PHARMALEX
CONFIDENCE BEYOND COMPLIANCE

Bioassays:

Assay Reproducibility versus Compound Precision using Bayesian Statistics

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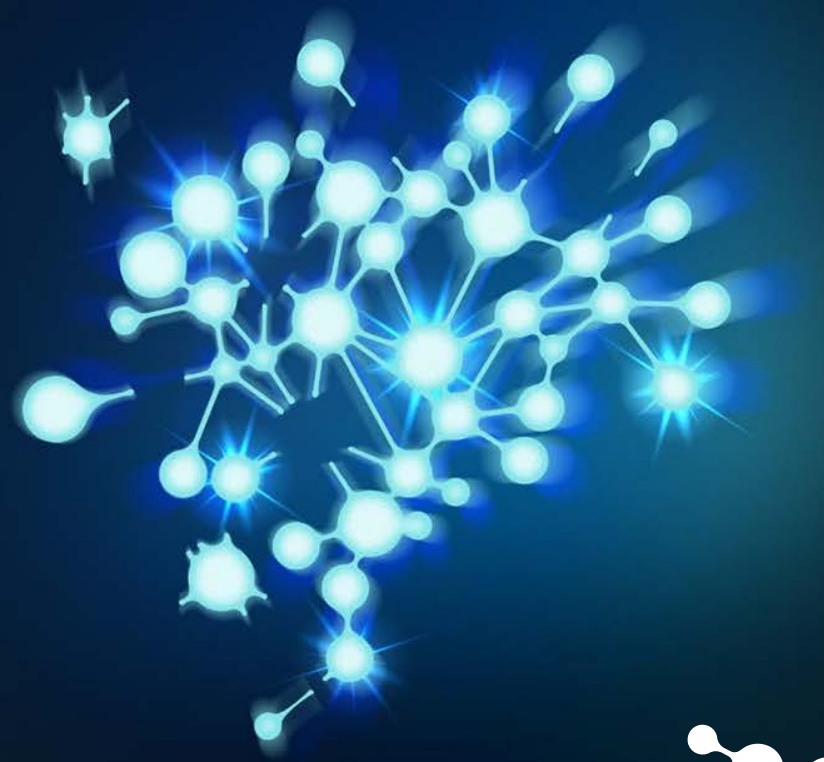


- Introduction
- Bayesian methodology
- Application in Bioassays
- Conclusion

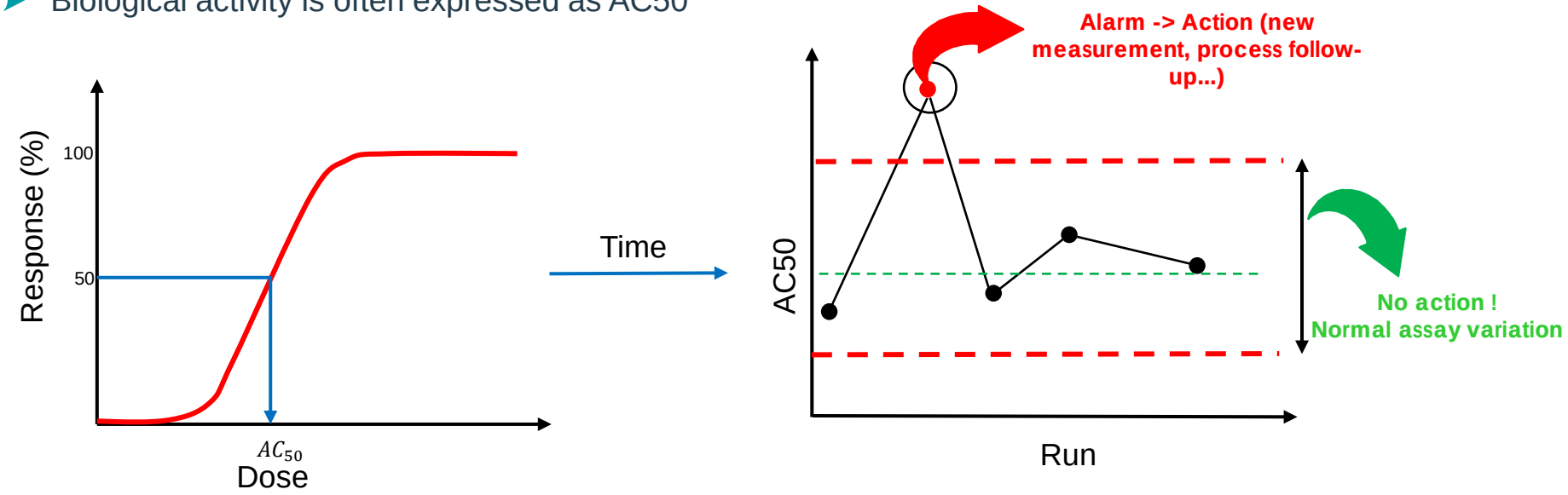
Introduction

What is the question?

- In drug development, assays are developed for the screening of potent compounds. In the event of compound repeat, how do scientists discriminate between true differences in potency and random noise?

- How can we verify?

- Biological activity is often expressed as AC50



- Due to inherent process and assay variability, estimates between runs are bound to differ.

Why differences in compound potencies?

- Sources of variation include:
  - Reagent preparation
  - Pipetting errors
  - Location on plate
  - Reader uncertainty
  - Environmental conditions
  - Different operators
  - Time effect

Methods implemented in practice

- **0.5log:** Expected threshold for within and between assay variability (EMAE and ICH guidelines)
  - Difference in log potency < 0.05 (3-fold change in potency)

- Disadvantage: ignores true assay variability (fixed cut-off)

Methods implemented in practice

- **MSR:** The smallest ratio between two potencies that is considered to be statistical significance,
  - captures variability.

MSR	Requirement	Estimate	Implementation
Replicate Experiment MSR	• Two independent runs of 20 – 30 compounds • Potency should span the concentration range to be tested by assay	$MSR = 10^{2*S_d}$	• Captures **within run variability** • Assay validation for production • <3 for a good assay
Control compound MSR	• Log-potency (logAC50) estimates of control compound • Minimum of 6 runs is considered adequate to estimate between-run variability	$MSR = 10^{2*\sqrt{2}*sd}$	• Captures **between-run variation** (within-run) • Monitor assay reproducibility over time
Database MSR	• logAC50 estimates of repeated compounds • Minimum of 6 runs	$MSR = 10^{2*\sqrt{2}*sd}$	• Captures **within and between-run variation** • Monitor assay reproducibility over time

Haas, J. V et al.; 2017

Application of MSR and limitations

- MSR is recommended to check for overall reproducibility of an assay (prior and post-production monitoring): MSR < threshold (dependent on practical experience).

- MSR as threshold for potency ratios, but literature is confusing…
  - Not recommended to evaluate potency precision of individual compounds (Haas et al. 2017): **With sufficient repeats**, current suggestion is to report geometric mean (backed transformed log potency) with 95% CI for the mean.

  - Compound specific MSR :
    - minimum of 6 repeats required
    - Significant difference between repeats when ${}^{Y_1}/_{Y_2} > MSR_Y$
    - When more than two repeats n>2, current estimate is compared to geometric mean

- Limitation:
  - Insufficient repeats in practice
  - For n>2, variability between repeats are not considered (averaged out)

Proposal: Bayesian beta(β) – expectation tolerance interval

- Region within which β proportion of future observations are expected to lie given the variability observed in data.
  - Also known as prediction interval of a future observation with β probability
- Computed based on historical data

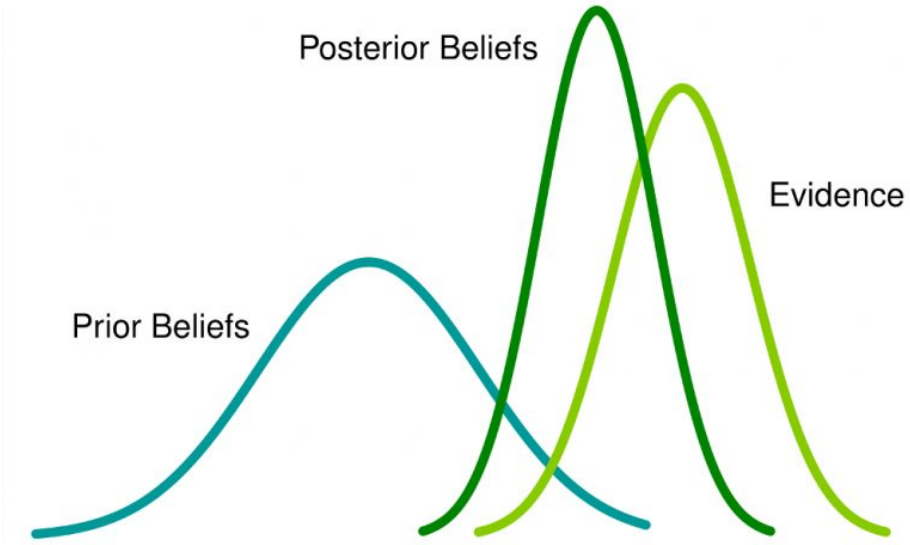
Use of information from reference compounds with multiple repeats

- Use of uncertainty in reference compounds estimates as prior
- Compute compound specific intervals upon first repeat
- Allows for assessment of compound repeat agreement

Bayesian methodology

Bayesian

- Bayesian statistics is a statistical approach that allow us to update our believes in the event of new data.
- Motivations for adopting Bayesian approach:
  - Natural and coherent way of thinking about science and learning



- Instead of point estimates (+/- standard deviation), we have a complete distribution for any parameter of interest

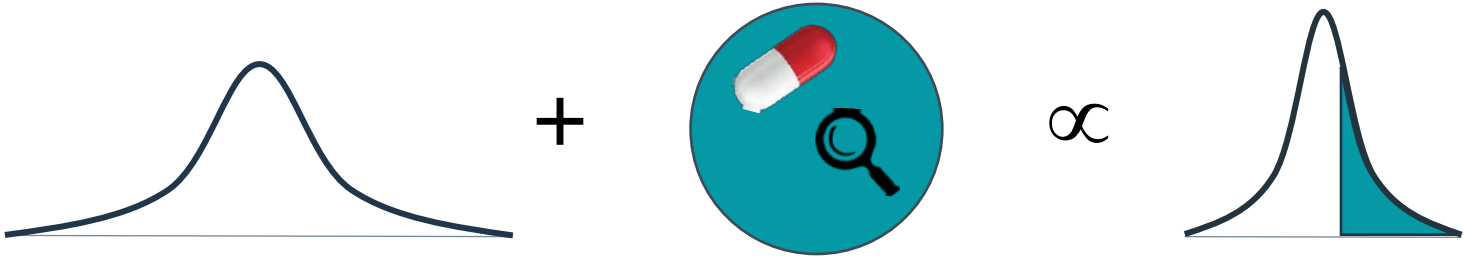
Bayesian principle

- Bayesian inference is the mechanism used to update the state of knowledge

prior information
$p(\theta)$

data information
$p(\text{data}|\theta)$

posterior information
$p(\theta|\text{data})$



Available Data + Observed Data = Total Data

"PRIOR DISTRIBUTION"
Information gained from reference compounds

"LIKELIHOOD"
compound repeats

"POSTERIOR DISTRIBUTION"
combination of both sources of information collected

Bayesian concepts

- Four major concepts:
  - Prior distribution
  - Likelihood
  - Posterior distribution
  - Predictive distribution

Prior belief

- Let's consider θ, the parameter of interest (ex: mean compound potency)

θ is treated as a random variable

- Prior distribution of parameter θ : $p(\theta)$
  - Distribution of θ before any data are observed
  - Reasonable opinion concerning the plausibility of different values of θ
  - Based on all available evidence/knowledge
  - Or non-informative prior

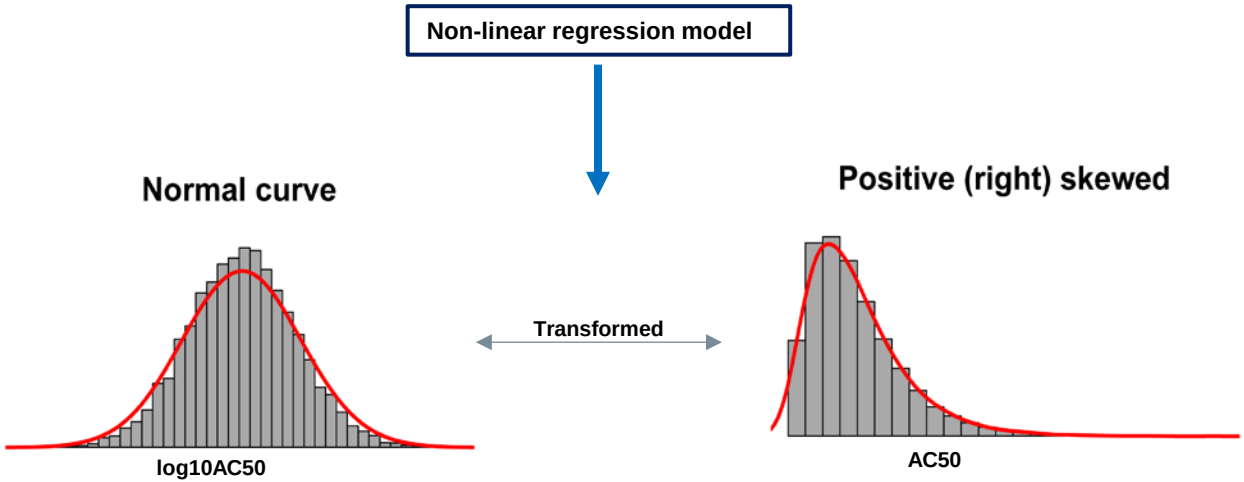
- Prior distribution ➔ Specify the domain of plausible values
  ➔ Specify the weights given to these values

Evident and posterior belief

- Likelihood:
  - Conditional probability of the data given θ: p(y| θ)
  - Based solely on data
- Posterior distribution:
  - Distribution of θ after observed data have been taken into account: p(θ|y)
  - Final opinion about θ
- Predictive distribution:
  - Given the model and the posterior distribution of its parameters, what are the plausible values for a future observation y*?
    p(y*| θ)

Derivation of posterior distribution

- Let's assume that the log potency of a compound y_i, i=1,…N, follow a normal distribution N(μ,σ^2) (= likelihood)



- Independent runs i.e fresh preparation of experimental materials

Derivation of posterior distribution

- Let's assume that the log potency of a compound y_i, i=1,…N, follow a normal distribution

  N(μ,σ^2) (= likelihood)

- We assume that the mean μ and variance σ^2 are unknown
- The objective is to estimate μ and σ^2 and predict a future measurement $\tilde{y}$
- We need to specify a prior distributions for the μ and σ^2:
  - We select priors distributions that lead to same family of posterior distributions
    - Normal conjugate family approach

Prior mean: $\mu|\sigma^2 \sim N(\mu_0, \sigma^2/\kappa_0)$

- We keep it non-informative

$\mu_0 = \bar{y}_0$

$\kappa_0 = n_0$

Prior variance: $\sigma^2 \sim Inv-\chi^2(v_0, \sigma_0^2)$

$\sigma_0^2 = s_0^2$

- Assay intermediate precision based reference compounds

- $\bar{y}_0$, n_0, s_0^2 and v_0 are the prior sample mean, sample size for the mean, sample variance and degree of freedom of the variance respectively

- Likelihood: Data

$$L(\mu, \sigma^2 | \boldsymbol{y}) = \frac{1}{(2\pi\sigma^2)^{\frac{n}{2}}} exp\left(-\frac{1}{2\sigma^2}[(n-1)s^2 + n(\bar{y}-\mu)^2]\right)$$

  - s^2 and $\bar{y}$ are sufficient statistics for μ and σ^2

- Posterior ∝ Prior * Likelihood

$$\bar{\kappa}_n = \kappa_0 + n,$$

$$\bar{\mu}_n = \frac{\kappa_0 \mu_0 + n\bar{y}}{\bar{\kappa}_n}$$

$$\bar{v}_n = v_0 + n$$

$$\bar{\sigma}_n^2 = \frac{1}{\bar{v}_n}\left(v_0\sigma_0^2 + \sum_i (y_i - \bar{y})^2 + \frac{n\kappa_0}{\kappa_0 + n}(\bar{y} - \mu_0)^2\right).$$

  - Posterior mean μ_n and variance σ_n^2 are a weighted averages of prior (μ_0, σ_0^2) and sample (s^2,$\bar{y}$) mean and variance
    - Weights depend on sample size

Marginal posterior distributions

- Mean: $p(\mu|\sigma^2, \boldsymbol{y}) = N(\mu|\bar{\mu}_n, \sigma_n^2/\bar{\kappa}_n)$
  $p(\mu|\boldsymbol{y}) = t_{v_n}(\mu|\bar{\mu}_n, \bar{\sigma}_n^2/\bar{\kappa}_n)$

- Variance: $p(\sigma^2|\boldsymbol{y}) = Inv - \chi^2(\sigma^2|\bar{v}_n, \bar{\sigma}_n^2)$

- The posterior distributions contains everything that can be said about μ and σ^2 .
- To summarize its information content:
  - Measures of location: posterior mode, posterior median, posterior mean
  - Measures of spread: Posterior variance
  - Bayesian credibility interval:
    - Get the quantiles of the distribution (2.5% and 97.5%)
    - An interval that contains 95% most plausible/credible values
  - Any probability on the values of the parameters

Posterior Predictive distribution

- Given the model and the posterior distribution of its parameters, what are the plausible values for a future observation $\tilde{y}$?

- Compute plausible values of $\tilde{y}$ conditionally on the available information:

$$p(\tilde{y}|data) = \int p(\tilde{y}|\theta)\, p(\theta|data)\, d\theta$$

Model Posterior

How to make predictions

1st , draw a mean and a variance from:

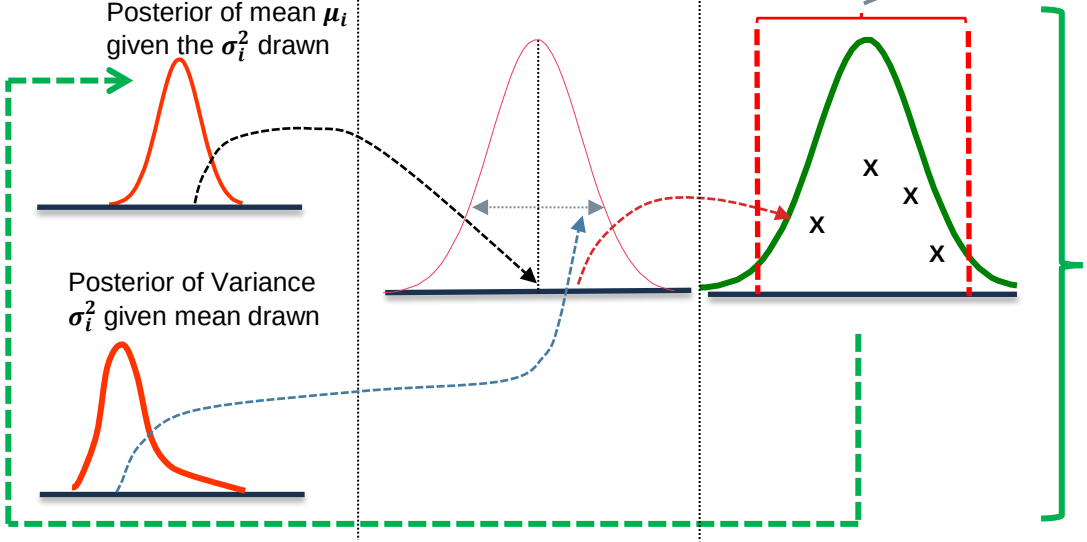
Posterior of mean $\boldsymbol{\mu_i}$ given the $\boldsymbol{\sigma_i^2}$ drawn

Posterior of Variance $\boldsymbol{\sigma_i^2}$ given mean drawn

2nd , draw an observation from the resulting distribution

$$\boldsymbol{y_i \sim N(\mu_i, \sigma_i^2)}$$

3rd , repeat a large number of time to obtain the predictive distribution



$\boldsymbol{\beta}$ – expectation tolerance interval

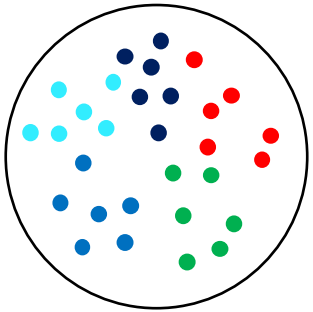
- After mathematical development,

$$p(\tilde{y}|\boldsymbol{y}) = t_{\bar{\nu}_n}\left[\tilde{y}|\bar{\mu}_n, \frac{\bar{\sigma}_n^2(1+\bar{\kappa}_n)}{\bar{\kappa}_n}\right]$$

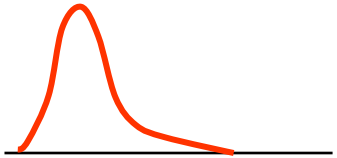
Application in Bioassays

Application in precision of compound repeats in assays

Potencies of 5 reference compounds

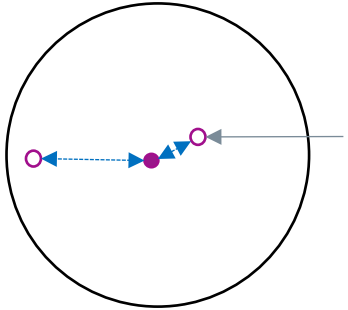


Intermediate precision

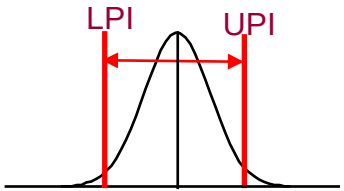


Prior information

Test compound



Compound repeat



Prediction

How to estimate assay variability: Intermediate precision (sd)

- Mixed- effect model:
  - Fixed effect for ref. compounds and random effect for run.

$$y_{ij} = \mu_i + \gamma_j + \varepsilon_{ij}$$
$$\gamma_j \sim N(0, \sigma_\gamma^2)$$
$$\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$$

y_{ij} = log potency of compound i in run j

μ_j = mean log potency of compound i

γ_j = random effect of run j

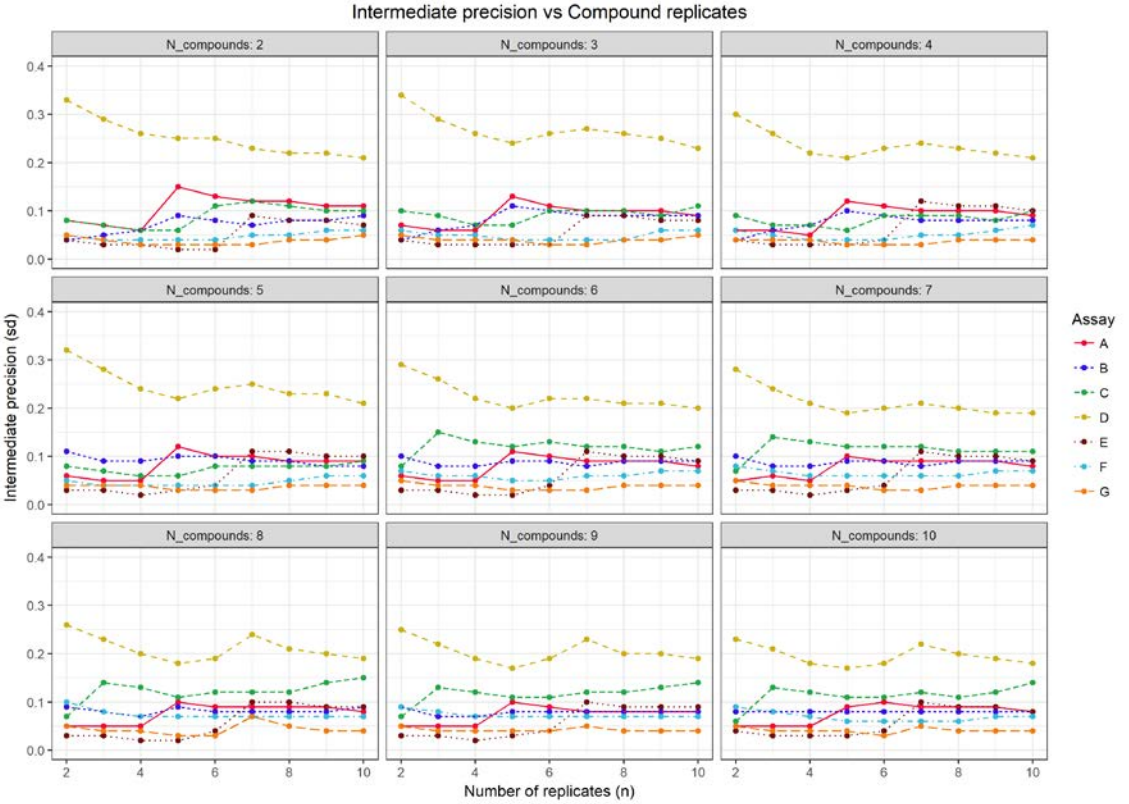
ε_{ij} = measurement error for compound i in run j

σ_γ^2 and σ_ε^2 are the run and measurement error variances respectively.

$$s_0 = sd = \sqrt{\sigma_\gamma^2 + \sigma_\varepsilon^2}$$

Sample size requirement

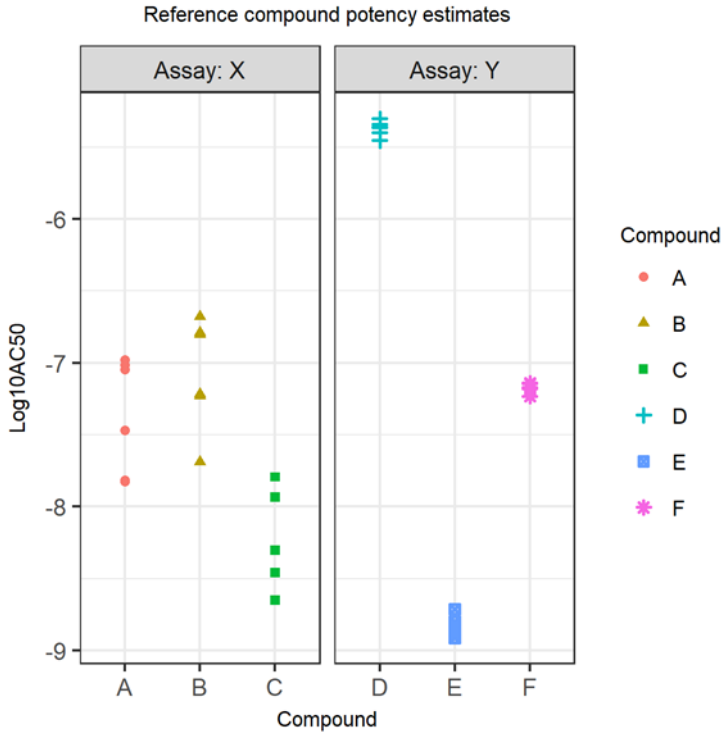
- Use of log potency of reference compound accrued over time
- A minimum of 6 repeat is proposed for Control compound and Database MSR.
- Is this a large enough sample?
  - This was assessed using real assay data
- Minimum recommendation:
  - 3 reference compounds with 6 repeat each (n = 18)
- This statistics does not depend on the actual log potencies of the compounds but if possible, compound selection may span the log potency range of assay



Example: Real assay data

- Compound **A** had a log potency estimates **-5.2** and a repeat of **-6.1**. Is the difference a true difference or noise from the assay?
- Consider two assays **X** and **Y**.

Run	Assay: X		Assay: Y	
	Ref. compound	Log10AC50	Ref. compound	Log10AC50
1	A	-6.98	D	-5.36
1	B	-6.79	E	-8.88
1	C	-7.93	F	-7.14
2	A	-7.82	D	-5.36
2	B	-7.69	E	-8.83
2	C	-8.65	F	-7.23
3	A	-7.04	D	-5.40
3	B	-6.68	E	-8.71
3	C	-7.79	F	-7.18
4	A	-7.82	D	-5.30
4	B	-7.23	E	-8.80
4	C	-8.30	F	-7.17
5	A	-7.01	D	-5.45
5	B	-6.80	E	-8.91
5	C	-7.93	F	-7.17
6	A	-7.47	D	-5.34
6	B	-7.22	E	-8.86
6	C	-8.46	F	-7.14



Example analysis

- Assay intermediate precision

Assay	Random effect	Variance	Intermediate precision (*sd*)
X	Run	0.1290	0.3755
	Residual	0.0119	
Y	Run	0.0002	0.0541
	Residual	0.0027	

- Prior:

$$\mu_0 = 0$$

$$\kappa_0 = 0.0001$$

$$\sigma_0^2 = 0.3755^2 = 0.1410$$

$$v_0 = 17$$

- Data : -5.2

$s^2 = 0$ and $\bar{y} = -5.2$

- Posterior:

$$\bar{\kappa}_n = 1 + 0.0001 = 1.0001$$

$$\bar{\mu}_n = \frac{(0.0001 * 0) + (1 * -5.2)}{1.0001} = -5.2$$

$$\bar{v}_n = 17 + 1 = 18$$

$$\bar{\sigma}_n^2 = \frac{1}{18}(2.3970 + 0 + 0.0027) = 0.1333$$

- Posterior predictive distribution:

$$p(\tilde{y}|\boldsymbol{y}) = t_{18}[-5.2, 0.2666]$$

- $\boldsymbol{\beta}$(95%) – expectation tolerance interval

[- 6.29, - 4.11]

- -6.1 is therefore within interval in assay X

Demo in R-Shiny

- Let’s go shiny…….

Bioassay: Compute beta-expectation tolerance interval of a test compound

Prior Distributions

Mean parameters

Mean

0

N(Mean):

0.0001

Variance Distribution

SD

0.0541

N(SD):

18

x-axis limits (min,max):

0,0.2

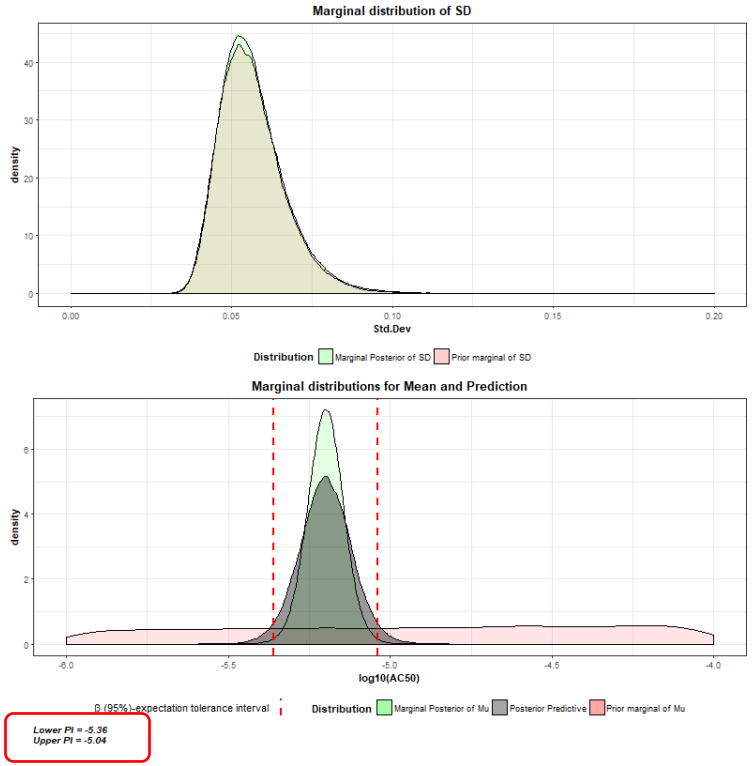
Data

Observations (comma delimited):

-5.2

x-axis limits (min,max):

-6,-4



With n=3 repeats

- Example where posterior shifts, due to more estimates (strength of bayesian)

Bioassay: Compute beta-expectation tolerance interval of a test compound

Prior Distributions

Mean parameters

Mean

0

N(Mean):

0.0001

Variance Distribution

SD

0.0541

N(SD):

18

x-axis limits (min,max):

0,.

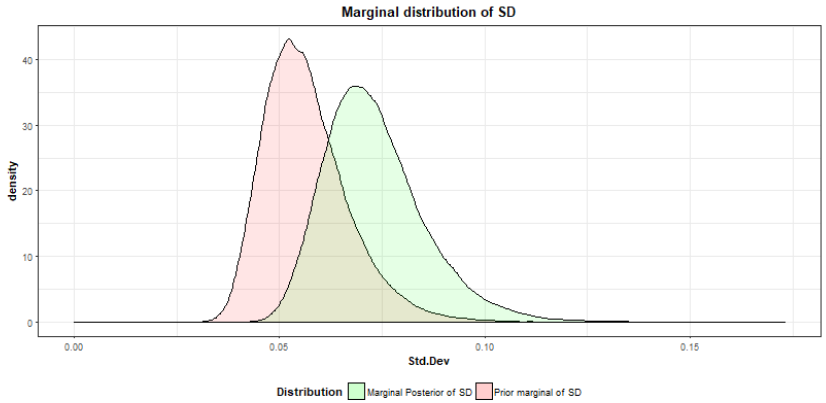
Data

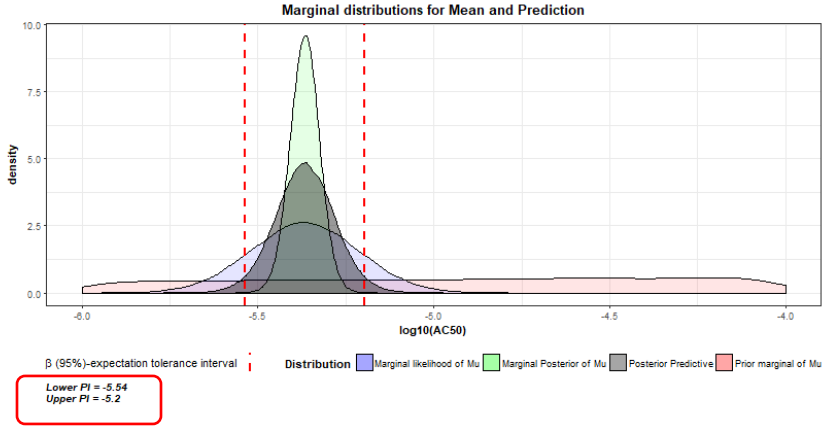
Observations (comma delimited):

-5.2,-5.5,-5.4

x-axis limits (min,max):

-6,-4





Conclusion

Summary

- Bayesian $\boldsymbol{\beta}$ – expectation tolerance interval approach:
  - An extension of Database MSR concept to capture Assay variability via reference compounds
  - Allows for compound specific prediction intervals to be computed upon the first repeat and updated as repeats are accrued.
  - Interval provides a save region within which differences in individual compound log potencies are considered to be within the normal assay variation limits.

Acknowledgement

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Comparison

- Comparison Bayesian-Frequentist interval
  - Bayesian : Prediction interval : 95% most plausible/credible values of future observations.
  - Frequentist : Prediction interval : “If we repeat the same experiment a large number of times, the prediction interval will contain a future observation 95% of the cases.”