AlbuVoid™ Enrichment & Antibody Depletion - Solving the Challenges of Serum Proteomics

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Poster first presented at the 67th ASMS Conference, June 2-6, 2019 in Atlanta, GA USA

Abstract

Proteomic workflows that support serum proteomics can be especially challenging for two reasons: 1) the presence of highly abundant proteins, Albumin alone accounts for about 50% of the total protein mass, and 2) a particularly proteolytic resistant sample type due the large concentration of antibodies present. Many proteomic enrichment strategies employ the use of immuno-affinity depletion to remove one or more high abundance proteins. Some common limitations of immuno-affinity however are high costs, regeneration requirements which may result in a diminished and inconsistent performance, as well as a required marriage of species to antibody. Because of these limitations, researchers need ways to enrich differently. We have previously reported a variety of tools that can bias towards or against select sub-proteomes of serum without the use of immuno-affinity. Now we report the serum proteome bias characteristics of **AlbuVoid™** & **NuGel™ Protein A**, alone or in serial combination, using LC-MS reporting metrics. Products and digest conditions produce different proteome qualitative and quantitative windows of observation. For biomarker discovery, we solicit the value for enrichment of categorical sub-proteomes to provide mechanistic insight into disease pathologies. For this, a knowledgebase of over 1000 serum proteins is now available to help proteomic researchers choose the best available products and methods for their particular needs.

Introduction

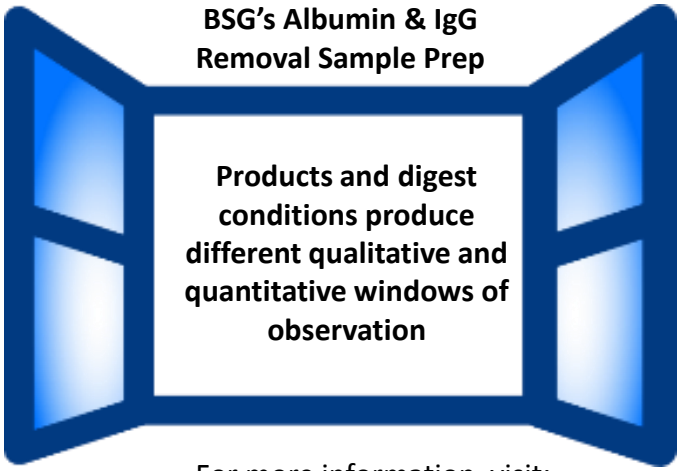
In a previous paper, we reported on the adaptation of the BSG product **AlbuVoid™**, with a simple on-bead digestion workflow of the Albumin-depleted sub-proteome[1]. With some modest workflow adjustments, Viaralet et al. recently concluded that this method proved to be faster and more cost-effective than antibody-based methods to improve quantitative clinical proteomics[2]. We now consider the advantages of first reducing the influence of IgGs, by using a Protein A depletion, based on a NuGel™ dry powder format for which buffers adapt seamlessly to **AlbuVoid™**. With this new workflow, we compared the performance of on-bead digestion (trademarked as BASP™ for Bead-assisted Sample Prep), and off-bead (eluent workflow) using a common strong denaturing digest method conventionally called FASP.

Methods

The vast majority of the plasma proteome falls into functional categories; by mass content these are: Albumin 50-60%; Immunoglobulins 10-20%; Transport (Transferrin, Apo) 5-10%; Complement related Proteins 3-5%; Protease Inhibitors 2-5%; and all others 2-5%. While these categorical sub-proteomes are required for normal body homeostasis, they nevertheless become dysfunctional during acute-phase and chronic stimuli. Herein, we report the serum proteome bias characteristics, both qualitative and quantitative, of the products **AlbuVoid™** & **NuGel™ Protein A**, alone or in serial combination, using LC-MS reporting metrics. An overview of the methods is presented below, the detailed LC-MS has been previously reported[3].

AlbuVoid™
An Albumin Depletion reagent kit. Selectively voids Albumin, binds to and enriches low abundance serum proteome.

AlbuVoid™ Plus
An Albumin & IgG Depletion reagent kit. Combines optimized immobilized Protein A with AlbuVoid™ in seamless workflow.

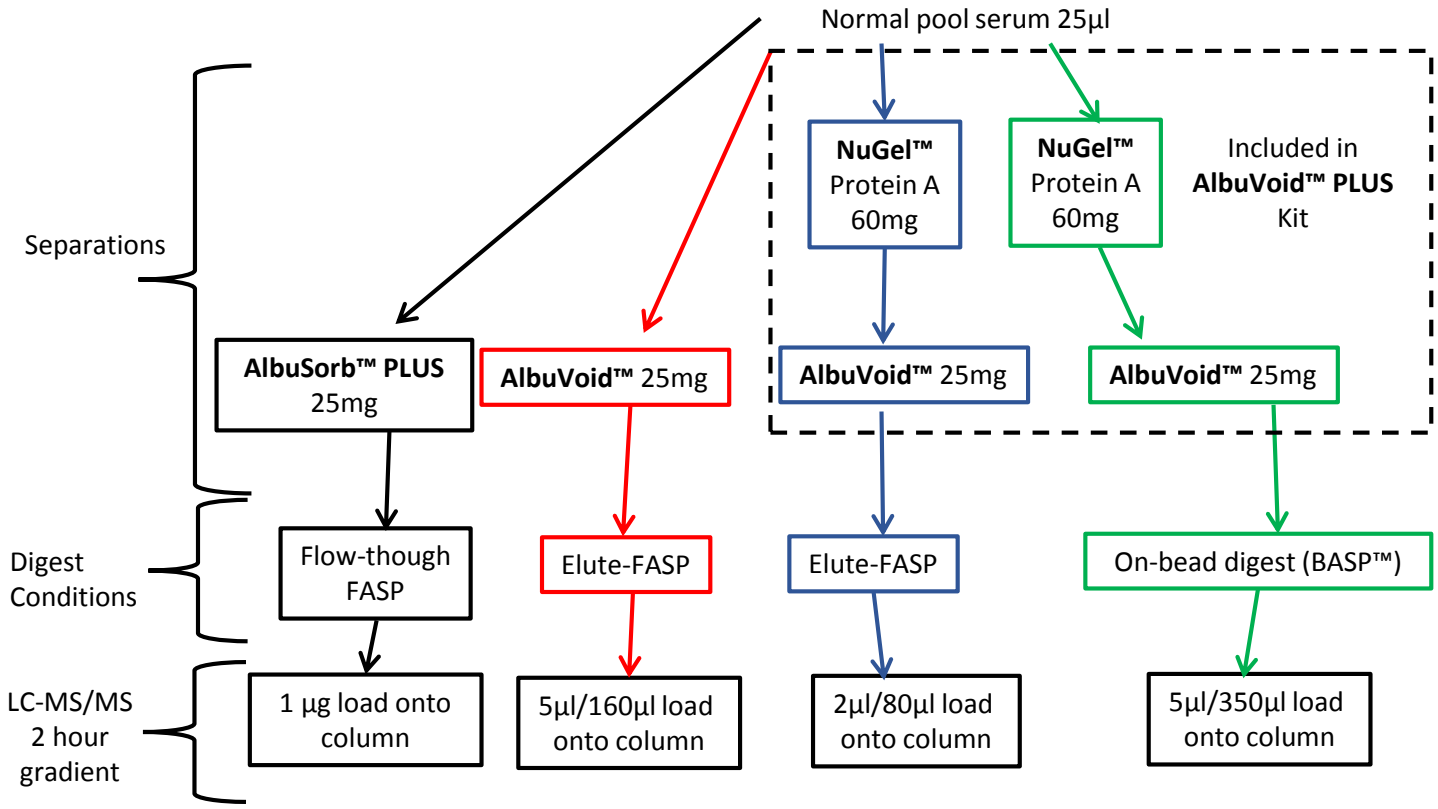


For more information, visit:
https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

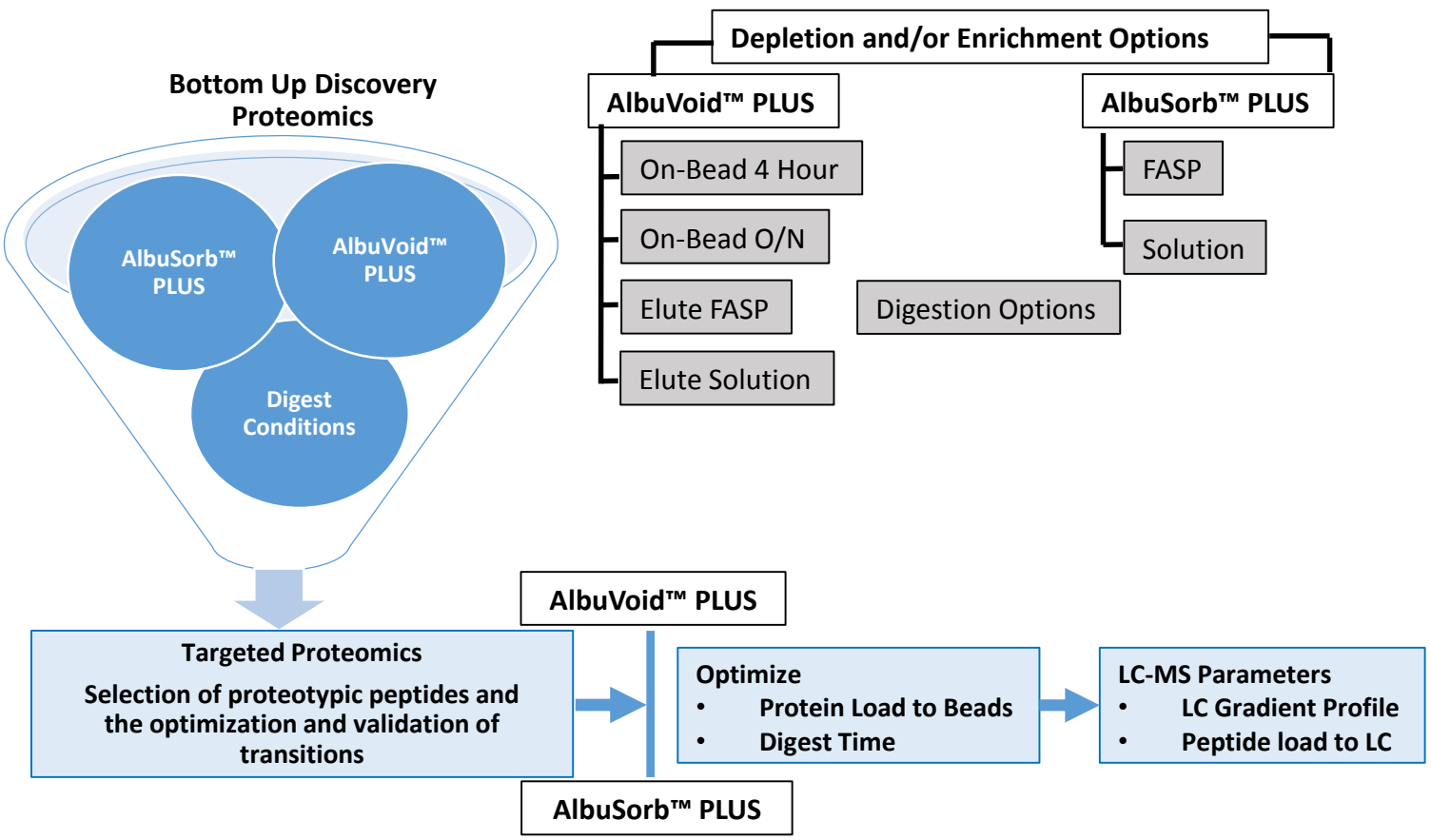
AlbuSorb™
An Albumin Depletion reagent kit. Selectively binds Albumin, not based on blue-dye or immuno-affinity.

AlbuSorb™ Plus
An Albumin & IgG Depletion reagent kit. Combines optimized immobilized Protein A with AlbuSorb™.

Overview of Workflow Methods



Sample Prep Workflow Considerations for LC-MS Proteomics



Results

On Table 1, we compare workflows with **AlbuVoid™**, along with another product in our Albumin Removal catalog, that selectively binds both Albumin and IgGs, called **AlbuSorb™ PLUS**[4]. IgGs, accounting for 70-80% of the total Immunoglobulin sub-proteome are very efficiently removed by both **AlbuSorb™ PLUS** and **AlbuVoid™ PLUS**. Note that the on-bead digest methods greatly diminishes the spectral counts and protein IDs associated with the Immunoglobulin sub-proteome; a particularly hard-to-digest class of proteins. In the new methods, we highlight that the Complement-related sub-proteome, being highly enriched with **AlbuVoid™**, can be deeply investigated at the tryptic peptide level for sub-forms differentially regulated in disease, Table 3.

Table 1	Apprx. plasma conc. %	AlbuVoid™ On-Bead 4 hr[3]	AlbuSorb™ PLUS[3]	AlbuVoid™ FASP	AlbuVoid™ PLUS / FASP	AlbuVoid™ PLUS / On-Bead
Total Spectral Counts (SC)		8969	18890	19388	23575	23389
Total Protein ID (≥2 SC)		235	370	568	467	350
% SC Albumin	50%	1%	14%	3%	5%	5%
% SC Immuno-globulins	20%	15%	6%	26%	8%	6%
% SC Apolipoproteins	4%	11%	5%	4%	5%	5%
% SC Transport Proteins	8%	17%	18%	16%	27%	23%
% SC Protease Inhibitors	6%	12%	21%	6%	9%	10%
% SC Complement related	5%	28%	8%	22%	26%	31%
% SC Coagulation / Fibrinolysis	4%	4%	1%	5%	4%	5%
% SC Other / Low Abundance	3%	12%	26%	18%	16%	15%

On Table 2, we highlight representative proteins with particular bias towards either AlbuSorb™ PLUS or AlbuVoid™ PLUS. As one of these proteins (Extracellular Matrix Protein 1) is part of BSG's patent pending Stroma Liquid Biopsy™ panel[5] of cancer biomarkers, it demonstrates the advantage of evaluating multiple windows of observation.

Table 2 (spectral counts)	AlbuSorb™ PLUS	AlbuVoid™ PLUS On-Bead
sp|P01023|A2MG_HUMAN	1918	73
sp|P00738|HPT_HUMAN	1355	94
tr|D9IWP9|D9IWP9_HUMAN	23	311
sp|Q16610|ECM1_HUMAN[5]	0	40[5]

As can be seen from Table 3, the Complement related sub-proteome is especially enriched with AlbuVoid™, >5X enrichment. Complement is a cascading protein interaction system that acts as an early alert and response mechanism to thwart exposure to infectious agents. Under-appreciated however, is its evolutionarily conserved link to coagulation to eliminate damaged tissues. We have previously reported this dysregulation as part of a panel of pan-cancer biomarkers called Stroma Liquid Biopsy™[5]. Observations from those studies supported many concepts surrounding the study of functional sub-forms of highly abundant proteins by differential observation at the peptide level[6].

As the highly abundant Complement related proteins exist in the circulation in a variety of functional sub-forms or proteolytically generated split products, we considered that our new methods might bias towards observing functional sub-forms differently. Such is the case here, where the native C3 is quantitatively different from the activated C3b sub-form when observed through the peptide features before amino acid 748 (the cleavage of C3a by C3 Convertase), and after amino acid 748.

C3 sub-forms (split products) report quantitative differences in normal vs. disease comparison with different digest methods.

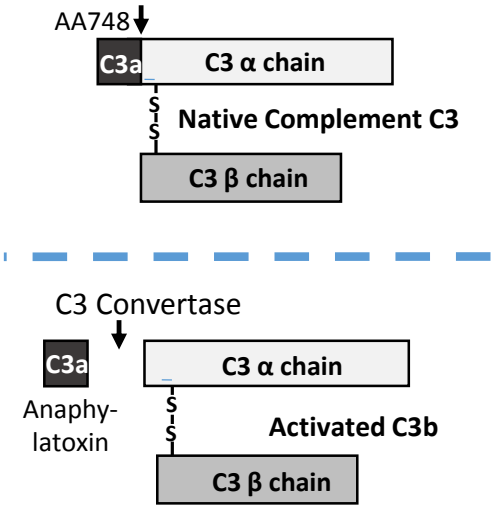


Table 3	ProA / AlbuVoid™ / FASP	ProA / AlbuVoid™ / FASP	ProA / AlbuVoid™ / On-Bead	ProA / AlbuVoid™ / On-Bead
Peptide level features before AA748 (from C3 β chain)				
	Pooled Normal	**Cancer**	**Pooled Normal**	**Cancer**
# Tryptic peptide IDs (>2 SC)	**15**	**14**	**17**	**16**
Spectral Counts	**238**	**223**	**258 (1.7x cancer)**	**151**
Peptide level features after AA748 (from C3 α chain)				
# Tryptic peptide IDs (>2 SC)	**10**	**11**	**12**	**11**
Spectral Counts	**154**	**339 (2.2x normal)**	**133**	**140**

Discussion & Conclusions

Products and digest conditions produce different sub-proteome windows of observation. So, depending on the needs of the investigation, it can be valuable to consider that one or more of these categorical sub-proteomes is simply background noise whereby depletion is beneficial. While in other cases, these same categorical sub-proteomes might provide new data and information and consequently, should not be depleted. Categorically the acute-phase sub-proteomes differentiated in disease may vary greatly from those associated with chronic sub-proteomes. So there is great benefit in having options to enrich or deplete one or more of these sub-proteomes. BSG's Albumin and IgG Removal Kits offer many such options:

- The '**PLUS**' products substantially deplete Immunoglobulins through separations at the protein level.
- The variable regions of Immunoglobulins are extremely heterogeneous, generating a background noise across the full LC gradient. On-bead digestion (BASP™) with **AlbuVoid™** substantially reduces the influence of such Ig peptide features. So in addition to workflow simplicity, BASP™ can be advantageous utilized in targeted proteomic workflows whenever the target proteins do not require strong denaturing conditions.
- With the exception of Immunoglobulins whereby FASP generates many more spectral features, both strong denaturing conditions (FASP) and on-bead digest (BASP™) conditions produce similar protein profiles. For certain proteins, a particular method can produce more spectral counts. So for targeted proteomics, please contact the corresponding author – Matt Kuruc, as we have a knowledgebase of over 1000 serum proteins to help select the best method(s) for particular protein(s).
- The Complement sub-proteome is especially enriched by **AlbuVoid™ PLUS**. The digest conditions may bias towards one or more functional sub-populations, likely due to conformational transitions and protein-protein interactions (i.e., Factor Bb, Properdin) that occur upon activation. This needs further investigation.
- The low abundance sub-proteome (~ng/ml) is enriched 5+ fold with **AlbuVoid™ & AlbuVoid™ PLUS** and 13+ fold with **AlbuSorb™ PLUS**.
- The same consumable products and methods used for discovery can be used for targeted proteomics, making the transitional goal easier, to quantitative LC-MS clinical proteomics.

References

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