# Characterization of the Antibody Quantitation Assay with the OctetRed

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**ForteBio Workshop**

**August 25, 2011**

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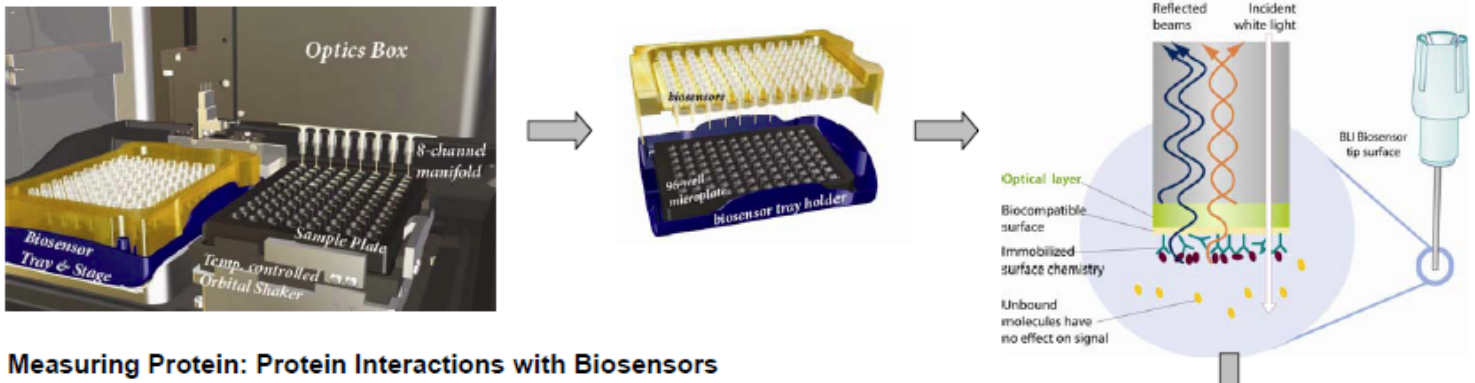
San Francisco

# Bio-Layer Interferometry (BLI)




**Measuring Protein: Protein Interactions with Biosensors**

- Optical layer reflects simple white light; second reflection from tip of biosensor, both reach detector

- Analyte binding changes thickness of bio-layer, which is measured at detector

❖Slide Courtesy of ForteBio

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# Features we love

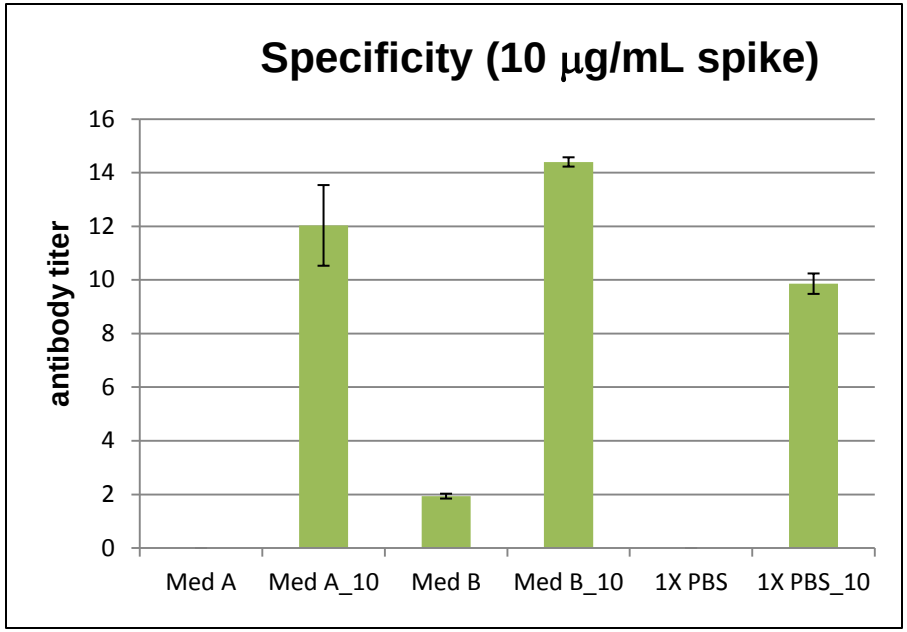
- Utilize the Protein A-coated Biosensor:
  - Regeneration of the ProA biosensor (up to 10 uses per sensor)
  - Load crude supernatant (assuming within the assay's dynamic concentration range)
  - Samples are not destroyed
  - No need to make a standard curve with each plate/assay
  - Load a plate and walk away (80 samples done in ~30 minutes)
  - Generates report as an Excel Spreadsheet

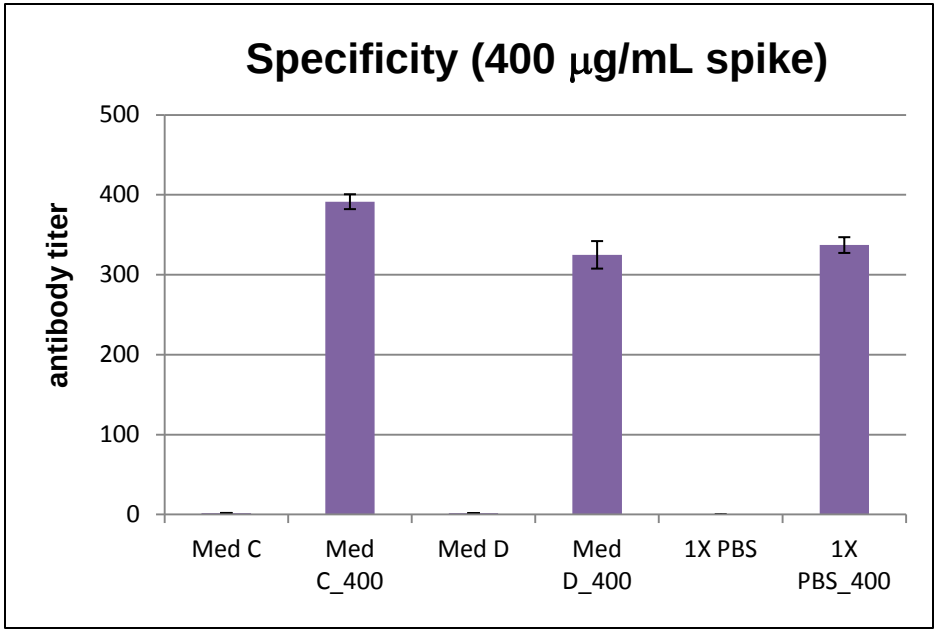
# Characterization Parameters (taken from ICH Q2-R1)

- **Specificity:**
  - "…the ability to measure unequivocally the analyte in the presence of components which may be expected to be present"
    - Cell debris
    - Antibody fragments
    - Interference from matrix
- **Precision:**
  - "…the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions"
    - Repeatability: Sample replicates and biosensor variability
    - Intermediate Precision: Day to day, operator to operator, and standard curve variability
- **Accuracy:**
  - "…the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found"
    - HPLC vs. OctetRed correlation

# Specificity

- Generated 4 spent supernatants by growing host cells in each medium under appropriate production conditions
- Ran each spent medium with and without antibody spike




❖No matrix effects seen for all media

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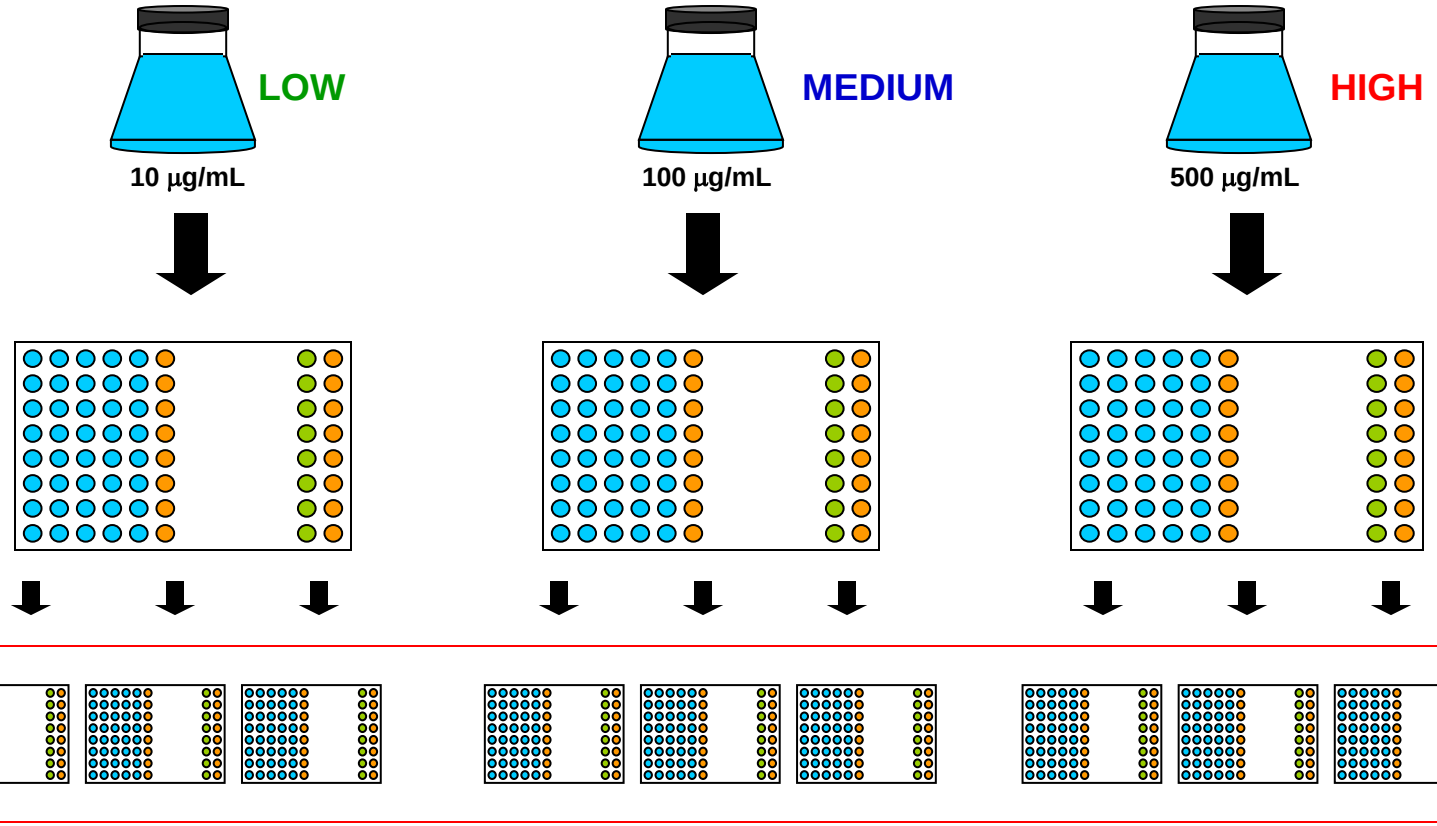
# Precision-Experimental Design

- Made up bulk solutions of 3 different concentrations from purified mAb material:
  - 10 μg/mL **LOW TITER**
  - 100 μg/mL **MEDIUM TITER**
  - 500 μg/mL **HIGH TITER**
- Loaded each concentration:
  - 8 biosensors
  - 5 replicates
  - 3 separate days
  - 2 operators

# Precision-Design Schematic

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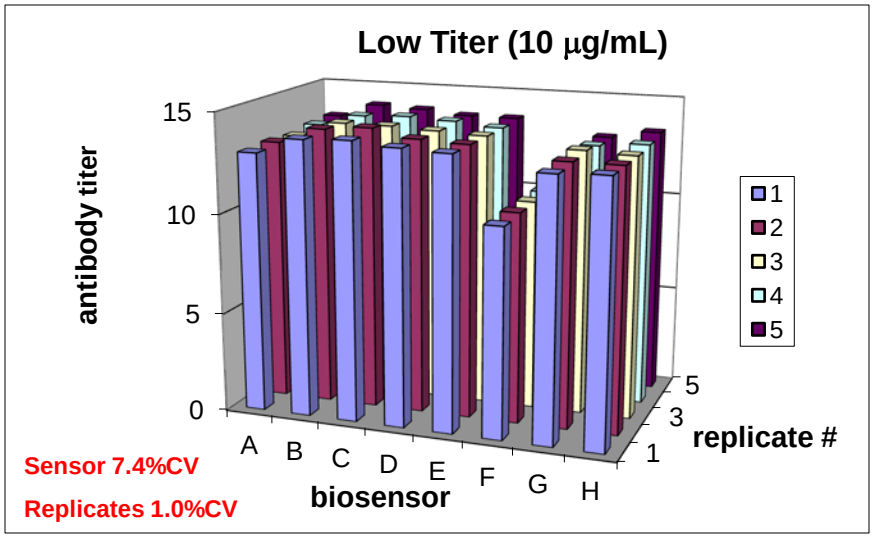
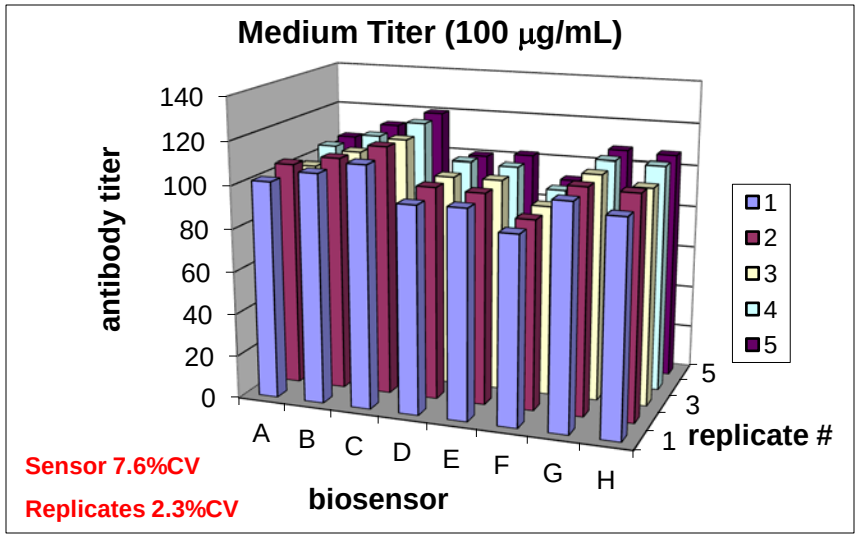
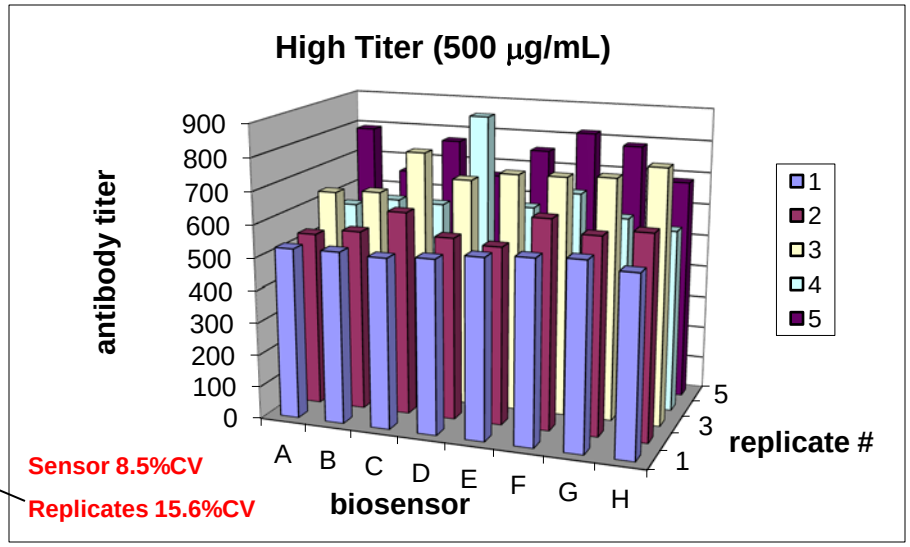
# Precision-Repeatability




*Note: Higher %CV values were not addressed by assay development due to ability to achieve lower %CV value and conserve sample volume through 10X dilution.to 10-100 ug/mL. Octet RED is capable of achieving %CV <10% for concentrations up to 2000 ug/mL*

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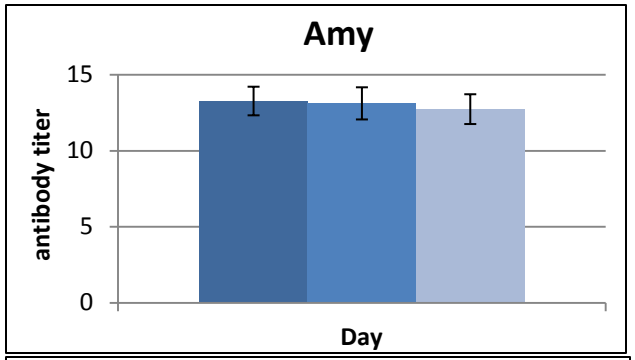
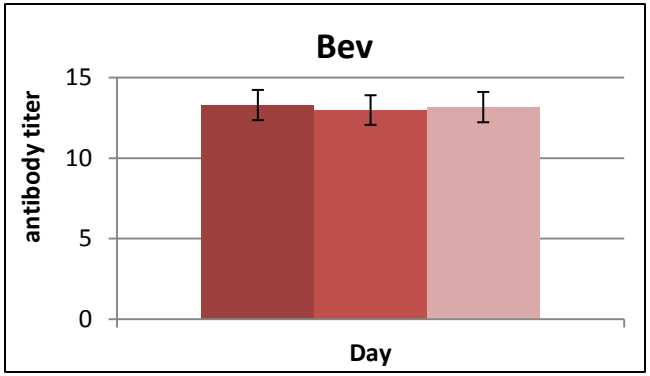
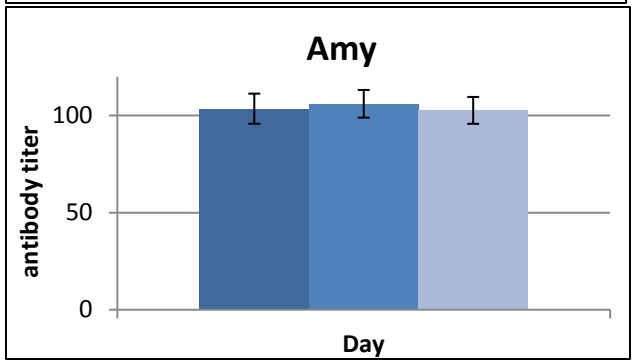
# Precision-Day to Day

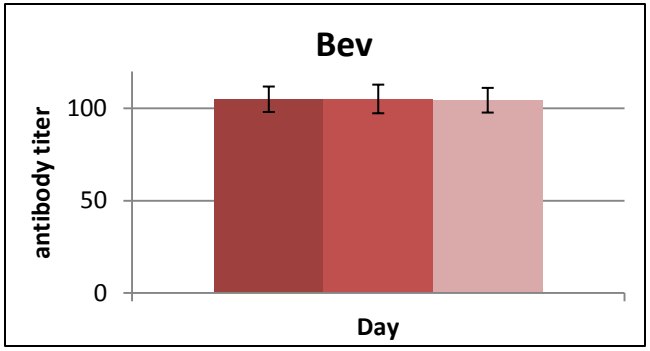


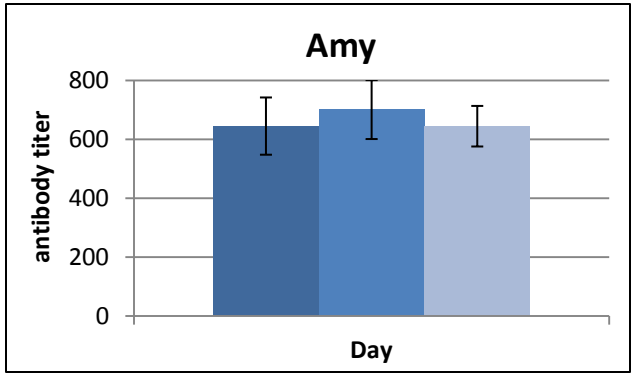

LOW



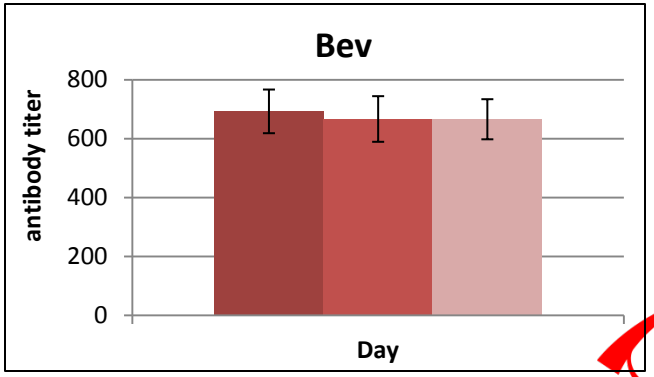




MEDIUM




HIGH

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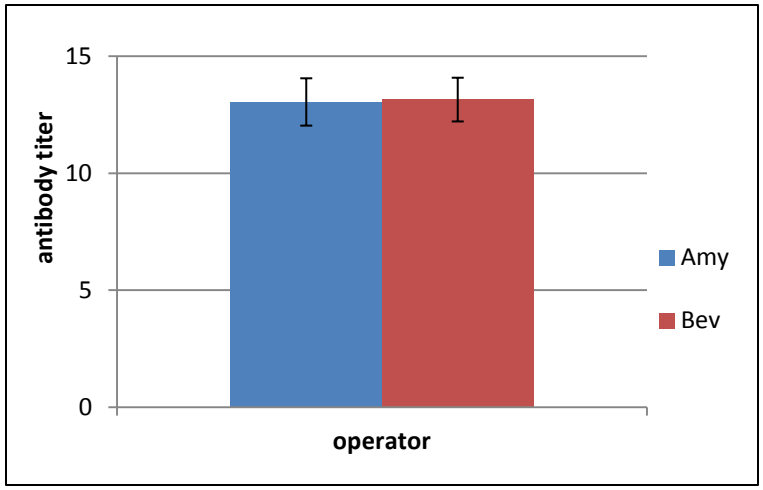
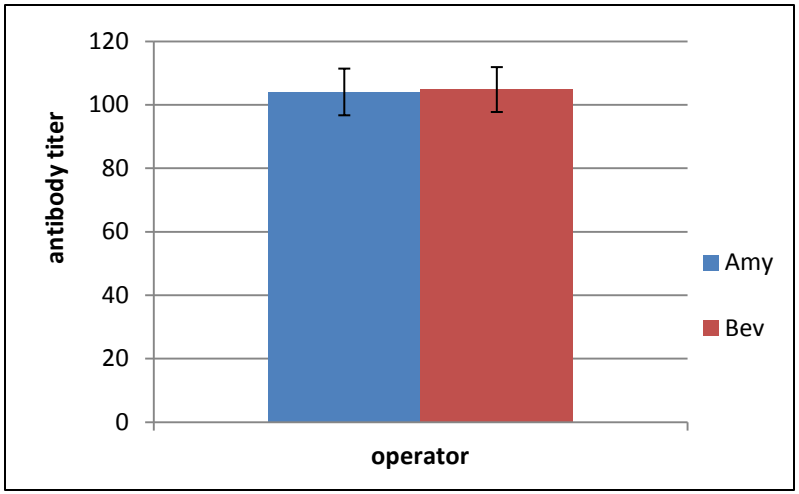
# Precision-Operator to Operator

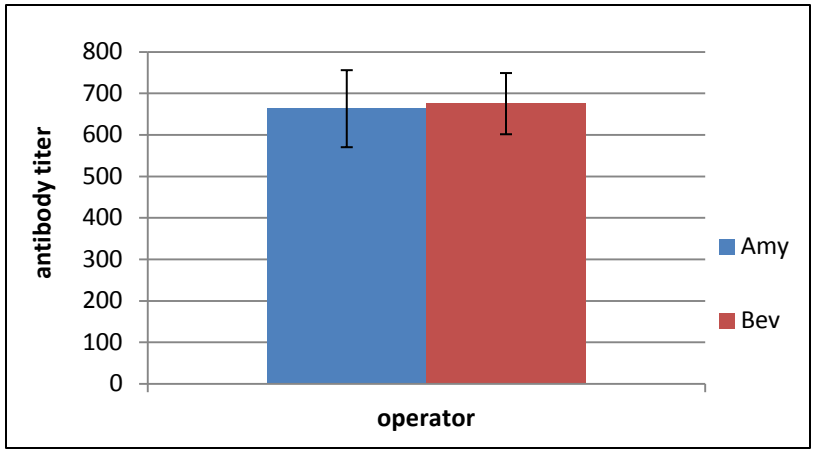
**LOW**




**MEDIUM**




**HIGH**

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# Precision-Summary

| | % CV | | |
|---|---|---|---|
| **Precision** | **Low** | **Medium** | **High** |
| Replicates | 1.0% | 2.3% | 15.6% |
| Biosensor | 7.4% | 7.6% | 8.5% |
| Operator #1 | 7.8% | 7.1% | 14.0% |
| Operator #2 | 7.1% | 6.7% | 10.9% |
| Operator | 0.5% | 0.5% | 1.3% |
| Overall | 7.4% | 6.9% | 12.5% |

*Note: Higher %CV values were not addressed by assay development due to ability to achieve lower %CV value and conserve sample volume through 10X dilution.to 10-100 ug/mL. Octet RED is capable of achieving %CV <10% for concentrations up to 2000 ug/mL*

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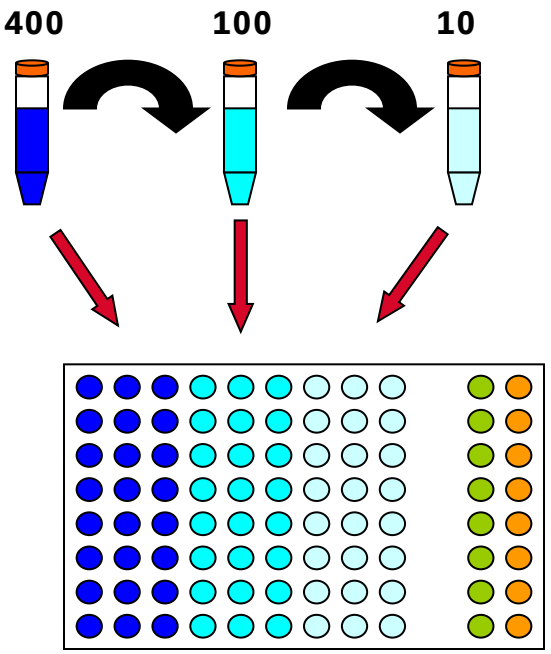
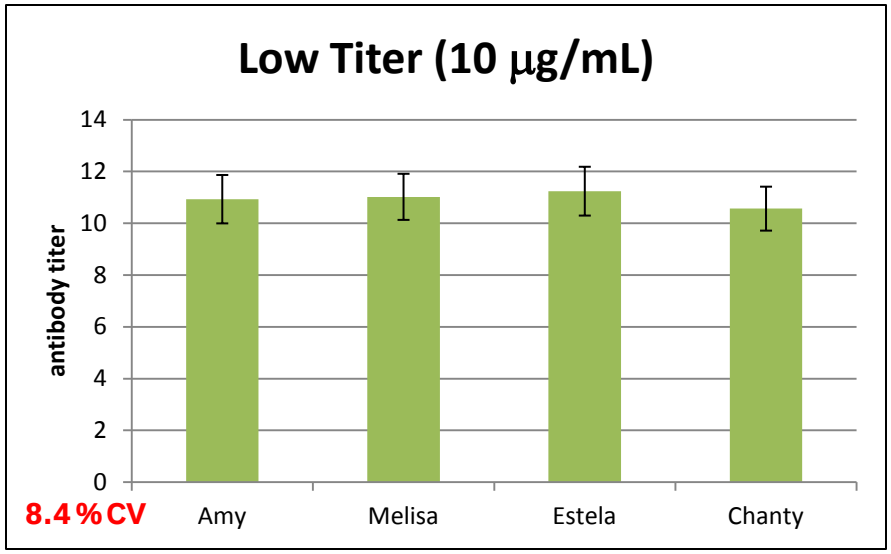
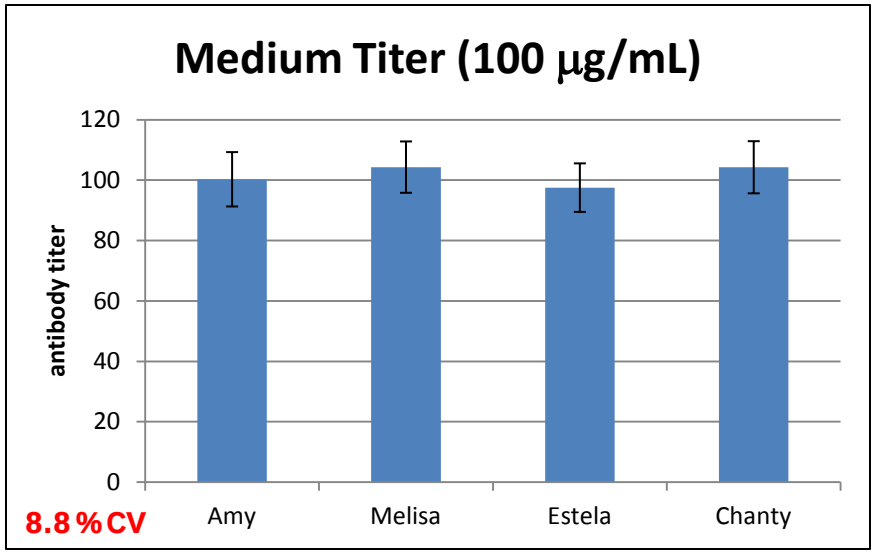
# Precision-Worst Case Scenario

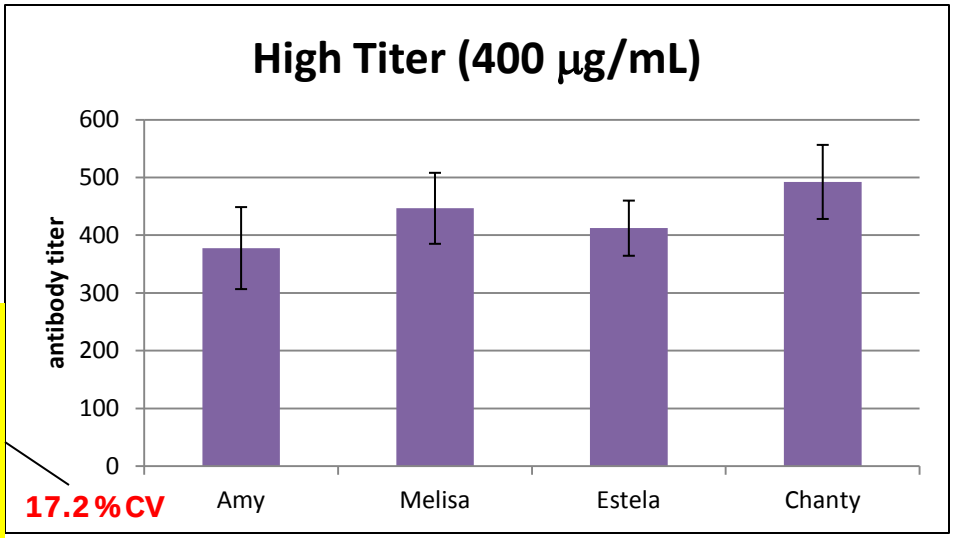
- **Each** operator made a serial dilution of purified mAb material to the following concentrations:
  - 10 μg/mL
  - 100 μg/mL
  - 400 μg/mL
- Loaded each concentration:
  - 8 biosensors
  - 3 replicates
  - 4 operators
  - separate days



# Precision-Worst Case Scenario

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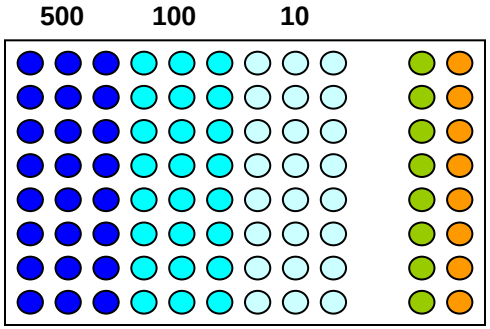
# Robustness-Time Course

- Plate was made and run at T = 0 hours
- Plate was subsequently sealed and stored at room temperature and rerun at:
  - T = 1.5 hours
  - T = 3.0 hours
  - T = 6.0 hours

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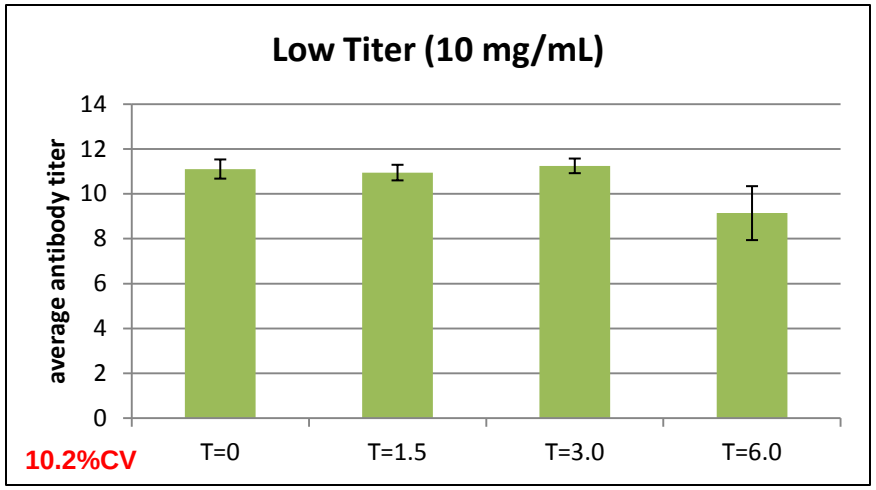
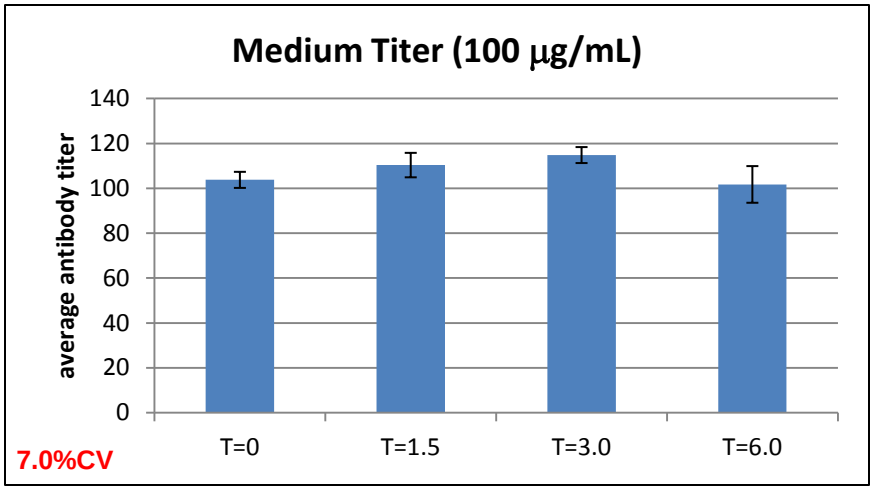
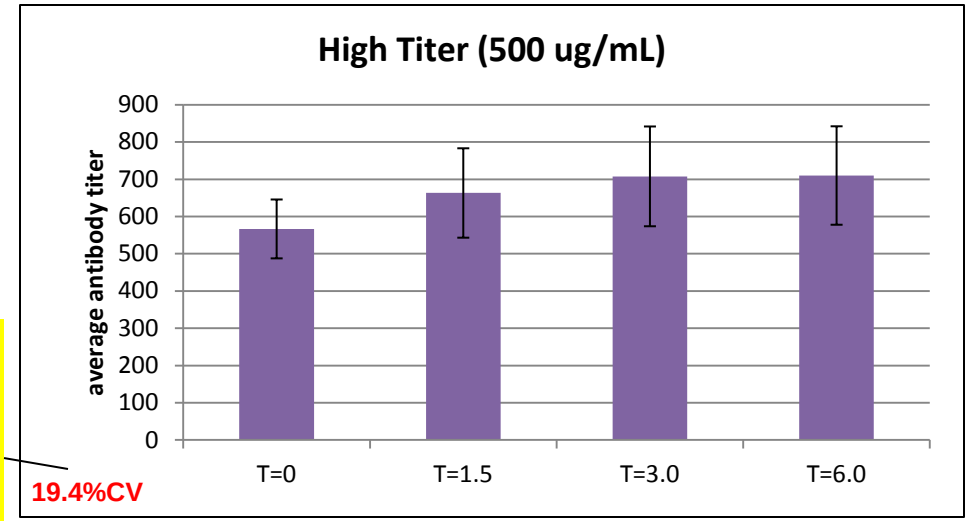
# Robustness-Time Course

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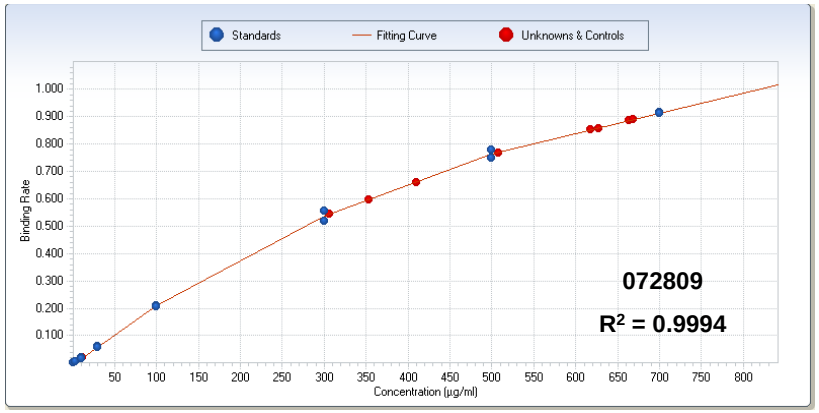
# Precision-Standard Curve Variability

- Generated a standard curve with ForteBio's pre-made calibrators:
  - Titer range: 1 to 700 ug/mL
- After ~1 year of using this standard curve, checked variability by making new standard curves (new lots of calibrators and biosensors) and analyzing existing data set on "old" and "new" curves

# ForteBio Standard Curve Variability

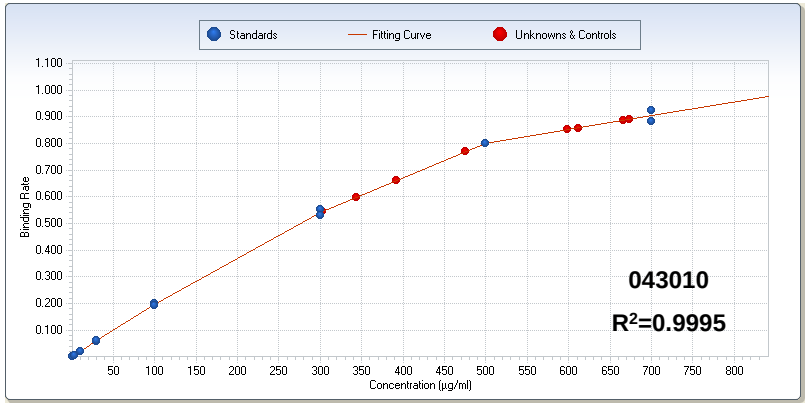
**OLD**



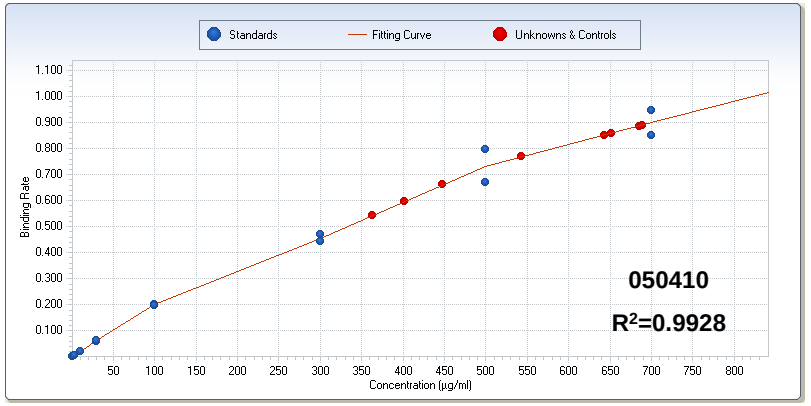

❖Average 3.4% CV for the unknowns analyzed by all 3 ForteBio standard curves

❖Noticeable “kink” at 500 µg/mL; titer range used is1-500 µg/mL

**NEW 1**




**NEW 2**

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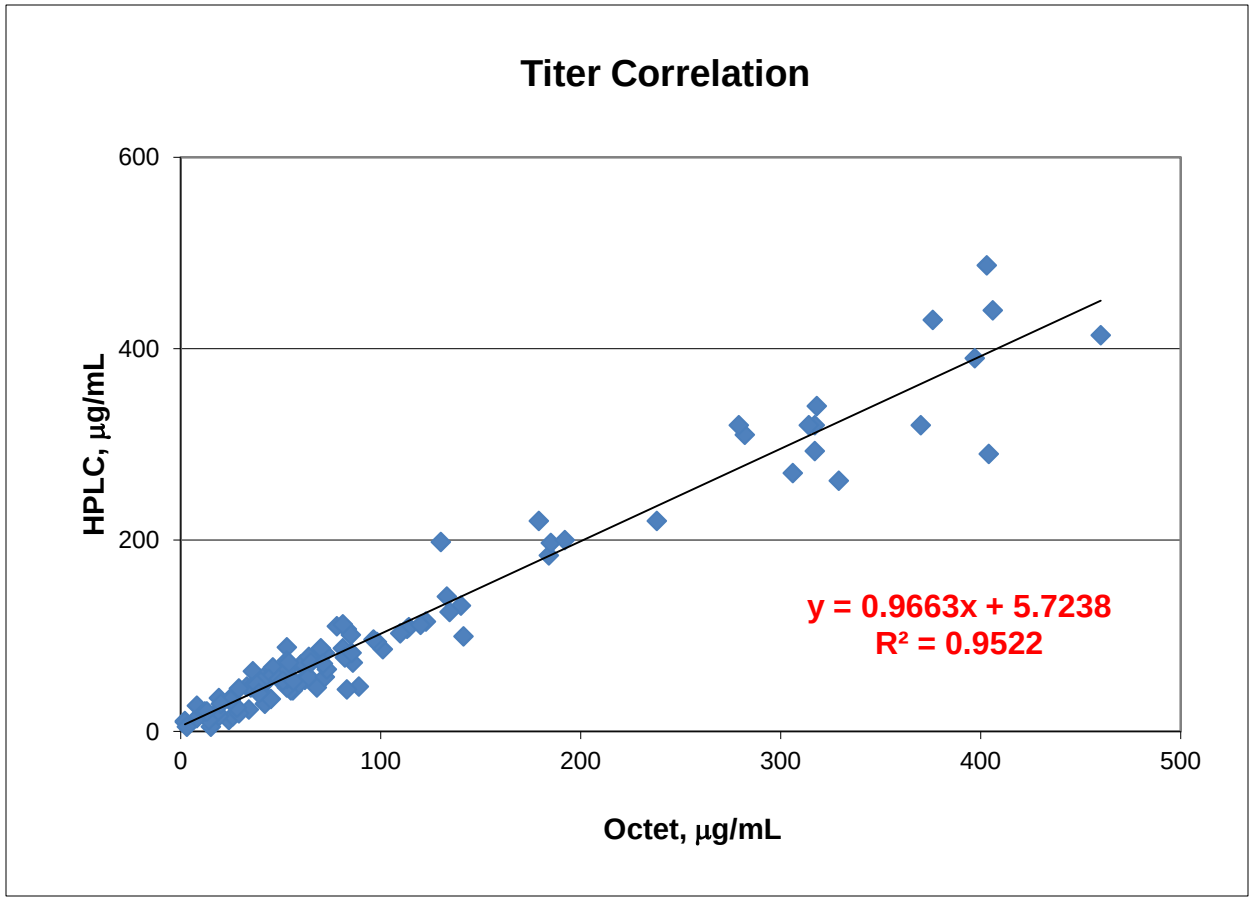
# Accuracy-ProA HPLC vs. OctetRed Correlation

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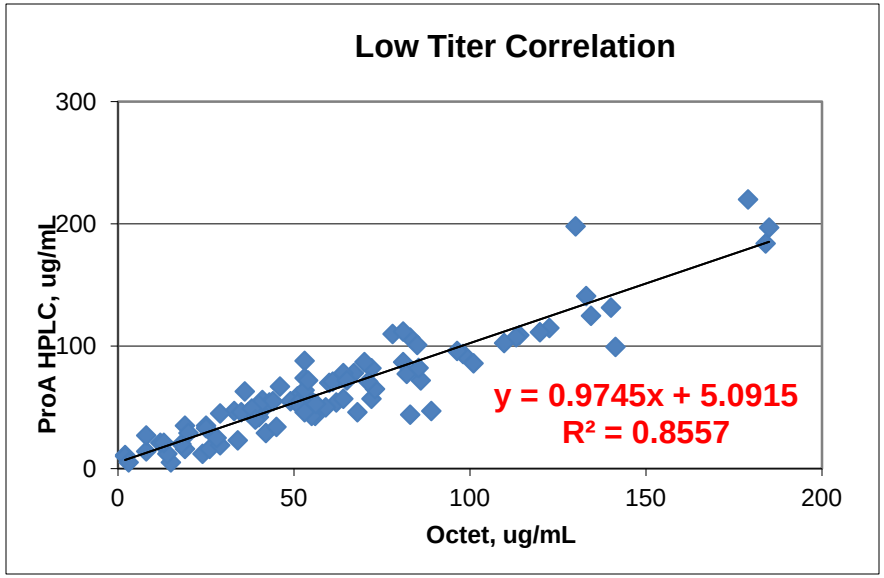
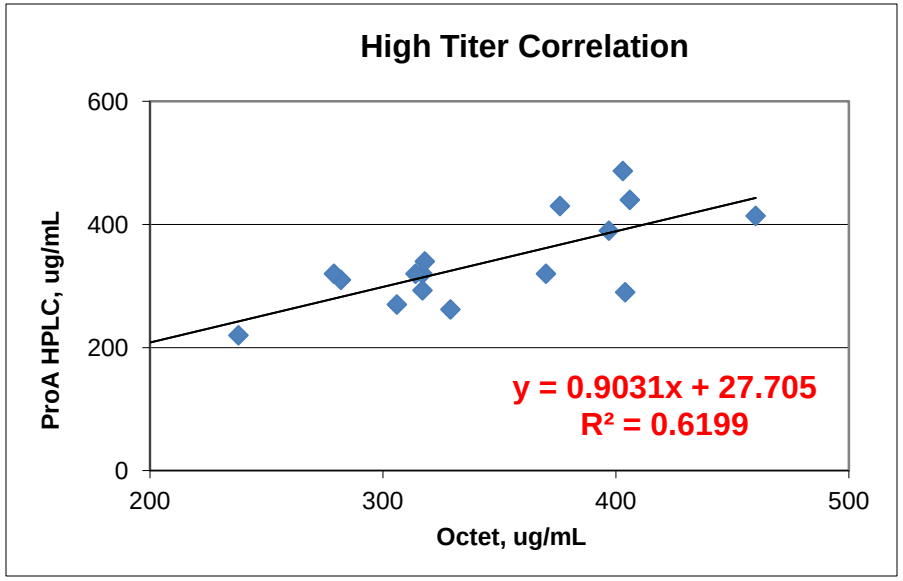
# Accuracy-ProA HPLC vs. OctetRed Correlation

➢Separated the data into 2 groups:

- Low titer: values <200 ug/mL
- High titer: values >200 ug/mL

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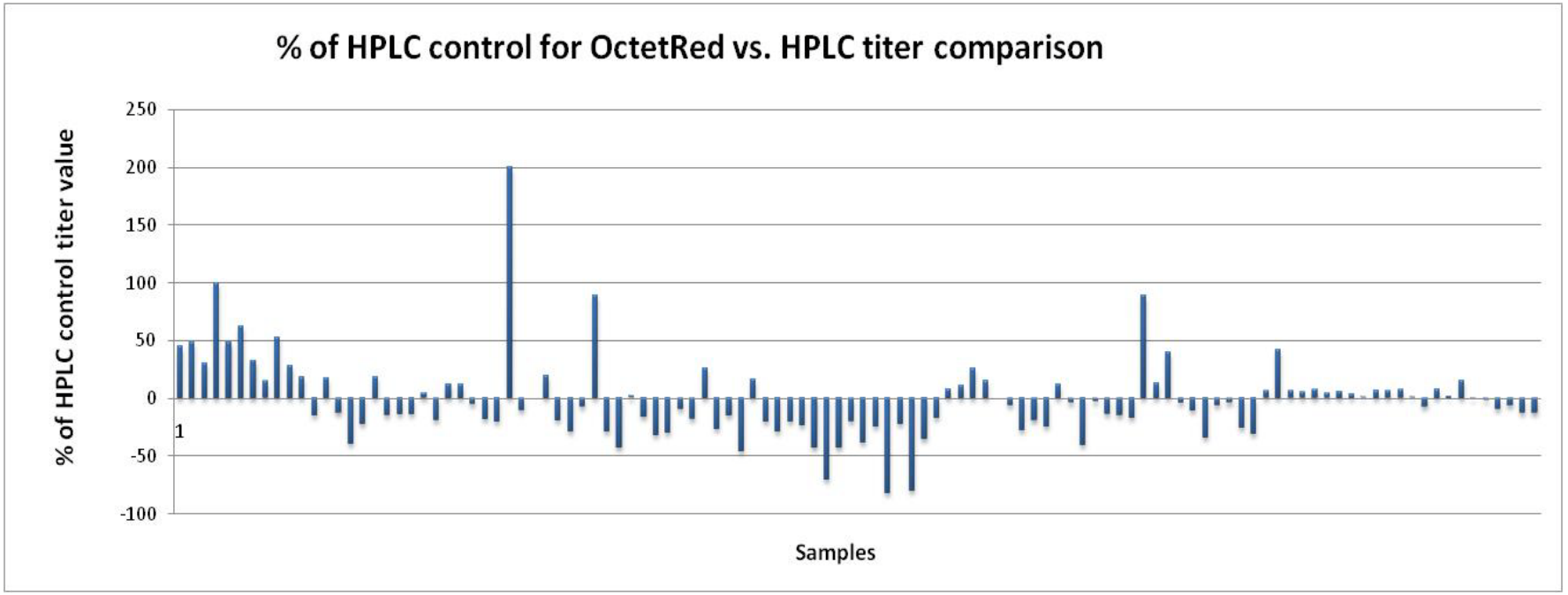
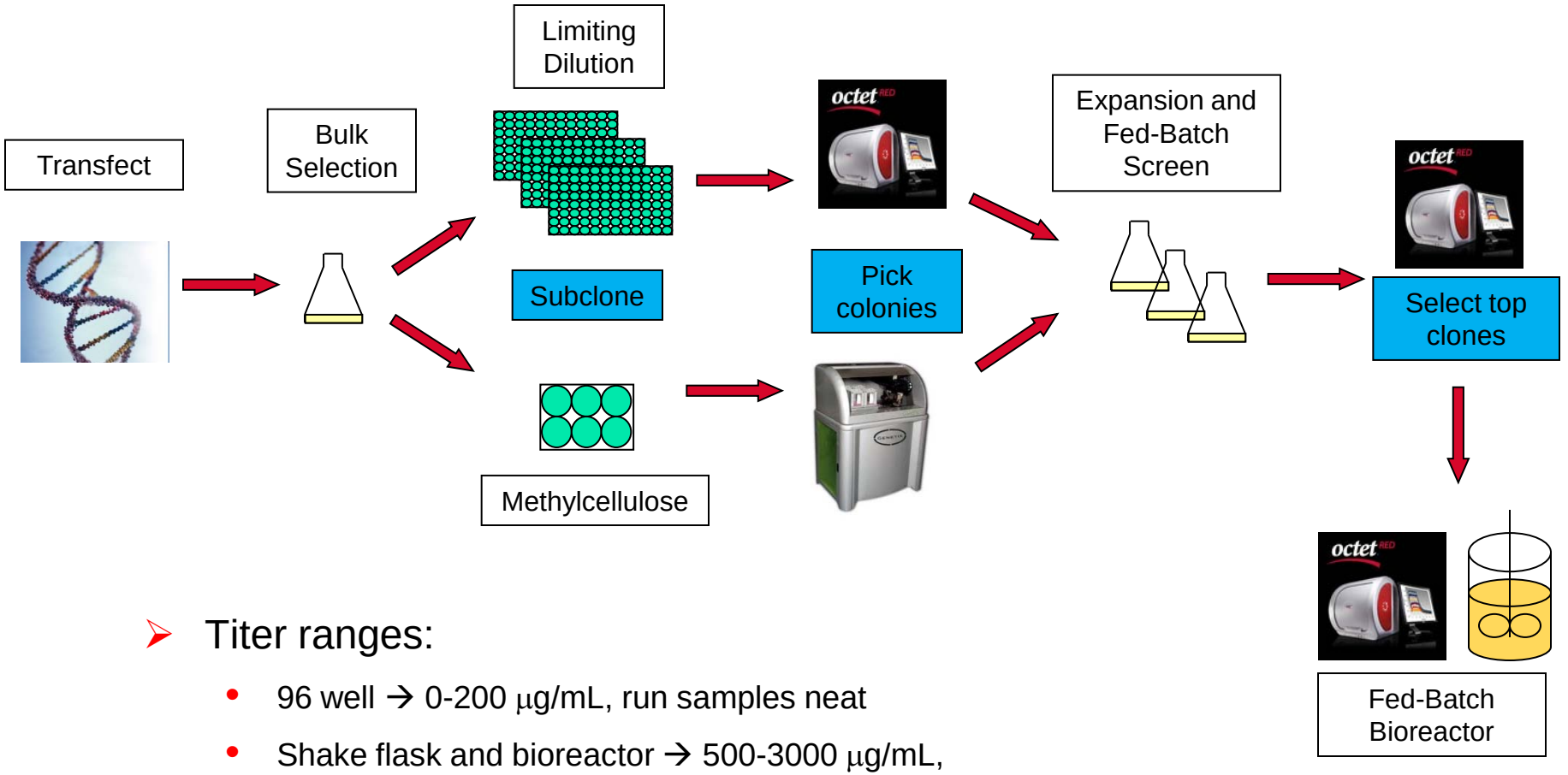
# Accuracy-% of control

- HPLC value is the control value
  - Positive percentage means OctetRed value is **higher** than HPLC value
  - Negative percentage means OctetRed value is **lower** than HPLC value



# Cell Line Development Workflow




- Titer ranges:
  - 96 well → 0-200 μg/mL, run samples neat
  - Shake flask and bioreactor → 500-3000 μg/mL, run samples at 1:10 dilution

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# Summary

- Incorporated the ForteBio Antibody Quantitation Assay using Protein A biosensors into Takeda San Francisco's Cell Line Development Platform
- Assay characterization shows:
  - High specificity with no matrix effects observed
  - High precision for repeatability between biosensors and replicates
  - High precision comparing operator to operator, day to day and standard curve to standard curve
  - Robustness and therefore flexibility when loading and running supernatants within the same workday
  - High accuracy when correlated to Protein A HPLC ($R^2 = 0.95$)
- Routinely Run System Suitability controls at the low, medium and high titer concentrations:
  - Assay is acceptable if values are within ± 2 Standard Deviations

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# Acknowledgements


- Cell Engineering:
  - Angeles Estelles
  - Amy Bass
  - Estela Garcia-Murillo
  - Melisa Carpio
  - Raffaella Briante
  - Bev Potts
  - Nicole Lapuyade-Baker
  - Chanty Mariategue
  - Sanjay Patel
- Analytical:
  - Tamara Gelzleichter
  - Samadhi Vitharana

❖Thanks ForteBio!


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