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Sample size considerations in IM assays

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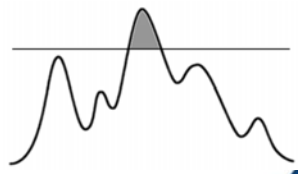

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Outline

- Introduction
- Homogeneous population
- Mixed effects model
- Conclusions

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Immunogenicity Assays

- Immunogenicity (IM)
  - Immune response to therapeutic proteins
  - Clinical effect: no effect at all to extreme harmful effects
  - Drug development effect: product safety and efficacy.
- IM assays
  - Analytical method for assessment of IM
  - Valid, sensitive
  - Evolving through different development stages

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Risk-Based Strategy

- Three groups of variables*
  - affecting the incidence of antidrug antibodies (ADA)
  - affecting the risk of consequences of ADAs
  - affecting patient safety
- IM assessment based on risk levels
  - Low risk products: titer and relative concentration of ADA may be sufficient
  - Medium risk products: neutralizing antibody (Nab) assay should be considered
  - High risk products: high sensitivity of ADA and Nab assays

*Shankar, Pendley, Stein (2007)

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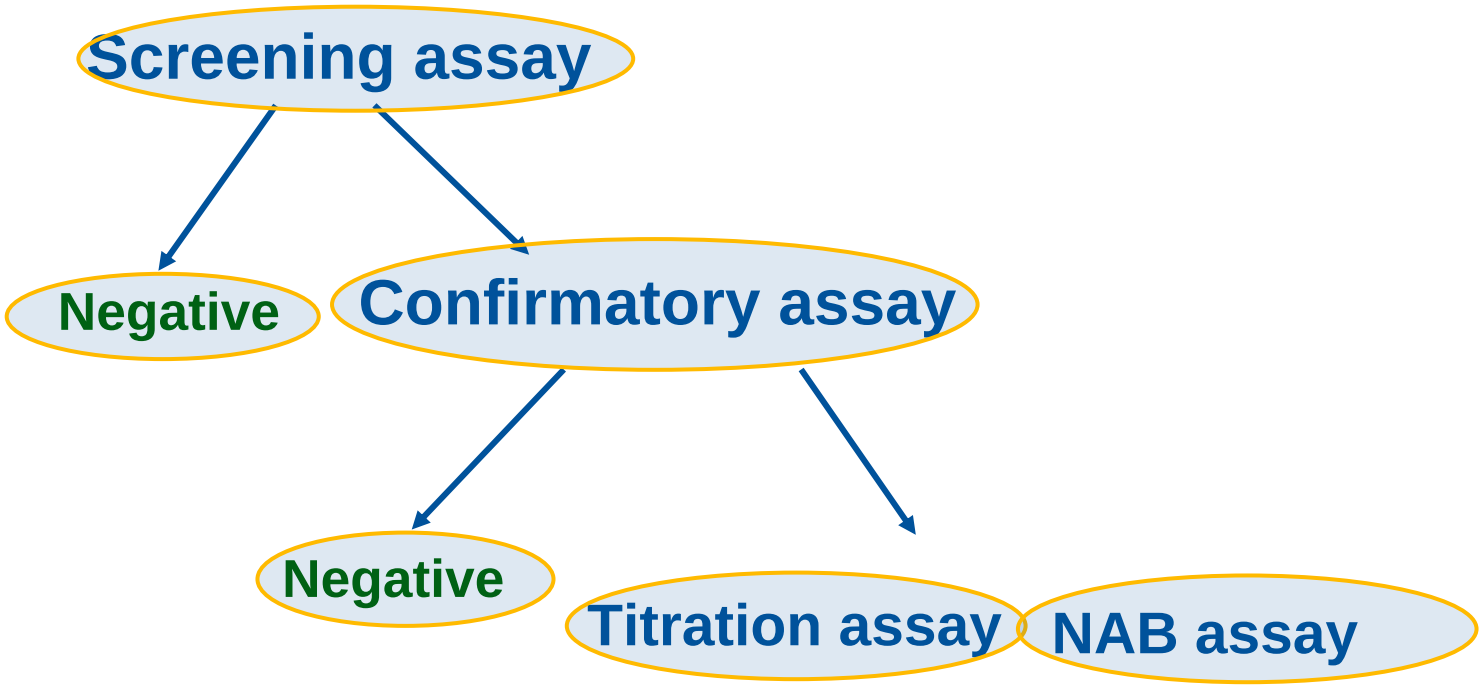
A Multiple-Tiered Approach



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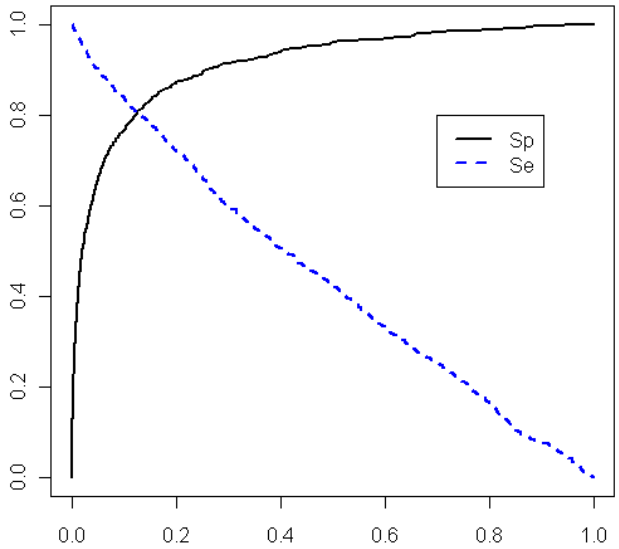
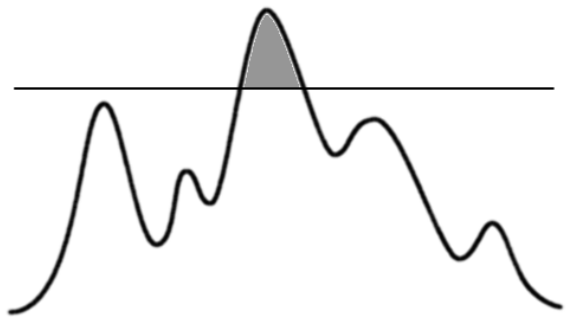
Cut Point

- The cut point is defined as the level of response of an assay at and above which a sample is defined to be a positive (or reactive) for the presence of ADA, and below which it is probably negative.
  - Screening cut point
  - Confirmatory cut point

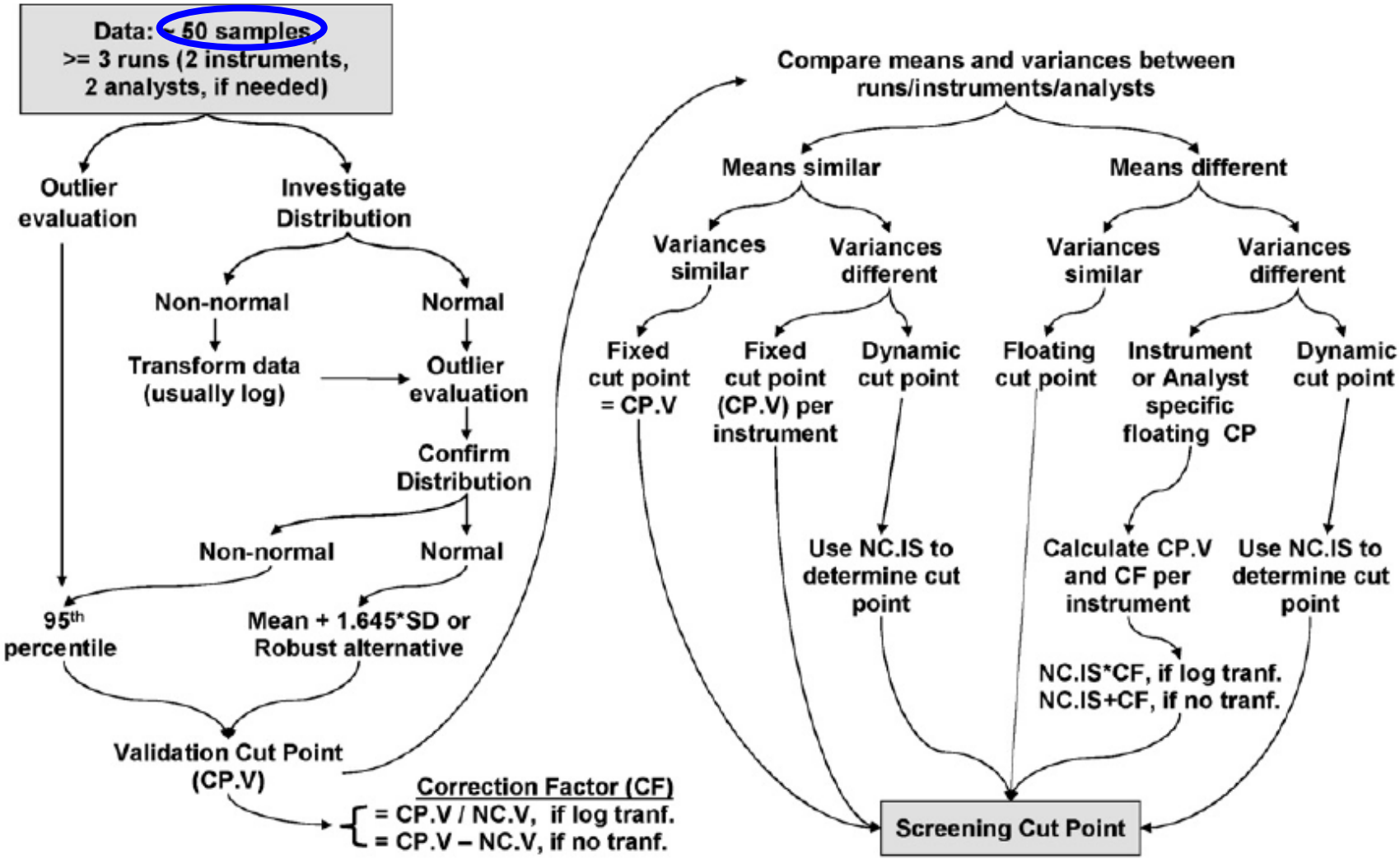


- Ideally we should use ROC analysis to guarantee a certain level of specificity and sensitivity



- Usually based on negative samples due to the lack of positive samples
  - Reduce to quantile estimation

Cut point analysis



*Shankar et al (2008)

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Experiment design format

- Design format (Shankar et al, 2008)

	Operator 1		Operator 2	
	Day 1	Day 2	Day 1	Day 2
Sample 1				
……				
Sample n				

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Sample Size

- At validation stage,
  - How many samples (=n) are needed?
  - How many replicates (=r) per sample are needed?
- A simplified version*:
  - How many data points (=nxr) are needed for cut point evaluation?

*Parish, Finco, and Devanarayan (2010)

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Sample Size

- Literature/Guideline for number of samples:
  - FDA (2009): development 5-10; validation 50-100
  - EMA (2010): NA
  - Shankar et al (2008): nonclinical ≥15; clinical ≥50
  - Schlain et al (2010): Pre-study ≥30; In-study 60-240
  - Parish et al (2010): same as Shankar

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How?

Guesstimation

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General Idea of Our Approach

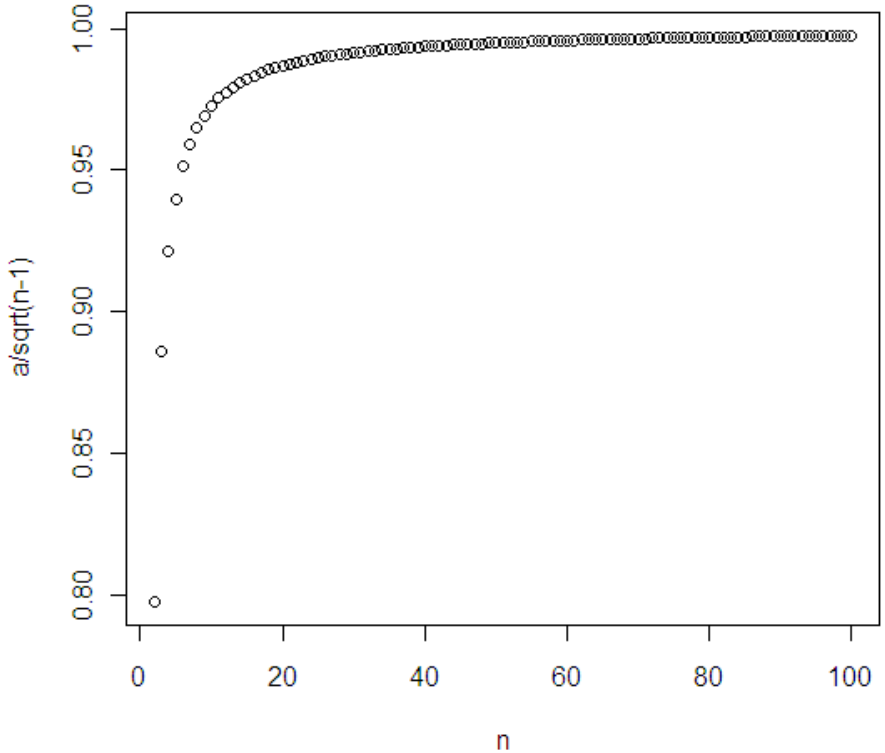
- The same idea as sample size estimation in clinical trial design
- Set up an acceptance criterion
- Determine sample size ***n*** to meet the acceptance criterion

Cut Point Estimation: Normal data

- Data (to be collected): $x_1, \ldots, x_n$
- Mean and SD: $\mu,\ \sigma$
- True cut point: $Q_\beta = \mu + z_\beta \sigma$
- Estimated cut point:

$$\hat{Q}_\beta = \bar{x} + z_\beta s\sqrt{n-1}/a$$

$$a = \sqrt{2}\frac{\Gamma(n/2)}{\Gamma((n-1)/2)}$$



Cut Point Interval Estimation: Normal data

- Interval estimate*

$$\hat{Q}_\beta \pm t_{\alpha/2,n-1}\left\{\frac{1}{n}+z_\beta^2\left(\frac{n-1}{a^2}-1\right)\right\}^{1/2} s$$

*Chakraborti and Li, 2007

Acceptance criterion

- Interval width:

$$2t_{\alpha/2,n-1}s\left\{\frac{1}{n}+z_{\beta}^{2}\left[\frac{n-1}{a^{2}}-1\right]\right\}^{1/2}$$

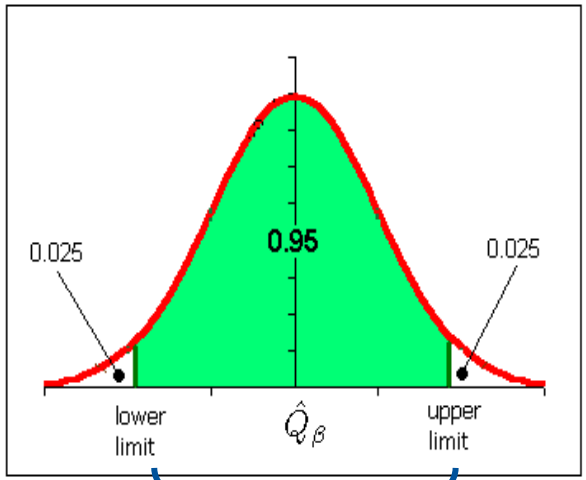
- Precision:

$$\frac{2t_{\alpha/2,n-1}s\left\{\frac{1}{n}+z_{\beta}^{2}\left[\frac{n-1}{a^{2}}-1\right]\right\}^{1/2}}{\hat{Q}_{\beta}}$$

- Set a precision threshold **d (=20%, 10%, 5%, etc)**

- Acceptance Criterion:

$$\frac{2t_{\alpha/2,n-1}s\left\{\frac{1}{n}+z_{\beta}^{2}\left[\frac{n-1}{a^{2}}-1\right]\right\}^{1/2}}{\hat{Q}_{\beta}} \leq d$$



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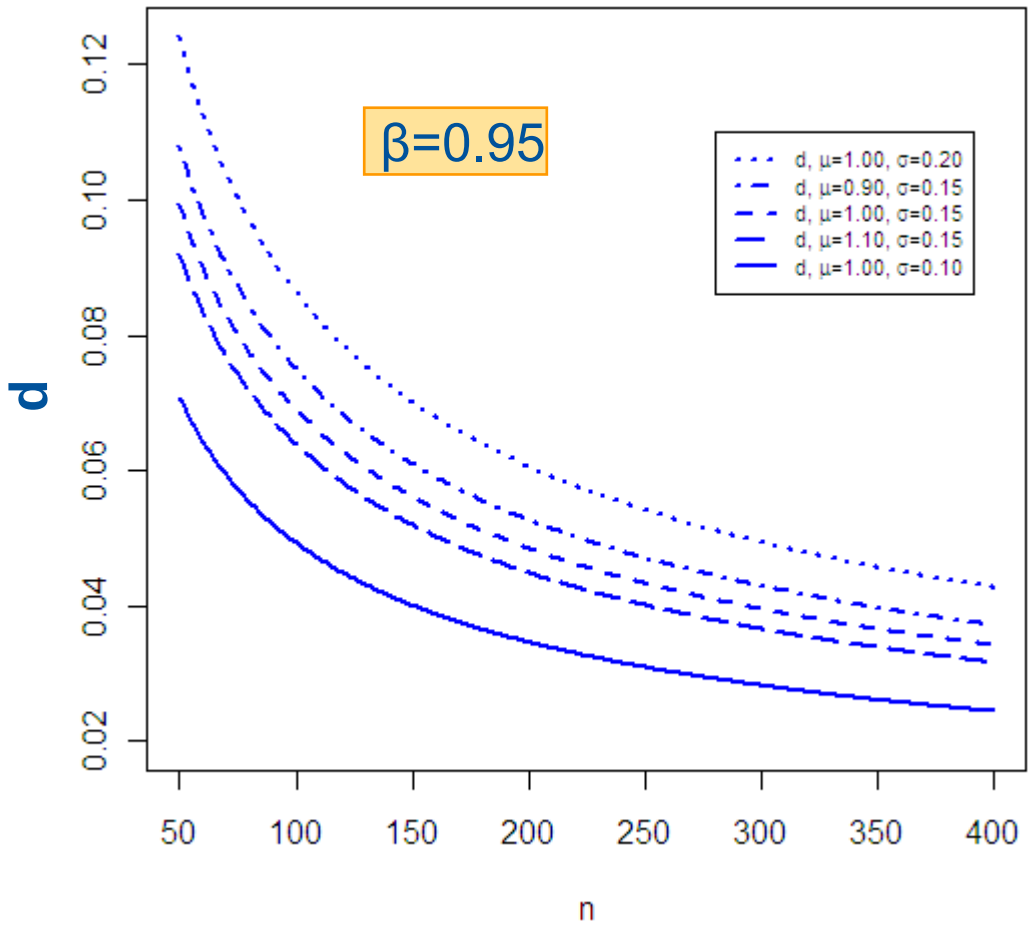
Sample Size Calculation

- Acceptance Criterion: $$\frac{2t_{\alpha/2,n-1}s\left\{\frac{1}{n}+z_{\beta}^{2}\left[\frac{n-1}{a^{2}}-1\right]\right\}^{1/2}}{\hat{Q}_{\beta}}\leq d$$
- Solve the equation for ***n***
- Need to have s and $\hat{Q}_{\beta}$
  - Estimate based on qualification data
- Proved that ***n*** can be uniquely determined.

Illustration

- Take a sample of size 30 from a normal distribution
- Estimate s and $\hat{Q}_\beta$
- Solve for ***n*** with different ***d***



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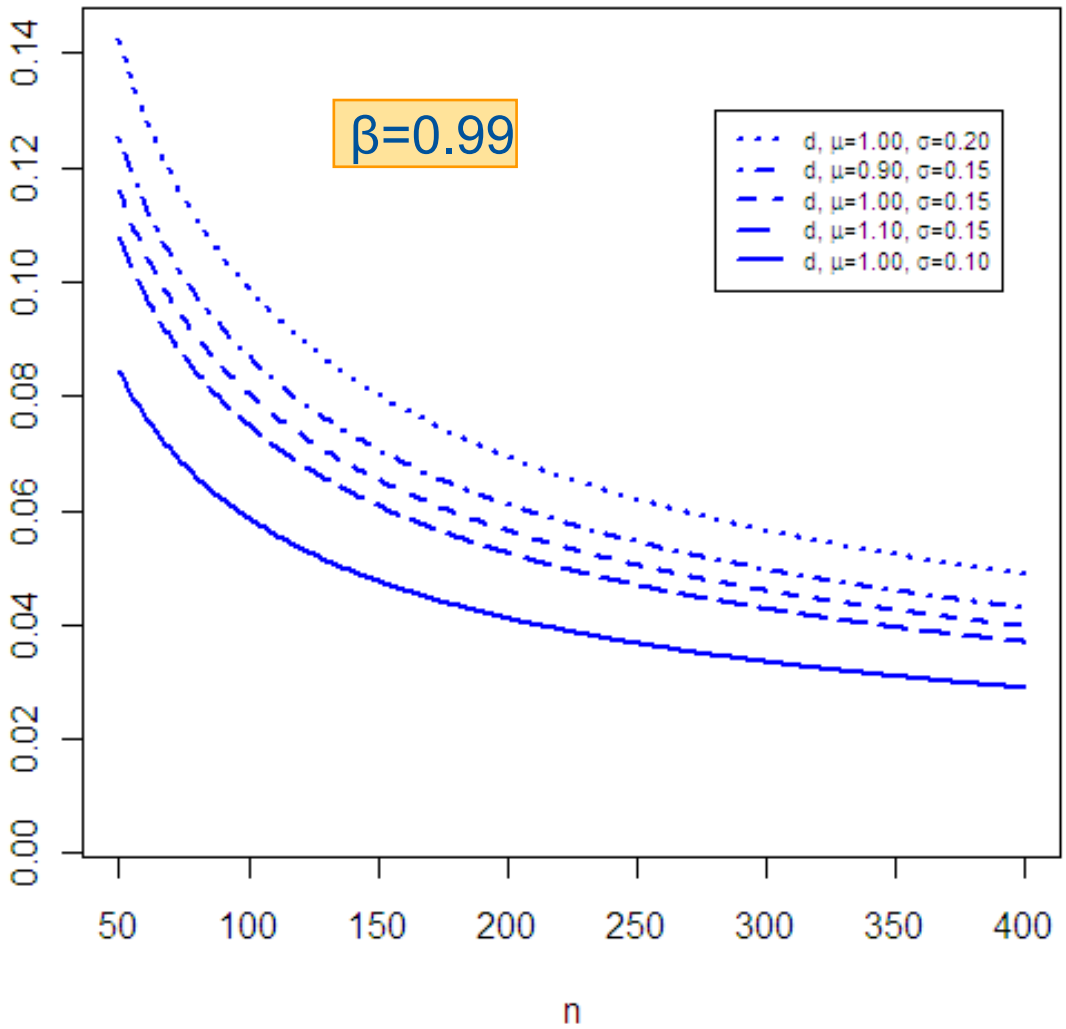

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Discussion

- We used confidence interval width scaled by the percentile estimate as our acceptance criterion
  - similar to the idea of %CV
  - The larger the percentile estimate is, the higher precision with the same confidence interval width
- An alternative acceptance criterion is the width of the confidence interval
  - The same width may have different implication when the cut point has different values

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Discussion

- Under this paradigm
  - Sample size determination is reduced to constructing an interval estimate for the cut point
- Data are often not normally distributed.
  - A gamma distribution may be useful (Schlain et al, 2010)
  - Experimental design is not considered in the data analysis

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Experiment design format

- Design format (Shankar et al, 2008)

	Operator 1		Operator 2	
	Day 1	Day 2	Day 1	Day 2
Sample 1				
……				
Sample n				

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Mixed Effects Model (variance components model)

- Without taking care of the data structure, the data points are assumed independent
  - The major reason for non-normality of the data
  - Also may result in a lot of outliers
- After taking care of the data structure by viewing factors as random, the data points from the same factor level are correlated;
  - Recommended in Shankar's paper
  - Fixed effects
    - > interest centers on the effects of the chosen factor levels
  - Random effects
    - > factor levels are a sample from a larger population;
    - > inferences are desired about the populations of factor levels
    - > Easy to construct

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Procedures of Cut Point Determination

- Fitting three-way random effect ANOVA (Analyst, day, sample)
- Residual analysis and outliers removal
- Refitting random effect ANOVA
- Estimation of total variability
- Determination of 95% quantile based on assumed normal distribution

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Cut point under mixed effects model

- The model

$$y_{ijk} = \mu + \tau_i + \beta_j + \gamma_k + \varepsilon_{ijk},$$

$$i = 1, \cdots, a,\ j = 1, \cdots b, k = 1, \cdots n$$

$$\tau_i \sim N(0, \sigma_\tau^2), \quad \beta_j \sim N(0, \sigma_\beta^2)$$

$$\gamma_k \sim N(0, \sigma_\gamma^2),\ \varepsilon_{ijk} \sim N(0, \sigma^2)$$

- The cut point

$$y_{ijk} \sim N(\mu, \sigma_\tau^2 + \sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2)$$

$$Q_p = \mu + z_p \sqrt{\sigma_\tau^2 + \sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2}$$

Cut point under mixed effects model

- One-way model

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij},\ i = 1, \ldots, n,\ j = 1, \ldots, r$$

$$\tau_i \sim N(0, \sigma_\tau^2),\ \varepsilon_{ij} \sim N(0, \sigma^2)$$

- Naïve method
  - Ignoring data structure

$$\mathbf{cp_N} = \hat{\mu} + z_\beta s, \quad s = \sqrt{\frac{\sum (y_{ij} - \bar{y}_{..})^2}{nr - 1}}$$

- Mixed effects model method

$$\mathbf{cp_M} = \hat{\mu} + z_\beta \sqrt{\hat{\sigma}_\tau^2 + \hat{\sigma}^2}$$

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Cut point under mixed effects model

The Naïve method Underestimates the cut point!

$$\frac{\mathbf{cp_M} - \hat{\mu}}{\mathbf{cp_N} - \hat{\mu}} = \frac{1}{1 - \frac{r-1}{nr-1}\hat{\rho}} > 1, \ \mathbf{if\ r > 1}$$

$$\hat{\rho} = \frac{\hat{\sigma}_\tau^2}{\hat{\sigma}_\tau^2 + \hat{\sigma}^2}$$

A simulation

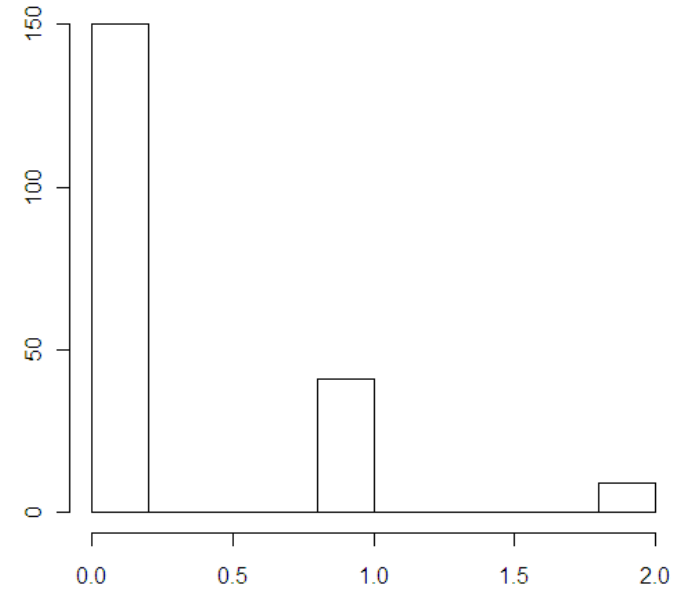
$\sigma_\tau^2 = 0.0824$ $\quad \hat{\sigma}_\tau^2 = 0.091$

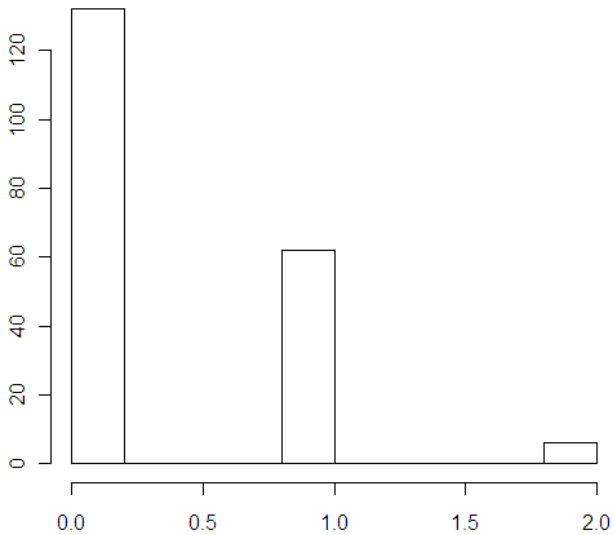
$\sigma^2 = 0.0089$ $\quad \hat{\sigma}^2 = 0.00796$

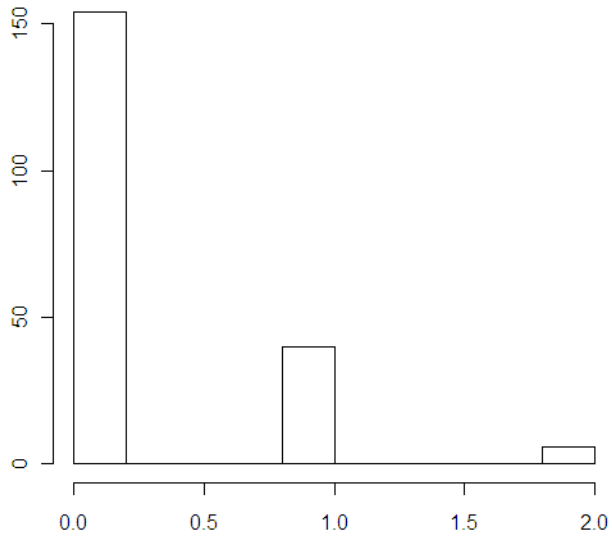
$\mu = 1$ $\quad \hat{\mu} = 1.01$

$\hat{\sigma}_y^2 = 0.0977$

n	r	CP_N	CP_M
10	4	1.728	1.751
20	4	1.623	1.634
50	4	1.503	1.507







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A real example

- N=50; r=4
- Naïve method:
  - 1.35
- Mixed effects model
  - 1.49
- It is of interest to consider sample size under the mixed effects model
  - Often all data are not normally distributed, even after log-transformation
  - A less biased estimator
  - Require statisticians' help

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CI for cut point under mixed effects model

- Given variability due to sample, analyst, day and random error, what is the sample size to achieve a specific precision for cut point estimate?

- How to construct confidence interval of a quantile under mixed effects model?
  - Asymptotic method
  - Hoffman method
  - Simulation

$$y_{ijk} \sim N(\mu, \sigma_\tau^2 + \sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2)$$

$$Q_p = \mu + z_p \sqrt{\sigma_\tau^2 + \sigma_\beta^2 + \sigma_\gamma^2 + \sigma^2}$$

$$\Pr(Q_p \le U) = 1 - \frac{\alpha}{2} \quad \Pr(Q_p \ge L) = 1 - \frac{\alpha}{2}$$

$$\Pr(L \le Q_p \le U) = 1 - \alpha$$

CI for cut point under mixed effects model

- Modified large sample method* (One-way model: Balanced case)

$$y_{ij} \sim N(\mu, \sigma_\tau^2 + \sigma^2)$$

$$Q_p = \mu + z_p \sqrt{\sigma_\tau^2 + \sigma^2}$$

$$UCL = \hat{\mu} + \left\{ Z_p \sqrt{\frac{S_1^2}{r} + (1-\frac{1}{r})S_2^2 + \left[\frac{H_1^2 S_1^4}{r^2} + \frac{(1-r)^2 H_2^2 S_2^4}{r^2} \right]^2} + Z_\alpha \frac{S_1^2}{nr} \right\}$$

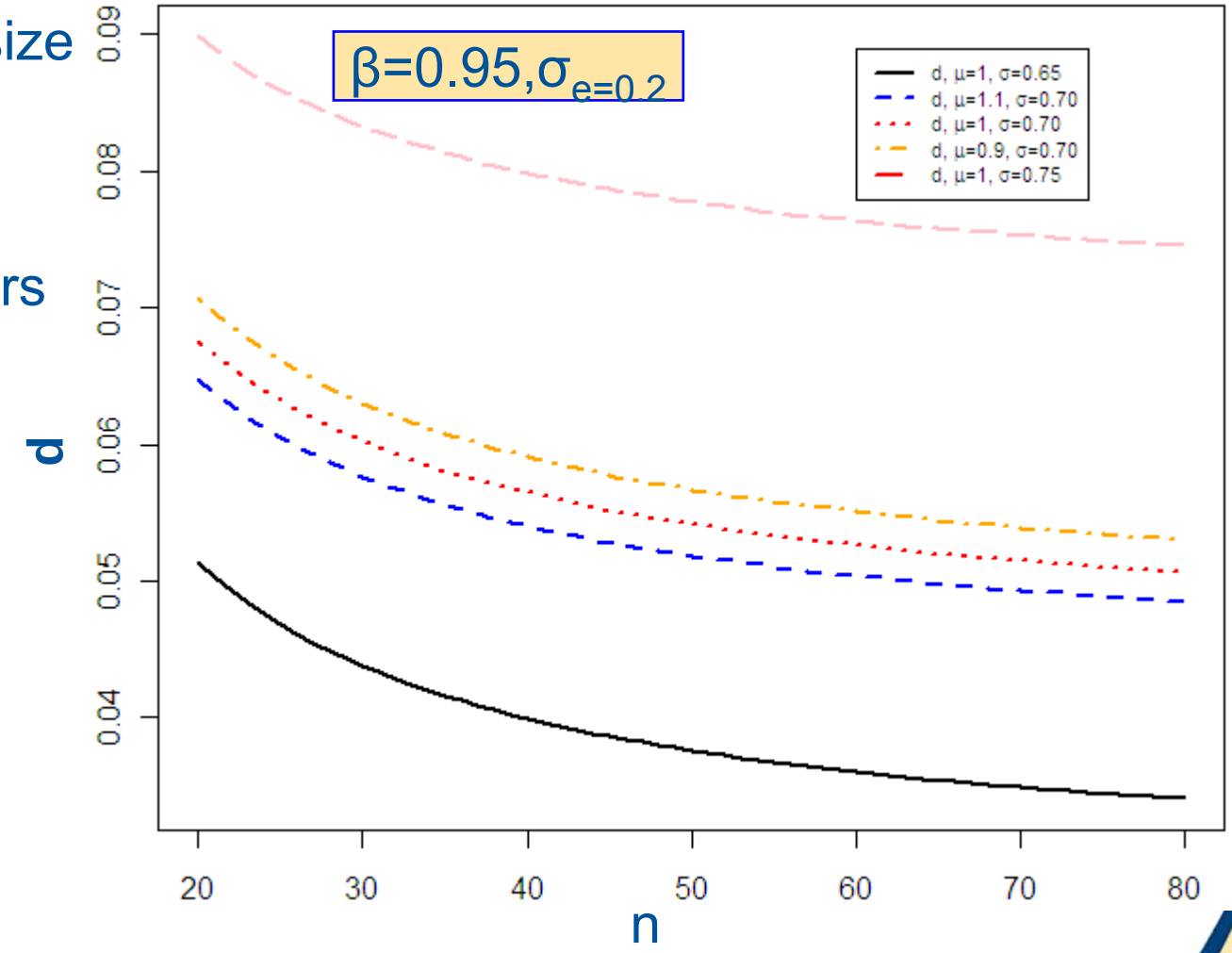
$$LCL = \hat{\mu} + \left\{ Z_p \sqrt{\frac{S_1^2}{r} + (1-\frac{1}{r})S_2^2 - \left[\frac{G_1^2 S_1^4}{r^2} + \frac{(1-r)^2 G_2^2 S_2^4}{r^2} \right]^2} - Z_\alpha \frac{S_1^2}{nr} \right\}$$

$$G_1 = 1 - \frac{1}{F_{\alpha;n-1,\infty}}, G_2 = 1 - \frac{1}{F_{\alpha;nr-n,\infty}}; H_1 = \frac{1}{F_{1-\alpha;n-1,\infty}} - 1, H_2 = \frac{1}{F_{1-\alpha;nr-n,\infty}} - 1$$

*Burdick and Graybill (1992)

Illustration: Sample size under mixed effect model

- Take a sample of size 30 from a normal distribution
- Estimate parameters
- Solve for ***n*** with different ***d***



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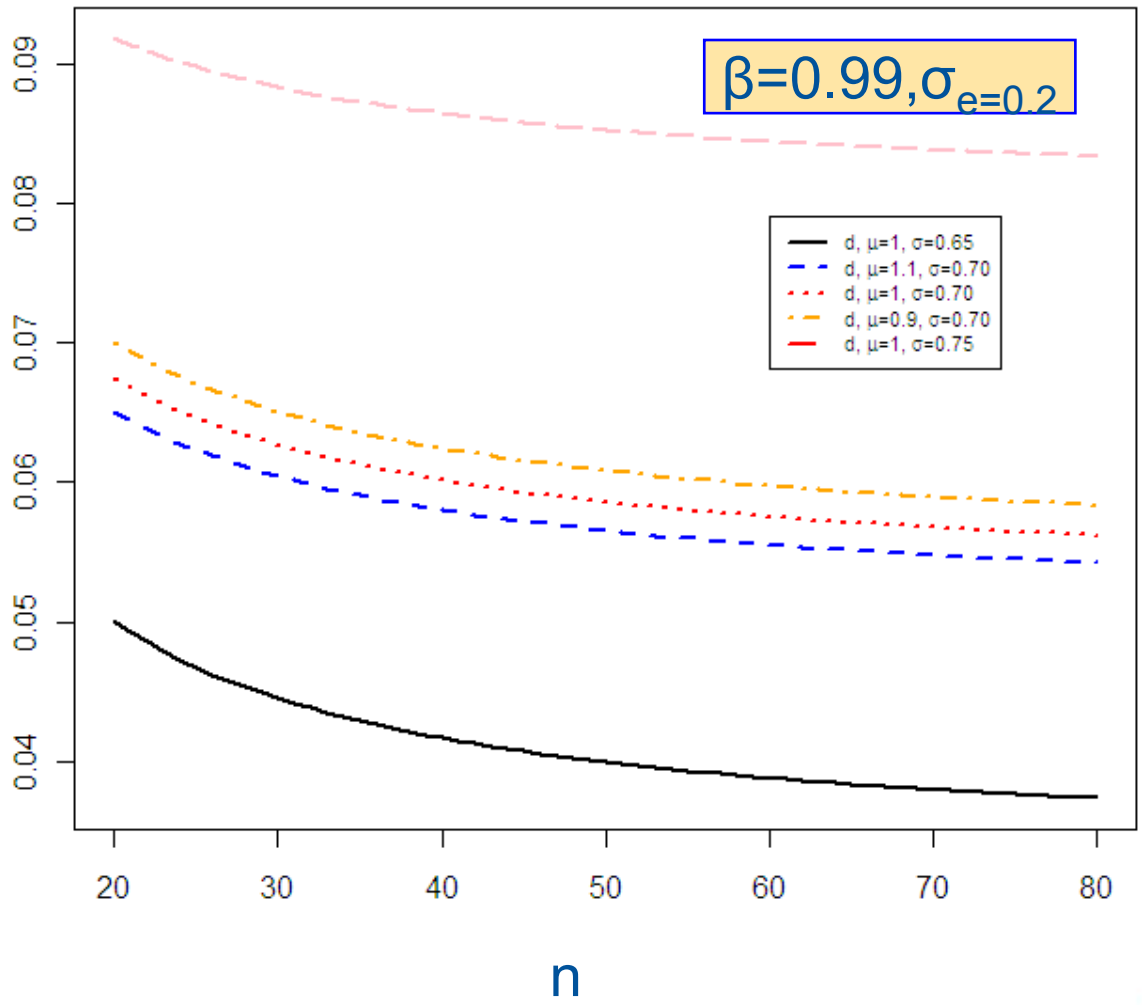

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Conclusions and Future consideraitons

- Sample size
  - There are no guidelines on sample size other than rules of thumb
  - A systematic approach to determine sample size
  - A desired precision needs to be prespecified
  - If some data (qualification) are available and normal distribution can be reasonably assumed, then sample size determination is straightforward
  - Mixed effects model can also be incorporated
  - Ignoring data structure has negligible effect on cut point analysis
- Future considerations
  - Non-normally distributed
    - > Nonparametric
    - > Gamma distribution (Schlain et al)
  - Unbalanced mixed effects models

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Acknowledgements

- Jason Zhang
- Harry Yang
- Lingmin Zeng
- Wei Zhao

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References

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