Case Studies: Bayesian Methods for Qualification of Flow Cytometry Methods in Cell Therapy

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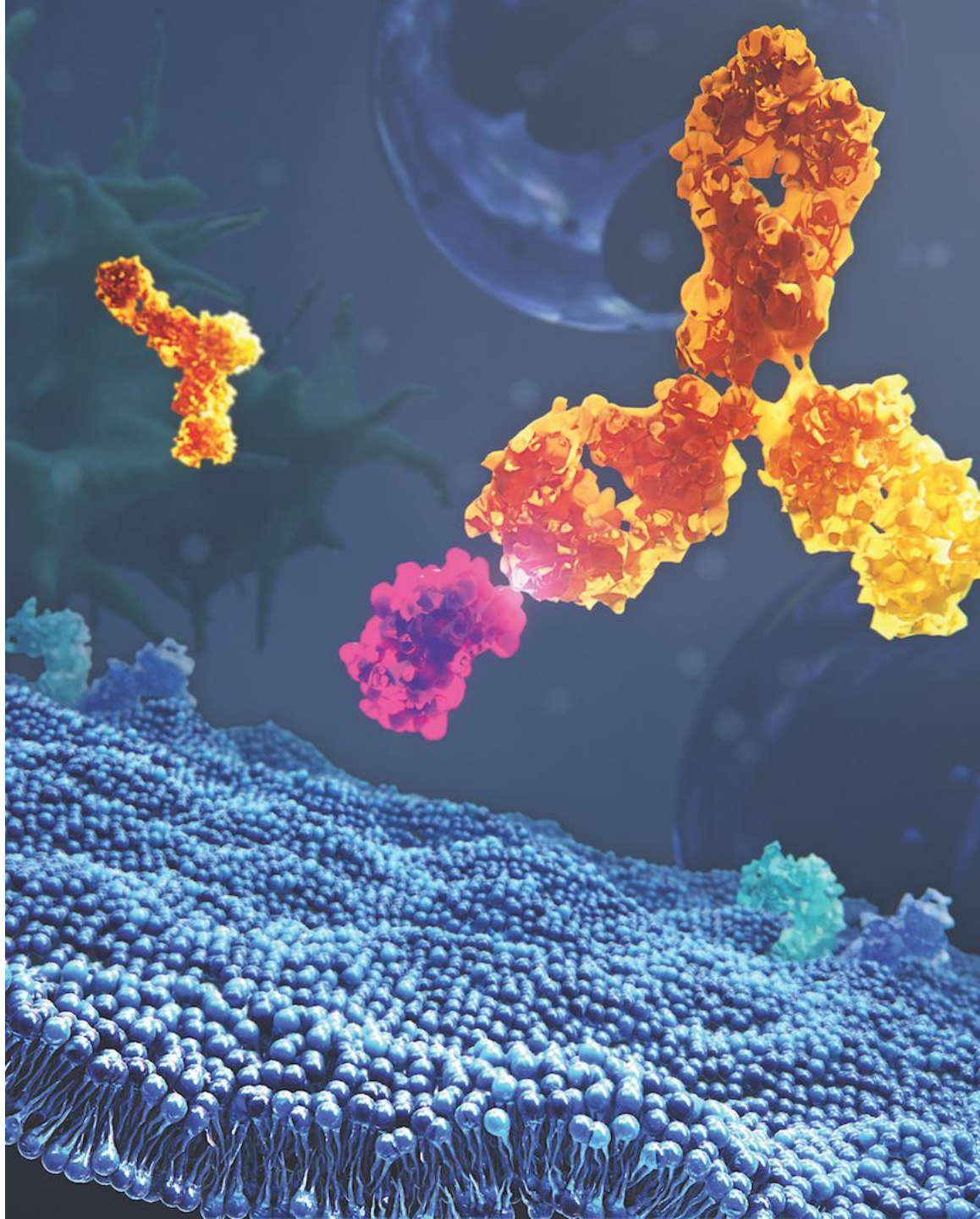
23Oct2025

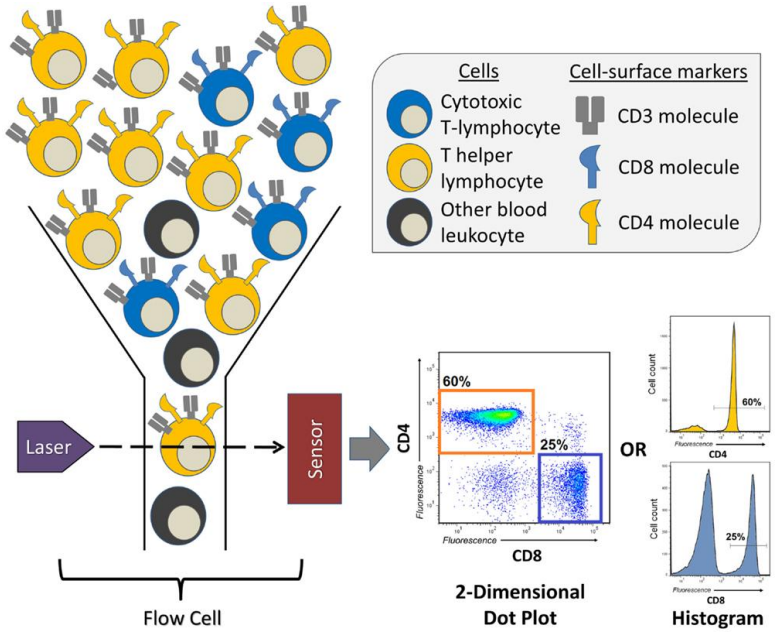


Introduction

- Cell therapies are populations of cells with altered phenotypes that can be used to treat diseases
- Flow cytometry methods are critical in characterizing the quality attributes of cell therapies by determining the phenotype and function of individual cells
- These methods pose unique statistical challenges when calculating typical analytical performance characteristics (APCs)
- Bayesian statistics offer several advantages when addressing these challenges
- Three flow cytometry methods qualified at AstraZeneca are analyzed here using Bayesian methods
- Solutions using the functions available to scientists in SAS are discussed, with an emphasis on accessibility

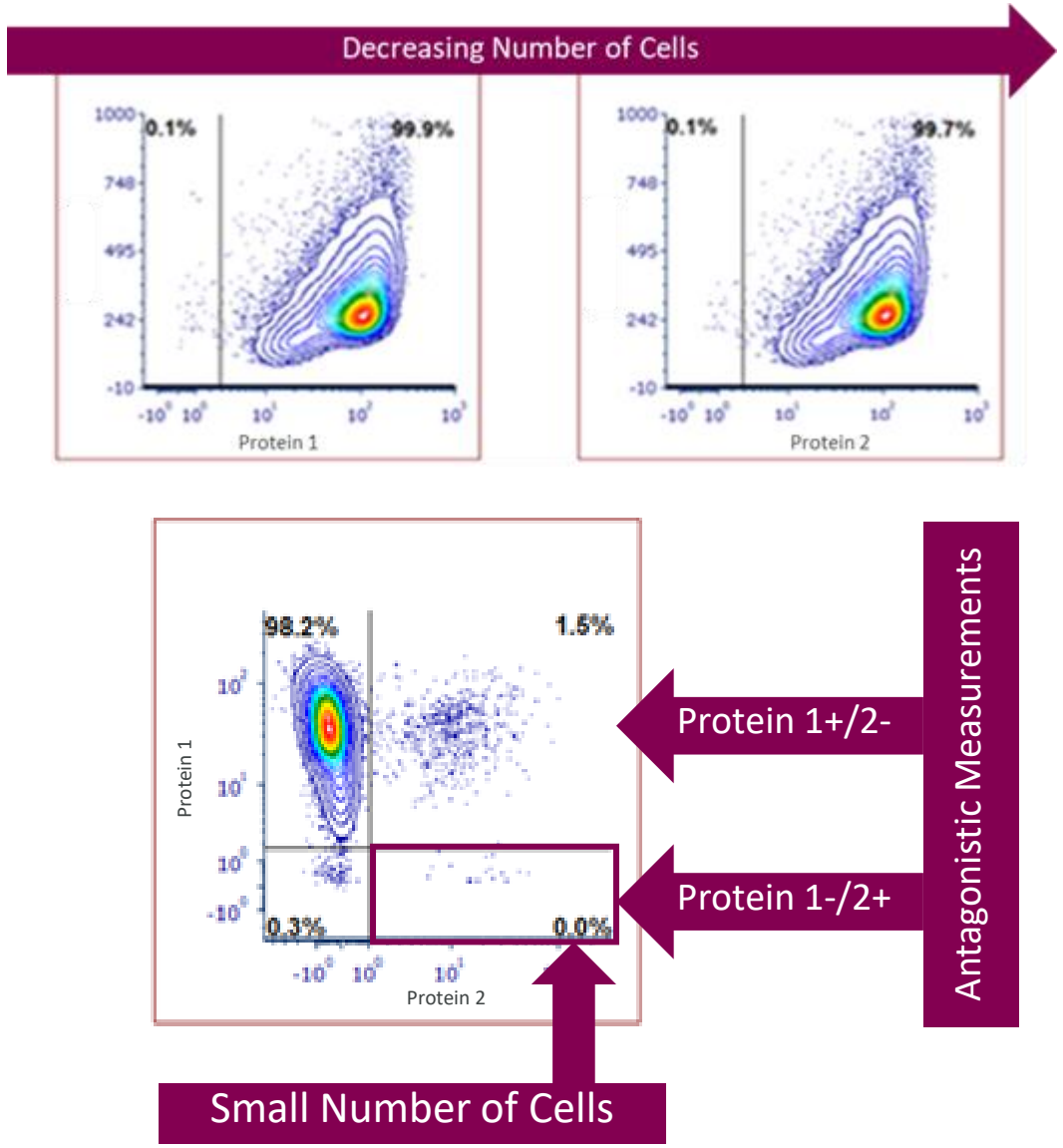
Background – Flow Cytometry Methods

- Flow cytometry is a critical method in evaluating the quality attributes of cell therapies
- Cells are typically stained with a fluorescent marker, singularly imaged, and analyzed on the population level
  - Typical results are the number or percent of cells with detectable levels of certain protein(s)
- These methods are low throughput and statistically challenging
  - Typical statistics used in method development are often sub-optimal when performing dilutional linearity designs from ICH Q2R2



Background – Flow Cytometry Challenges

- Flow cytometry measurements may violate two assumptions for typical analysis:
  1. Independent Measurements
     - All measurements are of a single cell
     - Impossible to separate out light from similar wavelengths completely
  2. Normal Likelihood Distribution
     - These measurements are unlikely to have an underlying normal likelihood distribution
- These problems have a similar solution with both Frequentist and Bayesian methods



Statistical Solutions in SAS

- We want to use a generalized linear mixed-effects model to analyze this data

Frequentist Solution: Glimmix Procedure

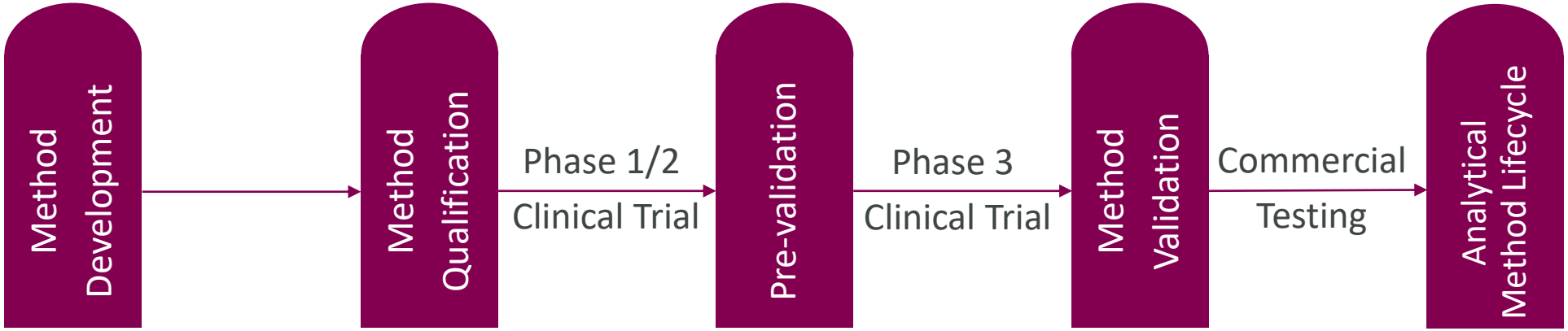
- Used to model count data
- Used to model multivariate data
- Can create hierarchal models with normal random effects
- Estimates the confidence of point estimates for analytical performance characteristics
- Does not leverage prior information

Bayesian Solution: MCMC Procedure

- Used to model count data
- Used to model multivariate data
- Can create hierarchal models with normal and non-normal random effects
- Estimates probability of analytical performance characteristic acceptability
- Leverages prior information

Bayesian Statistics in the Analytical Method Lifecycle

- The information we get in earlier parts of the analytical method lifecycle is valuable, but often is siloed off and never used



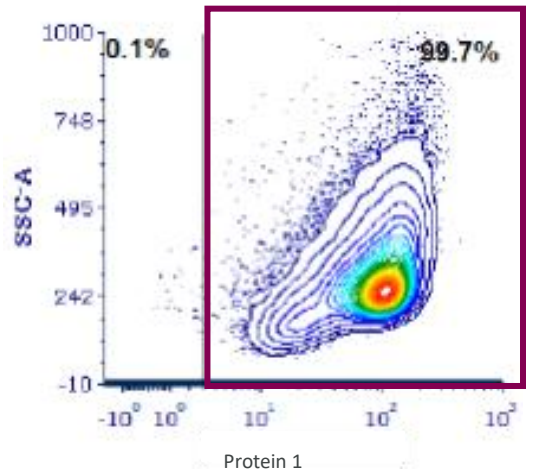
  - Bayesian methods allow us to use prior information from method development
    - By using prior data, we can reduce sample sizes (cost) and calculate better estimates
  - Bayesian methods can be interpreted in alignment with regulatory guidance
    - The probability of an analytical performance characteristic being acceptable is calculatable
  - We can calculate our estimates with complex likelihood models

Case Study 1: Simple Dilutional Linearity

- This assay seeks to characterize the number of cells expressing a single protein (P1+)
- This qualification was performed by one analyst at one laboratory over multiple runs
  - We have one random effect (run) for this qualification
- The likelihood distribution for this assay will be normal

$$Result = (\beta_0 + b_{Run}) + \beta_l + \beta_t Theoretical + \varepsilon$$

- We will feed in prior information to inform analyses
  - Relatively uninformed priors are used for pre-qualification
  - The pre-qualification results are used as priors for qualification

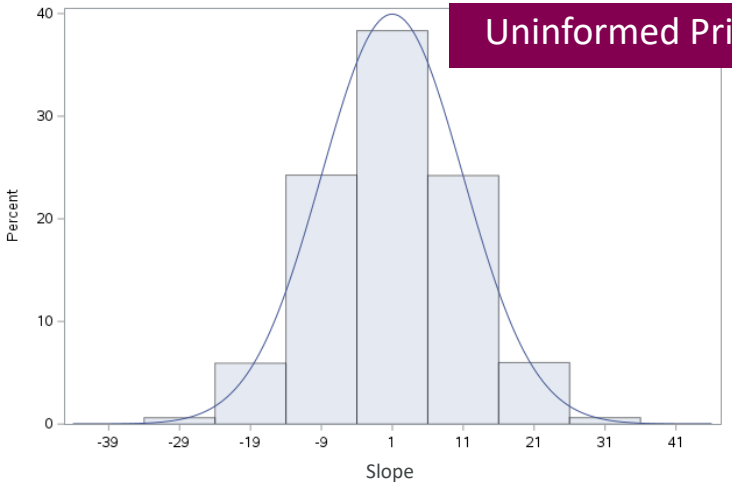


Results – Simple Dilutional Linearity

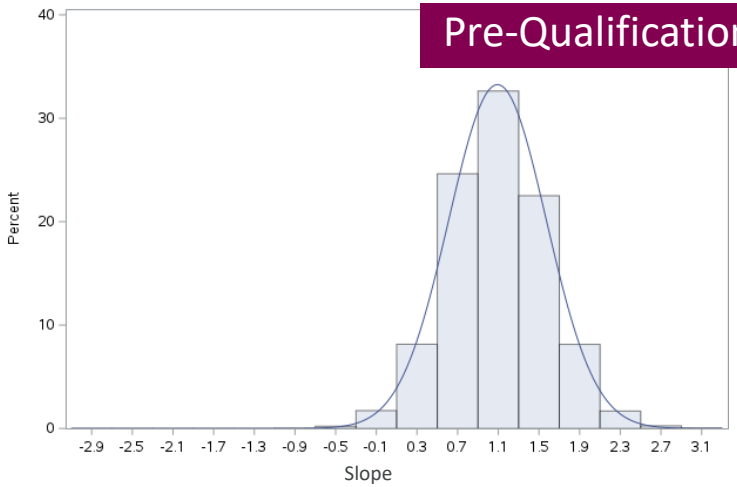
- For the likelihood distribution: $Result \sim N((\beta_0 + b_{Run}) + \beta_l + \beta_t Theoretical, \sigma_\varepsilon)$

Slope Estimate β_t Becomes More Precise

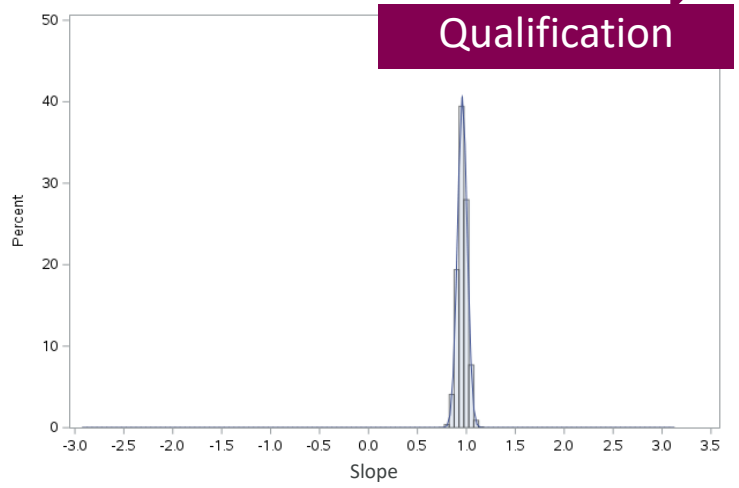
Uninformed Prior



Pre-Qualification



Qualification



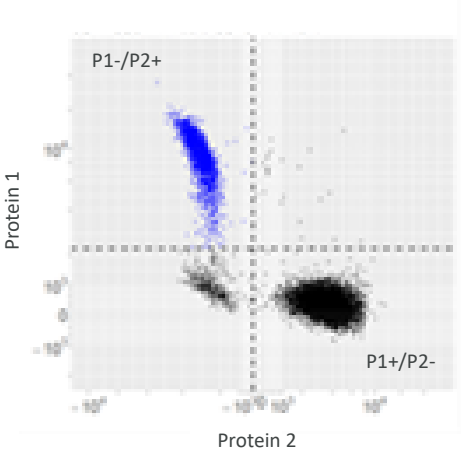
Model Parameter	Pre-qualification Prior	Qualification Prior	Qualification Average	Qualification 95% Credible Interval	Pr(Passing)
Linearity (β_1)	~N(1, 10)	~N(1.1, 0.5)	1.0	(0.9, 1.0)	N/A
Intercept (β_0)	~N(0, 10)	~N(3.6, 5.7)	3.1	(-5.5, 11.7)	N/A
Laboratory Difference (l)	~N(0, 10)	~N(0.2, 10.0)	2.0	(-6.6, 10.7)	N/A
Run Random Effect (b_{Run})	N/A	~N(0, σ_{Run})	-0.025	(-14.1, 14.3)	N/A
Between Run Variability (σ_{Run})	N/A	~igamma(5, 21.8)	5.0	9.9	N/A
Within Sample Variability (σ_ε)	~igamma(2, 50)	~igamma(0.1,21.8)	0.3	0.4	>0.99
Intermediate Precision $\sqrt{\sigma_{Run}^2 + \sigma_\varepsilon^2}$	N/A	N/A	5.0	9.9	>0.99
Accuracy (Percent Difference)	N/A	N/A	9.5	19.8	0.94

Case Study 2: Characterizing Antagonistic Relationships

- This assay seeks to characterize the number of cells expressing mutually exclusive proteins (P1+/P2- or P1-/P2+)
- This qualification was performed by one analyst at one laboratory over multiple runs
  - We have one random effect (run) for this qualification
- These cell types are related, in that a functional cell can only be protein 1 positive or protein 2 positive, but not both
- The likelihood distribution for this assay will be multivariate normal

$$\begin{bmatrix}\#P1\\ \#P2\end{bmatrix} \sim MVN\left(\left(\begin{bmatrix}\beta_{0P1}\\ \beta_{0P2}\end{bmatrix} + b_{run}\right) + \begin{bmatrix}\beta_{t\,P1}\\ \beta_{t\,P2}\end{bmatrix} Theoretical, \begin{bmatrix}\sigma_{P1} & cov\\ cov & \sigma_{P2}\end{bmatrix}\right)$$

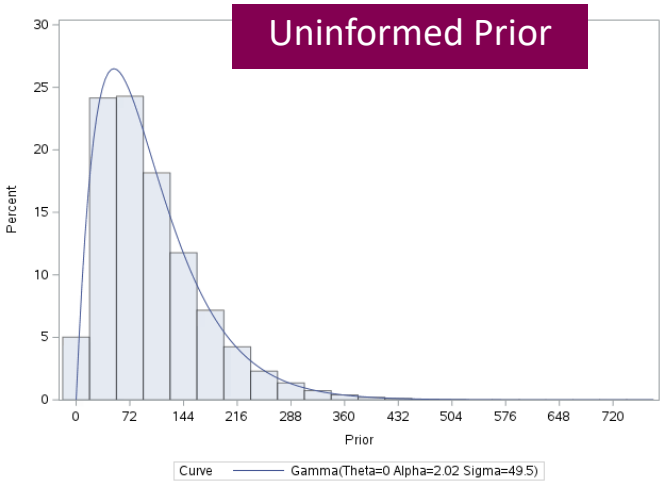
- Assuming random effect $b_{run} \sim MVN(\begin{bmatrix}0\\ 0\end{bmatrix}, \begin{bmatrix}\sigma_{r1} & cov\\ cov & \sigma_{r2}\end{bmatrix})$

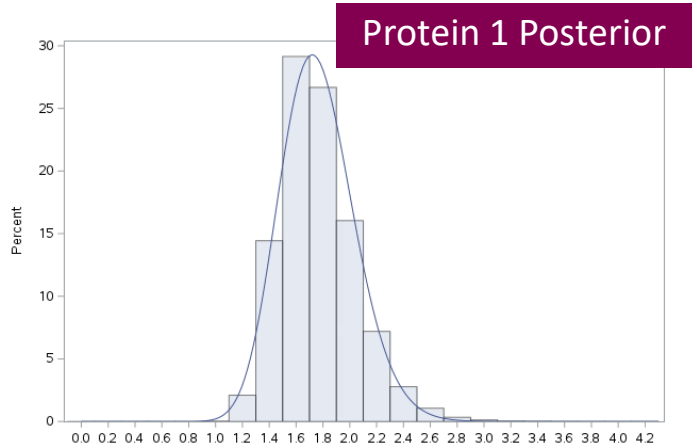


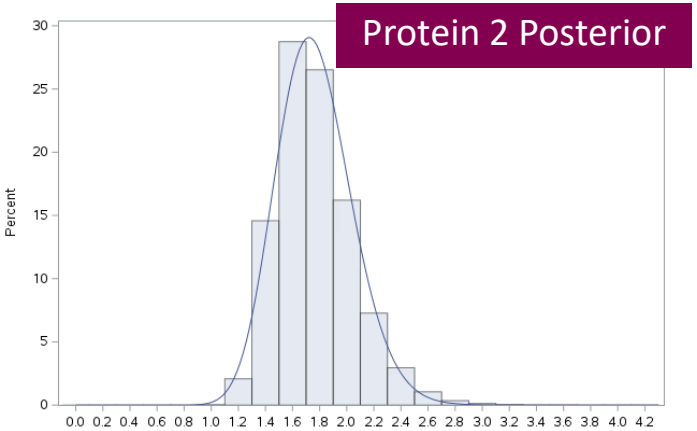
Results – Repeatability

- For the likelihood distribution: $\begin{bmatrix}\#P1\\ \#P2\end{bmatrix} \sim MVN\left(\left(\begin{bmatrix}\beta_{0P1}\\ \beta_{0P2}\end{bmatrix} + b_{run}\right) + \begin{bmatrix}\beta_{1\,P1}\\ \beta_{1\,P2}\end{bmatrix} Theoretical, \begin{bmatrix}\sigma_{P1} & cov\\ cov & \sigma_{P2}\end{bmatrix}\right)$
  - We are estimating cov, σ_{p1}, and σ_{p2} with the vague prior $\sim iwishart\left(2, \begin{pmatrix}0.02 & 0\\ 0 & 0.02\end{pmatrix}\right)$

Protein Target	Measurement	Covariance	Repeatability (Std. Dev)
Protein 1	Median	-3.0	1.8
	95% Credible Interval	-4.9	2.2
	Pr(Passing)	N/A	> 0.99
Protein 2	Median	-3.0	1.8
	95% Credible Interval	-4.9	2.2
	Pr(Passing)	N/A	> 0.99





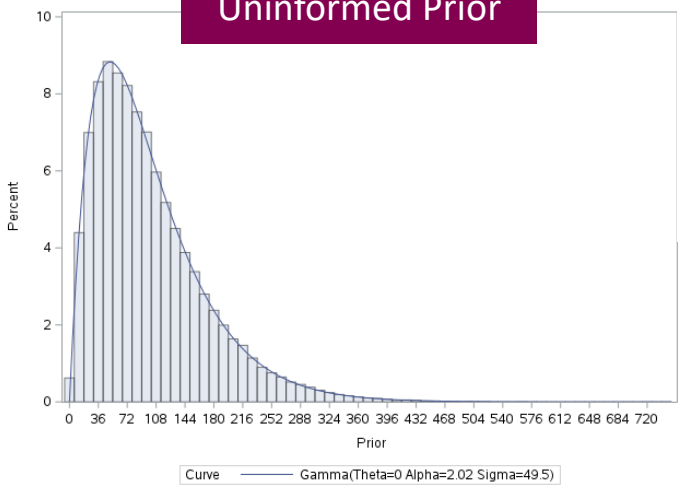


Results – Run-to-Run Variability

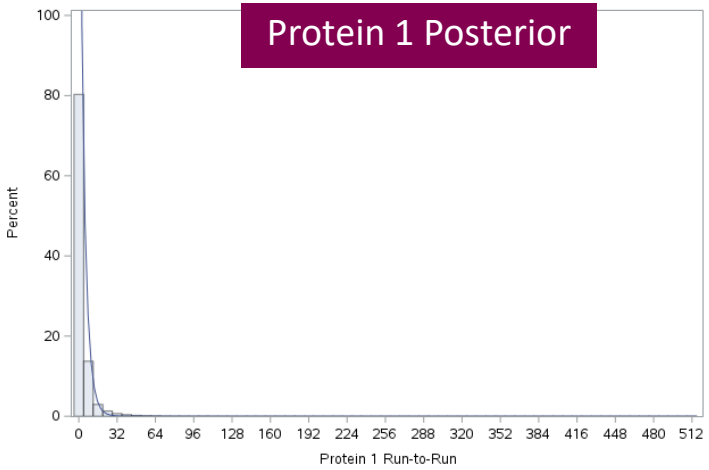
- For the likelihood distribution: $\begin{bmatrix}\#P1\\ \#P2\end{bmatrix} \sim MVN\left(\left(\begin{bmatrix}\beta_{0P1}\\ \beta_{0P2}\end{bmatrix} + \boxed{b_{run}}\right) + \begin{bmatrix}\beta_{1\,P1}\\ \beta_{1\,P2}\end{bmatrix} Theoretical, \begin{bmatrix}\sigma_{P1} & cov\\ cov & \sigma_{P2}\end{bmatrix}\right)$
  - We are estimating b_{run}~MVN($\begin{bmatrix}0\\0\end{bmatrix}$, $\begin{bmatrix}\sigma_{r1} & cov\\ cov & \sigma_{r2}\end{bmatrix}$, with the same uninformed iWishart prior

Protein Target	Measurement	Covariance	Variability (Std. Dev)
Protein 1	Median	-1.7	2.4
	95% Credible Interval	-9.4	11.7
Protein 2	Median	-1.7	3.3
	95% Credible Interval	-9.4	16.4

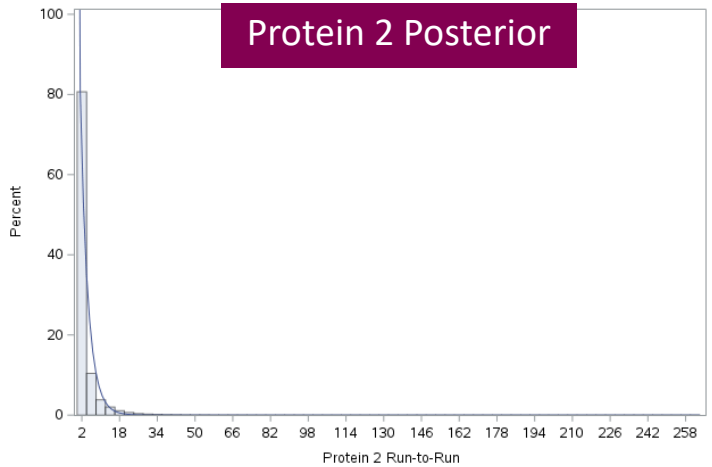
Uninformed Prior



Protein 1 Posterior



Protein 2 Posterior

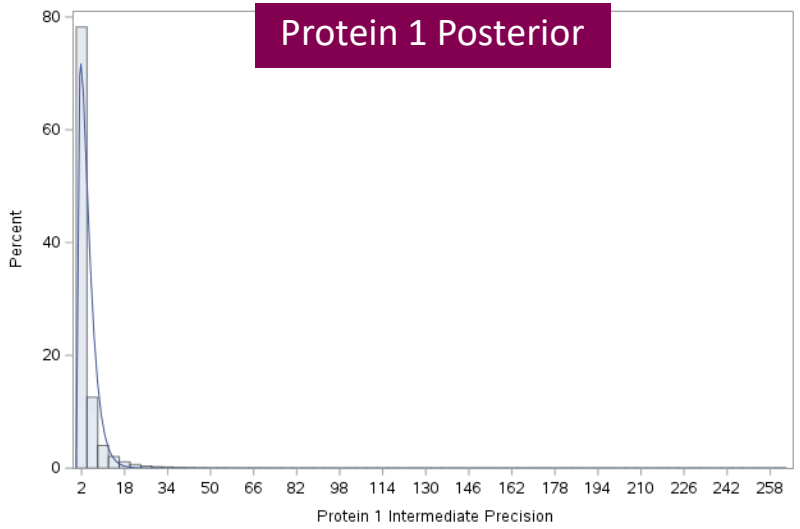


Results – Intermediate Precision

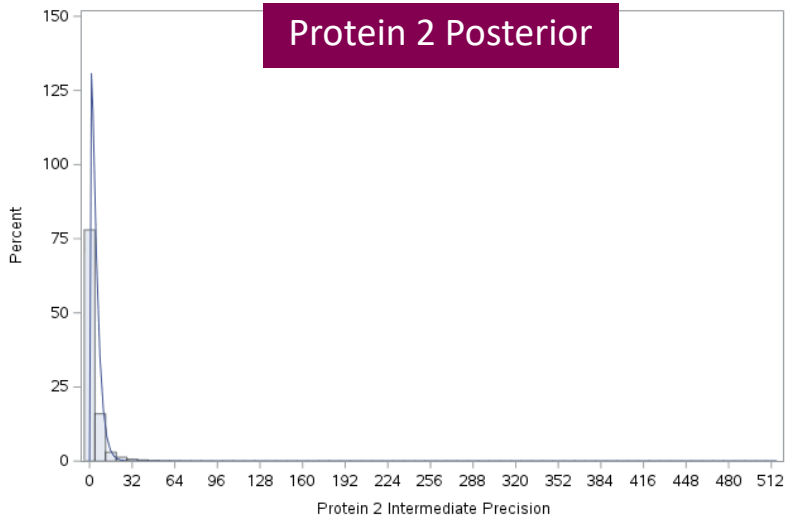
- For the Likelihood distribution: $\begin{bmatrix}\#P1\\ \#P2\end{bmatrix} \sim MVN\left(\left(\begin{bmatrix}\beta_{0P1}\\ \beta_{0P2}\end{bmatrix} + b_{run}\right) + \begin{bmatrix}\beta_{1\,P1}\\ \beta_{1\,P2}\end{bmatrix} Theoretical, \begin{bmatrix}\sigma_{P1} & cov\\ cov & \sigma_{P2}\end{bmatrix}\right)$
  - Intermediate Precision is the sum of the repeatability and between run variability

Analytical Performance Characteristic	Measurement	Protein 1 Result	Protein 2 Result
Intermediate Precision (Overall Standard Deviation)	Median	3.3	3.5
	95% Credible Interval	10.0	11.7
	Pr(Passing)	0.98	0.97

Protein 1 Posterior



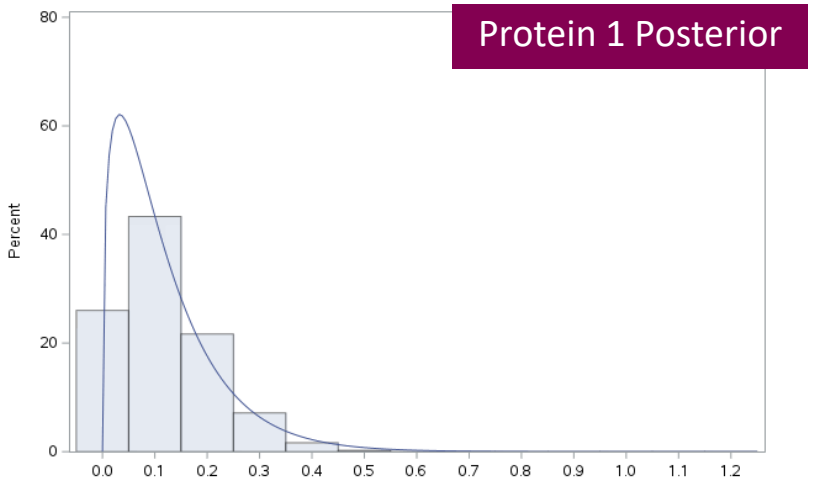
Protein 2 Posterior

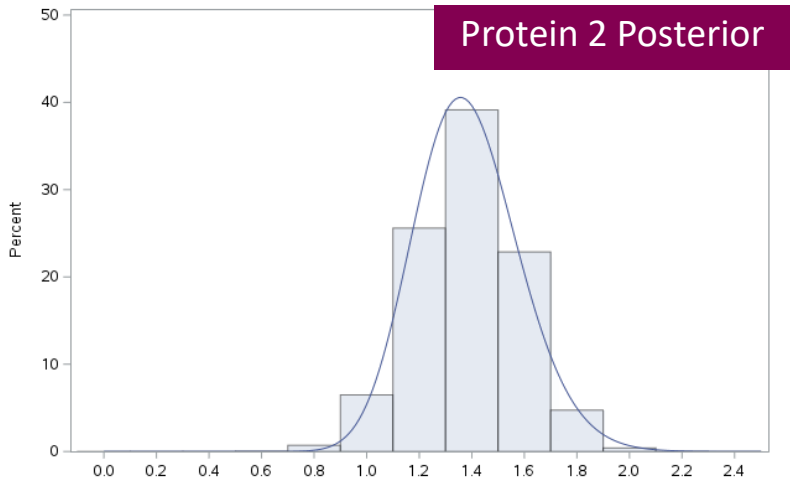


Results – Accuracy

- For the Likelihood distribution: $\begin{bmatrix}\#P1\\ \#P2\end{bmatrix} \sim MVN\left(\left(\begin{bmatrix}\beta_{0P1}\\ \beta_{0P2}\end{bmatrix} + b_{run}\right) + \begin{bmatrix}\beta_{1\,P1}\\ \beta_{1\,P2}\end{bmatrix} Theoretical, \begin{bmatrix}\sigma_{P1} & cov\\ cov & \sigma_{P2}\end{bmatrix}\right)$
  - Accuracy is calculated as the average difference of observed protein percentage against theoretical protein percentage for each protein across all target levels:

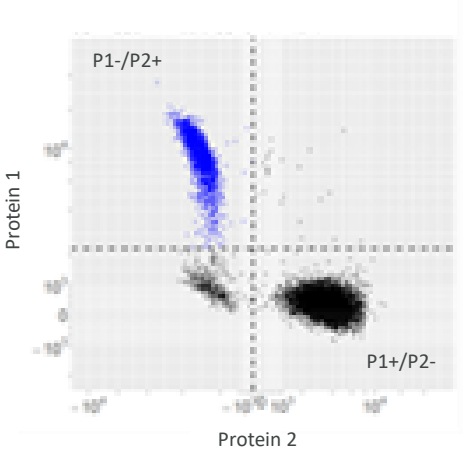
Analytical Performance Characteristic	Measurement	Protein 1 Result	Protein 2 Result
Accuracy (Average Absolute Difference from Theoretical)	Mean	0.1	1.4
	95% Credible Interval	0.3	1.7
	Pr(Passing)	> 0.99	> 0.99





Case Study 2 Takeaways

- For an assay characterizing the number of cells expressing mutually exclusive proteins
  1. We characterized the relationship between the two proteins
     1. Run-to-run relationships was represented as between run covariance
     2. Residual relationship was represented as within replicate covariance
  2. We successfully estimated the accuracy and precision of this assay with easily interpreted certainty
     - There was a probability of 0.98 that our method meets criteria for protein 1
     - There was a probability of 0.97 that our method meets criteria for protein 2



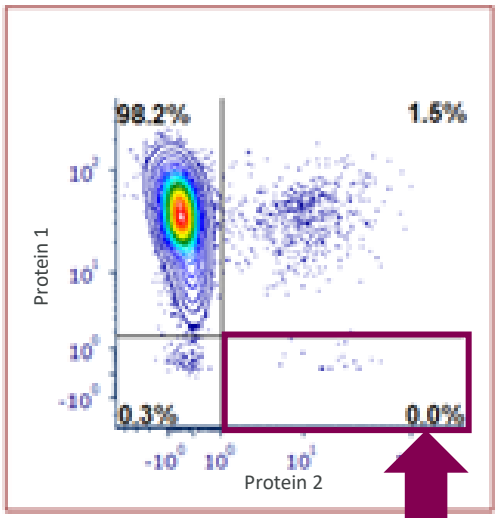
Protein Target	Pr(Repeatability Passes)	Pr(Accuracy Passes)	Pr(Intermediate Precision Passes)
Protein 1	> 0.99	> 0.99	0.98
Protein 2	> 0.99	> 0.99	0.97

Case Study 3: Rare Events Using Poisson Distribution

- This assay seeks to characterize the number of cells where the proteins of interest are not successfully knocked-out for a functional cell (P1-/P2+)
- This qualification was performed by one analyst at one laboratory over multiple days
  - We have one random effect (day) for this qualification
- Based on the dosage and counts, we are counting a few cells
- The likelihood distribution for this assay will be Poisson:

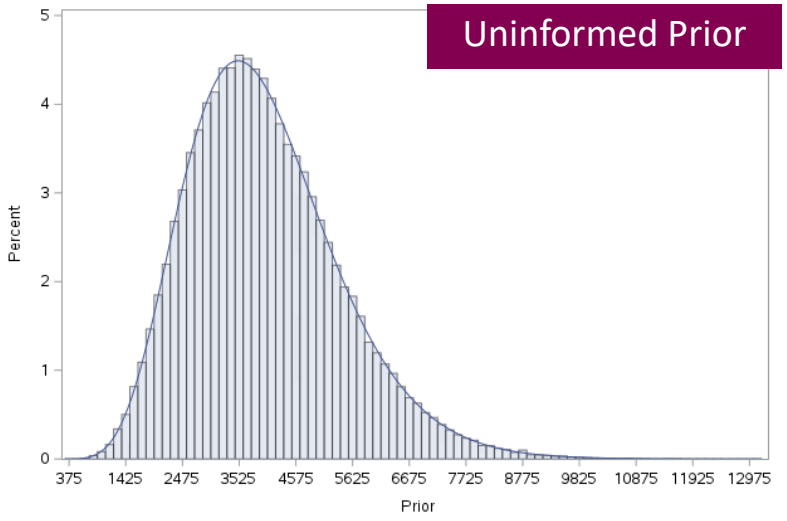
$$Result \sim Poisson(\lambda), \qquad \lambda = e^{(\beta_0 + b_d) + \beta_l + \beta_t Target_i}$$

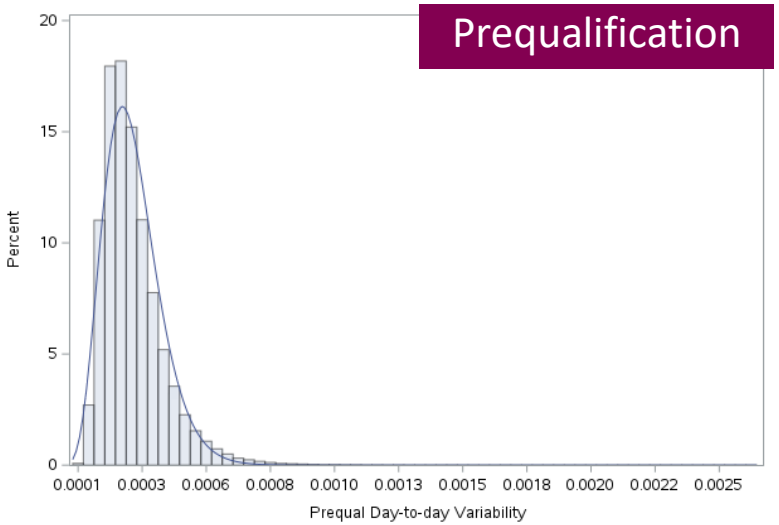
- Assuming the random effect for day b_d ~ N (0, σ_d), with fixed effects β_0 for intercept, β_l for between lab differences, and β_t for slope

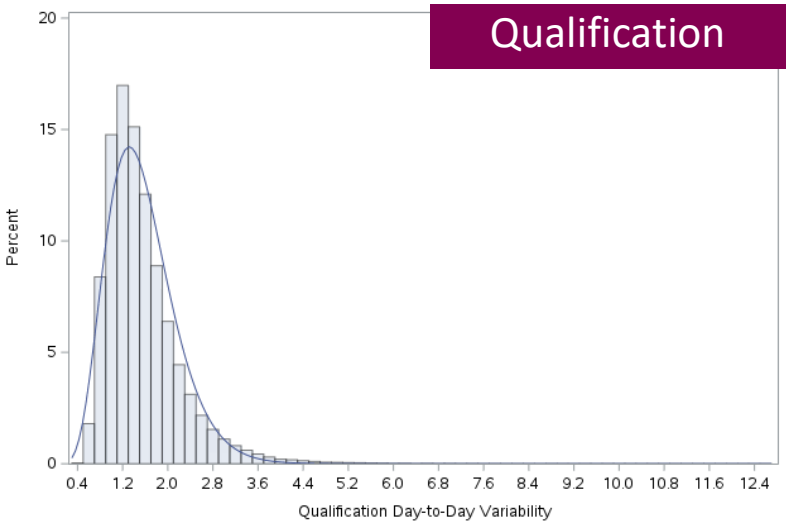


Results – Day-to-day Variability

- For the likelihood distribution: $Result \sim Poisson(\lambda), \qquad \lambda = e^{(\beta_0 + b_d) + \beta_l + \beta_t Target_i}$
- We are estimating σ_d with the relatively uninformed prior ~ igamma(8, 500)





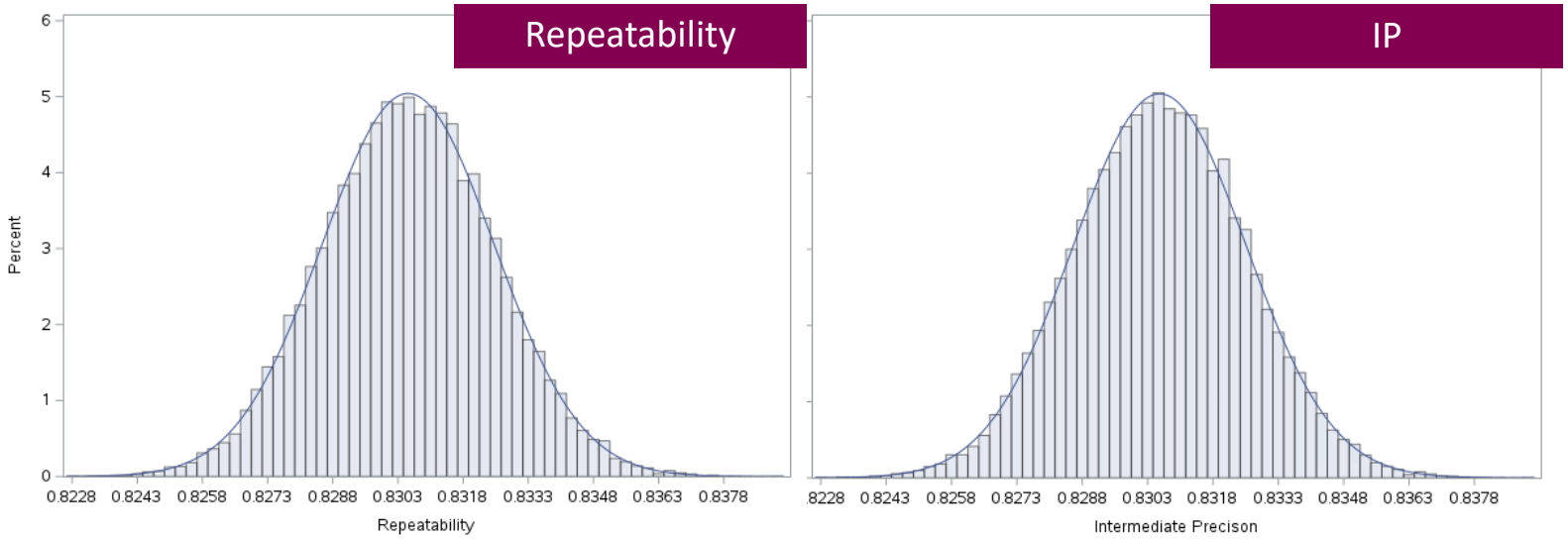


Analytical Performance Characteristic	Measurement	Pre-qualification Result (# of Cells)	Qualification Result (# of Cells)
Intermediate Precision (Overall Standard Deviation in Cells Counted)	Median	1	5
	95% Credible Interval	1	15
	Pr(Passing)	N/A	N/A

Results – Precision

- For the likelihood distribution: $Result \sim Poisson(\lambda), \quad \lambda = e^{(\beta_0 + b_d) + \beta_l + \beta_t Target_i}$
- We are estimating the Repeatability CV = $\sqrt{\lambda - \sigma_d^2}/\lambda$ and Intermediate Precision CV = $\frac{1}{\lambda}$

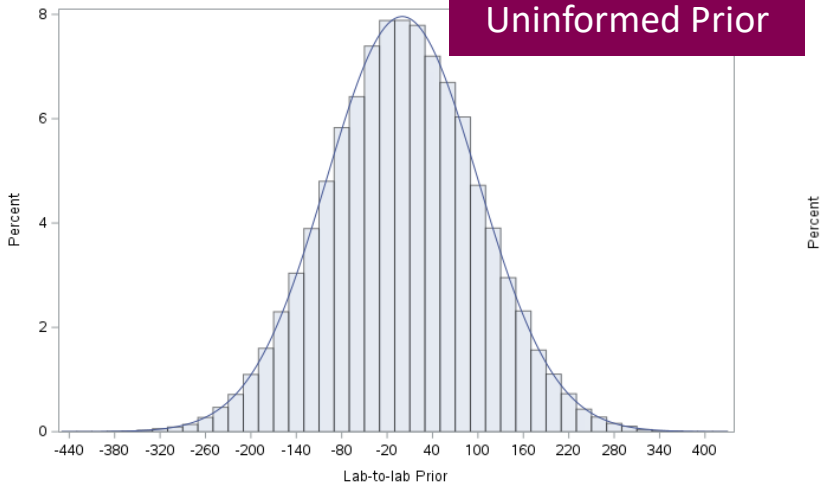
Analytical Performance Characteristic	Measurement	Result (% CV)
Repeatability (Percent Coefficient of Variation)	Median	0.8%
	95% Credible Interval	0.8%
	Pr(Passing)	> 0.99
Intermediate Precision (Percent Coefficient of Variation)	Median	0.8%
	95% Credible Interval	0.8%
	Pr(Passing)	> 0.99



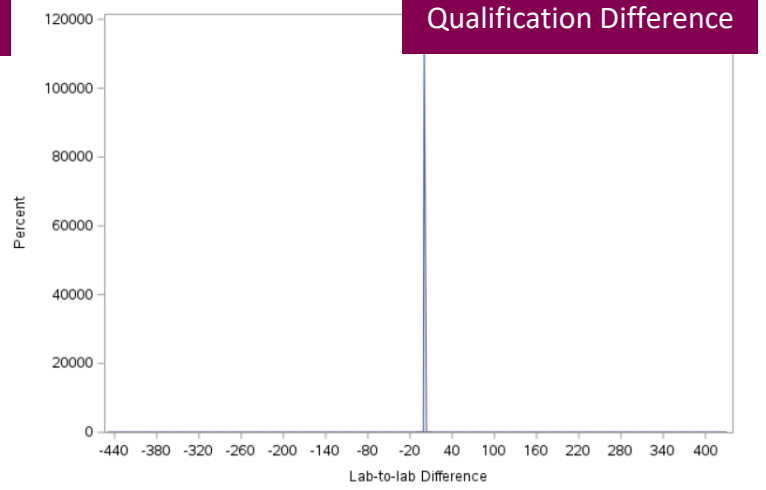
Results – Difference Between Labs and Accuracy

- For the likelihood distribution: $Result \sim Poisson(\lambda), \quad \lambda = e^{(\beta_0 + b_d) + \beta_l + \beta_t Target_i}$
- We are estimating the term *l* with the relatively uninformed prior ~N(0, 100) and the percent log(difference) from theoretical

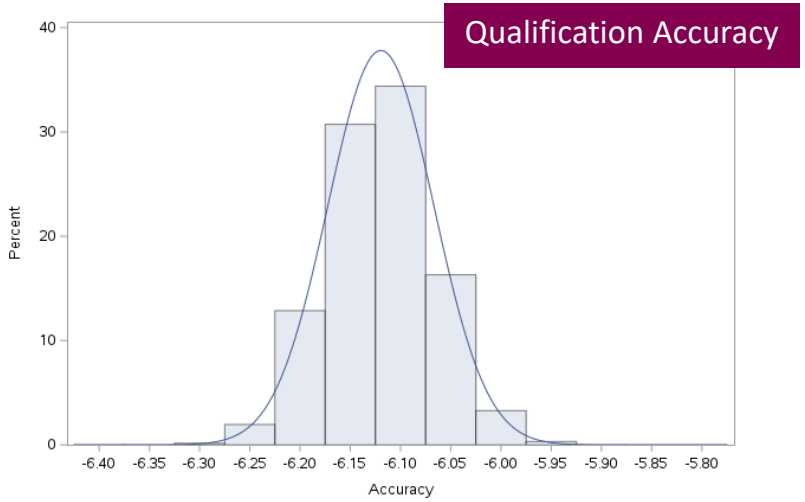
Uninformed Prior



Qualification Difference



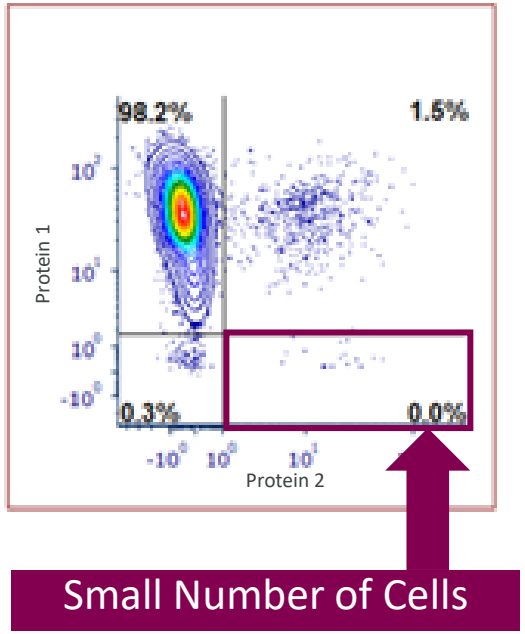
Qualification Accuracy



Analytical Performance Characteristic	Measurement	Result
Lab-to-lab Difference (Difference in Cells Counted from 2nd Laboratory)	Median	2
	95% Credible Interval	2
	Pr(Passing)	> 0.99
Accuracy (Percent Difference from Theoretical)	Median	-6.1
	95% Credible Interval	-6.0
	Pr(Passing)	> 0.99

Case Study 3 Takeaways

- For an assay characterizing the number of cells where the proteins of interest are not successfully knocked-out for a functional cell (P1-/P2+)
  1. We characterized the variance components when using a Poisson model
     - The between-day variability was large, as expected
  2. We successfully estimated the accuracy and precision of this assay with easily interpreted certainty
     - There was a probability > 0.99 that our method meets criteria

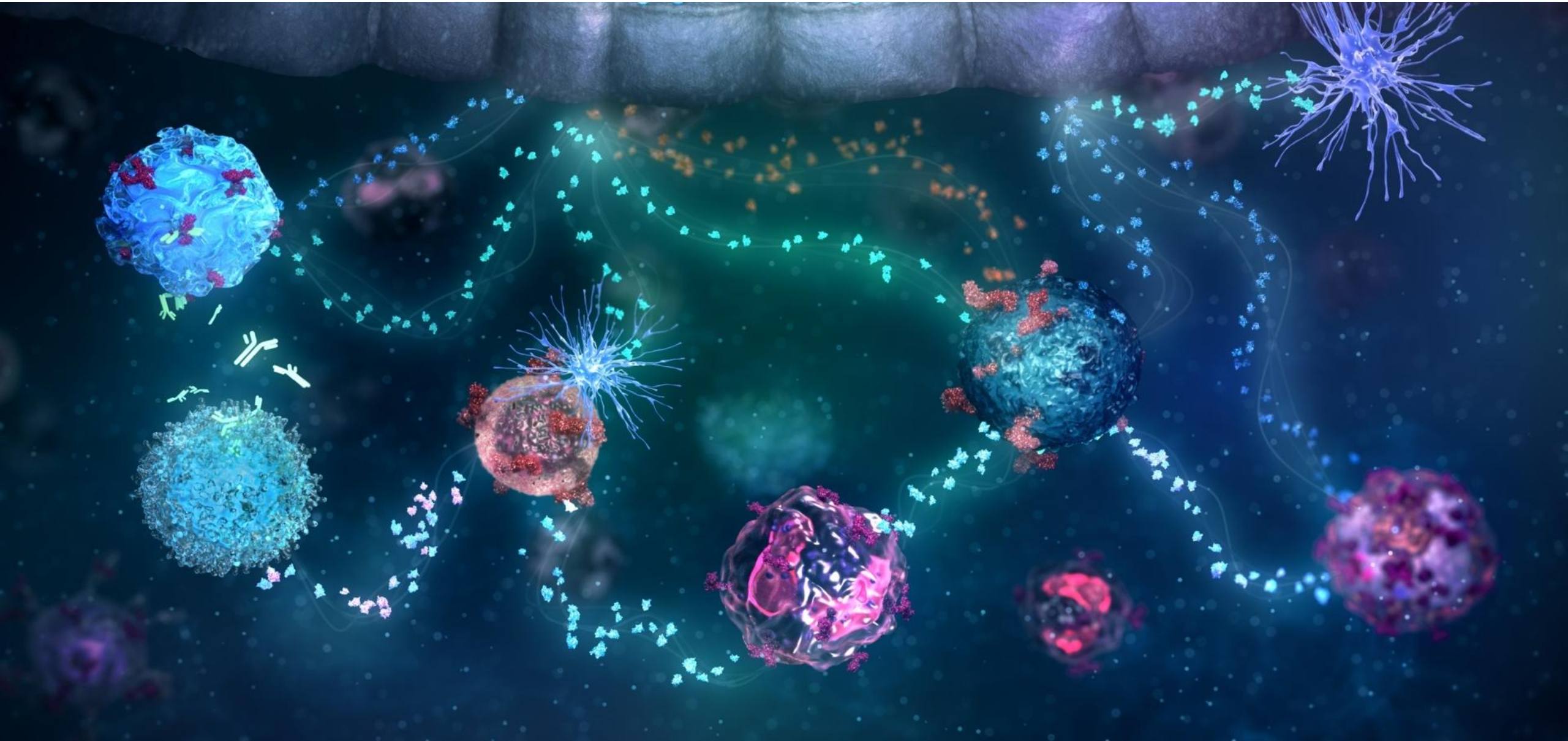


Pr(Repeatability Passes)	Pr(Accuracy Passes)	Pr(Intermediate Precision Passes)	Pr(Reproducibility Passes)
> 0.99	> 0.99	> 0.99	> 0.99

Conclusions and Next Steps

- We used Bayesian methods to solve some common problems with flow cytometry method qualification
  - Three assay types which violate typical analysis were analyzed with dilutional linearity designs described in ICH Q2R2
    - Simple dilutional linearity
    - Antagonistic relationships
    - Non-normal likelihood distributions
- Iterate on the procedures used to simulate the models
- As these products continue along their regulatory pathways, this analysis may be applied to pre-validation and validation data
  - More appropriate criteria and stage-gating may be applied to further analysis

Questions



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