Statistical Approaches to Meeting Emerging USP Guidelines for Bioassay Development, Analysis, and Validation

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Bioassay Background

- Parallel Line: Shapes similar assume:
  - same cmpd $\Rightarrow$ common slope & asymptotes
  - interpret horizontal shift $=$ log potency

- Slope Ratio: Intercepts similar assume:
  - same cmpd $\Rightarrow$ common $y$ intercept
  - interpret slope ratio $=$ potency
Similarity in assay

Distance between curves
Similarity in assay

Distance between curves

Do the two curves have the same shape?
Similarity in assay

Distance between curves

Do the two curves have the same shape?
Potency SD and Dose Range

- $SD_{\log \text{ potency}} \ll SD_{\log \text{ ED50}}$
- $\text{PGSD} \left(= 100(e^{SD} - 1)\right)$ 5%, 6x-15x
  ED50 range 68%-216%
- product potency spec. often 0.71-1.41
- Generally need 3 doses on steep part
- doses often 1:2 dilutions
- $SD_{\log \text{ ED50}}$ & potency range: need 5 dilutions
Transform not Weight

- Weight on dilution-specific variances?
- $\text{SD}_{\log \text{ED}50}$ inflates SD at middle dilutions
- Weighting confounds nonlinear mixed model
Outliers

- After transform (or weight)
- Fit model \((w/\text{Design Structure})\) to all data
- Avoid shape assumptions
- Separate outlier detection from model adequacy
Design Structure

- Old recommendation: keep design simple, drive bias into variance
- New: recognize design structure
  - Grouped (multi-channel) and serial dilution common
  - pseudo-replicates (multiple aliquots from a preparation)
  - strip-plot designs appear
  - (incomplete) block designs efficient
Strip-Plot Design

A  B  C  D  E  F  G  H

1.9 1.9 1.8 1.8 1.6 1.4 1.2 1.1

1.9 1.9 1.8 1.7 1.4 1.3 1.2 1.1

1.9 1.9 1.8 1.8 1.6 1.4 1.3 1.2
Strip-Plot Response
Strip-Plot Model

\[ y_{ijk} = \frac{A + a_k + a_{ik} - D}{1 + e^{-B(x_{ijk} - (C_i + c_k + c_{jk}))}} + D + \epsilon_{ijk} \]

independent: \( \epsilon_{ijk} \sim iid \ N \left(0, \sigma^2\right) \)

\( a_k \sim iid \ N \left(0, \sigma^2_{a_{block}}\right), a_{ik} \sim iid \ N \left(0, \sigma^2_{a_{row}}\right) \)

\( c_k \sim iid \ N \left(0, \sigma^2_{c_{block}}\right), c_{jk} \sim iid \ N \left(0, \sigma^2_{c_{col}}\right) \)

with \( i \) for sample (row in block), \( j \) for dilution (column in block), and \( k \) block.
Combine log Potencies

- "weighted" and "semi-weighted" assume between-assay $\sigma_{\text{potency}} = 0$ (Finney, 1978)
- sampling SD of log potency safe
- Link properties of reported value to clinical need
Validation Params & Methods

- Relative Accuracy
  - "Linearity" of log potency
  - Bias limit at each target
  - Bias trend limit
- Precision
  - Components (repeatability, intermediate precision, reproducibility)
  - Predict for various "formats"
- Specificity
  - (Robustness)
- Equivalence used broadly

USP <1032>, <1033>, and <1034> will appear in PF 36(4) (early July). These and <111> at http://www.usp.org/meetings/workshops/2010Bioassay.html
Why Equivalence?

- Statistical similarity tested
- Biological similarity assumed (stat. similarity necessary, not sufficient)
- Assume critical differences known:
  - Slope
  - Upper asymptote
  - Lower asymptote
- Equivalence tests: "Are reference and test sufficiently similar"
- USP -> equivalence
- Curve parameters have meaning
  - Critical quality attributes
  - Separate equivalence intervals
Similarity: What is Needed

- Slope ONLY: lot release
- Asymptote of max activity:
  - compare standards (i.e.; new lot)
  - change production
  - stability
- Asymptote of min activity: checks only matrix effects
Difference testing for Similarity

- **Practical Problems**
  - Assays w/low variance fail parallelism
  - Assays w/high variance pass parallelism

- **Theoretical Problem (one parameter)**
  - Difference test
    - $H_0 : \beta_{\text{Reference}} = \beta_{\text{Test}}$
    - $\alpha$ (Type I) controls $P(\text{Falsely rejecting } H_0)$
    - $\beta^*$ (Type II) controls $P(\text{Accepting } H_0 | \delta^*)$

- **Equivalence test**
  - $H_0 : |\beta_{\text{Reference}} - \beta_{\text{Test}}| > \delta^*$
  - $\alpha$ controls Type I error of $H_0$ : for $\delta^*$

- **In Practice: What is $\delta^*$?**
Difference test for Similarity

Incorrectly sensitive to variance

\[
\text{Response} \quad \text{Log(dose)}
\]

-1 0 1
20 40 80 160 3 20
R R R R
T T T T
p< 0.03612
assay

R R R R
T T T T
p< 0.00577
assay

R R R R
T T T T
p< 0.53529
assay

R R R R
T T T T
p< 0.07369
assay

-1 0 1
20 40 80 160 3 20
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p< 0.53529
assay

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p< 0.07369
assay
Equivalence test for Similarity

Correctly sensitive to variance
90% CI inside indifference zone ⇒ equivalence
95% CI does not include 0 ⇒ difference
What can we say about $\delta^*$?

- What if we knew $\delta^*$?
- Assay dependent
- Scale dependent
- Parameter link to quality attribute weak
- Standardize meaning of $\delta$?
Seeking Scale Invariance

- Assays have different responses
- Asymptotes in response units
- Slope units \( \frac{\text{logit(response)}}{\text{log(dose)}} \)
Simple Scale Invariance

\[ y^* = \frac{A_i}{1 + e^{-B_i(\log(x)-C_i)}} + D_i \]

with \( C = \log(\text{ED50}) \) and \( i = [\text{Ref}|\text{Test}] \)

(Ratkowsky & Reedy, 1986)

**Range**
\( A \):
\[
\%\Delta_A = 100 \frac{A_{\text{Test}}-A_{\text{Ref}}}{A_{\text{Ref}}}
\]

**Lower Asy**
\( D \):
\[
\%\Delta_D = 100 \frac{D_{\text{Test}}-D_{\text{Ref}}}{D_{\text{Ref}}}
\]

**Slope**
\( B \):
\[
\%\Delta_B = 100 \frac{B_{\text{Test}}-B_{\text{Ref}}}{B_{\text{Ref}}}
\]

**Concerns:**
- Is meaning consistent?
- Are these useful across assays?
- Variances of \( \%\Delta_A, \%\Delta_B \), and \( \%\Delta_D \)
Interpretation of $\%\Delta_{A:B:D}$

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2A/3, A, 3A/2$, $%\Delta_A = 10$</td>
<td>$B/3, B, 3B$, $%\Delta_B = 50$</td>
<td>$2D/3, D, 3D/2$, $%\Delta_D = 10$</td>
</tr>
</tbody>
</table>

A in $\{2A/3, A, 3A/2\}$, $\%\Delta_A = 10$
B in $\{B/3, B, 3B\}$, $\%\Delta_B = 50$
D in $\{2D/3, D, 3D/2\}$, $\%\Delta_D = 10$
Interpretation of $\%\Delta_{A:B:D}$

- scaling mostly works
- Requires some explaining
50% slope change seems small in comparison
%Δₐ:ₐ:B:D 5:35:5

D=-5

D=0

D=5

35% slope change small?
5% range small vs. 5% on lower asy?
Experience with $\%\Delta_{A:B:D}$

- excellent assays can use 5:35:5
- many cell fail 5:35:5, ok w/10:50:10
- noisy assays struggle with 15:50:15
- Equiv. in linear: longer subsets
Summary

- Transform
- Detect outliers (w/smooth) model
- Use Design Structure
- Assess similarity with equivalence
- Asymptote sim. needed at times
- Assess Validation with equivalence
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