



Raven biotechnologies, inc.

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Determining IgG Concentration by Bio-Layer Interferometry

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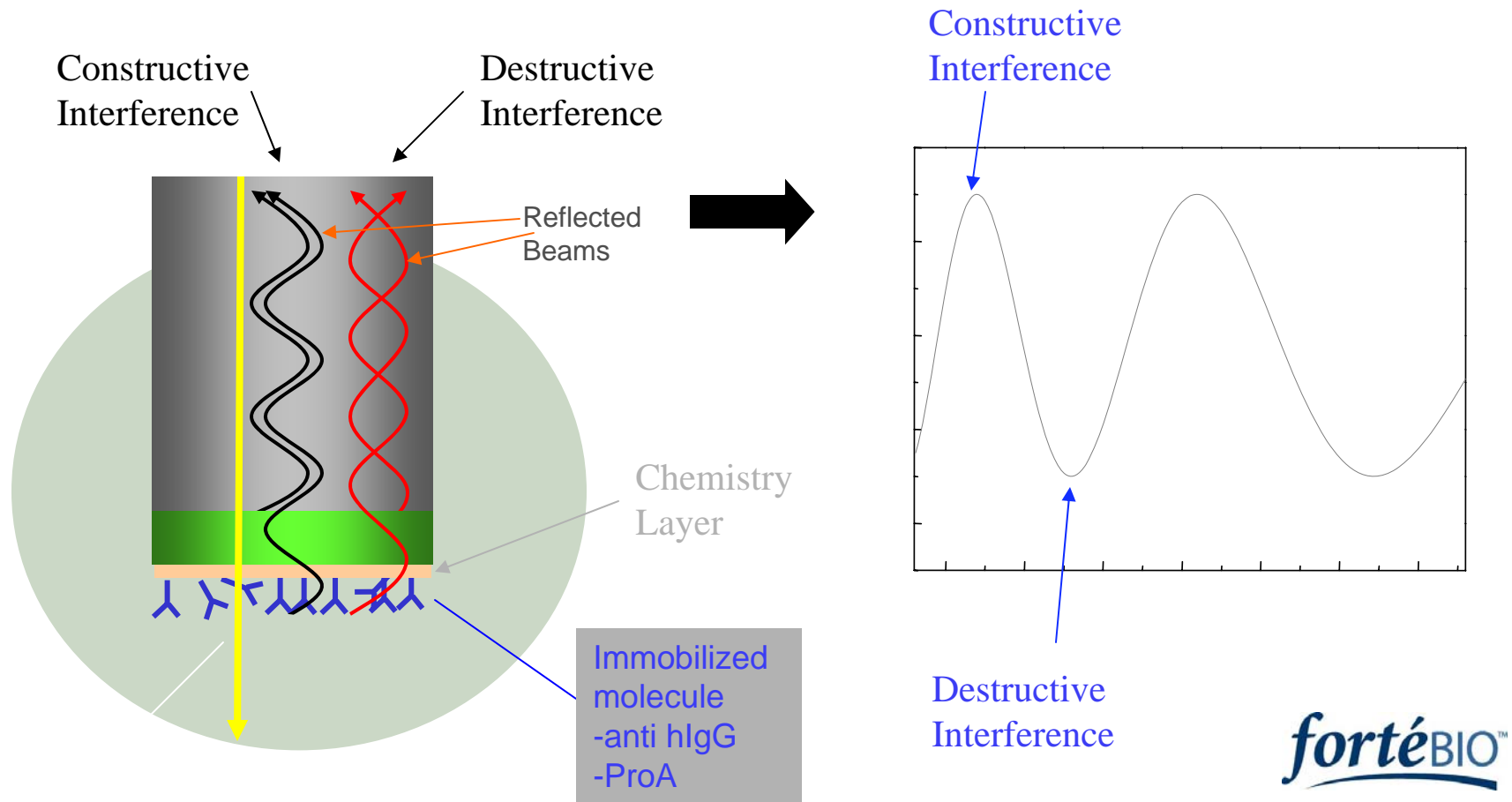
South San Francisco CA USA

Using BLI (Octet) to Determine [IgG]

- Principles of operation
- Qualification of the method
- Anti-IgG tips vs. Protein A tips
- Comparison with other methods
- Applications at Raven

Bio-Layer Interferometry (BLI)

A layer of molecules attached to the tip of an optical fiber creates an interference pattern at the detector.

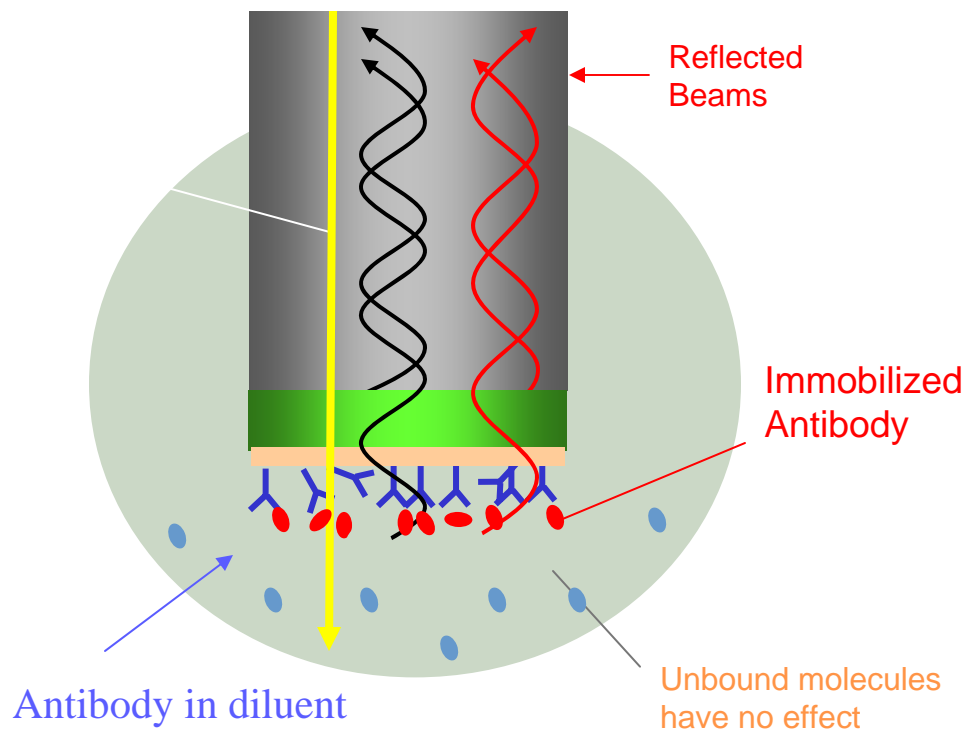


Bio-Layer Interferometry (BLI), continued

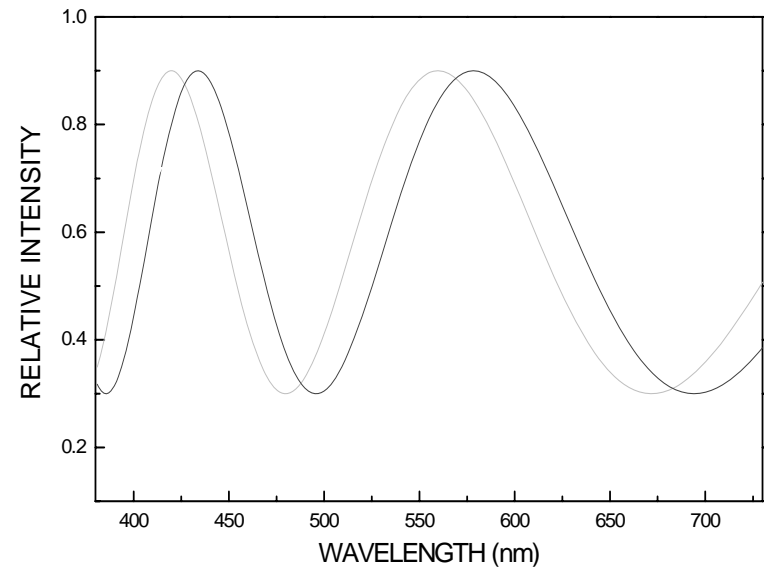
Proprietary new technology for label-free detection

- Any change in the number of molecules bound causes a measured shift in the wavelength pattern

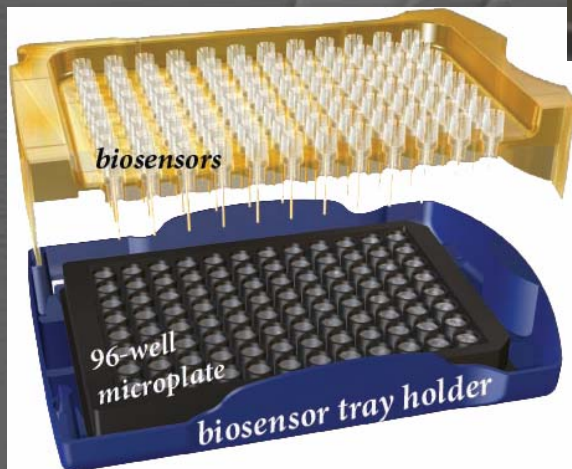
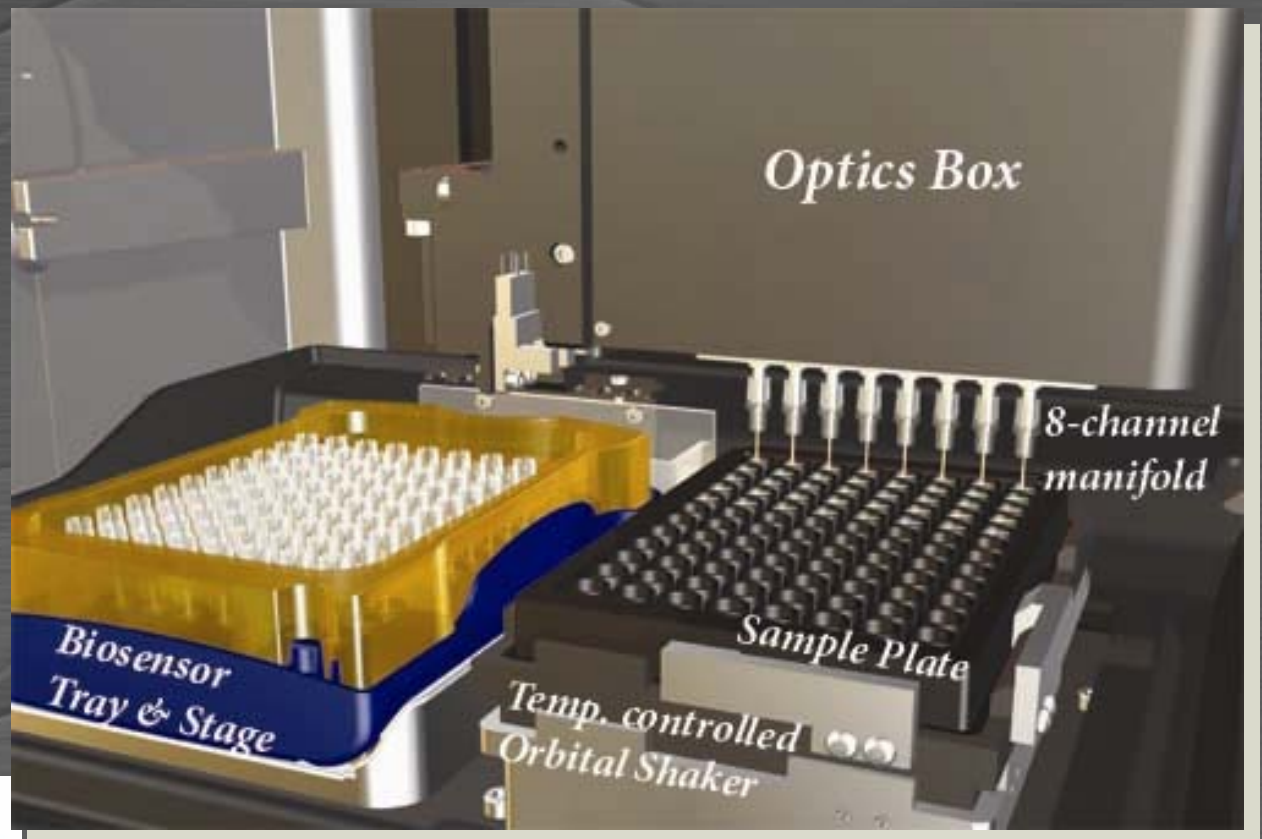
fortéBIO™



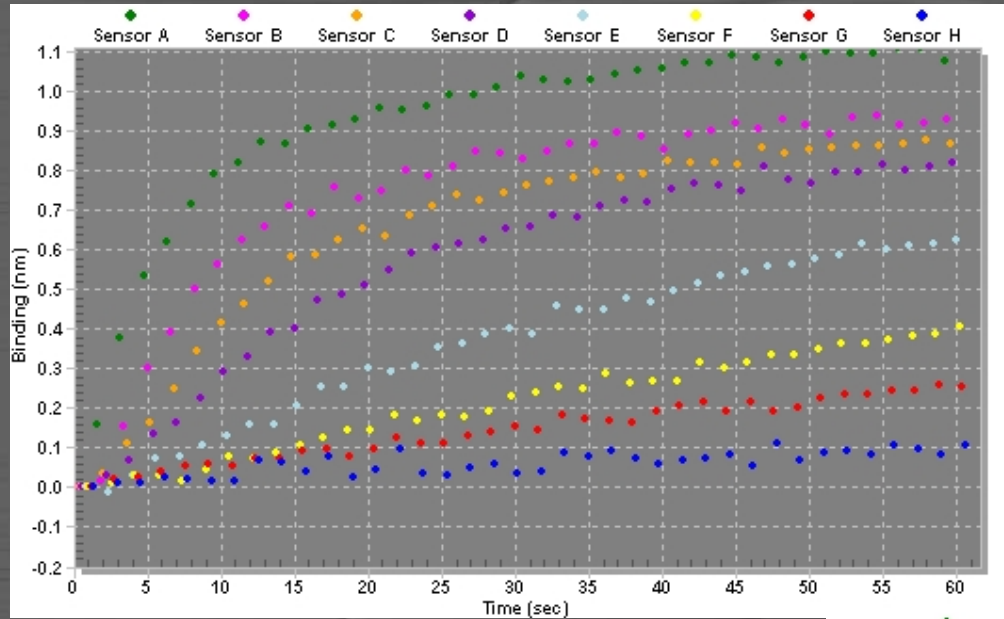
$$\Delta\lambda = \text{Layer thickness}$$



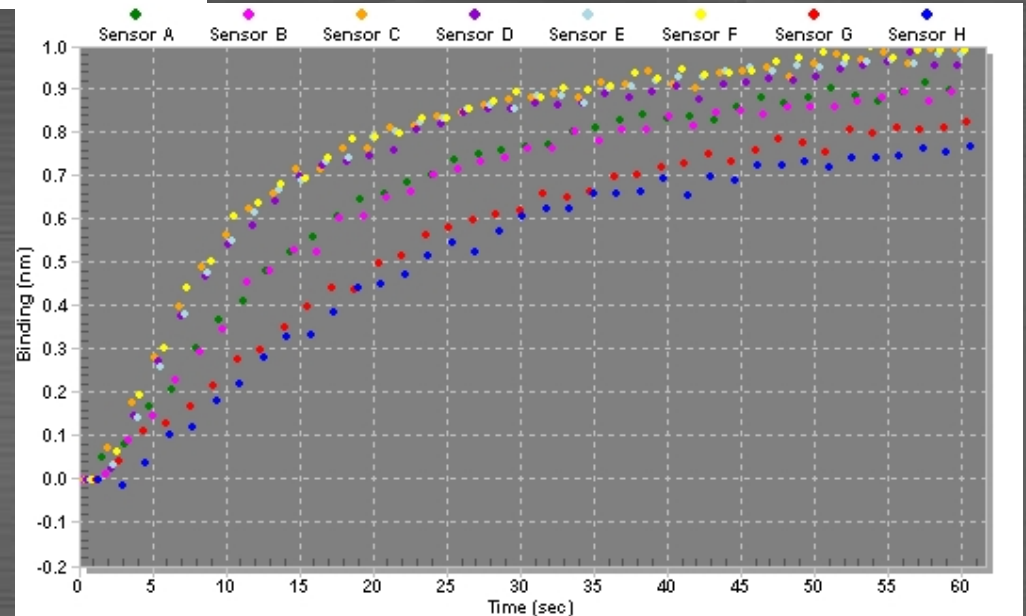
1nm wavelength shift = 0.97nm thickness



Data Acquisition

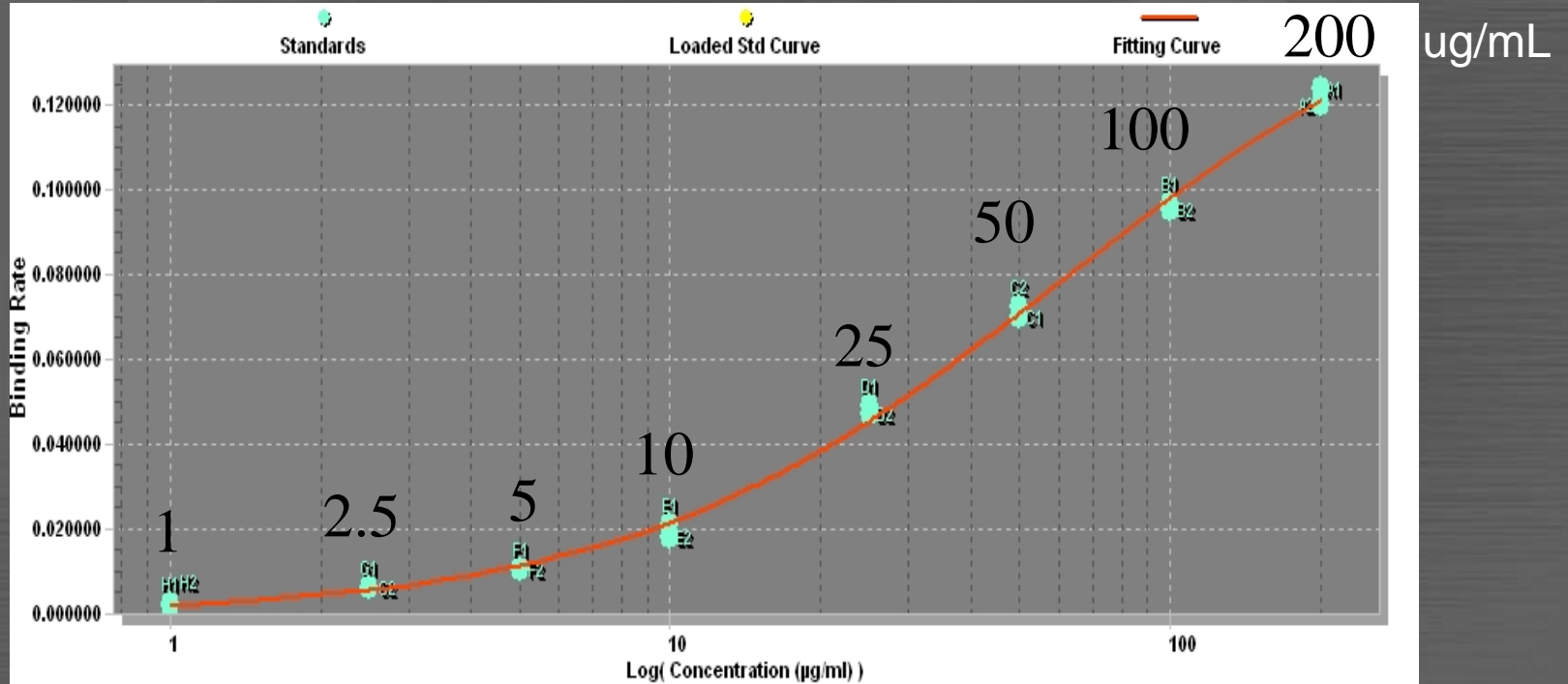


Standards

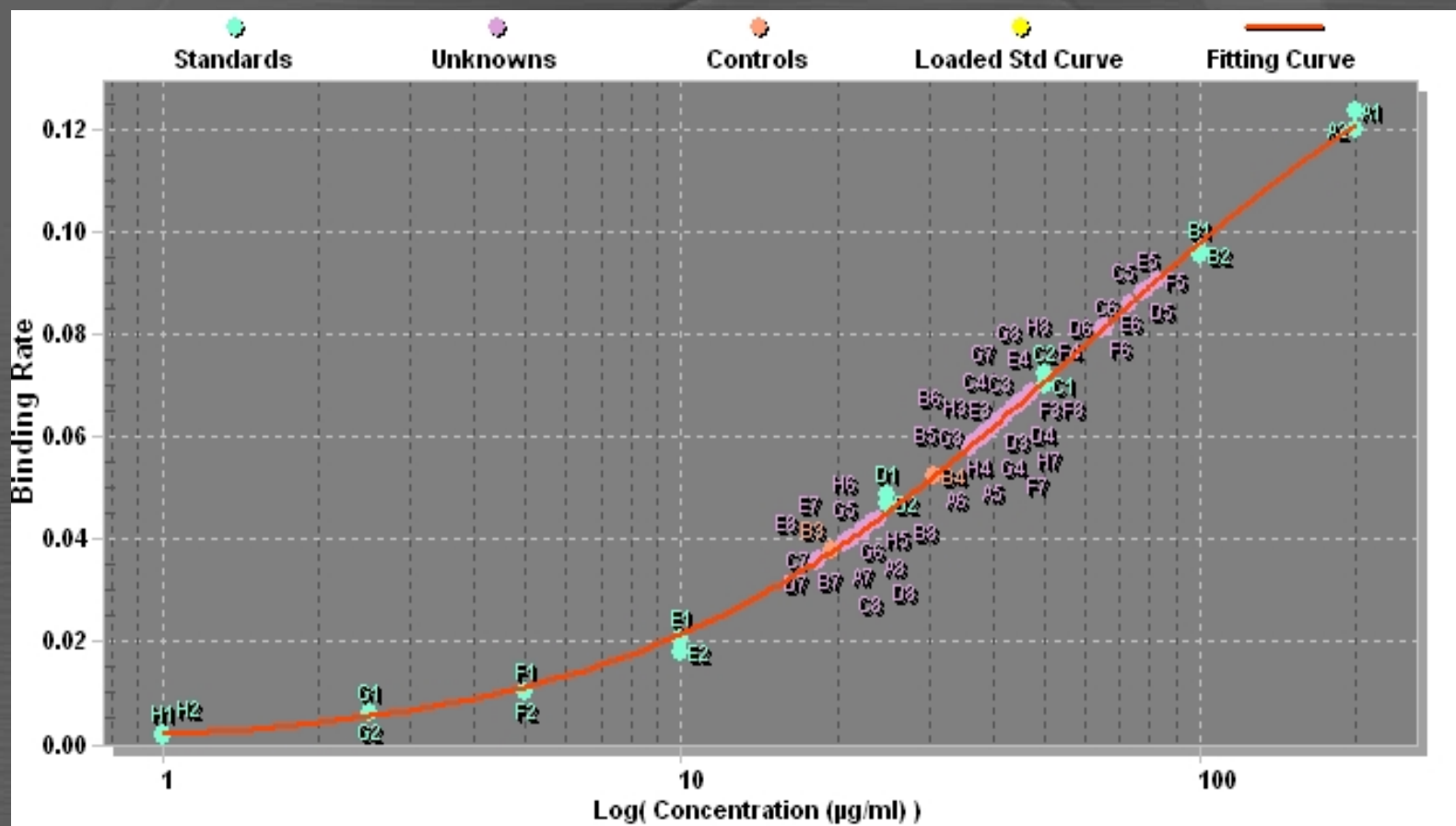


Samples

Standard Curve

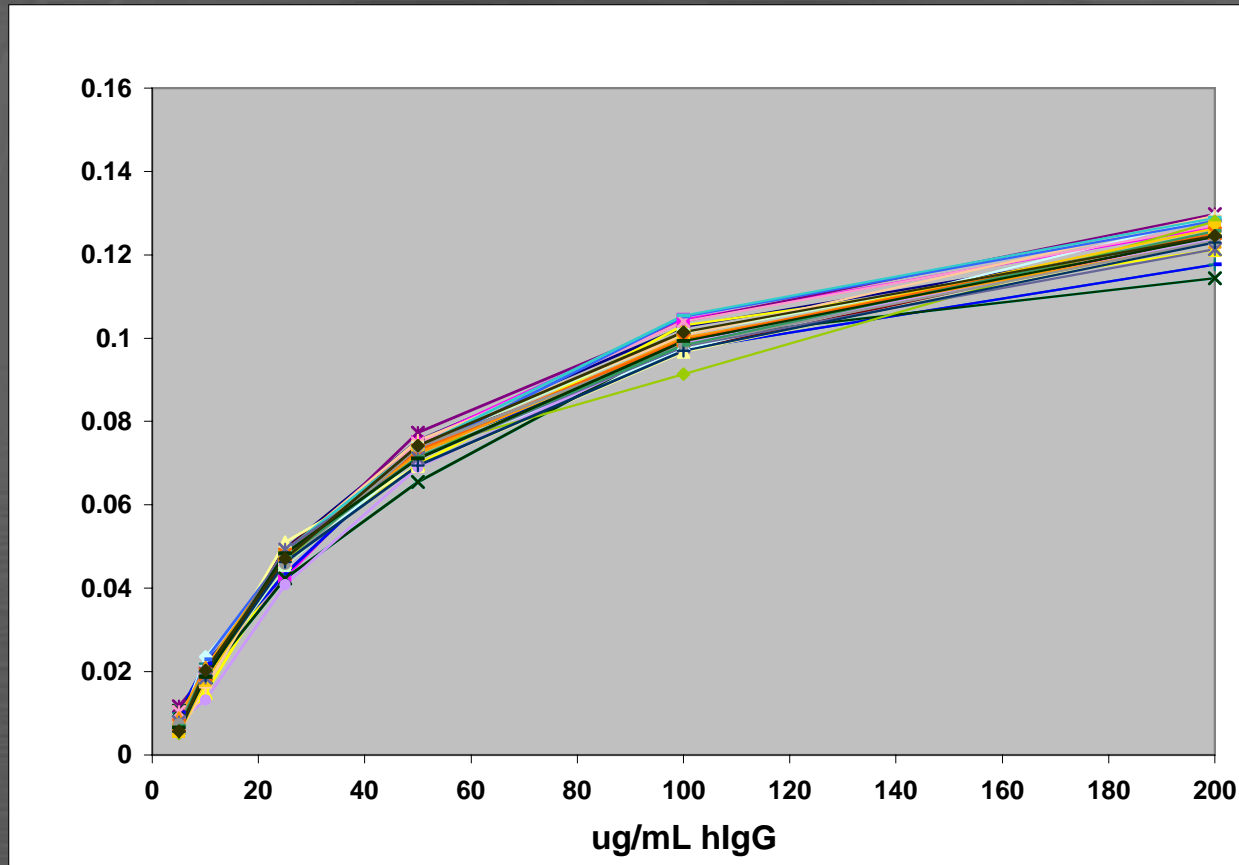


Concentrations Based on Binding Rates of Standards

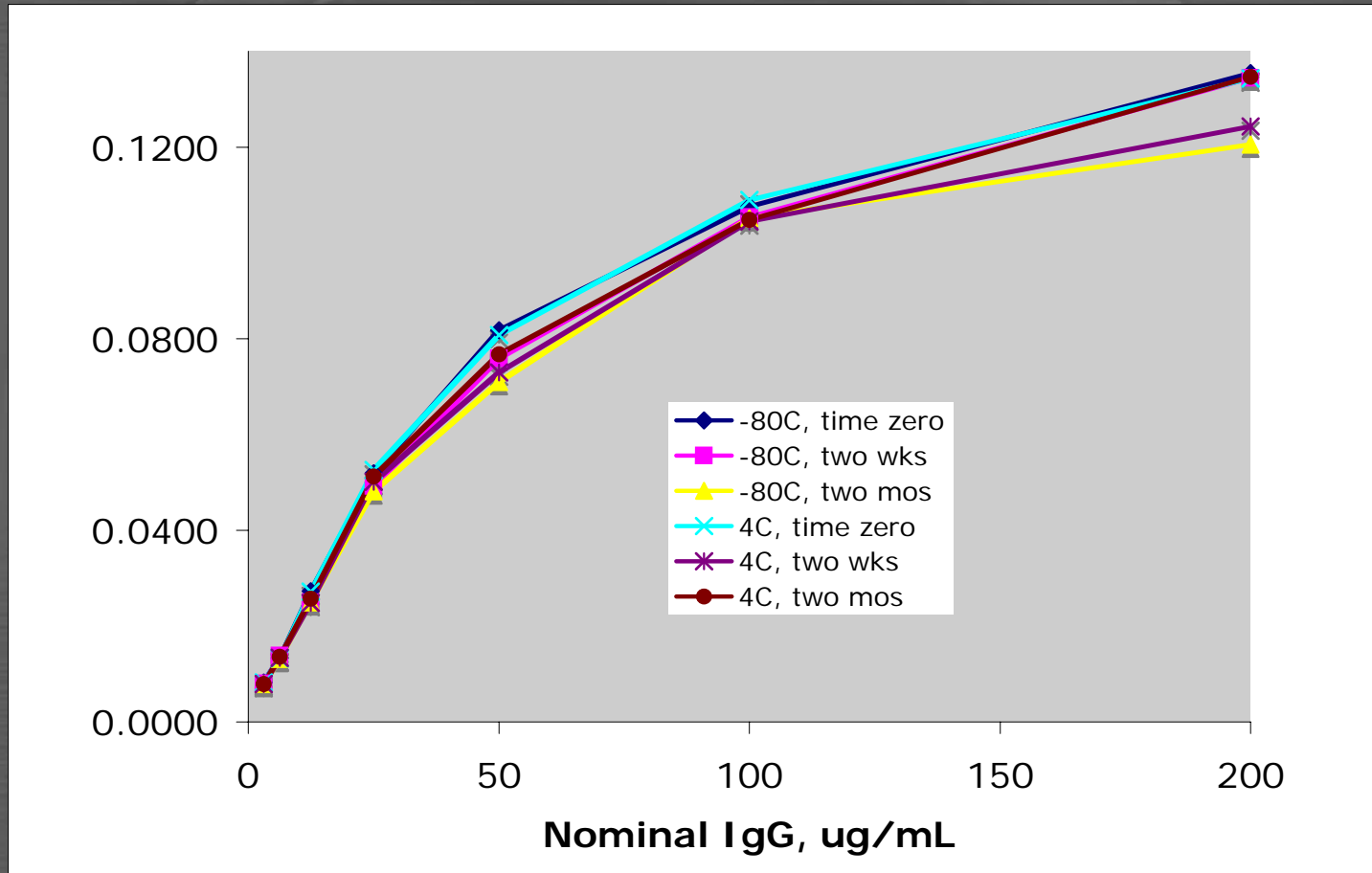


ForteBio Polyclonal Standards

27 runs, 4 users, 12 days



Raven MAb Standards

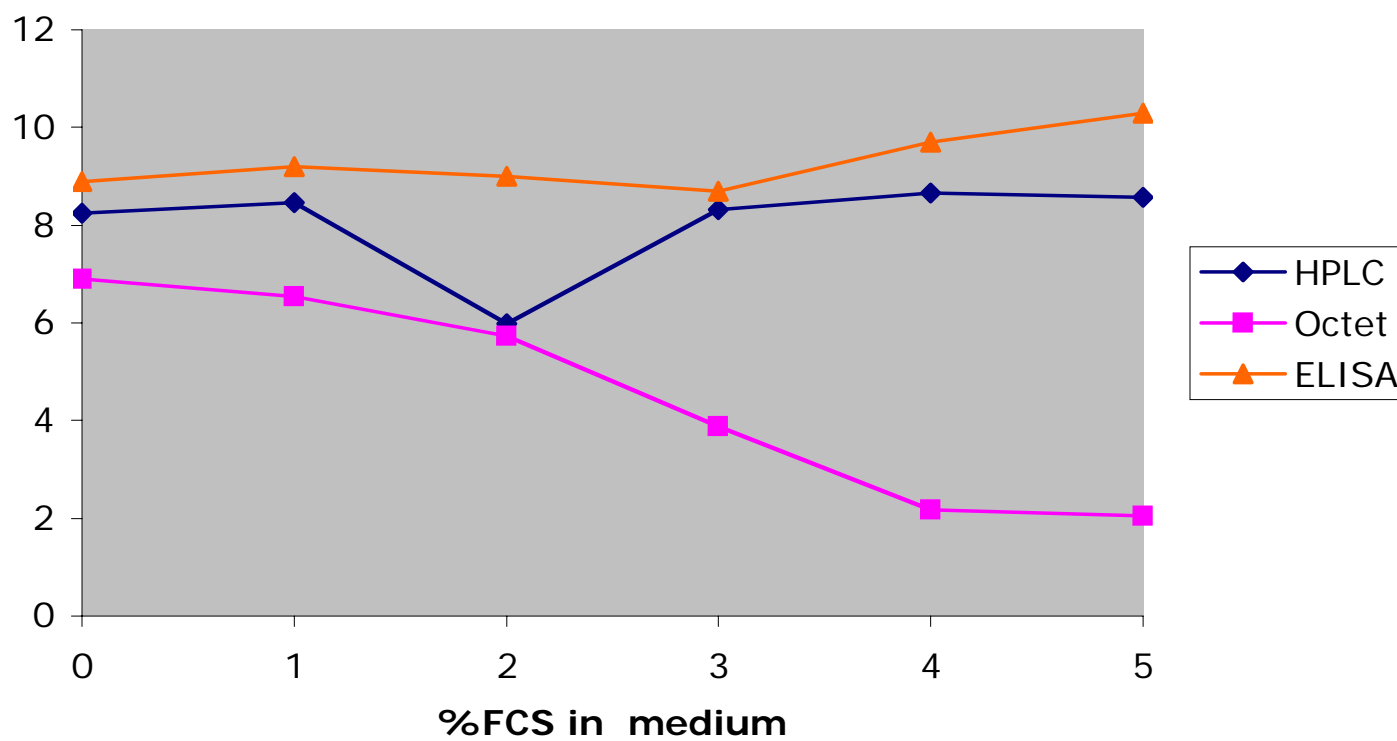


%CV's ranged from 2 - 7% (n=3)

Specificity

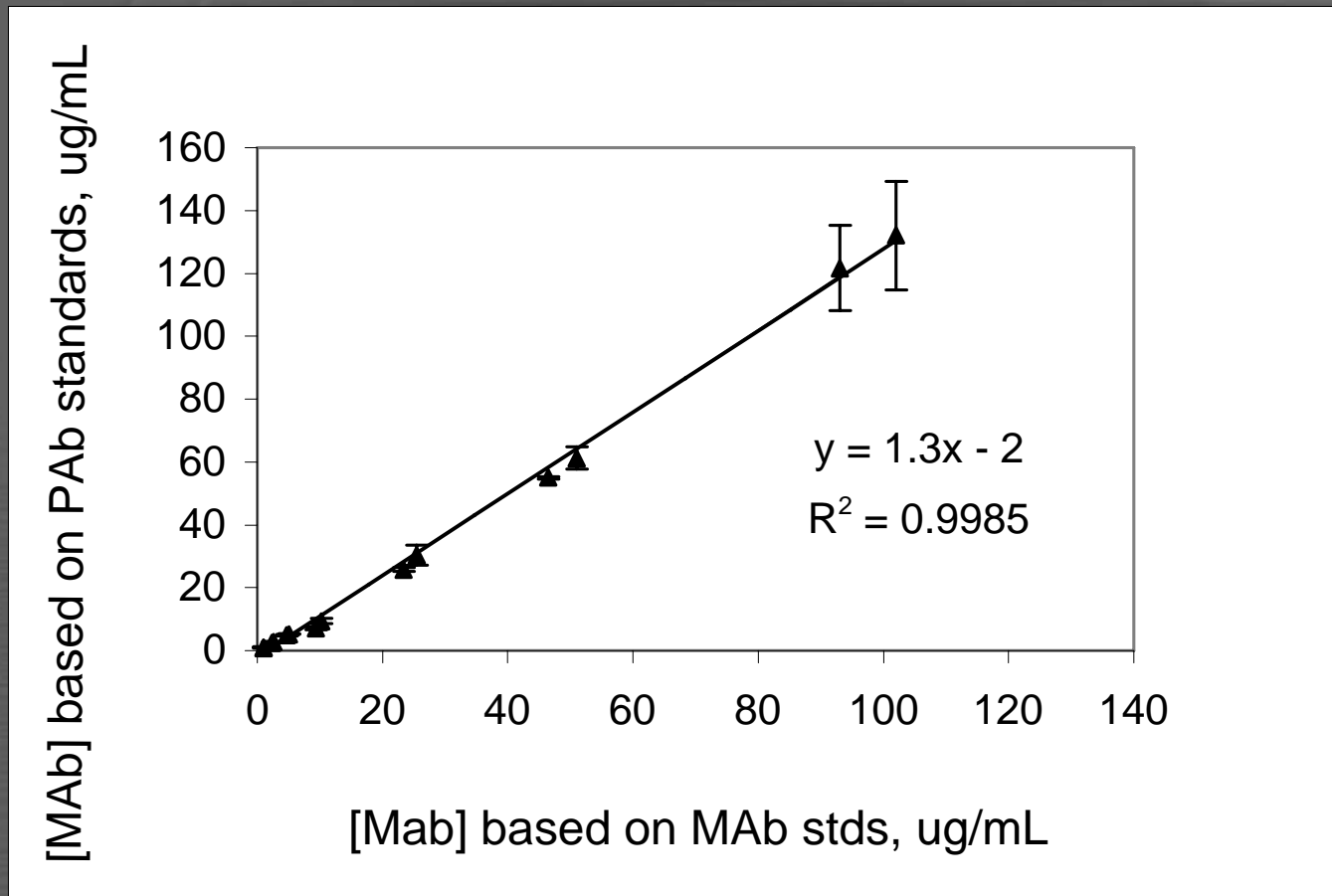
- Fiber optic tips coated with polyclonal anti-huIgG
- No signal from murine IgG1, IgG2a, IgG2b
(tested up to 100 ug/mL)
- No signal from neat FBS, but serum can suppress binding
- Background problems reduced by prewetting tips in appropriate matrix

Determination of 10ug/mL MAb in Presence of FCS

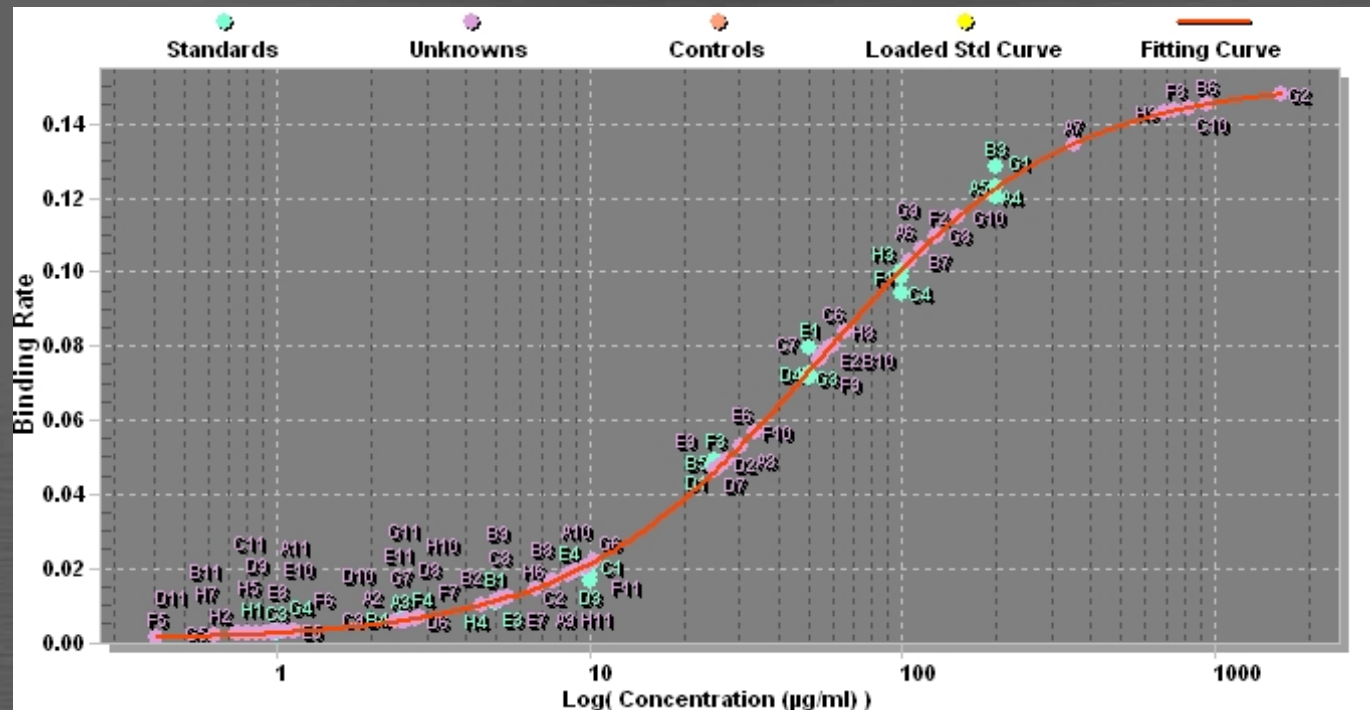


Polyclonal vs. Monoclonal Standards

Values are consistently 30% higher



- Raven std is IgG1; ForteBio std is polyclonal, multiple isotypes which may have different binding rates.
- Because of semi-log plot, small difference in binding rate at high end translates into larger difference in apparent concentration.

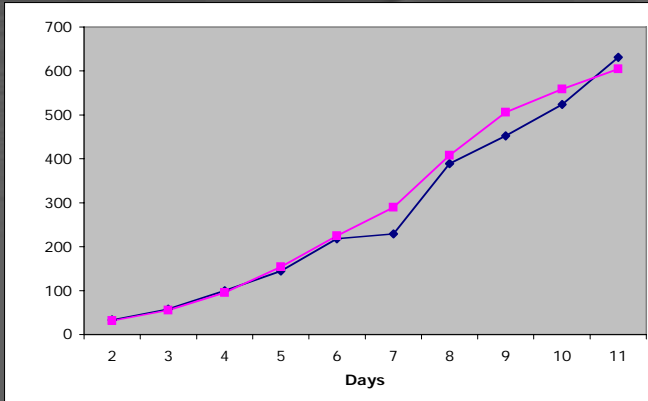


ForteBio stds in **green**
MAb stds in **pink**

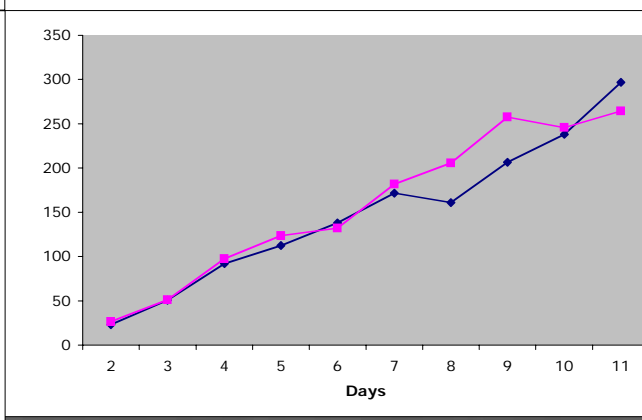
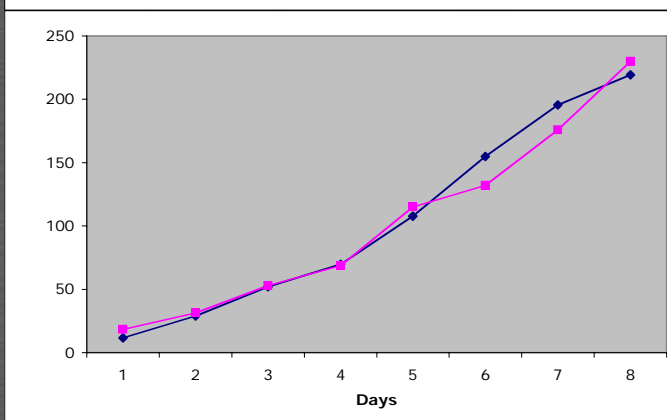
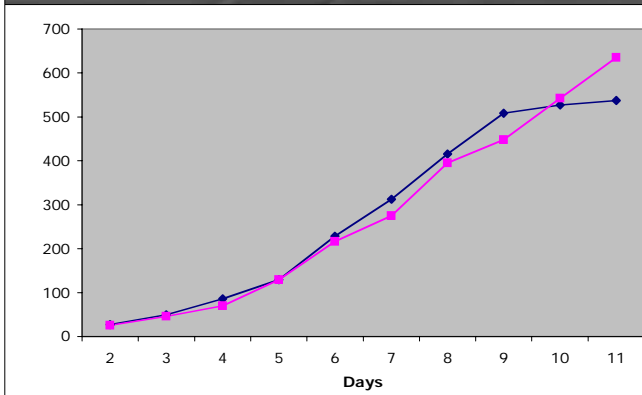
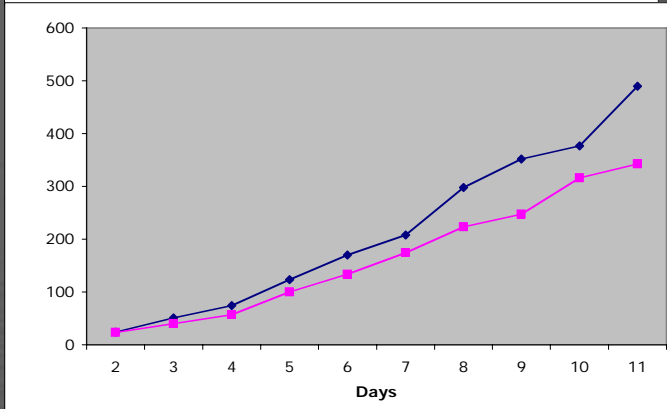
Applications at Raven

- Productivity in cell culture supernatants
- Downstream
 - Pro A yields
 - Dynamic binding capacity

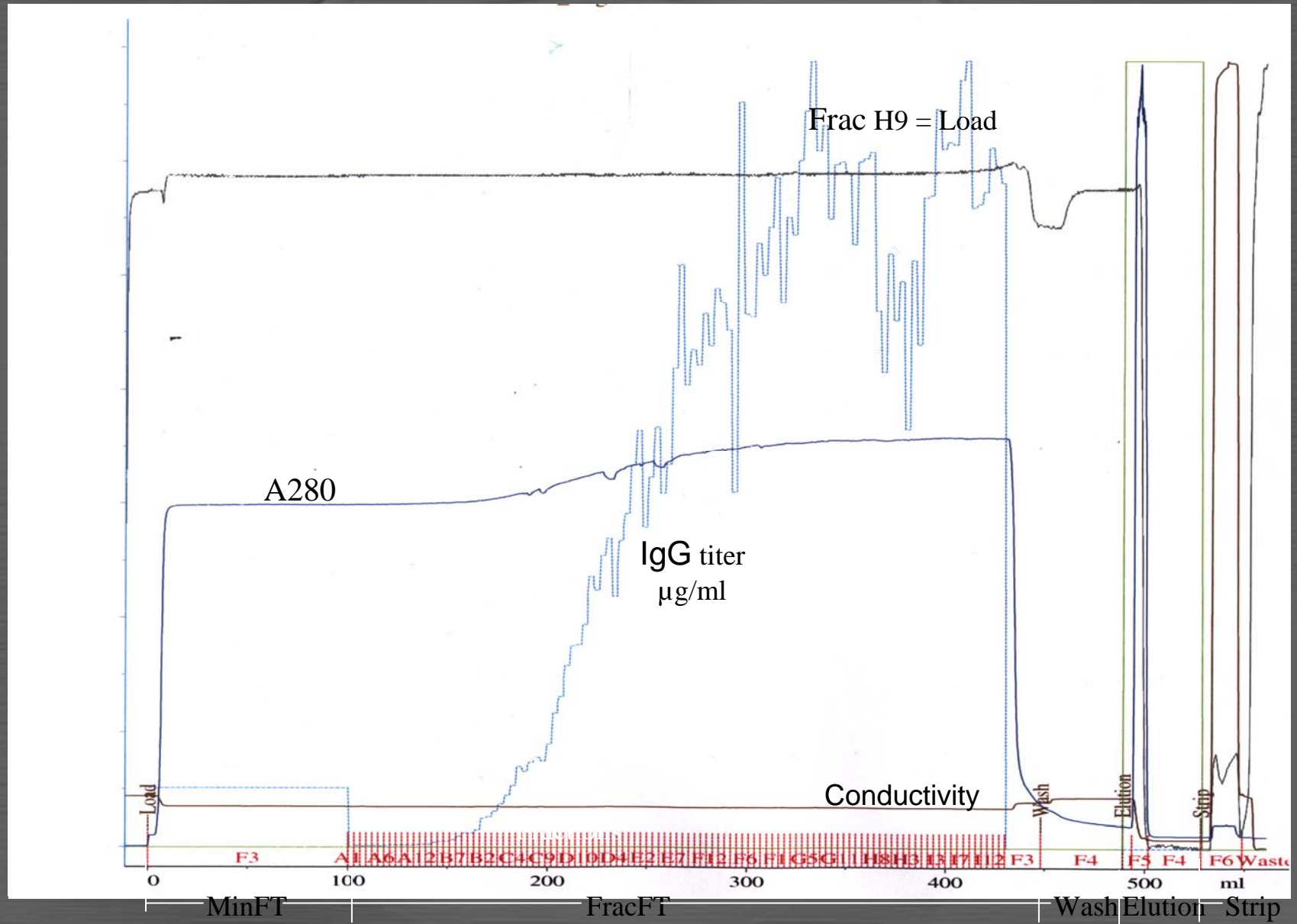
ELISA and Octet comparison - Bioreactor Supes



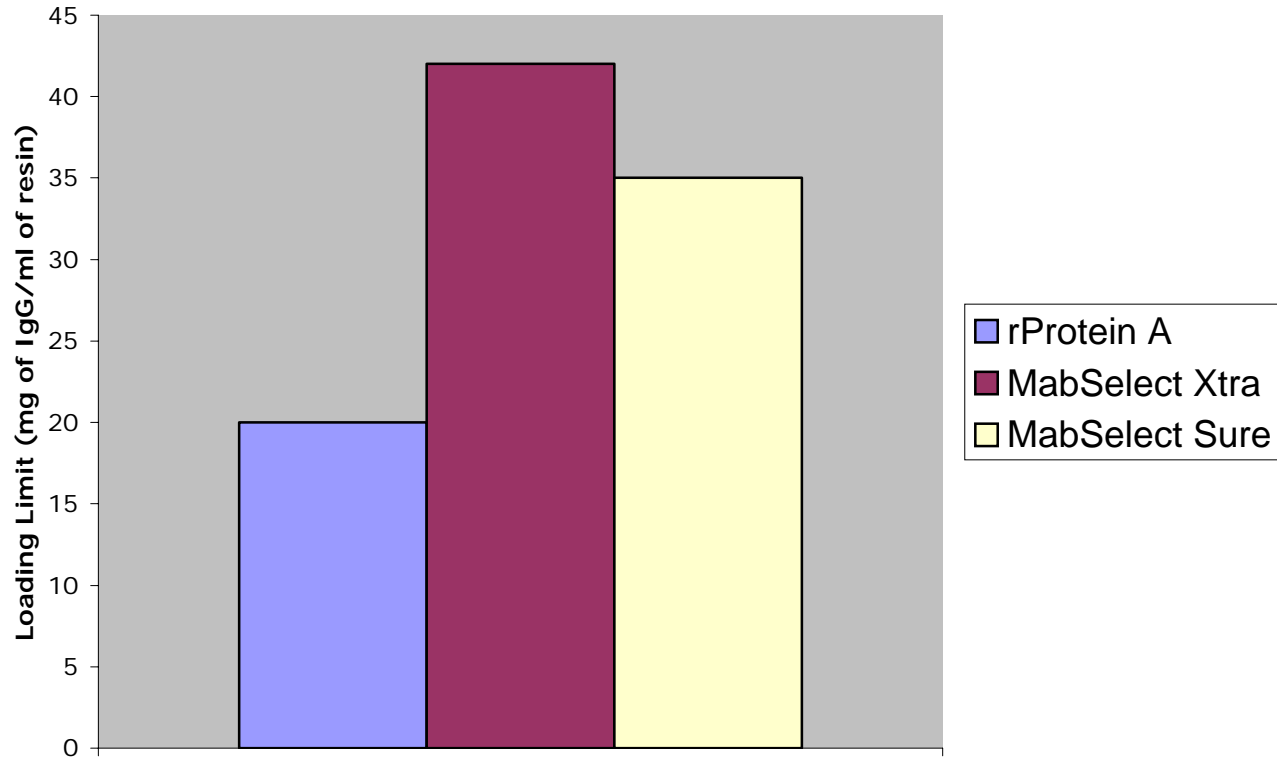
blue- Octet
pink - ELISA



Dynamic Binding Capacity of a Pro A Resin



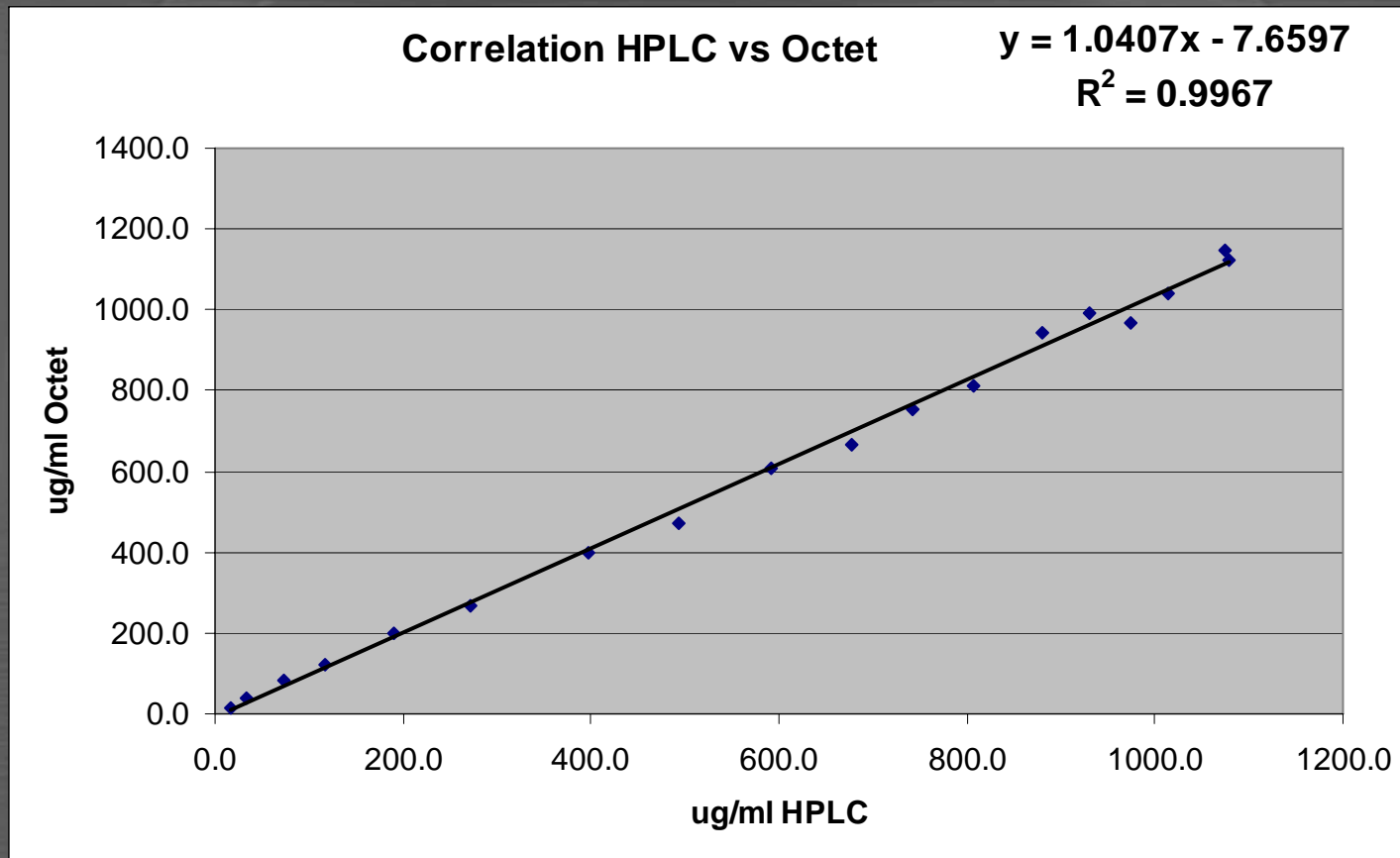
Resin Binding Capacity



ProA Tips vs. anti-huIgG Tips

- Wider dynamic maximum range
(1 - 750 vs. 1 - 200 ug/mL)
- More uniform binding rate than polyclonal
- May reflect recovery on proA resin
- Not restricted to human IgG's
Mouse, fusion proteins, etc.

Alpha Testing - Correlation with proA HPLC



- All samples were assayed neat
- Tips were pre-wet using fresh medium

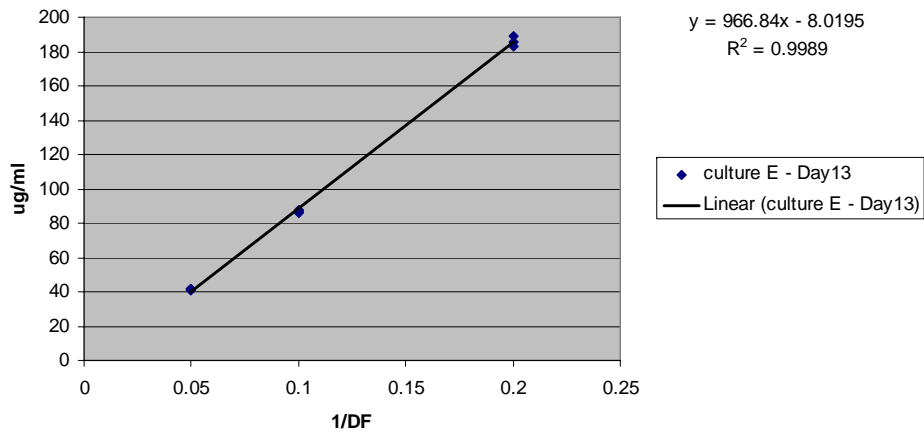


Dilution Linearity in Media

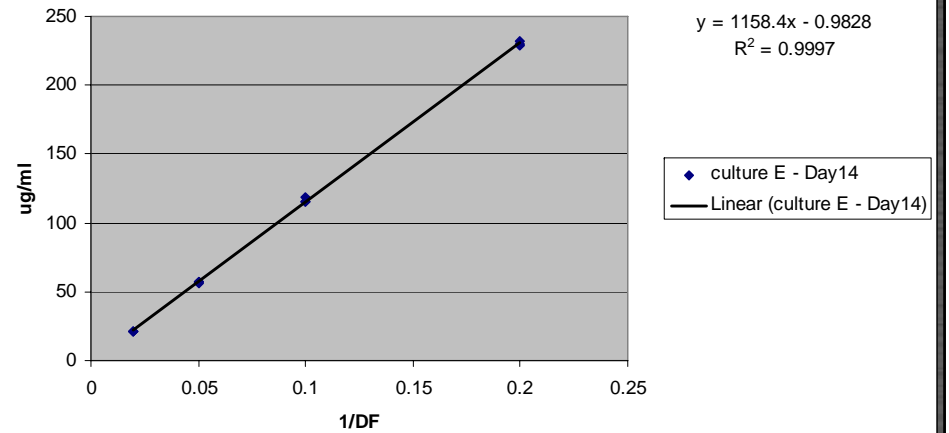
Day13 – 1:5, 1:10, 1:20

Day14 & 15 - 1:5, 1:10, 1:20, 1:50

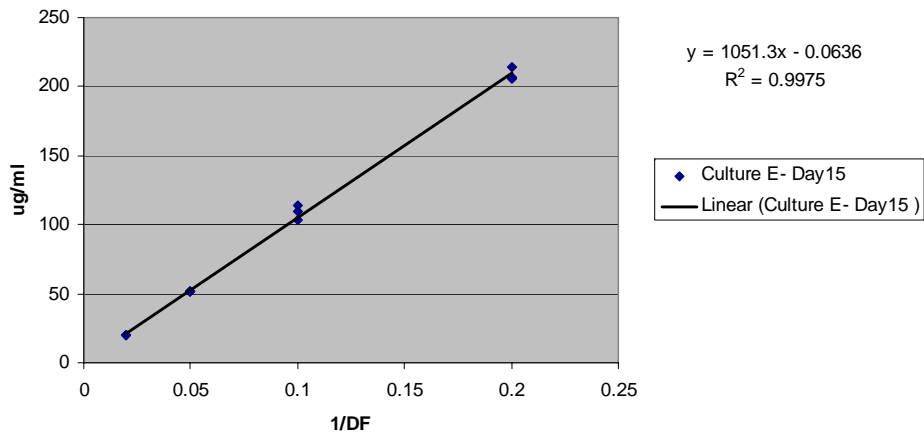
dilution linearity sample E Day13



dilution linearity sample E Day14



dilution linearity sample E Day15



Polyclonal IgG Standards and Pro A Tips

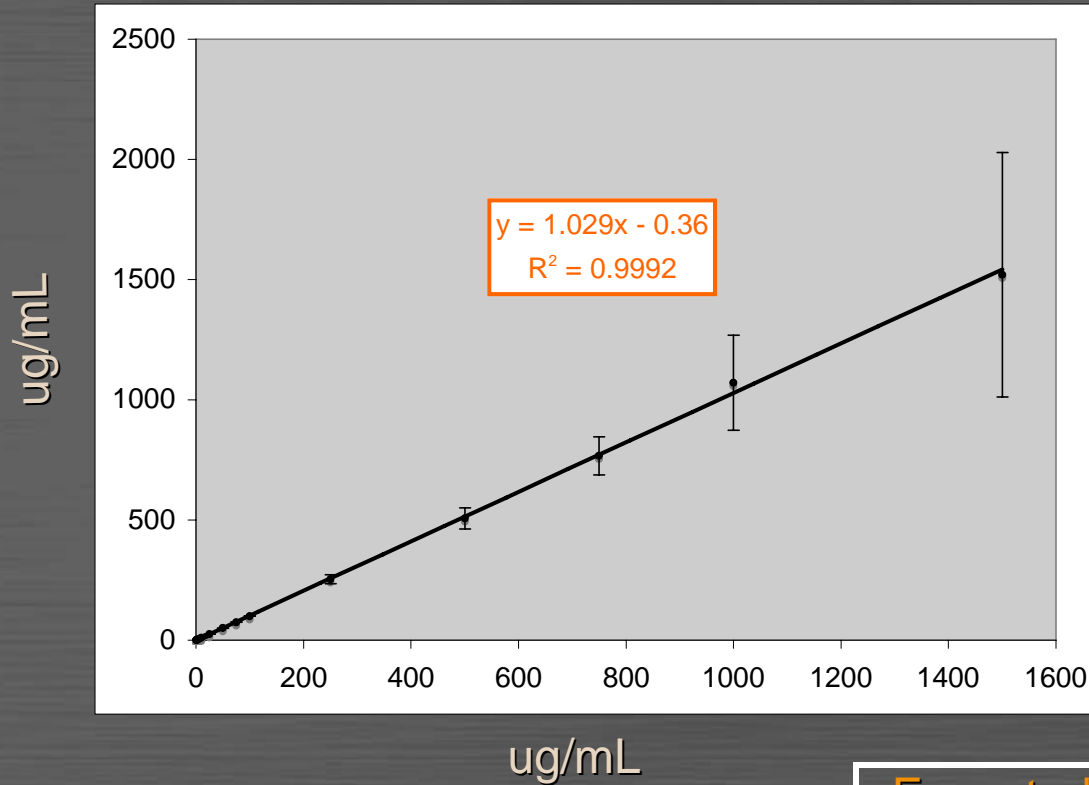
Standard Curve

Expected ug/mL	Avg. measured ug/mL	%sd (n=3)
1	1.0	3%
2.5	2.5	5%
10	10.0	0.6%
100	100.2	1%
250	251.0	2.5%
500	501.6	2%
1000	1014.2	7%
1500	1500	15%

Samples

Expected ug/mL	Avg. ug/mL	%sd (n = 8)	Recovery
2	1.8	2.5%	89%
194	204	3%	105%
905	1013	11%	112%

Monoclonal IgG Standards and Pro A Tips

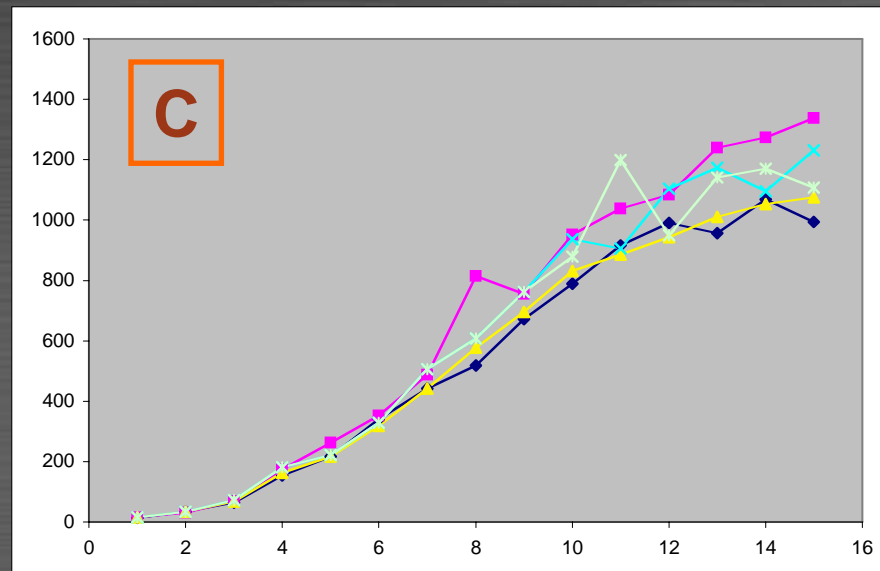
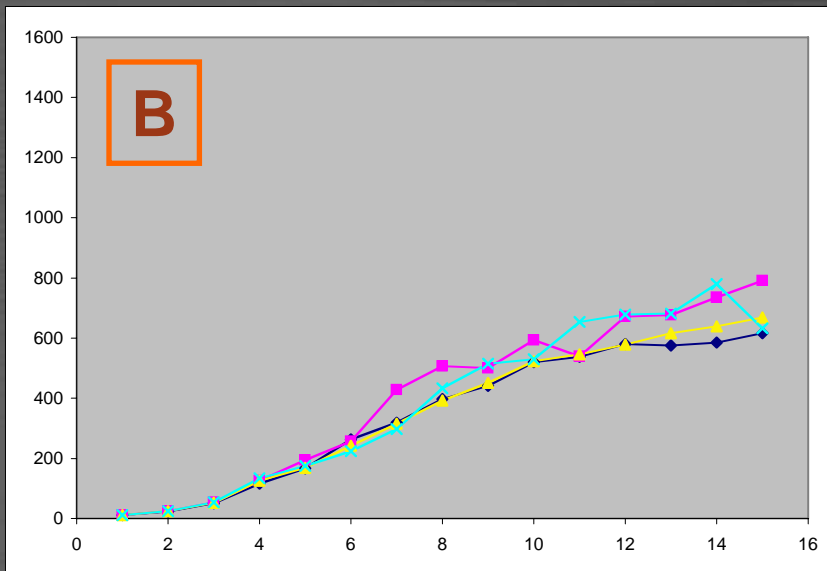
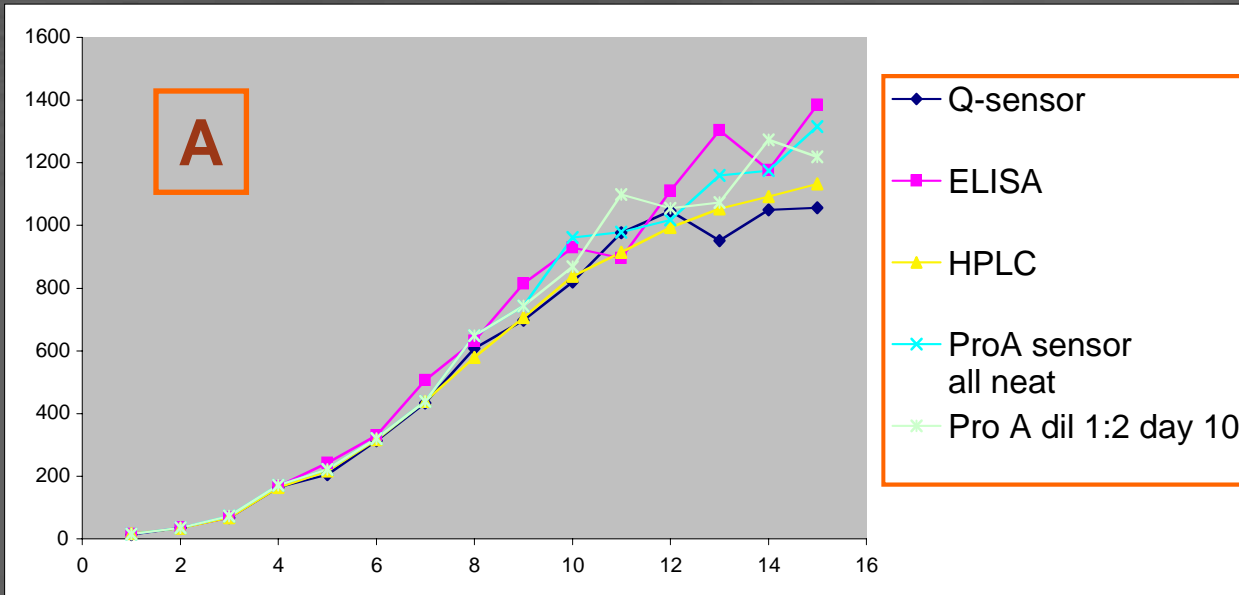


Standards

Samples

Expected ug/mL	Avg. ug/mL	%sd (n = 8)	Recovery
20	18	4%	88%
200	201	5%	101%
900	859	19%	95%

Bioreactor Titer: Comparison of Quantitative Methods



Summary

Bio-Layer Interferometry

- Rapid
- Easy
- Linear
 - 1 - 200 ug/mL with anti-hulgG tips
 - 1- 750 ug/mL with proA tips
- Robust
- Excellent correlation with proA HPLC and ELISA

The Best Method? It depends...

Octet

- Fast turnaround
- Low skill requirement
- Simple or no dilution required

ELISA

- High throughput
- Labor intensive (or use robot)
- Very sensitive
- Can distinguish between intact MAbs and fragments

At Raven

- Octet for monitoring upstream and downstream processes
- ELISA for high-throughput clone screening

Acknowledgments

- Raven Cell Culture Group

- ForteBio

Krista Witte

Bettina Heidecker

Janette Phi-Wilson

Joy Concepcion