfee, Jessica-Ann Taylor, Enoch Shum, and Qi Yang have equal contribution.

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Example

- Developed sensitive drug tolerant ADA assays to ensure patient safety
- Sensitivity screening assay is below 10.0 ng/mL
- Drug tolerance is at least 800 µg/mL
- Technically the ADA assays are ok

- Validated Excel spreadsheets available
- Validated CP calculations in LIMS available

Choice of calculation method/publication

Sponsor is not fully aware of multiple guidances/papers methods I, II, III

Sponsor does not know details - CRO starting points of CP calculation

Same assay format as clinical assay

Differences in approach Clinical vs preclinical ADA

Very little discussion with Sponsor
-
Lot of discussion in CRO BA lab

Exceptional ADA data sets

Need for knowledge Excel and CP calculation

LIMS bugs Multiple Tickets to CRO BA lab IT

Time and costs to program and update Excel/LIMS

Lot of internal discussion CRO BA lab

CRO to partner with customer

- Reduce costs

  Fit-for-purpose CP calculations

  Appropriate statistical methods for appropriate data interpretation

- Sponsor to compare immunogenicity data to previous and future studies

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CRO general approach

Phase II
Devanarayan since new clinical program

Phase III
Shankar in case ongoing clinical program (5 years)

EIP survey

Preclinical
Shankar/Devanarayan

- SCP only
- SCP+CCP
- SCP+CCP+Titer

Not required, appropriate/ scientific needs?

Research study
Ratio pre-postdose

Responsibility of CRO or Sponsor

- Partnership
  - Opportunity for CRO to be good strategic partner for pharma companies
  - Providing strategic input and accommodate pharma companies' needs
- CRO to work in appropriate way and to scientific needs
  - Long-term contract arrangements

Conclusions

- Appropriate statistical methods for appropriate data interpretation - challenging
- CRO prefers to be good strategic partner for pharma companies
- Drive development speed with fit-for-purpose quality
- Partnership between CRO and pharma could lead to cost reduction in drug development
- Awareness that flexibility of CRO is required to have multiple statistical methods programmed and validated

Thank You

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