

PRG2009 Research Study

Relative Protein Quantification in a Clinical Matrix

<http://www.abrf.org/prg>



*Proteomics Research
Group*

PRG Members 2008-2009

- Michael MacCoss (Chair) – *University of Washington*
- Allis S. Chien - *Stanford University*
- David B. Friedman - *Vanderbilt University*
- Jeroen Krijgsveld - *Utrecht University*
- David Hawk – *MD Anderson Cancer Center*
- Kathryn Lilley – *University of Cambridge*
- Michael MacCoss – *University of Washington*
- Robert E. Settlage – *VBI Virginia Tech*
- Nicholas Sherman – *University of Virginia*
- Chris Turck (EB Liaison) - *Max Planck Institute*



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Proteomics Research Group Mission

- The PRG is a volunteer scientific organization dedicated to sharing knowledge about the analysis of proteins.
- The PRG aims to assist protein scientists and resource facilities by sponsoring annual research studies that examine current techniques and capabilities.
- Through the promotion of broad participation and scientific excellence, the PRG aims to raise awareness, knowledge and education about modern methods of protein analysis.



Past Research Studies

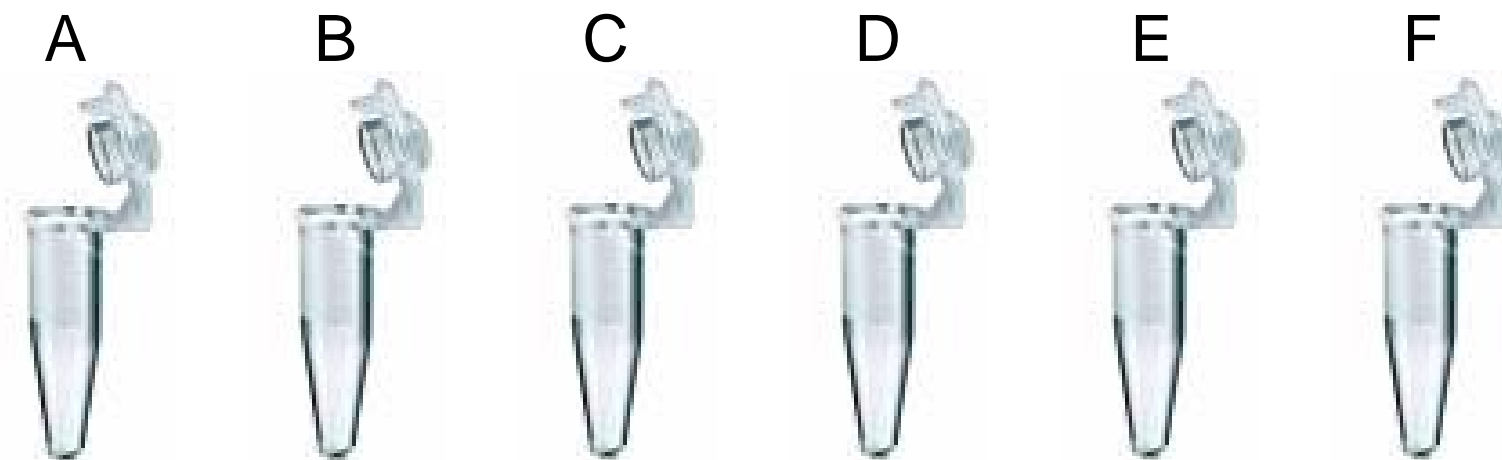
- **PRG2002:** Identification of Proteins in a Simple Mixture
 - Task: Identify components of a 5 protein mixture
- **PRG2003:** Phosphorylation Site Determination
 - Task: Identify 2 phosphopeptides and sites of phosphorylation
- **PRG2004:** Differentiation of Protein Isoforms
 - Task: Discrimination of 3 closely related proteins
- **PRG2005:** Sequencing Unknown Peptides
 - Task: *De novo* sequence analysis of 5 peptide mixture
- **PRG2006:** Quantification of Proteins from a Simple Mixture
 - Task: Relative Abundance of 8 Proteins Between 2 Different Samples
- **PRG2007:** Quantification of Proteins from a Complex Mixture
 - Task: Relative Abundance of 12 Proteins Spiked into an *E. coli* Lysate Between 3 Different Samples
- **PRG2008:** Identifying Differences in Protein Isoforms Between Samples
 - Task: Identify differences between two samples that have different lengths of the same protein sequence

PRG2009 Study Objectives

- What methods are used in the community for assessing differences between complex mixtures?
- How well established are quantitative methodologies in the community?
- Do groups handle samples differently when they have specific proteins they are targeting?
- How well do labs handle samples in a clinical matrix?
- Is there a common sample preparation protocol that is used within the community?

PRG2009 Sample Design

Six Lyophilized Plasma Samples Were Shipped
3 Samples in Duplicate



Each Tube Contains

- 40 μL of Female plasma
- Prostate specific antigen (human)
- Beta chorionic gonadotropin (human)
- Glycogen phosphorylase A (rabbit)
- Glycogen phosphorylase B (rabbit)

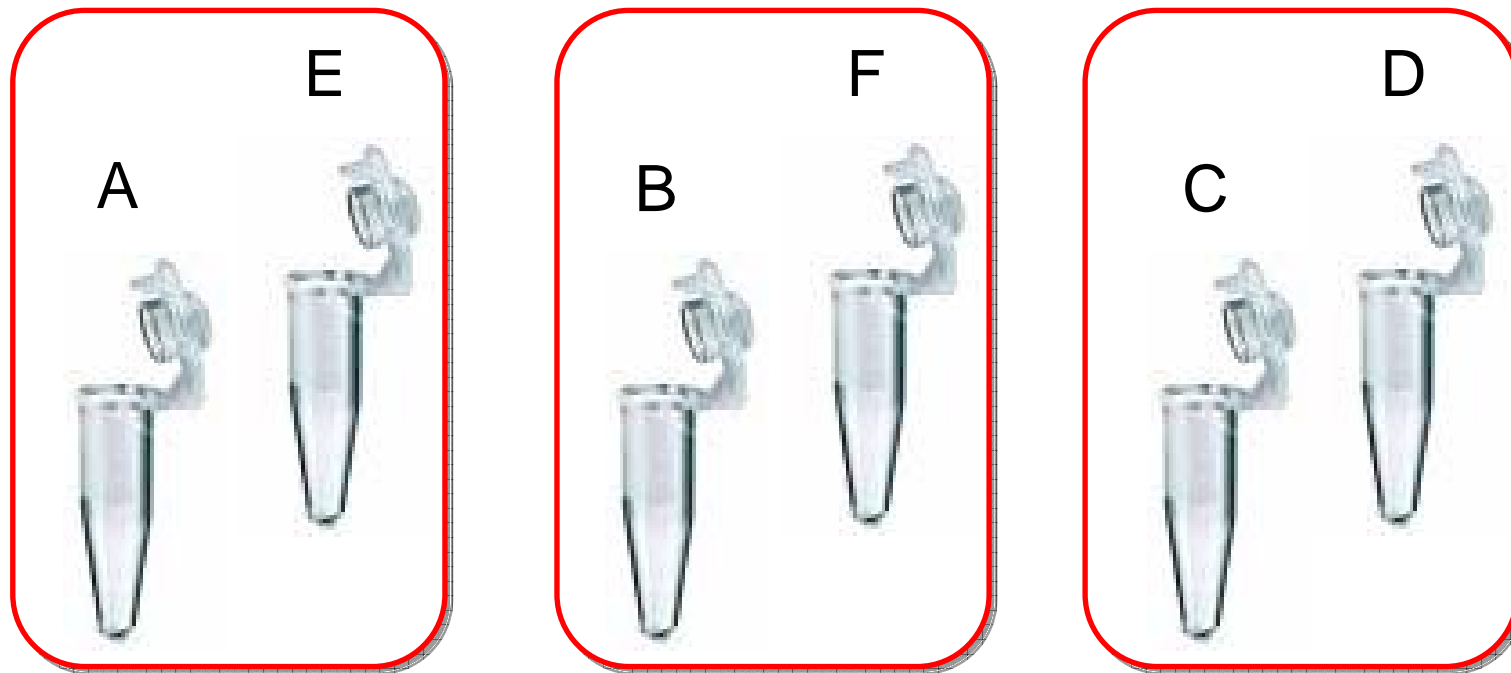
Spiked in a range
of 2.5 fmol/ μL to
1.25 pmol/ μL

PRG2007 Asked to Provide

- Relative quantification of the four specified proteins between the 6 samples in human plasma
 - Which samples were pairs?
- Information about methods used to analyze the samples
- Information about the experimental design used for relative quantification

PRG2009 Sample

Samples were in Pairs – Participants Blind to the Grouping

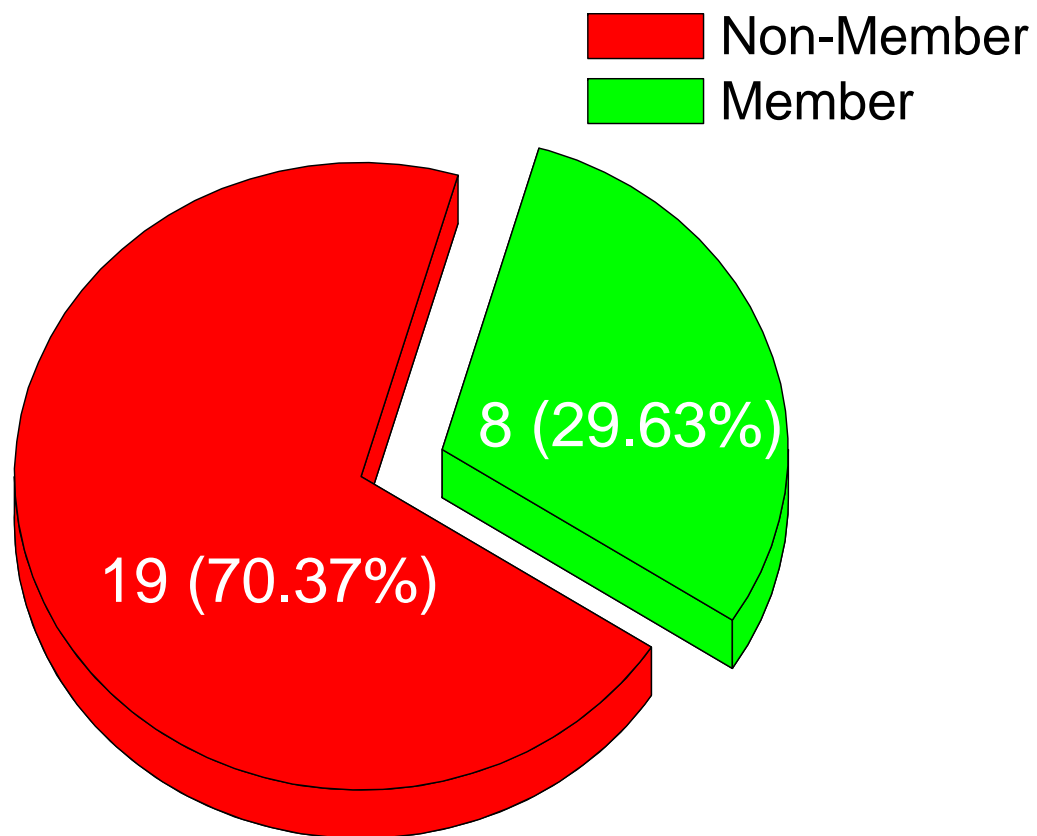


Proteins in PRG2009 Sample

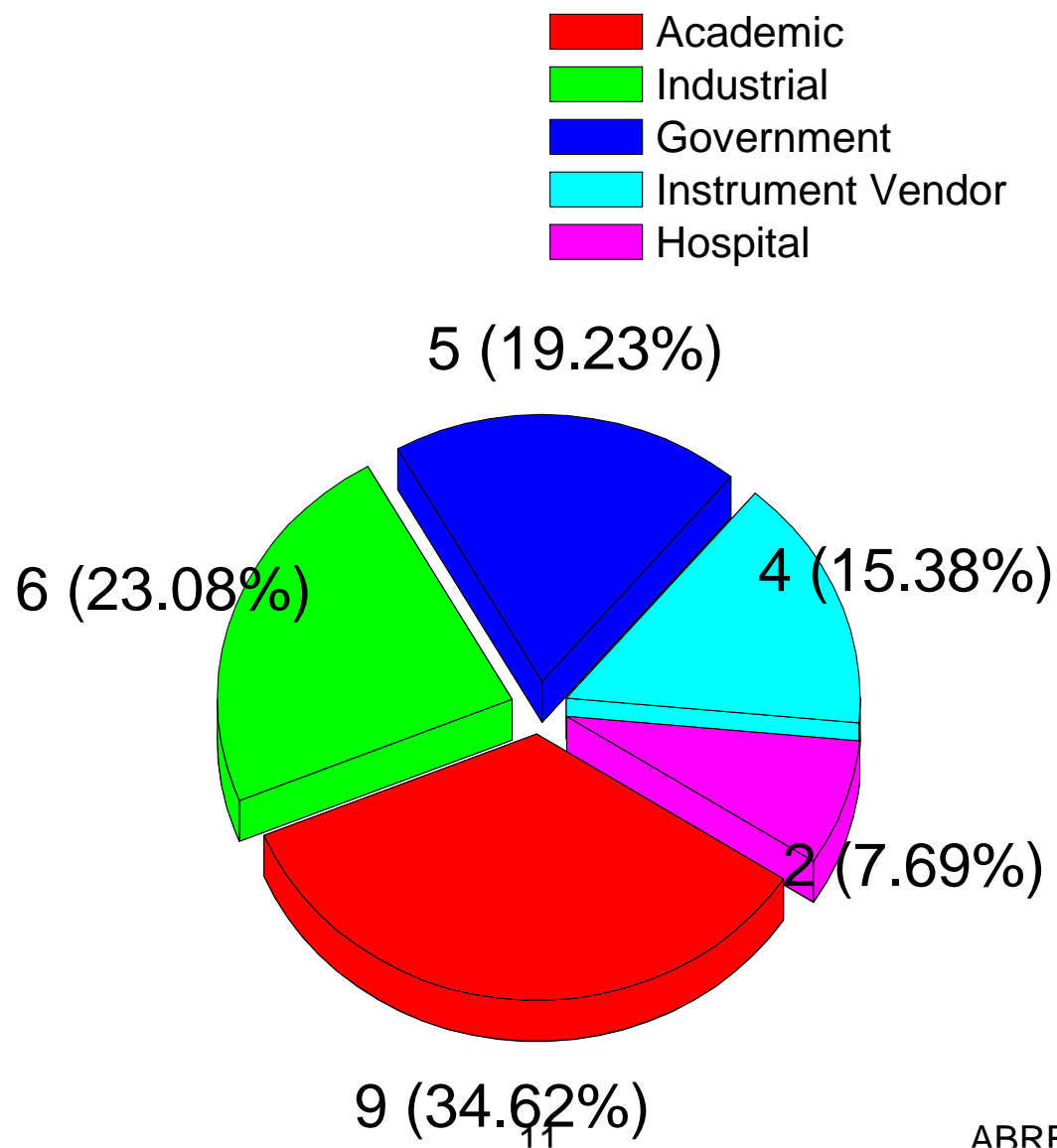
Tube	PSA <i>pmol</i>	B-hCG <i>pmol</i>	GPA <i>pmol</i>	GPB <i>pmol</i>	Total GP <i>pmol</i>
A and E	5	15	2.5	0.5	3
B and F	1	50	1	2	3
C and D	10	1	0.5	2.5	3

The above amount was spiked into 40 μ L of female plasma

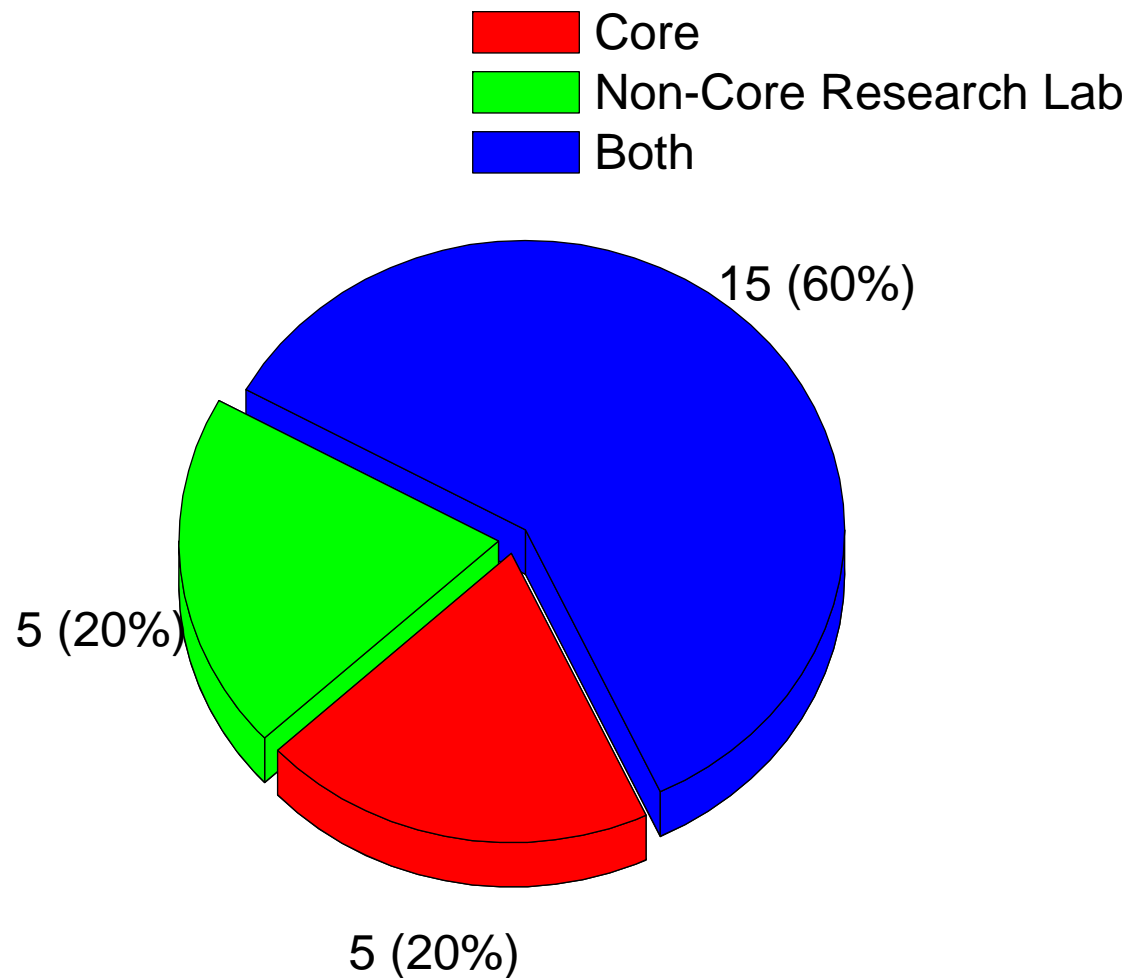
Demographics of the Participants



Demographics of the Participants

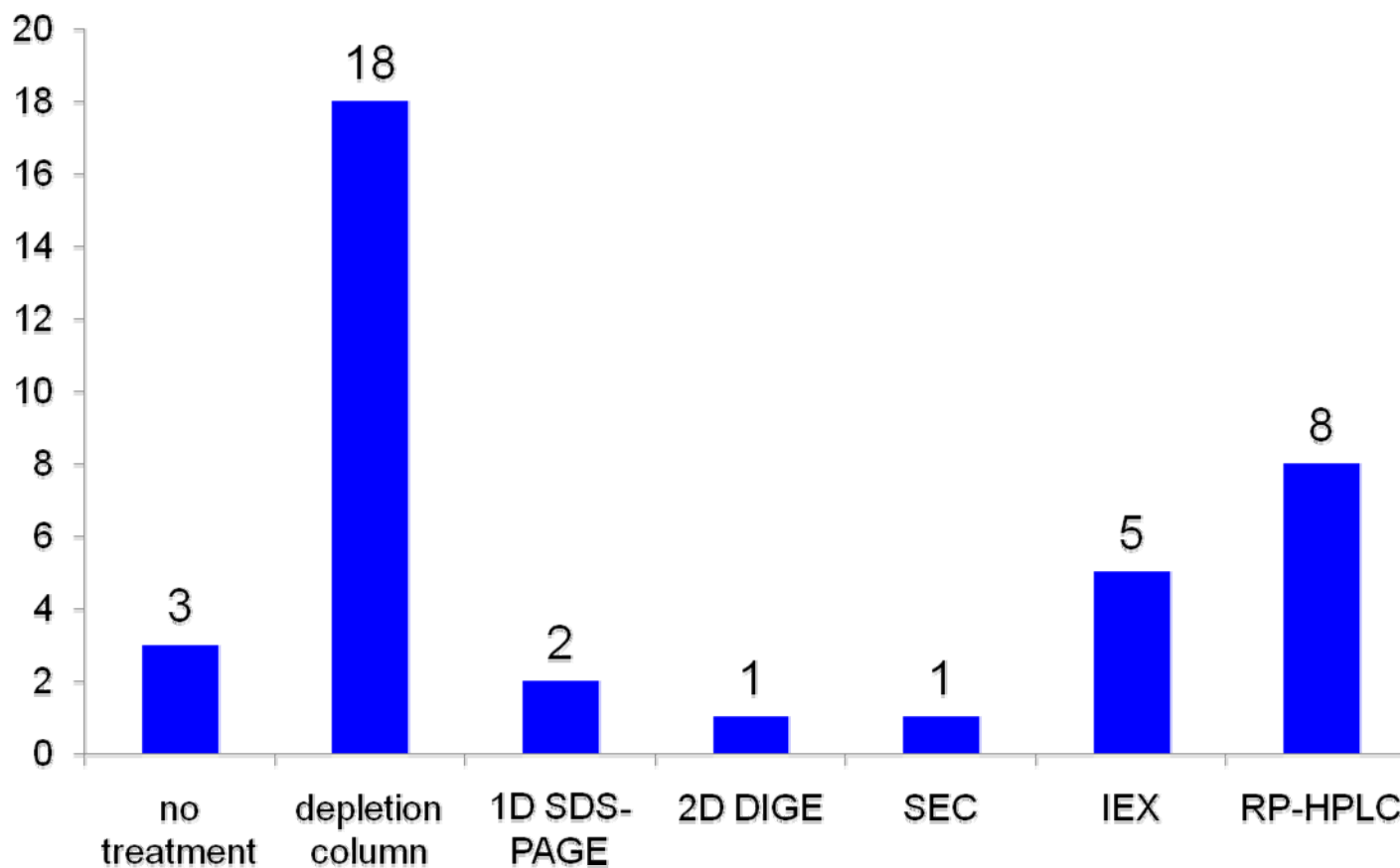


Demographics of the Participants





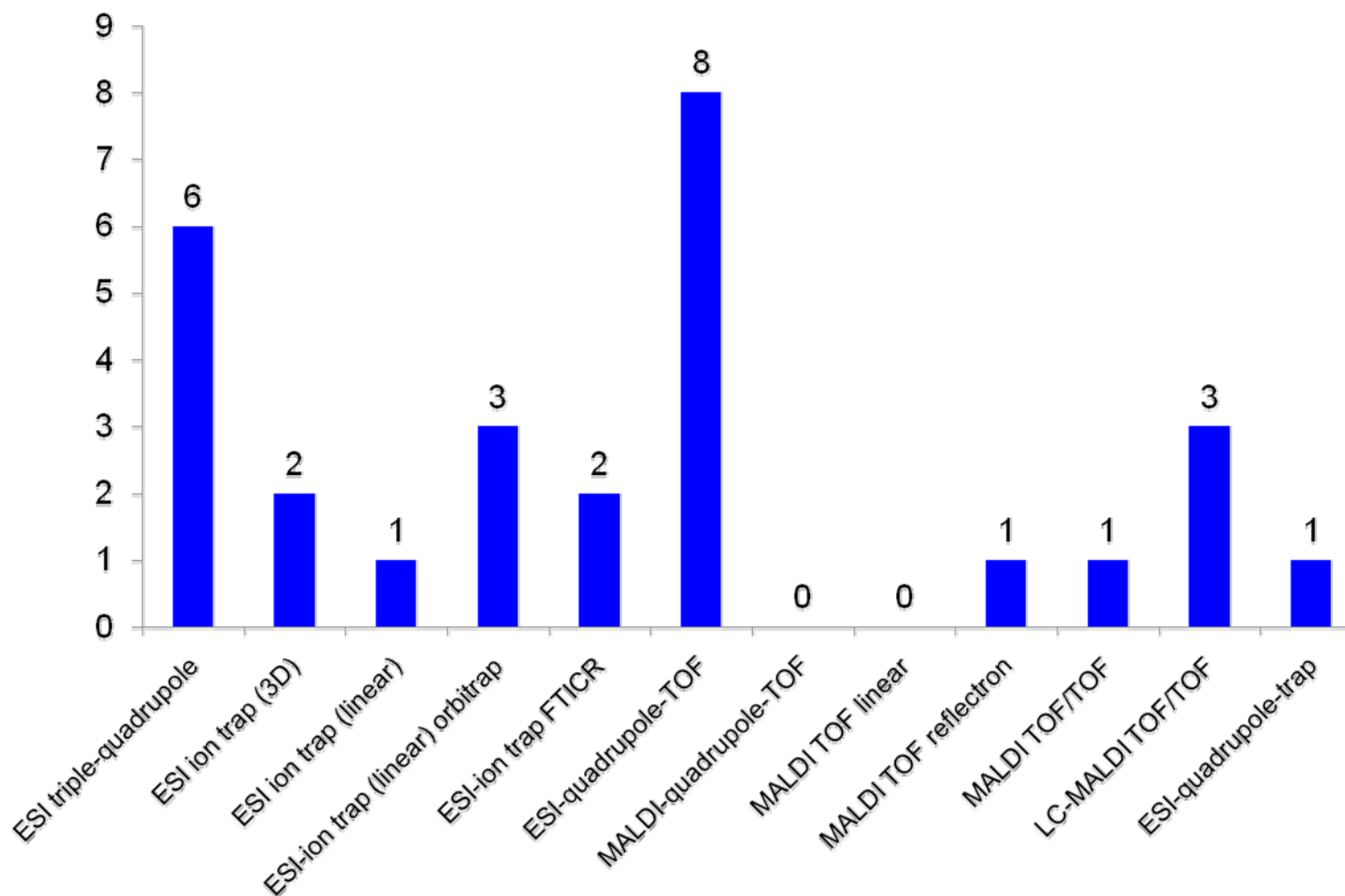
Sample Prep Methods Used





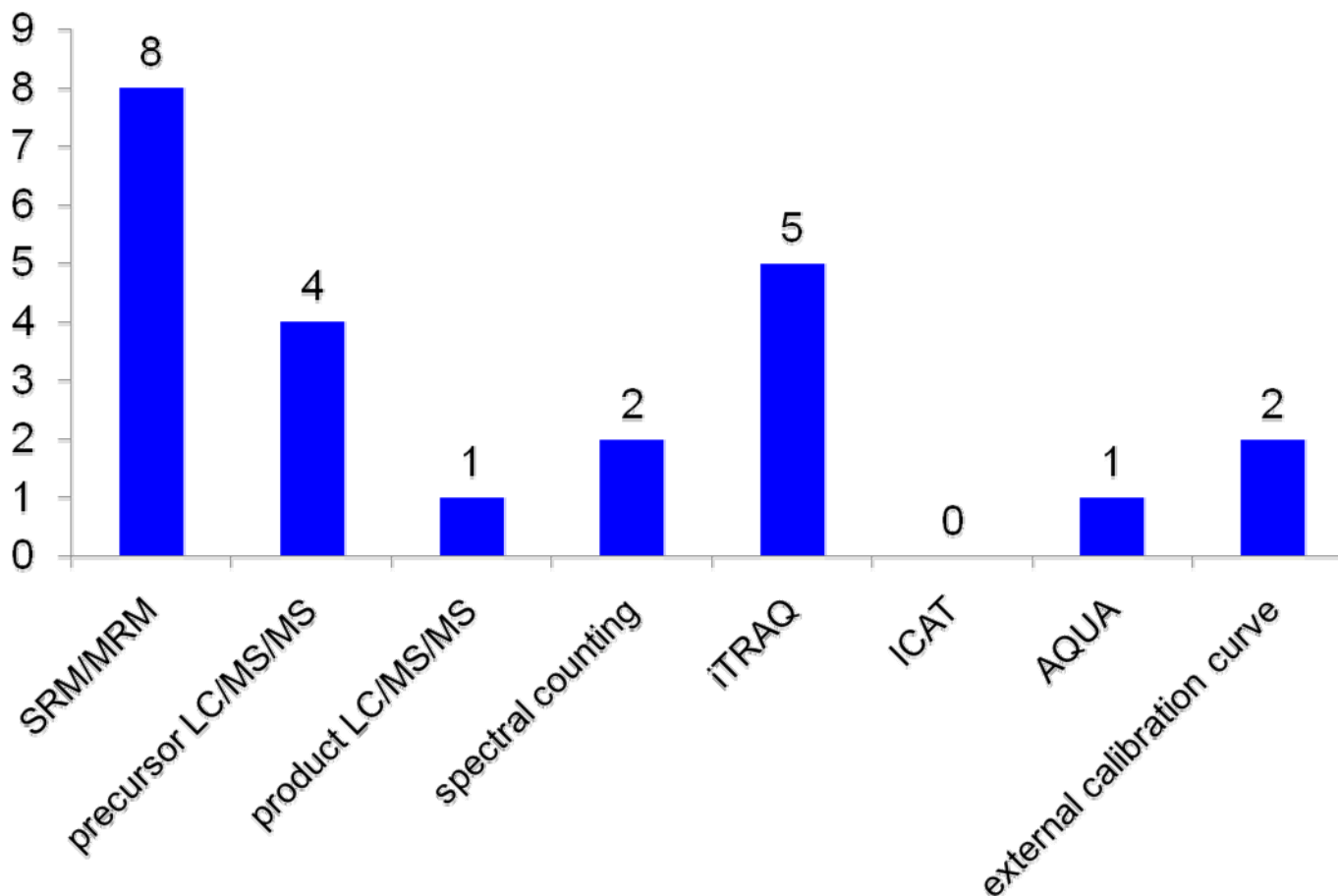
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Mass Spec Type Used





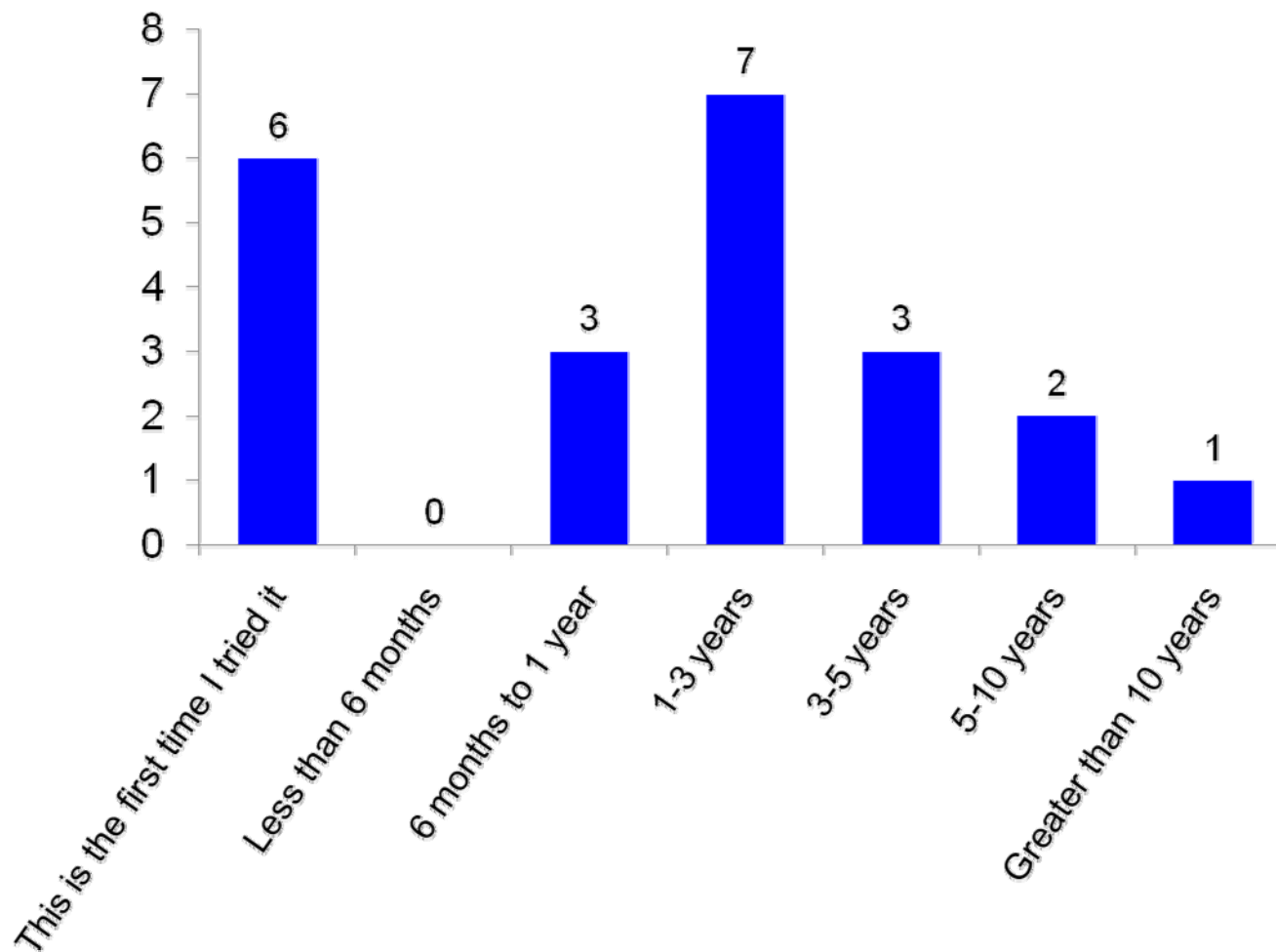
Quantitation Strategy Used



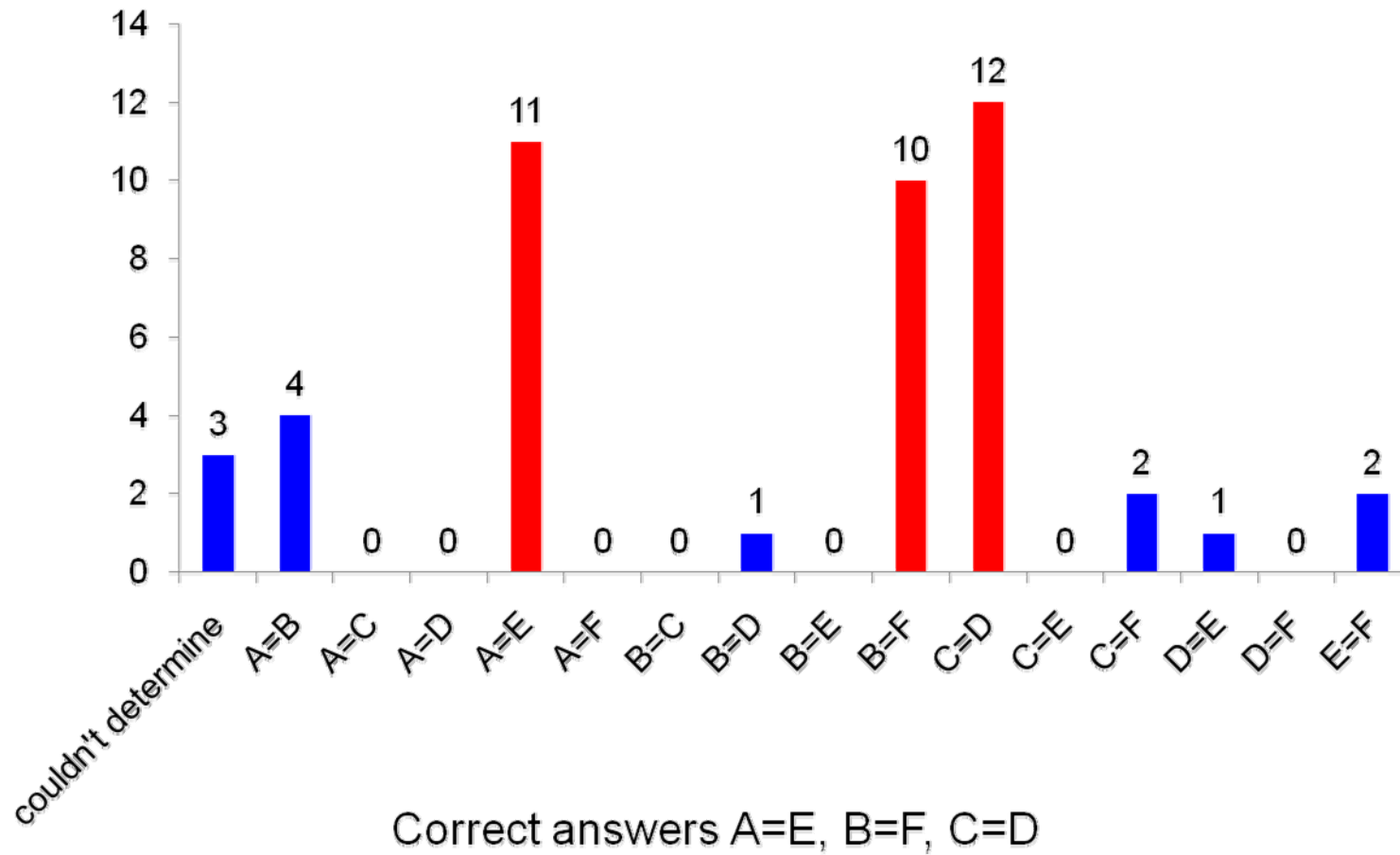


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Experience with this Type of Analysis



Results: Assignment of Duplicate Samples

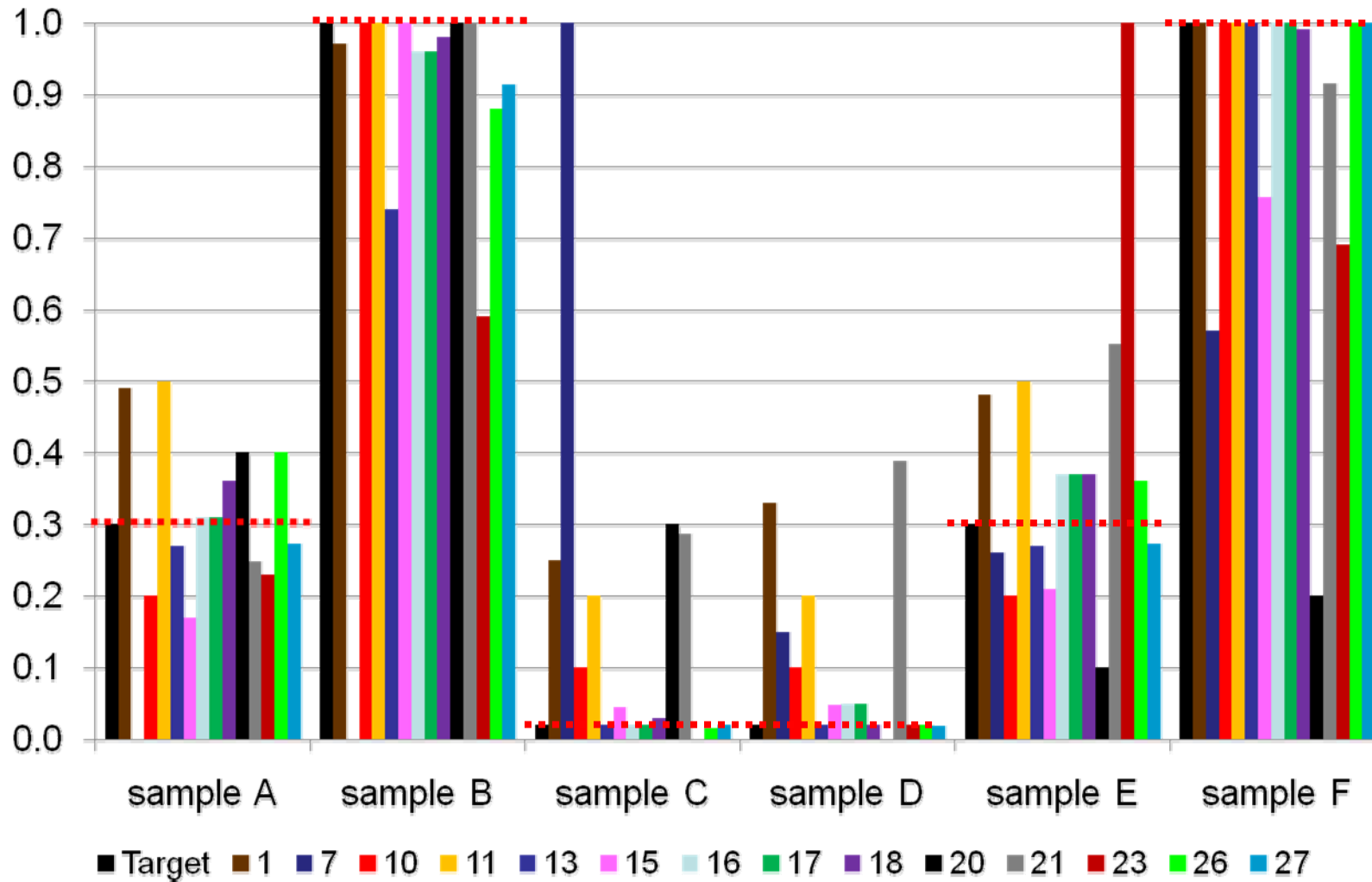




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Results: Beta-hCG

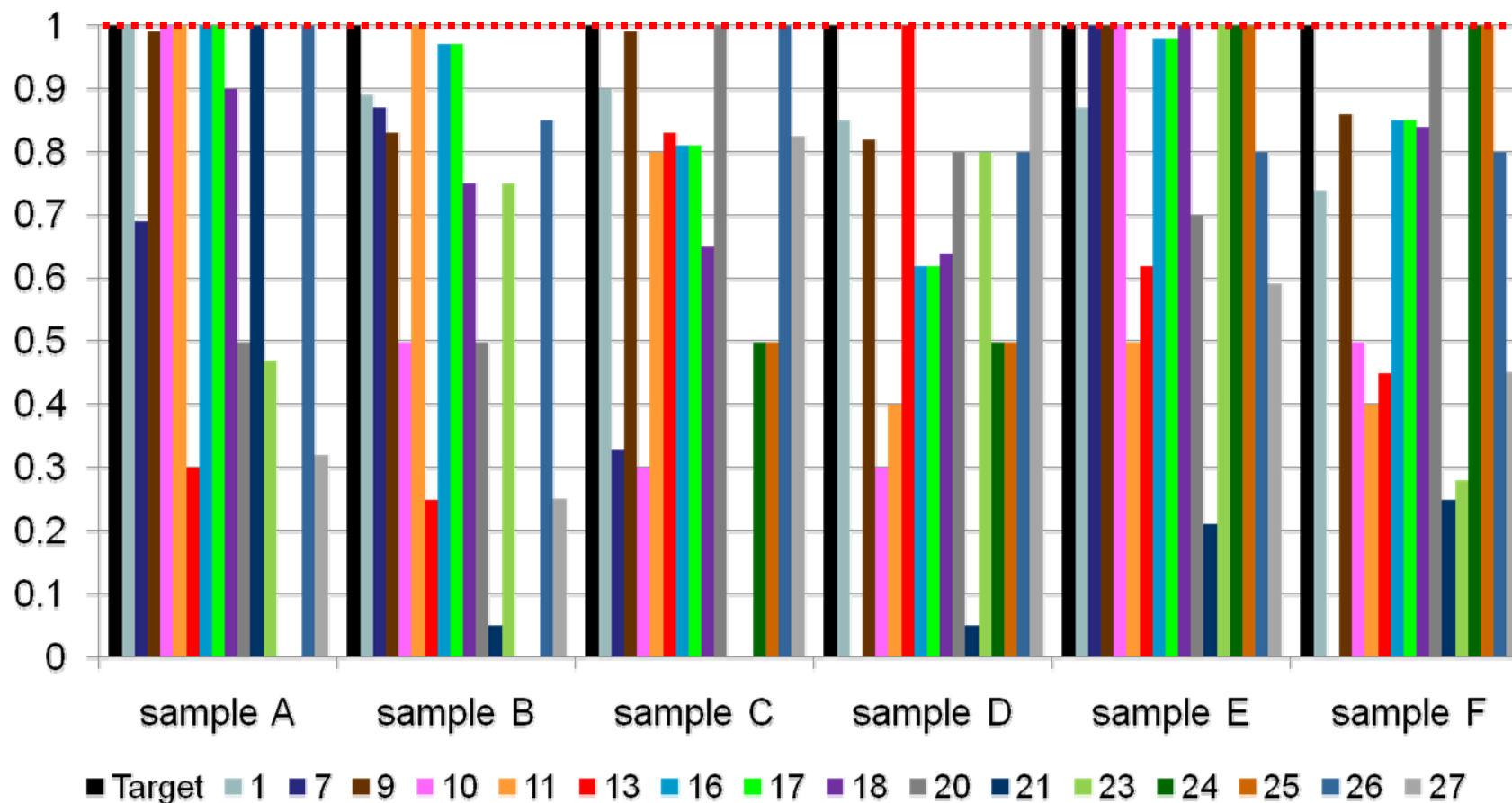
Normalized b-hCG levels





Results: Total Glycogen Phosphorylase

Normalized total GP-(a+b) levels





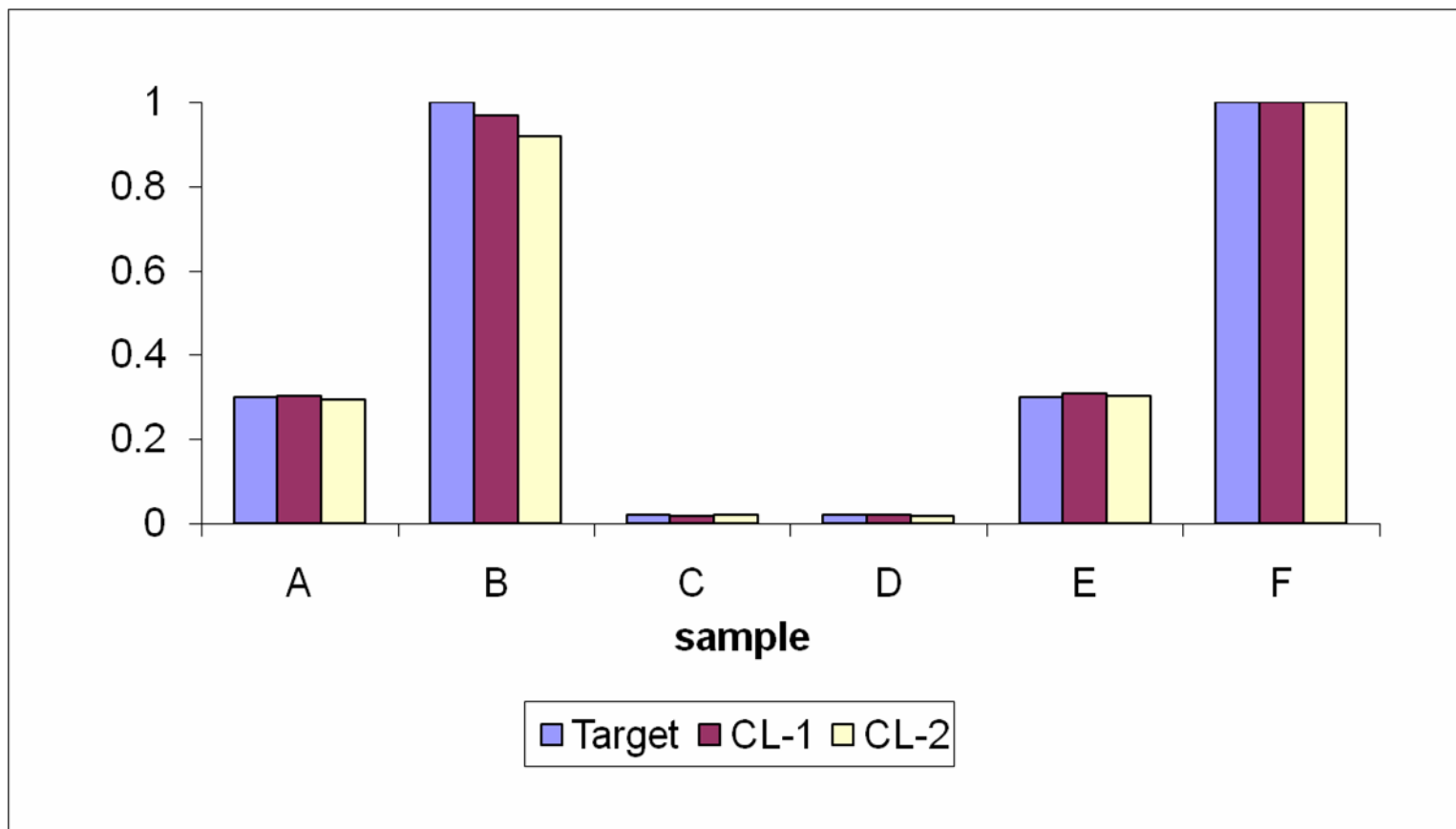
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Results: PSA Analyzed by Two Clinical Labs





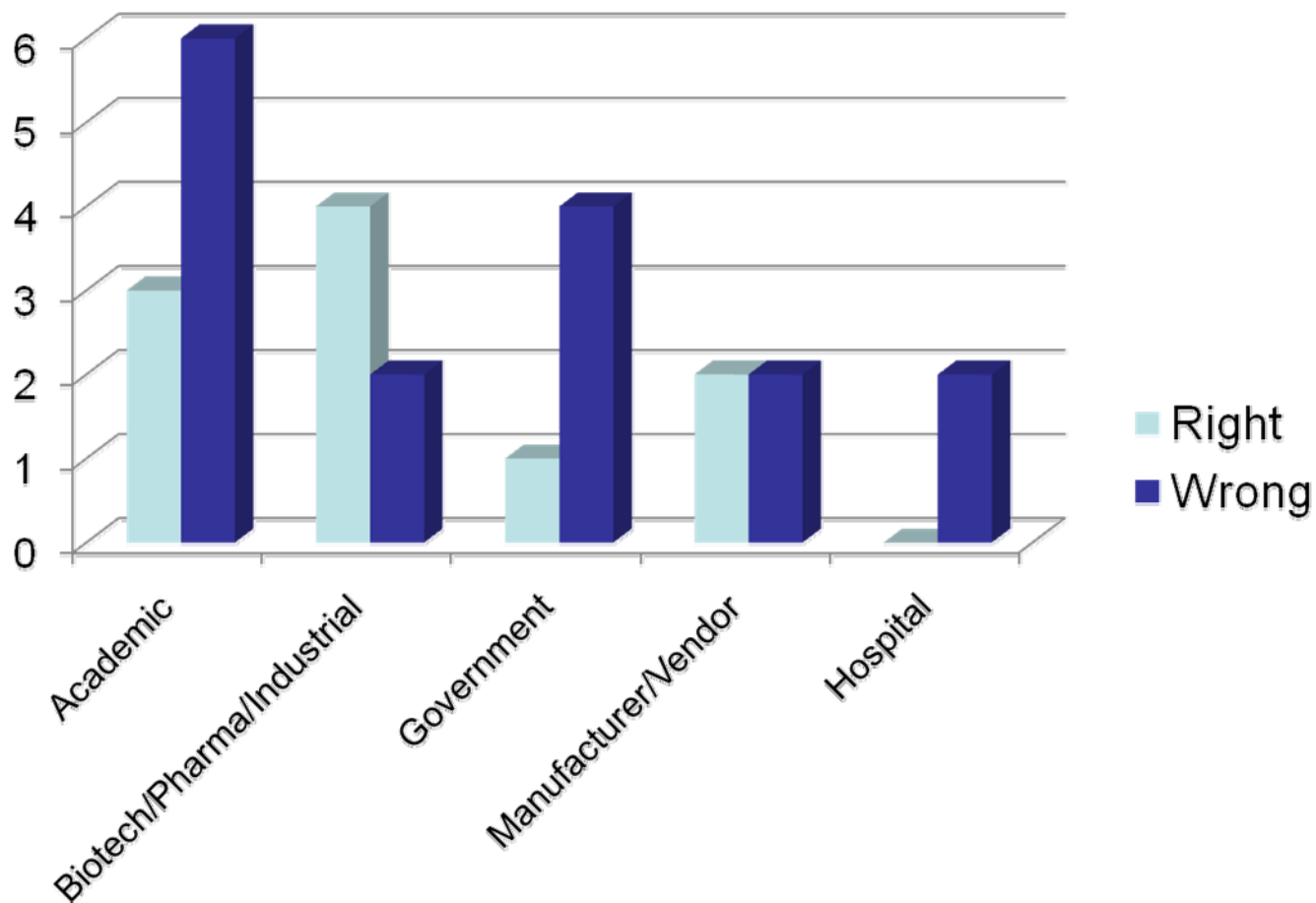
Results: Beta-hCG Analyzed by Clinical Labs





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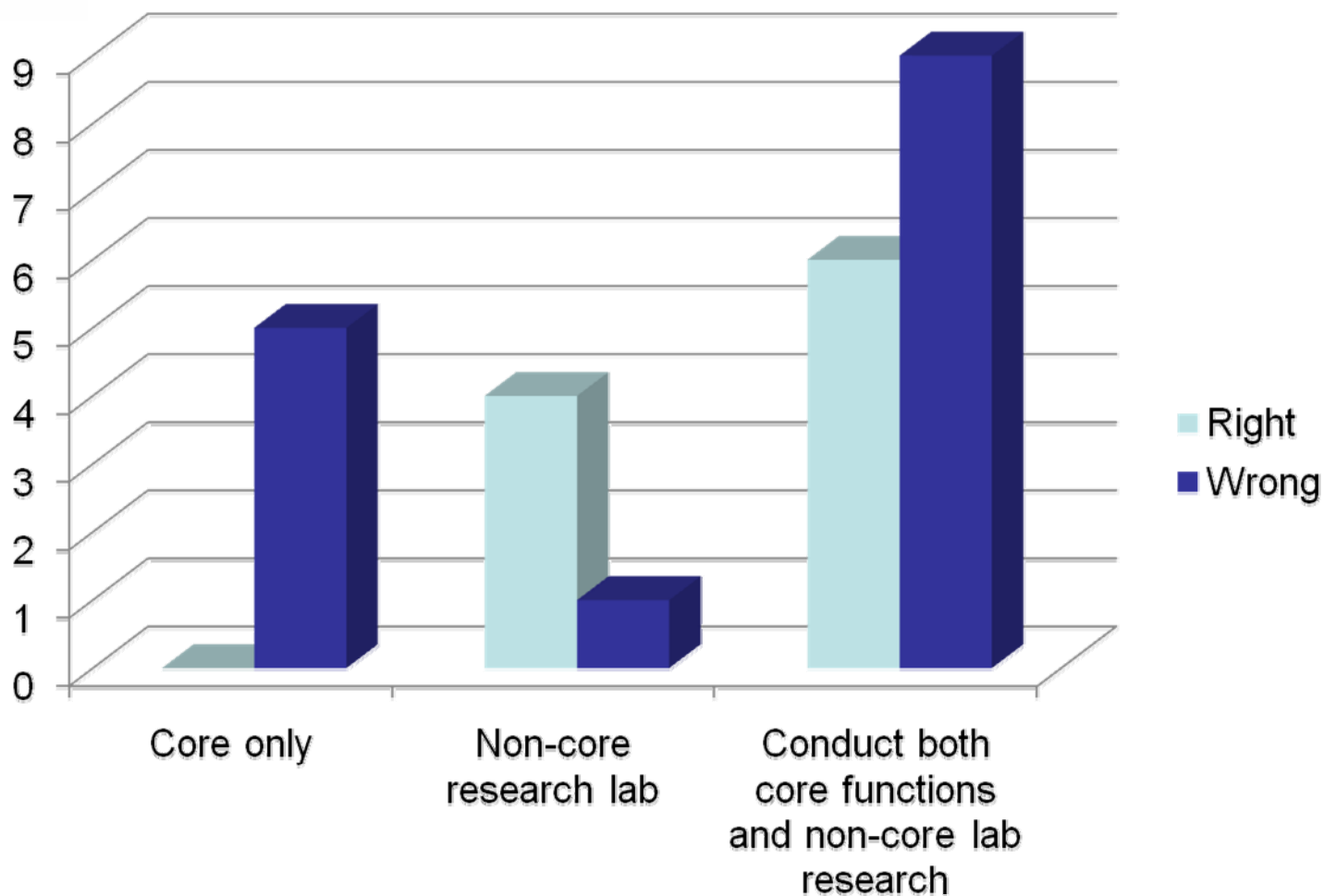
Results: Correct Pairing





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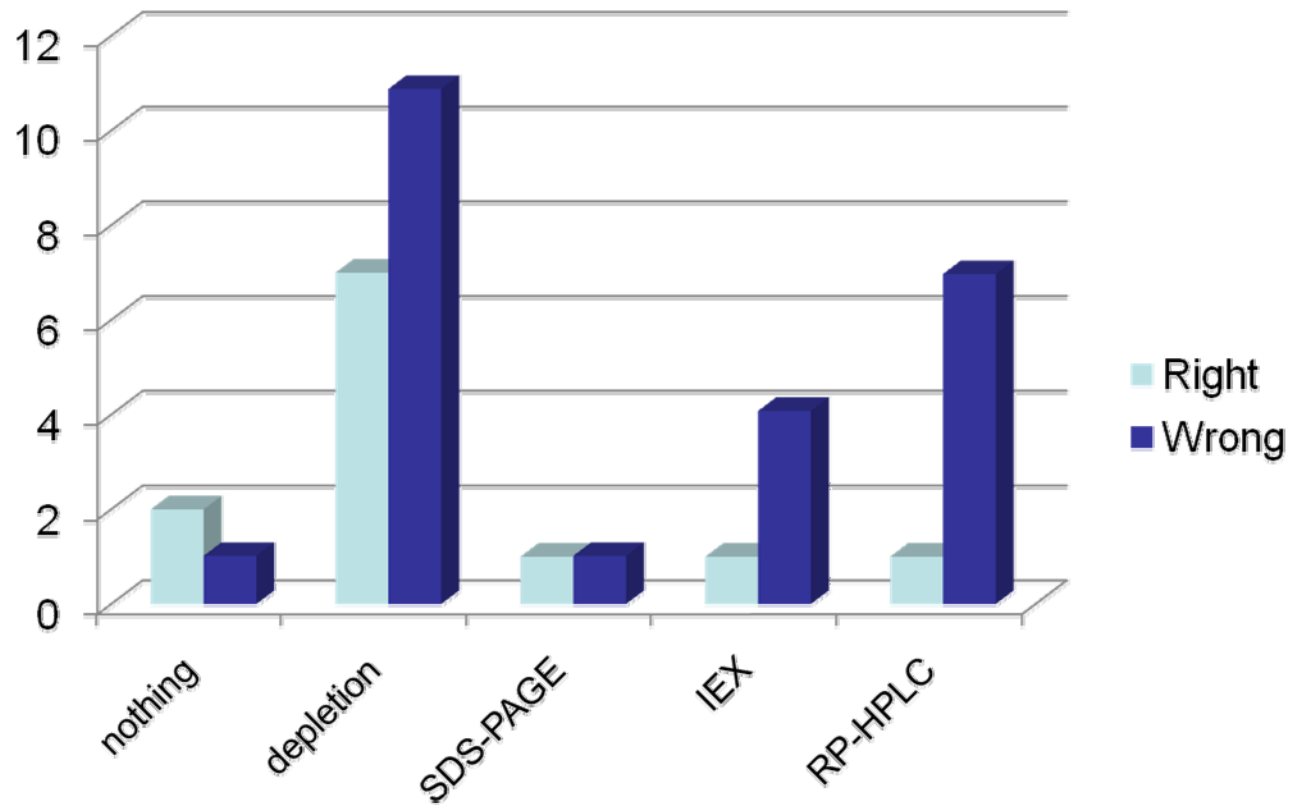
Results: Correct Pairing





Results: Correct Pairing

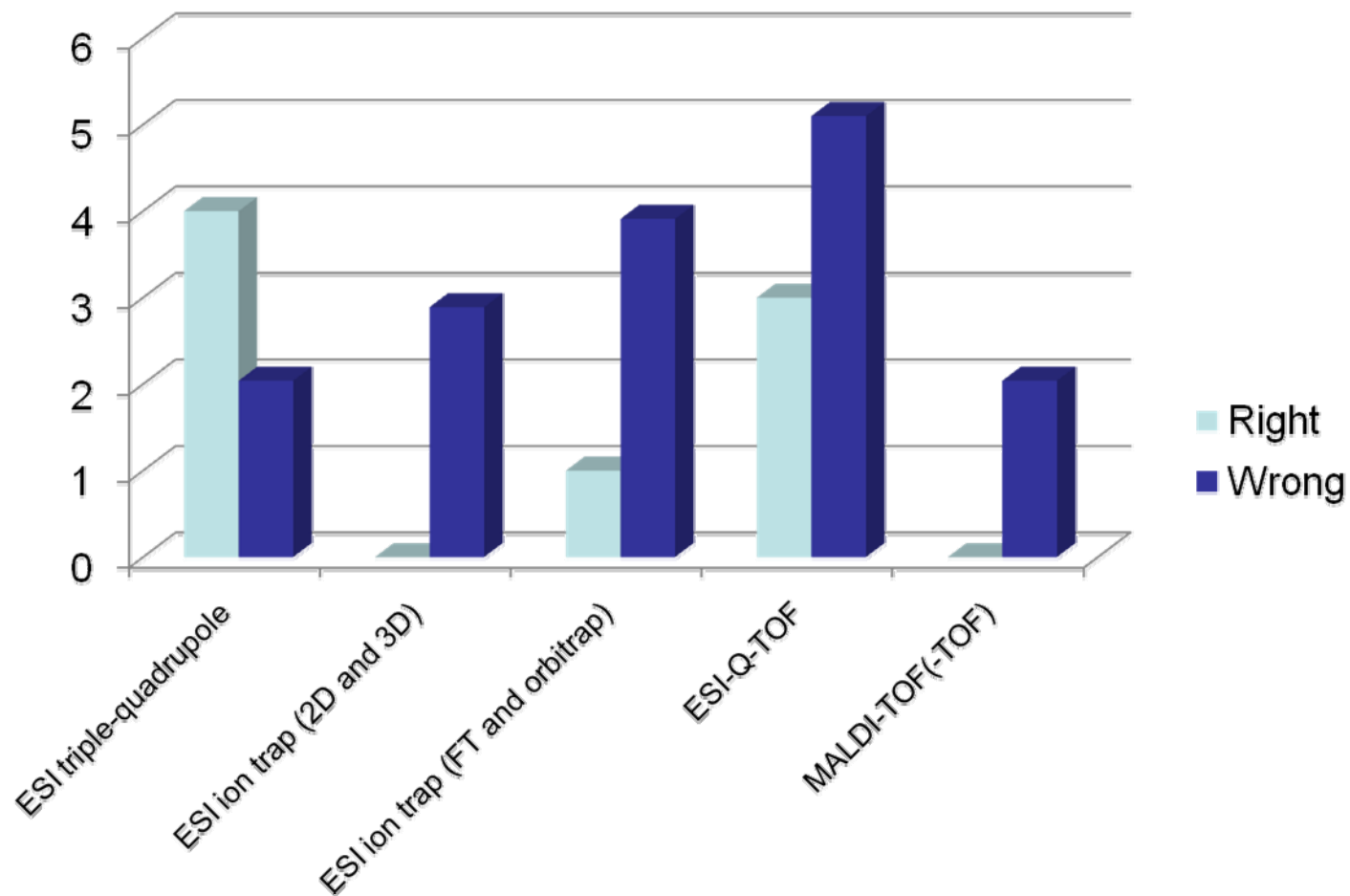
Sample Preparation





Results: Correct Pairing

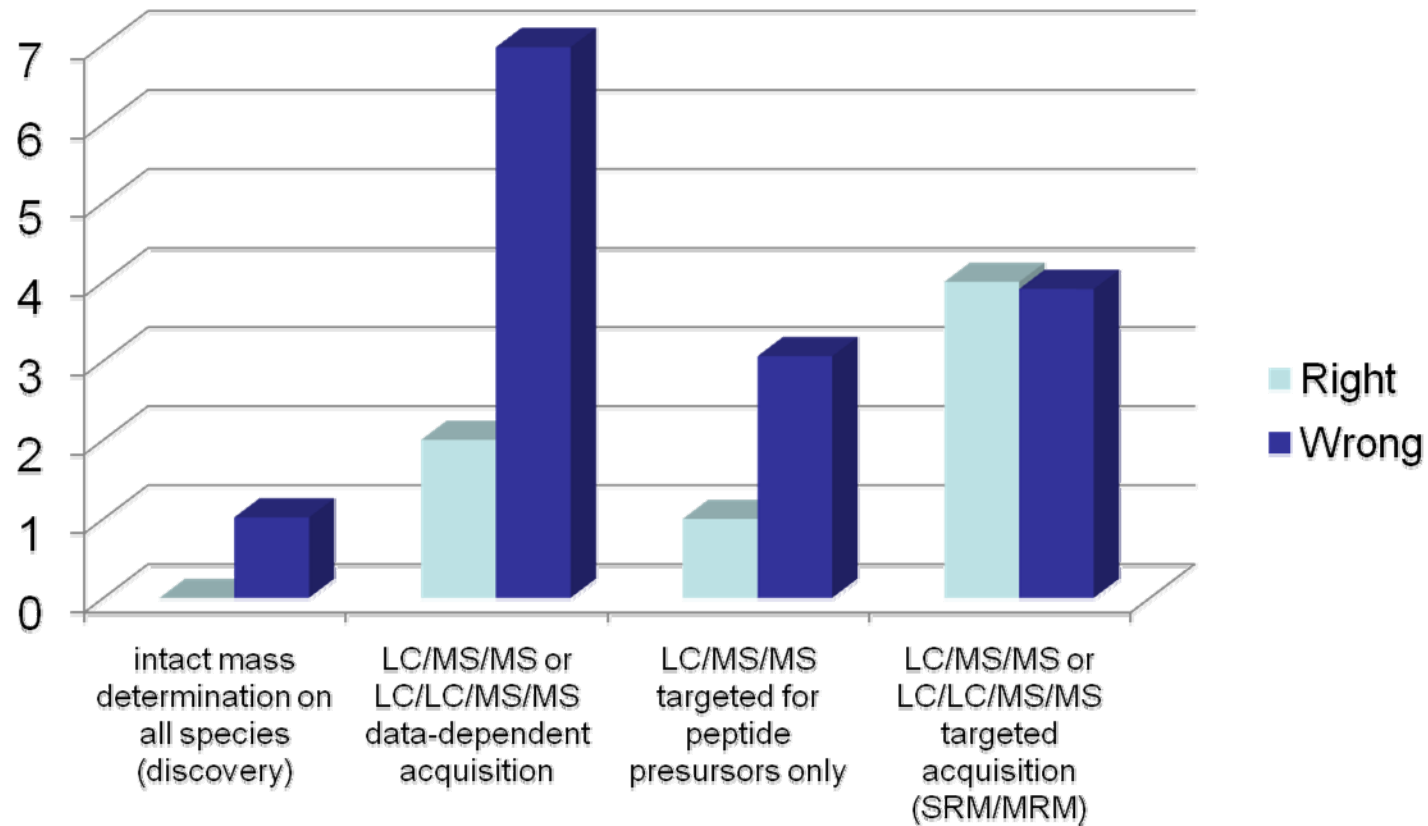
Instrumentation Used





Results: Correct Pairing

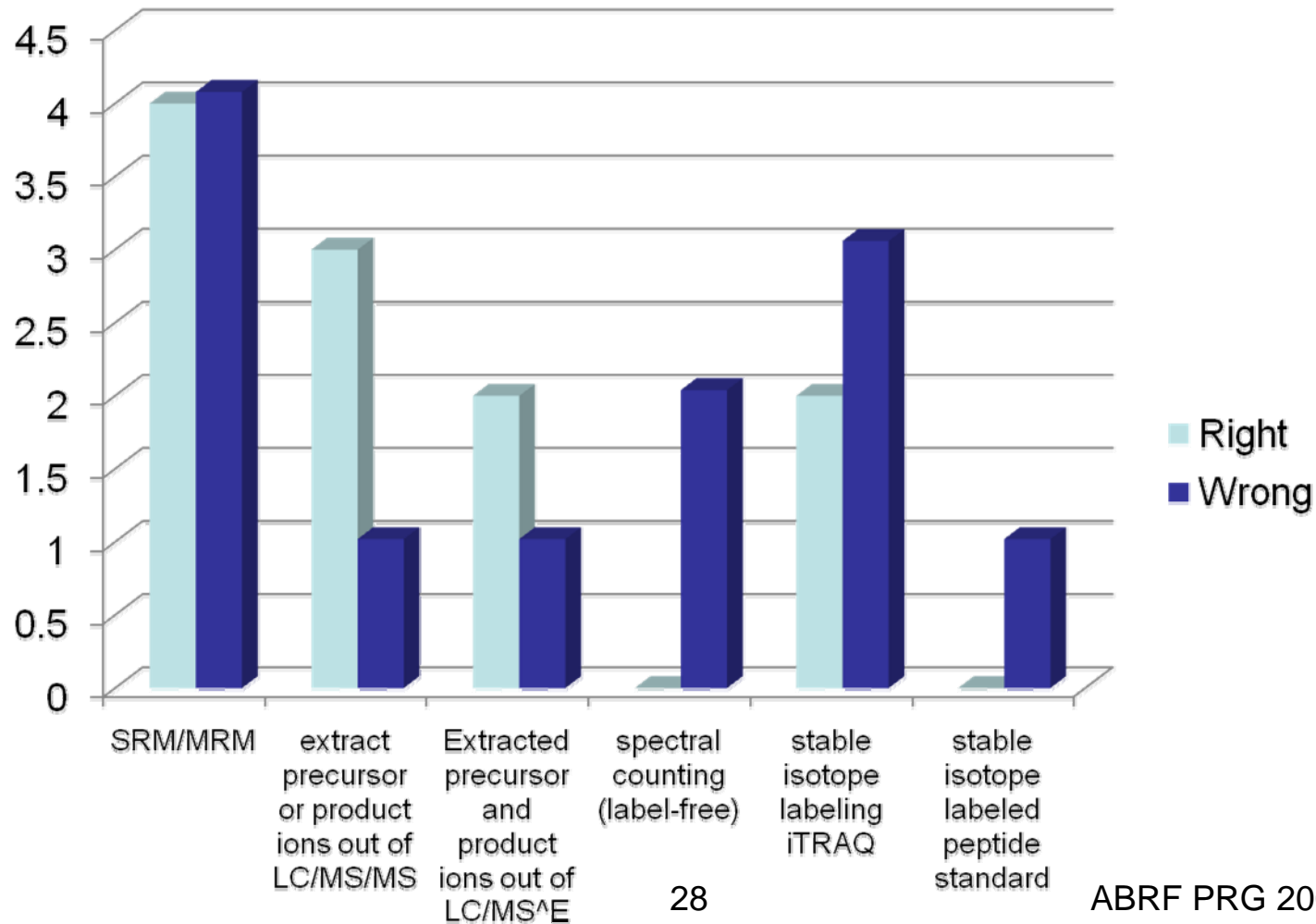
MS Scan Type





Results: Correct Pairing

Approach Used for Relative Abundance





Comments and Challenges

- The biggest challenge we faced was certainly the time in which the study needed to be completed.
- We tried MRM method without knowing the MS/MS spectra of the proteins were on ABRF web. Without the MS/MS information, our MRM was not successful.
- We wish the samples were sent to us in Sept. or Oct. and we wish we were informed in e-mail that the MS/MS spectra of the proteins were published online so we could be successful in MRM.
- Instrument problems and not enough previous experience with designing MRM analyses.



Comments and Challenges - Continued

- Solubilizing the samples was a major problem for us and thus we suggest that for next study, the samples are provided in solution.
- Phosphorylase B – weren't able to identify suitable peptides for use on the 5500 QTrap. The limited mass range (below 1000 m/z really hindered this analysis)
- The small peptide with the phosphorylation site was challenging because it was so hydrophilic after digestion with trypsin. Perhaps we should have used another enzyme.
- working with lyophilized plasma, no heavy peptide standards
- Dynamic range and sensitivity of instrumentation and LC columns. Defining relative concentration differences of different proteins within one sample. Instrument and software performance.
- Identifying low level proteins in a complex and dynamic mixture. Quantifying relative amounts of different proteins within one sample.
- Find good ionizable peptides.



Conclusions

- Quantitative proteomics experiments are complex and require many factors for success
- A handful of participants reported excellent results indicating that quantitative results are achievable
- Clinical laboratory analyses also varied for PSA analyses
- Participants using similar techniques did not obtain similar performance and suggests that expertise can be an important factor
- Head to head comparisons of different approaches is not possible because of the high dependence on expertise
- Interest in this area is high and many labs appear to be developing these capabilities



Acknowledgements

- Peter Sharratt, University of Cambridge, for amino acid analysis;
- Giuseppina Maccarrone and Christiane Rewerts, Max Planck Institute of Psychiatry, for preparing test samples;
- 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.

Acknowledgements

A huge thanks to all the labs that participated in this year's study!



Results: Glycogen Phosphorylase B/A Ratio

