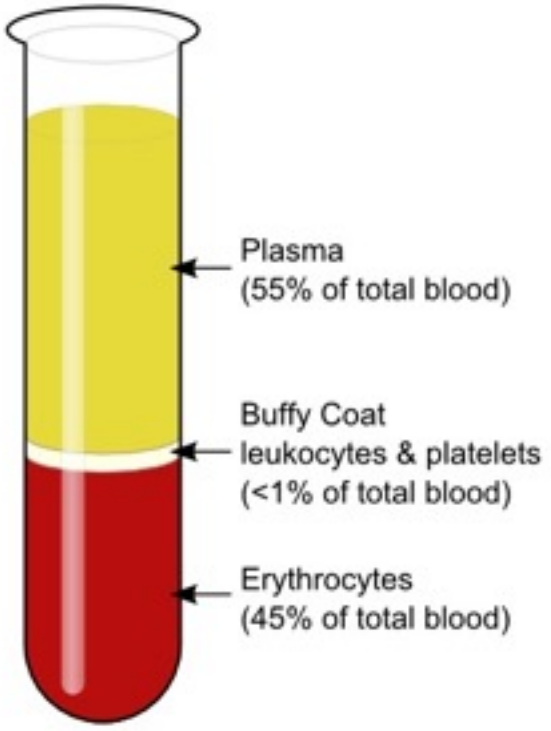
After removing the noise from Hemoglobin, your signal is so much clearer!

Hemoglobin accounting for ≥ 95% of the protein mass in erythrocytes, is often an interfering factor for many types of analysis, both proteinaceous (i.e., immunoassay) and non-proteinaceous (i.e., PCR). With the rising use of dried blood cards and point-of-care devices, analytical interferences from Hemoglobin continues to present problems.

Our specialized application specific products are designed for all these challenges. If you do not see a solution within our standard products, come talk to us and we can design custom specific dry powder or suspension formulations.



Proteomic information can be derived from all blood compartments

>Plasma/serum proteins and other circulating factors directly regulate complex processes such as aging, the development of chronic diseases, and severe acute disease (i.e., acute respiratory distress syndrome).

>Activated leukocytes and platelets release granulocytic cargo proteins in response to local inflammatory stimuli, generating a protease storm, that if not resolved alters steady state homeostasis contributing to both acute and chronic disease.

>Erythrocytes carry more than just oxygen and are now under investigations for many chronic conditions including Malaria and Parkinson's Disease.

BSG's products and methods can help proteomic investigators explore all these blood compartments.

Key Applications:

There have been 40+ citations for successful use of our products for a variety of diseases and challenging applications for hemoglobin removal. Here are some of the highlights.

Human Proteome Project - Annotation of Erythrocytes

HemoVoid™ “protein-level pre-fractionation proved helpful in identifying additional proteins and N-termini”. 778 proteins were identified from the cytosolic fraction, 171 of which were not represented in either the soluble non-depleted fraction or the membrane fraction.

LEARN MORE

Malaria infected erythrocytes

The analysis of Plasmodium falciparum schizont phospho-proteome followed HemoVoid™ treatment.

LEARN MORE

For drug interaction and discovery

“The intact-cell cellular thermal shift assay protocol features a HemogloBind™ - based sample processing step, which provides a relatively fast, reliable and inexpensive method to deplete >90% of hemoglobin…it leads to a 40-50% increase in the number of peptide spectrum matches (PSMs) for *P. falciparum* and non-hemoglobin human proteins.”

LEARN MORE

Erythrocyte Proteomic Methods

Careful investigation of different strategies for … proteome profiling by 2-DE was carried out, paying particular attention to hemoglobin removal. The paper concludes that “a simple, quick, and satisfactory approach for hemoglobin depletion of erythrocyte cells based on HemogloBind™ reagent is shown here to satisfactorily analyze the cytosolic sub-proteome by 2-DE without major interference.”

LEARN MORE

Whole Blood Lysates

The article investigates Total cholinesterase activity of whole blood samples with HemogloBind™ treatment prior to Ellman method. It concludes that, the HemogloBind™ protocol is consistent, and simple with only one incubation and a short, low speed centrifugation step.

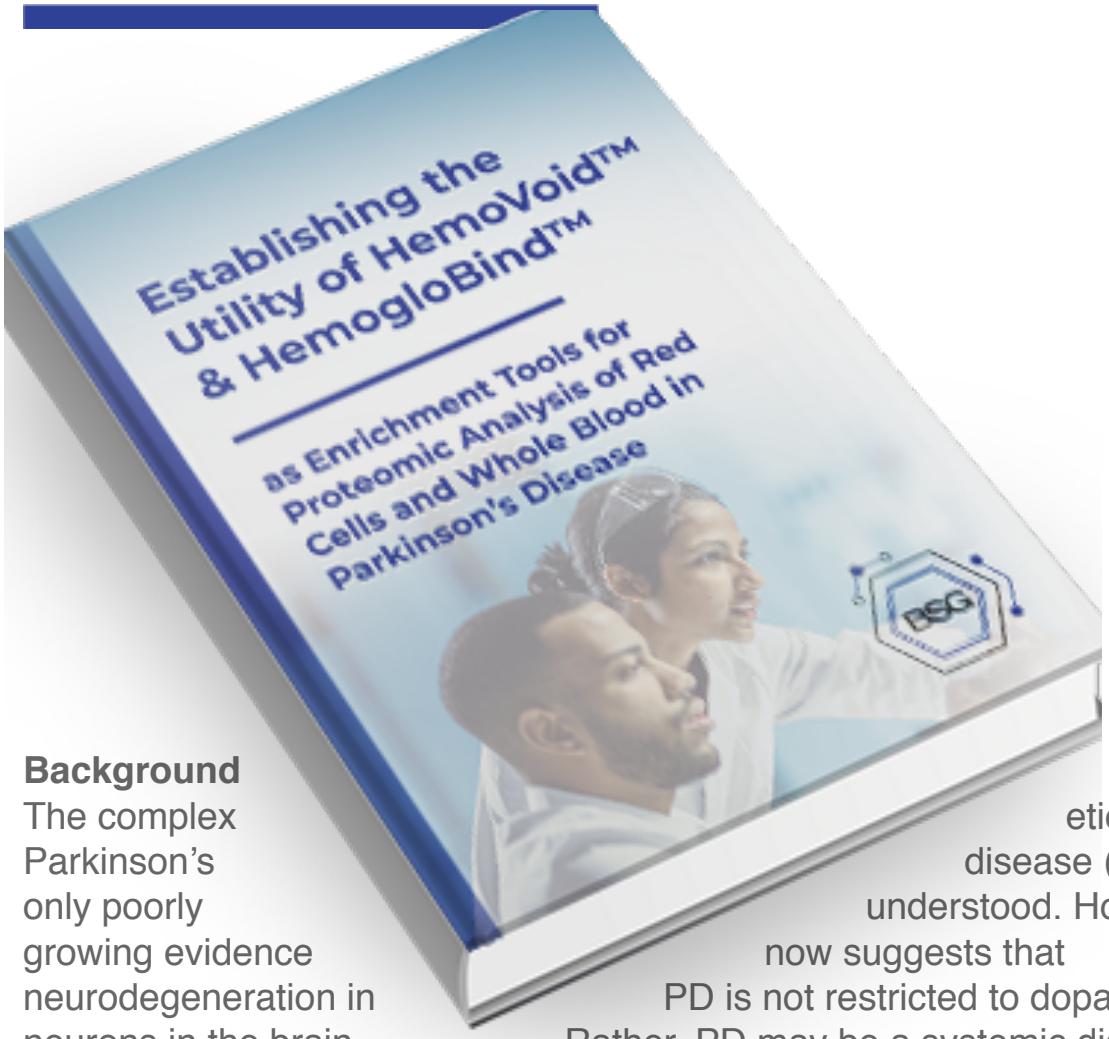
LEARN MORE

Reticulocyte transitioning

From a Science article, the study used multiplexed quantitative proteomics to identify candidate substrates of UBE2O, an E2 (ubiquitin-conjugating) enzyme, in an unbiased and global manner. Because of the overly abundant presence of Hemoglobin, selective depletion of Hemoglobin using HemogloBind™ was employed.

LEARN MORE

Case Study



Background

The complex etiology of Parkinson's disease (PD) is only poorly understood. However, growing evidence now suggests that neurodegeneration in PD is not restricted to dopaminergic neurons in the brain. Rather, PD may be a systemic disease, involving peripheral tissues and may include oxidative, metabolic, or inflammatory processes. So blood based biomarkers may prove useful in early detection of PD as well as to assess the progression of disease in response to medical interventions.

Challenge

a-Synuclein (Gene: **SNCA**, a-Syn) is a major protein constituent of Lewy pathology in the central nervous system (CNS); the pathological hallmark of PD. Consequently, a-Syn in blood is under investigation as a biomarker for PD, the vast majority of which in circulation, comes from red blood cells (RBCs), by far the most abundant cell type found in blood. Lacking a

nucleus, RBCs might serve as important reservoirs for biomarker research as, they are unable to synthesize new proteins in response to outside stimuli. Thus, any changes in the RBC proteome content or structure due to disease, should be measurable once a baseline of normal/healthy controls is established. However, there has been limited biomarker discovery programs conducted on RBCs because of their large dynamic range of proteins, high abundance of lipids, and most especially the super-abundance of hemoglobin, accounting for >95% of the RBC protein mass. Hemoglobin thus presents a major interfering factor in proteomic analyses of whole blood and sedimented erythrocytes.

Solution

As one of the key BSG Advantages, BSG offers a choice of two strategies for proteome enrichment, to get the best results: either selectively binding high abundance proteins (the “Bind” products), or choosing not to bind (the “Void” products) thereby enriching the underlying proteome. To achieve this complementary product line, we first developed a chemical library of general non-specific adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. Without the use of antibodies, progressive displacement at or near saturation, allows the beads to bias for or against certain proteins. So each bead product was empirically characterized to meet the needs of the application.

HemoVoid™, is designed to remove hemoglobin from erythrocyte lysates in a simple and efficient manner, through a negative selection strategy, meaning the Hemoglobin voids out through the beads, and the vast majority of the remaining proteome binds to the beads. For subsequent analysis, the bead-bound enriched proteome can either be eluted, or for LC-MS/MS, digested on-bead. This latter process is called Bead-assisted Sample Prep or BASP™.

HemogloBind™, binds hemoglobin, with a high degree of selectivity that does not cross-react with most other serum components. It is available as either a suspension reagent product or in a dry powder NuGel™-based format. In the former, HemogloBind™ is supplied in a bottle and aliquoted at suitable v/v ratios depending upon the Hemoglobin concentration. In the

NuGel™ powder format, the powder is first weighed and dispensed into SpinX filters according to use guidelines supplied with the product.

The Outcomes

Three reports describe the utility of BSG's Hemoglobin Removal products to study potential biomarkers for Parkinson's Disease in Blood

1) Klatt, Stephan, et al. " Optimizing red blood cell protein extraction for biomarker quantitation with mass spectrometry." Analytical and Bioanalytical Chemistry (2020): 1-14.

The article describes the advantage of HemoVoid™ in detection of low abundance proteins when comparing their amounts (in percent) between four alternative extraction conditions, stating "… Most peptides, following HemoVoid™ extraction, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols". With respect to potential biomarkers for Parkinson's Disease, the article states "For example, PRDX6 accounts for 0.4% of the total ion abundance after DOC (deoxycholate) extraction, whereas following HV (HemoVoid™) extraction, this increases to 8%, a 20-fold enrichment". The authors conclude that the HemoVoid™ method significantly reduces the concentration of hemoglobin, resulting in an increased signal-to noise of the remaining red cell proteins. The article describes methods to digest the HemoVoid™ bead-bound proteome, greatly simplifying the workflow for LC-MS/MS analysis.

2) Elhadi, Suaad Abd, et al. " α-Synuclein in blood cells differentiates Parkinson's disease from healthy controls." Annals of Clinical and Translational Neurology.

The goal of this study was to determine whether blood cells expressing α-Synuclein can differentiate Parkinson's disease (PD) from healthy controls. Two proteoforms - PSer129 a-Syn (phosphorylated pathological form in Lewy bodies) and Oxidized a-Syn levels are observed in blood cells, but both at considerably lower concentration than total a-Syn, so the extremely high abundance of hemoglobin interferes with their analysis. To compensate, the article states for PSer129 α -Syn & Oxidized α -Syn

detection by immunoassay, “followed from hemoglobin clearance with HemoVoid kit (Biotech Support Group LLC, NJ, US)”.

3) Lahut, Suna, et al. "Blood RNA biomarkers in prodromal PARK4 and REM sleep behavior disorder show role of complexin-1 loss for risk of Parkinson's disease." Disease Models & Mechanisms (2017): dmm-028035. http://dmm.biologists.org/lookup/doi/10.1242/dmm.028035 In this study, the authors studied blood samples from a large pedigree with SNCA gene duplication (PARK4 mutation), to identify effects of SNCA gain-of-function as potential disease biomarkers. The article states for whole blood analysis, “For protein extraction from the EDTA tubes, 300 µl blood were lysed …The blood lysates were rotated at 4 °C for 30 min and centrifuged at 4 °C for 30 min. The supernatants were depleted in hemoglobin content using a commercial kit (HemogloBind™) following the manufacturer’s instructions”. After hemoglobin depletion, quantitative immunoblots of protein extracts from corresponding whole blood PARK4 samples showed a ~1.5-fold accumulation of SNCA monomer, but no SNCA aggregates were detectable.

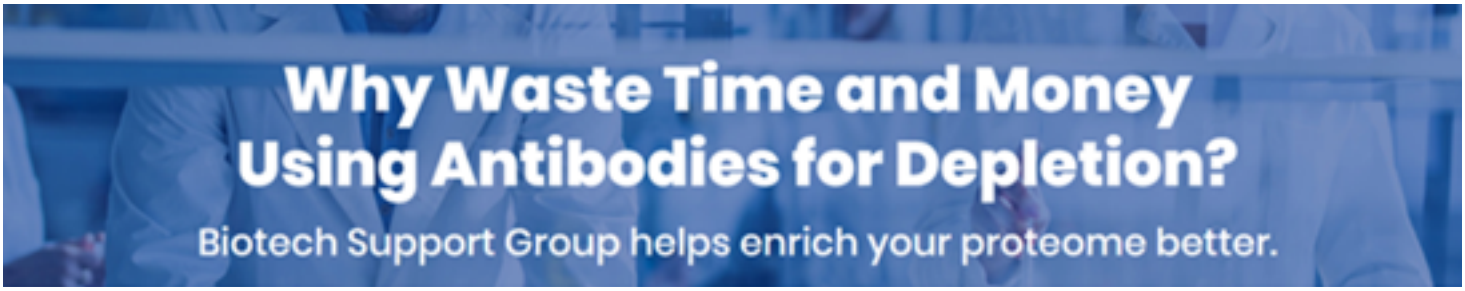
These three references illustrate how BSG’s products can efficiently remove Hemoglobin enabling proteomic analyses of whole blood and sedimented erythrocytes. HemogloBind™, binds hemoglobin, which is engineered for a high degree of selectivity that does not cross-react with most common serum components. And, HemoVoid™ works inversely, binding the underlying sub-proteome to the beads, and voiding Hemoglobin in the unbound fraction.

Efficient Sample Prep Enrichment Brings Better information from Blood

Proteomics started with coverage annotation and Venn diagrams but now the field demands robust quantitation. This has been our focus; how to address quantitative differences between proteins in blood samples representing a challenge or disease state, vs. samples representing a

normal or control state.Through reduction of background noise, enrichment of low-abundance biomarker proteins improves linearity between the peptide ion signals and protein abundances. A consumable sample prep product line, not reliant on immuno-affinity, provides the efficiency necessary for clinical proteomic biomarkers derived from blood.

- ➢ BSG's enrichment products have proven to be robust, reproducible and quantitatively linear across >4x log of LC-MS/MS signal intensity data.
- ➢ BSG's unique Bead Assisted Sample Prep (BASP™) digestion protocols minimize inconsistencies of proteolytic digestion, greatly simply workflows, and can often speed the time to analysis.



"AlbuVoid™ method proved to be faster and more cost-effective than antibody-based methods". Current Topics in Peptide & Protein Research 19. 53-62.

"The advantage of HemoVoid™ in detection of low abundance proteins can be seen when comparing their amounts (in percent) between HemoVoid™ and the other four extraction conditions.… Most peptides, following HemoVoid™, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols.". Analytical and Bioanalytical Chemistry Feb. 2020

"To obtain purified exosome fractions for proteomic analysis,… albumin was depleted … using AlbuSorb™ - Albumin Depletion Kit" Scientific Reports 8.1 (2018): 7227

"Reticulocytes were lysed…HemogloBind™ suspension was added to the samples, …The supernatants, which contain hemoglobin-depleted sample, were … processed for TMT quantification." Science 357.6350 (2017)

"If the elimination of lipids…is necessary, the sample can by treated with lipid removal (Cleanascite™)…" Proteomics for Biomarker Discovery. Humana Press, New York,

BIOTECH SUPPORT GROUP LLC and PRO TEST DIAGNOSTICS AB Enter Supply Agreement for HemoVoid™ for Blood Doping Tests

MONMOUTH JUNCTION, NJ , April 4, 2019 -- Biotech SupportGroup (BSG, Monmouth Junction, NJ) and Pro Test Diagnostics AB (Umeå, Sweden) jointly announce that they have entered a supply agreement for the BSG product HemoVoid™. BSG produces products for simple and efficient removal of hemoglobin from red cell lysates. Pro Test Diagnostics AB is a developer of rapid and accurate detection of blood doping through molecular fingerprints. Learn more>

The BSG Advantages

Cost Effective & Efficient

Sample prep methods essential for expanding proteomic biomarkers into routine healthcare

Consumable Research Products

Supporting the expanding installation of LC-MS instruments & computational infrastructure

Serves All Proteomic Analytical Platforms

Mass Spectrometry (LC-MS/MS, MALDI), Immunoassays, ELISAs, Western blots, 1 & 2 DE, Enzyme & Functional Assays

Species Agnostic

Not derived from immuno-affinity, all products work for all species, validated on human, mouse, rat, bovine, porcine, sheep, guinea pig

Knowledgebase of 1000+ Serum Proteins

Supports targeted & quantitative protein markers from serum/ plasma

Selection Criteria For Proteomic Sample Prep and Enrichment Products for LC-MS/MS

	Albumin & IgG Removal		Hemoglobin Removal		Lipid Removal
	AlbuVoid™ PLUS	AlbuSorb™ PLUS	HemoVoid™	HemogloBind™	Cleanascite™
Discovery LC-MS	Recommended for Serum	Recommended for Serum	Recommended for Red Cells, Whole Blood and Dried Blood Cards	Recommended for Red Cells, Whole Blood and Dried Blood Cards	Depletes Lipid associated proteins
Quantitative Targeted SRM/MRM	Recommended for Serum May be target(s) specific	Recommended for Serum May be target(s) specific	Recommended for Red Cells, Whole Blood and Dried Blood Cards May be target(s) specific	Recommended for Red Cells, Whole Blood and Dried Blood Cards May be target(s) specific	Depletes Lipid associated proteins
Innate Immune Response Scoring (requires Total Complement Enrichment)	Recommended				
Low Abundance Serum Proteome Enrichment (Complement depleted)		Recommended			
Bead-assisted Sample Prep (BASP™)	Recommended		Recommended		Suitable, but not yet validated

For Albumin & IgG Removal Kits, visit

https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

For Hemoglobin Removal, visit

https://www.biotechsupportgroup.com/Hemoglobin-Removal-s/312.htm

For Lipid Removal & Clarification, visit

https://www.biotechsupportgroup.com/Articles.asp?ID=456

Additional Resources

BSG ebook "Categorization of Blood Based Biomarkers – Unleashing the Power of Proteomics To Better Understand the Innate Immune Response to Infectious and Non-infectious Inflammatory Stimuli", April 17, 2020

Proteomics started with coverage annotation and Venn diagrams but now the field demands robust quantitation. This ebook describes our focus; how to address quantitative differences between proteins in blood samples representing a challenge or disease state, vs. samples representing a normal or control state. For this purpose, BSG's products and methods can help proteomic investigators explore all blood compartments. By adopting BSG's products to enrich potential protein biomarkers of low-abundance to mid-abundance, we can improve linearity between targeted peptide MS ion signals and protein abundances. Furthermore, as unresolved inflammatory stimuli can lead to chronic disease and pre-dispose individuals to severe acute responses, we describe new categorization strategies for selection of innate immunity biomarker proteins. Finally, we describe how past proteomic analysis based on antigen recognition can lead to egregiously misleading information on the status of protease regulation in the general blood circulation. The resulting convergence of proteomic technologies has made the task immediately available to understand the role of the innate immunity proteome within the pathogenesis of pandemic infections, cancer, and autoimmune disease.

Kuruc M, Zheng H, Sowerhardy A, Avadhani S, Roy D, et al. (2020) New Strategies to Categorize Blood for Proteomic Biomarker Discovery. Proteomics Bioinformatics, 2(2): 90-107.

Although much effort has gone into genomic sequencing to define disease, the downstream products of gene sequences-proteins, nevertheless remain the master regulators of biology. Many proteins are measurable in blood, making it a rich resource for biomarkers. Yet for reasons largely unrelated to analytical limitations, this resource remains largely untapped. In this review, we describe how chronic illness manifests itself in blood and how we might study innate immunity to understand mechanisms that can potentially translate into new biomarkers and therapeutic modalities. We draw upon our own knowledgebase of proteome information reportable after using depletion or enrichment products in LC-MS/MS workflows and how this knowledge can be utilized in new strategies for biomarker discovery from blood samples. We note that BSG's products have simply and efficiently reduced the complexity of the serum proteome allowing for cost-effective workflows, without the use of antibody-based depletion methods. Finally, we discuss how patterns of Serpins, a superfamily of protease inhibitors, may serve as a surrogate measure of the progressive stages of the innate immune systems' response to both infectious and non-infectious disease. This convergence of strategies and LC-MS/MS technologies has made the task immediately available to investigators to now develop the next generation of molecular tests for more precise and personalized treatment of patients.

This poster was presented at The Serpins2019 Conference, September 19-22, 2019 in Sevilla, Spain, and entitled "Loss of Functional Alpha-1-Antitrypsin and Heparin Cofactor II in Inflammation and Cancer". Authors were: Ingrid M. Verhamme, Vanderbilt University Medical Center; Nashville TN, Swapan Roy, Sowmya Avadhani, Matthew Kuruc, Biotech Support Group LLC, Monmouth Junction NJ.

The poster describes that in various cancer types, blood serum levels of Serpins have been reported as altered. Significantly however, is that in these reports, concentrations were measured by immunological methods (ELISA) rather than by functional activity. This can lead to egregiously misleading interpretation of a host's systemic response to cancer, as the immunological assay measures in aggregate both the intact and cleaved Serpin forms. In contrast to these methods, we demonstrate that with albumin depleted (AlbuVoid™) serum samples, combined with LC-MS/MS, there is potential of distinguishing between active and cleaved subpopulations of Serpins. Cleaved Serpins are the product of the substrate pathway in the bifurcated Serpin suicidal mechanism, which can become more pronounced due to Serpin mutations or changes in the micro-environment.

Zheng et al., "AlbuVoid™ Coupled to On-Bead Digestion - Tackling the Challenges of Serum Proteomics". J Proteomics Bioinform 2015, 8:9 DOI: 10.4172/0974-276X.1000373

Using 2 different allotted digestion times - 4 hours, and overnight, each with a singular 3 hour gradient LC-MS run, between 400-500 total proteins were observed for both human and rat sera, with mostly overlapping but also with distinct sub-populations observable at each digest time. These results support that the described methods gain efficiencies over antibody depletion and in-solution digestion workflows, for both discovery and quantitative serum proteomic applications.

Application Note entitled “AlbuVoid™ PLUS & AlbuSorb™ PLUS - Evaluating Different Windows of Observation Solves The Many Challenges of Serum Proteomics”
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/PLUS%20Application%20Report%2007212019%20v1.pdf

For serum, 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, and regeneration requirements which may result in a diminished and inconsistent performance. Because of these limitations, proteomic researchers need ways to enrich without immuno-affinity. This report considers the advantages of first reducing the influence of IgGs- a heterogeneous and proteolytically resistant class of proteins, along with Albumin depletion. Two products - AlbuVoid™ PLUS & AlbuSorb™ PLUS support depletion of both Albumin and IgG, through different strategies and workflows. Using LC-MS reporting metrics, the report highlights the serum sub-proteome bias characteristics of these products. Some examples of their selective utility for biomarker discovery in cancer are also presented.

Whitepaper entitled “Stroma Liquid Biopsy™ - Blood-based biomarkers to monitor stromal conditioning in cancer.” Published February, 2019.
http://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/StromaLiquidBiopsyWhitepaper022519.pdf

The whitepaper describes that tumors are more than simply a collection of immortalized cells as the supporting microenvironments or stroma also contributes to pathogenesis. Because of this, tumor characterization cannot be fully characterized solely through the analyses of the tumor cell genome – the current emphasis of liquid biopsy platforms. So because tumors are more than just a mass of proliferating cells, cancer progression must take into consideration the influence of the multiple cell types and networks of host response proteins dynamically interacting in active tumorigenesis. These are not simply passive bystanders. The unique significance of the Stroma Liquid Biopsy™ pan-cancer profile is that dysregulation in blood was categorically intertwined with the most rudimentary needs of cancer: space, nutrients and immune evasion. Moreover, the changes within the 13 biomarker panel all occur within an interdependent network of cascading proteolytic events. Because proteolysis is irreversible, all species of life have evolved molecular regulatory systems to control aberrancies. The most distinguished is a protease inhibitory family of regulators known as SERPINs.

Chapter from Book: Functional Proteomics – Methods and Protocols, publisher Springer 2018. “Methods to Monitor the Functional Subproteomes of SERPIN Protease Inhibitors”.

https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/FunctionalProteomicsBookSERPINChapter.pdf

Conformational variants of the unique family of protease inhibitors annotated as SERPINs are most often under-represented in proteomic analyses. This limits understanding the complex regulation that this family of proteins presents to the networks within the protease web of interactions. Using bead-based separation provided by a family of proteomic enrichment products—notably AlbuVoid™ and AlbuSorb™, we demonstrate their utility to satisfy investigations of serum SERPINs. We also suggest their use to develop functional profiles of the SERPIN proteoforms, and how those can establish relationships to disease phenotypes, gene mutations, and dysregulated mechanisms.

About Biotech Support Group LLC
Converging with cultural and technological disruptions forthcoming in healthcare, Biotech Support Group develops methods for cost effective and efficient sample prep essential for these expanding markets. Following a tiered business strategy, the company continues its growth in the consumable research products area supporting the rapidly expanding installation of LC-MS instrument and computational infrastructure. From these innovations, the company has acquired knowledgebase and biomarker intellectual property assets that support discoveries of protein markers from blood. For business development, commercial and research partnerships and collaborations, contact: Matt Kuruc 732-274-2866, mkuruc@biotechsupportgroup.com

www.biotechsupportgroup.com