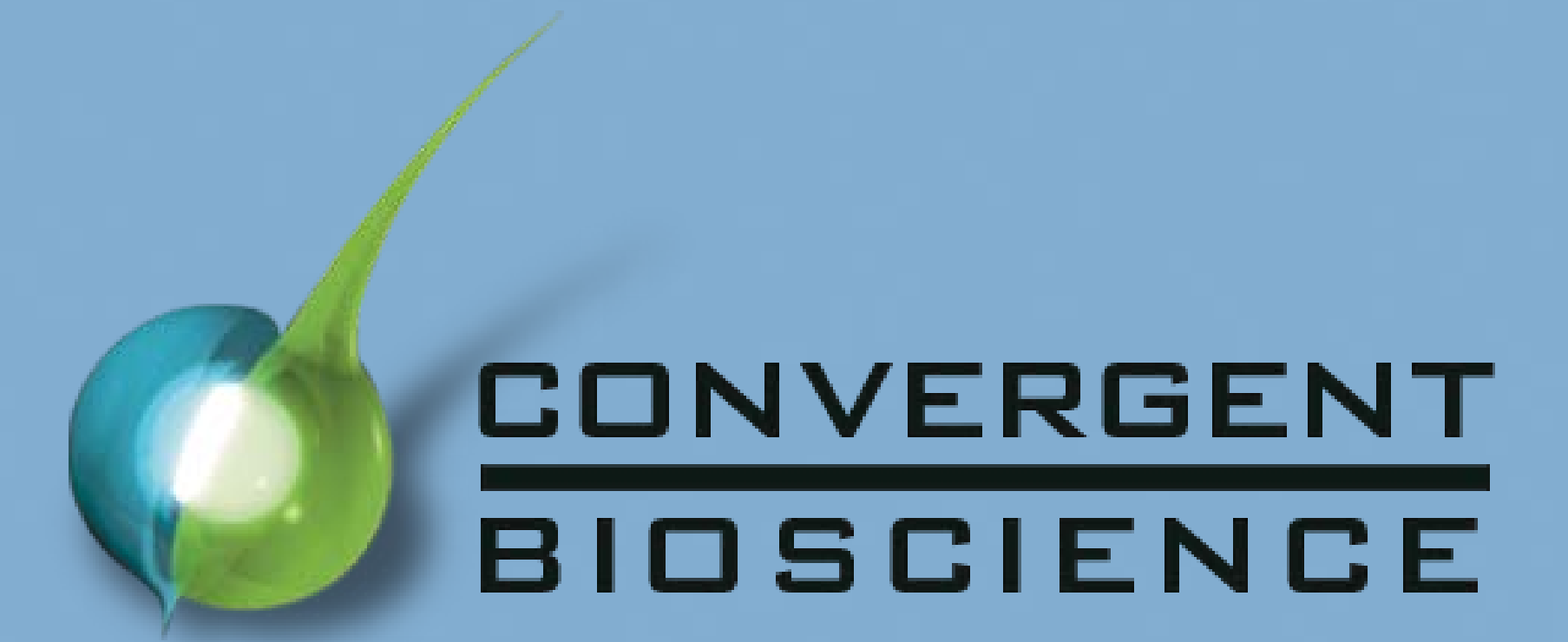


# Lot to Lot Variation of Carrier Ampholytes Used in Isoelectric Focusing (IEF)

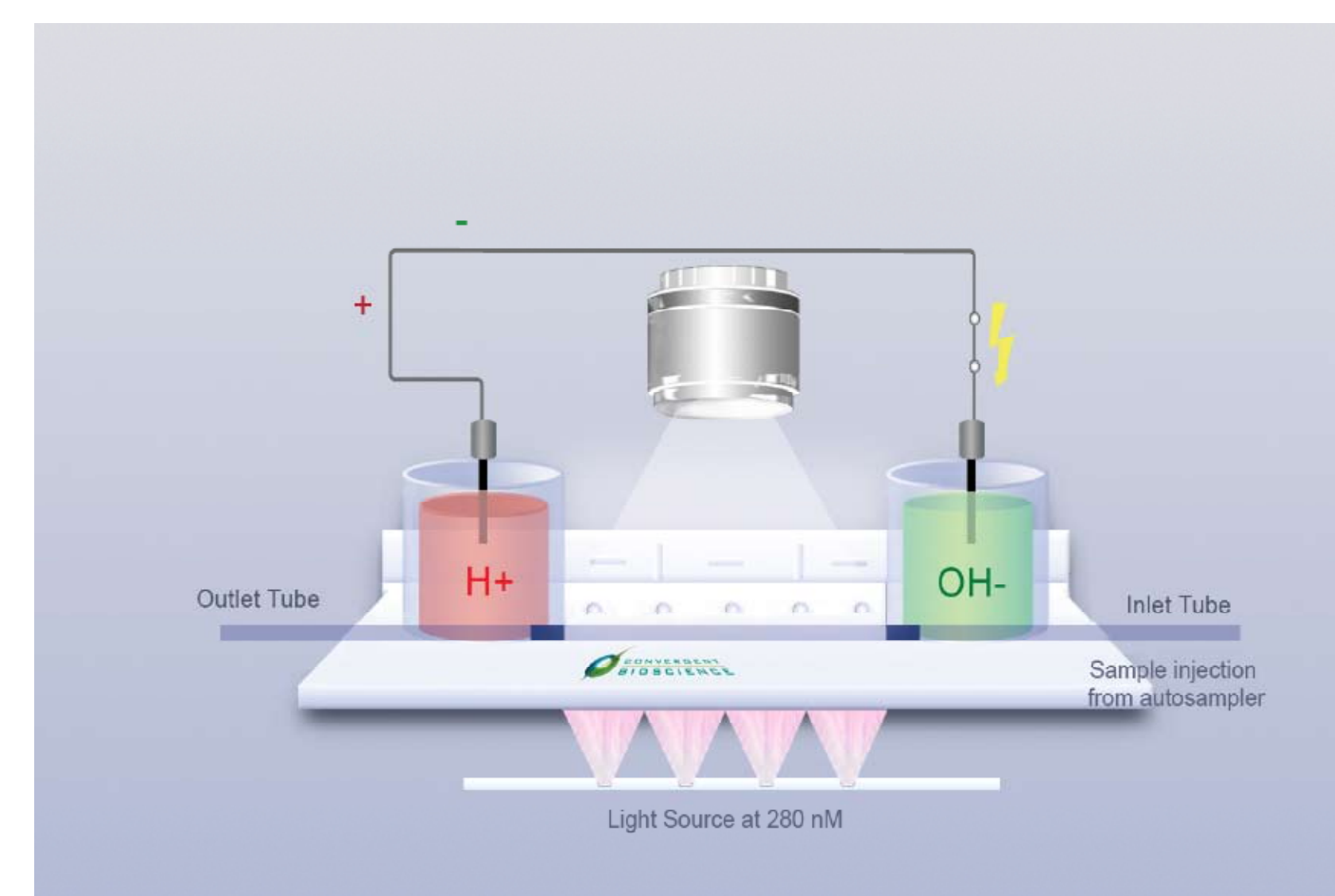
Jiaqi Wu, Convergent Bioscience Ltd., Toronto, ON, Canada



## INTRODUCTION

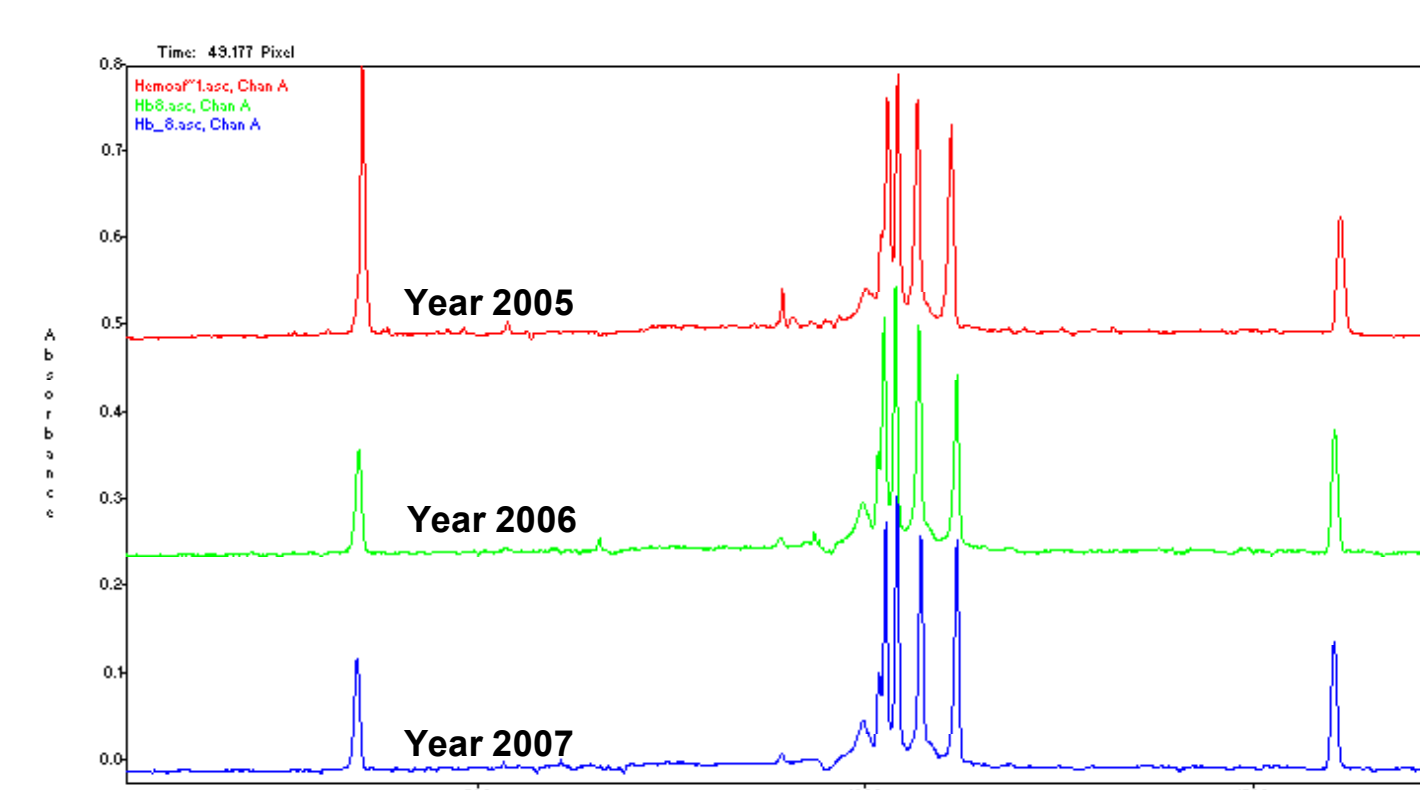
At Convergent Bioscience, the final testing for our whole-column detection capillary isoelectric focusing (cIEF) analyzer, the iCE280, includes running a standard sample in Pharmalytes pH 3-10 (by GE Healthcare). The data collected from the final testing is a good record of variation of Pharmalytes pH 3-10. In this presentation, data obtained using different lots of Pharmalytes pH 3-10 over a three year period (2005 – 2008) are analyzed. Knowledge about the variation between different lots of carrier ampholytes is important in cIEF method development and validation since it partly determines the design space in the cIEF methods.

## INSTRUMENT USED IN THE EXPERIMENT – THE ICE280 ANALYZER



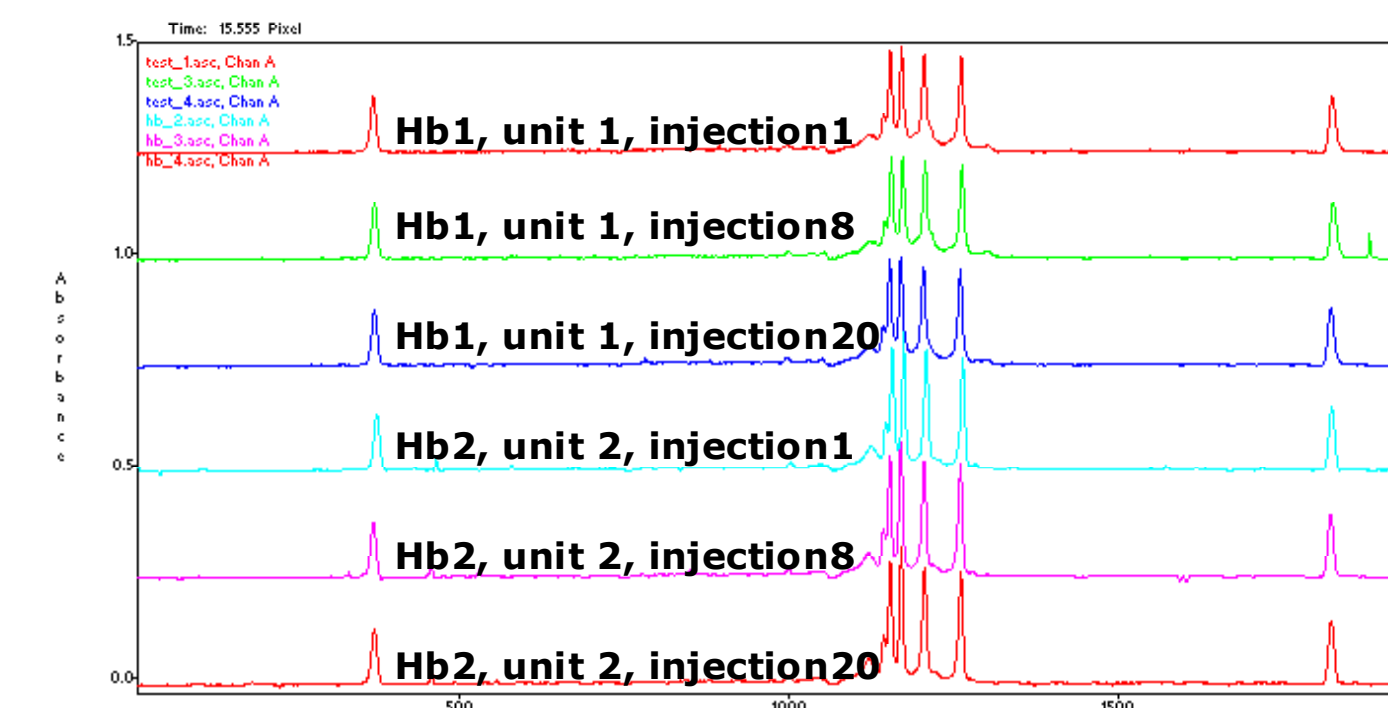
## CONFIRMATION OF METHOD REPRODUCIBILITY

Human hemoglobin A, F, S and C (three different lots):  
in 8 % pH 3-10 Pharmalytes (same lot), 0.35% methyl cellulose. Data was obtained from three different iCE280 analyzers.



## DIFFERENCES IN DIFFERENT LOTS OF PHARMALYTES

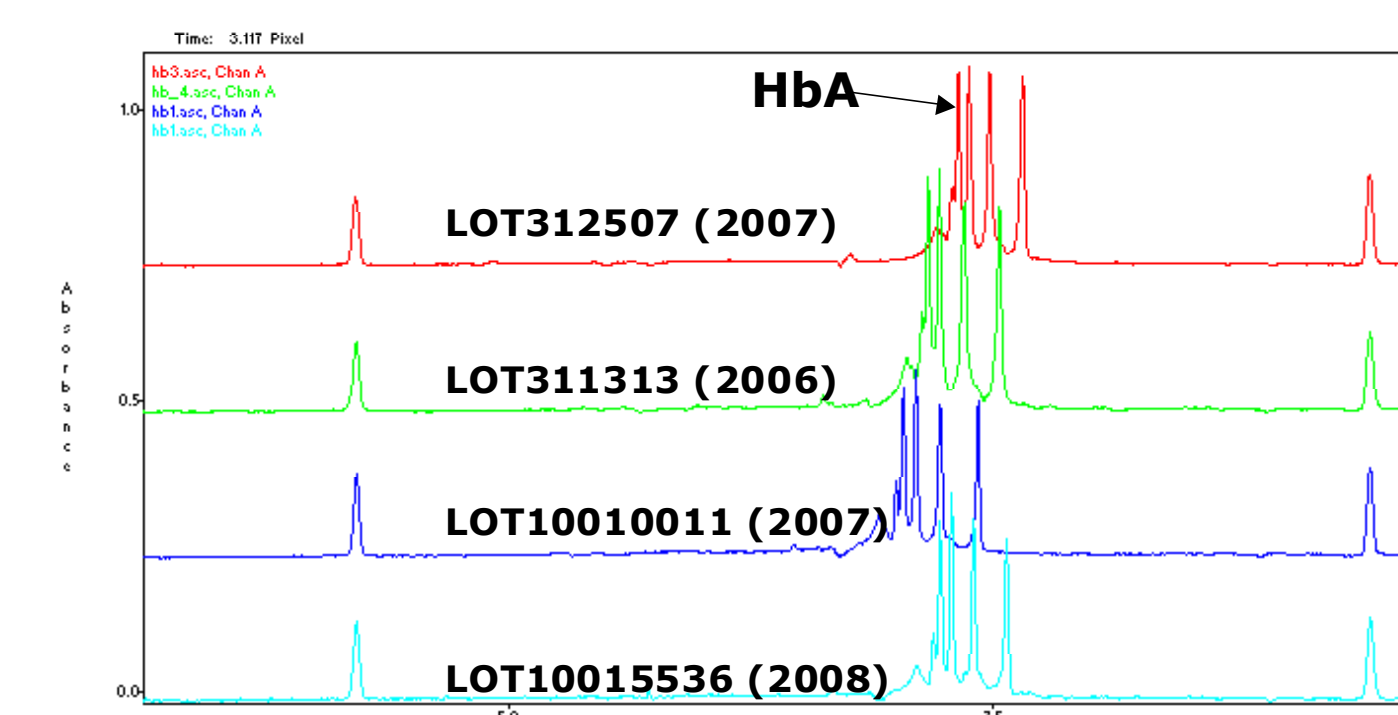
Human hemoglobin A, F, S and C (two different lots):  
in 8 % pH 3-10 Pharmalytes (same lot), 0.35% methyl cellulose. Data was obtained from two iCE280s.



Reproducible peak positions were obtained as long as a single lot of Pharmalytes was used.

## PROBLEMS USING MULTIPLE MARKERS – LINEAR LEAST SQUARES METHOD

Human hemoglobin A, F, S and C:  
in 8 % pH 3-10 Pharmalytes (four different lots), 0.35% methyl cellulose. Data was obtained from four iCE280s.



Differences in pH gradients were observed for different lots of Pharmalytes.

## TRACKING THE CHANGES OF THE PHARMALYTES

### Method:

Record the measured pI value of the HbA peak (the left side of the first major peak, as shown in the above figure) after e-grams are calibrated using the two spiked pI markers (pI 4.22 and pI 9.4).

### Results:

The table below shows seven lots of Pharmalytes pH 3-10 that were used in the final testing at Convergent Bioscience in three years.

Month/Year	Pharmalytes Lot	Calibrated pI of HbA
April, 2006	305023	7.12
June, 2006	311313	7.25
March, 2007	312507	7.33
April, 2007	10006151	7.17
August, 2007	10010011	7.05
December, 2007	10010492	7.25
September, 2008	10015536	7.12

### Observations:

1. The pH gradient differences between these lots are obvious.
2. The changes are random.
3. Some later lots may be close to some earlier lots.

## SUGGESTIONS FOR METHOD DEVELOPMENT AND VALIDATION

Due to the lot to lot variation in carrier ampholytes, enough space should be given for calibrated pI values of samples in cIEF method development and validation.

### In method development:

1. Try as many different lots of carrier ampholytes as possible.
2. Evaluate the differences between different lots.

### In method validation:

1. There should be enough room left in the specification of calibrated pI values of the samples.  $\pm 0.2$  should be adequate based on the results above.
2. All the calibrated pI values should be compared to the reference sample run in the same batch.

## CONCLUSIONS

1. **Lot to lot variation in Pharmalytes pH 3-10 was observed when results run under different lots of Pharmalytes in 3 years were compared.**
2. **This variation is a risk in a cIEF method lifecycle.**
3. **This can be overcome by providing adequate space for the specification of the calibrated pI values of the samples.**