Welcome to **Session 1** of FasTrain's

Statistical Method in Bioassay Course:

Introduction to Bioassay

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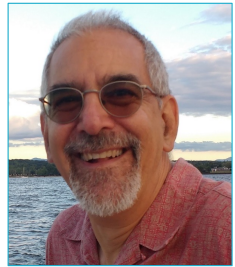
Introduction

Me: David Lansky, Ph.D.

Experience: Dr. Lansky has been learning about bioassays and statistics for bioassays (and statistics for other non-clinical applications in Pharma) for over 30 years. His training includes a year of Electrical and Computer Engineering (University of Michigan), a BS in Botany (San Francisco State), an MS in Entomology (Cornell) and finally both an MS and Ph.D. in Biometry (both Cornell). His work experience includes 10 years at Searle/Monsanto/Pharmacia and owner of Precision Bioassay, Inc. since 2002. David is part of the team revising the USP bioassay chapters.



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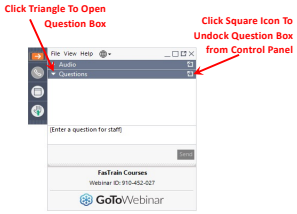

Questions?

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Introduction to Bioassay

Session 1

FasTrain's Statistical Method in Bioassay Course

David Lansky, Ph.D.

Poll 1:

- What is your background in bioassay?
  - A New to bioassay (less than 6 months)
  - B Some experience (6 months - 2 years)
  - C I've been at this a while (2+ years), not in management
  - D Bioassay management

Poll 2:

- What is your current relationship to bioassay?
  - A I'm at the lab bench doing bioassays
  - B I'm a bioassay manager
  - C I'm focused on validation with bioassay responsibilities
  - D I'm a statistician working on bioassay
  - E Other

Poll 3:

- What is your statistics background?
  - A I had a course (or two) long ago
  - B Some training, and struggling with it now
  - C none
  - D I am a statistics user, reasonably comfortable with it
  - E I have substantial training and practice

Session 1 Outline

- A Brief History
  - Bioassay history
  - Statistics history
- Types of Bioassays
- Key Ideas
- Recent Big Changes
  -
  -
- Current Challenges
  -
  -

Early 1900s

- Experimental goal: compare 'activity' of compounds (e.g.; toxins)
- Early discovery: EC50 varies widely with day, batch of animals, etc.
- Early steps towards bioassay:
  - Use a reference sample in each assay
  - Use probit (later logit) vs. log concentration (metameters)
  - Recognize that 'potency' behaved like relative concentration
  - Recognize that 'potency' didn't make sense if conc-response not similar
  - Potency defined from assay or clinic: functional response in living system
- For more history, see Finney's book and/or Stan Demings book

1940s - Pertussus

- 'Poster child' for why bioassays are so important (Laureen)
- Pertussus vaccine in broad use, disease still endemic
- Lots passed QC, but some lots didn't protect patients
- Dr. Margaret Pittman (director of the Biological Control Lab)
  - Developed a mouse bioassay that distinguished between good and bad lots
  - Potency from a bioassay became a Critical Quality Attribute (name came later)

What is a bioassay?

Finney (1978): A bioassay is an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows is application to living matter.
Finney(1978): The potency of any test preparation of the stimulus is assayed by finding the ratio between equivalent doses of it and the standard preparation, equivalence being intrepreted as equality of the corresponding mean responses; experimentation with several different doses of one or both preparations is almost always needed in order to accomplish this satisfactorily.

What is a bioassay? (2)

21 CFR 600.3(s): The word potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.
ICH: The measure of the biological activity using a suitably quantitative biological assay (also called a potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.

Selected Bioassay Statistics History

- Finney (1947, 1952) Probit Analysis
- Chester Bliss (1950s <111>) used mixed model concepts (no software)
- Old <111> advised: recognize groups in assay during analysis
- Systematic and Randomized Arrangements in Experiments (F. Yates, 1939)
- Split-unit/Strip-unit designs and analyses (1930s-1940s? - Yates?)
- Statistical Method in Biological Assay (David Finney, 1952, 1964, 1971, 1978)
- Non-linear models theory & software 1970s-1980s
  - Nonlinear Regression (George Seber & C. Wild, 2005)
  - Nonlinear Regression and its Applications (Bates & Watts, 1988 & 2007)
- Equivalence tests for non-similarity 2000s-current
- 'Modern' Statistical Methods
  - Generalized Linear Models (P. McCullagh & J. Nelder, 1989)
  - Transformation and Weighting in Regression (Raymond Carroll & David Rupert, 1988 & 2017)
  - Nonlinear models for Repeated Measurement Data (M. Davidian & D. Giltinan, 1995, 2003)
  - Mixed-Effects Models in S and S-plus (J. Pinhiero & D. Bates, 2000)
  - Modern Applied Statistics with S (W.Venables & B.Ripley, 1994, 1997, 1999, 2002)

Central Concepts

- RELATIVE potency (need a reference, uses blocking)
- Metameters (abstractions of measures of dose and response)
- Similarity required for relative potency to be meaningful, interpretation as 'same active compound'
- Bioassay requires collaboration between lab biologists and statisticians

Course Goals

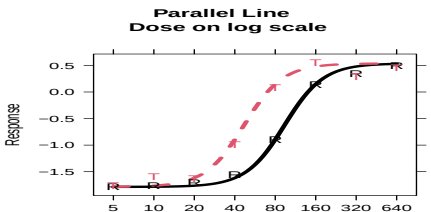
- Focus on the parts of bioassay that are probably less familiar
- Focus on the statistical ideas and high level tools (some risks here)
- Why and how to use better bioassay designs
- Why and how to use (adapt) DOE to bioassays
- NOT: how to analyze bioassays
- Design structures (CRD, RCBD, split- & strip-unit) in bioassay (& experimentation)
- Link design structures to what mixed (or multilevel) models do for us
- How to get more from your statistician
- A collection of strategic ideas about how to approach bioassay
- Bioassay requires great 'animal husbandry' and good statistics; easy to over-focus on details
- Carefully worked examples, go through them step by step (pre-publication)

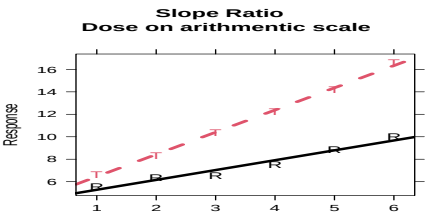
Poll 4:

- Your thoughts on these course goals:
  - A Sounds right to me
  - B I want to learn how to do bioassay analyses myself (and I have a strong background in statistics and programming)
  - C I wish I could do bioassay analyses myself, but my statistics and programming background is pretty limited
  - D I'm worried about how much of the statistics I'll be able to follow
  - E Not what I'm looking for

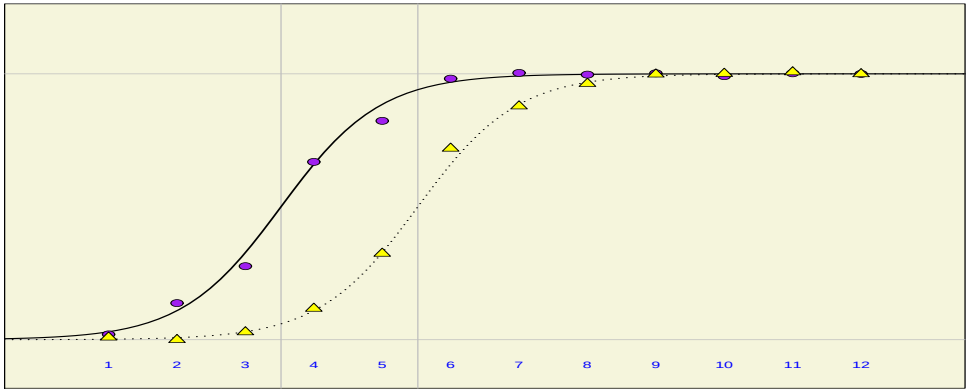
Key Idea: Potency is Relative

- Parallel Line: If shapes similar, assume
  - same cmpd
  - horizontal shift is log potency
- Slope Ratio: Intercepts similar, assume
  - same cmpd
  - slope ratio is potency





Bioassay in Action



Why Bioassay?

- $\sigma^2_{\text{Log EC50}} >> \sigma^2_{\text{Log Potency}}$ (Blocks)
- Multi-dose test needed (not single-point w/calibration curve) to assess similarity
- High variance system need replicates
- Parallel line log relative potency is:
  - Linear: $\frac{\text{intercept difference}}{\text{slope}}$
  - Nonlinear: difference in Log EC50s

Types of Bioassays

1. Direct (random dose, fixed response)
2. Indirect (fixed doses, random responses)
   - Discrete response (logit/probit-log)
   - Quantitative response
     - Slope Ratio
     - Parallel Line
       - straight line
       - nonlinear $\Leftarrow$
       - smooth

Poll 5:

- What kind of bioassays are you running?
  - A Animal bioassay
  - B Continuous cell culture based bioassay
  - C Frozen ready-to-use cell based bioassay
  - D Two of the above
  - E All of A, B, C

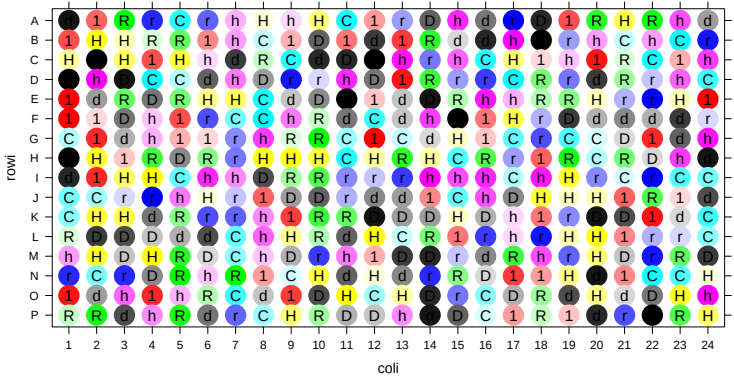
Poll 6:

- What types of bioassays /analyses are you using?
  - A Slope ratio
  - B Parallel straight line
  - C Parallel four parameter logistic
  - D logit or probit-log
  - E Something else

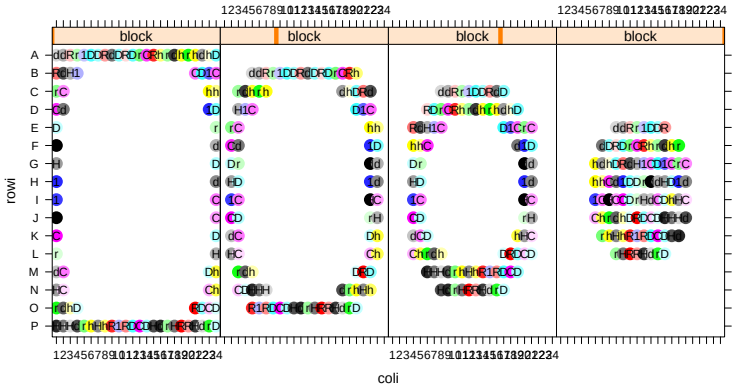
A Statistician's View of 'Types of Bioassays'

- Discrete or Continuous Response
- Linear or non-linear model
- Slope ratio or parallel line (concentration or log concentration)
- Design structure:
  - Completely Randomized Design (CRD)
  - Randomized (Complete) Block Design (RCBD)
  - Split-unit (split-plot)
  - Strip-unit (split-block)
  - Split-lot

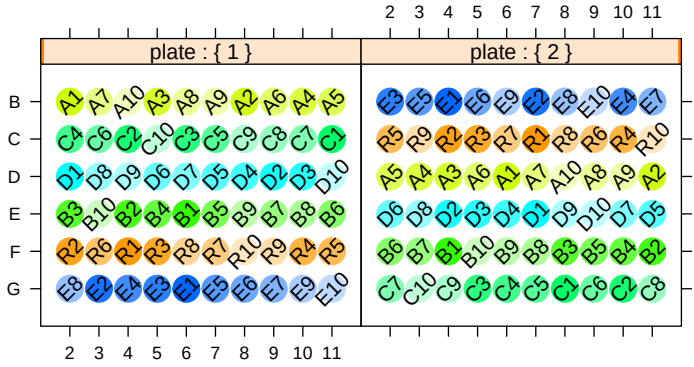
Completely Randomized Design, n=4 (384 well)



4-Block 384 Well Design



Split-unit Design

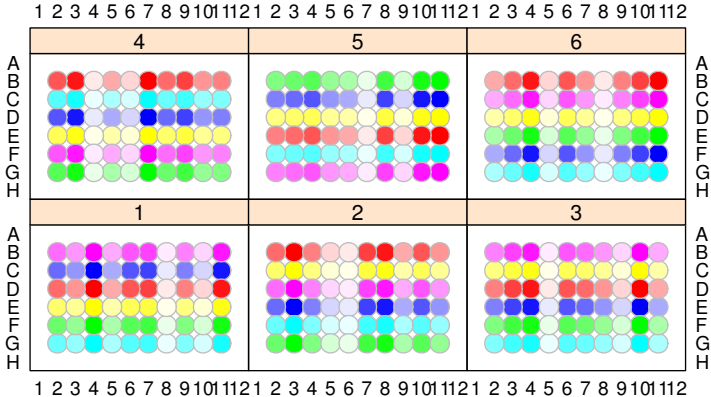


Color and letter indicates sample
Color intensity and number indicates dose

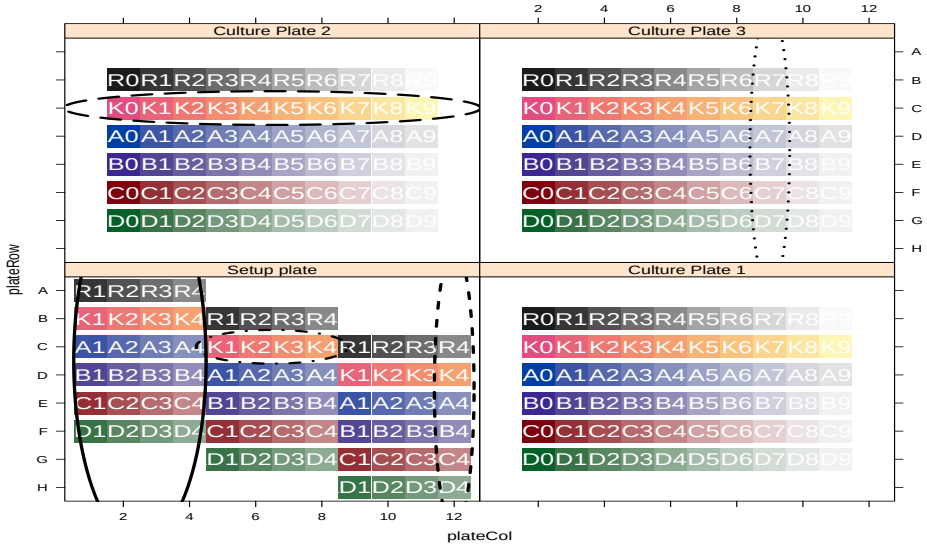
Split-unit Design 2

- USP monographs for Heparin and antibiotic bioassays prescribe a split-unit design
  - Make a stock dilution for each of reference standard and test
  - Make replicate dilution sets for each of reference and test from each stock
- This method SOP creates a split-unit
  - with sample assigned to stock
  - with dilution assigned to tube within dilution set
- It is surprisingly easy to create split-unit (and more complex) design structures because it is hard to change levels of some factors

6-Block Randomized Strip-Unit Design



3-Block Non-Randomized Strip-Unit Design



USP Big Changes (official in 2012)

- USP <111> minimized, new chapters <1030>, <1032>, <1033>, <1034>
- Address statistical assumptions in development (don't test each assay)
- Transform rather than weight
- Outliers: after transform, via all-data minimal-assumptions model
- Discourage use of R^2 to assess model adequacy or linearity
- Equivalence testing for similarity
- Explain and emphasize design structure
- Routinely combined potencies with 'simple' method
- Validation:
  - Performance requirements from needs (e.g.; Cpm)
  - Reportable value (geometric mean potency)
  - Variance components approach
  - Focus on method (IP) rather than procedure

More Recent Changes

- Unfortunate Trends
  - Continued use of R^2 to assess model adequacy or linearity
  - Efforts to justify estimating potency from unrestricted model
  - Over-use of control samples (equiv bounds, assay acceptance)
  - Rejecting blocks (e.g.; plates) based on sample similarity failure
  - Setting equivalence bounds based on assay capability
  - While assay designs are better (e.g.; pseudo-replicates in adjacent wells less common) analyses are still overly simplistic (e.g.; assuming a CRD when the assay has a much more complex structure)
- Good Directions
  - Life cycle approach
  - Analytic Target Profile (assay performance requirements from intended use)
  - Recognition that allowed non-similarity can induce bias in potency
  - Recognition that equivalence bounds for similarity tests should be based on impact of non-similarity

Bioassay Challenges

- Typically complex in lab, attention to many details
- Analyses require broad collection of statistical tools
- Combine good lab work and good statistics
- Design and analysis goal - turn potential bias into variation
- Set equivalence bounds based on impact of non-similarity (e.g.; potency bias) not assay capability
- Lack of understanding of design structure / strategic approaches
- Preparing for or transition to lab automation
- Software is generally inadequate, but better software alone isn't the solution
- Limited access to sufficient statistical expertise

Bioassay Challenges - 2

- Would help to discard some commonly applied notions:
  - CV
  - The analysis of a sample must 'stand alone' (not use other data)
  - Tight limits on reference (& control) make an assay more precise
  - Assay=Method=Procedure
  - Need within-plate replicates to assess outliers
  - Equivalence test bounds should be set based on data from the assay
  - Sensible to fail a plate (in a multi-plate assay) on non-similarity
  - It may be reasonable to estimate potency from unrestricted model fit
- Suggestions:
  - stop using CV, use PGSD (%GCV)
  - fit each all test & reference samples together
  - use historical data to help estimate difficult things
  - shift emphasis from 'control samples' to trending references & assay
  - method as instance of assay, procedure as replicates of assay
  - set equivalence test bounds based on the effects of non-similarity
  - without evidence of similarity, don't report potency (use NFXX)

Strategic Directions

- Big picture design and analysis strategy/goals:
  - Develop assay performance targets from intended use
  - Use life-cycle strategies starting in early development
    - development/qual/validation experiments are a series
    - DOE is essential
    - knowledge space grows with expt & experience
    - operating space selected within knowledge space
    - monitoring, periodic review, periodic updating
  - A successful product's assay will run for decades
- Design and analysis must protect against bias
  - Known and unknown sources
  - Measured (and monitored) sources
    - Location effects
    - Target-specific or overall bias
    - Bias trend
  - Not measured (hard to monitor) sources
    - Unknown sources of bias
    - location/sequence effects
    - bias due to allowed non-similarity

Strategic Methods

- Randomize (at least rotate)
- Use designs that are easy to move to robots (samples in rows)
- Use analyses that fit the design
- Minimize pseudo-replication
- Replicate at high level (e.g.; assays, plates) not low level (rows, wells)
- Use a wide concentration range to reliably capture both asymptotes
- Fit full curves
- For development & validation, if practical 4+ plates/assay
- Set equivalence bounds to (at least) limit potency bias
- Choose assay size to have similarity CI narrower than 1/2 the equivalence region
- Choose number of assays/reportable value for precision of potency

Good Designs

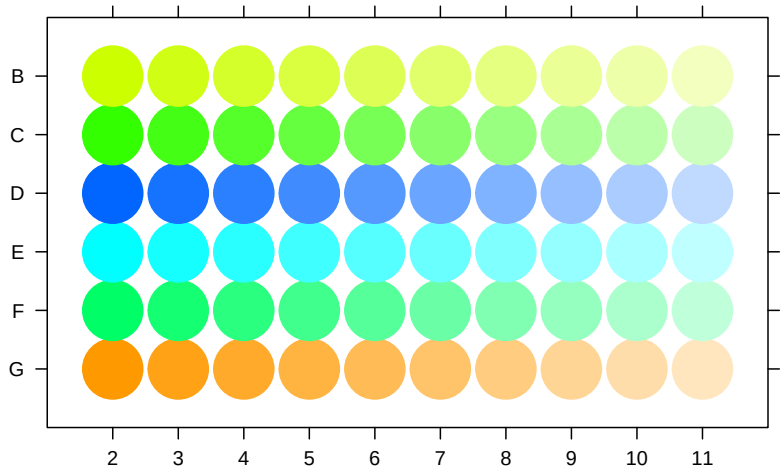
- Important to coordinate design & analysis strategy
- Simple analyses on complex designs often yield
  - wide CI for similarity
  - poor precision of potency
  - need for more lab work
- Randomization or rotation are easily adapted to robotics, provide some protection against location effects, and support monitoring
- Illustrate common good and poor designs

Finney (1978)

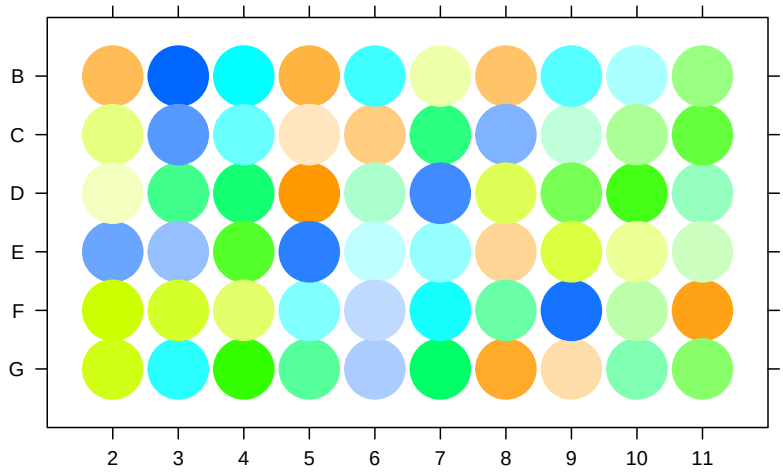
A biochemist, pharmacologist, or microbiologist whose own statistical expertise is small will perhaps object to some of the designs in later chapters: ...because when he had obtained the data he would have no idea how to analyze them. This difficulty illustrates the need for close collaboation between the experimental scientist and the statistician. ...the right policy is surely to learn how to analyze the data or to obtain assistance from a professional statistician.

In framing his advice, the statistician needs to remember that a simple design can give better results ...

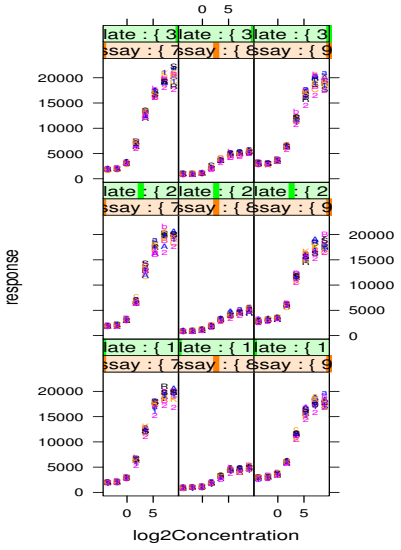
Simple Design?

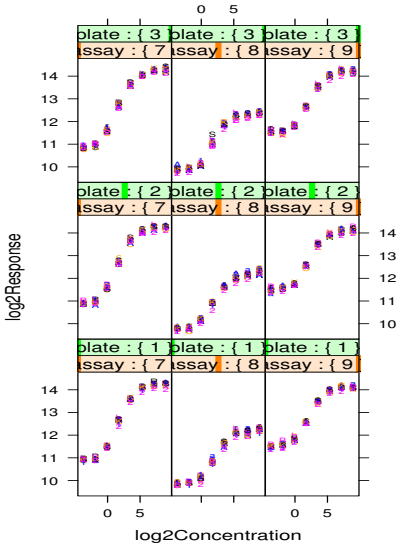


Simple Design?

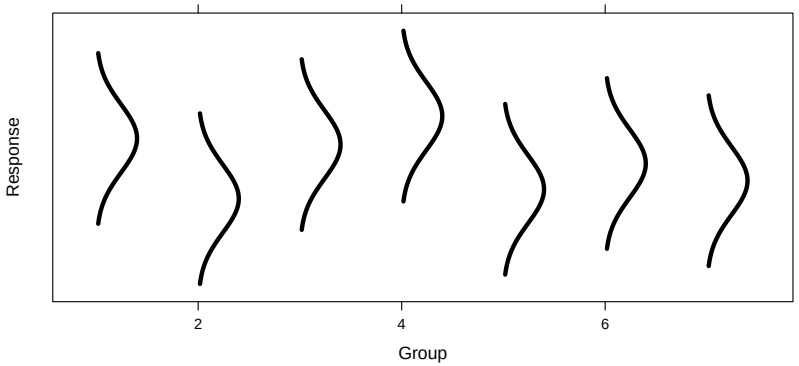


Why Transform?

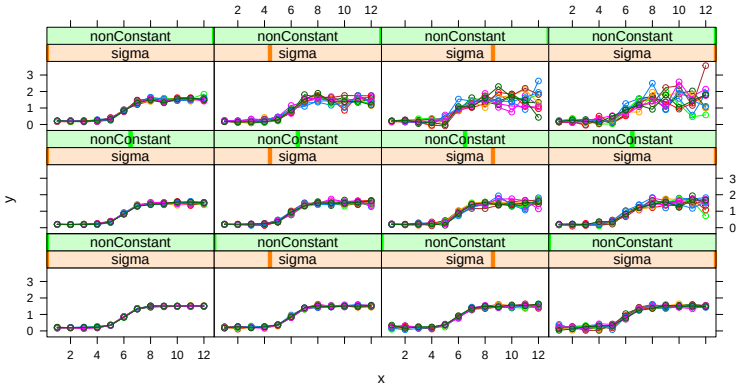




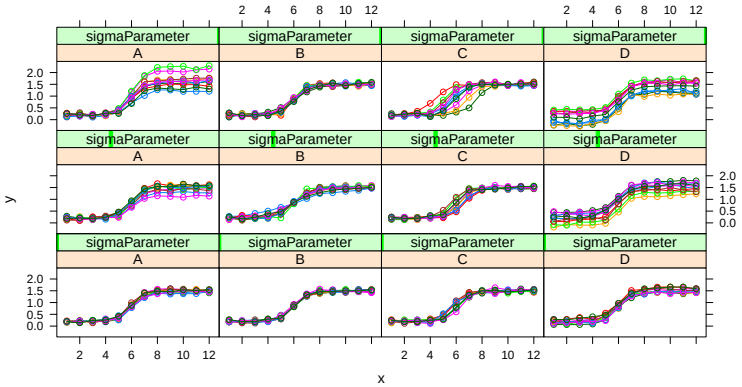
Constant Variance



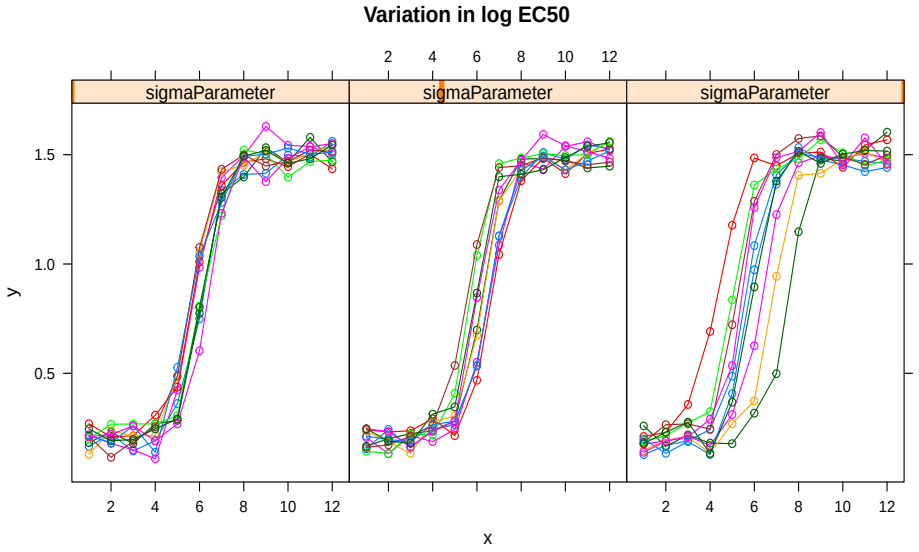
Simple Non-Constant Variance



Variance of Curve Parameters

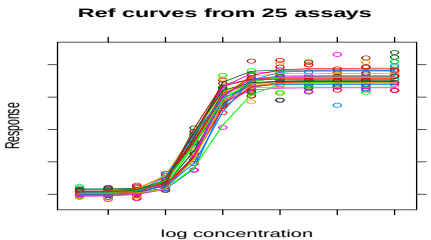


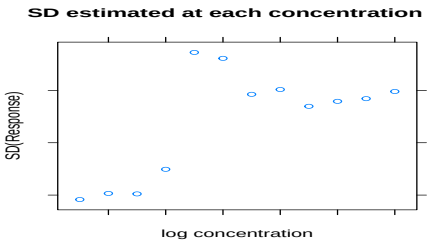
Reference with Two Sources of Variation: log EC50 and residual



Many Potential Causes of $\neq$ Variance

- 25 typical reference curves
- 4 Processes that can cause a common variance pattern:
  - variation changes with response
  - $\sigma^2_{\text{Log EC50}}$ large
  - (serial dilution error)
  - (underlying binomial response)





Big Statistical Challenges

- 'Rearguard battles'
  - Education (randomization, R^2, equivalence testing, design structure, CV)
  - Bring modern QC and statistical methods into routine practice in bioassay
- 'Available Now or Soon'
  - Document links between allowed non-similarity and potential potency bias
  - Practical ways to set similarity bounds that limit potency bias
  - Software that handles the groups in practical (complex) designs + robotics
  - Generate, fit, and select within large collections of RE models for complex designs
  - Develop better ways to compute fits for complex nonlinear mixed models
  - In linear model bioassay, choose subsets
  - What to do with partial curves or no relevant controls?
- 'Future directions'
  - Bayesian methods for borrowing historical information & monitoring
  - Incomplete block designs (several ideas: sample, dilution set, etc.)

Homework 1 - for Friday 2/25, email to david@precisionbioassay.com

- Describe an assay system that you work with
- Omit ALL confidential and proprietary information (so we can discuss/share)
- Please focus on the big picture (a 1 page summary)
  - Biology:
    - animals or cells?
    - if cells: suspension or adherent / proliferation or ?
    - what is the analyte (protein, vaccine, etc.)?
    - what is the readout?
  - Statistical Design:
    - How much effort/experimental material is there in what you call 'an assay' or 'a run?'
    - If there are plates (or other groups) how many/assay and what do you call them?
    - How many samples/assay (e.g.; reference, test lot 1, stability sample, etc.)
    - How many dilutions of each sample, what is the dilution step size & range?
    - How many replicates of each dilution of each sample?
    - What are the experimental units for sample & dilution?
    - Are the replicates independent? Why or why not?
    - How many assays in a reportable value?
- List two questions you hope the course will address

Next Session

- ***Session 2:***

  Statistical Fundamentals, Part 1

- ***Content:***
  - Inference
  - Randomization
  - Experimental Units
  - Pseudoreplication
  - Blocks
  - Reference Distributions
  - Assumptions

Upcoming Statistical Method Sessions

1. ✓ ***Introduction to Bioassay***
2. Statistical Fundamentals (part 1)
3. Statistical Fundamentals (part 2)
4. Intro to Bioassay Design
5. Intro to Bioassay Analysis
6. Setting Equivalence Bounds for Similarity Assessment
7. Mixed Models for Bioassay
8. Intro to DOE
9. Development & Robustness Experiments
10. Qualification & Validation Experiment Design & Analysis
11. Bioassay Stability Experiment Design & Analysis
12. Product Specifications & Process Control Limits
13. Monitoring Bioassays
14. Questions, Discussion and Additional Resources

Any Last Questions?

Contact me at: david@precisionbioassay.com