Cut-point determination

Prediction limit as an alternative to the 95th percentile ?

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OUTLINE

- Context and study designs
- Variance components analysis
- Weaknesses of simple parametric & non-parametric percentiles
- Interest of prediction limit approach
- Open considerations

Context and study designs

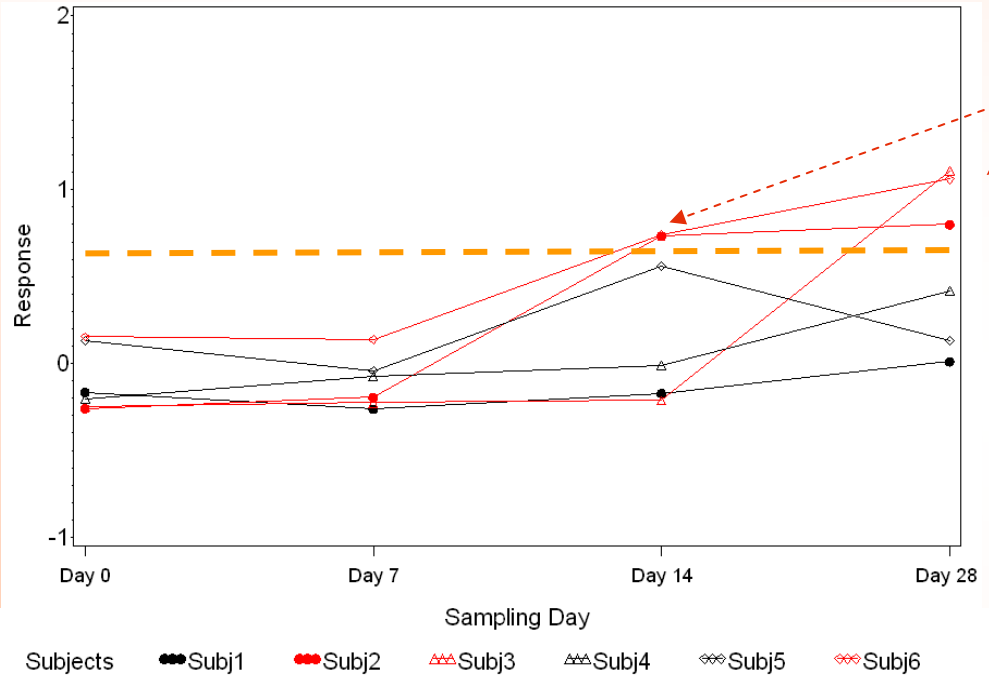
- Some drugs are biological proteins
- We need to know if the body is going to develop antibodies to these proteins
  - Like for vaccines
  - Although here we hope for no antibodies activation
- The actions of antibodies on the drug may cause
  - Risk of adverse events
  - Less efficacy
  - Clinical sound implications/complications

Context and study designs

- **Controlling the presence of antibodies has become a regulatory concern**
  - **FDA : *Draft Guidance for Industry on Assay Development for Immunogenicity Testing of Therapeutic Proteins***
    - Called for comments in Jan 2010
  - **EMEA : *Concept Paper On Immunogenicity Assessment Of Monoclonal Antibodies Intended For In Vivo Clinical Use***
    - Issued for comments in March 2009 by the Committee For Medicinal Products For Human Use (CHMP)

Context and study designs

- **Pharmaceutical industry develops specific bioanalytical methods to detect the presence of antibodies**
  - The idea is to detect a meaningful change or level in the subject sample immunoassay signal



Context and study designs

Objective of cut-point determination studies

- Determine a signal level above which samples are suspected "positive" = may contain antibody
- Any clinical study sample above that level will be re-tested for confirmation
  - The confirmatory assay is a different assay with a more specific test
  - ⇒ Cost in time and money
- How to determine a biologically meaningful signal level ?
  - ⇒ find a statistically meaningful level

Context and study designs

Objective of cut-point determination studies

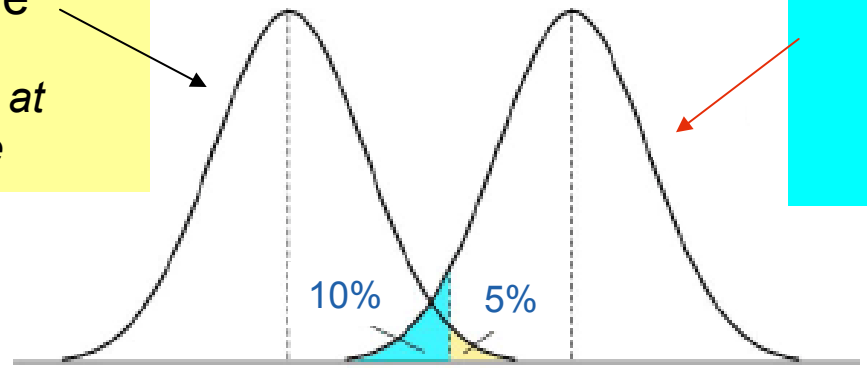
- **Cut-off = Trade-off :**

Distribution of true negative

Estimable at predose

Distribution of true positive

Not estimable



False positive rate

False negative rate

Cut-point=trade off

Numbers are just for illustration, not potential target values

Context and study designs

Cut-point determination

- **Based on some percentile of the distribution of negative samples (**Negative = without antibodies**)**

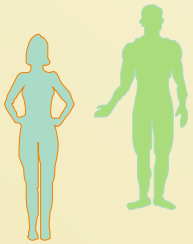
False Positive Rate = what we want to control

Context and study designs

Study design for cut-point determination

Some validation method designs at sanofi-aventis (on negs):

- Animals : 20 subjects measured in 3 times in 3 runs.
  Total = 60 observations



- Humans : 50-100 subjects measured in 3-6 times in 3-6 runs.
  Total = 150-600 observations

Context and study designs

- **Cut-point determination : more complicated than just the 95th percentile**
  - **At least 3 sources of variability to be considered in clinical studies**
    - Run-to-run variability
      - Chip, day, plate …
    - Biological subject-to-subject variability
    - Analytical variability
  - **Cut-point calculation: should incorporate at least these 3 sources**

Context and study designs

Study design for cut-point determination

- **Associated model :** 2 crossed random factors ANOVA model

  - $Y_{ij} = \mu + RUN_i + SUBJ_j + ERROR_{ij}$

where

Y_{ij} = response for subject j in run i ~ $N(\mu, \sigma^2_{TOT})$

RUN_i = random Run term ~ $N(0, \sigma^2_{RUN})$

$SUBJ_j$ = random Subject term ~ $N(0, \sigma^2_{SUBJ})$

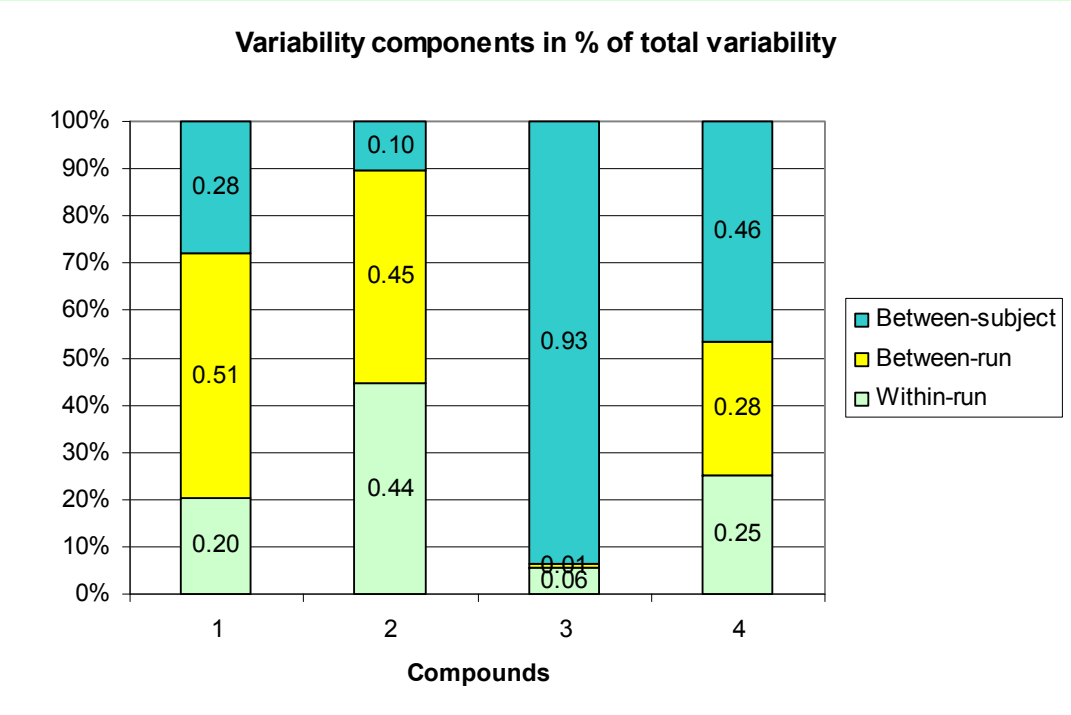
$ERROR_{ij}$ = random Error term ~ $N(0, \sigma^2_{ERROR})$

  - $\sigma^2_{TOT} = \sigma^2_{RUN} + \sigma^2_{SUBJ} + \sigma^2_{ERROR}$

Variance components analysis

Proportions of variability components

Examples of 4 studies on 4 compounds



- **"adjusted" response does not remove all the run-to-run variability**
  - Not always perfect correlation between subject samples and negative control plasma pool

Weaknesses of simple parametric & non-parametric percentile

"Simple" Parametric (SP) percentile based on normal distribution

- Cut-point = mean + $z_{1-\alpha}$* SD
  - α = probability that the sample will be considered positive while it is negative
  - For α = 5% $\Rightarrow$ $Z_{1-\alpha}$ = 1.645
  - SD = total SD from ANOVA model

Non-parametric (NP) percentile based on free distribution

- Cut-point = (1-α)% observed percentile

Other approaches possible

- Example: Robust parametric method
  - Cut-point = median + $z_{1-\alpha}$*(1.483*MAD)
    - MAD= median absolute deviation
    - 1.483*MAD $\approx$ SD for normally distributed data

Weaknesses of parametric & non-parametric percentile

- **Not for prediction**
  - Just tells you that 95% of the sample data are below this value
  - Does not tell you anything about any future value
  - Does not ensure you to capture 95% of the true negative in future assays
    - Does not ensure you control the false positive rate
- **For non-parametric and semi-parametric approaches**
  - Does not take into account the correlation between the data

Interest of prediction limit approach

Prediction interval (PI) approach

- **An interval in which a future observation will fall, with a certain probability (i.e. 95%), given what has already been observed**
  - on repeated experiments, any new observation (X_{n+1}) shall fall in this interval the desired percentage of the time

Prediction distribution (simple random sample)

- We know from previous experiment that

$$X_{n+1} \sim N(\mu, \sigma^2) \quad \text{and} \quad \overline{X} \sim N(\mu, \sigma^2 / n)$$

- If we take the difference of these two and center, we have :

$$\frac{X_{n+1} - \overline{X}}{\sqrt{\sigma^2 + \sigma^2 / n}} \sim N(0,1)$$

- Therefore:

$$X_{n+1} \sim N\left(\overline{X}, \sigma^2 * (1 + 1/n)\right)$$

Interest of prediction limit approach

Prediction distribution

- With unknown σ, we have:

$$\frac{X_{n+1} - \overline{X}}{\sqrt{SD^2 + SD^2 / n}} \sim T^{n-1}$$

- For a simple random sample :
  - Cut-point = mean + $t_{df,\ 1-\alpha}$*SD*$(1+1/n)^{0.5}$
    - Mean = overall mean of the experiment
    - df = n-1
    - n = sample size of the experiment
    - α = probability that an observation will be considered as positive while it is negative
    - SD = standard deviation from the experiment

Interest of prediction limit approach

- **However, we don't have a simple random sample**
  - **Recall: correlation + multiple variance components**
    - Use total SD from ANOVA
    - Use Ne : effective sample size

Interest of prediction limit approach

Ne : effective sample size

- With a simple random sample, we have: $\sigma^2_{\bar{Y}} = \sigma^2_Y / N \Rightarrow N = \dfrac{\sigma^2_Y}{\sigma^2_{\bar{Y}}}$

- In our case, we have:

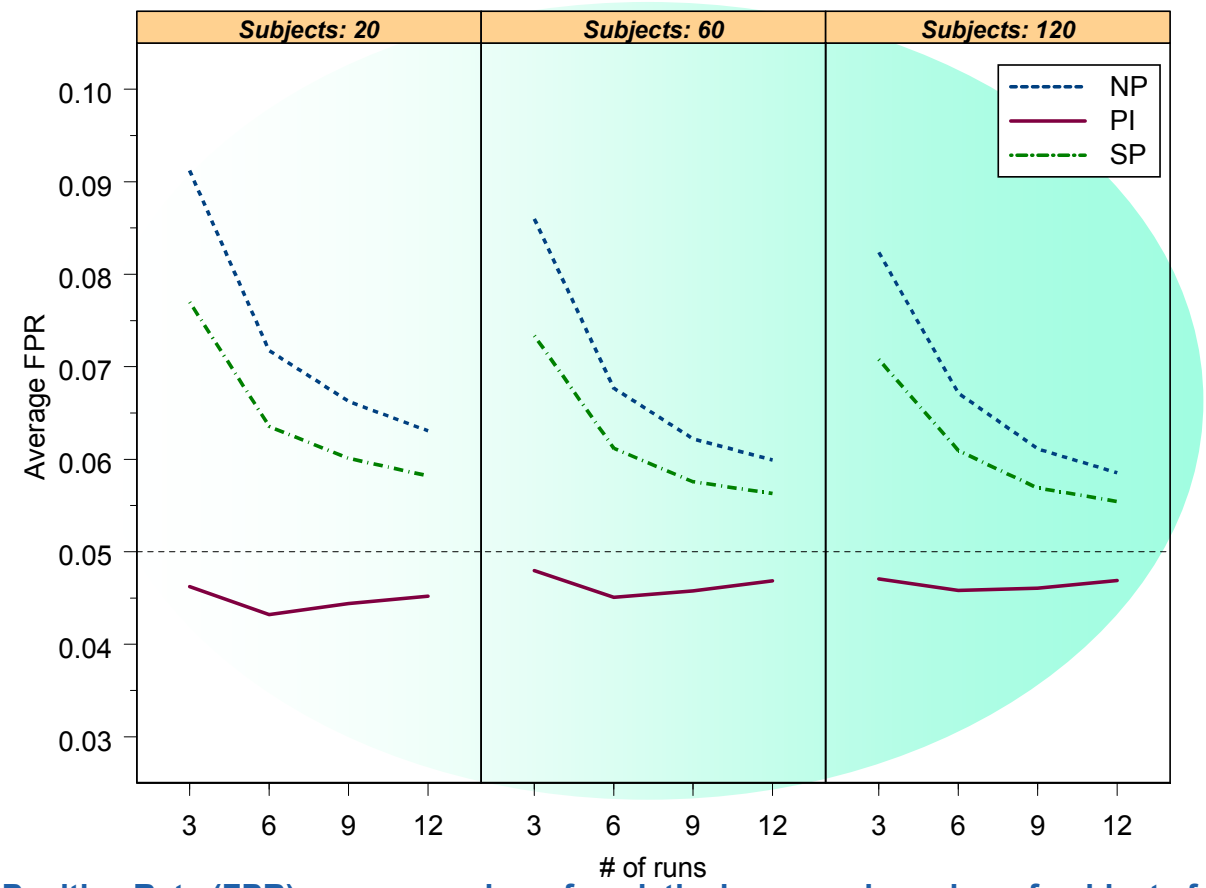
$$\sigma^2_Y = \sum_{j=1}^{q-1} \sigma^2_j + \sigma^2_e = \sigma^2_{RUN} + \sigma^2_{SUBJ} + \sigma^2_{ERROR}$$

$$\sigma^2_{\bar{Y}} = \sum_{j=1}^{q-1} (\sigma^2_j / I_j) + (\sigma^2_e / N) = \frac{\sigma^2_{RUN}}{nb\ of\ runs} + \frac{\sigma^2_{SUBj}}{nb\ of\ subj} + \frac{\sigma^2_{ERROR}}{total\ nb\ of\ obs}$$

  - with j variance terms in the model and I_j observations/modalities for the factor j
  - N : total number of observations

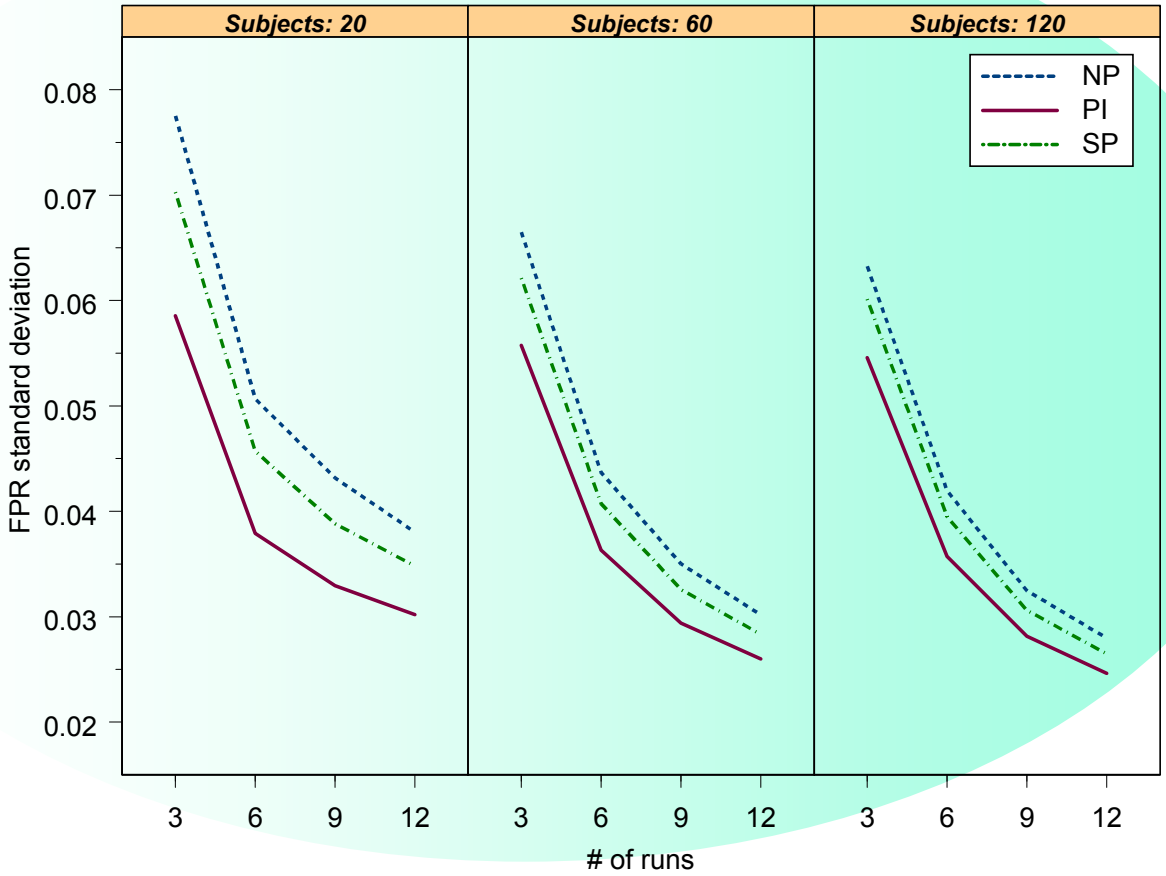
- Then $$Ne = \sigma^2_Y / \sigma^2_{\bar{Y}}$$

Interest of prediction limit approach
Simulation results



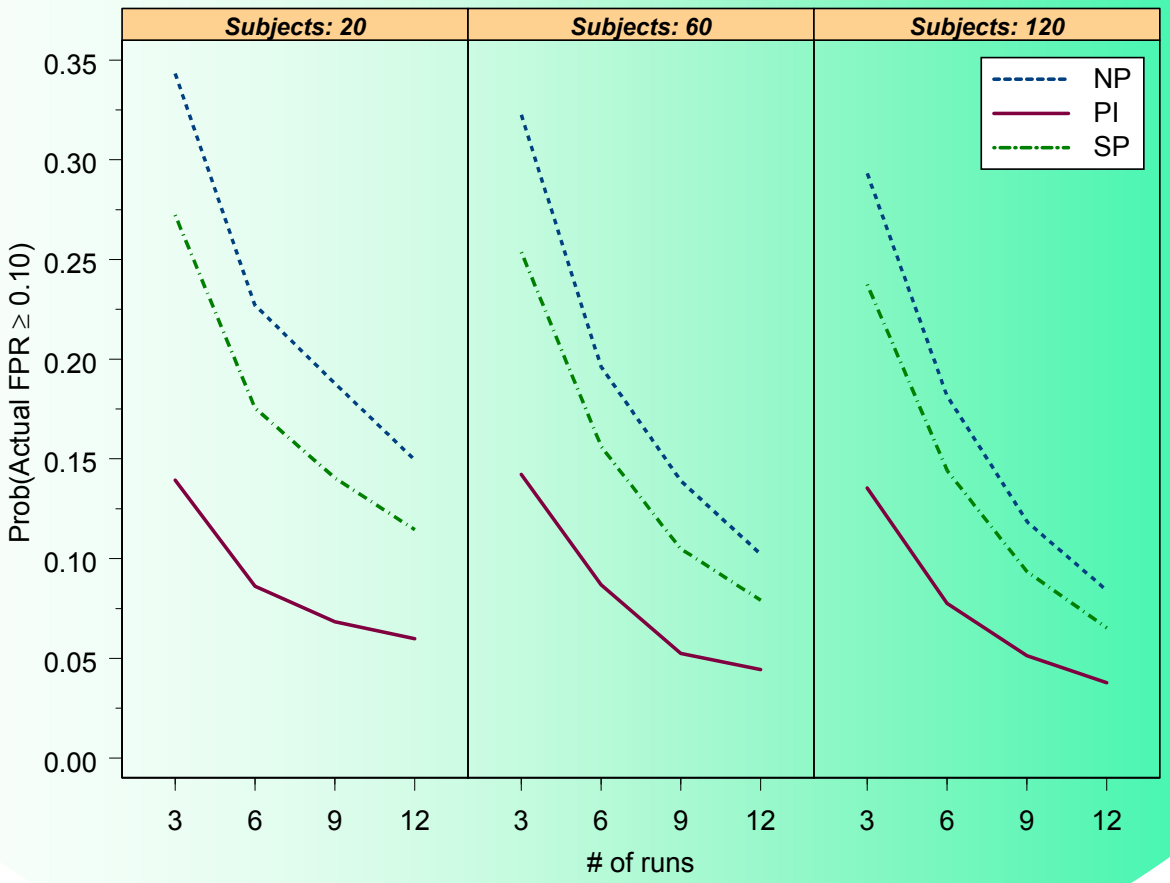
Average False Positive Rate (FPR) versus number of analytical runs and number of subjects for nonparametric (NP), simple parametric (SP), and prediction interval (PI) approaches. Variance component ratio $\sigma^2_{SUBJ:} : \sigma^2_{RUN} : \sigma^2_{ERROR}$ fixed at 2:2:1. Reference line at nominal FPR=0.05.

Interest of prediction limit approach
Simulation results



False Positive Rate (FPR) standard deviation versus number of analytical runs and number of subjects for nonparametric (NP), simple parametric (SP), and prediction interval (PI) approaches. Variance component ratio $\sigma^2_{SUBJ:} : \sigma^2_{RUN} : \sigma^2_{ERROR}$ fixed at 2:2:1.

Interest of prediction limit approach Simulation results



Probability of obtaining actual False Positive Rate (FPR) ≥ 0.10 versus number of analytical runs and number of subjects for nonparametric (NP), simple parametric (SP), and prediction interval (PI) approaches. Variance component ratio $\sigma^2_{SUBJ:} : \sigma^2_{RUN} : \sigma^2_{ERROR}$ fixed at 2:2:1.

Interest of prediction limit approach

- **May not see advantage on a study-by-study basis**
- **But**
  - **Will provide least bias**
    - the closest FPR from the target FPR on average
  - **With least variability**
    - Won't vary from study to study as much as the others

Open considerations

Weaknesses of prediction limit approach

- **Need normal distribution**
  - Usually overcome with appropriate transformation (Box-Cox, log)
- **In some studies, will yield very low false positive rate**
  - Can be less than 1%
  - Risk of detecting less true positive ?
    - Used on real cases: risk unchanged

Advantages

- **Consistent theoretically if goal is to confirm 5% of screened samples on average**
- **Least variability compared to the FPRs estimated from the other approaches**
- **Save resources, so costs and money**

References

Books

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- **Confidence intervals on variance components**, Richard K. Burdick, Franklin A. Graybill, 1992, Marcel Dekker, Inc.

Articles

- **Two-sided tolerance intervals for balanced and unbalanced random effects models**, David Hoffman, Robert Kringle, 2005, Journal of Biopharmaceutical Statistics, 15: 283-293
- **Tolerance limits for linear regression**, W.A. Wallis, 1951, Proceedings of the second Berkeley symposium of mathematical statistics and probability, 43-52.
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