Development of an Imaged Capillary IEF Method for a BiTE® Antibody

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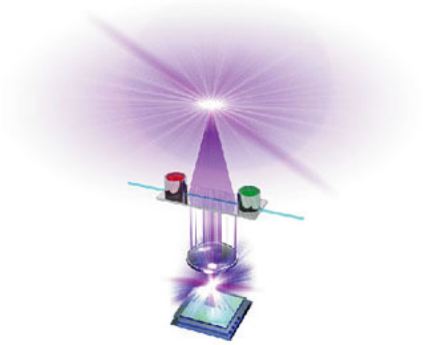
Why Automate Electrophoresis Assays?

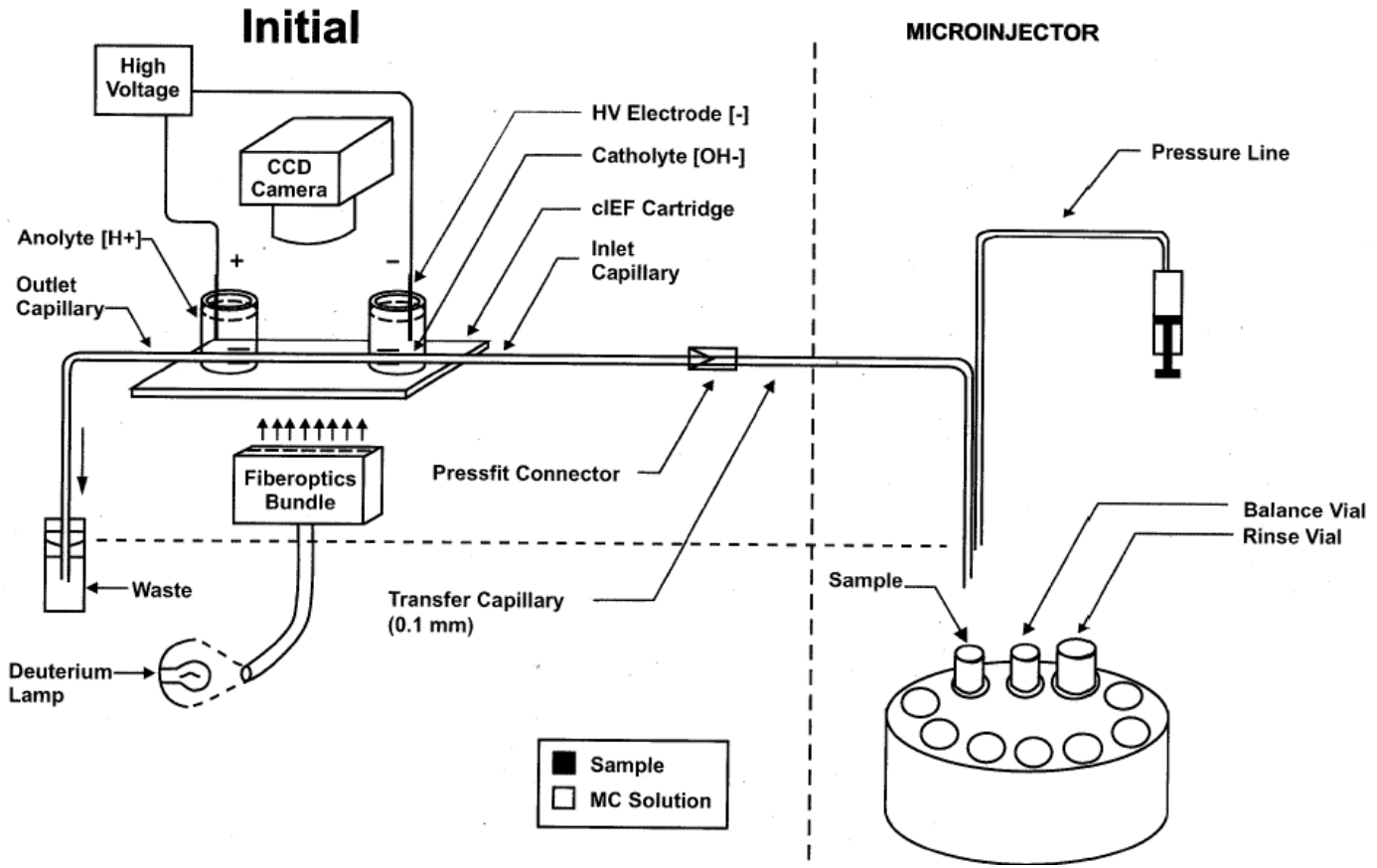
- Increased accuracy and reproducibility
- Smaller reagent/sample volumes
- Generate less waste
- Allows analyst to work on other tasks while assay is running

Convergent iCE280

- Imaged capillary isoelectric focusing:
  - Molecules placed in pH gradient; under influence of electric current, molecules migrate to their isoelectric point
- iCE280 instrument uses whole column detection:
  - Entire length of capillary illuminated at 280 nm
  - Digital camera takes snapshots of entire capillary in real time
- After sample preparation (30 minutes), instrument can process 72 samples and deliver results in approximately 24 hours



Convergent iCE280



Focusing Progression

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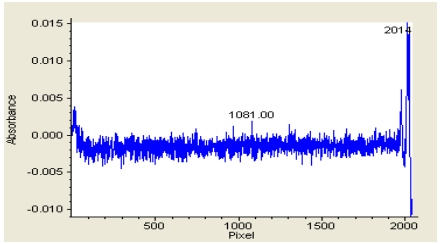

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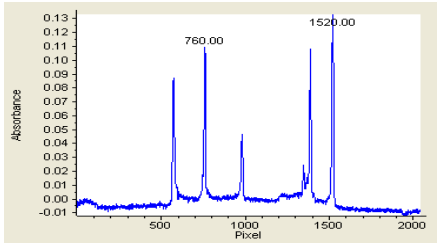

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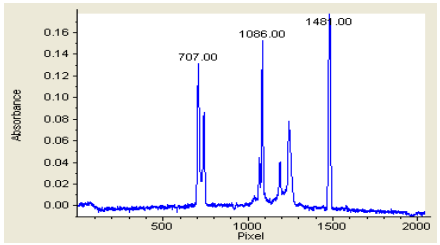

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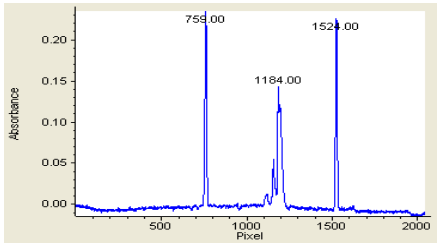

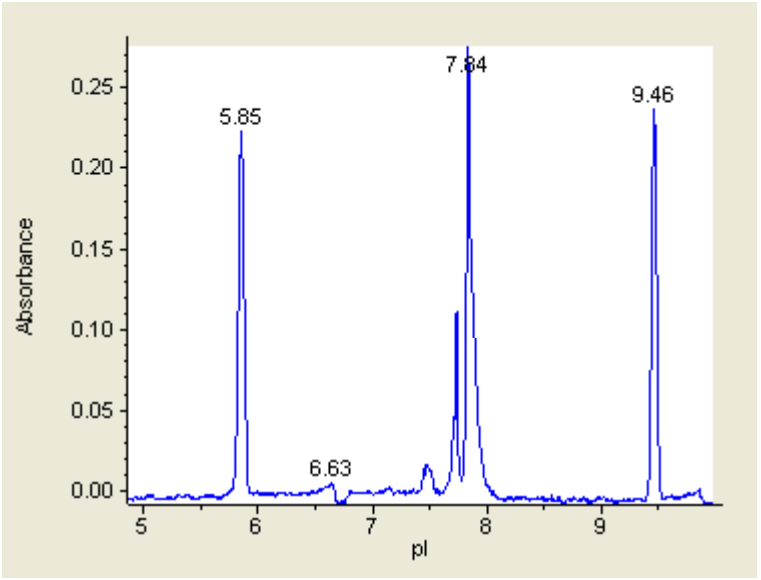

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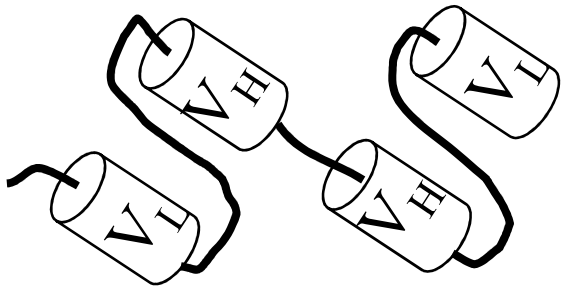
BiTE

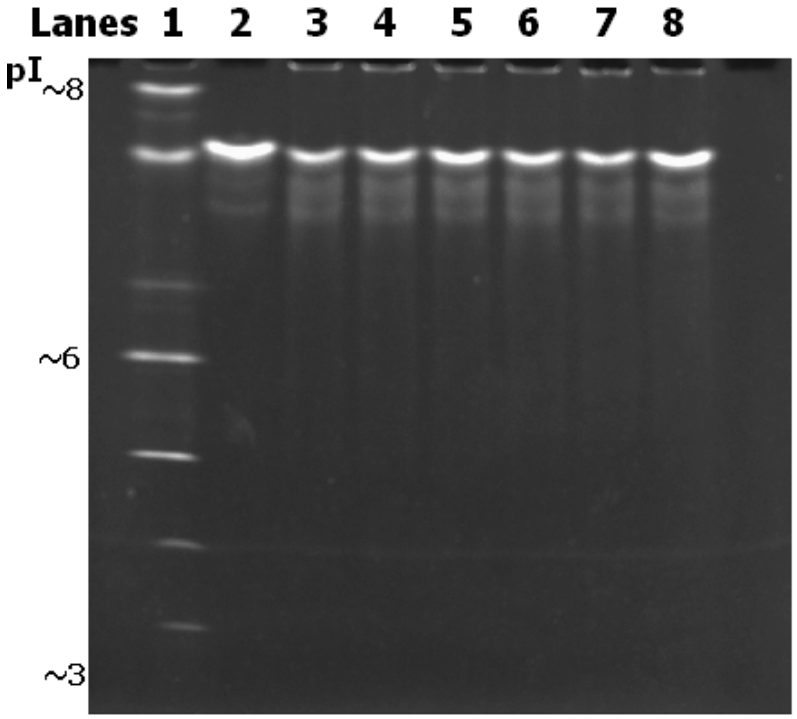
- BiTE antibodies are bi-specific T-cell engaging molecules
- Single chain antibody derivative
  - Has anti-CD19 and anti-CD3 moieties at opposite ends of the molecule
  - Works by recruiting CD3-expressing T cells to target/destroy CD19 expressing cells

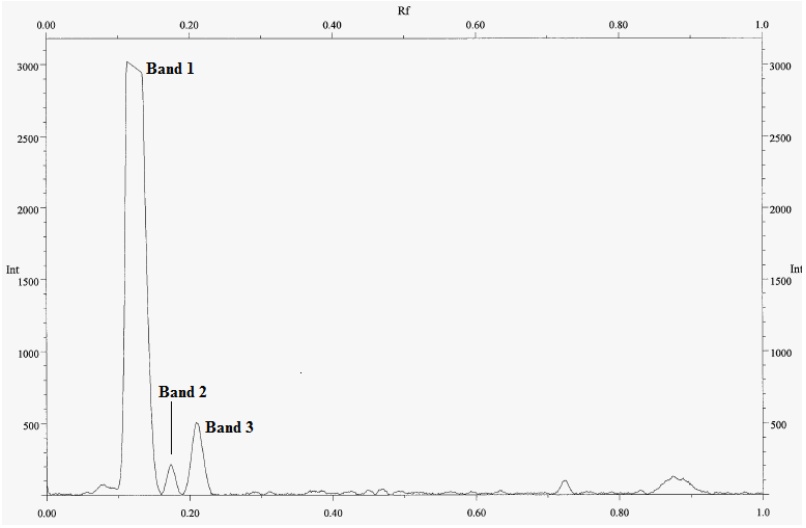


Slab Gel IEF of BiTE antibody

- Current slab gel IEF assay uses fluorescent staining followed by imaging
- Assay time (start to logging in results): approximately 36-48 hours.
- Throughput: 5 samples/gel; up to 4 gels processed in 36 hours
- Variability in imaging of pI markers makes pI assignment difficult/impossible
- Sample Suitability: Comparability with Reference Standard

BiTE antibody on Slab Gel IEF





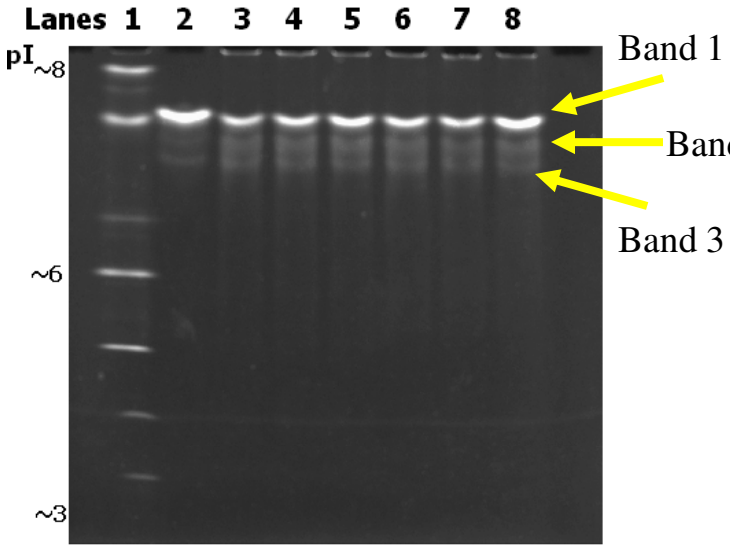
iCE280 Assay

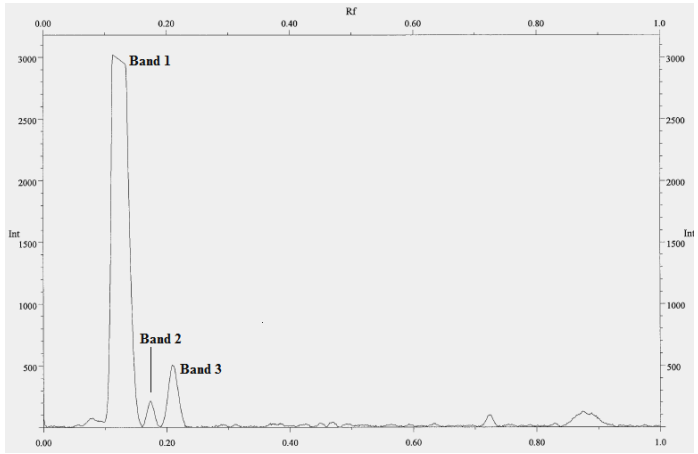
- Two SOPs prepared:
  - Assay
  - Instrument operation and maintenance
- Sample and System Suitability Criteria (proposed):
  - System suitability: marker pIs
  - For new projects, sample suitability will be Consistent with Reference Standard
  - As we collect and trend data, use pI of major peak(s) and major peak area as sample suitability
  - Also have the option of using a ratio of major peaks
- Currently in the process of qualifying the iCE280 method for multiple projects

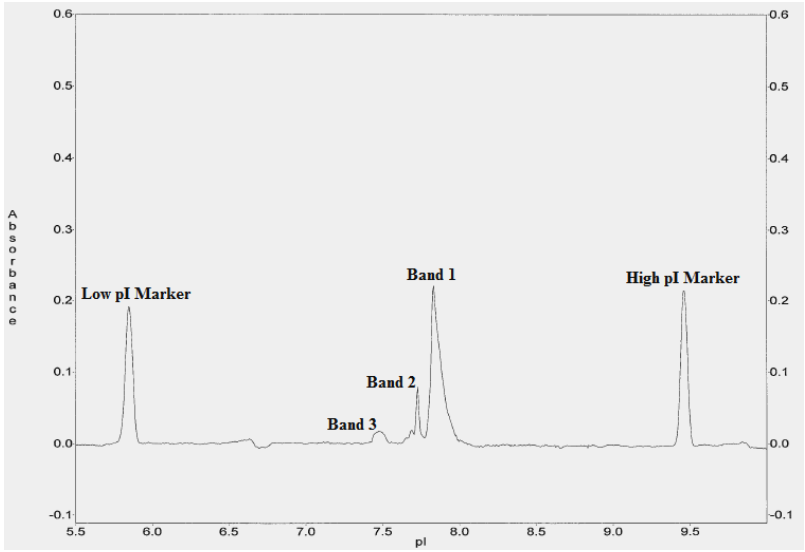
iCE280 assay for BiTE antibody

- Concentration of BiTE antibody samples adjusted to approximately ~0.3 mg/mL
  - Using spin-concentrators has dual function of increasing protein concentration and removal of interfering substances
- Following concentration, Pharmalyte 3-10 ampholines, 1% methylcellulose and pI markers (5.85 and 9.77; Convergent) are added
- Samples focused at 1500V for 1 min followed by 3000V for 6 min

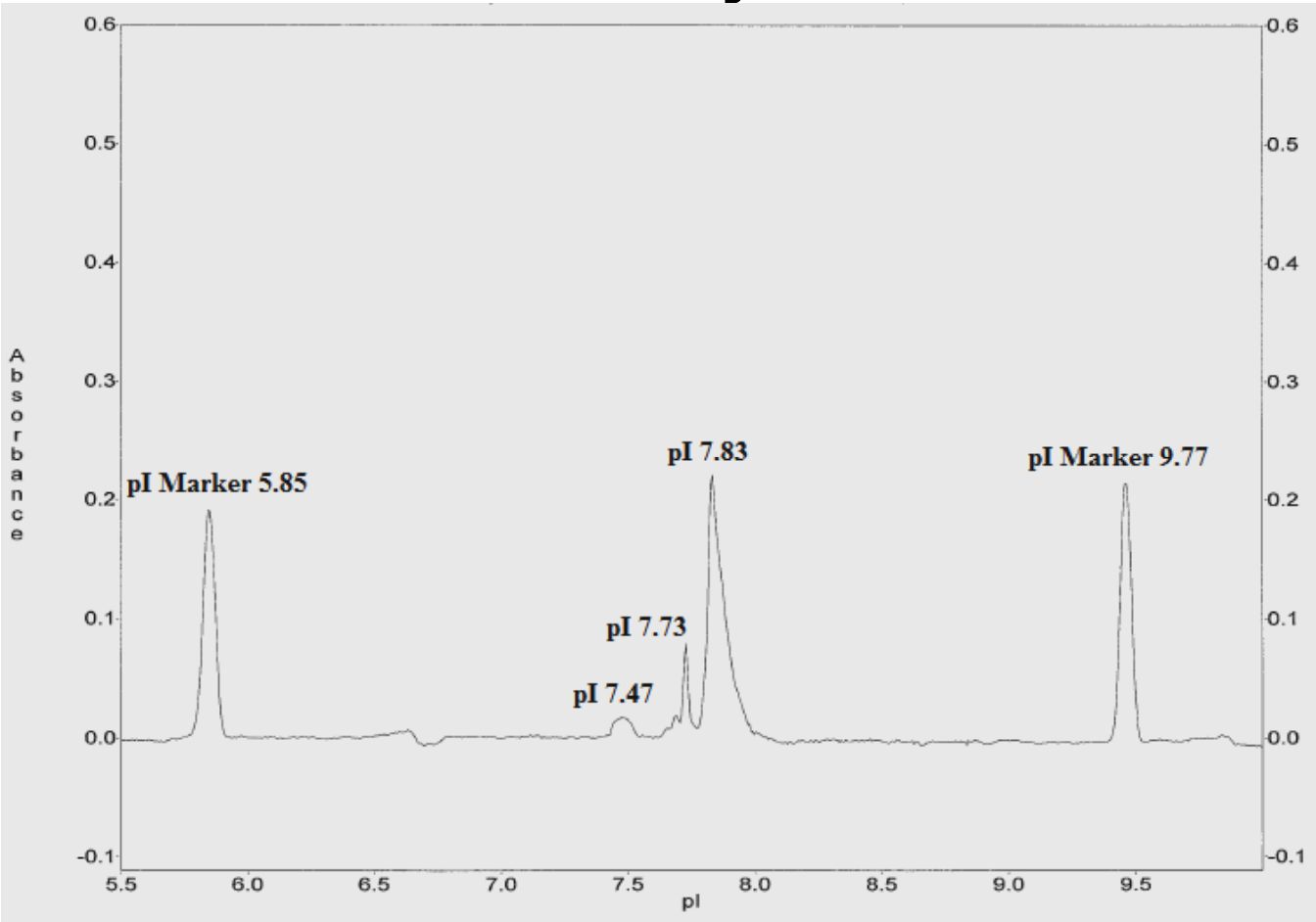
Slab Gel IEF vs. iCE280 for BiTE antibody







BiTE antibody on iCE280



Accuracy of iCE280 Assay for BiTE antibody and Humanized Monoclonal Antibody

		Experimental pI (iCE280 or densitometry)	Theoretical pI (ExPASy)	Difference
Slab gel IEF	BiTE	n/a	7.22	n/a
	Antibody 1	9.28	8.36	9.9%
iCE280	BiTE	7.85	7.22	8.0%
	Antibody 1	8.98	8.36	6.9%

Precision of iCE280 Assay for BiTE antibody

Assay Precision		Band 1	Band 2	Band 3
Analyst (n = 3)	Mean	*7.85*	*7.74*	*7.49*
	SD	*0.02*	*0.01*	*0.02*
	%CV	*0.25*	*0.11*	*0.31*
Day (n = 3)	Mean	*7.84*	*7.73*	*7.47*
	SD	*0.00*	*0.00*	*0.01*
	%CV	*0.00*	*0.00*	*0.11*
Reproducibility (n = 6)	Mean	*7.85*	*7.73*	*7.49*
	SD	*0.02*	*0.01*	*0.01*
	%CV	*0.23*	*0.07*	*0.16*

Summary: Slab Gel vs. iCE

ATTRIBUTE	Slab gel IEF	iCE280
Detection	Indirect (based on dye binding)	Direct (UV)
Technology acceptance	Acceptable Industry Standard	Arising New Industry Standard
Throughput	20 samples/36 hours	72 samples/24 hours
Waste generation per run	1.5 L	<0.06 L
Sample Preparation	45 minutes	45 minutes
Run time	3 hours	0.3 hours
Post-run processing time	28 hours (staining, destaining, densitometry)	0.25 hours (for manual integration)
Total sample turnaround time	2.5 days	1.3 hours

Conclusions

- A capillary IEF assay has been established for a BiTE antibody using the iCE280 Analyzer that is:
  - Accurate
  - Precise
  - High throughput

Future work with iCE280 Analyzer at MedImmune

- Continue with the qualification of the iCE280 method for various projects
- Evaluate new High Throughput iCE from Convergent:
  - Larger/more efficient autosampler
  - Microtiter plate capacity: up to 200 samples in 60 hours

Acknowledgements

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