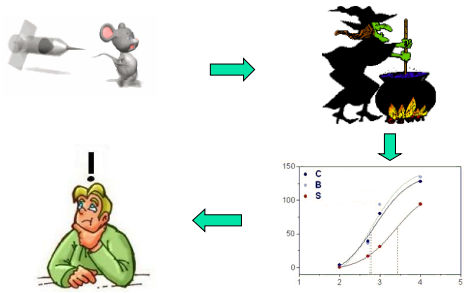
Random effect models and their application in qualification and validation of biological methods

Review

- What is a biological method?
- Accuracy and precision
- "The way we used to do it"
- Estimation of accuracy and precision
  - Modeling
  - Experimental design
  - Reporting

Biological method for dummies



Definitions – ICH Guideline Q2A

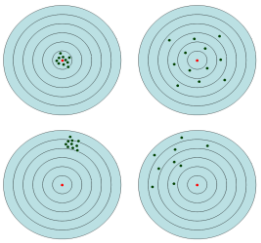
The accuracy … expresses the closeness of agreement between … an accepted reference value and the value found.

The precision … expresses the closeness of agreement (degree of scatter) between a series of measurements

Definitions – ICH Guideline Q2A

- Repeatability expresses the precision under the same operating conditions over a short interval of time
- Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.
- Reproducibility expresses the precision between laboratories

A look at accuracy and precision



Accuracy=Bias *Precision=Variance*

"The way we used to do it"

1. Measure accuracy and repeatability using 6 runs by the same analyst on the same day – report CV
2. Measure reproducibility using another 6 runs by another analyst on another day – report "Reproducibility Difference"

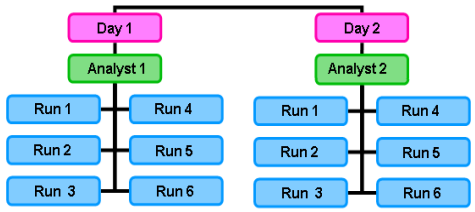
"The way we used to do it" advantages

- No experimental design
- No modeling
- No complex calculations
- Simple reporting

"The way they do it" problems

- Biological methods are more complicated to implement, therefore the numbers of possible runs in a single day is limited
- Variation of biological methods is generally higher compared to chemical methods
- Measuring intermediate precision is not enabled
- No statistical sense

"The way they do it" experimental design



Example - biological data

Day/ Analyst	Run 1	Run2	Run 3	Mean	STD
1	0.768	0.601	0.887	0.752	0.144
2	0.460	0.398	0.519	0.459	0.061

$$\text{Accuracy} = 100 \cdot \frac{0.752}{0.7} = 107.4\%$$

$$\text{Repeatability} = 100 \cdot \frac{0.144}{0.752} = 19.1\%$$

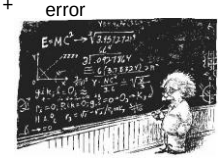
$$\text{Reproducibility Difference} = 100 \cdot \frac{|0.752 - 0.459|}{\frac{0.752 + 0.459}{2}} = 48.4\%$$

The way we would do it, at Biostatistics

$$Y_{ij} = \mu + b_i + c_j + \varepsilon_{ij}$$

Signal = fixed parameter + random effects + random error

Assumptions:
- Independence
- Normal distribution
- Zero mean deviations
- STDs: $\sigma_b, \sigma_c, \sigma$



Results that make statistical sense

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
day	0.03887	0.06077	0.64	0.2612	0.05	0.007062	175.52
Residual	0.01215	0.008592	1.41	0.0786	0.05	0.004362	0.1003

Repeatability

Between Day precision

Solution for Fixed Effects								
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
Intercept	0.6055	0.1465	1	4.13	0.1511	0.05	-1.2560	2.4670

Accuracy

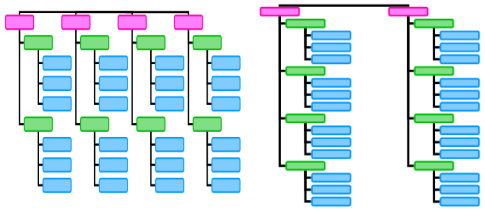
Results that make biological sense

$$\text{Accuracy} = 100 \cdot \frac{0.6055}{0.7} = 86.4\%$$

$$\text{Repeatability} = 100 \cdot \frac{\sqrt{0.01215}}{0.6055} = 18.2\%$$

$$\text{Reproducibility} = 100 \cdot \frac{\sqrt{0.03887 + 0.01215}}{0.6055} = 37.3\%$$

DOE to measure intermediate precisions



4 Days, 2 Analysts or 2 Days, 4 Analysts

Example 2

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
Analyst	0.000548	0.000796	0.69	0.2455	0.05	0.000106	0.8027
Day	0.002582	0.002132	1.21	0.1130	0.05	0.000821	0.03765
Residual	0.000177	0.000057	3.08	0.0010	0.05	0.000102	0.000377

Repeatability

Between Day precision

Between Analyst precision

Solution for Fixed Effects								
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
Intercept	0.5621	0.03045	1	18.46	0.0344	0.05	0.1753	0.9490

Accuracy

Example 2 results

$$\text{Accuracy} = 100 \cdot \frac{0.5621}{0.7} = 80.3\%$$

$$\text{Repeatability} = 100 \cdot \frac{\sqrt{0.000177}}{0.5621} = 2.4\%$$

$$\text{Between Day Precision} = 100 \cdot \frac{\sqrt{0.002582 + 0.000177}}{0.5621} = 9.0\%$$

$$\text{Between Analyst Precision} = 100 \cdot \frac{\sqrt{0.000548 + 0.000177}}{0.5621} = 4.8\%$$

$$\text{Overall Precision} = 100 \cdot \frac{\sqrt{0.002582 + 0.000548 + 0.000177}}{0.5621} = 16.1\%$$

Confidence Intervals for :

$$\mu, \quad \sigma, \quad \sqrt{\sigma^2_{\text{Day}} + \sigma^2}, \quad \sqrt{\sigma^2_{\text{Analyst}} + \sigma^2}, \quad \sqrt{\sigma^2_{\text{Day}} + \sigma^2_{\text{Analyst}} + \sigma^2}$$

Resources

- ICH Q2A -Text on Validation of Analytical Procedures
- ICH Q2B - Validation of Analytical Procedures
- Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules, DeSilva et. al. (*Pharmaceutical Research, Vol. 20, No. 11, November 2003)*