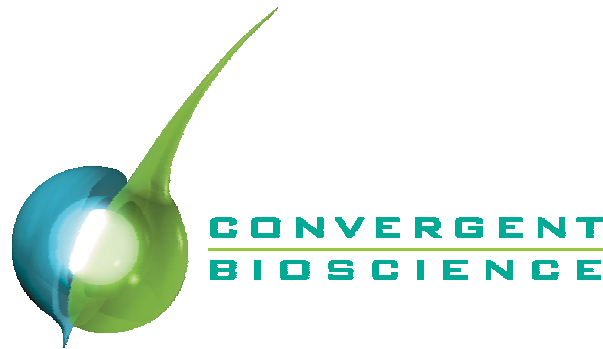


Fast pI Determination for Viruses Using Whole-Column Detection cIEF

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Why pI Information is Important for Viruses

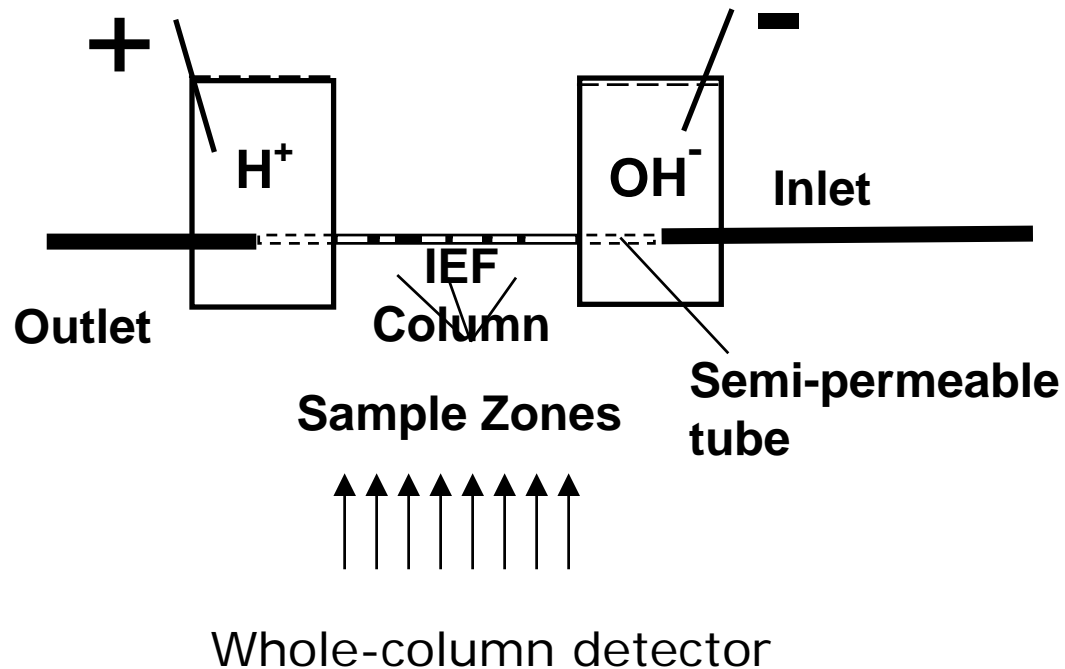
- ◆ Formulation for vaccines
 - pI information is required in formulation development for viruses *
- ◆ Product processing for vaccines
 - pI values of viruses need to be monitored in the whole deactivating process
 - Vaccines' pI change is an indicator of production condition changes
- ◆ Fast identification of viruses * *

* **Journal of Pharmaceutical Sciences, 2006, DOI 10.1002/jps20675**

** **Analytical Chemistry, 76, 2004, 48-52**

Instrument Used in Experiments

- Whole-Column Detection cIEF
- iCE280 Analyzer



iCE280 Analyzer Features

- ◆ Mobilization process is eliminated
 - Only focusing time needs to be optimized
- ◆ Whole column detector constantly monitors IEF process in entire column
- ◆ Free solution IEF allows large molecular weight samples to be loaded
- ◆ Wide pI range (pH 2-11)

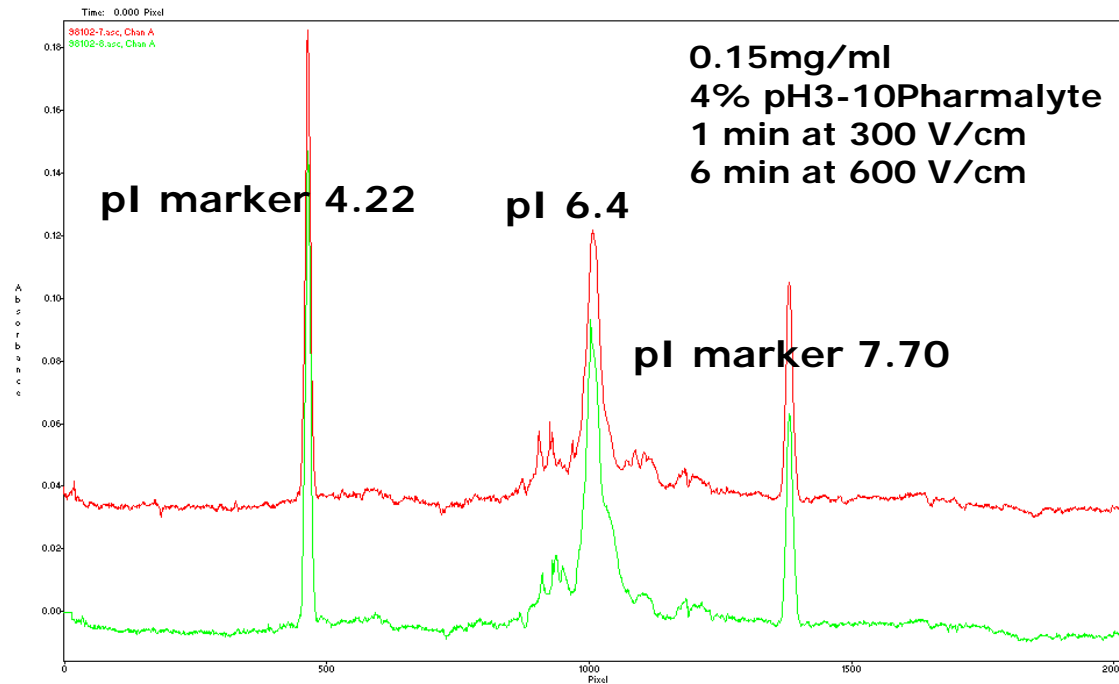
Proposed Conditions for Fast pI Determination for Viruses

- ◆ 4% pH 3 - 10 Pharmalyte
 - pI range covers most viruses
 - Lower background noise at 280 nm compared to other brand name carrier ampholytes
- ◆ 6 min focusing time at 600 V/cm
 - 1 min pre-focusing at 300 V/cm may apply
- ◆ Sample concentration needs to be adjusted for each individual virus

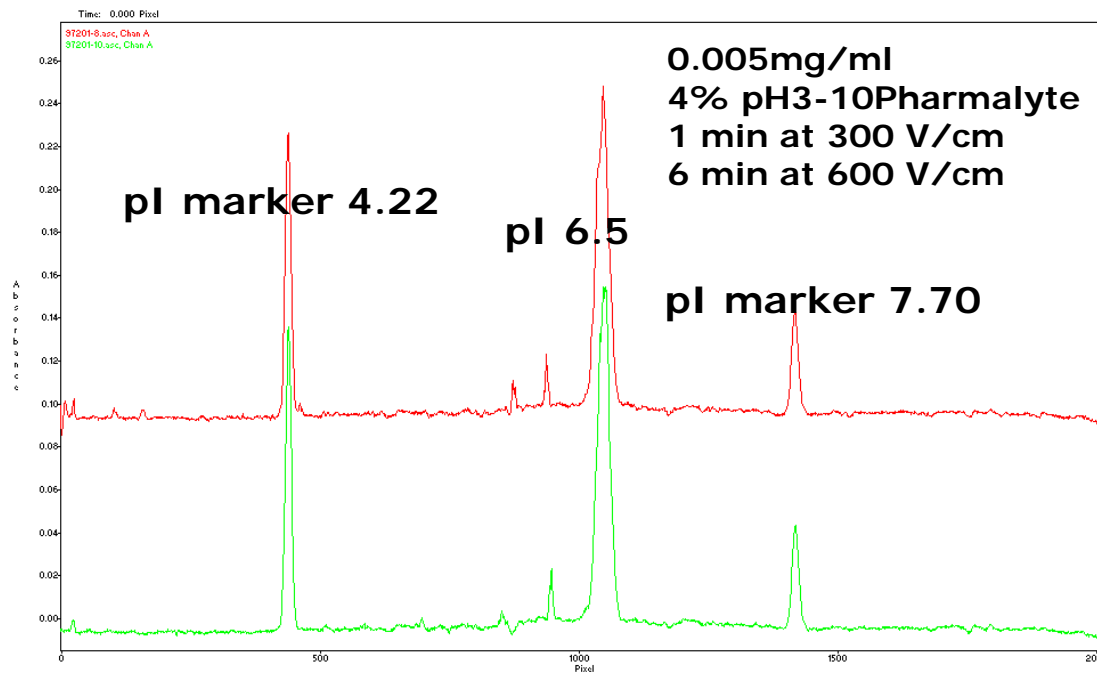
Example - Deactivated Polio Virus

- ◆ The deactivated viruses are used as polio vaccines
- ◆ Different types of viruses may be identified by their pI values

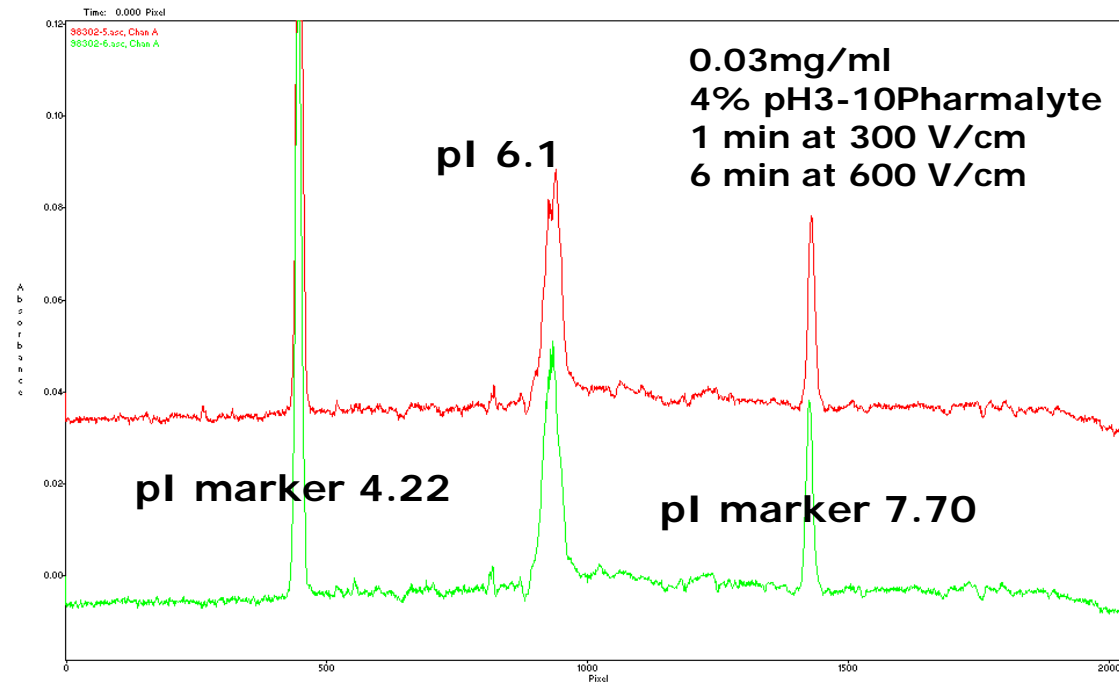
Deactivated Polio Virus Type 1



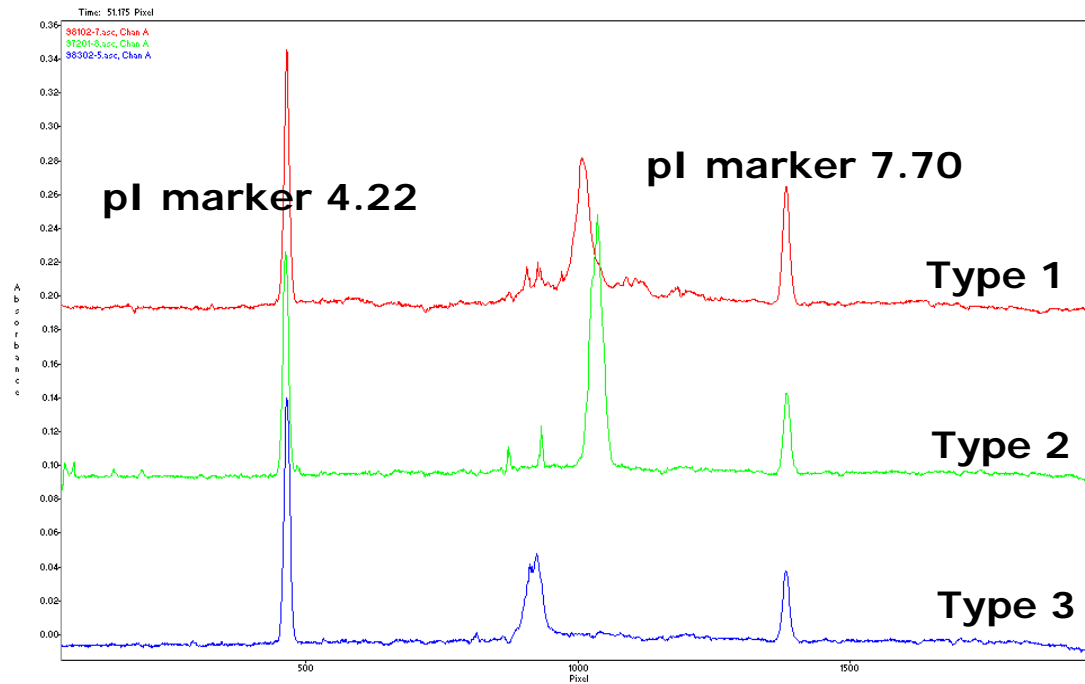
Deactivated Polio Virus Type 2



Deactivated Polio Virus Type 3



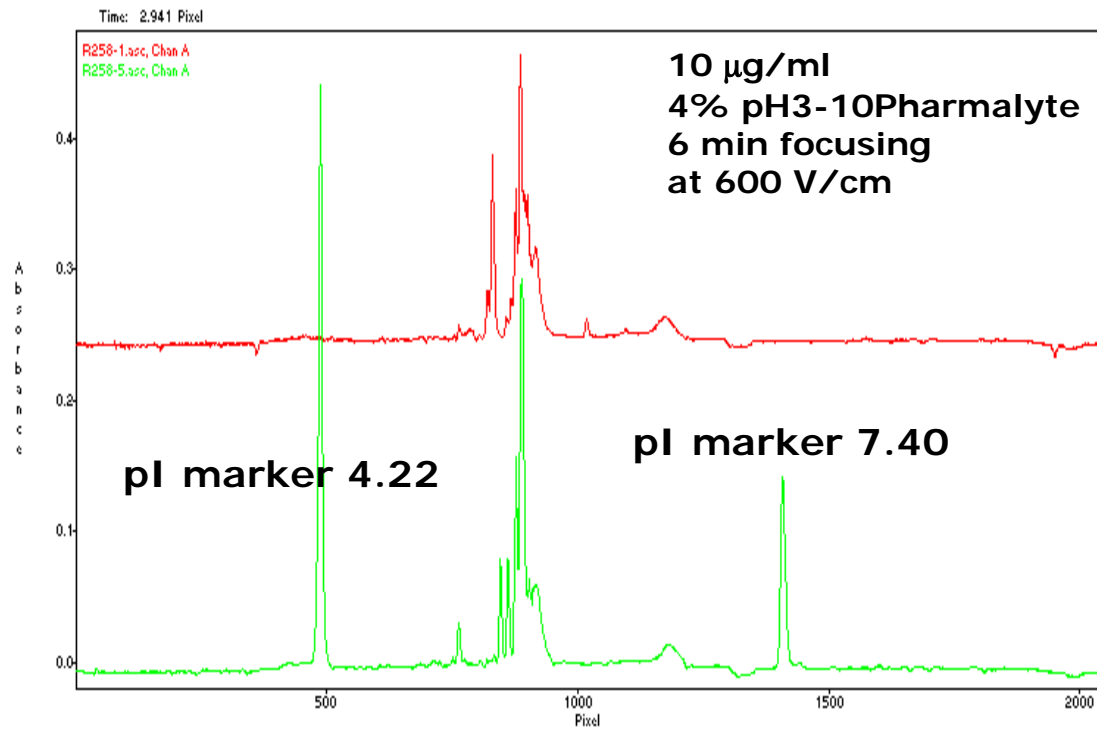
Deactivated Polio Viruses



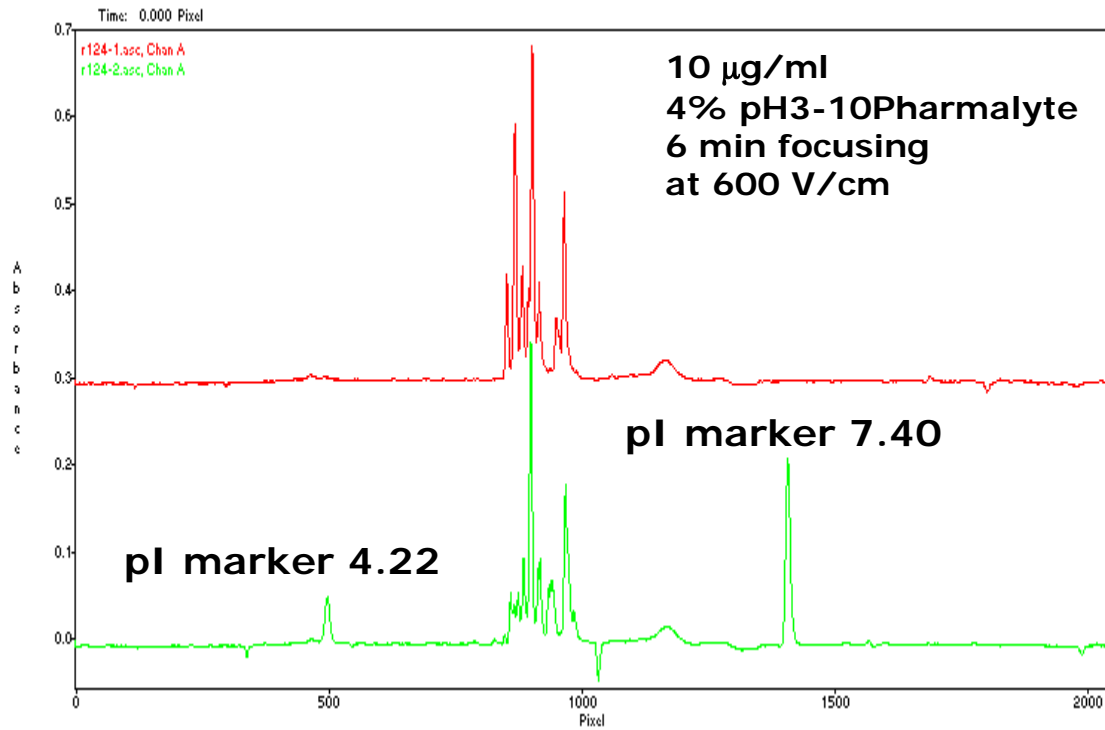
Example – Norovirus Virus-Like Particles (VLPs)

- ◆ Norovirus is the main source of nonbacterial food poisoning
- ◆ Problem with Traditional identification methods: False negative
 - Enzyme-Linked Immunosorbent Assay (ELISA)
 - PCR
- ◆ Possible identification based on pl

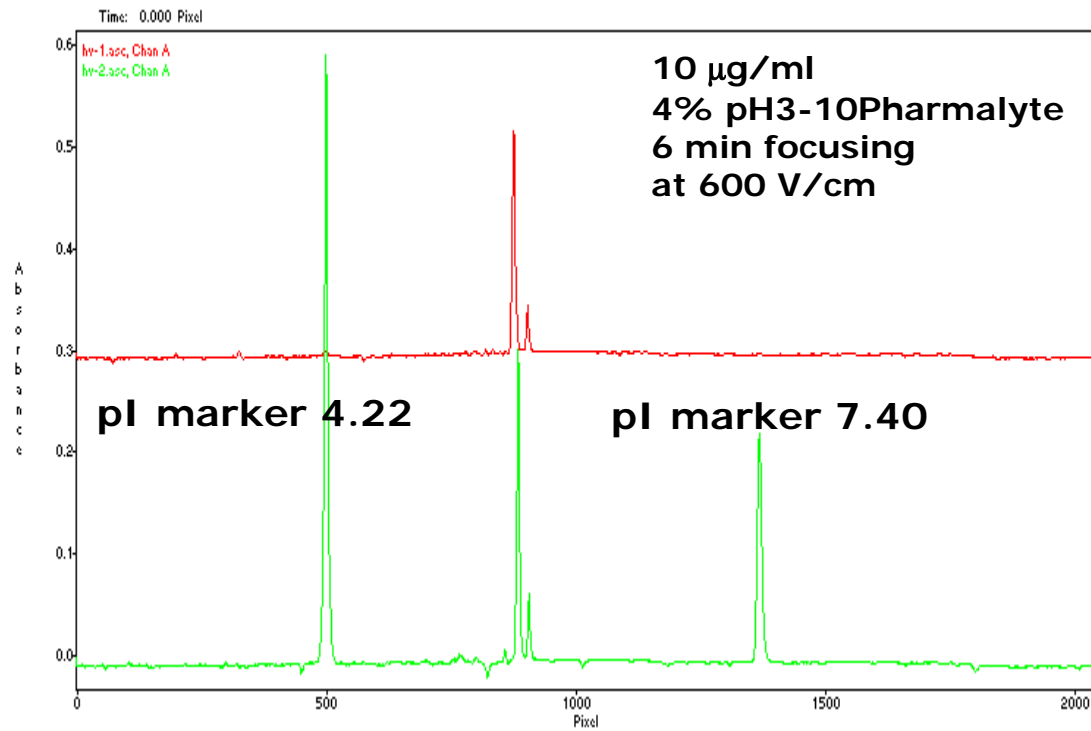
Norovirus VLP Funabashi



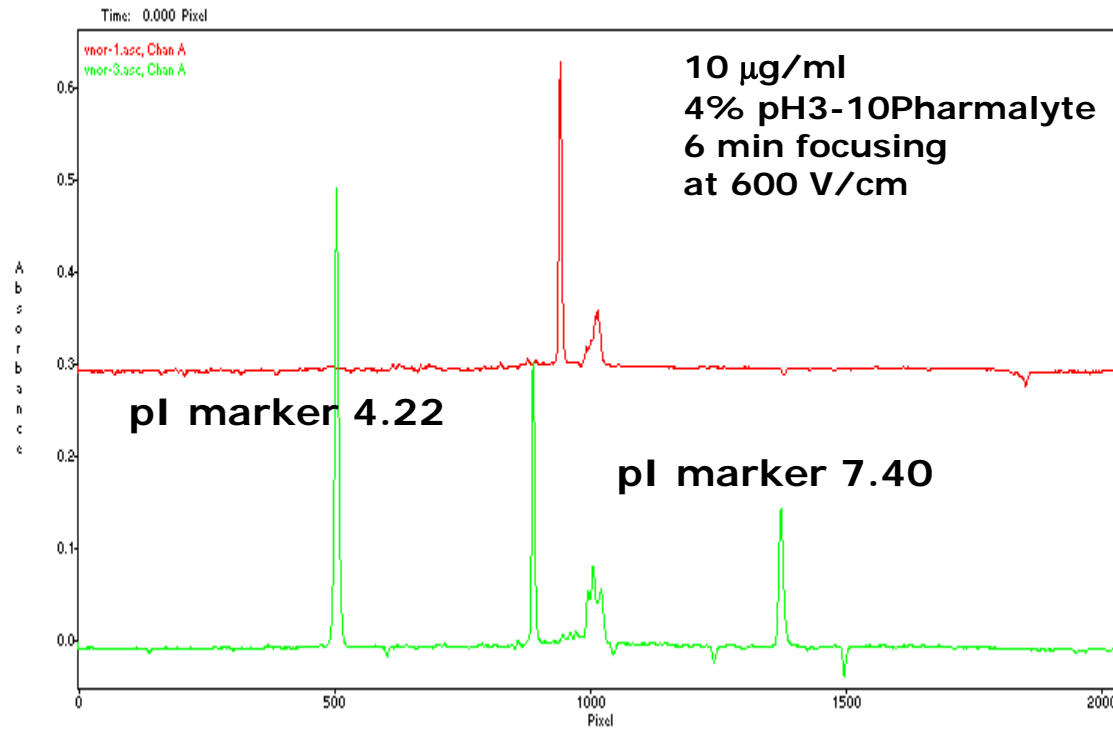
Norovirus VLP Seto



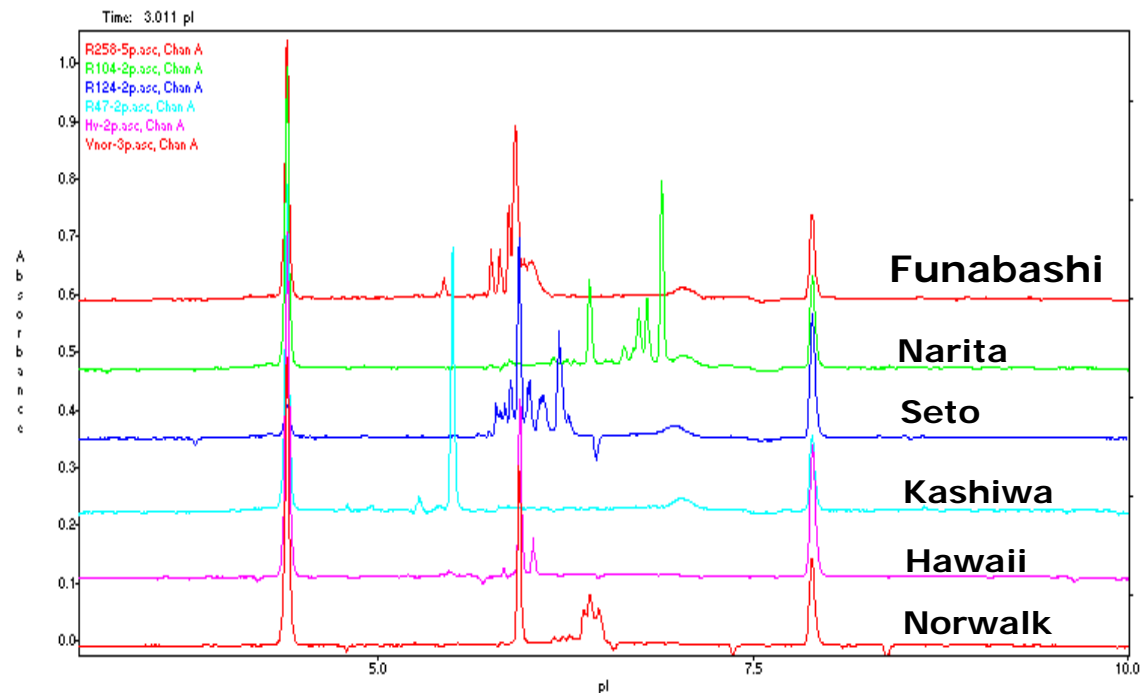
Norovirus VLP Hawaii



Norovirus VLP Norwalk



Norovirus VLPs



Conclusions

- ◆ Fast method development
 - Less than 3 days for Viruses
- ◆ Reproducible measured pI values
 - ± 0.05 pH units from run to run
- ◆ High throughput for virus analysis
 - Up to 5 samples/hour