The 2012 PRG study: Assessing longitudinal variability in routine peptide LC-MS/MS analysis

Maureen K Bunger[1],Tracy M Andacht[2], Keiryn L Bennett[3], Cory Bystrom[4], Larry Dangott[5], John Leszyk[6], Henrik Molina[7], Robert Moritz[8], J. Will Thompson[9], David Tabb[10] Matt Chambers[10] Brett S Phinney[11]

[1]North Carolina Central University, [2]Centers for Disease Control and Prevention, [3]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, [4]Quest Diagnostics, [5]Texas A&M University, [6]University of Massachusetts Medical School; [7]The Rockefeller University; [8]Institute for Systems Biology, [9]Duke University, [10]Vanderbilt University Medical Center, [11]Unviersity of California Davis

Introduction

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) is a staple of both proteomic core facilities and research laboratories. It is well known that many factors that affect LC-MS/MS are prone to change over time. Some of these factors include sensitivity, retention time, peak shape, mass accuracy, just to name a few. These factors are even more pronounced as flow rates and column sizes decrease.

The importance of quality control in the proteomics lab has recently been illuminated in publications by the CPTAC group (e.g., Molecular Cellular Proteomics (2010) Volume: 9, Issue: 2, Pages: 242-254). Most labs have developed their own quality control procedures to assess data acquisition performance but may rarely perform longitudinal analysis to monitor instrument performance and variability. Measuring and documenting these changes is important as core facilities need to ensure that their LC-MS/MS systems are functioning properly and any drift in analytical performance is within the suitability of purpose of what they are attempting to measure. Currently there is no standard quality control measure used by core facility laboratories nor has there been any large studies that measure instrument performance over time.

Goals

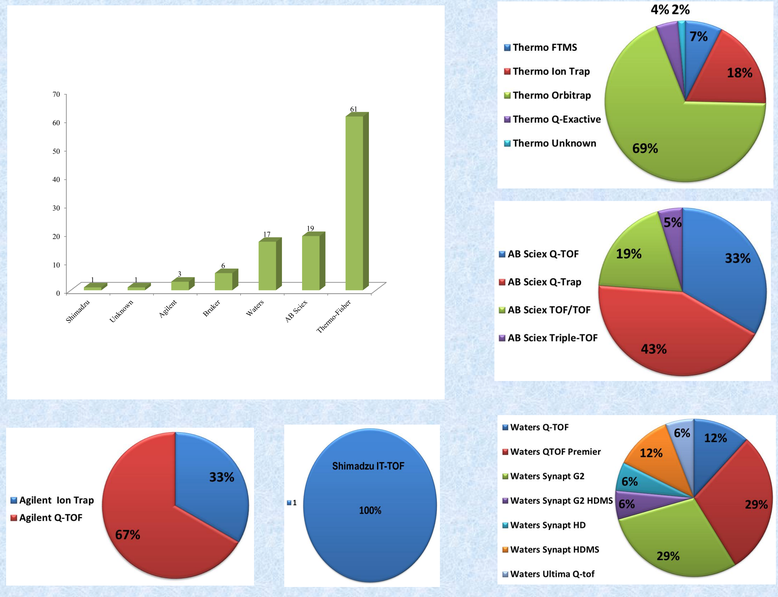
The 2012 PRG study is ambitious. We are performing a nine month longitudinal assessment (March 2012 – November 2012) of intra-laboratory instrument performance using regular runs of a tryptic digest of six proteins. The goal of the study is not directed toward maximizing protein identifications, or for determining best practices for quality control procedures. Rather, our aim is to determine a number of performance metrics that represent the overall performance of an LC-MS system and then monitor these metrics over time. We expect that the results from this study will provide participants with a performance benchmark within their own lab and will allow the opportunity to see the range of performance metrics from a broad sampling of labs across the globe.

Materials and Methods

Each participating laboratory will receive 9 lyophilized aliquots of 6 bovine proteins digested with trypsin (Michrom 6 bovine equimolar mix). Samples will simply require re-suspension and LC-MS/MS data acquisition. Because this study is an intra-laboratory comparison, participating laboratories are free to use their standard LC-MS/MS configuration with data-dependent acquisition settings of their choice. However, the study does require that the method and instrumentation of choice is kept constant during the test period. The PRG anticipates that the samples can be run in any proteomics lab equipped with standard LC-MS/MS instrumentation.

Each lab is asked to run each sample once per month during course of this study and upload the data to our server. After data upload, a brief questionnaire will be used to determine the general conditions of the system at the time of acquisition. All subsequent data processing and analysis will be provided by the PRG.

Figure 1. Instrument Diversity (includes laboratories running multiple instruments).



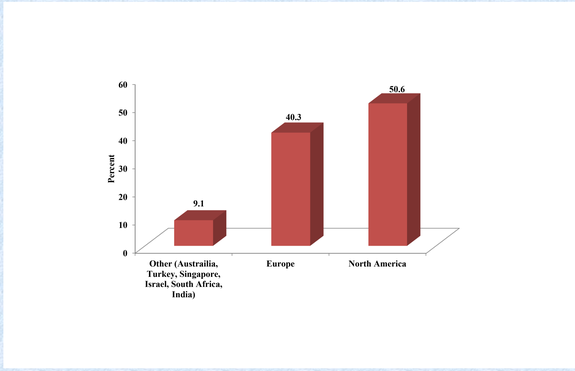


Figure 2. Demographics

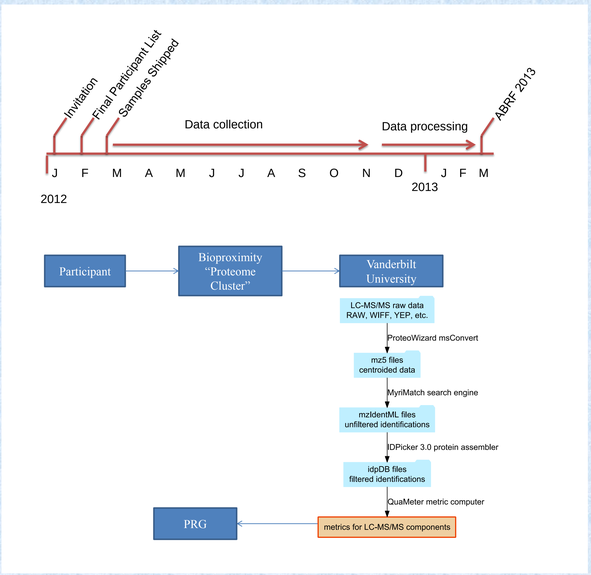


Figure 4. Data analysis Storage and Pipeline

ProteoWizard reads LC-MS/MS data in native formats from most mass spectrometry vendors. Peaklists can be identified through database search in the MyriMatch database search engine (PubMed 17269722). IDPicker can then determine which spectra have been identified at a reasonable FDR (PubMed 19522537). The QuaMeter engine connects raw data to identifications to generate the metrics. These values, modeled after the set published by NIST (PubMed 19837981), characterize chromatography, ionization, mass spectrometry, precursor selection, and identifications for each LC-MS/MS experiment.

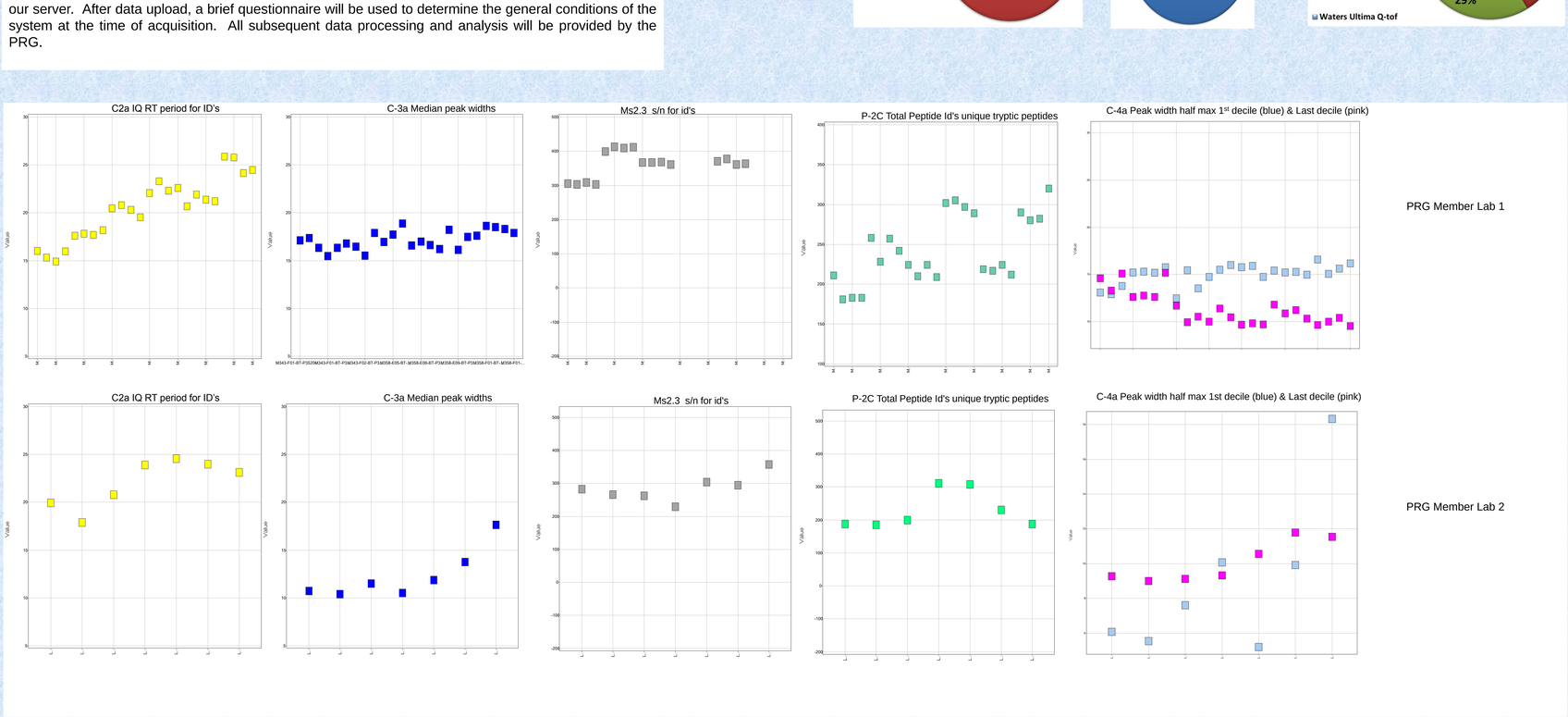


Figure 3. Sample Data analysis

Samples were injected over multiple days. Top row, PRG member 1 ran 4 technical replicate injections of either 10fmol or 40fmol quantity of the digested mix on 3 different days. Total run time was 100 minutes on a Thermo Velos-Orbitrap. Bottom row: PRG member 2 ran single replicates of 100 fmol on 7 different days over 1.5 months. Total run time was120minutes on a Thermo LTQ. Shown are 5 of the 42 metrics analyzed in QuaMeter and a sampling of the type of expected output.

Results

Results will be collected over the next 9 months and the data will be presented at the next ABRF meeting in Palm Springs!

Acknowledgements

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Please visit www.abrf.org/PRG for more in-depth examination of the methods used and the experimental results obtained.