Proteomic Sample Preparation Method: Rodent Blood High Abundant Protein Removal

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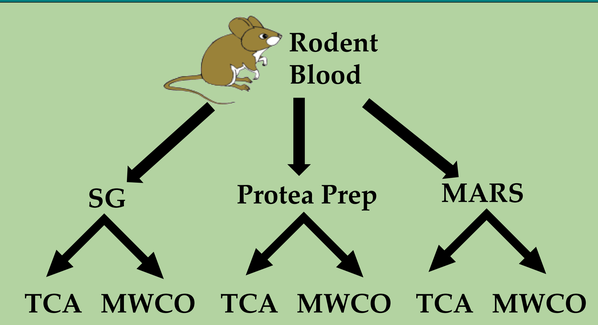
Introduction

One of the biggest challenges for the study of proteomic biomarkers in blood plasma and blood serum is the broad dynamic range of its protein constituents. For example, 70-95% of all the rat plasma proteins are comprised of albumin, Immunoglobulin (IgG), and transferrin. Therefore, a successful system of proteomic sample preparation to remove these high abundant proteins is needed to examine lower abundant proteins of interest. Researchers have developed successful ways to remove these proteins from human blood samples, but many high abundant protein removal kits for mouse and rat models vary in the efficiency of actual targeted protein content that is removed. In addition, there are different systems for high abundant protein removal, such as antibody based approaches and newer resin/bead based constructs. In this study, three different methods for high abundant protein removal were compared on rat blood plasma and mouse blood serum.

Methods



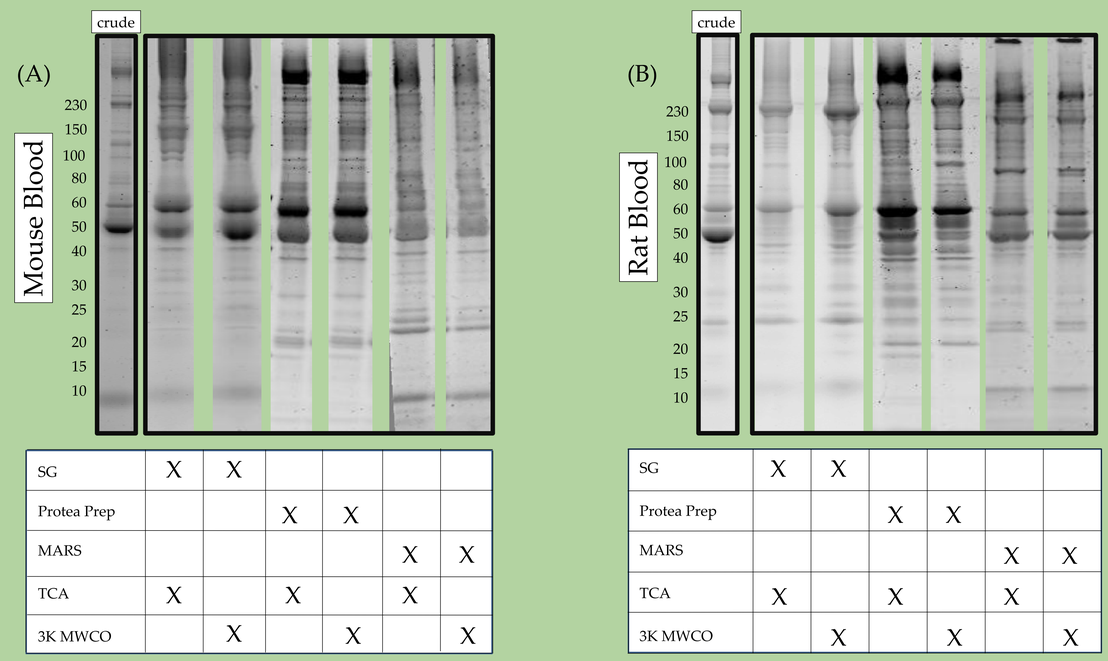
High abundant protein removal methods	Proteins removed
Swell Gel blue kit (SG) Pierce #89845	Albumin
Protea Prep Protea #SP-200	Albumin
Multiple affinity removal system (MARS) Agilent #5188-5217 (mouse-3)	Albumin, IgG, transferrin

Concentration and buffer exchange methods
Trichloroacetic acid/acetone precipitation (TCA)
3K Molecular weight cut-off (MWCO)

Results

Table 1: Shows start/end amount of protein for each sample prep method combination, as well as % remaining protein.

	ug of crude starting sample	High abundant protein removal method	Buffer exchange/ method	average remaining protein (ug)	average % remaining protein
Mouse serum	1756.09	SG	TCA	75.50	4.30%
			MWCO	54.24	3.09%
	1062.53	Protea Prep	TCA	64.20	3.66%
			MWCO	15.83	0.90%
	1756.09	MARS	TCA	80.50	4.58%
			MWCO	47.88	2.73%
Rat Plasma	540.42	SG	TCA	50.42	2.87%
			MWCO	37.43	2.13%
	540.42	Protea Prep	TCA	60.47	3.44%
			MWCO	83.66	4.76%
	540.42	MARS	TCA	38.16	2.17%
			MWCO	79.95	4.55%



SG	X	X				
Protea Prep			X	X		
MARS					X	X
TCA	X		X		X	
3K MWCO		X		X		X

SG	X	X				
Protea Prep			X	X		
MARS					X	X
TCA	X		X		X	
3K MWCO		X		X		X

Figure 1: All sample prep combinations were analyzed by SDS-PAGE (Invitrogen 4-20% Tris-Glycine). (A) Shows mouse serum samples. (B) Shows rat plasma samples.

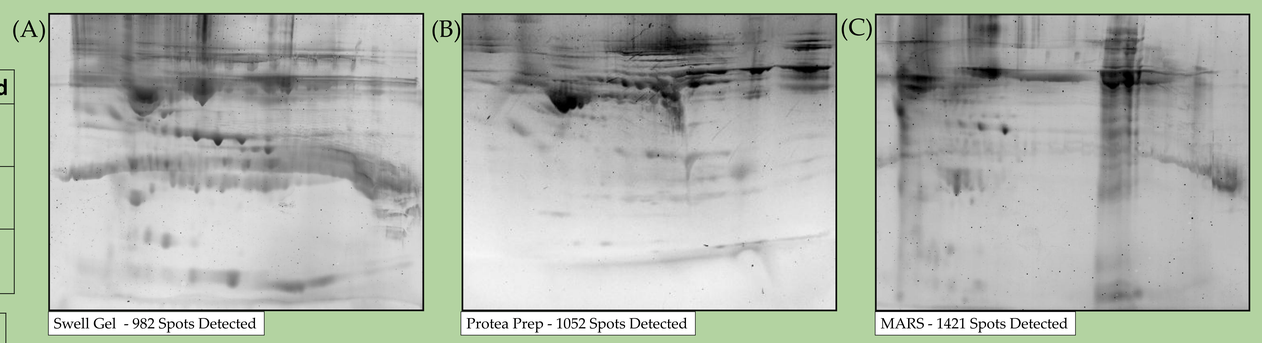


Figure 2: Mouse blood in conjunction with MWCO preparation was selected and used to compare the 3 high abundant protein removal methods, Swell gel (A), Protea kit (B), MARS (C) by 2D gel separation. Spot patterns were evaluated using DeCyder 7.0 and total number of spots detected are noted for each method.

Conclusion

This preliminary study aims to compare methods of high abundant protein removal, as well as concentration/buffer exchange methods. For mouse serum, the best high abundant protein removal method was achieved using the Mouse-3 Multiple affinity removal system. This gave the greatest appearance of lower abundant spots by SDS-PAGE, as well as the greatest number of spots detected on 2D gel. Less efficient albumin removal was seen via the swell gel and Protea Prep methods.

For rat plasma, SG and MARS shows some high abundant protein depletion, but the Protea Prep kit demonstrates the greatest increase in the appearance of lower abundant protein spots by SDS-PAGE.

Trichloroacetic Acid/acetone precipitation and 3K molecular weight cut-off preparations showed little difference.

References

Brett A. Chromy, Arlene D. Gonzales, Julie Perkins, Megan W. Choi, Michele H. Corzett, Brian C. Chang, Christopher H. Corzett, and Sandra L. McCutchen-Maloney. Proteomic Analysis of Human Serum by Two-Dimensional Differential Gel Electrophoresis after Depletion of High-Abundant Proteins *Journal of Proteome Research* **2004**, 3, 1120-1127.

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