Statistical Considerations for Defining Cut Points and Titers in Anti-Drug Antibody (ADA) Assays

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Outline

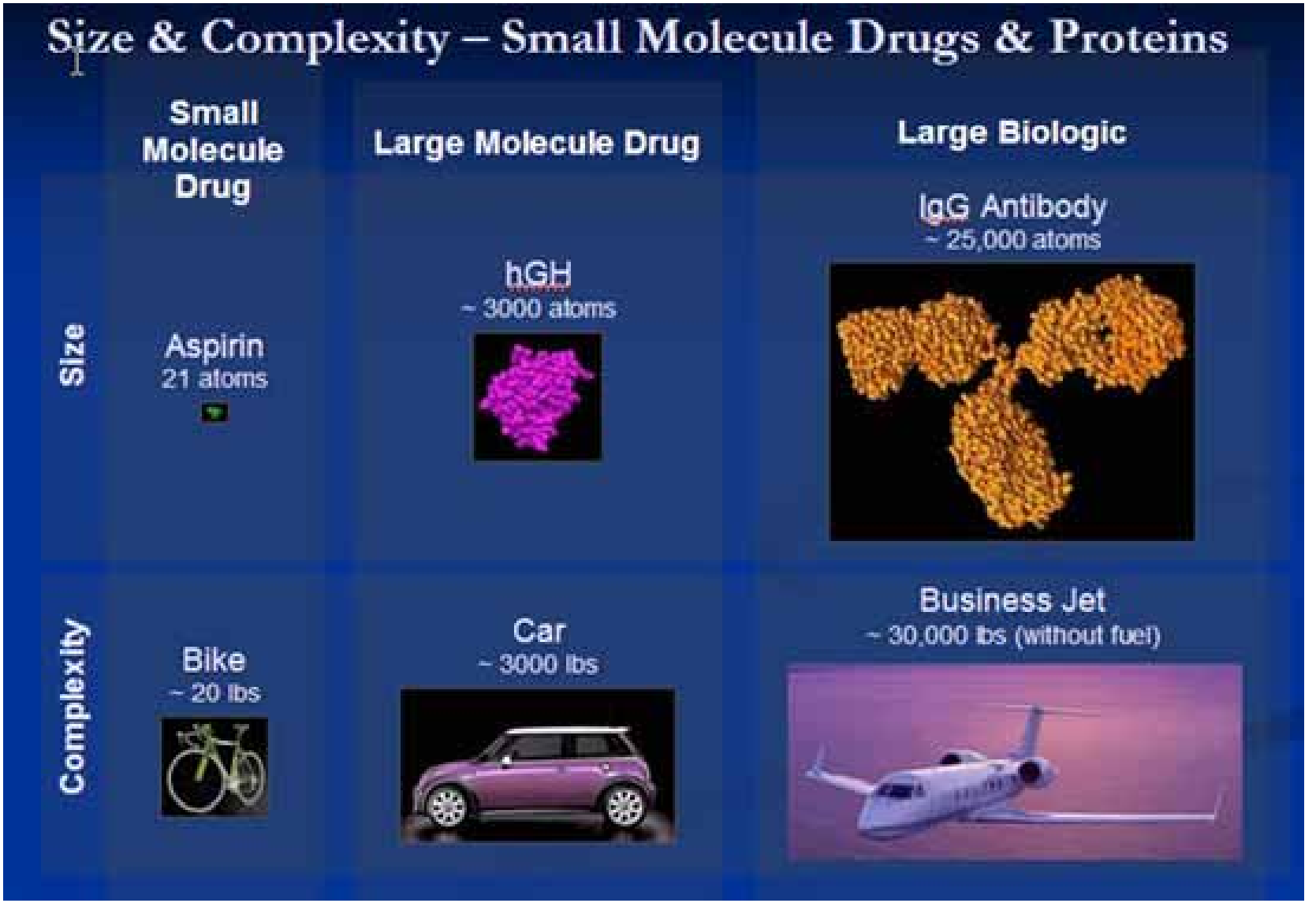
- Introduction
  - Why are ADA and IR assays important?
- Two case studies
  1. RIA: How to define %binding?
  2. ECL: How to define titration cut point?
  3. Both use a Huber 3-parameter nonlinear logistic regression

Immune Response (IR) Assay

- Primary question: ADA, Yes or No?
- Every biologic must be evaluated.
- Safety and Efficacy concerns.
- Too much IR can kill a compound.

Biological Drug Products are Different than Traditional Small Molecule Drugs

- Made by cells not chemists
- Complicated manufacturing process
- Small & simple vs large & complex chemical structures



Reference: Genentech, Inc. http://www.gene.com/gene/about/views/followon-biologics.html

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Adverse Clinical Sequelae

- Hypersensitivity & autoimmunity
- Altered PK
  - Drug neutralization
  - Abnormal biodistribution
  - Enhanced clearance rate

➔ Regulatory bodies require ADA evaluation for all biologics

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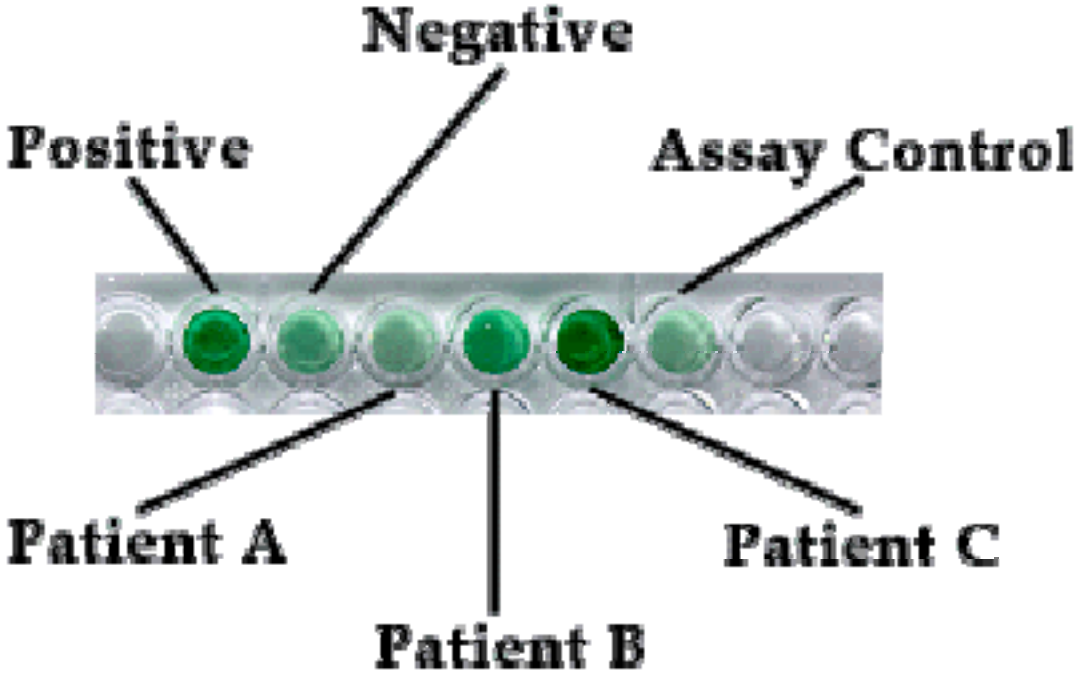
Immune Response (IR) Assay Challenges

- Cut Point for confidence that screening bioassay response (eg, ECL, OD, RLU, CPM) reflects immunogenicity
- Statistical issues of variance components, distributions, outliers, …

Screening Cut Point Flags 5% of Naïve Samples as False Positive

- Use Mean + 1.645 x SD with caution
  - Only for normally independently distributed data without outliers
  - Usually requires at least a transformation like logs
- Nonparametric often easier
  - Simply use 95th percentile
  - Caution if unbalanced design

ELISA Activity



Positive Control	Negative Control	Patient A	Patient B	Patient C	Assay Control
1.689	0.153	0.055	0.412	1.999	0.123

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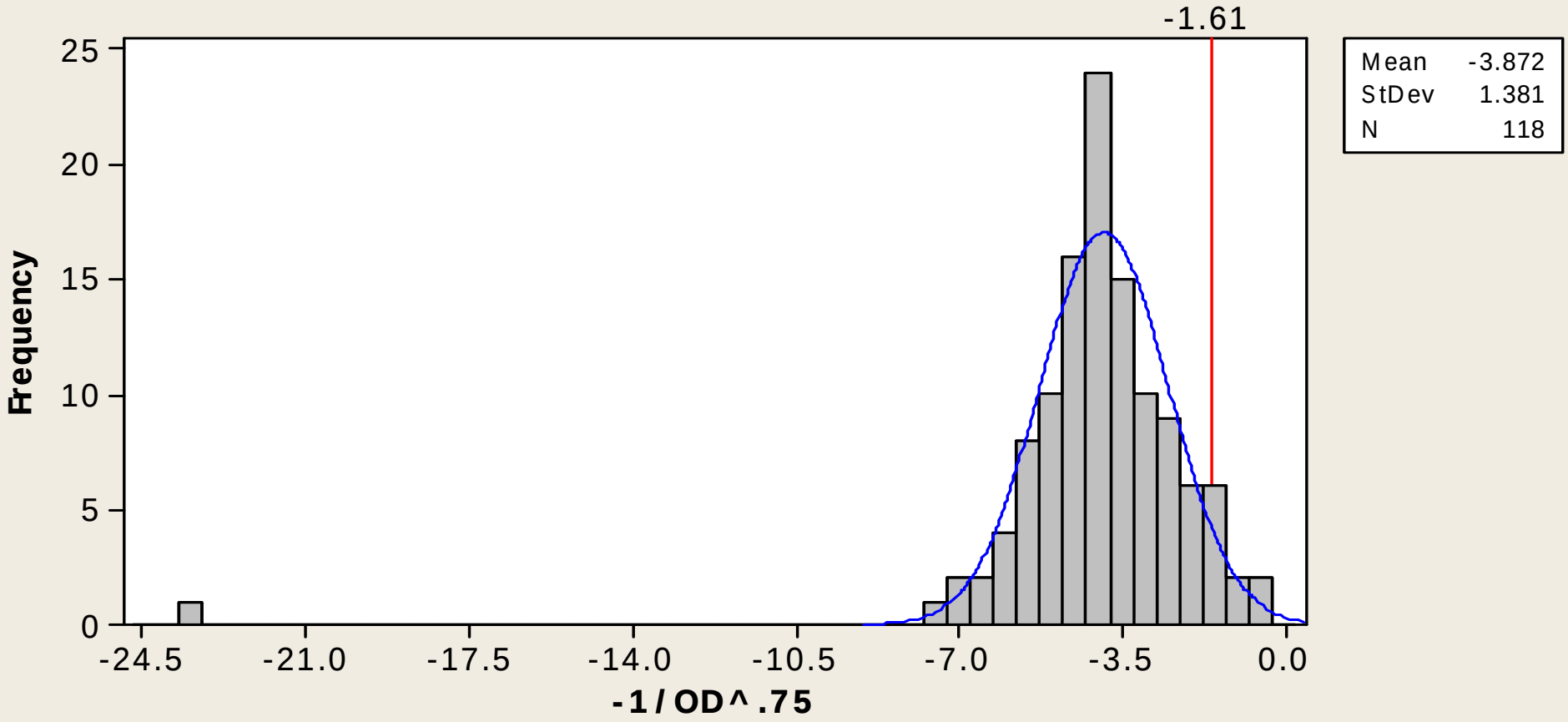
ELISA Cut Point Example



Mean and Standard Deviation based on mixed effects analysis of 117 non-outliers.

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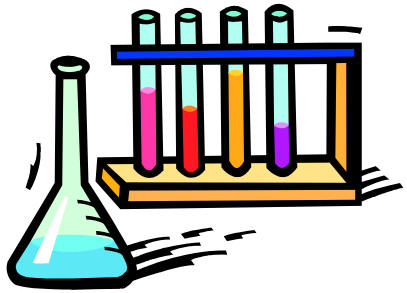
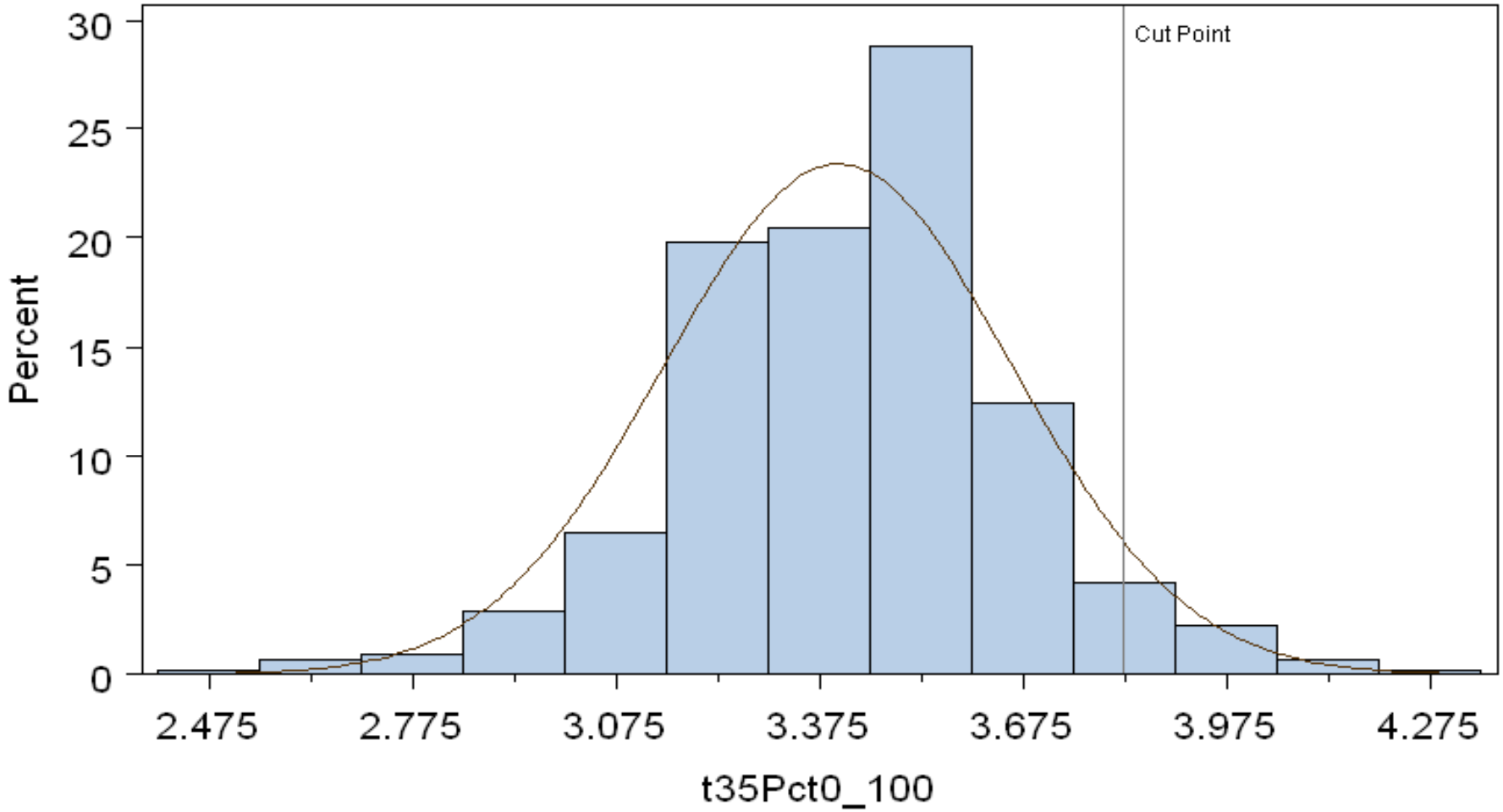
Analysis of an RIA Cut Point Assay Validation Experiment

- 6 Assay controls
- 2 Analysts with 3 assays each
- 2 Populations (Normal and Diabetes)
- 75 Naïve Human Serum samples
- Nonnormal data
- Unequal variances



RIA Histogram of 450 Naïve Sample Results

Transformed %Binding = ln(35+%Binding). Parametric Cut Point = 10.757 ± 3.524.
Transformed Cut Point = 3.823 based on adjusted mean = 3.402 and total standard deviation = 0.256.



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RIA Normal Probability Plot of 450 Naïve Sample Results

Transformed %Binding = ln(35+%Binding). Parametric Cut Point = 10.757 ± 3.524.
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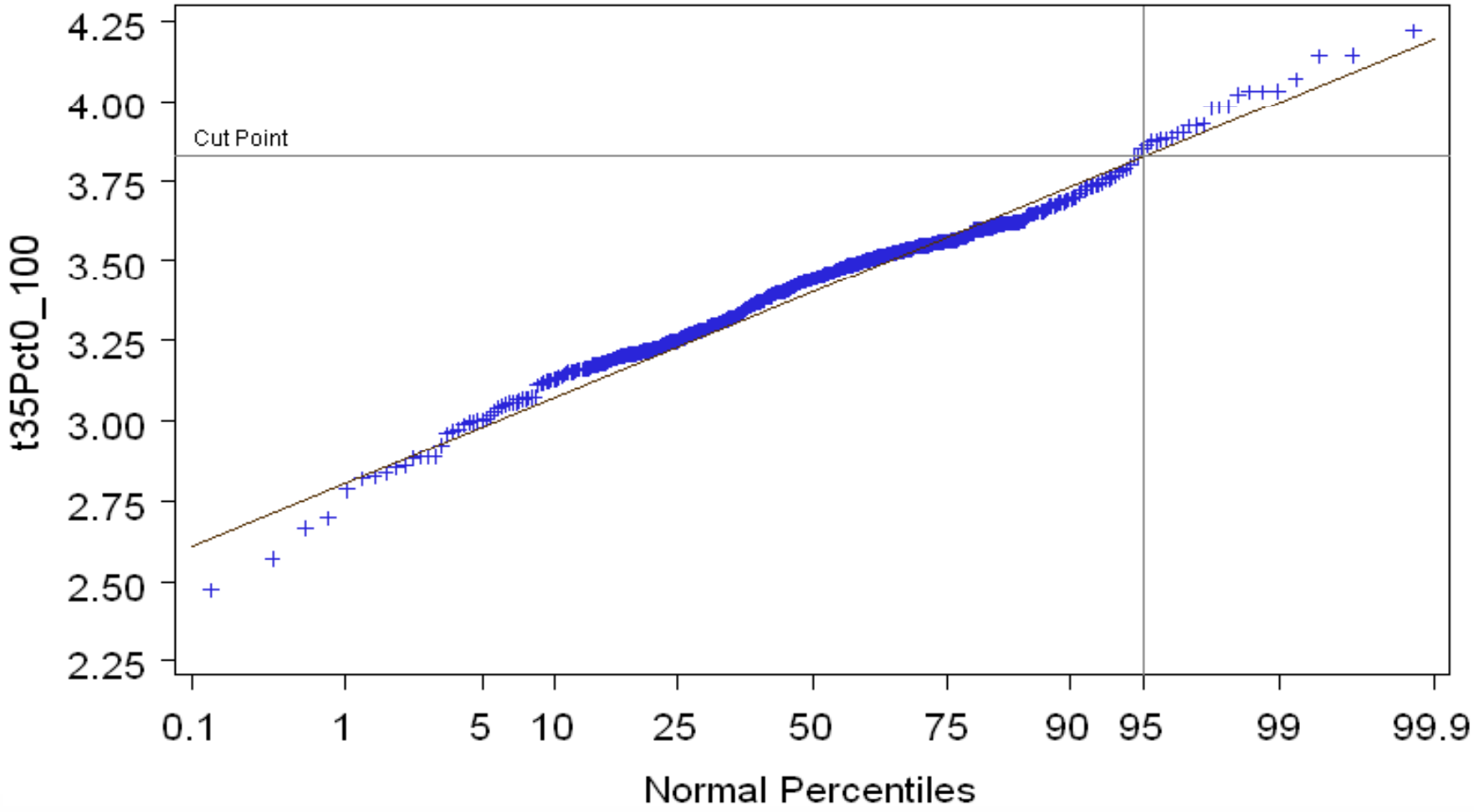

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My RIA Notation

MinCPM = Minimum of the 2 Sample CPMs
MaxCPM= Maximum of the 2 Sample CPMs
AvgCPM = Average of the 2 Sample CPMs
CV = Coefficient of Variation of the 2 Sample CPMs

B0 = Average of all 6 "Validation sample 0 ng/mL" CPMs
B100 = Average of all 6 "Validation sample 100 ng/mL" CPMs
B250 = Average of all 6 "Validation sample 250 ng/mL" CPMs
B1000 = Average of all 6 "Validation sample 1000 ng/mL" CPMs
NSB = Average of all 2-6 "NSB" (Non-Specific Binding) CPMs
TC = Average of all 2-6 "TC" (Total Count) CPMs

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Some RIA %Binding Definitions

Response	%CV Limit	Sample N	Sample Mean	Sample SD	Addend[1]	Sample %CV[1]
(MinCPM-B0)/(B100-B0)*100		450	-3.490	7.968	65	13.0
(AvgCPM-B0)/(B100-B0)*100	25	420	-1.173	8.373	85	10.0
(MinCPM-NSB)/(TC-NSB)*100		450	1.249	0.841	4.4	14.9
MinCPM/NSB		450	1.321	0.218	-0.7	35.0
AvgCPM/(TC-NSB)*100	**20**	**403**	**5.459**	**1.119**	**3**	**13.2**
MinCPM-B0		450	-59.339	151.356	1000	16.1
MinCPM/sqrt(B100*B0)		450	0.549	0.084	0	15.3

[1]CV of (Response + Adder) = Standard Deviation / (Mean + Adder) x 100%. Addend chosen so that CV is not related to control concentration.

How to Choose the RIA %Binding Definition?

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New versus Old RIA %Binding Definitions

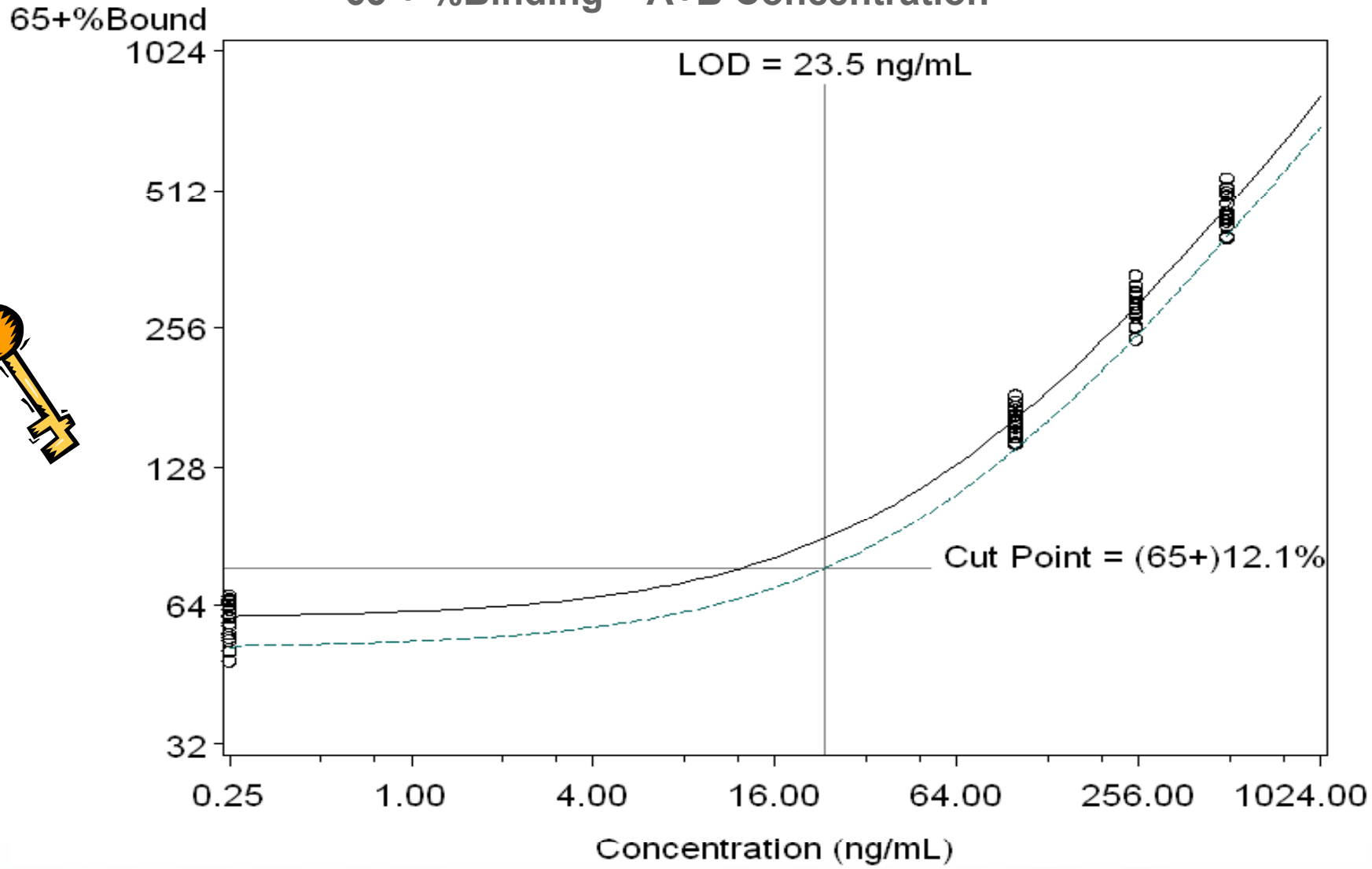
- **New: (*MinCPM – B0*) / (*B100 – B0*)**
  - **Repeat if CV > 25% and (*MaxCPM – B0*) / (*B100 – B0*) > 12.0% (the Cut Point)**
- **Old: (*AvgCPM – NSB*) / *TC***
  - **Repeat if CV > 20%**

Attributes of Selected RIA %Binding Definitions

Response	%CV Limit	Cut Point	LOD (ng/mL)	0 ng/mL %Pos.	N
(MinCPM-B0)/(B100-B0)		.120	23.5	0.04	450
(AvgCPM-B0)/(B100-B0)	25	.149	25.5	1.29	420
(AvgCPM-B0)/(B100-B0)	20	.153	25.0	0.10	403
(AvgCPM-NSB)/TC	20	3.380	31.7	0.112	403

RIA Validation Control Curve with Lower 1-sided 95% Prediction Limit

$65 + \%Binding = A+B \cdot Concentration^{C}$



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A Logistic Curve with an Infinite Plateau is Linear wrt X^{λ}

$$\underline{C + R X^{H} / (M^{H} + X^{H})} =$$

Substitute $\alpha = C$, $\lambda = H$, and $R/\beta = M^{H}$

$$\alpha + R X^{\lambda} / (R/\beta + X^{\lambda}) =$$

Multiply second term by β/β

$$\alpha + \beta R X^{\lambda} / (R + \beta X^{\lambda})$$

Apply L'Hopital's rule

$$\lim_{(R \to \pm\infty)} [\alpha + R \beta X^{\lambda} / (R + \beta X^{\lambda})] = \underline{\alpha + \beta X^{\lambda}}$$

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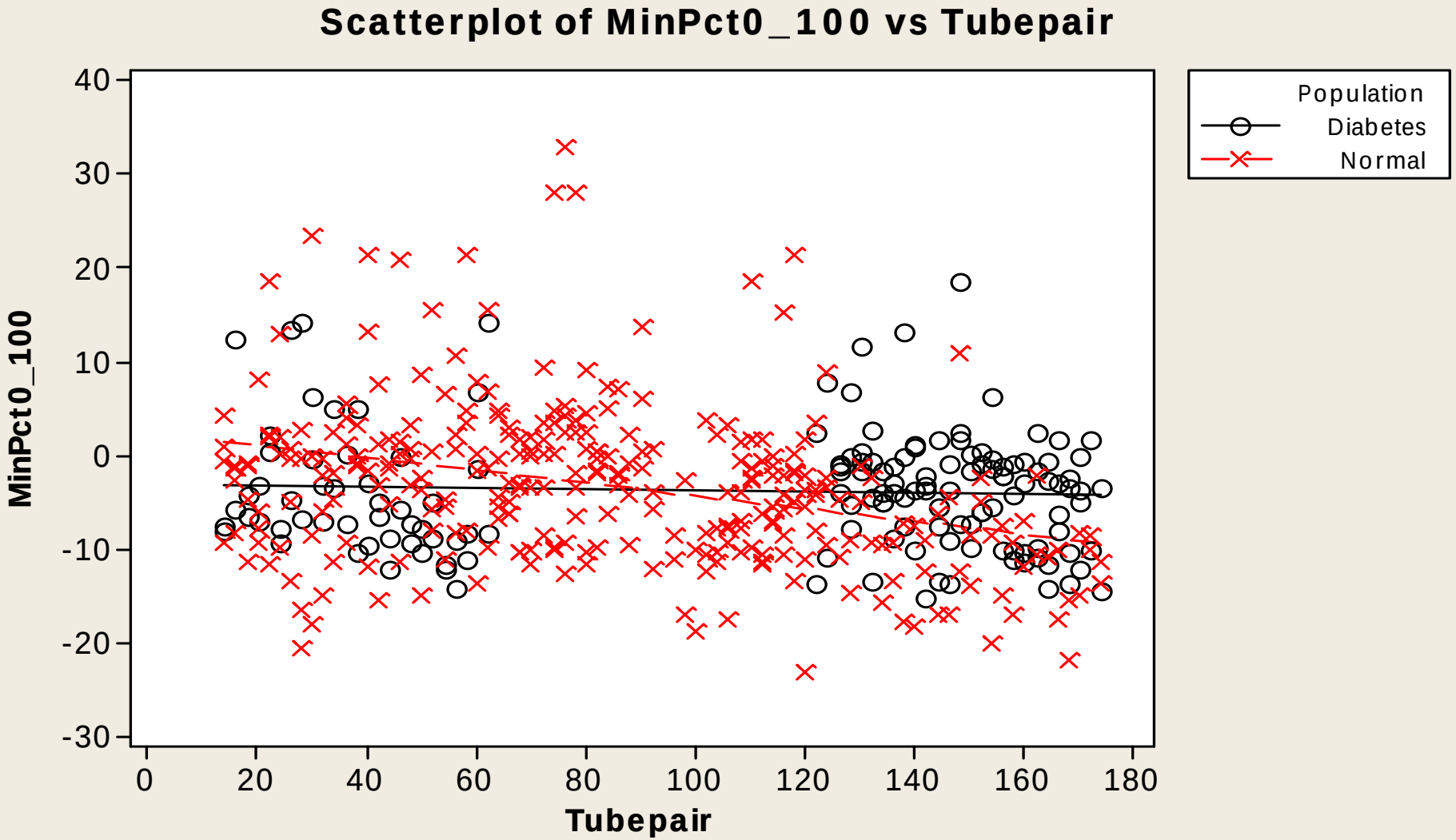
RIA Naïve Sample %Binding vs Test Tube Order by Population



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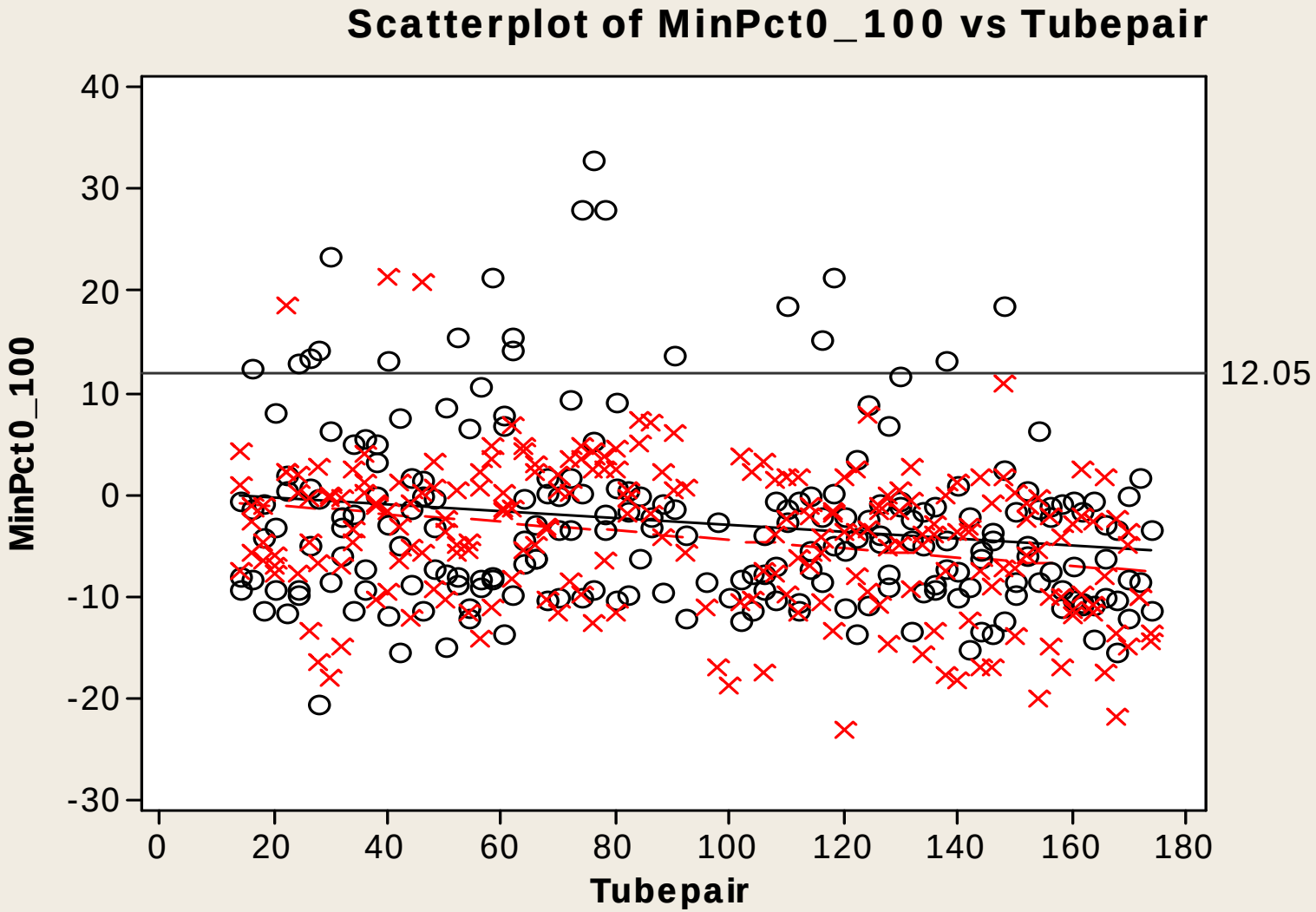
RIA Naïve Sample %Binding vs Test Tube Order by Analyst



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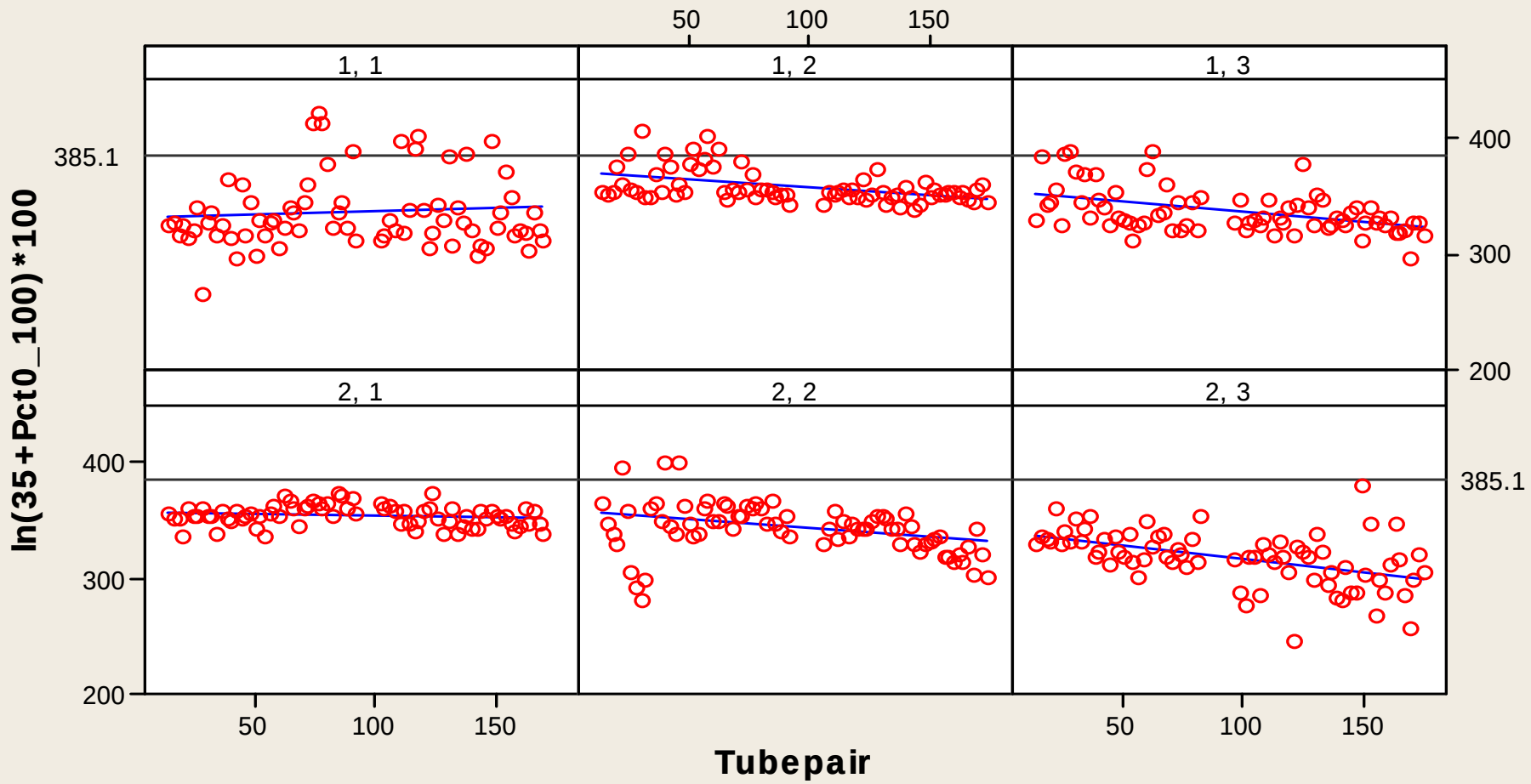
RIA Naïve Sample %Binding vs Test Tube Order by Analyst and Run



Panel variables: Analyst, Run

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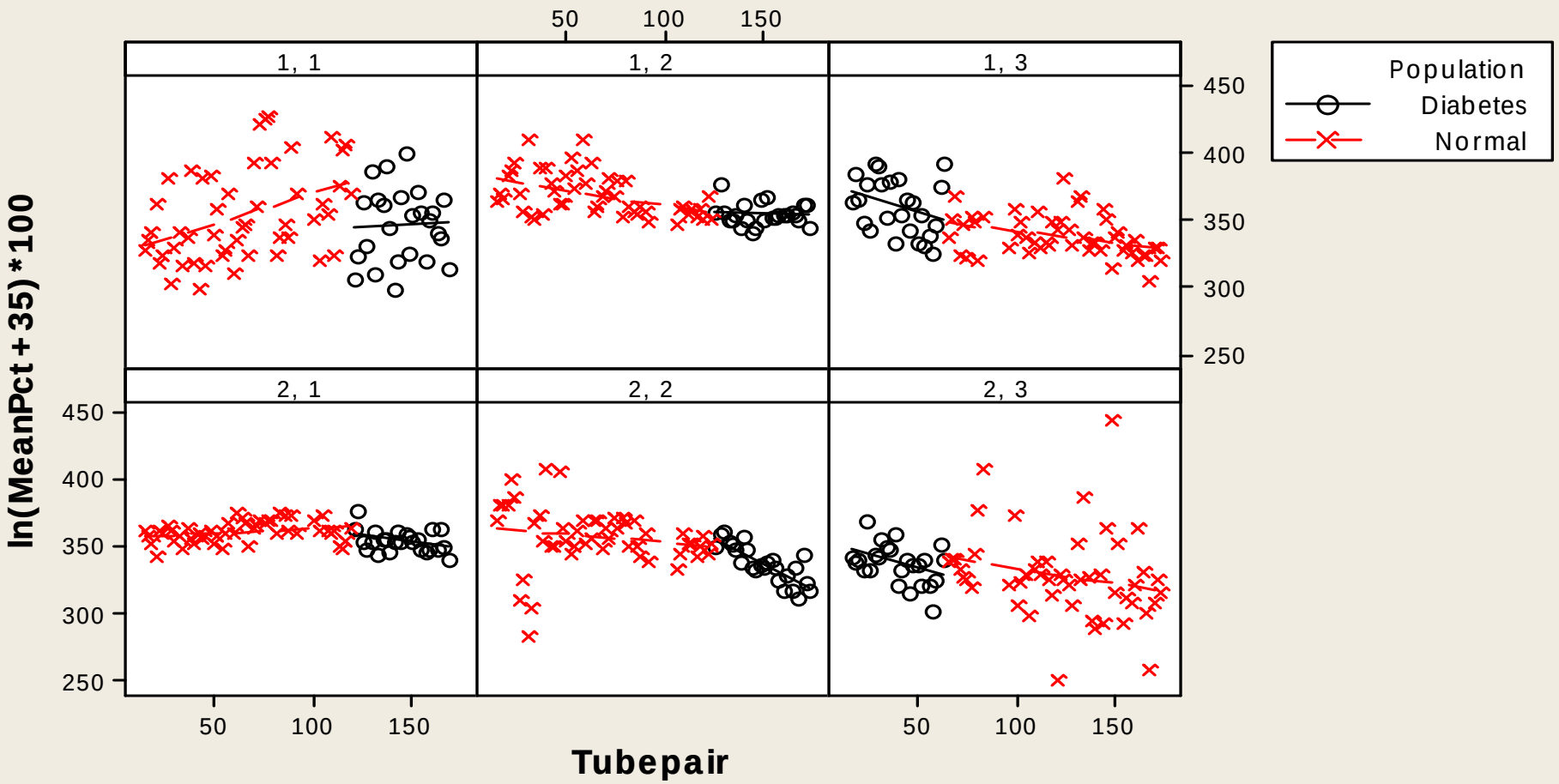
RIA Naïve Sample Means vs Test Tube Order by Population, Analyst and Run



Panel variables: Analyst, Run

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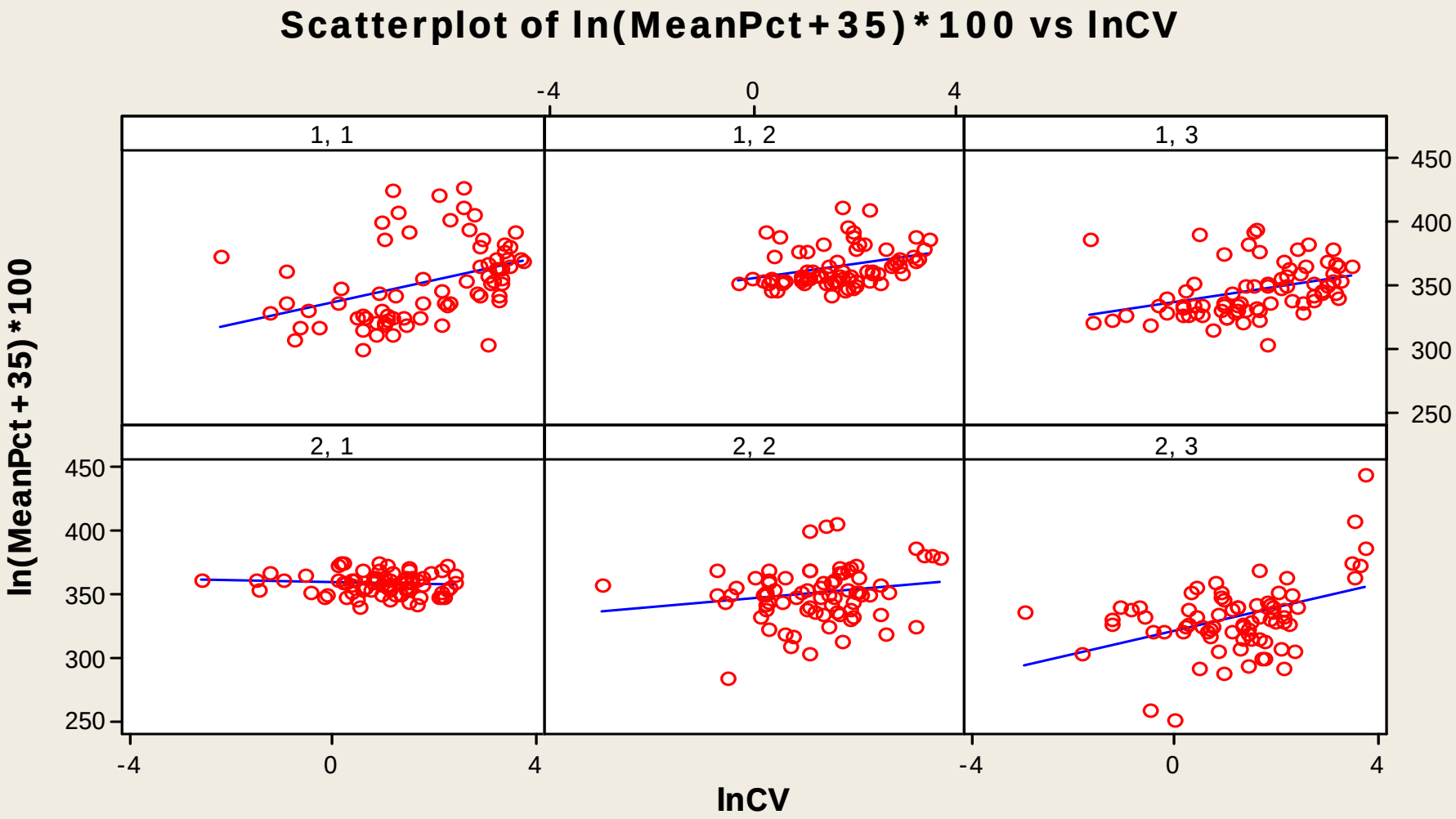
RIA Naïve Sample Mean %Binding vs CV by Analyst and Run



Panel variables: Analyst, Run

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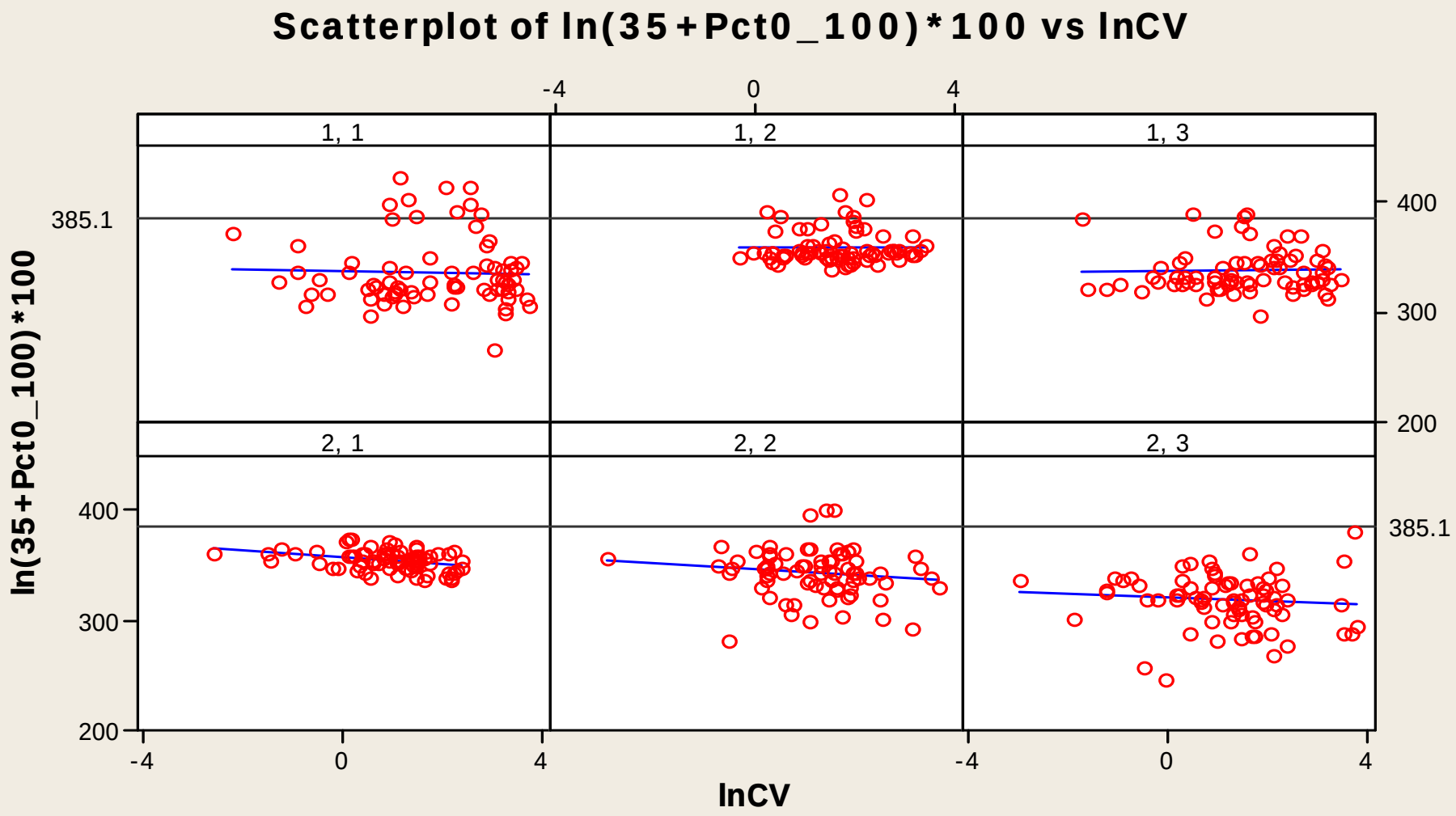
RIA Naïve Sample Minimum %Binding vs CV by Analyst and Run



Panel variables: Analyst, Run

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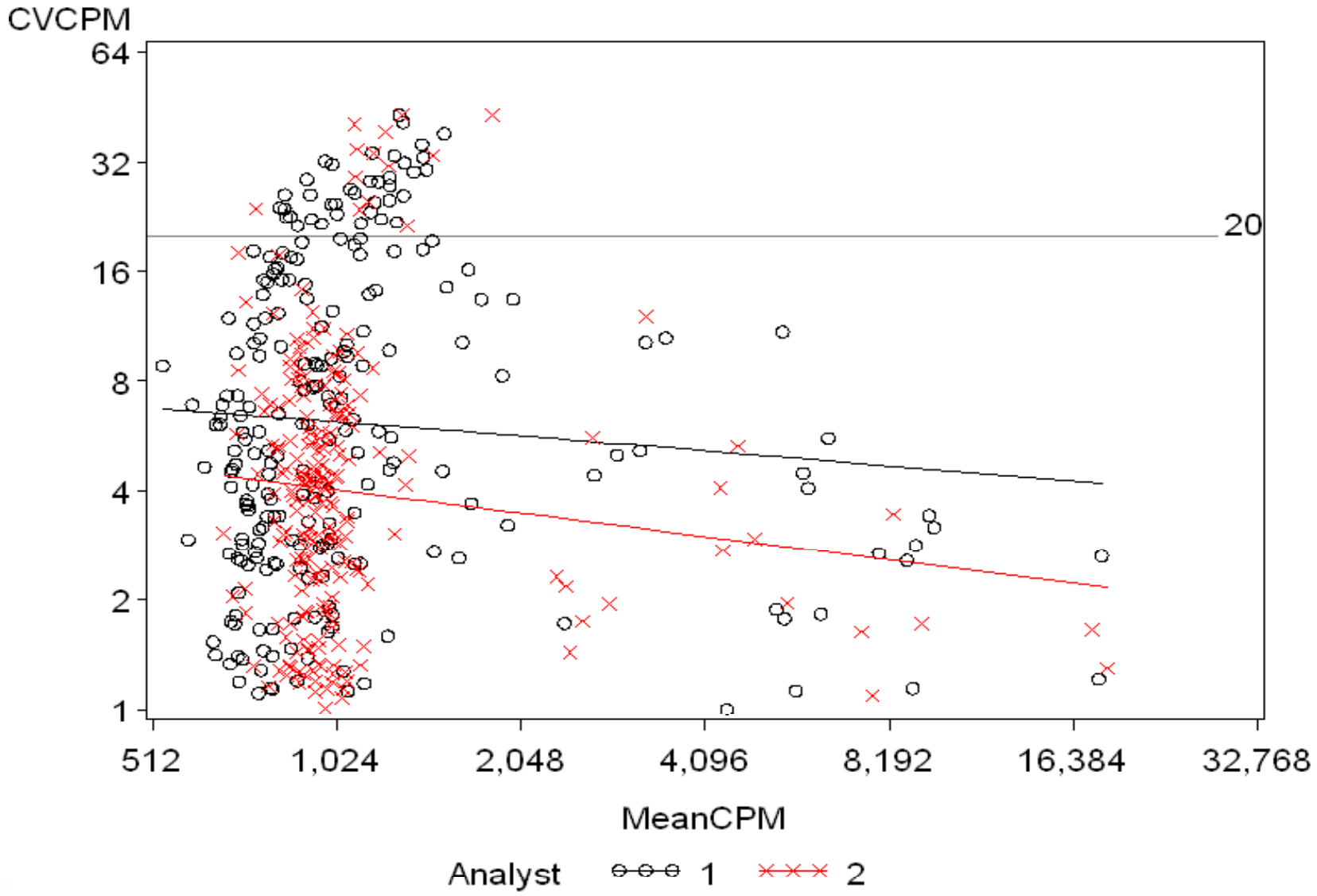
RIA Naïve Sample CPM CV vs Mean by Analyst



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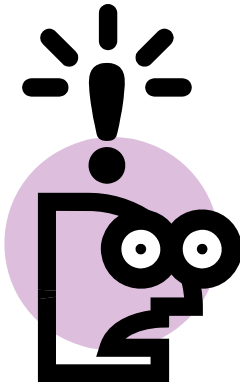
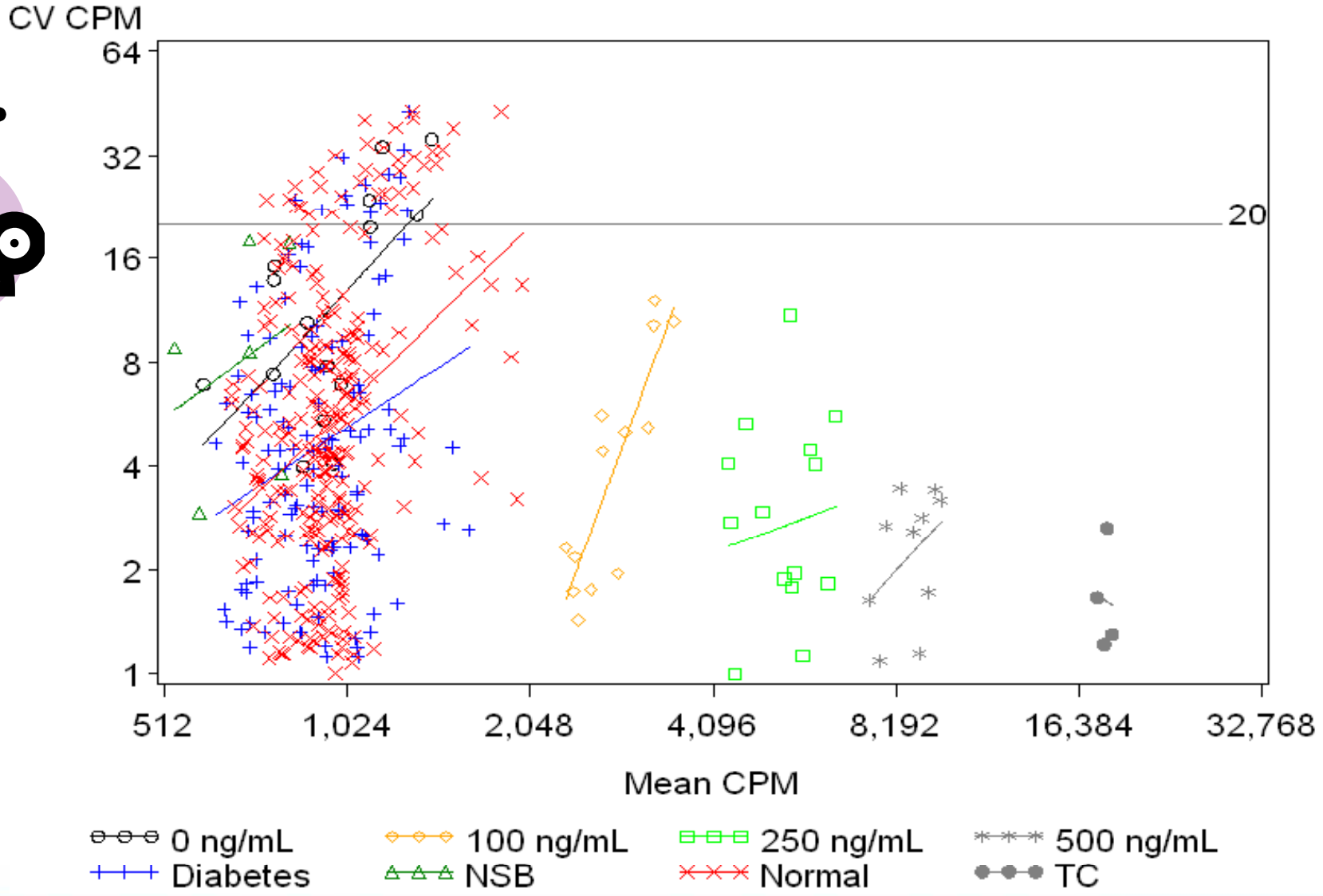
RIA Naïve Sample CPM CV vs Mean by Population and Control





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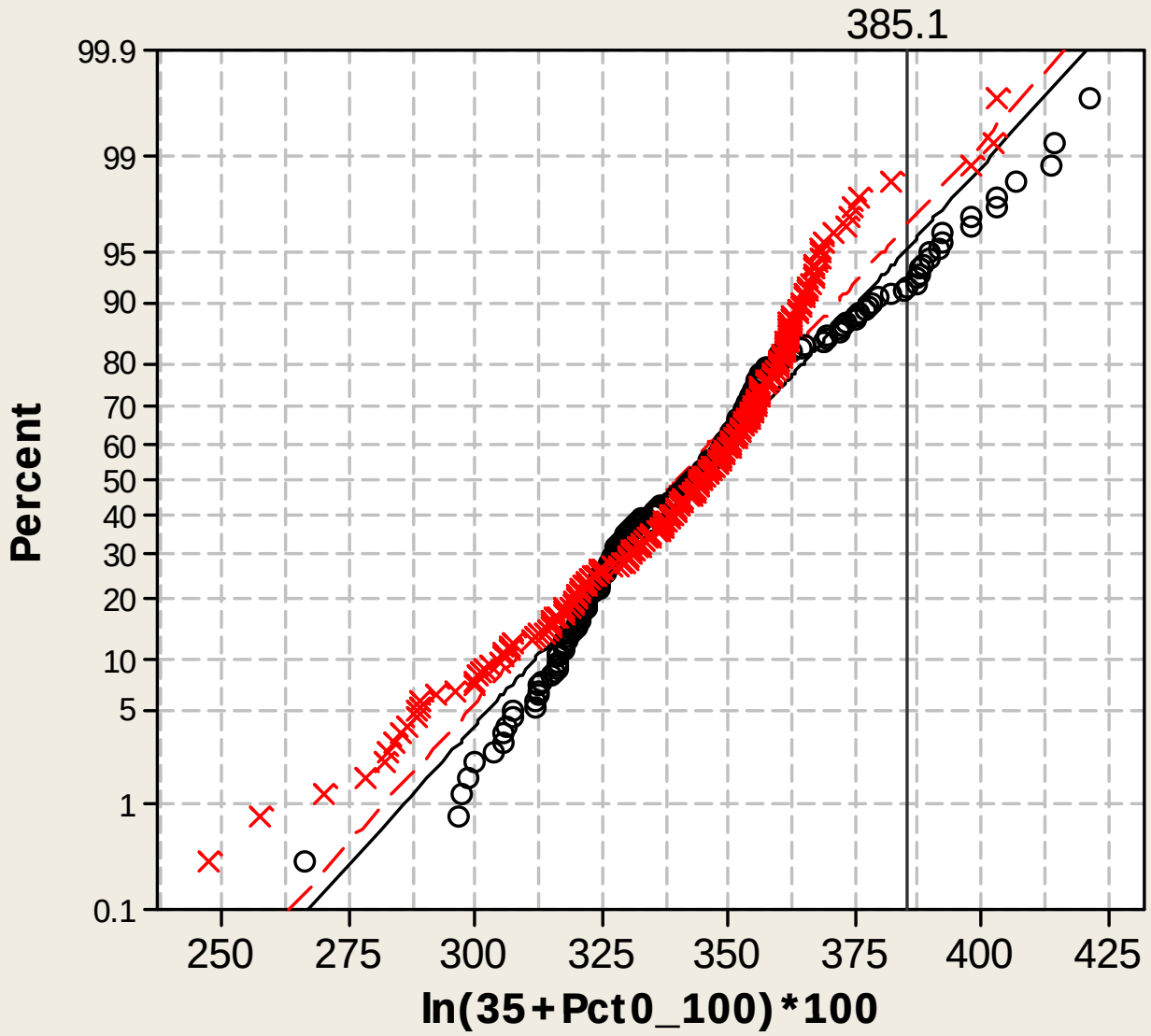
RIA Probability Plots of ln(35+%Binding)•100 by Analyst



Analyst
1
2

Mean	StDev	N	AD	P
344.0	24.90	225	2.052	<0.005
339.9	24.84	225	3.863	<0.005

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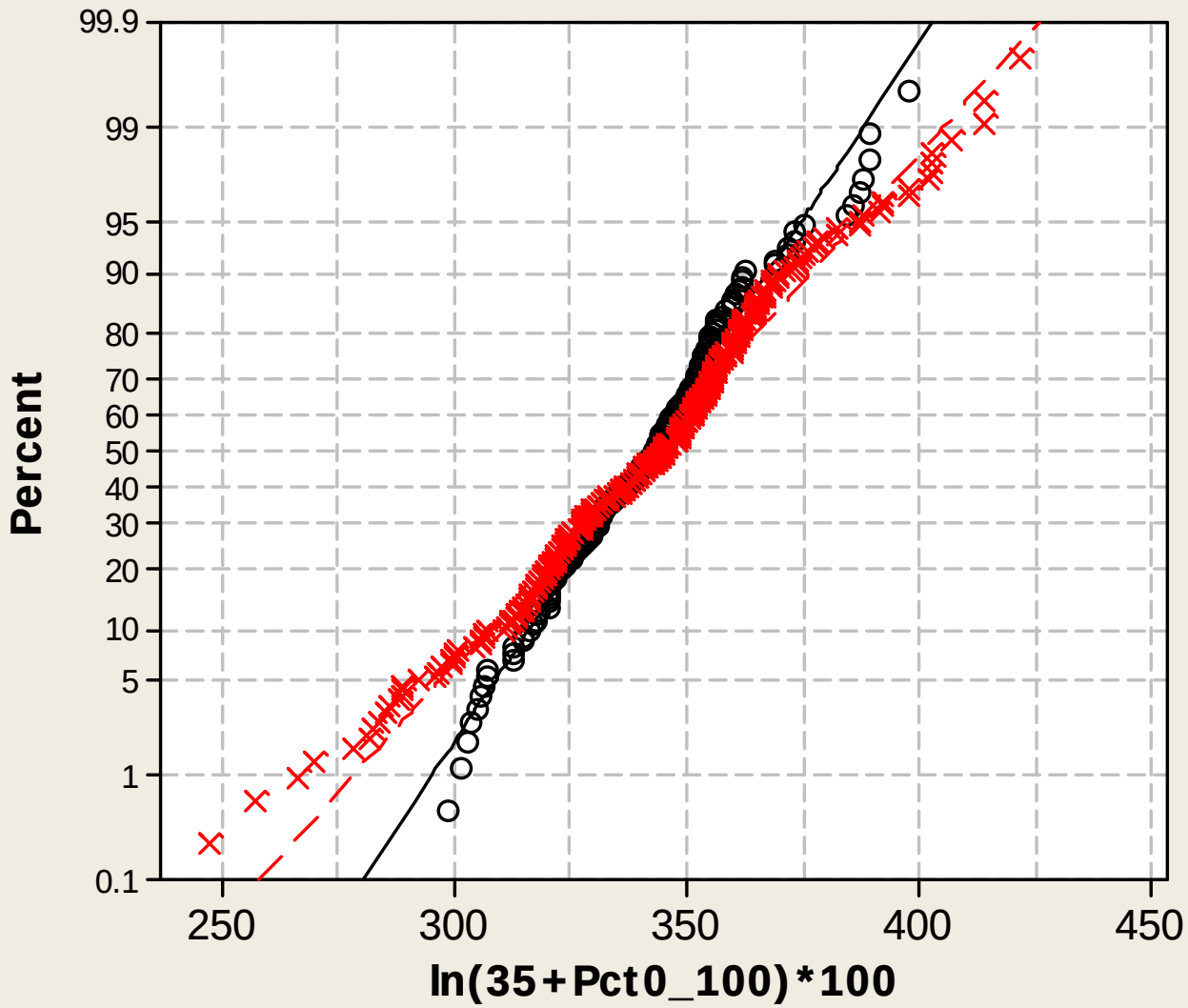
RIA Probability Plots of ln(35+%Binding)•100 by Population



Population
Diabetes
Normal

Mean	StDev	N	AD	P
341.8	19.87	150	0.542	0.162
342.0	27.14	300	1.573	<0.005

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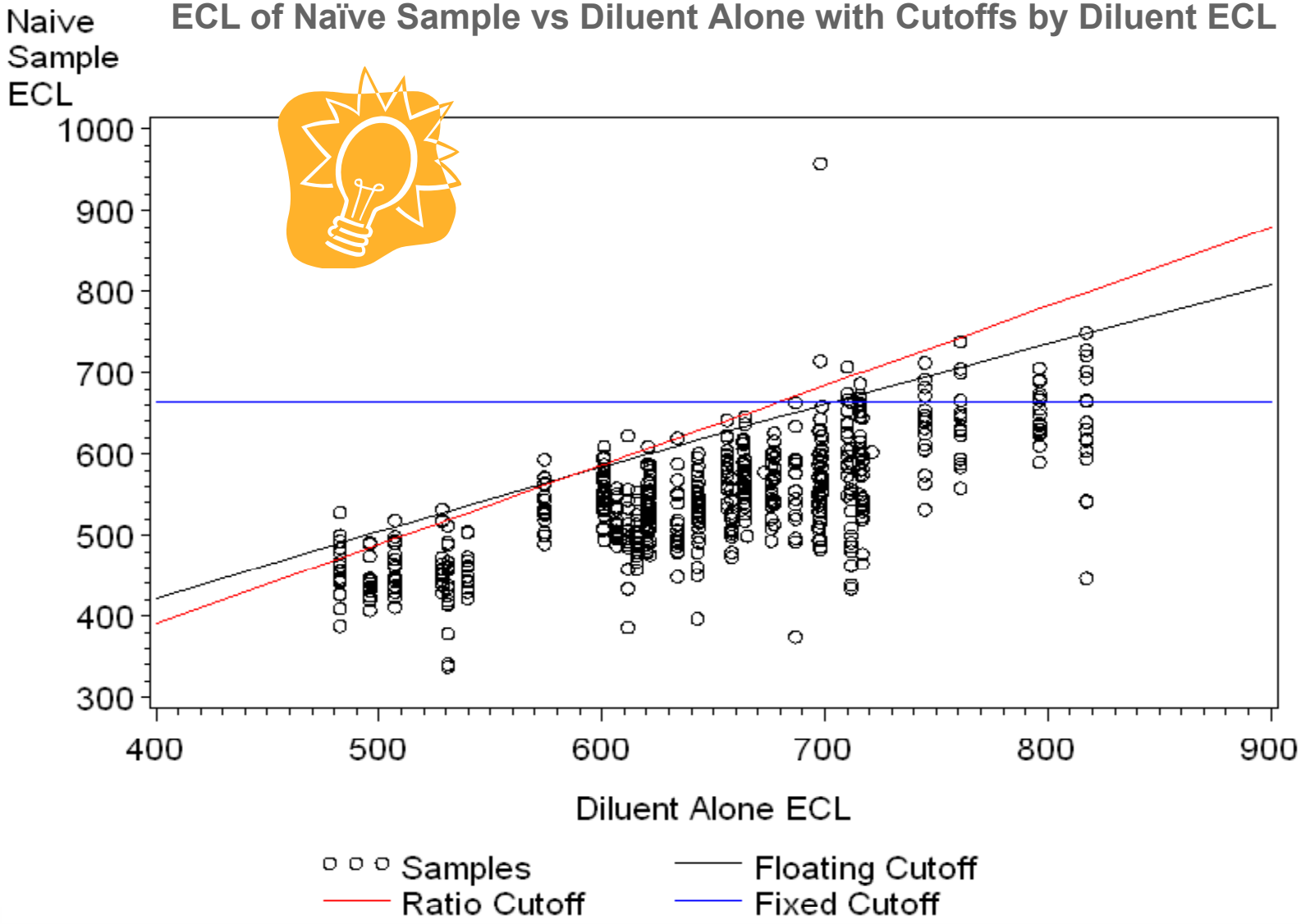
Electrochemiluminescence (ECL) BioVeris Assay

- New way to determine screening cut point (Data = naïve samples)
- New way to determine titration Cut Points (Data = positive samples' Titration series)
- Estimator of Titer within-assay CV

Screening Cut Point Determination



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Titer Definition

- **Smallest distinct dilution in a titration series with a negative response**
  - **Response is Sample ECL mean / Diluent Control ECL mean in this case study**

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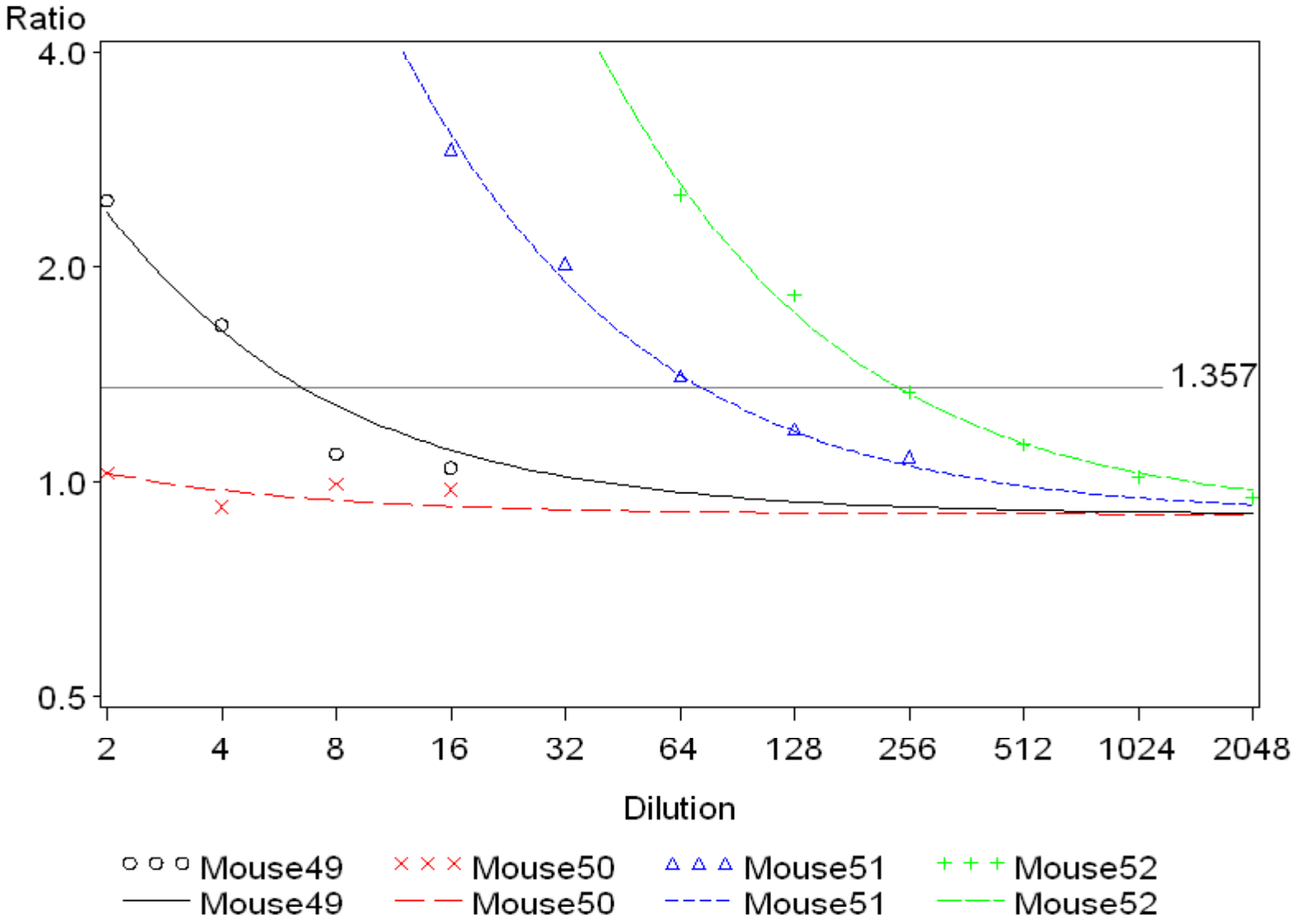
Plot where Sample/Diluent Control ECL Ratio < 4 for 1 Selected Plate out of 24



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Potential Problems with a Common Screening and Titration Cut Point

- Highly diluted samples tend to be positive!
  - The opposite would not be a problem
- Titration curve too flat at cut point
  - Makes the titer highly variable
  - Common

Titration Cut Point Defined

- The continuous titer inverse predicted from it has **CV ≤ 30.0%** with 95% confidence
  - **30.0% makes best case CV = worst case CV with cut point = ∞**
  - Continuous titer is exact dilution giving cut point (only as a theoretical concept)

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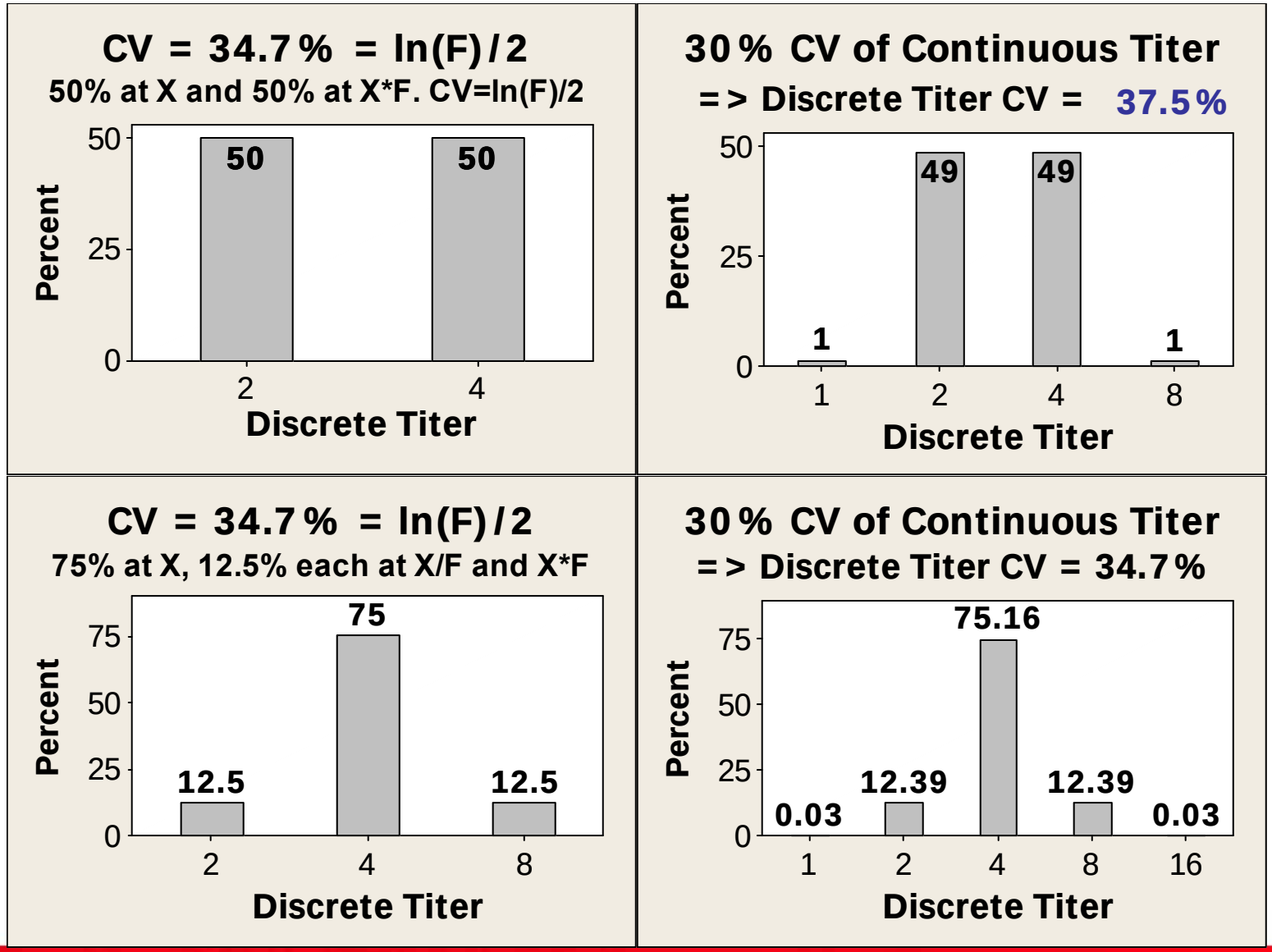
Asymptotic CV

- CV ≈ Standard deviation of natural log ratio or titer
- CV of dilution@ratio ≈ CV of ratio / slope of titration curve@ratio
- CV of dilution decreases as ratio and slope increase
- These CVs are within-plate CVs

Four Theoretical Titer Distributions



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Titration Cut Point Defined

- A continuous titer inverse predicted from it has CV<30.0% with 95% confidence
  - Exact dilution giving cut point (eg, 1.357 ratio) is the continuous titer
  - Continuous titer used here only as a theoretical concept
  - Our cut-point 5 SD above diluent mean so false-positives of noncensored titers unlikely

Summary

- All biologics need ADA evaluation
- Use controls to adjust for plate-to-plate variance and minimize the LOD
- Define titration cut point so best case CV = worst case CV if cut point = ∞

Acknowledgements:

- Sheng Dai
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- Gopi Shankar
- Bill Pikounis

Reference:

Shankar, G. et al, (2008). Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. *Journal of Pharmaceutical and Biomedical Analysis*. 48:1267–1281.