Mechanistic and Statistic Partitioning the Technical Variability of Ligand Binding Assays in Distinct Formats

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Outline

- ICH M10 LBA Guideline
- Distinct LBA assays
- LBA (PK, Biomarker, ADA)
- Partitioning technical variabilities
- Deterministic effect
- 4PL model
- Linear Mixed Models
- LLOQ
- Gyros-Lab
- SIMOA
- Zero-inflated models

ICH M10 LBA Guideline

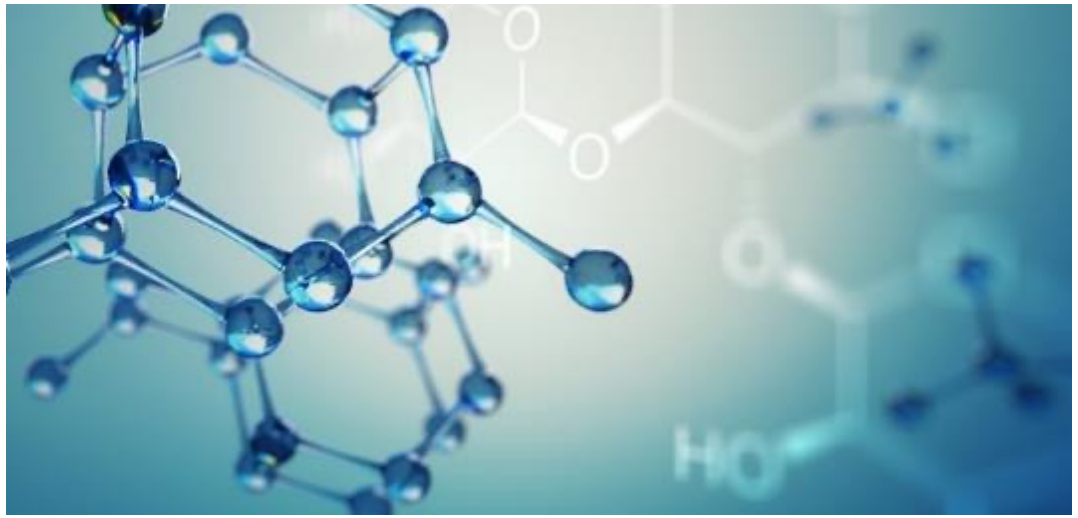
- ±20% for RE & CV, except for the LLOQ and ULOQ, where it should not exceed 25%.
- For non-accuracy and precision validation runs, at least 2/3 of the total QCs and at least 50% at each concentration level should be within ±20% of the nominal values.
- TE should not exceed 30% (40% at LLOQ and ULOQ).
- Detailed regression models including weighing factors for LBA assays, which are deterministic factors for technical variability for LBAs, are not available in ICH M10.
- Stochastic factors, including batch effects, are not detailed in ICH M10.
- Both distinct formats of LBAs and inherent Ab-Ag affinity and avidity are crucial for technical variability.

25 July 2022 EMA/CHMP/ICH/172948/2019

Committee for Medicinal Products for Human Use

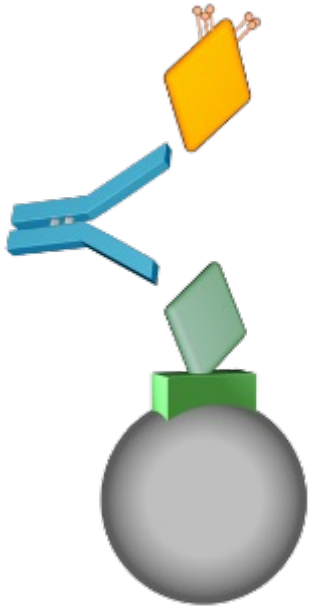
ICH Guideline M10 on Bioanalytical Method Validation and Study Sample Analysis

Step 5



Iba Assays

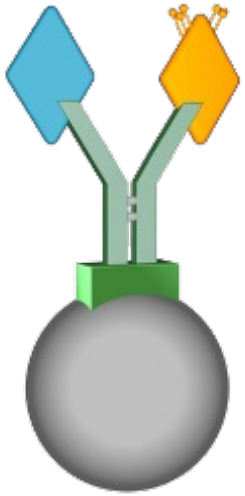
Sandwich Immunoassay



Bridging Immunoassay

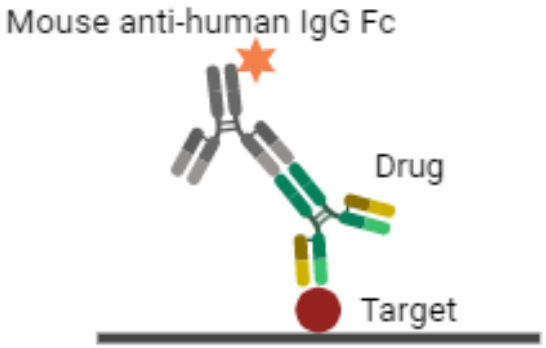
Indirect Antibody Assay

Reversed Antibody Assay

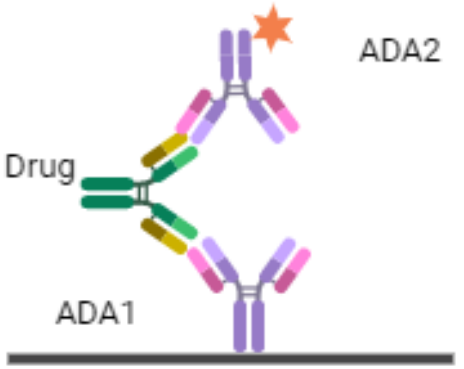


Sequential Assay Procedure

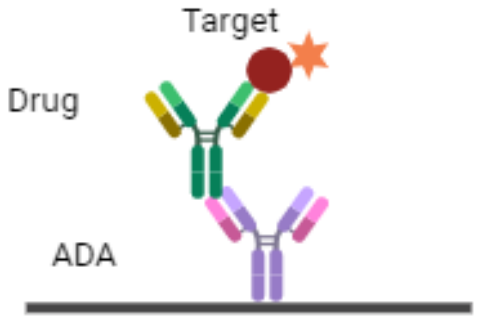
Classical PK Assay Formats



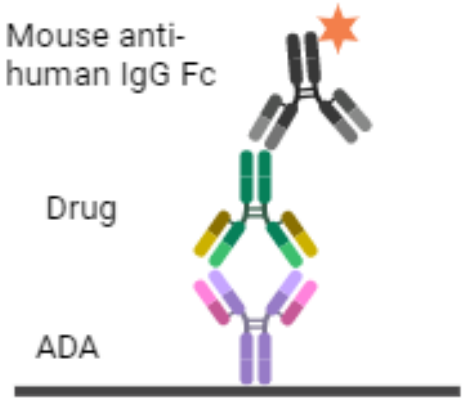
Antigen Capture



Anti-ID Bridging



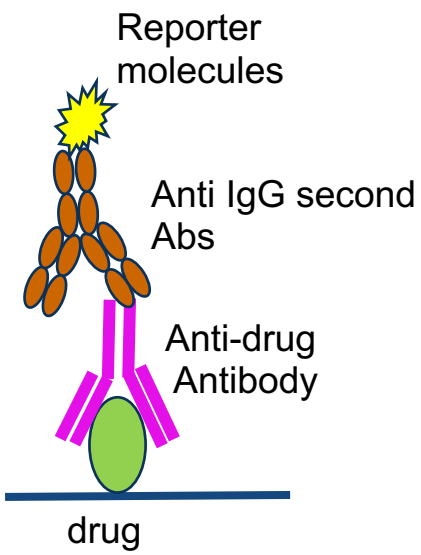
Anti-ID Antigen Bridging



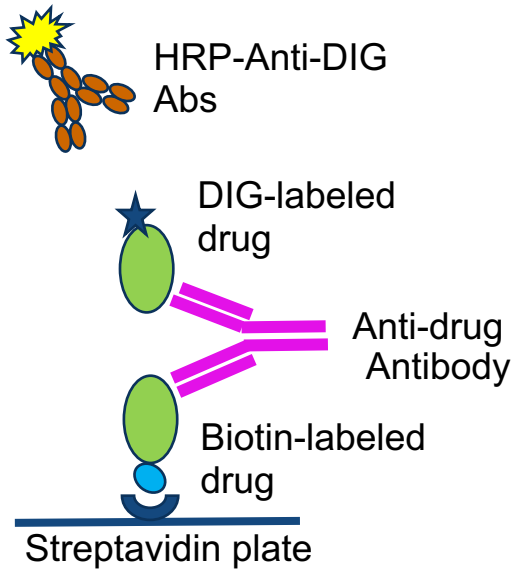
Anti-ID Capture Sandwich

Distinct Formats for Anti-Drug Antibodies Assays

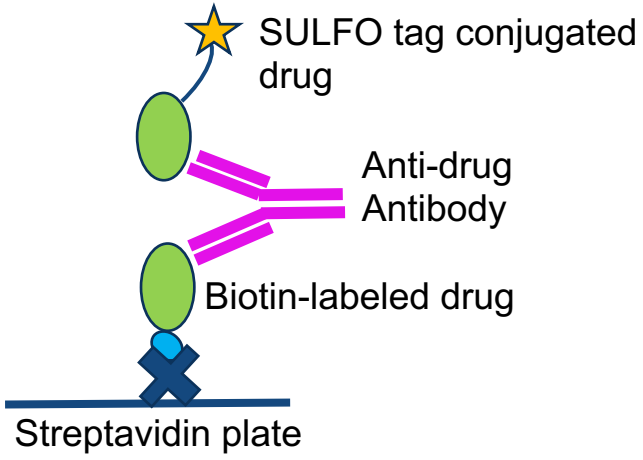
ELISA
Absorbance



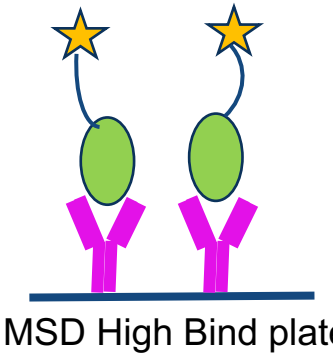
ELISA
Absorbance



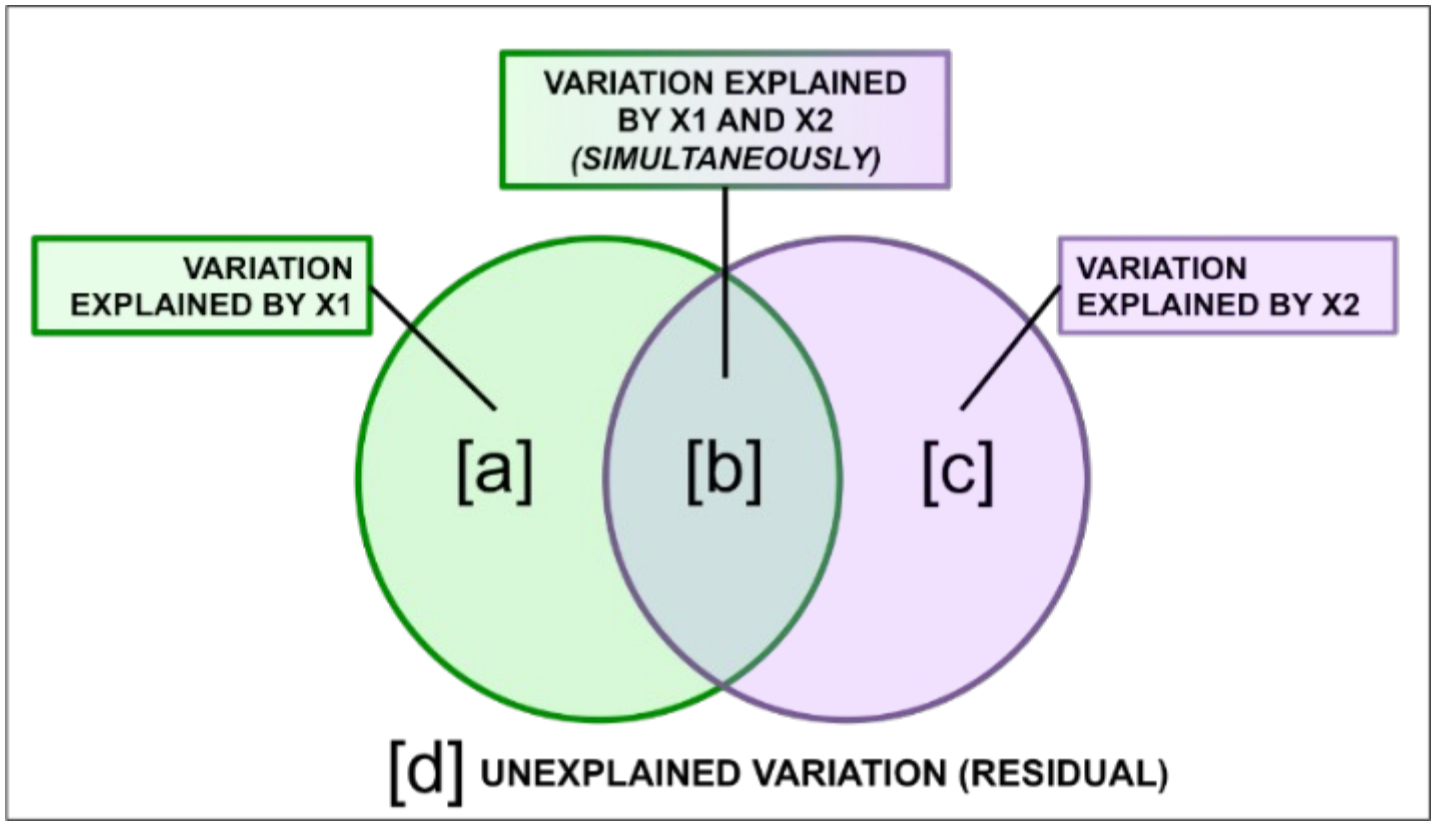
MSD
Bridge Assay



MSD
Direct format



Partitioning Technical Variabilities (Stochastic)



Y ~X1 +X2

$$R^2 = \frac{\text{variance of fitted model values}}{\text{variance of response values}}$$

(Coefficient of determination)

Variability and LLOQ are Not Independent

In-Well (Without X Dilution Factor)

Deterministic Factors

Stochastic Factors

Platform	LLOQ	CV%
ELISA	~1 ng/mL	<15%
MSD	~2 pg/mL	<15%
ELLA	~1 pg/mL	<20%
Gyros-Lab	~1 pg/mL	<25%
SIMOA	~0.2pg/Ml	<30%

In general, LLOQ and variability are negative correlated.

Deterministic Effect

$$\frac{[RL]}{[R][L]} = \frac{k_{on}}{k_{off}} = K$$

K, the equilibrium affinity constant, has dimensions of M^{-1}. As K (analogous to the thirst of the delegates) increases, so the concentration of the receptor–ligand complexes increases at the expense of the free species. Alternatively,

$$\frac{[R][L]}{[RL]} = \frac{k_{off}}{k_{on}} = K_d$$

Model

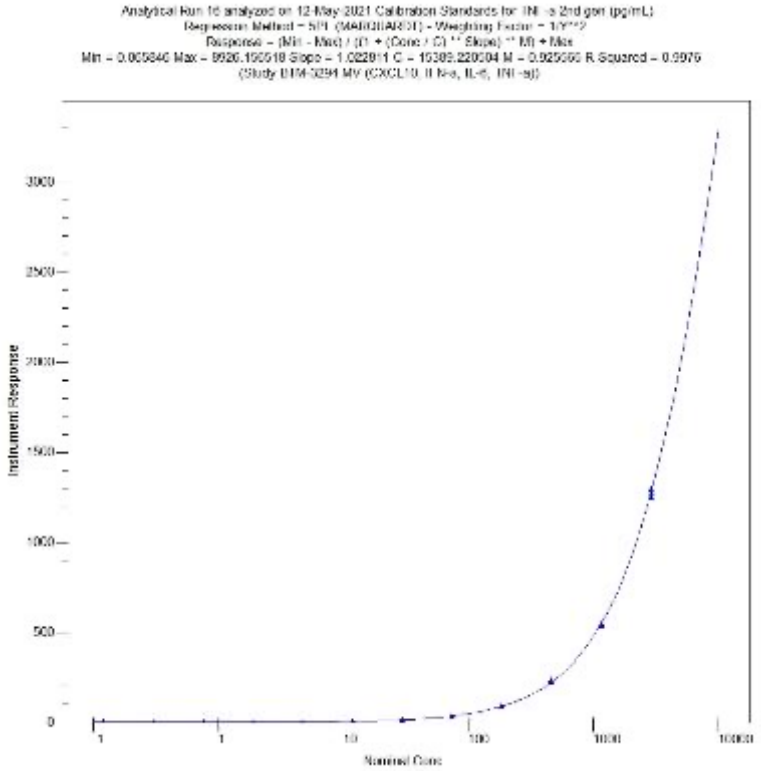
$$Y(t) = A + \frac{K - A}{(C + e^{-B(t-M)})^{1/\nu}}$$

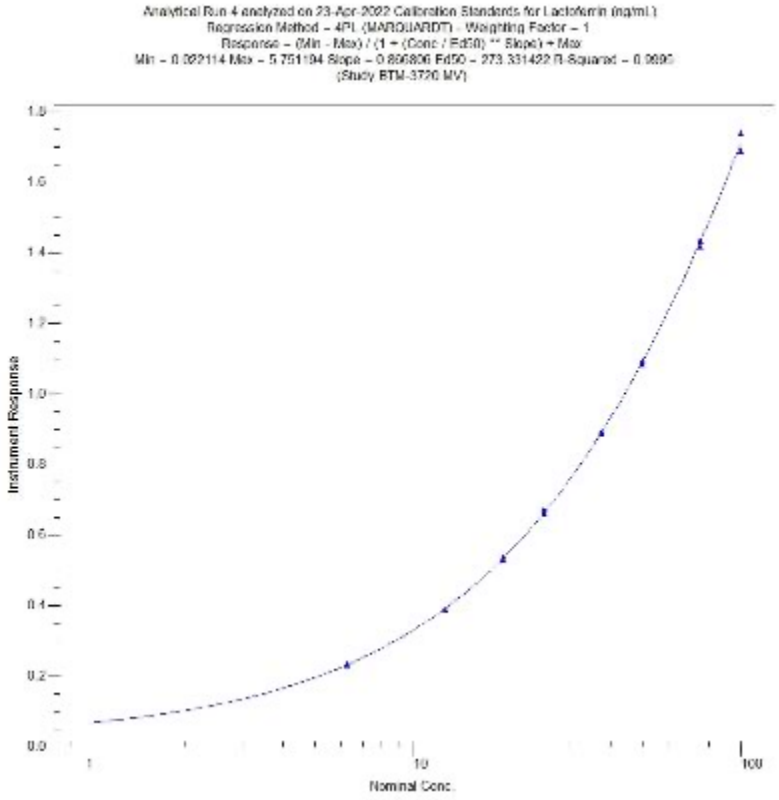
$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^{b}}$$

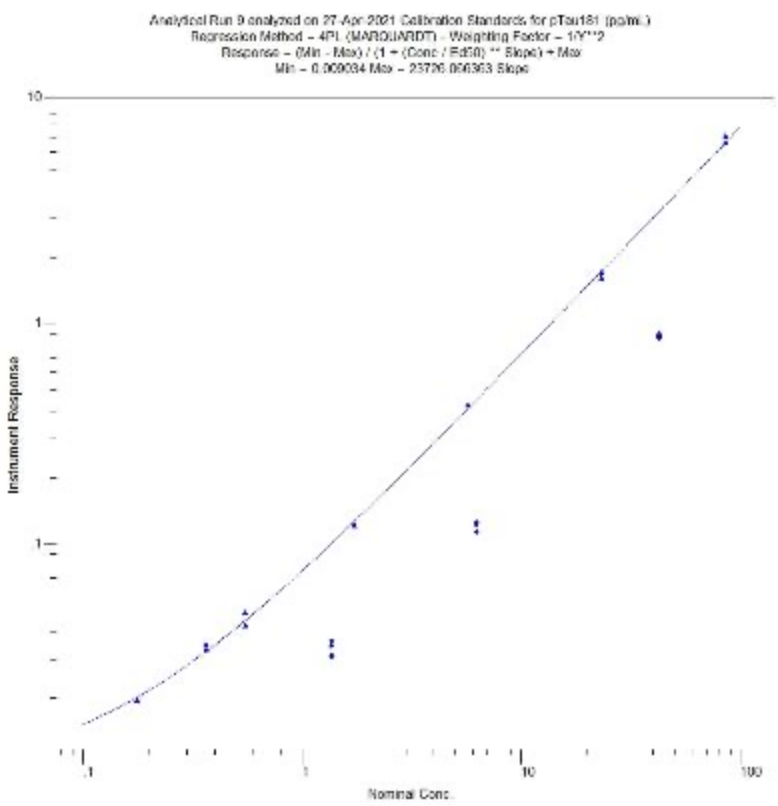
$$x = c\left(\frac{a - d}{y - d} - 1\right)^{\frac{1}{b}}$$

- Simple linear regression can work well for ELISA, especially for IVD kits;
- Weighing factor for 4/5 PL impact result, including CV% and RE%;
- Log-log transformation may be needed;
- For laboratory developed test (LDT), determined by LBA platforms.

Standard Curves (Data Transformation)







Linear Mixed Model

$$
\begin{aligned}
\mathbf{y}_i &= \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i + \boldsymbol{\varepsilon}_i \\
\mathbf{b}_i &\sim \mathbf{N}_q(\mathbf{0}, \boldsymbol{\Psi}) \\
\boldsymbol{\varepsilon}_i &\sim \mathbf{N}_{n_i}(\mathbf{0}, \sigma^2\boldsymbol{\Lambda}_i)
\end{aligned}
$$

where

- $\mathbf{y}_i$ is the $n_i \times 1$ response vector for observations in the ith group.
- $\mathbf{X}_i$ is the $n_i \times p$ model matrix for the fixed effects for observations in group i.
- $\boldsymbol{\beta}$ is the $p \times 1$ vector of fixed-effect coefficients.
- $\mathbf{Z}_i$ is the $n_i \times q$ model matrix for the random effects for observations in group i.
- $\mathbf{b}_i$ is the $q \times 1$ vector of random-effect coefficients for group i.
- $\boldsymbol{\varepsilon}_i$ is the $n_i \times 1$ vector of errors for observations in group i.
- $\boldsymbol{\Psi}$ is the $q \times q$ covariance matrix for the random effects.
- $\sigma^2\boldsymbol{\Lambda}_i$ is the $n_i \times n_i$ covariance matrix for the errors in group i.

Scientist
Lot
Batch
Instrument

Gyros-Lab (Deterministic)

High Analyte Concentration

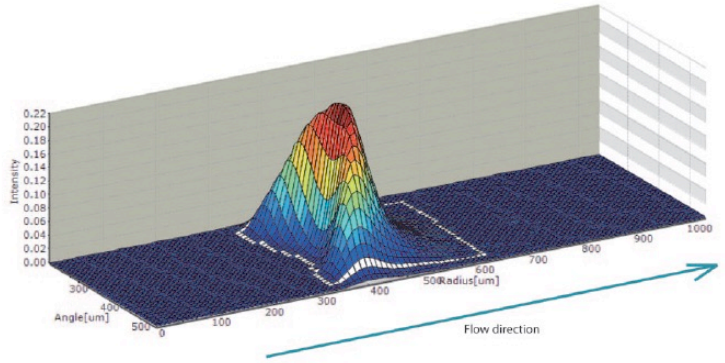


Figure 1: Example of desired binding profile in Gyrolab Viewer

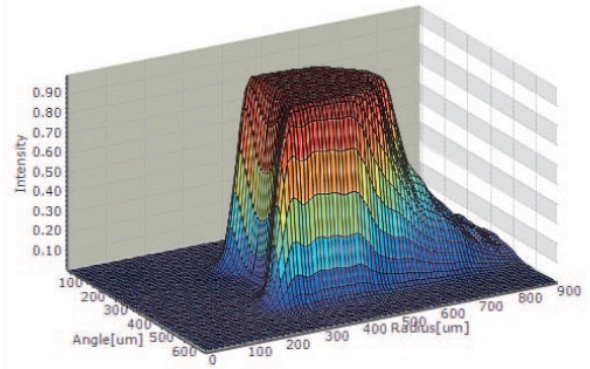


Figure 2: Example of binding profile that indicates saturated detector signal

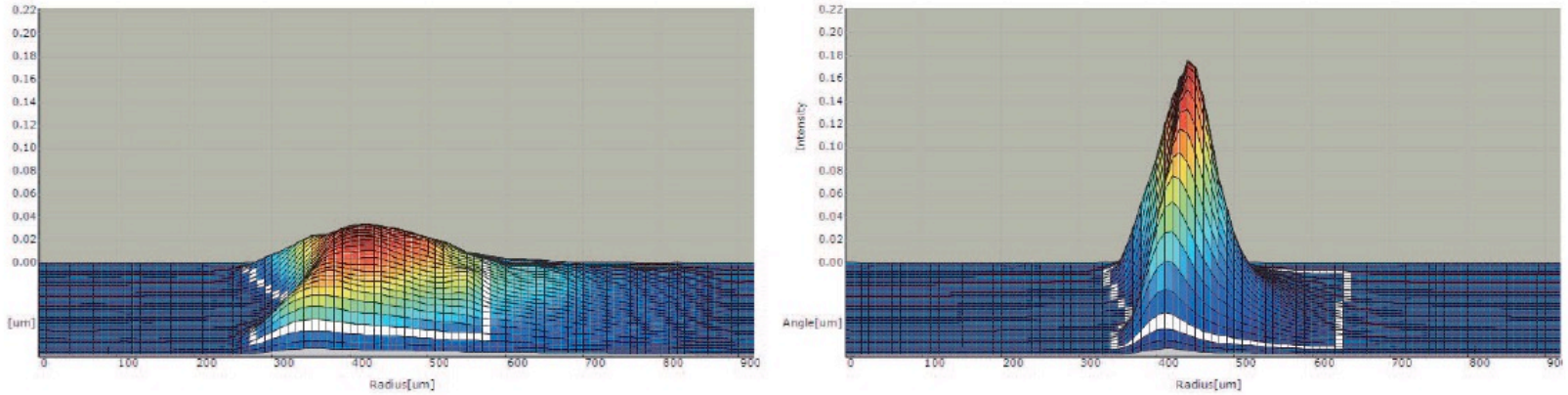
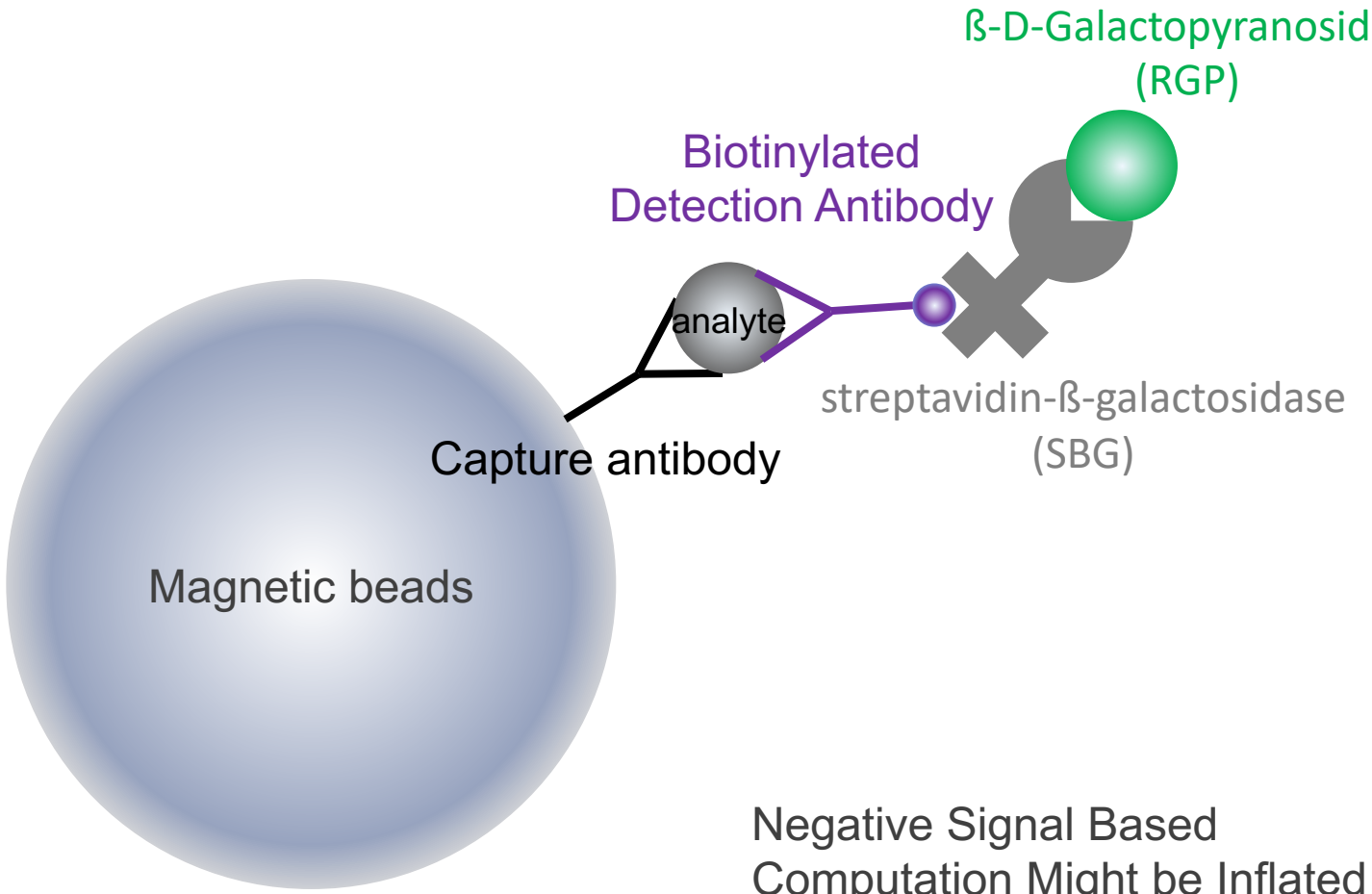
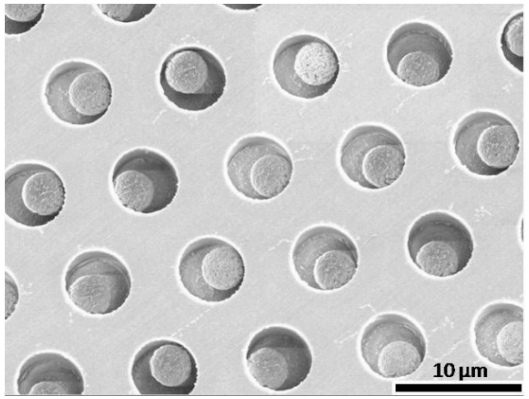
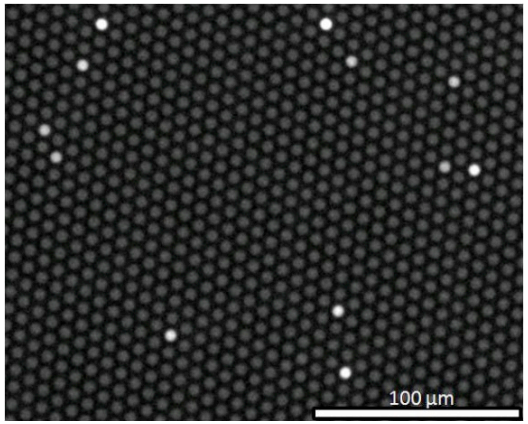


Figure 3: Example of binding profiles that indicate lower affinity (left) and higher affinity (right) between capture reagent and analyte

SIMOA (Digital-ELISA)







Negative Signal Based
Computation Might be Inflated

Zero-Inflated Models (SIMOA)

$$f(k;\lambda) = \Pr(X{=}k) = \frac{\lambda^k e^{-\lambda}}{k!}$$

λ=-ln(N-neg/N-total)

$$\lambda = \mathrm{E}(X) = \mathrm{Var}(X).$$

CV = std/mean = $\sqrt{\lambda}/\lambda$

$$f_{Y|\mathbf{X},\mathbf{W}}(y;\mathbf{x},\mathbf{w},\boldsymbol{\beta},\boldsymbol{\vartheta},\boldsymbol{\alpha}) = \begin{cases} \pi + (1-\pi)p_{Y|\mathbf{X}}(0;\mathbf{x},\eta,\boldsymbol{\vartheta}), & \text{for } y = 0; \\ (1-\pi)p_{Y|\mathbf{X}}(y;\mathbf{x},\eta,\boldsymbol{\vartheta}), & \text{for } y \in \mathbb{N}^+, \end{cases}$$

For Fit-for-Purpose Design, Relaxed Criteria Might be Needed.

Summary

- Technical variability control (TVC) is the prerequisite for assess biological variabilities;
- A better understanding the deterministic and stochastic partitioning LBA holds the promise to foster validation accomplishment;
- Distinct formats my have specific criteria for validation as the sensitivity might be at the cost of technical variabilities;
- LBA platforms should be carefully chosen, given the characteristics of analytes;
- Linear mixed model can be employed to dissect technical variability;
- Zero-inflated model can be applied to SIMOA platform;
- ICH M10 Guideline may require science-based adaptation, namely, fit-for-purpose.

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Thank You

FRONTAGE