

# Use of ForteBIO Octet for Quantitative Titer Analysis in Biopharmaceutical Development

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**Wyeth**  
BioPharma

# Outline

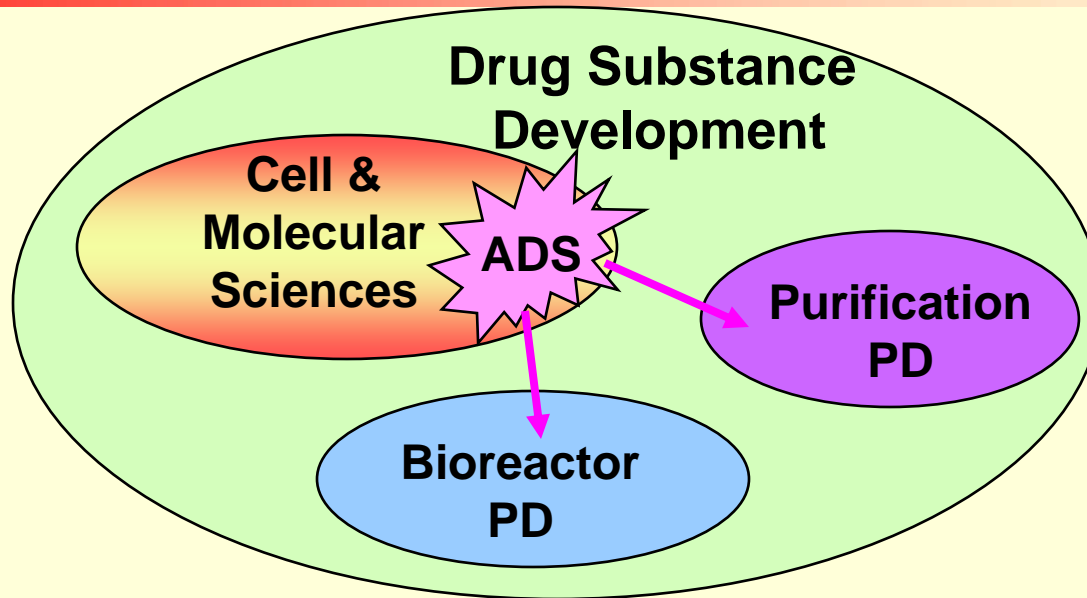
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- **Who we are and what we do**
- **Our interest in the the ForteBio Octet**
  - ▶ Ease of use
  - ▶ Efficiency
  - ▶ Throughput
- **Comparability to ProA HPLC for diluted high concentration antibody cell culture samples**
- **Comparability to immunoassay methods for antibody cell line screening**
- **Octet performance for some non-antibody quantitation**
- **Conclusions**

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# Who we are and what we do

# Analytical Development and Support Group

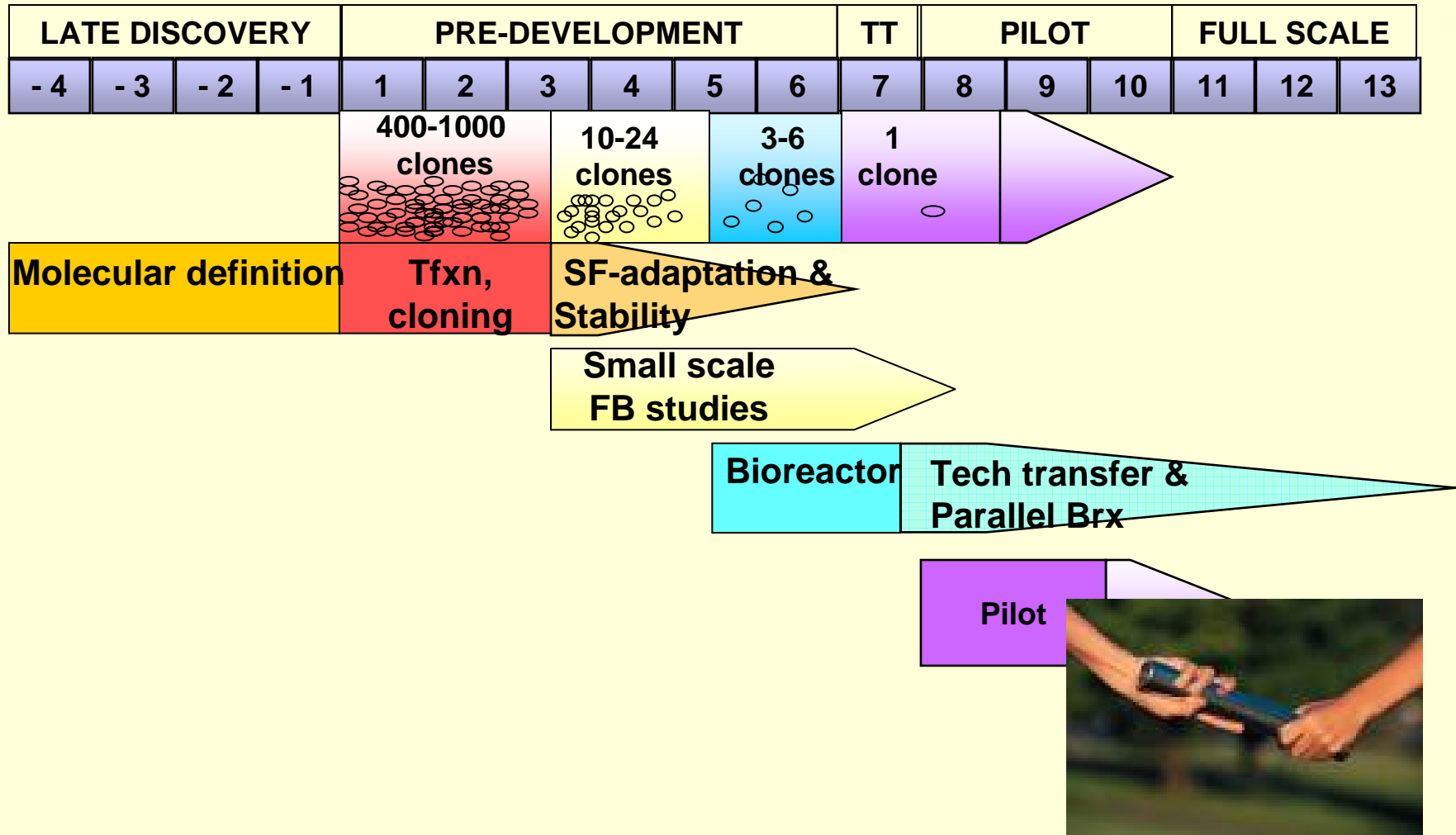


**Centralized, integrated group with analytical expertise**

**High-throughput and fastest turnaround possible**

**Centralization of equipment (use and maintenance)**

# Platform project timeline



ADS analytical development & support from (-)4 to 10 months

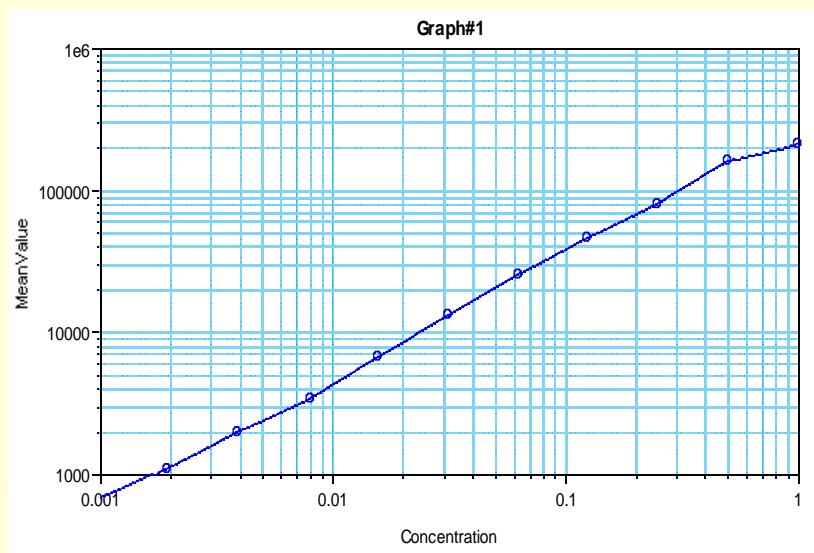
# ADS provides timely support for a broad spectrum of platform projects

- **Includes both antibody and some non-antibody biotherapeutics**
- **Platform rationale**
  - ▶ Aggressive timing enables more protein candidates in pipeline
  - ▶ Increases speed of getting molecules into clinic
  - ▶ Ensures minimal investment before clinical POC
- **ADS currently supporting 18 molecules**
  - ▶ Includes pipeline (6-7 new projects per year)
  - ▶ tech support
  - ▶ tech development
- **Diversity of sample matrices**
  - ▶ Serum-containing media, multiple serum free media

# Our existing methods for titer analysis

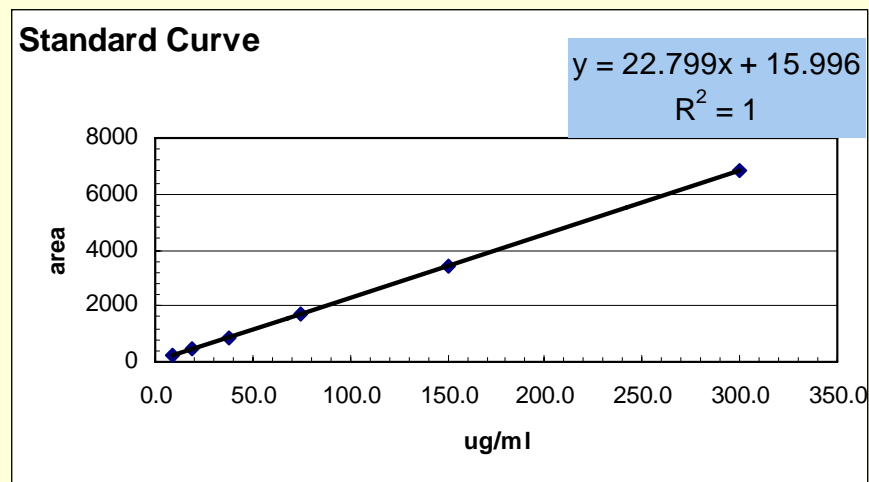
## • Bioveris and MSD

- ▶ Immunoassays with electrochemiluminescent detection
- ▶ Standard curve range 2ng/ml-1ug/ml
- ▶ Used for low concentration samples (under 300 ug/ml)
- ▶ Very sensitive, extensive sample dilution up to 1 in 3600
- ▶ Extensive data analysis



## • ProA HPLC

- ▶ Standard curve range 9.4ug/ml – 300ug/ml
- ▶ Used for diluted high concentration samples (over 300 ug/ml)
- ▶ Runs 8 min per sample, overnight run for 96 well-plate plate
- ▶ Extensive data analysis



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# Our interest in the the ForteBio Octet

Ease of use

Efficiency

Throughput



## We became interested in the Octet as a quantitative assay platform

- ▶ Even with extensive use of automation on both assay platforms, we were often running at or above capacity
- ▶ Requests for same day turnaround of critical process steps were increasing
- ▶ Opportunity existed in the laboratory for rapid, accurate titer assessment
  - Quick turnaround samples to support process development
  - Troubleshooting
  - High-throughput screens (HTS)
- ▶ Octet biosensor” technology attractive due to rapid per-plate “run time”
- ▶ Conducted a one-week assessment of Octet performance, focused on Pro A sensors for Ab titer
- ▶ Strong performance in demo led to instrument purchase
- ▶ Octet now in use for routine and *ad hoc* titer determinations and for certain screens of cell culture samples

# Protein A BioSensors used with Octet

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- **for this application, we are focusing on ProA BioSensors**
  - ▶ Streptavidin coated and amine reactive BioSensors are also available
- **we are focusing on quantitative analysis, a kinetic analysis instrument is also available**

# The Octet offers us:

- **standard curve range 1-500ug/ml,**
  - ▶ wider range than proA-HPLC
  - ▶ can use the same dilutions we have automated on the Tecan for ProA HPLC
  - ▶ low concentration samples can be run with little or no dilution
  - ▶ significant labor saving over immunoassay dilutions
- **set-ups of standards and controls similar to ProA-HPLC**
  - ▶ additional labor saving over immunoassay
- **8 samples analyzed at once**
- **one full plate = approx 30 minutes**
  - ▶ have results hours sooner than immunoassay or HPLC methods
- **data analysis automated in software, we need only to assemble**
- **holds one plate at a time**
- **requires minimum of 100ul sample**

# Octet system has small footprint

- required 18 inches of bench, plus PC

Related?



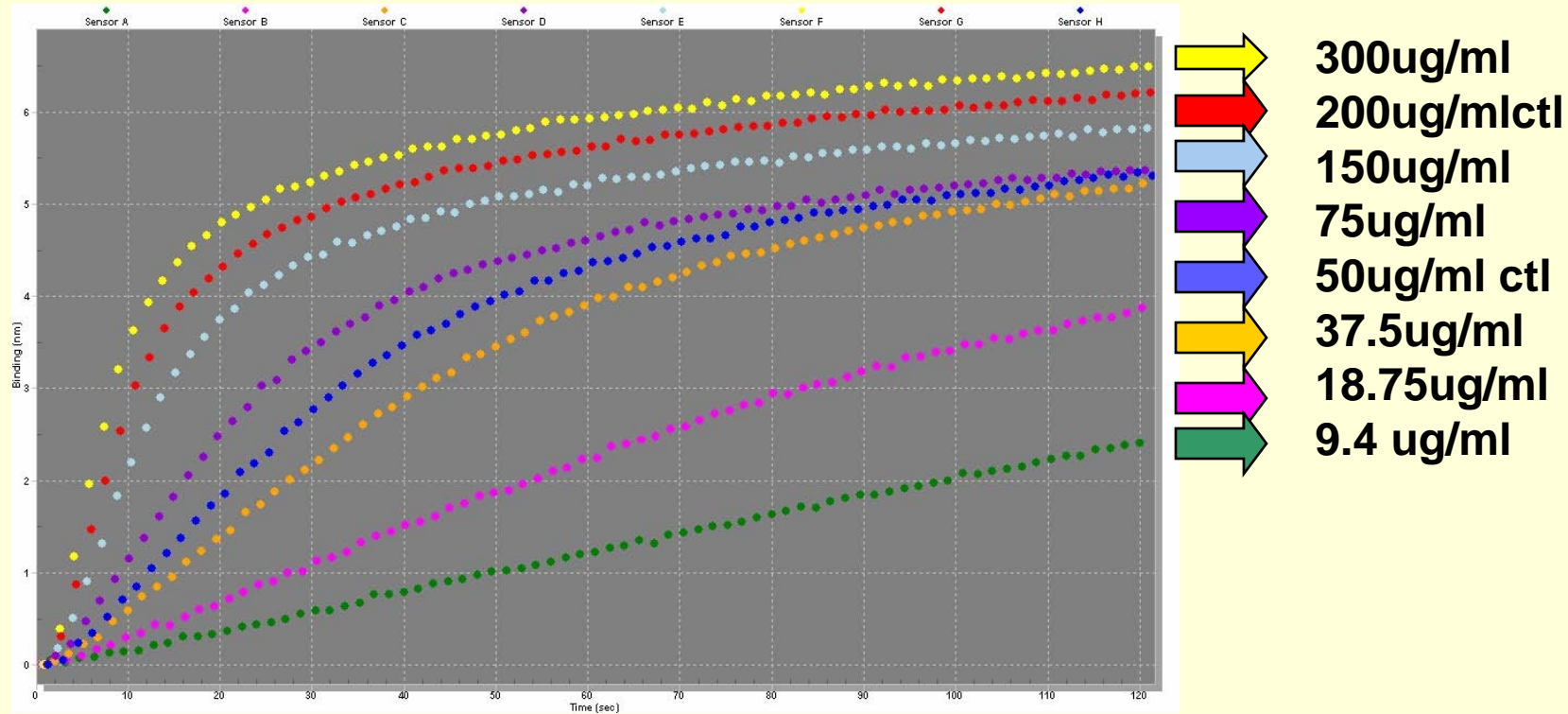
# Good well to well consistency for entire plate 6 % CV

- Same antibody sample in all wells

25.88	28.38	26.13	27.87	26.46	27.44	26.86	27.61	27.28	28.06	26.8	19.72
30.78	28.59	27.35	27.07	25.67	26.44	25.33	26.99	28.23	26.79	26.64	26.92
31.22	27.82	28.88	27.53	26.87	26.86	25.95	27.74	29.77	27.94	27.81	28.46
29.61	29.18	27.22	28.41	26.19	27.7	26.64	27.07	27.55	25.74	27.69	27.11
31.38	29.25	29.31	27.85	27.16	22.3	26.64	28.43	27.51	27.49	28.15	28.84
31.08	29.62	29.04	28.08	27.54	28.24	28.06	28.31	27.49	26.77	27.81	28.78
31.12	30	29.06	27.83	27.75	28.26	27.42	27.94	26.62	26.82	28.48	27.72
27.33	29.96	28.05	28.56	26.77	28.64	28.8	26.98	26.82	25.63	26.09	21.32

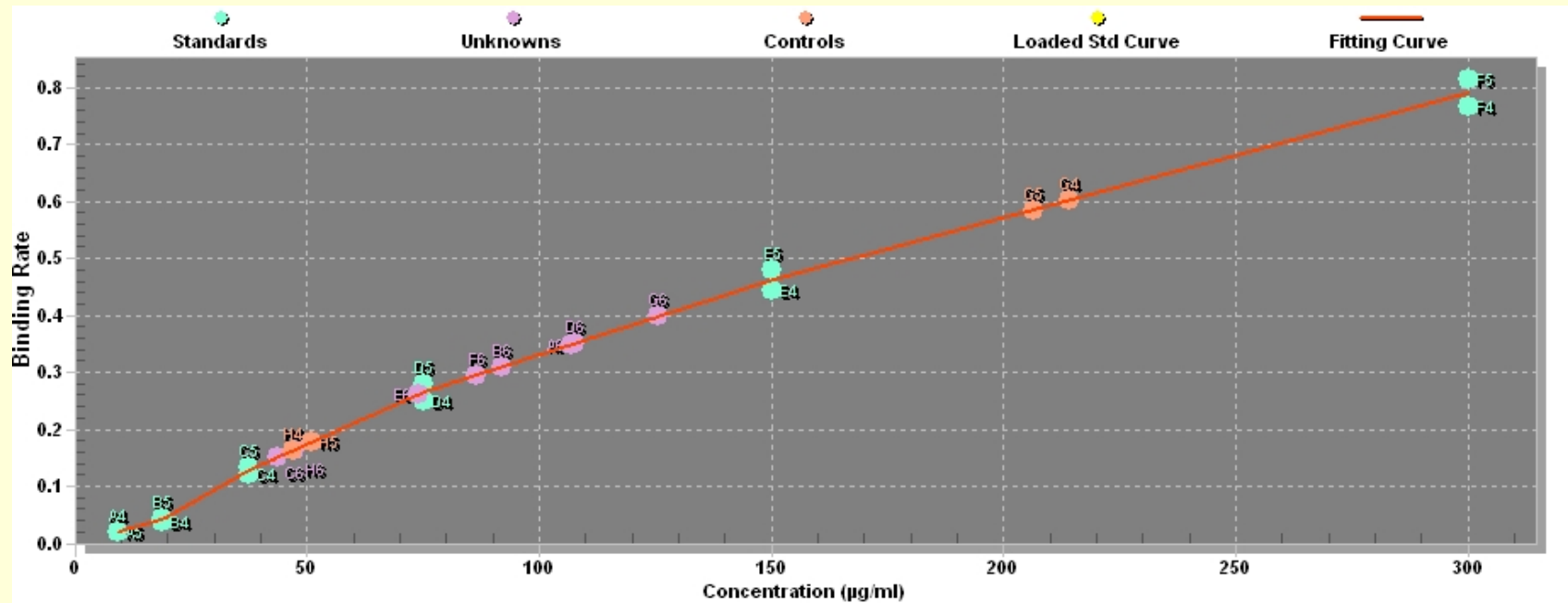
<b>High</b>	<b>over 31</b>
<b>Med-High</b>	<b>29-30</b>
<b>Medium</b>	<b>27-28</b>
<b>Medium-Low</b>	<b>25-26</b>
<b>Low</b>	<b>under 25</b>
<b>mean</b>	<b>27.6</b>
<b>%CV</b>	<b>6.3</b>

# Standard Curve Binding Rate Data on Octet



- Raw data is in the form of a binding rate
- Measured over a period of 120 seconds from the time the BioSensor is dipped in sample

# Octet software generates a plot of standards and samples (antibody)

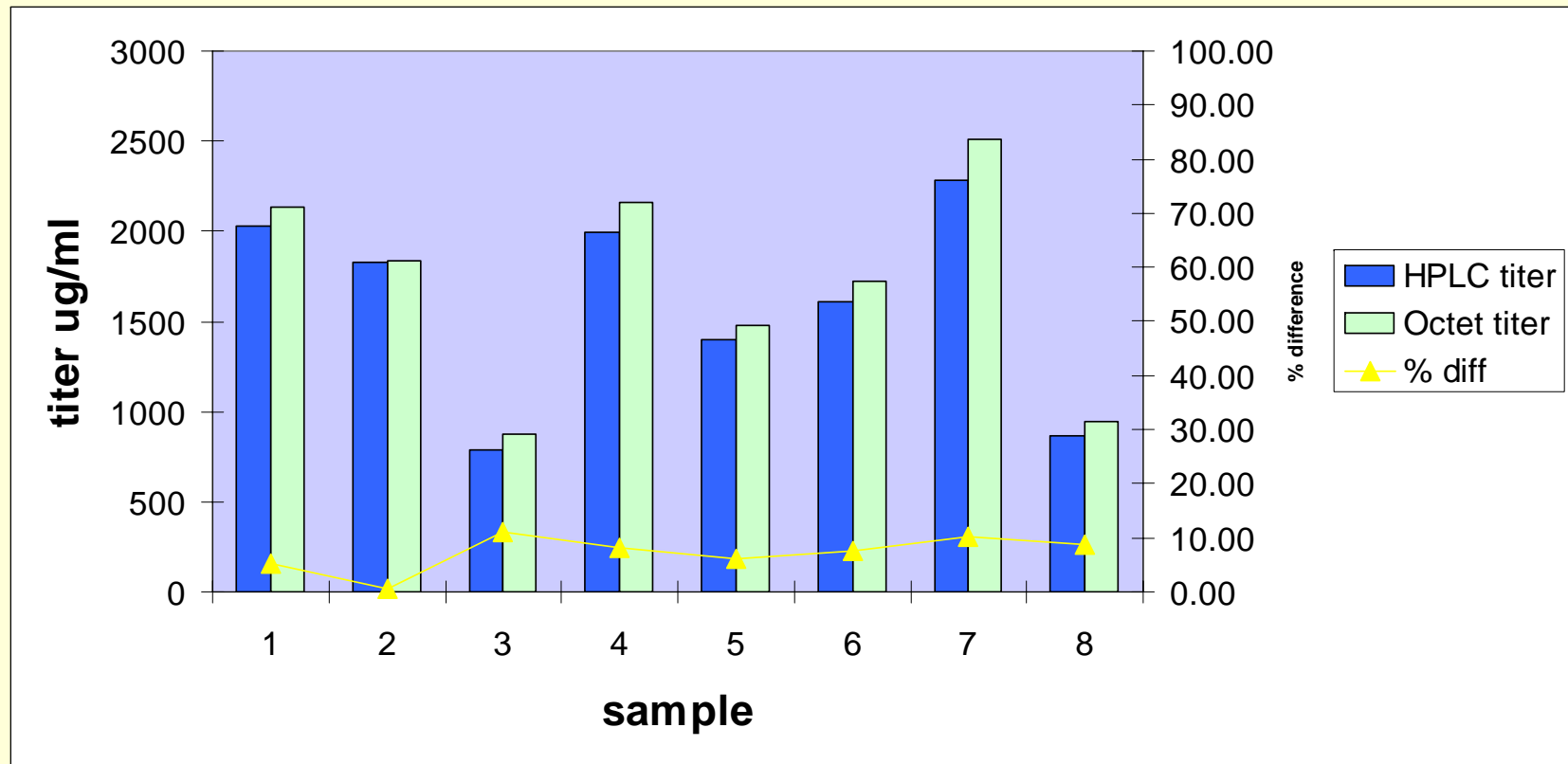


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# **Comparability to ProA HPLC for diluted high concentration antibody cell culture samples**



# Octet results are comparable to ProA-HPLC results

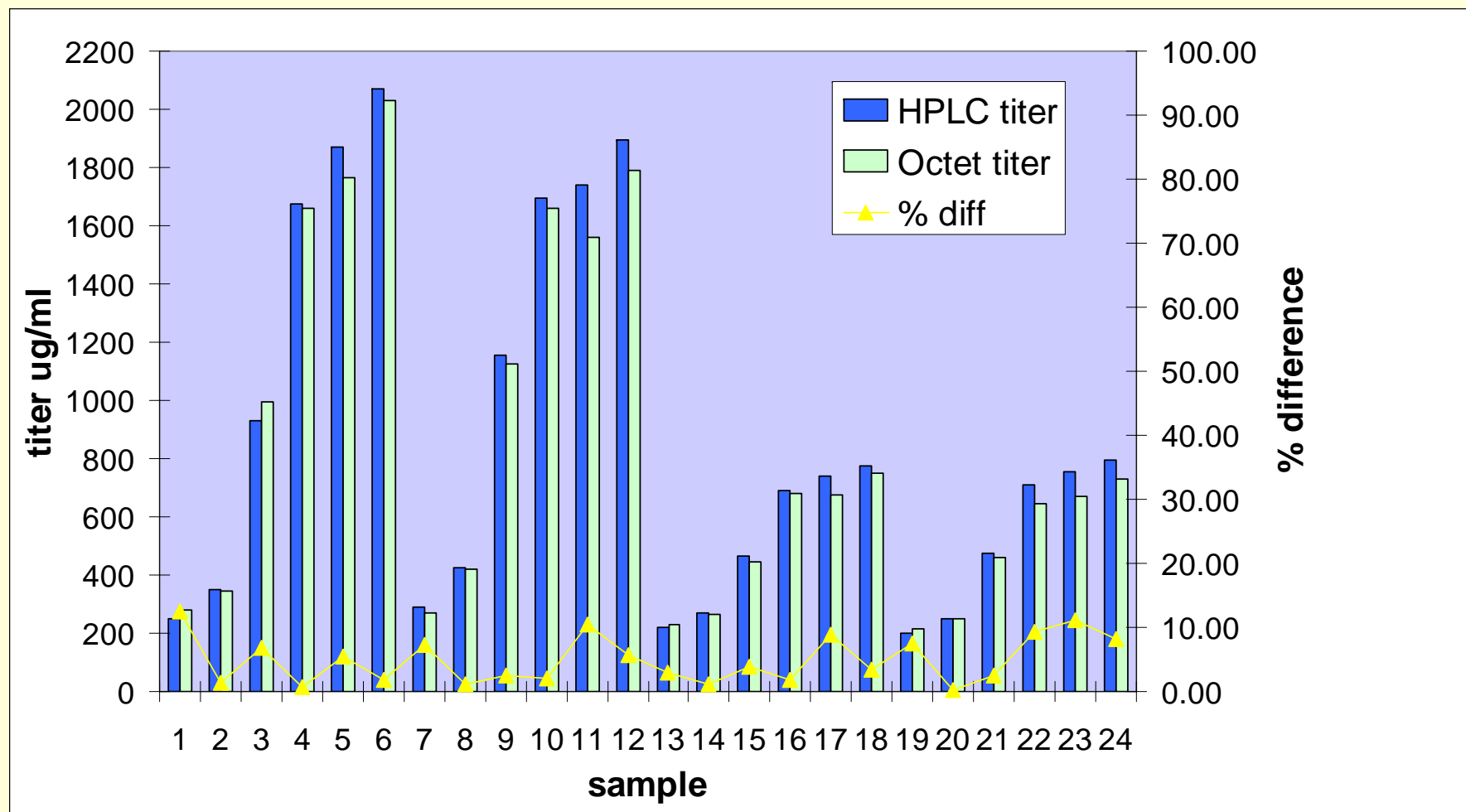


All samples dilutions are the same for both platforms

mAb # 1

# Comparable results for an additional antibody

## ProA-HPLC vs. Octet



Octet results n=2  
mAb #2

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# **Comparability to immunoassay methods for antibody cell line screening**

# High-throughput screening of cell lines

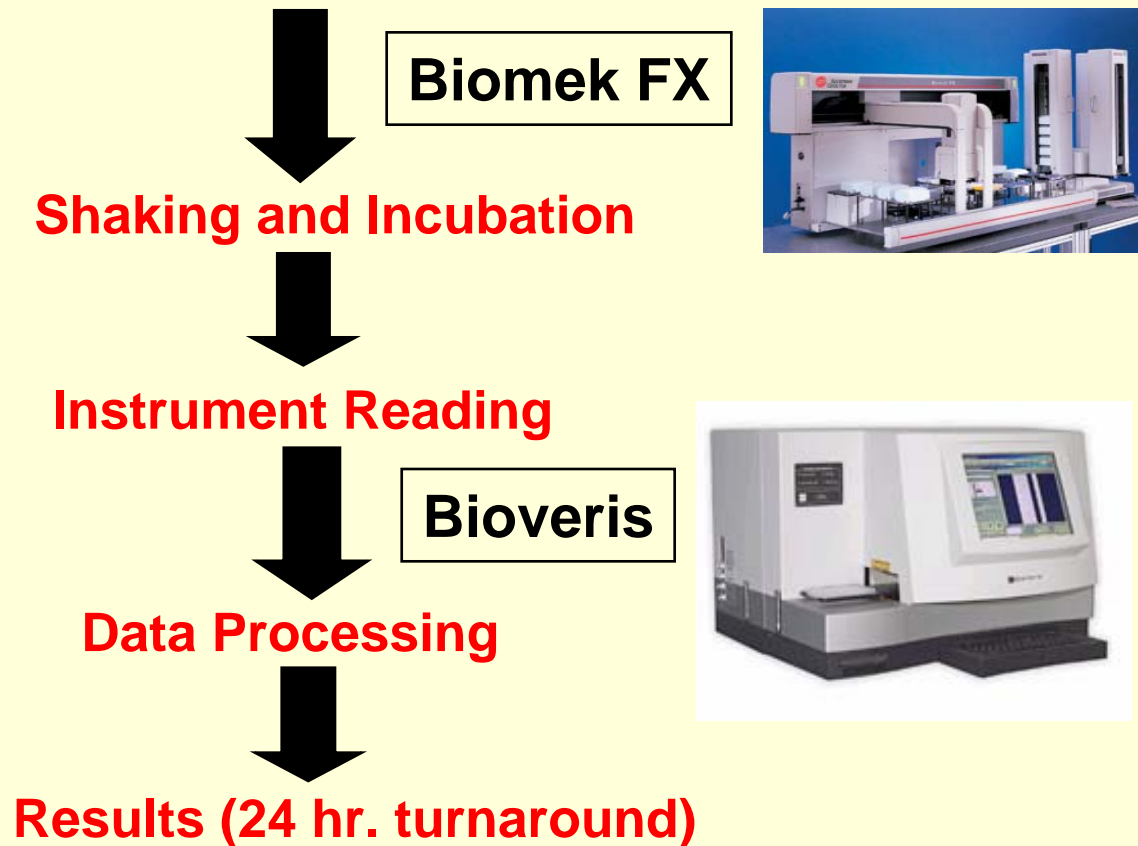
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- a typical screen is 720 low titer cell culture samples (under 300 ug/ml)
- samples may be in serum-containing medium
- limited sample volume, often 100 ul
- rapid generation of results reduces time required to maintain low producing cultures
- 90% of clones are winnowed with primary screen
- high quality assays enable meaningful comparisons among projects

**Existing High-throughput assay already developed:  
Automation employed to deliver next day results**

## **Biomek FX Station combined with Bioveris (IGEN)**

**Sample Dilution and Reagent Distribution  $\leq$  1500 samples**

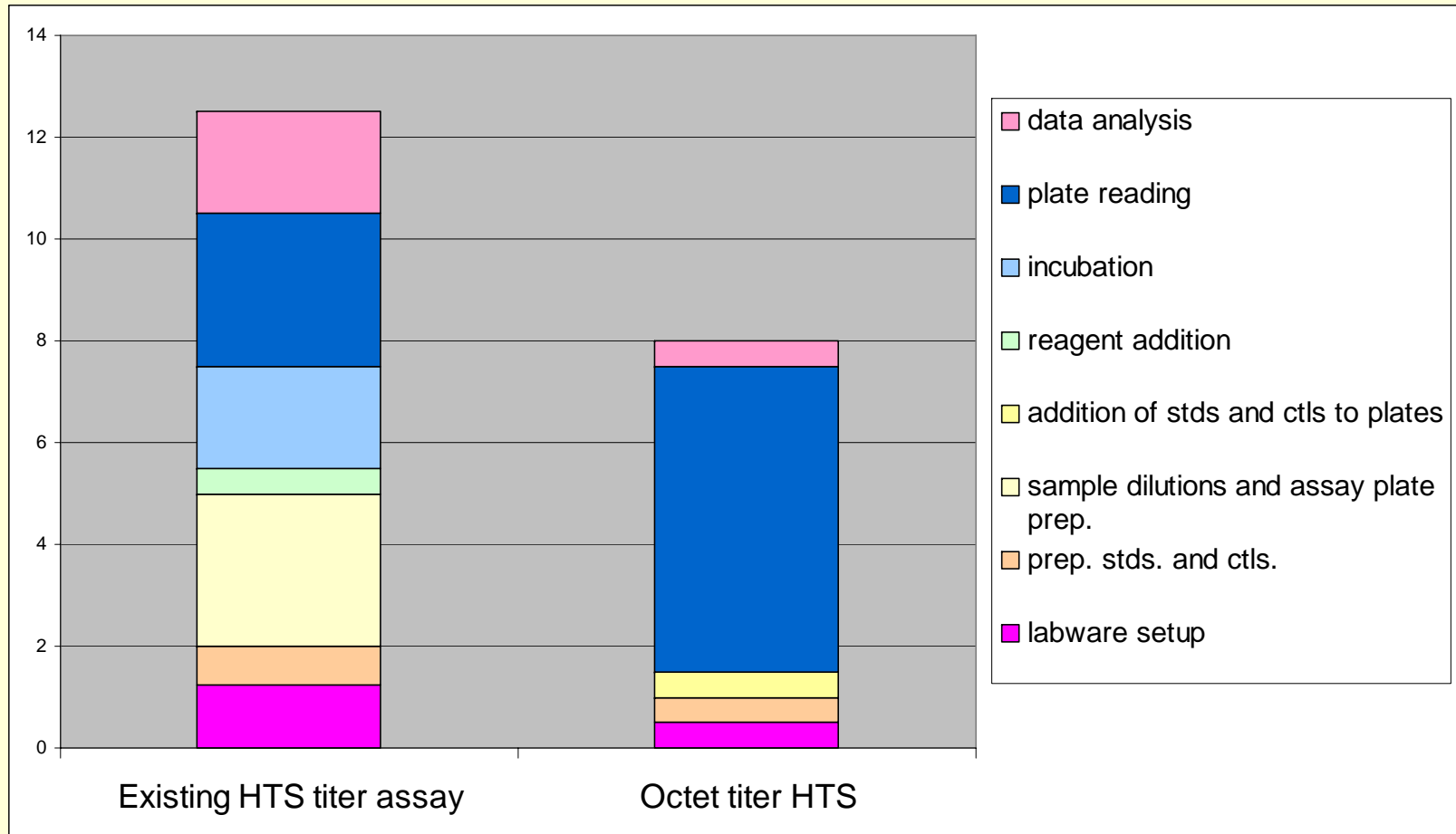


# **Octet allows even faster turnaround of results for primary screening of cell lines**

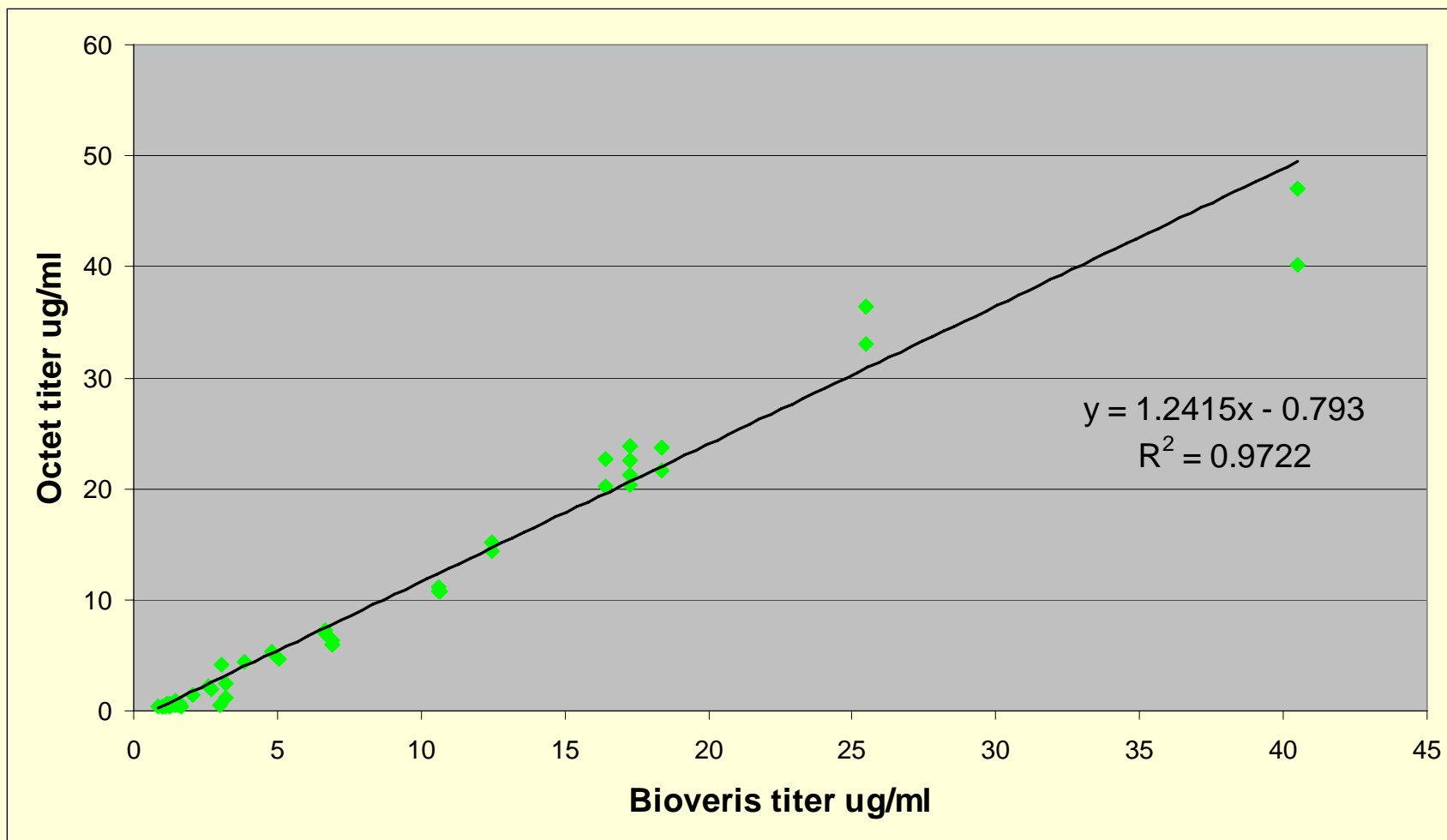
- **No sample dilution for cell culture screening samples from 1- 150 ug/ml**
- **30 min per plate read time**
- **Immediate and automatic data analysis**
- **Working toward automating the plate/biosensor loading with a stacker and arm for a fully walk-away assay**
- **100 ul sample req. with half area micro-titer plates**
- **Multiple projects can be run on same day**
- **One analyst can run screens on consecutive days**
- **Same day turnaround of results for 720-900 samples**

# Octet HTS titer assay: time and labor savings

Time hrs.



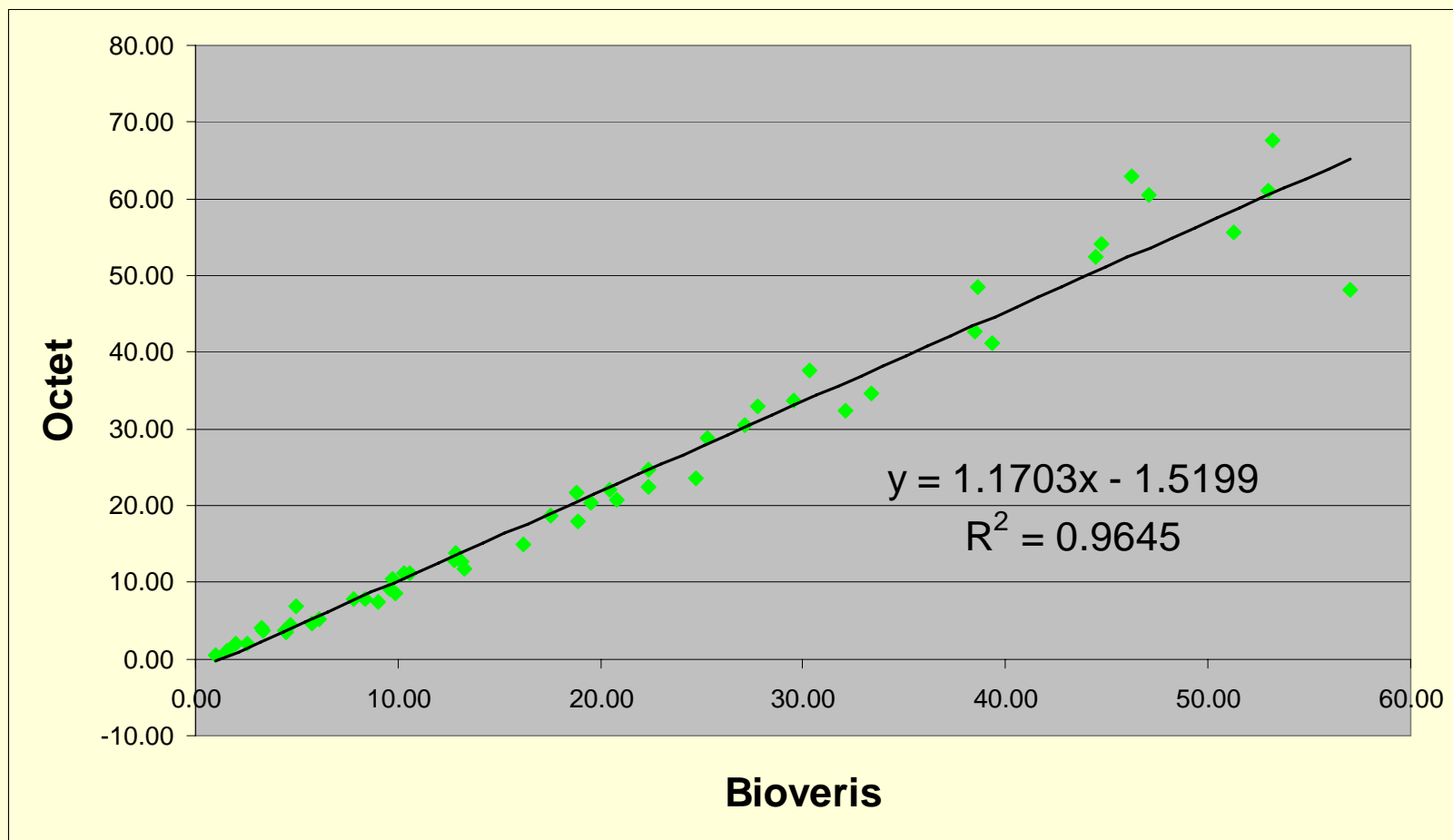
# HTS: good correlation BioVeris vs. Octet



mAb # 3

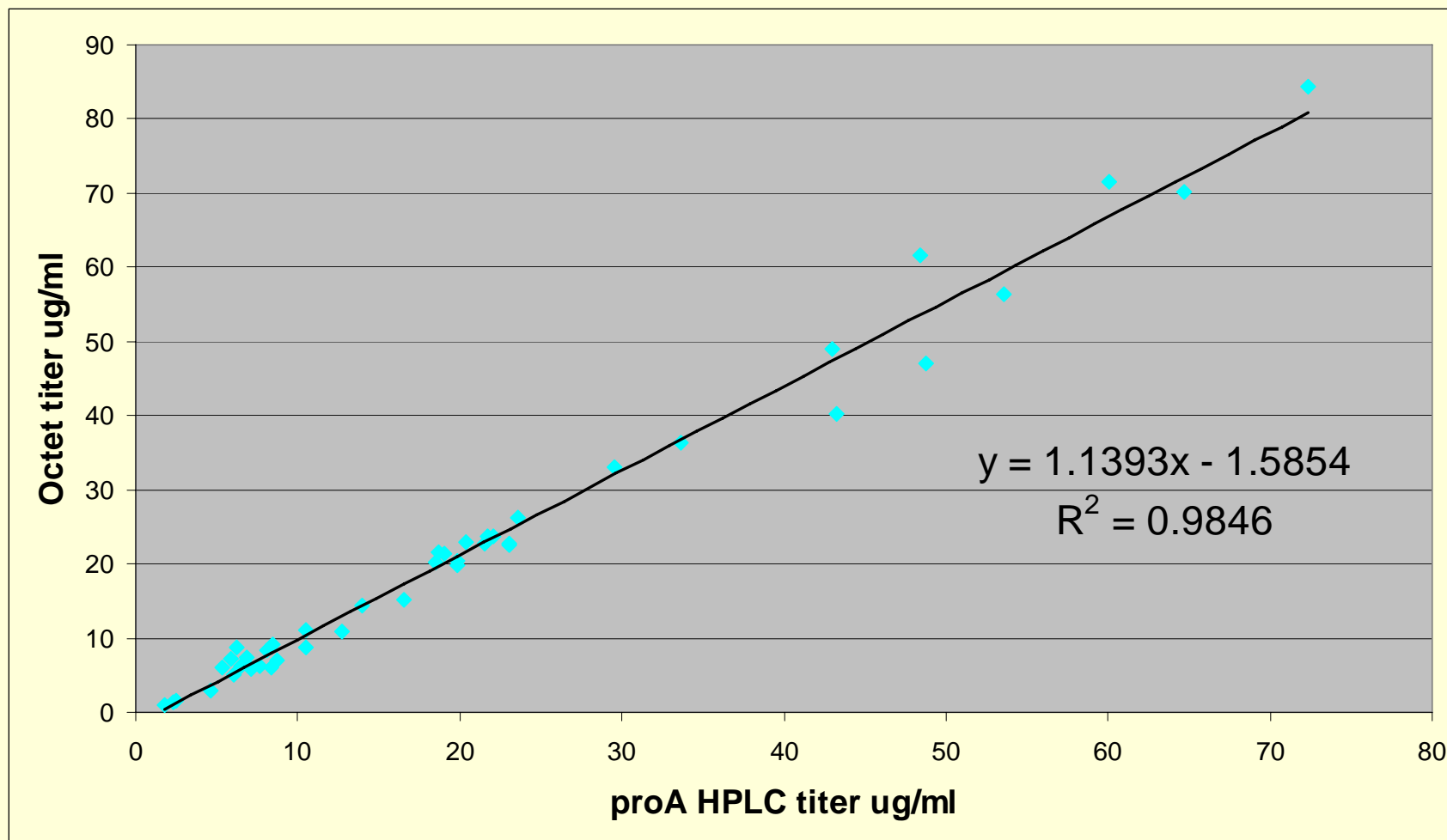


# HTS: another good correlation BioVeris vs. Octet



mAb # 4

# HTS: excellent correlation of ProA-HPLC and Octet results



mAb # 3

## Controls performed well throughout 10-plate screen on Octet

Controls	150	50
P1 row A	129.4	47.8
P1 row H	138.4	53.6
P2 row A	130.2	46
P2 row H	134.3	47.7
P3 row A	138	45.2
P3 row H	152.3	48.6
P4 row A	135.7	44.8
P4 row H	137.9	46.1
P5 row A	147.8	45.3
P5 row H	139.4	48.6
P6 row A	123.4	45.1
P6 row H	141.4	46.2
P7 row A	125.5	47.5
P7 row H	136.2	51.2
P8 row A	131.2	41.9
P8 row H	130	45.1
P9 row A	140.6	44.2
P9 row H	137.8	45.2
P10 row A	133.8	45.2
P10 row H	147.4	46.6

mean	137	47
% recovery	91	93
%CV	5.4	5.5

- Controls consistently met our acceptance criteria for recovery
- Low variability seen in controls 5-6 %CV

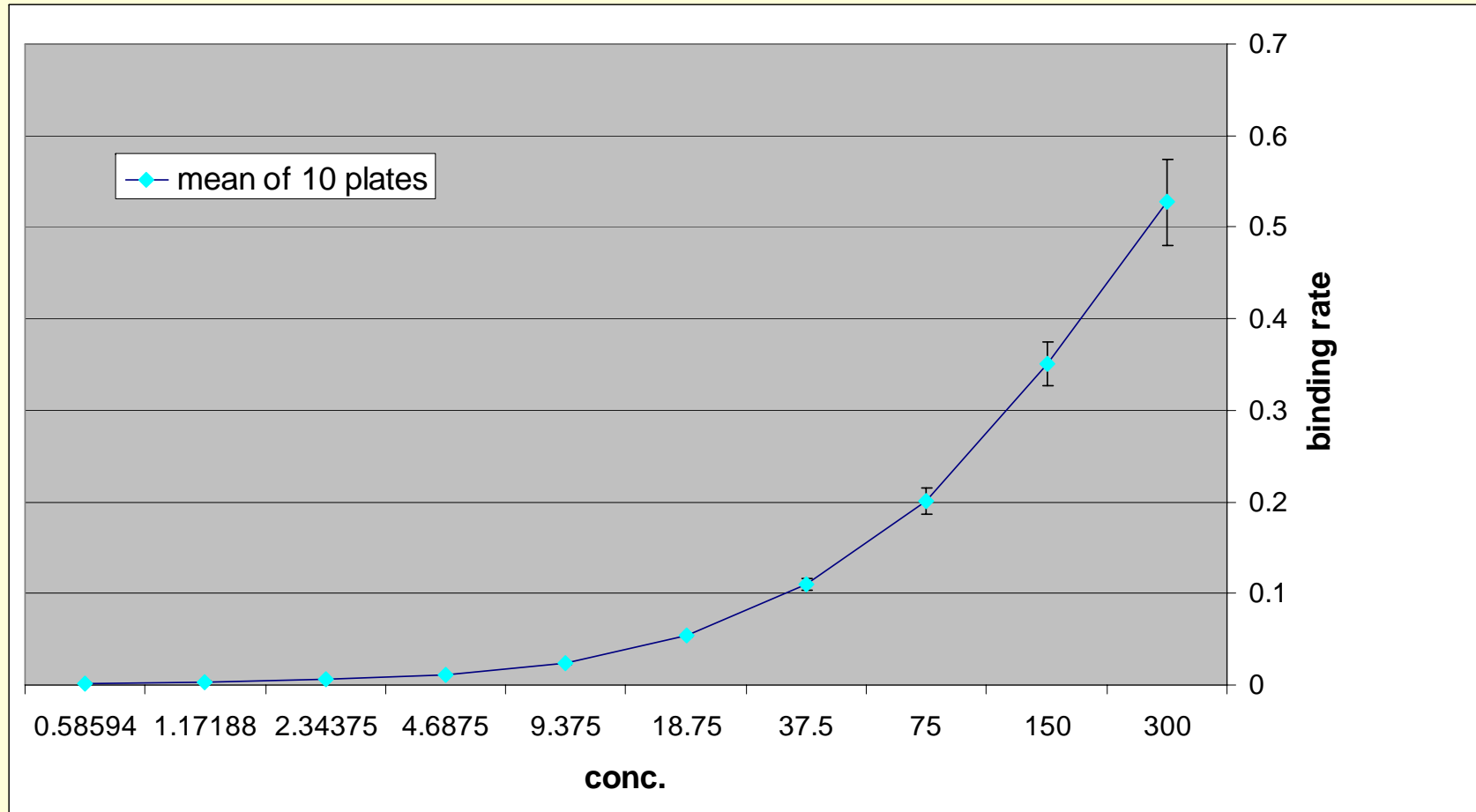
# Does each plate require a standard curve?

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- **Could standards be run once per assay and imported to each assay plate?**
- **Each plate would contain controls**
- **Significant savings on reference material usage**
- **Cell line screening typically performed in early development when reference material is in limited supply**
- **More “seats on the plane” available for samples**

# For screening applications: No

## Mean of 10 standard curves for an antibody



16 %CV at 150 ug/ml

mAb # 3

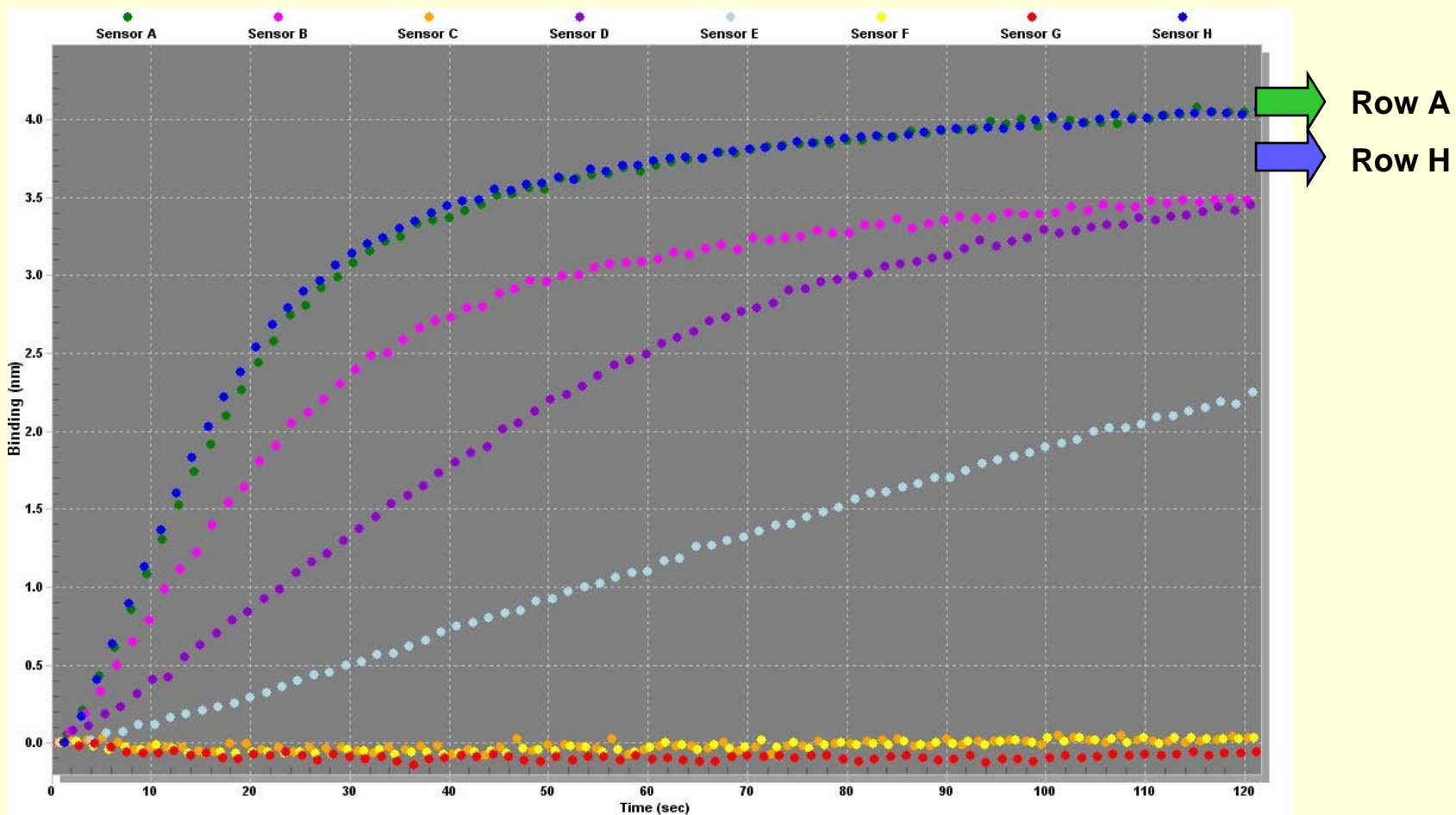
# Observations to consider at high concentrations

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- 8 BioSensors are read in rapid sequence throughout the 120 second read time
- Read starts with row A and ends with row H
- A small amount of time elapses between A and H readings
- This only becomes apparent at high concentrations when a significant amount of binding has taken place during that delay
- We “bracket” our samples by placing them between row A standards and row H standards for optimum accuracy
- For antibodies, we find the most accurate range is below 150 ug/ml
- Some added variability above that range

# Binding curves for 75 ug /ml standard

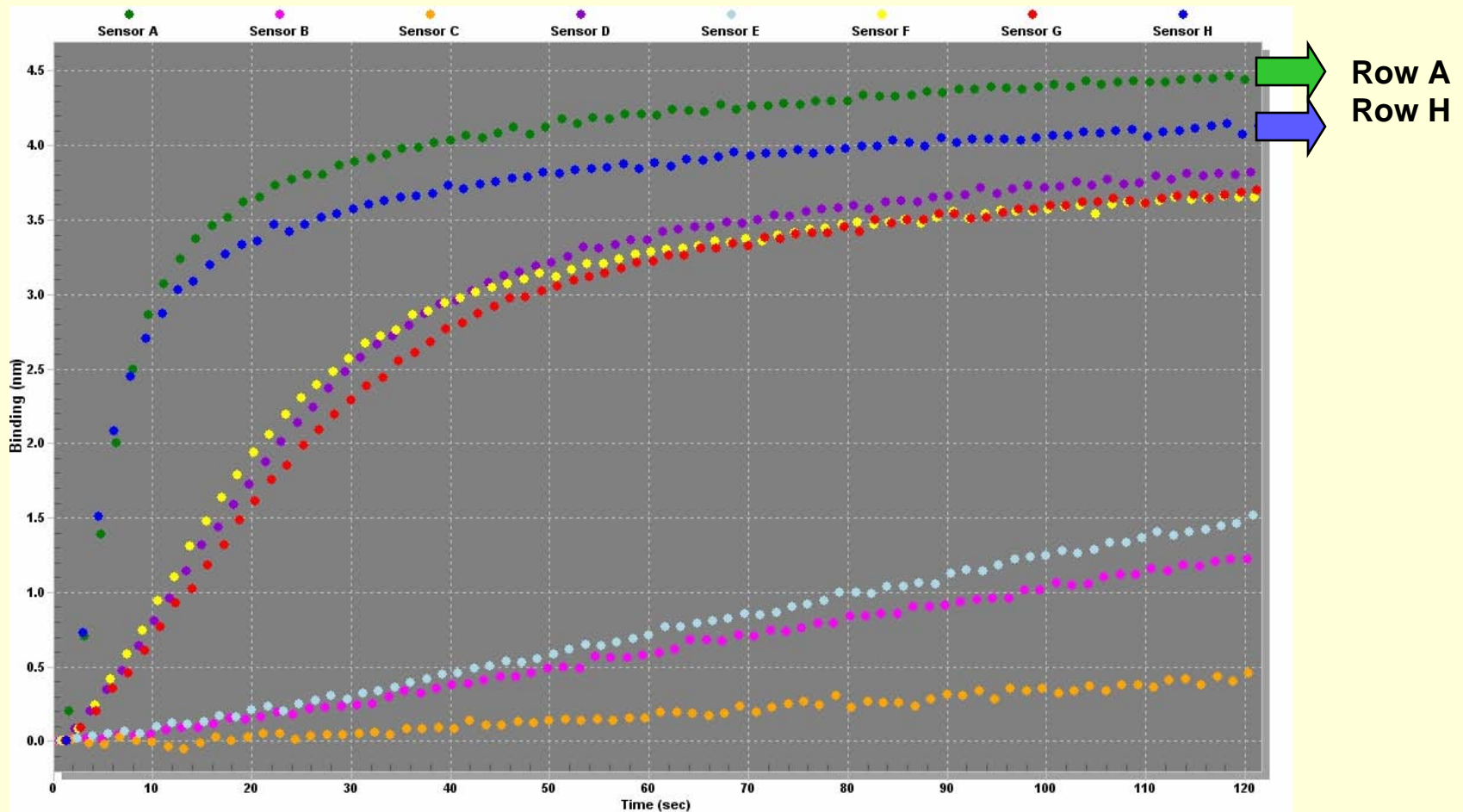
Standards Row A (green), Row H (blue)



mAb # 3

# Binding curves for 300 ug /ml standard

## Standards Row A (green), Row H (blue)

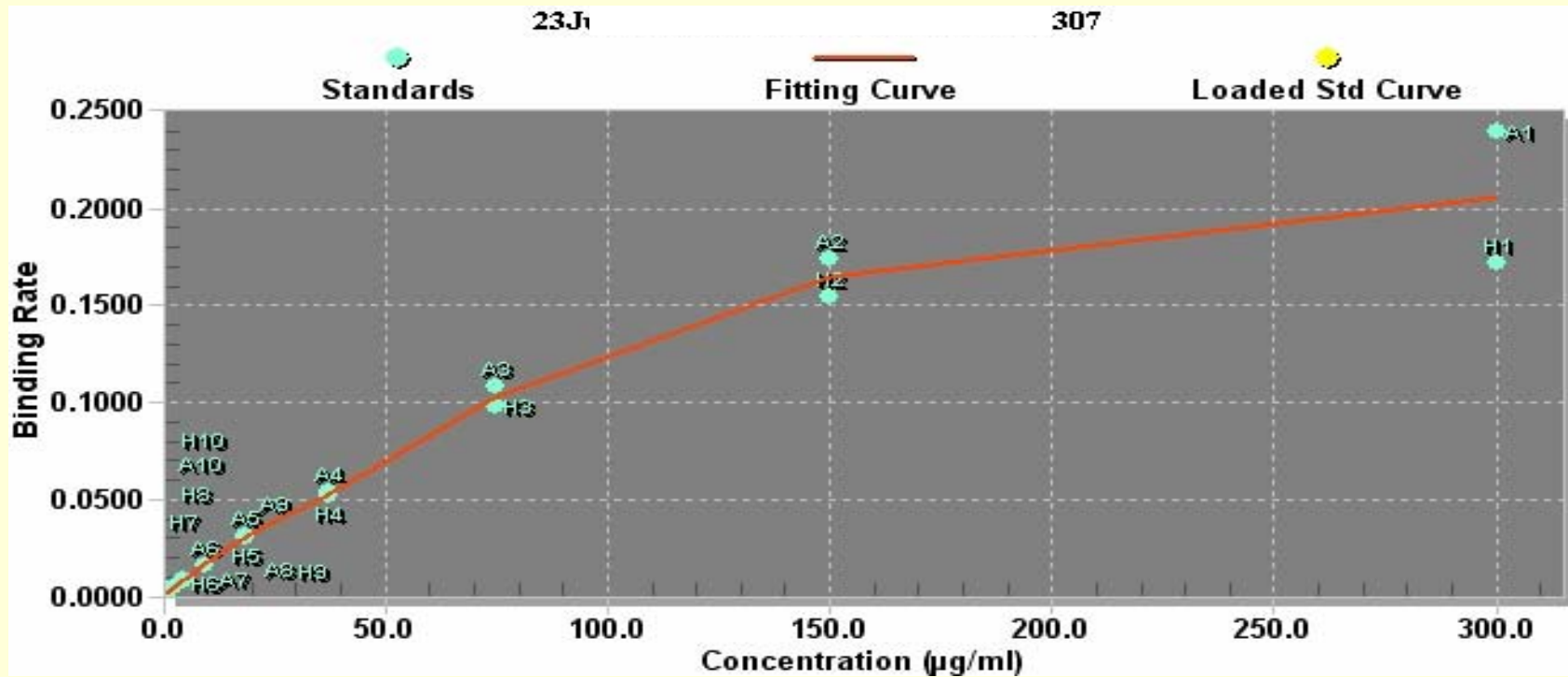


mAb # 3

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BioPharma



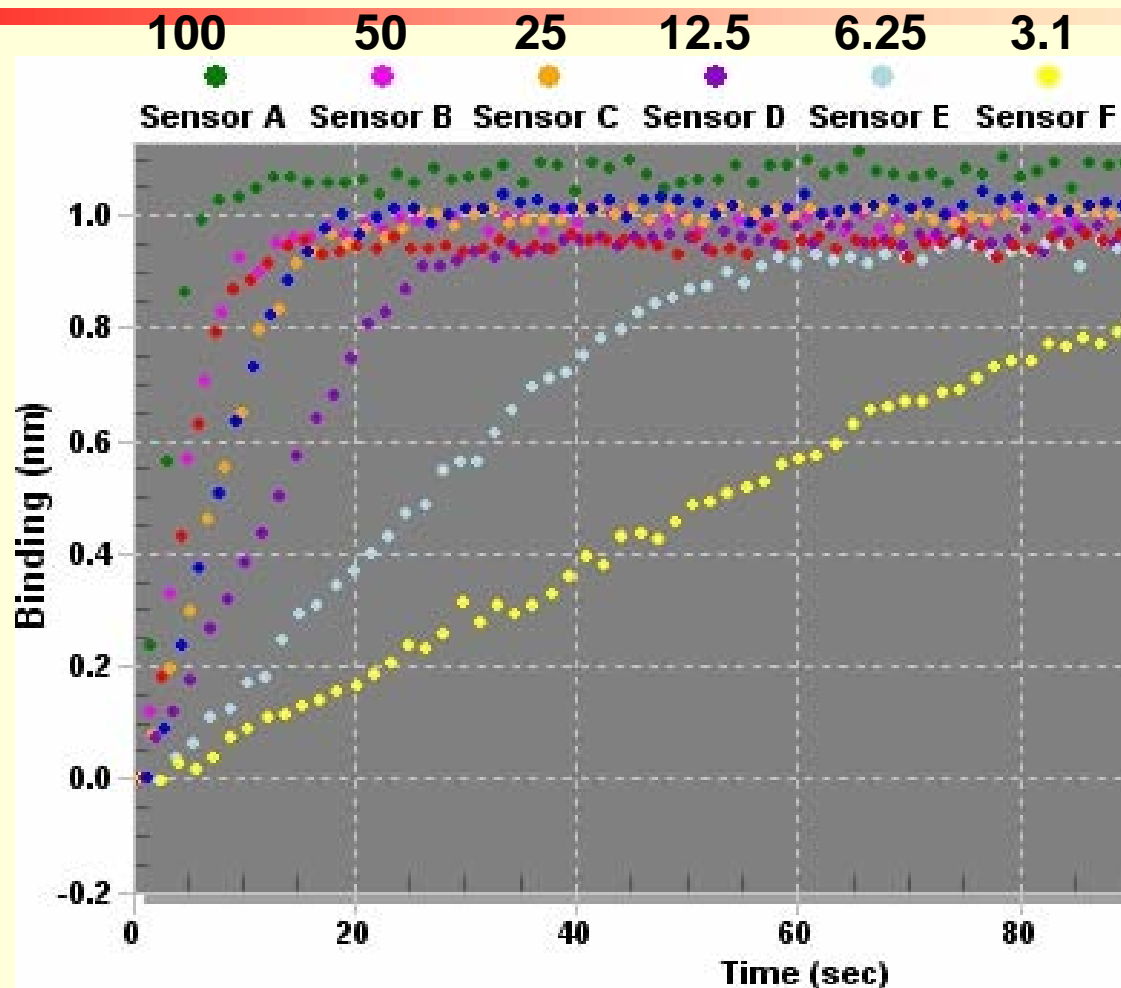
# An extreme case of the offset between row A and row H at higher concentration



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# **Octet performance for some non-antibody quantitation**

# A Fourth Case: Binding Rate “Too Fast” for Octet



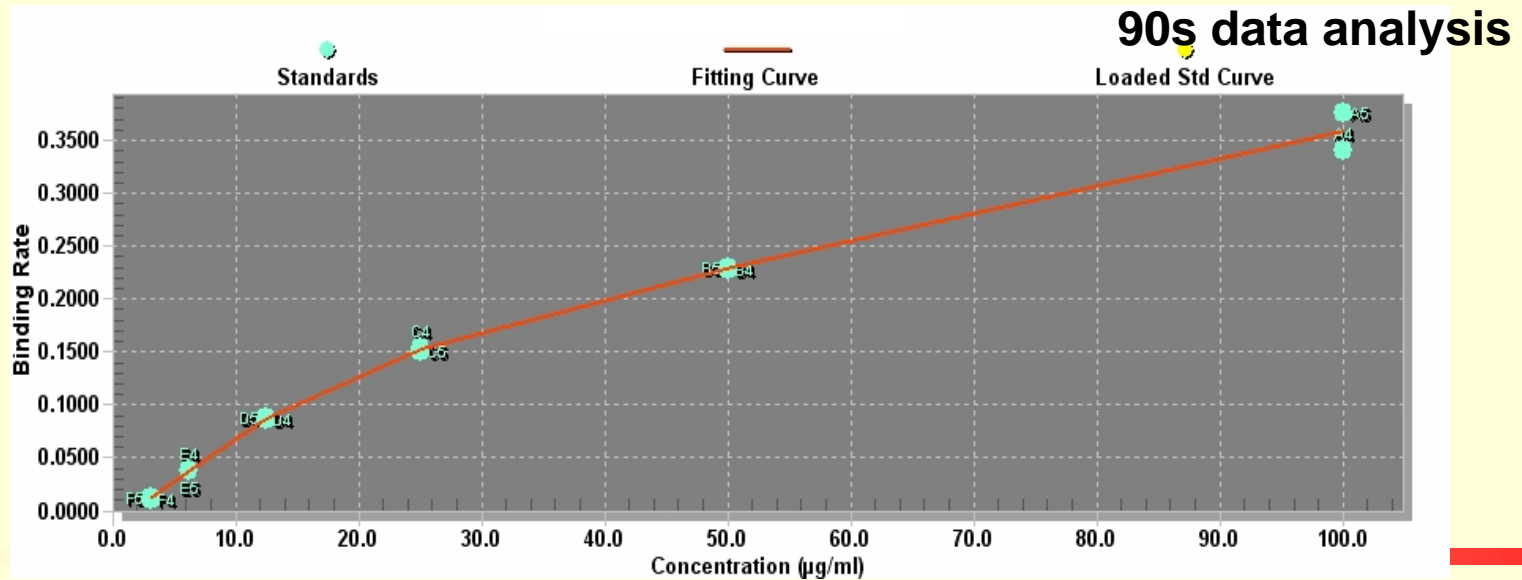
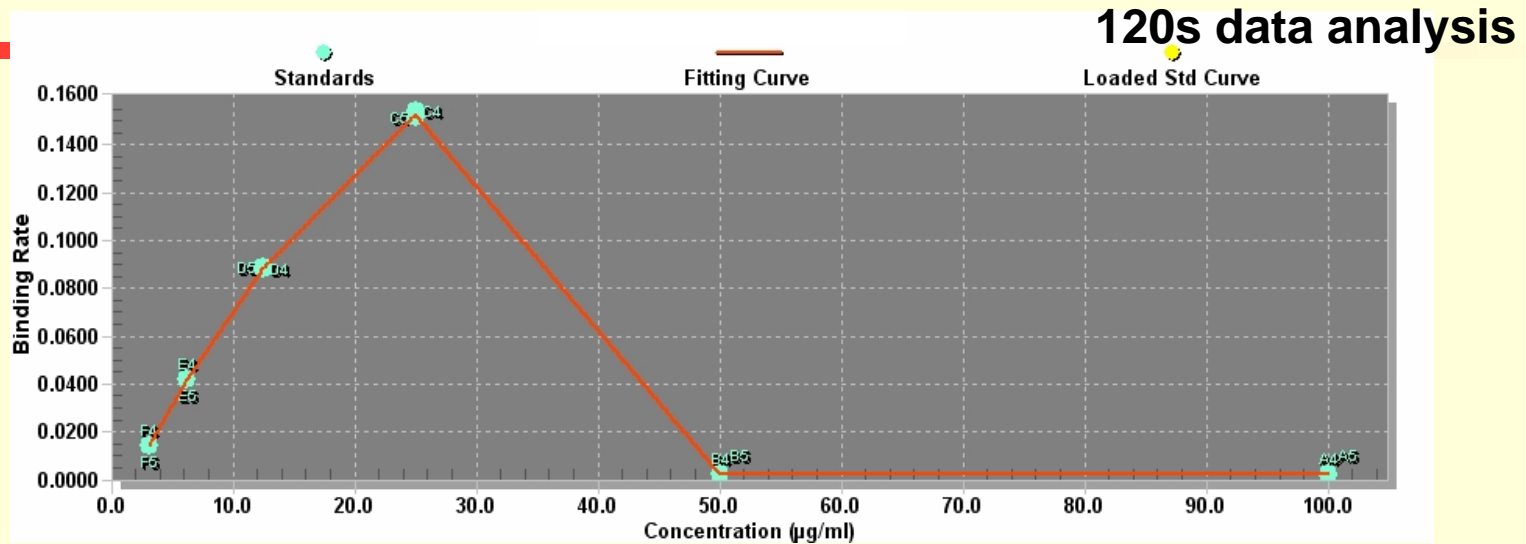
non-antibody protein #5

## **Data analysis for binding rate for non-antibody #5**

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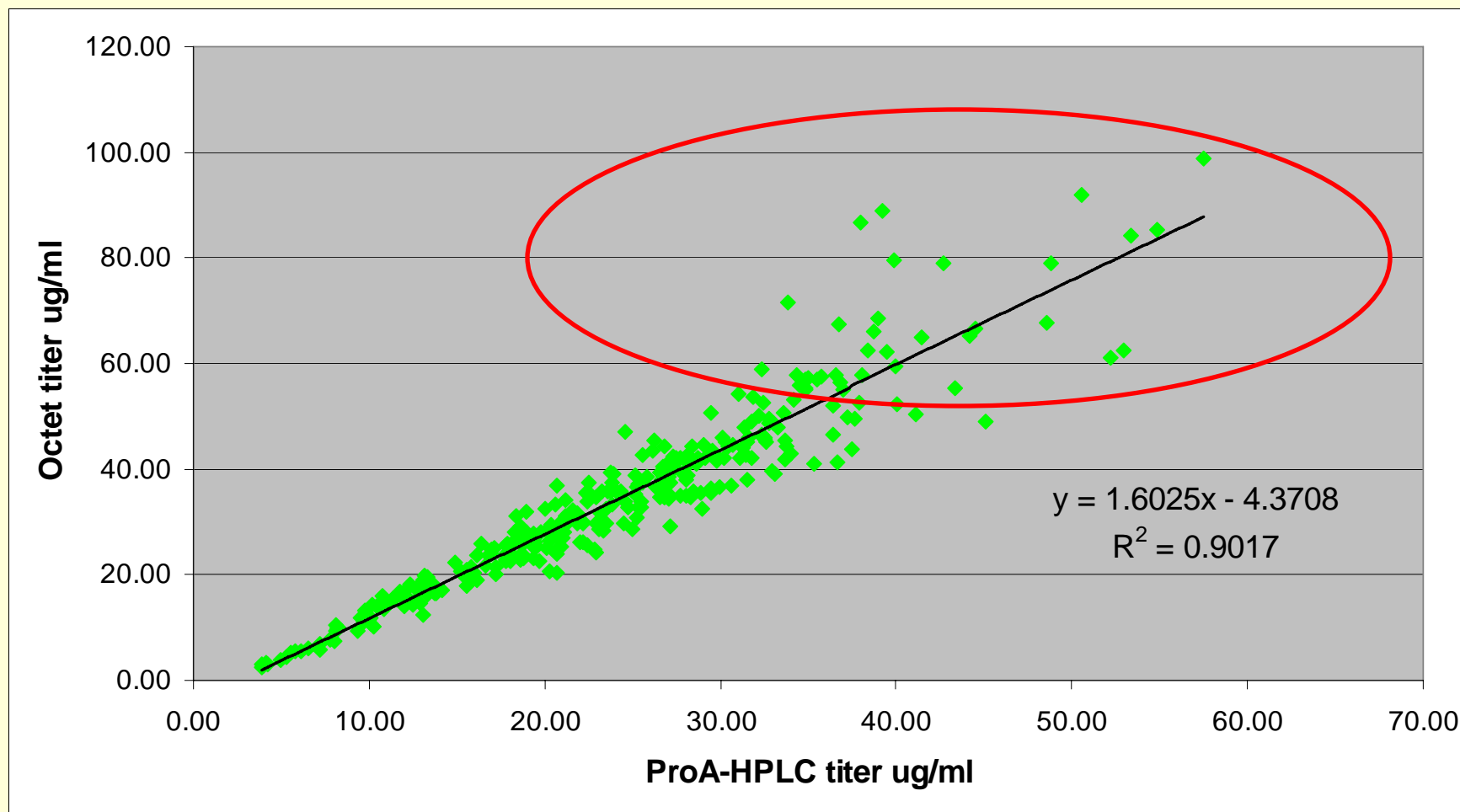
- **Binding curve at high concentration may have too few points in the sloped region relative to the plateau region**
- **Solution 1: diluted samples to fall in the lower range on the standard curve**
- **Solution 2: reduce data analysis time to shorten plateau region**

# Quick Fix: shorten data analysis time to 90 s.



non-antibody protein #5

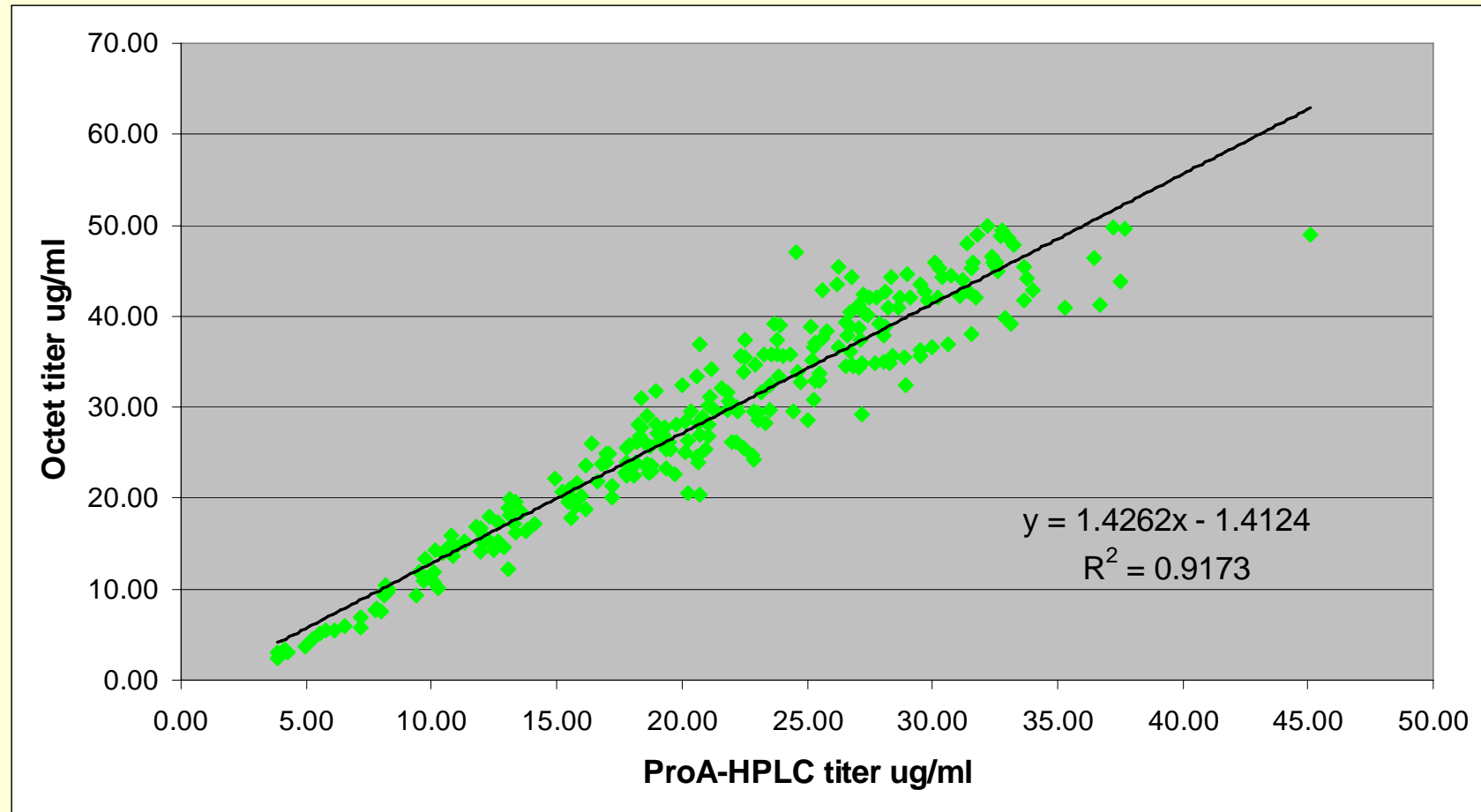
## Correlation of Octet vs ProA-HPLC results shows some variability in the region over 50 ug/ml (Octet)



non-antibody protein #5

# Desirably tight correlation when re-plotted showing only results under 50 ug/ml (Octet)

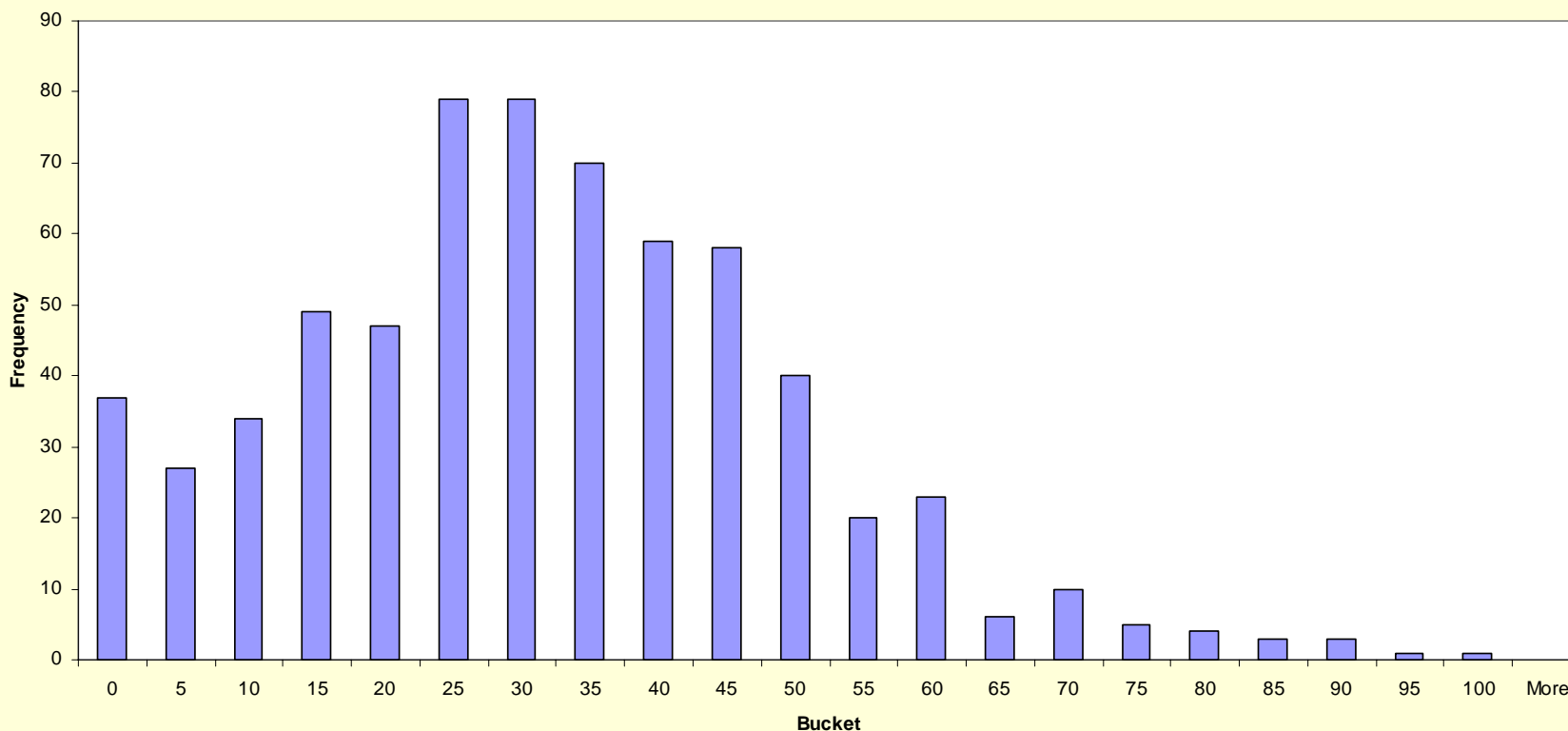
**Slope of 1.4 shows significant offset in values.**



**non-antibody protein #5**

# Large screen of cell lines successfully performed for our rapid-binding non-antibody

Distribution of HTS titer results for protein #5 (non-antibody)





# Conclusions

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- **Octet instrument was successfully used for quick turnaround ProA-based titer analysis of diluted, high concentration, in-process cell culture samples**
- **Octet performed successfully for primary titer screening of 720 cell lines with same day turnaround of results**
- **Strong linear correlation seen between Octet assay results, and HPLC and BioVeris /IGEN results**
  - ▶ Occasional offset in values
  - ▶ Allows us to select assay platform based on desired use of the data
- **Best assay performance for antibodies was in the range of 1-150 ug/ml standard curve**
- **Standard curve range may need to be adjusted on a case-by-case basis for non-antibodies**
- **Octet offers considerable time savings over both Pro A HPLC and BioVeris for quantitative titer analyses**

# Acknowledgements

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- **Marty Sinacore**
- **Tim Charlebois**
- **Jeff Deetz**
- **DSD**
  - ▶ Cell and Molecular Sciences
  - ▶ Bioreactor process development
  - ▶ Purification process development