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MAb Quantitation: Protein A HPLC vs. Protein A Bio-layer Interferometry

Alexander Martino

Mark Schofield

Rene Gantier

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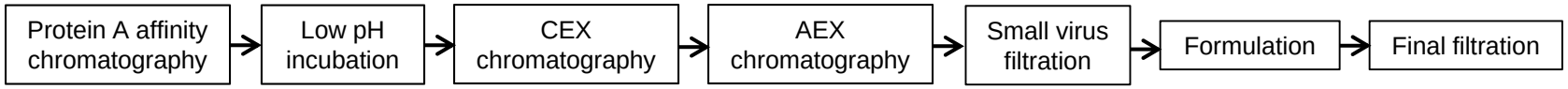
Agenda

- Goal:
  - Compare Protein A based monoclonal antibody (Mab) quantification: **HPLC vs Bio-Layer Interferometry**
- Context:
  - MAb process development
- Accuracy
  - Bias
  - Precision
- Dynamic range
  - LoD/LoQ
- Assay time
- Assay cost
- Conclusions

MAb purification

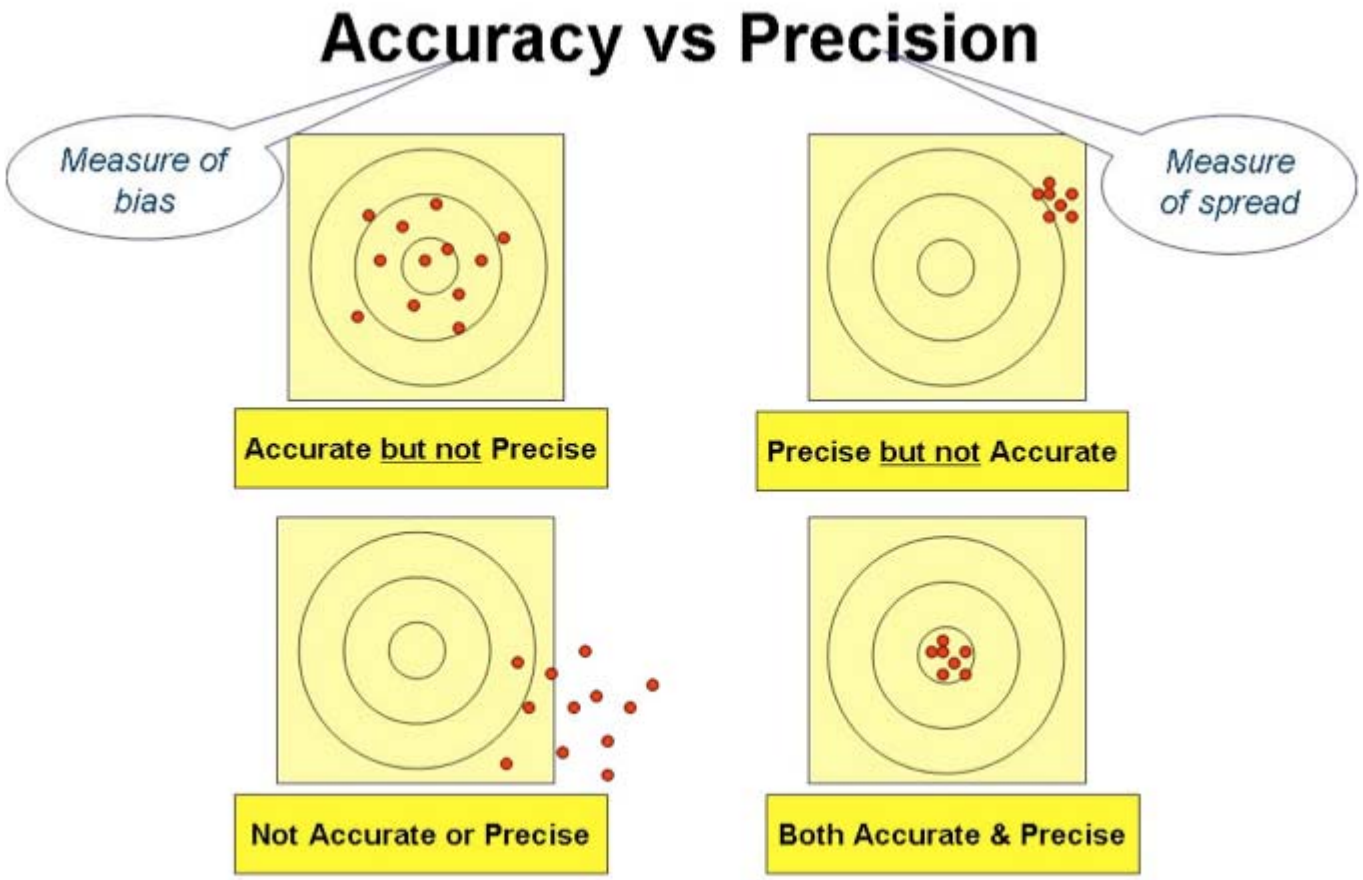
- Mab Process development
  - Consensus Mab purification



  - Majority of MAb purification processes employ Protein A as a capture step
    - Process development may include capacity optimization of this step for:
      - Protein A resin
      - Residence time (the amount of time any particle spends in the column)
      - MAb titre
  - Capacity in this context is normally defined as the breakthrough of 10% of product
    - This cannot be accurately followed by U.V. as the feedstock has a high UV absorbance

- Therefore we need an accurate, precise, cheap and high-throughput method for MAb concentration determination.
  - Traditionally this has been performed by Protein A HPLC

Data quality



Data quality

- Bias
  - A standard curve based on triplicate data was prepared for each system within the assay dynamic range
  - An additional three standard curves were then compared to the average standard curve

$$\%Bias = \frac{|Experimental - Expected|}{Expected} * 100$$

HPLC		Octet	
Expected Value (µg/mL)	Bias (%)	Expected Value (µg/mL)	Bias (%)
2000	0.67	300	3.83
1000	2.51	150	0.87
500	0.25	75	0.27
250	1.45	37.5	0.80
125	2.79	18.8	0.35
62.50	4.38	9.38	0.25
31.25	6.08	4.69	1.42
27	4.40	1.3	0.96

Data quality

- Precision
  - All 6 curve replicates were used to produce an average standard curve
  - Each of the 48 points used to generate this curve could then be assigned a concentration.
  - The six measurements at each of the 8 known concentrations could then be used to calculate the coefficient of variance (CV)

$$\%CV = \frac{\text{Standard Deviation}}{\text{Average Value}} * 100$$

HPLC		Octet	
Expected Value (µg/mL)	CV (%)	Expected Value (µg/mL)	CV (%)
2000	1.56	300	6.52
1000	1.66	150	3.19
500	1.05	75	1.57
250	1.68	37.5	1.70
125	0.99	18.8	2.42
62.50	1.45	9.38	0.70
31.25	5.43	4.69	0.64
27	0.64	1.3	0.79

LoQ and LoD

- Limit of quantitation $$LoQ = \frac{10\sigma}{M}$$

- Limit of detection $$LoD = \frac{3.3\sigma}{M}$$

Where:
σ = the standard deviation of the response
M = the slope of the calibration curve

- Ref: ICH 1996 Validation of Analytical Procedures: Methodology

HPLC		Octet	
LoD (µg/mL)	LoQ (µg/mL)	LoD (µg/mL)	LoQ (µg/mL)
20.69	29.58	0.11	1.32

Verifying theoretical LoD and LoQ

- 6x replicate measurements at close to the LoD and LoQ

HPLC				
	Loaded Conc. (µg/mL)	Average Value (µg/mL)	Bias (%)	CV (%)
LoD	17	21.53	26.67	2.33
LoQ	27	28.19	4.40	0.64
Octet				
	Loaded Conc. (µg/mL)	Average Value (µg/mL)	Bias (%)	CV (%)
LoD	0.1	0.18	82.37	39.63
LoQ	1.3	1.29	0.96	0.79

- In-line with expectations
  - % bias and %CV unacceptably high at LoD, but acceptable at LoQ.

Linear dynamic range

- Dynamic range (based on a CV<5%)
  - **HPLC**: 5000 ug/ml to 62.5 ug/ml
  - 80 fold range (1.9 orders of magnitude)
  - **Octet**: 150 ug/ml to 1.3 ug/ml
  - 115 fold range (2.1 orders of magnitude)

HPLC			Octet		
Expected Value (µg/mL)	Bias (%)	CV (%)	Expected Value (µg/mL)	Bias (%)	CV (%)
2000	0.67	1.56	300	3.83	6.52
1000	2.51	1.66	150	0.87	3.19
500	0.25	1.05	75	0.27	1.57
250	1.45	1.68	37.5	0.80	1.70
125	2.79	0.99	18.8	0.35	2.42
62.50	4.38	1.45	9.38	0.25	0.70
31.25	6.08	5.43	4.69	1.42	0.64
27	4.40	0.64	1.3	0.96	0.79

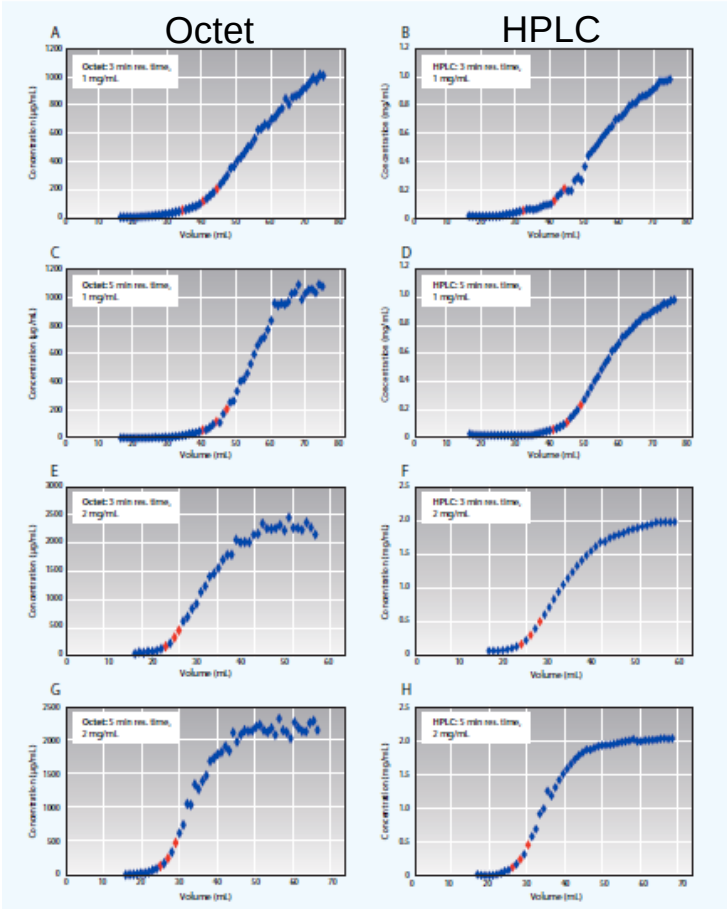
- Max value determined previously
- Low value discrepancy: (27ug/ml) value determined by 6 sequential loadings
  - Other values measured in context of standard curve
- HPLC assay error influenced by previous sample loaded

Summary of accuracy and precision

- HPLC and Octet
  - Have similar dynamic ranges
    - 1.9 vs 2.1 orders of magnitude
  - Have similar % bias and %CV over the range of the assay measured
    - Bias HPLC 0.3-4.4%
    - Bias Octet 0.3-3.3%
    - CV HPLC 1-1.7%
    - CV Octet 0.7-3.2%

Experiment details

- Performed breakthrough (BT) assay using 1ml column
  - 2 level 2 factor DoE
    - Residence time 3 and 5 min
    - MAb titre 1 and 2 mg/ml
  - Toward the end of the BT, MAb concentration exceeds the assay range for the Octet
- BTs analysed by HPLC and Octet
  - 272 vs 276 measurements
- BT measured at 5, 10 and 20% very similar for both techniques
- Highest capacity found at longest residence time and highest concentration



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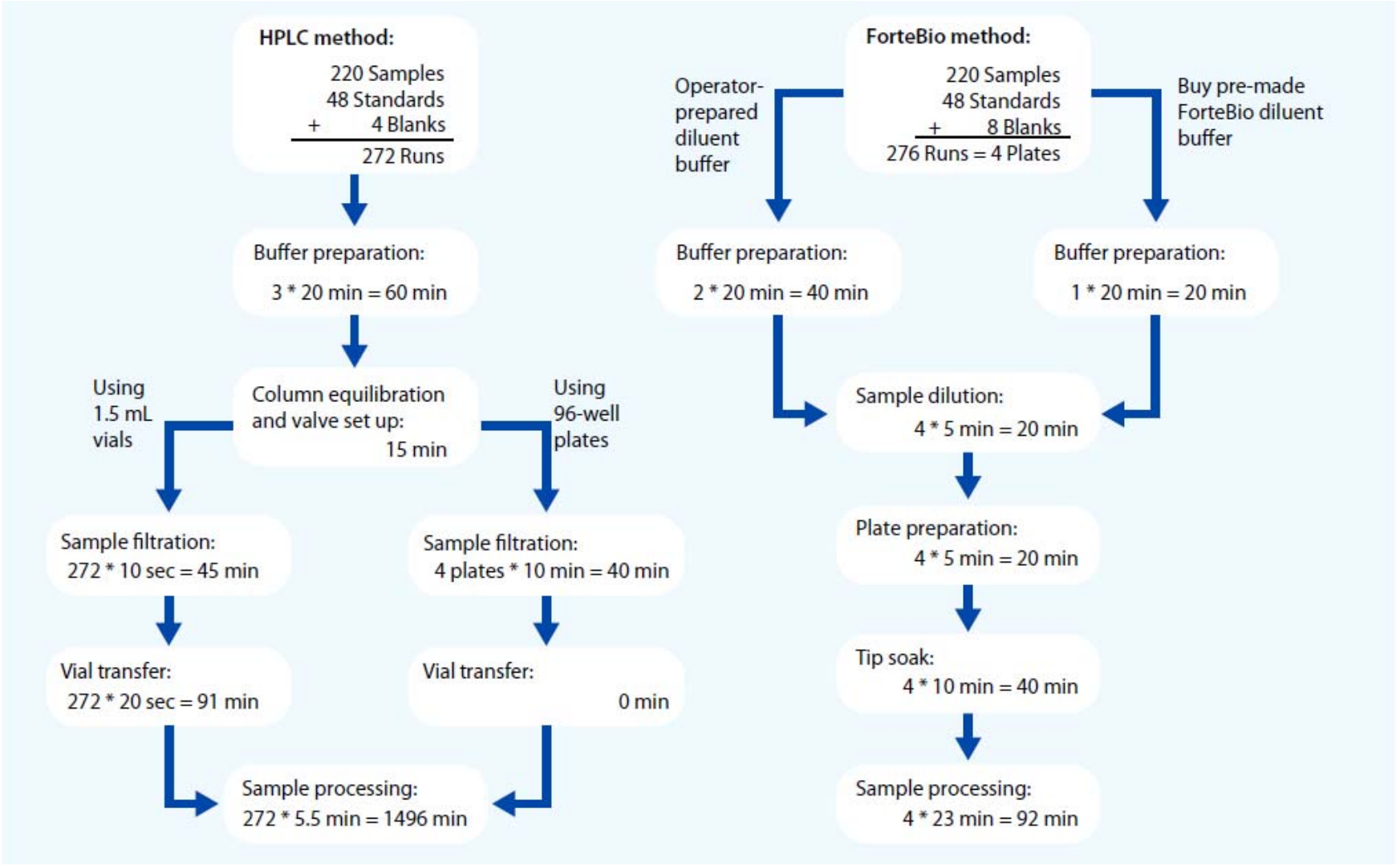
Comparison of assay cost and time



Assay time

- Total assay time remarkably reduced using the Octet
  - Reduces assay from 26 hours to just over 3 hours
    - **Octet 8.4x quicker**
  - This is a major advantage when results are needed as soon as possible to guide the next experiment/step

	HPLC w/ Vials	HPLC w/ Plates	Octet (Prepare Diluent)	Octet (Buy Diluent)
Preparation Time (min)	211	115	80	60
Process Time (min)	1496	1496	132	132
Total Time (min)	1707	1611	212	192

Assay cost

- Estimated assay cost based on operator at $100/hour

	HPLC w/ Vials	HPLC w/ Plates	Octet (Prepare Diluent)	Octet (Buy Diluent)
Buffer Cost	$ 0.0028	$0.0028	$ 0.0042	$0.3478
Consumable Cost	$1.58	$0.50	$ 0.44	$ 0.44
Operator Cost	$ 1.29	$0.70	$0.48	$0.36
Total	$2.87	$1.21	$ 0.93	$ 1.15

- Octet assay is cheapest
  - >20% savings over the least expensive HPLC assay

Conclusions

- Forte Bio Octet provides a fast, accurate, cost effective and high-throughput method for Mab quantification

- Octet can compete with HPLC in all metrics
  - Assays are >20% cheaper
  - Assays can be performed considerably quicker with the Octet
    - Each HPLC run takes 5.5 minutes
    - Octet can read a whole plate in 23 minutes