

# Instrument-to-Instrument Variability of Convergent Bioscience iCE280

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Late Stage Analytical Development

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## Introduction

Imaged capillary isoelectric focusing (icIEF) has been recognized by recognized by pharmaceutical companies as a powerful separation method to determine the charge heterogeneity of recombinant recombinant monoclonal antibodies (rMAbs). Within the last year, last year, Genentech has implemented, seven Convergent Bioscience Bioscience iCE280 instruments within various departments including including quality control. The initial evaluation of a single instrument instrument showed very low run-to-run variation in reproducibility (< reproducibility (< 2% RSD main peak area). However, as more more instruments were installed, instrument-to-instrument performance performance was inconsistent. Studies determined the source of this source of this variation was due to the height difference in the inlet the inlet buffer vial and the outlet waste vial levels. The variation in vial variation in vial levels came from two sources. The first source was source was attributed to the poor performance of the overflow waste vial, overflow waste vial. The second source came from variation between the between the height of the iCE280 instrument and the height of the height of the balance vial in the Prince auto-sampler. In this report, we this report, we demonstrated that when proper balancing was established, was established, instrument-to-instrument variation reduced reduced dramatically. With properly balanced systems in place, we have place, we have validated Genentech molecules on multiple quality quality control instruments.

## Instrumentation and Multi-Product Method

**Ampholyte Solution**  
50µg Protein Sample  
4% (w/v) Ampholytes  
0.30% (w/v) HP-β-CD  
pI Markers

Vortex, centrifuge, and load on iCE280

Focusing time 10 min

Data Analysis

**Hydroxy propyl methyl cellulose**

## Instrument-to-Instrument Variation of rMAB

The performance of seven iCE280 instruments showed distinct variation in variation in charge heterogeneity and peak area percent. (Figure 1 and Table 1).

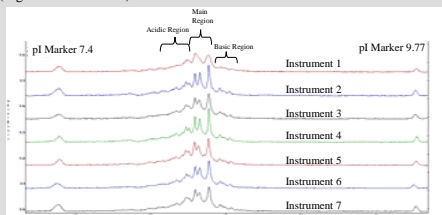


Figure 1: icIEF variability in seven iCE280 instruments for rMAB.

Table 1: Peak Area Percent from Seven Instruments

	Acidic Region	Main Region	Basic Region
Mean	39.1	49.3	11.5
RSD(%)	5.3	4.3	23.4

### Investigation of Variation

Experiments to determine the loss of resolution included included multiple cartridges, different ampholytes lots, and focusing focusing times. The results showed that instruments with poor resolution poor resolution continued to have poor resolution and instruments with instruments with good resolution continued to maintain good resolution, good resolution. At this point, bulk flow was investigated by replacing replacing the over-flow design with balance vials marked with fill lines with fill lines (Figure 2). These fill lines are used to establish consistency establish consistency of the meniscus. The new waste and balance vials balance vials were filled at the same height as the standard; then then conditions were changed so that the waste vial level was filled to was filled to either 5mm above or below the balance vial. All three three conditions were then run using the same sample and method. method. (Figure 3). The results demonstrated that the presence of bulk presence of bulk flow can severely effect resolution for rMAbs. rMAbs.

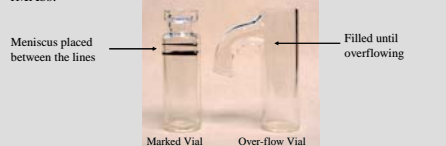


Figure 2: The over-flow waste vial (left) was replaced using a standard vial marked with fill marked with fill lines (right).

## Effect and Sources of Bulk Flow Within the Capillary

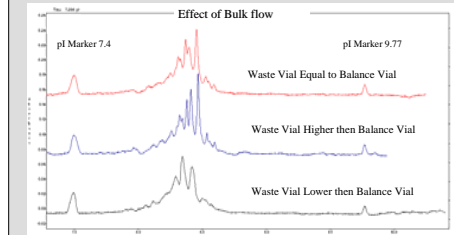


Figure 3: Effect of waste vial level on peak resolution for rMAB.

The source of the bulk flow within the capillary was determined to be from two determined to be from two sources:

- 1) Variation in the drain-off height as indicated by different different water levels in 3 over-flow waste vials (below).



- 2) Inaccurate height alignment between the iCE280 instrument and the Prince auto-sampler.

To isolate the effect of bulk flow induced by a height difference between the between the iCE280 and auto-sampler, the levels of the marked waste vial and waste vial and the balanced vial were filled to the same level. To monitor the To monitor the bulk flow, the pI 9.46 marker was used as a reference to reference to measure its final pixel position over multiple injections for seven seven injections for seven instruments (Figure 4). Final position of pI marker marker represents the influence of bulk flow in the capillary.

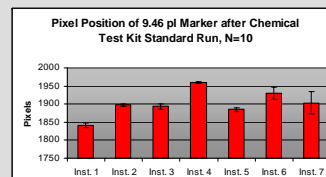


Figure 4: Effect of bulk flow on pixel position for pI 9.46 marker.

## Initial Quality Control Assessment of rMAB

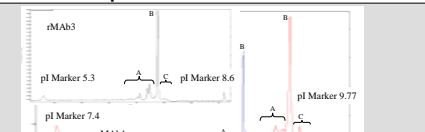
With the two main sources of variation minimized, the assay was transferred was transferred to Quality Control (QC) Analytical Technologies for initial initial Technologies for initial evaluation (Figure 5). Three iCE280 instruments instruments were used. This initial assessment showed high reproducibility reproducibility and generated an %RSD < 2% (n = 18) for the main peak main peak region (Table 2).

Table 2: Peak Area Percent from Three QC Instruments

	Acidic Region	Main Region	Basic Region
Mean	34.8	54.7	10.5
RSD (%)	4.7	1.7	5.8

Note: 3 samples, 2 injections per instrument; all samples were treated with carboxypeptidase B prior to analysis.

## icIEF Method Reproducible with Additional rMAbs



Peak Area %	A) Acidic Region		B) Main Region		C) Basic Region	
	Mean	%RSD	Mean	%RSD	Mean	%RSD
rMAB1 (N=18)	27.4	2.0	66.5	0.8	6.2	5.8
rMAB2 (N=112)	27.0	2.0	71.1	0.8	2.0	10.9
rMAB3 (N=48)	38.7	1.2	59.3	1.0	2.2	11.2

## Conclusions

The iCE280 delivers excellent reproducibility for protein analytes. analytes. However to produce low RSD's between multiple instruments, instruments, bulk flow must be minimized.

The over-flow waste vials should be replaced with the marked waste vials waste vials and the batch be periodically passed to allow reduction of the reduction of the waste water level to the marked level.

A standard should be implemented to both assess the relative balance of the balance of the instrument to the auto-sampler as well as compare the compare the performance of multiple iCE280 systems. This will help will help ensure consistent charge heterogeneity profiles and peak area peak area percents across multiple systems.

## Acknowledgments

The authors wish to thank: Zara Safarian, Toby Reichenberg of Genentech, Genentech, QC Analytic Technology

And Jiaqi Wu, Tiemin Huang, Ed Chase and Ravi Mandke of Convergent Convergent Bioscience