

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

# Thanks!

## 2012 PRG

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Cory Bystrom - *Quest Diagnostics*

Larry Dangott - *Texas A&M University*

John Leszyk - *University of Massachusetts Medical School*

Henrik Molina - *The Rockefeller University*

Robert L Moritz - *Institute for Systems biology*

Brett S Phinney (EB liaison) - *Proteomics Core UC Davis Genome Center*

## PRG adhoc members and other supporters

J. Will Thompson - *Duke University*

Matthew Chambers - *Vanderbilt University*

David Tabb - *Vanderbilt University*

Paul Rudnick - *National Institutes of Standards and Technologies*

## To Our Participants!

### RG LC-MS/MS

Dr Rich Eigenheer - *UC Davis Genome Center*

Christian Knoll - *CeMM,*

### Companies that contributed



# Key Goals

- Measure intra-laboratory variation in LC-MS performance over time
- Survey types of QC procedures implemented in proteomics facilities
- Survey elements of system design/setup that correlate with variability
- Educate and Inform

# Study Design Principles

- Provide labs with purified, digested, aliquotted protein mixture
- Expect one run per month for 9 months
- Expect fresh aliquot to be used each time
- Expect user to implement same settings for each run, but settings they determine based on their typical setup/comfort
- User only needs to upload raw data

# Other study designs that were considered

- Providing labs with both complex (biological organism) and simple protein mixtures
  - Too time consuming and expensive
  - labs may not want a complex biological system
  - sample availability limited
- Requiring targeted MS1 level approach (e.g. monitoring 5-10 peptides)
  - May be out of comfort zone for many
  - Uncertain how results would translate to a more standard DDA identification analysis

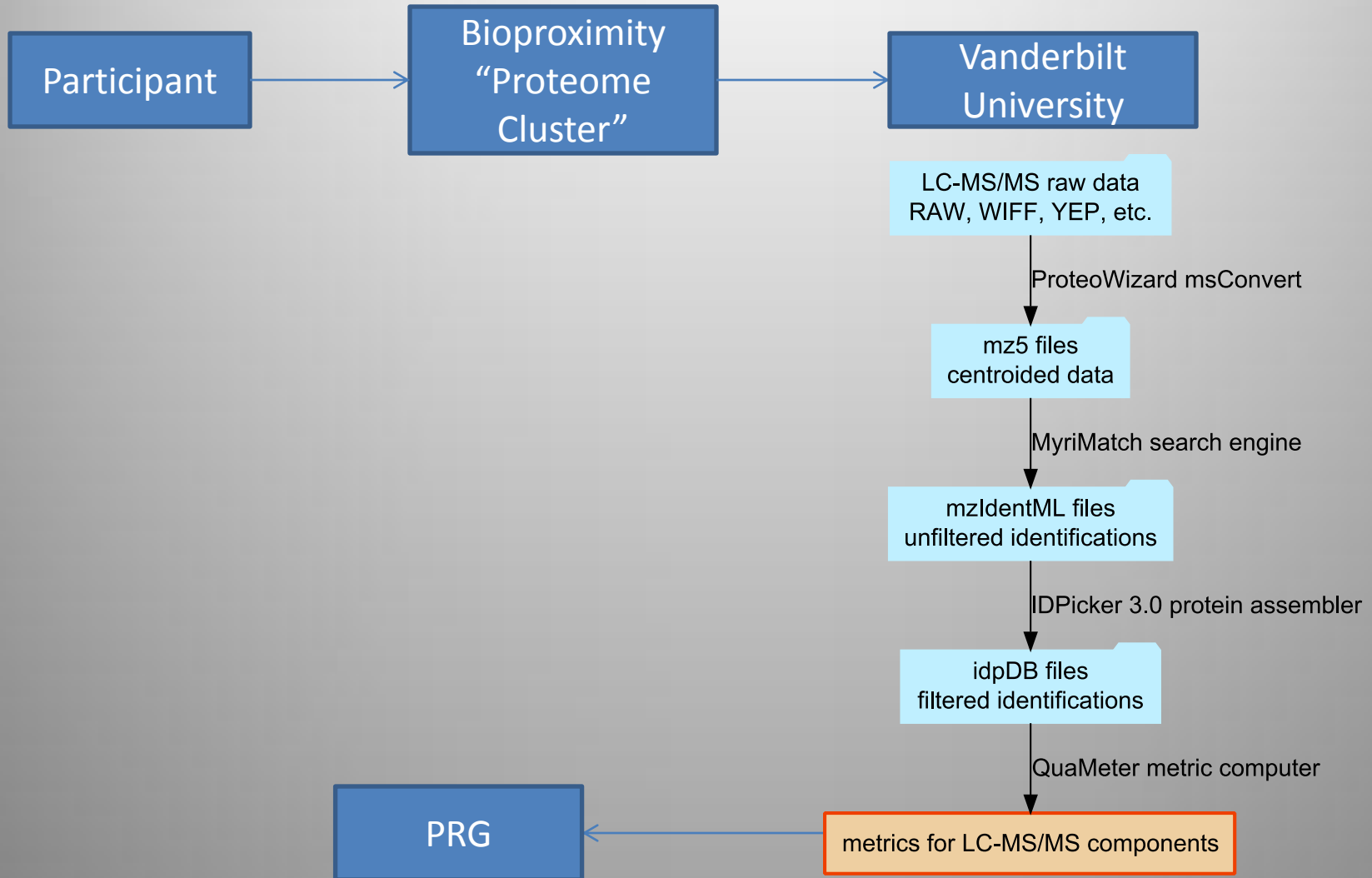
# Bovine 6 protein mix (Michrom)

- Tryptic digest of the following Proteins:
  - glutamate dehydrogenase 1, mitochondrial precursor
  - serum albumin precursor
  - beta-lactoglobulin precursor
  - carbonic anhydrase 2
  - lactoperoxidase precursor
  - alpha-S1-casein precursor
- 1 pmol (total material) equimolar mix provided in each aliquot (approx 166 fmol of each protein)

# Timeline



# Data pipeline





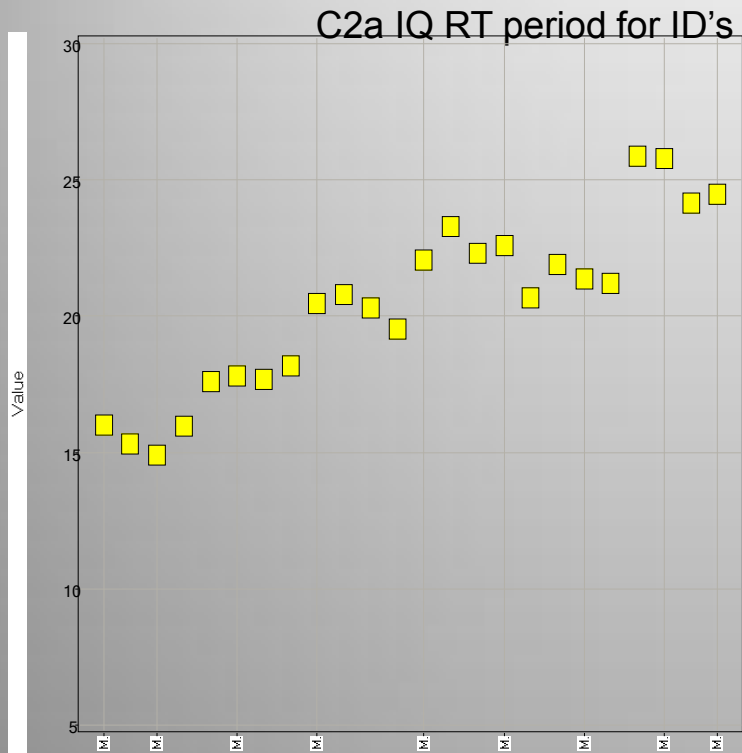
# QuaMeter Metrics

- MS2.4C Fraction of total MS2 scans ID'd in the third quartile of peptides sorted by MS1 max intensity
- MS2.4A Fraction of total MS2 scans ID'd in the first quartile of peptides sorted by MS1 max intensity
- MS2.4D Fraction of total MS2 scans ID'd in the fourth quartile of peptides sorted by MS1 max intensity
- MS2.4B Fraction of total MS2 scans ID'd in the second quartile of peptides sorted by MS1 max intensity
- C.1A Fraction of all peptides ID'd at least 4 minutes earlier than max MS1 for ID
- C.1B Fraction of all peptides ID'd at least 4 minutes later than max MS1 for ID
- IS.3A Number of 1+ peptides over 2+
- IS.3C Number of 4+ peptides over 2+
- IS.3B Number of 3+ peptides over 2+
- MS2.1 MS2 ion injection time in ms (multiplied by 0.01)
- MS1.1 MS1 ion injection time in ms
- IS.1B Number of times where MS1 signal greatly increased between adjacent scans more than 10 fold
- IS.1A Number of times where MS1 signal greatly decreased between adjacent scans more than 10 fold
- MS1.2A Median signal-to-noise value (ratio of maximum to median peak height) for MS1 spectra
- MS1.2B Median TIC value for ID'd peptides up to and including C.2A (time period)
- MS1.3A Ratio of 95th over 5th percentile MS1 maximum intensity values for ID'd peptides
- MS1.3B Median maximum MS1 value for ID'd peptides
- DS.3A Ratio of MS1 maximum to MS1 value at sampling for median decile of peptides by MS1 maximum intensity
- DS.3B Ratio of MS1 maximum to MS1 value at sampling for bottom 50% of peptides by MS1 maximum intensity
- DS.2B Number of MS2 scans taken of C.2A (times 0.001)
- C.2B Ratio of peptides per minute ID'd during C.2A
- DS.2A Number of MS1 scans taken over C.2A (times 0.01)
- C.2A Time period over which 50% of peptides were ID'd (times 0.1)
- C.3B Measure of the distribution of the peak widths
- C.3A Median peak widths for all ID'd unique peptide(s)
- P.3 Ratio of semi/fully tryptic peptide IDs (times 100)
- IS.2 Median m/z for all ID'd peptides(unique ions) times 0.001
- DS.1A Ratio of peptides ID'd by one spectrum to number ID'd by two spectra
- DS.1B Ratio of peptides ID'd by two spectra to number ID'd by three spectra
- P.2C Number of unique tryptic peptide sequences ID'd
- P.2B Number of tryptic peptide ions ID'd; ions differing by charge state and/or modification are counted separately
- P.2A Spectral count
- \* Taken from Rudnick P.A., et al. 2009. Molecular and Cellular Proteomics. p.225

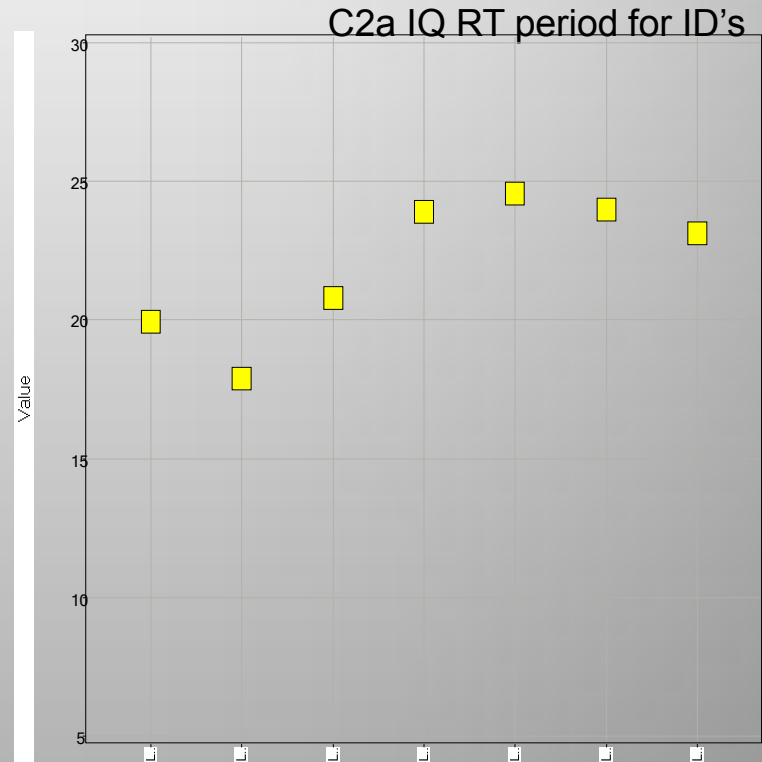
# Metrics part II: The Survey

Variables	
Maintenance	Calibration Tuning Preventative Maintenance Column change
Chromatography	Gradient Flow rate Column Specifics Mobile Phases
Mass Spectrometry	DDA settings Exclusion list settings
Sample	Load amount Dissolution solution

# Testing Data example: IQ RT period for IDs

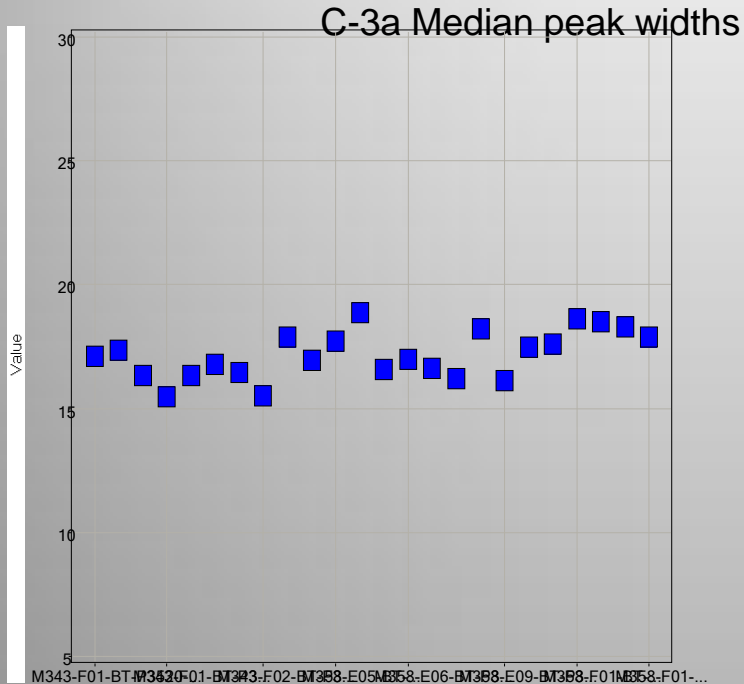


RG participant 1  
Velos-Orbitrap

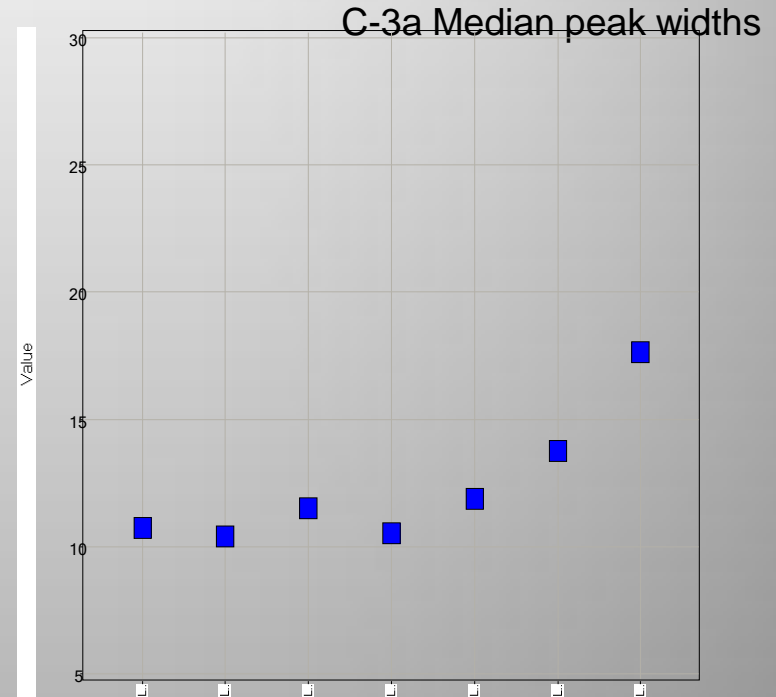


RG participant 2  
LTQ

# Testing Data Example: Median Peak widths



RG participant 1  
Velos-Orbitrap



RG participant 2  
LTQ

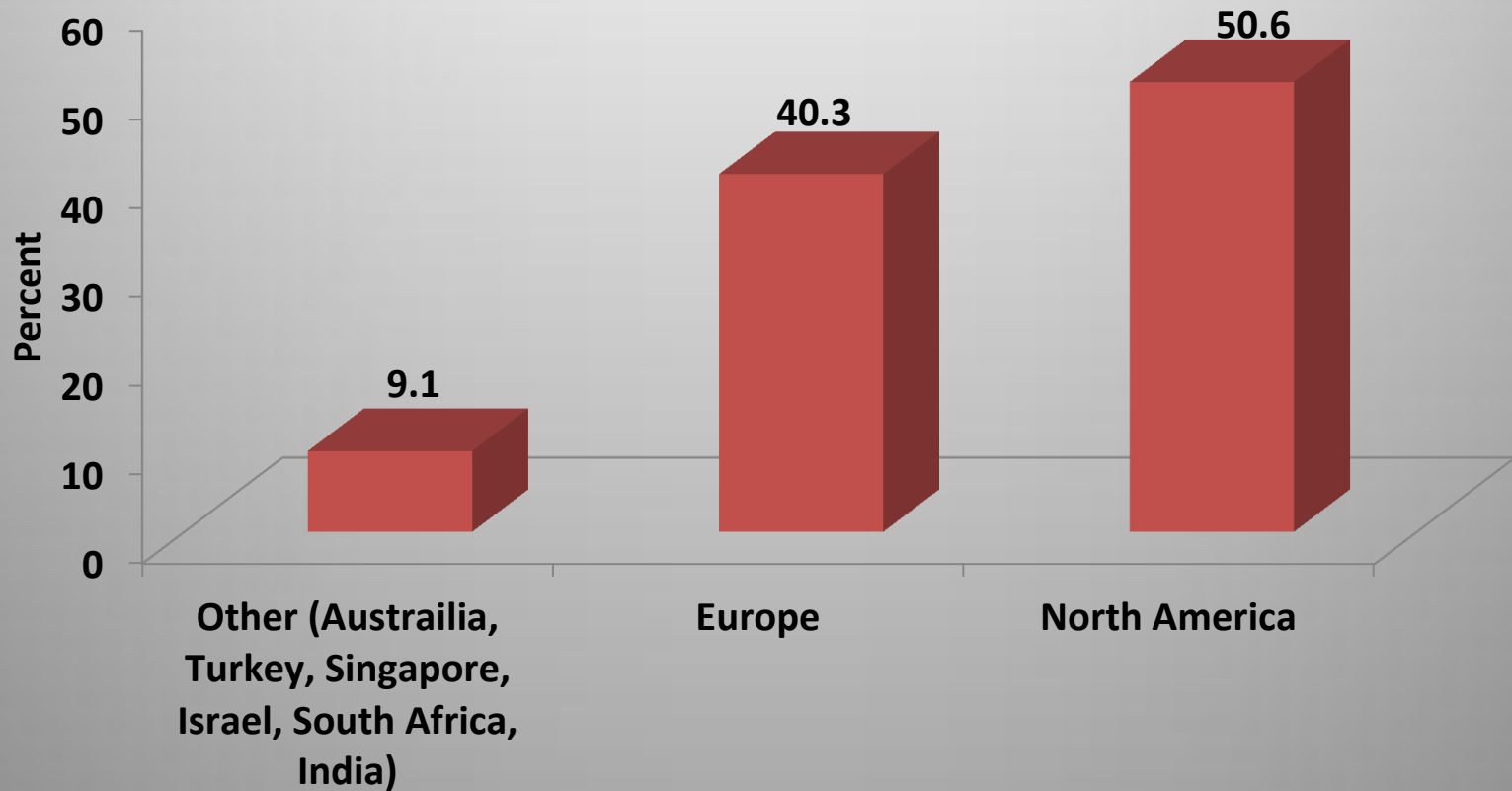
# Contaminants

- lactotransferrin precursor [Bos taurus]
- alpha-S2-casein precursor [Bos taurus]
- superoxide dismutase [Cu-Zn] [Bos taurus]
- cationic trypsin precursor [Bos taurus]

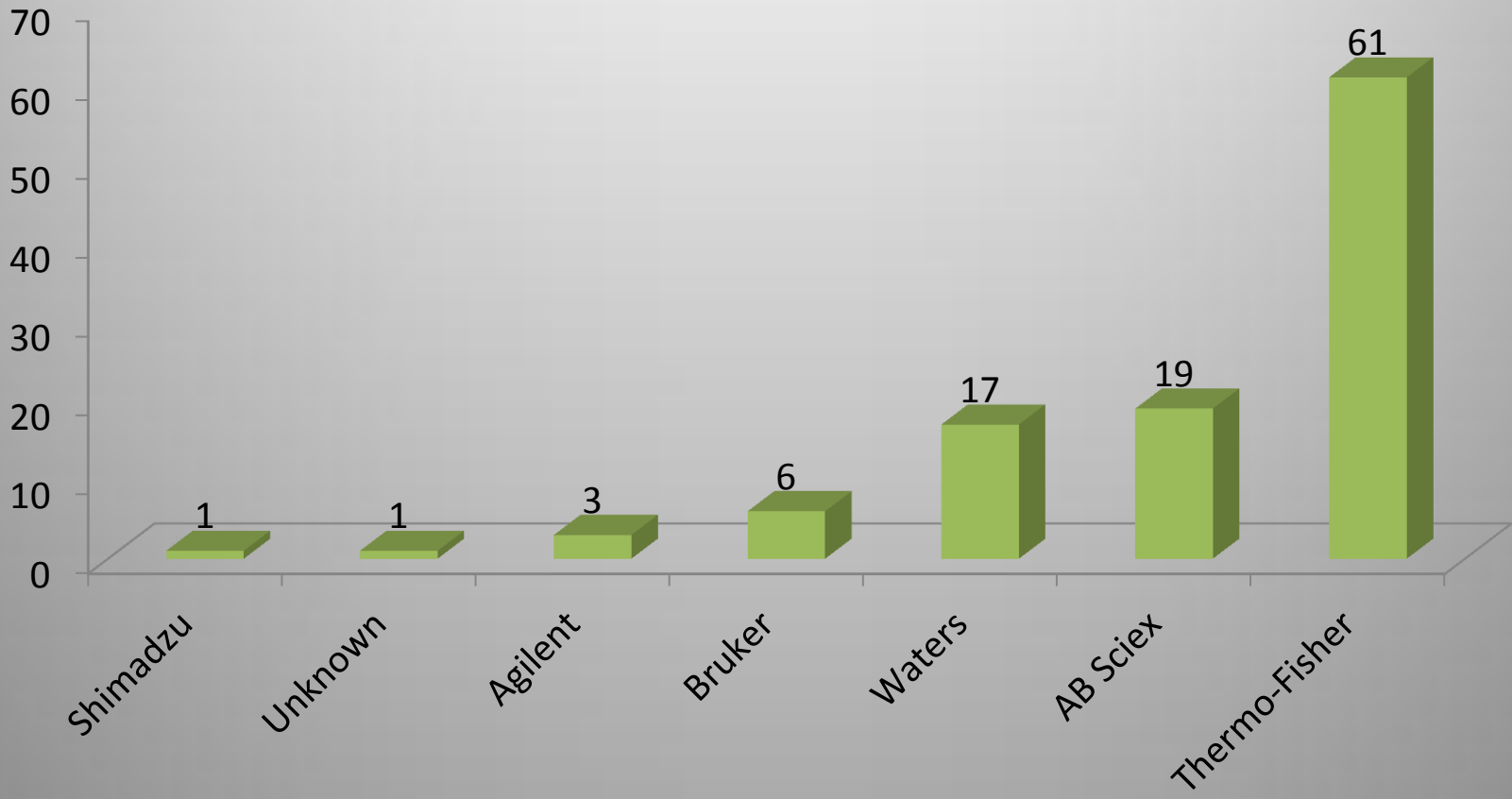
# Number of Participants

- Started with approximately 100 requests
- Scared about 30 people away by sending out an e-mail that required a response before we would send out the samples.
- Approximately 70 remaining

# Participant Demographics

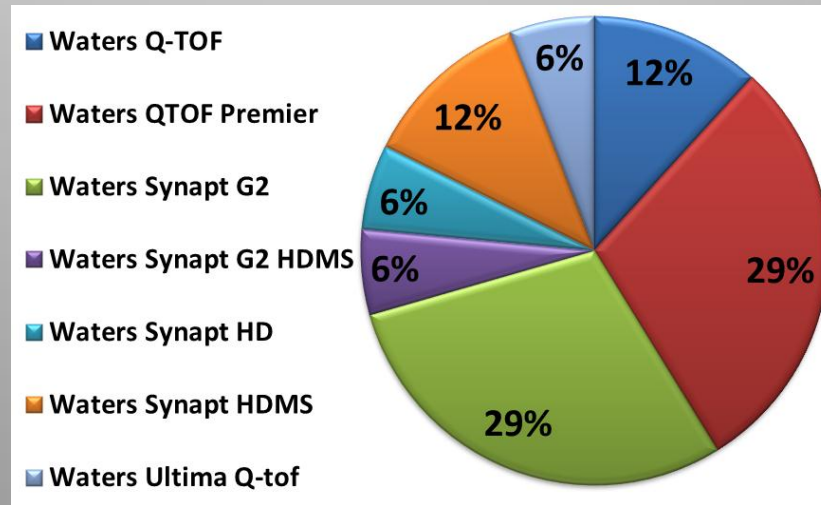
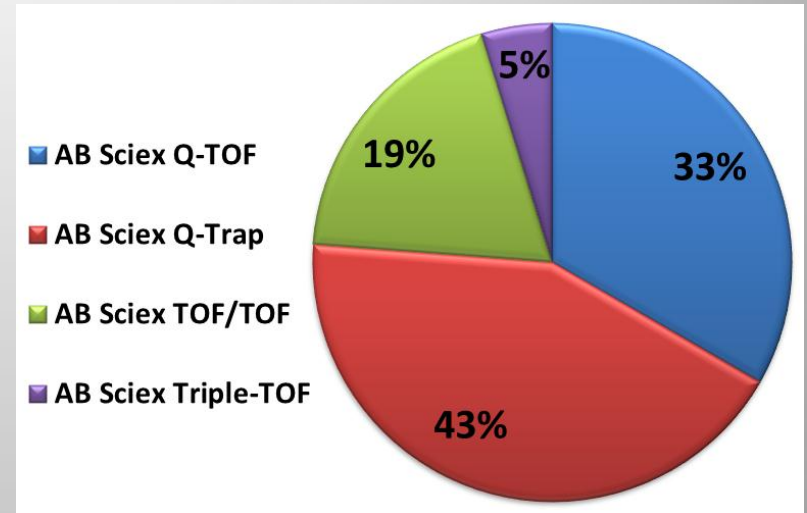
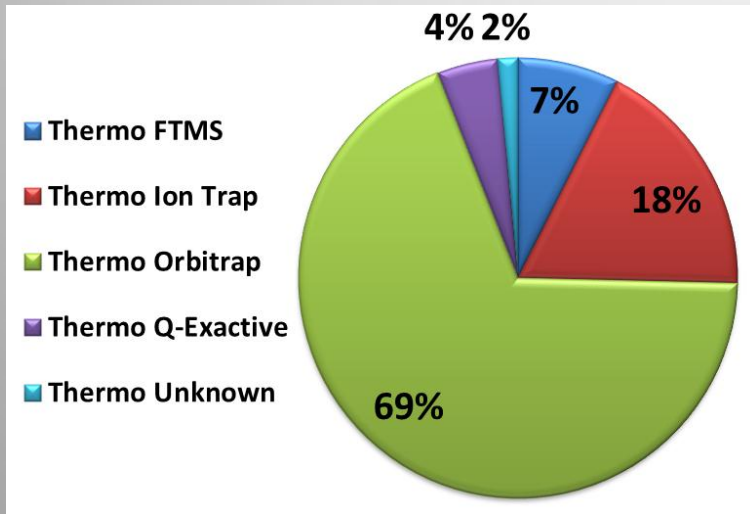


# Instrument diversity

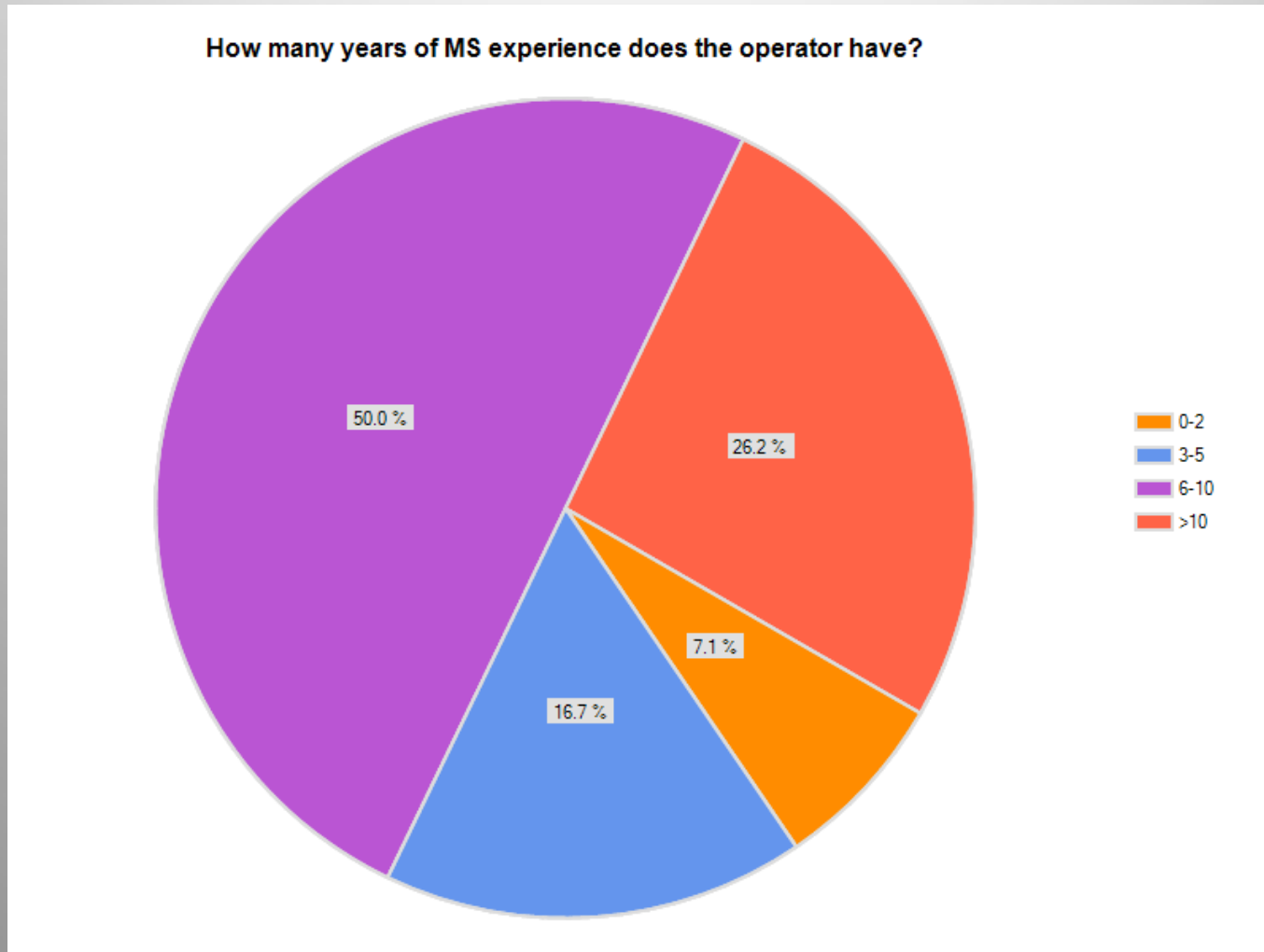




# Instrument Diversity part II

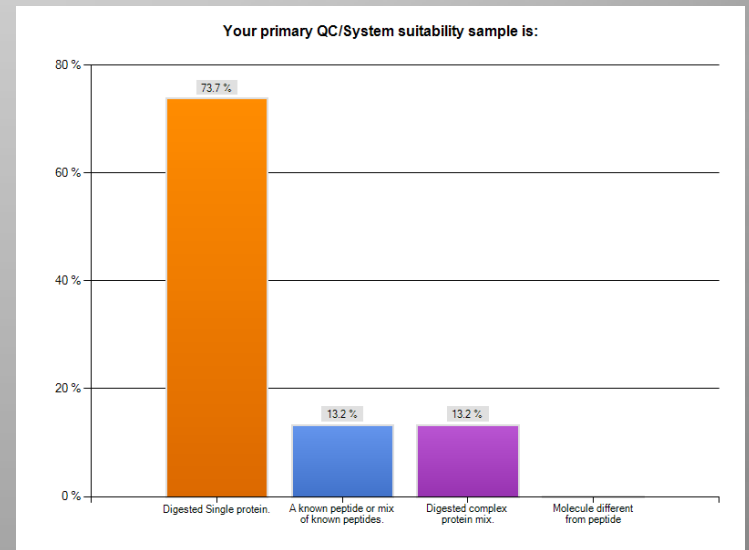
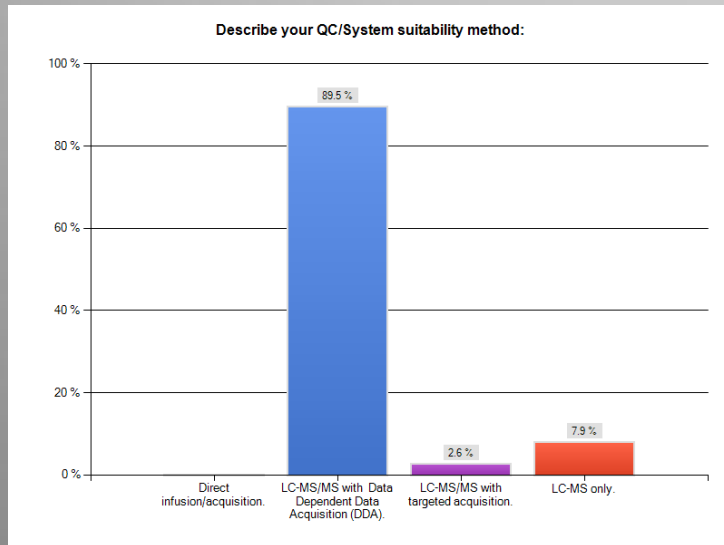
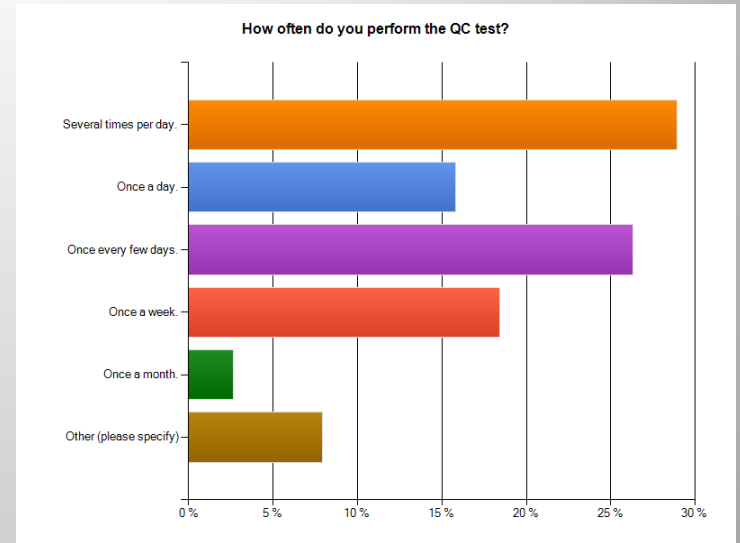
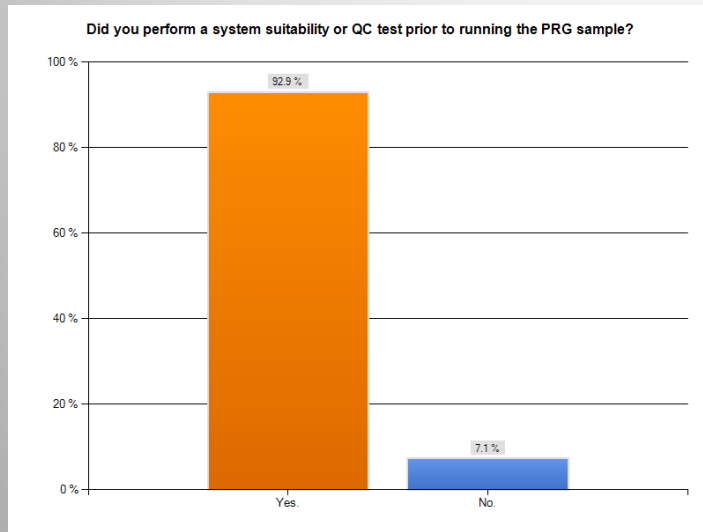


# Initial upload info: 42 Survey entries



75% of participants have more than 5 years experience

# QC variability among participants



# Other Variables

- Flow rate: range 100nL/min – 2uL/Min (one reported 150uL/min)
- Amount injected: range 40fmol – 1pmol
- Resolving gradient: range 11 min – 100 min

# Goals for next year:

- Collect Data
- Harass participants
- Decide what Metrics vary most across labs
- Try to determine what metrics correlate with each other
- Correlate survey data with variability data
- Try to figure out a long term solution to make sure the raw data is available for future studies or different tools (1-2TB)

