Estimating Precision of Analytical Methods in Biologics Development and Manufacturing

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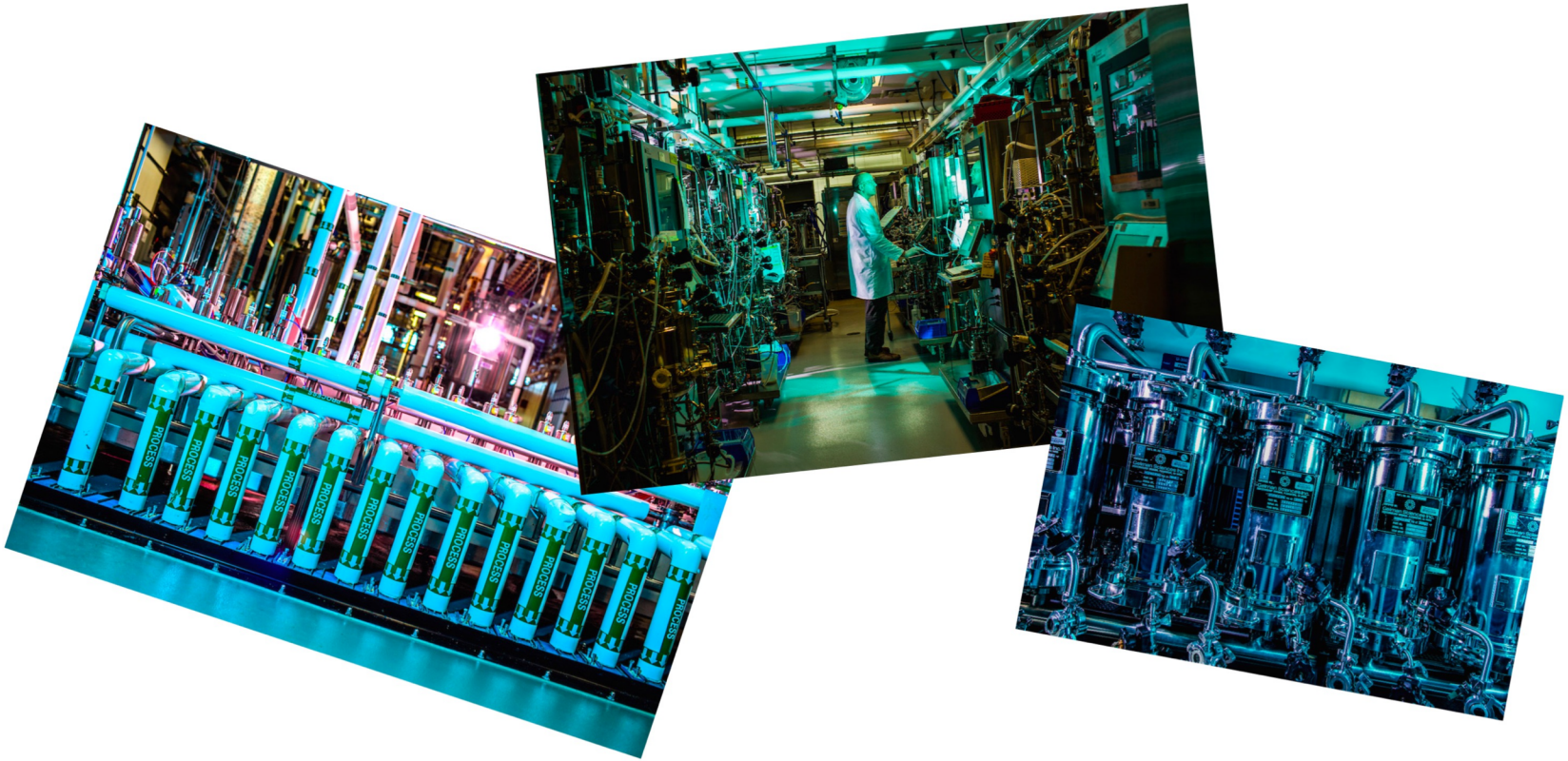
* Opinions expressed in this talk are those of the authors and do not necessarily reflect those of Roche/Genentech

What is the True Process Variation?



Process or Assay (Measurement)?

Observed Process Variation =

True Process Variation

+

Assay Variation

Types of Assay Variability

- Repeatability
  - Within assay-run variability
  - In reality: change nothing, test same sample multiple times in the same assay run
- Intermediate Precision (IP)
  - Total of variation (between + within assay-run variability)
  - In validation: change analysts, reagent lots, machines, etc.
  - In reality: variation in re-assaying a sample weeks or months later
- Reproducibility
  - IP + between site variability
  - In reality: sites, climate, training, machinery, etc.
- Repeatability (SD) <= Intermediate Precision (SD) <= Reproducibility (SD)

Many Uses of IP

- Inform specifications (e.g., release specifications)
- Inform acceptance criteria for experiments (e.g., comparability)
- Investigations (e.g., process or assay?)
- Risk assessment (e.g., probability of OOS due to assay variability)

Estimation of IP in Practice

- In practice, IP is estimated by validation experiments
- *Estimates of IP from validation have poor precision due to low degrees of freedom and are likely biased downwards due to motivated analysts performing the validation.*
  – Anonymous Statistician
- *The variation of assay controls run during development may provide a better estimate of IP.*
  – Anonymous Statistician

Is there data other than validation data that can be used to estimate IP?

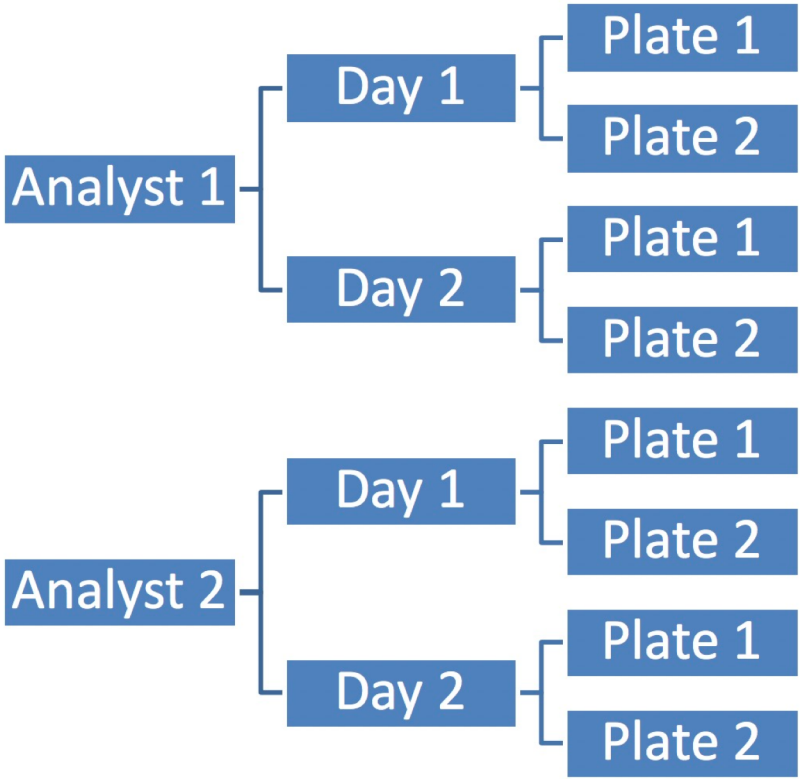
Data for IP Estimation

- Assay Validation Data
- Stability Data
- Method Monitoring (MM) Data

Will these data provide consistent estimates for IP?

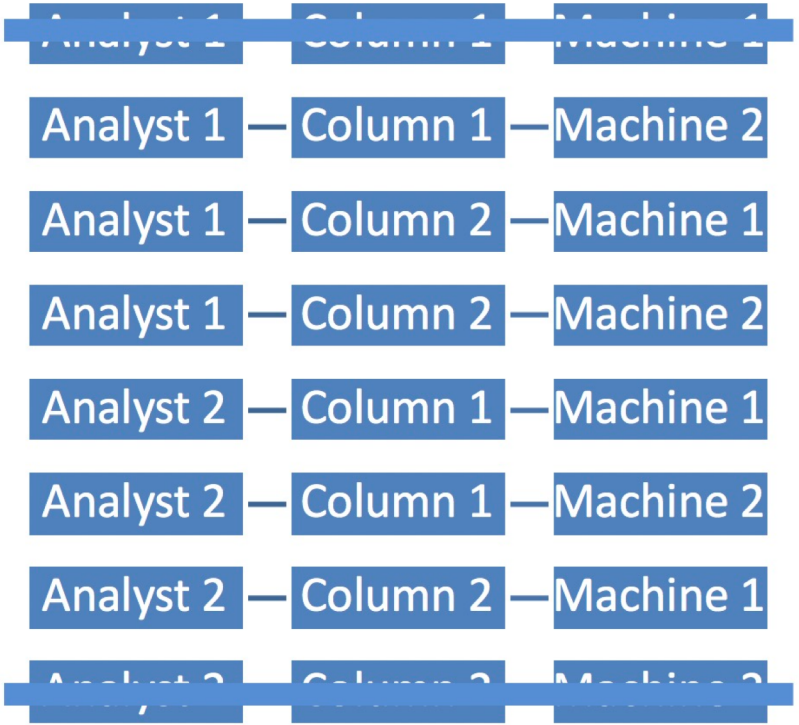
Assay Validation Experiment – Potency

- Cell based potency and ELISA methods (e.g. CHOP, ECP, DNA)
  - Potential sources of variation: Analyst, Day, Plate
  - Variation due to assay run and preparation of reagents, samples and cells is included in Day
- The appropriate sources of variation depends on the method, the technology, etc.
- Modeling: Variance Components Proposal
- Estimation method: Restricted Maximum Likelihood (REML)



Assay Validation Experiment – HPLC

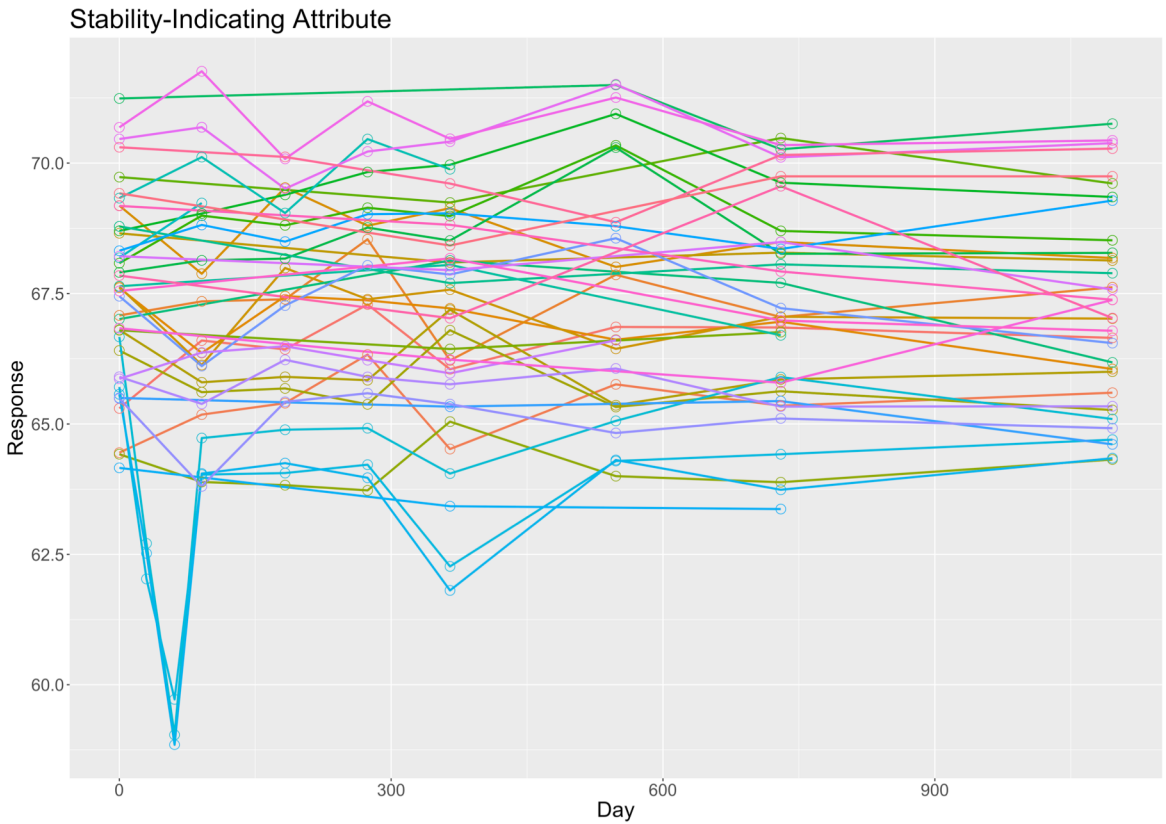
- 8 run full factorial design
- Reduced design removes runs 1 and 8
  - “Sufficient” for fitting variance components



Stability Data at -20°C

- Originally, stability studies investigate the degradation rate of Critical Quality Attributes (CQAs) that are closely related to efficacy and safety
- For drug substance (DS) stored at -20°C, degradation rates are almost negligible, which means they are highly stable
- Data source:
  - Before approval, 3 lots of DS are put on stability at -20°C and are tested 7 times over a period of time
  - After approval, a lot is put on stability at -20°C every year or campaign

IP from Biologics Stability



In reality: IP is observed in variation of re-assaying sample weeks or months later.

In stability,
IP = average within-lot variation

Method Monitoring (MM)

- Part of Continuous Process Verification
- Monitor **assay controls** for drift in method performance
- Ensures consistent method performance over time and across testing sites
- IP can be estimated from assay controls
  - Method variance is computed periodically within each site
  - We took **weighted average variance** across different sites and different periods for the same method

Results: Products with High Concordance

(difference within 0.015)

Assay:

Potency Assay

Product	IP from Stability Data (SD)	IP from MM Data (SD)	IP from Validation Data (SD)
Product 1	0.035		0.037
Product 2	0.029	0.029	0.024
Product 3	0.033	0.028	0.02
Product 4	0.066	0.05	0.052
Product 5	0.202		0.173
Product 6		0.029	0.019
Product 7		0.009	0.023

Results: Products with Low Concordance

(difference greater than 0.015)

Product	IP from Stability Data (SD)	IP from MM Data (SD)	IP from Validation Data (SD)
Product 8	0.049	0.029	
Product 9	0.063	0.057	0.106
Product 10	0.075	0.052	0.019
Product 11	0.063	0.016	0.011

Discussion

- Data from Stability and Method Monitoring can be used to confirm or potentially improve the estimation of IP
  - Provide insights to Assay Validation in estimating IP
  - Help answer the true process variability question
- Concordance of IP estimates across these data exists in many products
  - Samples that did not satisfy system suitability (SST) criteria were removed from data analysis
- Challenges and future directions
  - Methods in scope of MM are limited
  - Potential autocorrelation of MM data may lead to underestimation of IP
  - Historical data are sometimes hard to retrieve, e.g., paper copies, .pdf files
  - Collaborative effort from different functional groups to build a data pool

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Doing now what patients need next

Appendix

Assay Validation Data Modeling

Variance Components Proposal

- Model all factors as random effects*

$$Y_{itk} = \mu + a_i + b_{t(i)} + \epsilon_{k(itp)},$$

  - μ describes the grand mean
  - $a_i \sim \text{Normal}(0, \sigma_a^2)$ is analyst random effect. Describes analyst-to-analyst variation
  - $b_{t(i)} \sim \text{Normal}(0, \sigma_b^2)$ is assay instance random effect. Describes day-to-day variation within the same analyst
  - $\epsilon_{k(it)} \sim \text{Normal}(0, \sigma_e^2)$ is random error term. Describes plate-to-plate variation (within-assay variability)

- The total IP is estimated by $\sqrt{\hat{\sigma}_a^2 + \hat{\sigma}_b^2 + \hat{\sigma}_e^2}$

- Estimation method: Restricted Maximum Likelihood (REML)
  - Handles imbalance, e.g., staggered designs, missing data

Variance Components Proposal

- Model all factors as random effects*
- Use REML
  - Handles imbalance, e.g., staggered designs, missing data
- Use parametric bootstrap to make CI for IP and CV
  - Resamples both random effects and residuals and takes into account the hierarchical structure of nested designs
  - Described in *Fitting linear mixed-effects models using lme4*, Bates et-al, submitted to Journal of Statistical Software

* For reproducibility, laboratory is fixed effect

Why bootstrap CIs?

- REML on small data sets can be numerically unstable due to flat likelihood
    - Software implementations may balk at making likelihood based CIs when this is the case.
- Not sufficient data in IP experiment to support asymptotic results.

Stability Data Modeling

- LMM model

$$Y_{it} = \mu + a_i + \epsilon_{it},$$

  - μ describes the grand mean
  - a_i is lot-specific random effect. Describes deviation of the ith lot mean from grand mean
  - e_{it} is random error. Describes deviation of the itth measurement from the ith lot mean.

- Assumptions
  - $a_i, i = 1, \dots, I$ are i.i.d. from $Normal(0, \sigma_a^2)$
  - $\epsilon_{it}, i = 1, \dots I; t = 1, \dots, T$ are i.i.d. from $Normal(0, \sigma_e^2)$
  - $a_i \perp \epsilon_{ij}$

- Assay variation → within-lot variation, σ_e^2
  True process variation → between-lot variation, σ_a^2

- Estimation method: Restricted Maximum Likelihood (REML)