PRG-2009: Relative Protein Quantification in a Clinical Matrix

R.E. Settlage[1], A.S. Chien[2], D.B. Friedman[3], D.H. Hawke[4]; J. Krijgsveld[5], K.S. Lilley[6], M.J. MacCoss[7], N.E. Sherman[8], C.W. Turck[9]

[1]Virginia Bioinformatics Institute, Blacksburg VA; [2]Stanford Univ., Stanford CA; [3]Vanderbilt Univ., Nashville TN; [4]Univ. of Texas, MD Anderson Cancer Center, Houston TX; [5]Utrecht Univ., Utrecht, The Netherlands; [6]Univ. of Cambridge, Cambridge, UK; [7]Univ. of Washington, Seattle WA; [8]Univ. of Virginia, Charlottesville VA; [9]Max Planck Institute of Psychiatry, Munich, Germany

Proteomics Research Group

Proteomics Research Group

Introduction

The Proteomics Research Group (PRG) of the ABRF developed the 2009 study to assess approaches that individual laboratories would use to determine the relative abundance of target proteins in a complex mixture. An increasingly common request for proteomics laboratories is the detection of a specific target protein of interest in a complex mixture. Likewise, most of these requests are also interested in knowing the abundance of the target protein relative to that in a control sample. While this type of analysis has traditionally been addressed using Western blots or other immunoaffinity assay, recent advances in targeted mass spectrometry-based analyses are beginning to be reported in the literature as an alternative.

For this year's study, four different proteins were spiked into a plasma background matrix at three different levels. Two of these proteins are commonly measured plasma protein biomarkers, and the remaining two had identical primary structure and differed by only a single phosphorylation site. The participants were shipped six samples in total (three samples in blinded duplicate) and asked to report the relative abundances of the four target proteins in the six samples. Results from analysis of the samples and survey responses will be used to assess the different approaches that are used by the proteomics community to determine the relative abundance of a target protein of interest.

Objectives

The primary goals of this study are to document the breadth of approaches used by the scientific community and to highlight the type of information obtained. Participants were asked to provide the following:

- Relative quantification of four specified proteins in human plasma.
- Information about methods used to analyze the samples.
- Information about the experimental design used for quantification.

Sample Preparation

Human plasma was obtained from Analytical Biological Services Inc. (Wilmington, DE). Prostate Specific Antigen (PSA) was obtained from BIOTREND Chemikalien GmbH (Koeln, Germany), chronic gonadotrophin (β-hCG), and glycogen phosphorylase (GP-α/β) were obtained from Sigma Aldrich (St. Louis, MO). Protein stock solutions were prepared at a concentration of 1 mg/mL in 50 mM Tris-HCl, pH 8. Study samples were prepared by spiking appropriate volumes of stock solutions to give target concentrations shown in Table 1 and aliquots were prepared with the volumes shown. The study samples were then lyophilized to dryness and shipped to the study participants for analysis. The participant key is shown in Table 2.

Tube	PSA	b-hCG	GP-a	GP-b	GPA-(a+b)	GPA-(b/a)	Volume of sample aliquot shipped
	fmol/µL (norm)	fmol/µL (norm)	fmol/µL (norm)	fmol/µL (norm)	fmol/µL (norm)	absolute (norm)	µL
A and E	125 (0.5)	375 (0.3)	62.5 (1)	12.5 (0.2)	75 (1)	0.2 (0.04)	42.5
B and F	25 (0.1)	1250 (1)	25 (0.4)	50 (0.8)	75 (1)	2 (0.4)	41
C and D	250 (1)	25 (0.02)	12.5 (0.2)	62.5 (1)	75 (1)	5 (1)	40.5

Table 1: spike concentrations in samples (concentration and normalized)

Respondant ID	Code	#
716445809	12158	1
715653182	13010	2
713416067	15128	3
713984312	18017	4
702755472	18174	5
716558863	19055	6
716317338	24480	7
716421005	27150	8
714009992	29109	9
716266405	29850	10
716435514	32365	11
716733544	38146	12
716947344	38716	13
717002832	39180	14
714010786	40003	15
713769100	49441	16
715053722	49441	17
716958692	53104	18
714268559	55555	19
711969119	63103	20
716992416	92125	21
712946531	92421	22
715890133	93120	23
716474774	95959	24
716625285	95959	25
717550918	20577	26
717515952	38716	27
716768560	12882	28

Table 2: study participant key

Acknowledgements

We would like to thank:
Peter Sharratt, University of Cambridge, for amino acid analysis;
Giuseppina Maccarrone and Christiane Rewerts, Max Planck Institute of Psychiatry, for preparing test samples;
Ms. Karolina Krasinska, Stanford University, for anonymizing the study participants;
Bih-Fang Pan, MD Anderson Cancer Center, for performing Western Blots and other tests on initial samples to ensure sample quality;
Julie Coleman and Salisha Hill, Vanderbilt University, for sample testing;
Kristi Nelson, Virginia Commonwealth University for acquiring MS/MS spectra on the protein standards.

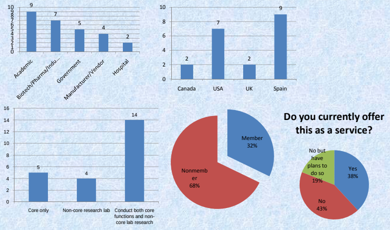
Target Proteins in the Study.

Figure 1: Proteins used in PRG-2009. Please note that all proteins were prepared by commercial vendors and the PRG does not have complete details on protein heterogeneity resulting from truncations, isoforms, post-translational modifications, etc.

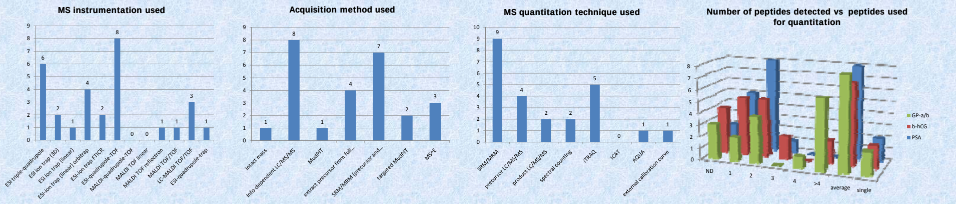
Prostate specific antigen from human seminal fluids (PSA)
-may include isoforms 1-6

human chorionic gonadotropin (recombinant protein from mouse cell line) (α-CG and β-CG)
-includes both α and β isoforms

Glycogen phosphorylase A/B (from rabbit muscle)
-includes both A and B isoforms, B isoforms is phosphorylated

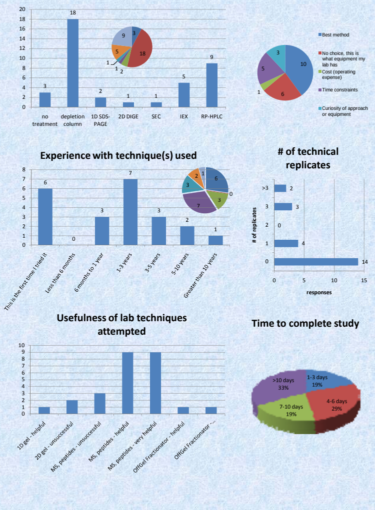


Participant demographics

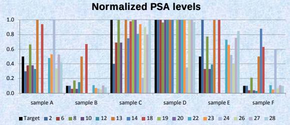


MS instrumentation and methods used

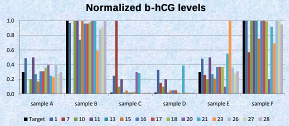
Lab methods used



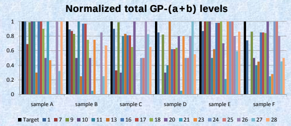
Results and Conclusions



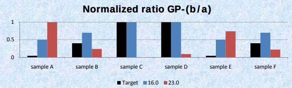
Respondent	sample A	sample B	sample C	sample D	sample E	sample F
Target	0.5	0.1	1.0	1.0	0.5	0.1
2	0.3	0.1	0.4	1.0	1.0	0.1
6	0.4	0.1	0.7	1.0	0.3	0.0
8	0.7	0.2	1.0	1.0	0.8	0.2
10	0.4	0.1	0.7	1.0	0.3	0.0
12	0.3	0.2		1.0	0.4	0.0
13	1.0	0.5	1.0	1.0	1.0	0.5
14			0.8	1.0		0.9
18	0.9	0.7	1.0		1.0	0.6
19			1.0	1.0		
20			1.0	1.0		
22	0.5	0.1	0.8	1.0	0.7	0.1
23	0.5	0.1	0.9	1.0	0.7	0.1
24	1.0	0.1	0.2	0.4	0.5	0.6
26	0.3	0.1	0.9	1.0	0.4	0.1
27	0.5	0.1	0.8	1.0	0.8	0.1
28	0.42	0.08	1	0.97	0.85	0.1



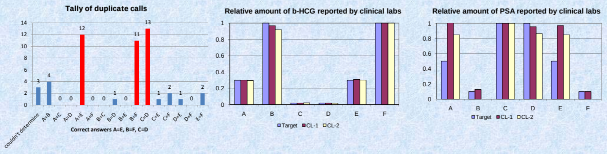
Respondent	sample A	sample B	sample C	sample D	sample E	sample F
Target	0.3	1.0	0.0	0.0	0.3	1.0
1	0.5	1.0	0.3	0.3	0.5	1.0
7			1.0	0.2	0.3	0.6
10	0.2	1.0	0.1	0.1	0.2	1.0
11	0.5	1.0	0.2	0.2	0.5	1.0
13	0.3	0.7	0.0	0.0	0.3	1.0
15	0.2	1.0	0.0	0.0	0.2	0.8
16	0.3	1.0	0.0	0.1	0.4	1.0
17	0.3	1.0	0.0	0.1	0.4	1.0
18	0.4	1.0	0.0	0.0	0.4	1.0
20	0.4	1.0	0.3		0.1	0.2
21	0.2	1.0	0.3	0.4	0.6	0.9
23	0.2	0.6		0.0	1.0	0.7
26	0.4	0.9	0.0	0.0	0.4	1.0
27	0.3	0.9	0.0	0.0	0.3	1.0
28	0.3	1	0.02	0.02	0.31	0.95



Respondent	sample A	sample B	sample C	sample D	sample E	sample F
Target	1	1	1	1	1	1
1	1.0	0.9	0.9	0.9	0.9	0.7
7	0.7	0.9	0.3		1.0	
9	1.0	0.8	1.0	0.8	1.0	0.9
10	1.0	0.5	0.3	0.3	1.0	0.5
11	1.0	1.0	0.8	0.4	0.5	0.4
13	0.3	0.3	0.8	1.0	0.6	0.5
16	1.0	1.0	0.8	0.6	1.0	0.9
17	1.0	1.0	0.8	0.6	1.0	0.9
18	0.9	0.8	0.7	0.6	1.0	0.8
20	0.5	0.5	1.0	0.8	0.7	1.0
21	1.0	0.1		0.1	0.2	0.3
23	0.5	0.8		0.8	1.0	0.3
24			0.5	0.5	1.0	1.0
25			0.5	0.5	1.0	1.0
26	1.0	0.9	1.1	0.8	0.8	0.8
27	0.3	0.3	0.8	1.0	0.6	0.5
28	1	0.67	0.65	0.55	0.86	0.5



Respondent	sample A	sample B	sample C	sample D	sample E	sample F
Target	0.04	0.4	1	1	0.04	0.4
16	0.5	0.7	1.0	1.0	0.5	0.7
23	1.0	0.2		0.1	0.7	0.2



Discussion/Conclusions

Study samples were prepared as shown in Table 1 and distributed to 67 participants. The study participants were asked to determine the relative abundance of 4 proteins spiked into the samples and report their methods and results.

As a control, two sample sets were analyzed for PSA and beta HCG by clinical laboratories using standard ELISA techniques. As shown above, the results of the clinical laboratories were largely in agreement with our theoretical values calculated using quantitation values from amino acid analysis. Both of the clinical laboratories did note some difficulty in analyzing PSA levels in samples A and E. In addition, PSA levels in samples B and F appeared to be below the detection limit of CL-2.

While most successful respondents found depletion of the most abundant proteins helpful, success was achieved using all instrument types interfaced with chromatographic separation with and without depletion. All successful participants used enzymatic digestion (trypsin). Successful MS scanning methods included SRM/MRM, peptide precursor/product signal extracted from full scan MS/MS, and MSᴱ methods. Most participants did not find sample amount limiting.

Several participants noted some difficulty with the lyophilized state of the samples. It should be noted that samples were lyophilized prior to shipping for the sole purpose of reducing the cost of the study.

This year's study was rated as difficult to very difficult by the participants with the average confidence level of the participants ability to perform the analysis remaining at confident to very-confident before and after the study. When asked whether they would repeat the study given an opportunity, we received a resounding 'yes, it was fun' with many requests for additional time to perform the study.