Proliant BIOLOGICALS

How Proliant's "Closed Loop" Has Revolutionized BSA Manufacturing

2425 SE Oak Tree Court | Ankeny, IA | 50021 | 515.480.3206 | www.proliantbiolocials.com

Introduction

Proliant Biologicals has emerged as the world leader in Bovine Serum Albumin (BSA) production as a result of unprecedented process control, from raw material collection through processing, drying and packaging. Proliant controls every aspect of BSA production to produce the world's most consistent and reliable Bovine Albumin. Our high-quality albumins are also a product of our thorough understanding of albumin chemistry and the history of its manufacturing.

From the beginning, we sought to build on the successful efforts of researchers and predecessors in albumin production, while improving upon outdated methodology and equipment. A brief history of albumin chemistry, isolation, uses and manufacturing history provides an overview of why Proliant produces the superior BSA of choice for a variety of applications.

I. History of *Albumin* Manufacturing

Throughout much of the 20th Century and beyond, human blood products have provided important life-saving medical treatments. But these highly perishable biologicals were sometimes in short supply. During World War II, there was an urgent need existed for material that could serve the life-saving functions of blood plasma, the liquid portion of blood. It would, however, need to be easier and more practical to store, inventory, transport and administer.

Figure A: Original Cohn Ethanol Fractionation

Fraction	Major Products	Supernate Condition
Plasma		8% EtOH, pH 7, -3° C
Fraction I Precipitate	Fibrinogen	25% EtOH, pH 7, -5° C
Fraction II & III Precipitate	IgG, Thrombin, Plasminogen	18% EtOH, pH 5.2, -5° C
Fraction IV-4 Precipitate	Transferrin	25% EtOH, pH 4.8, -5° C
Fraction V Precipitate	Albumin	

Methods were sought to divide, or "fraction," the blood plasma into its active components. The goal was to find stable substitutes for whole plasma in trauma situations. Dr. Edwin J. Cohn, a researcher at Harvard University, developed the "fractionation" procedure in the 1940s that now bears his name.

Dr. Cohn determined that different proteins in the blood could be separated from each other by using different temperatures and biochemical conditions and by the use of solvents like ethanol. His method used these biochemical techniques to separate the blood plasma into five fractions. The fifth fraction was albumin, the most abundant protein in the blood, which is why albumin is often referred to as "Fraction V." (See Figure A).

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Albumin was an excellent substitute for human plasma. When administered to wounded soldiers or other patients with blood loss, it helped expand the volume of blood and led to speedier recovery. Cohn's method was also gentle enough to retain the protein's biological activity.

BSA
fraction V
Bovine Serum Albumin

Recognizing the importance of this discovery, the government immediately "asked" the major pharmaceutical companies of the time to scale-up and commercialize Dr. Cohn's fractionation process.

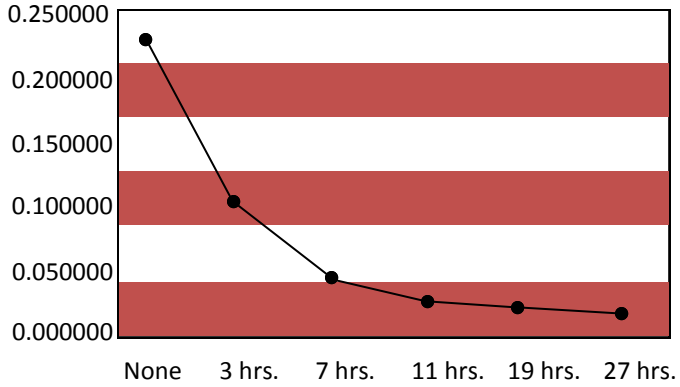
The often misunderstood terms "Fraction V" and "Cohn", as applied to albumin have their origins in this early development of albumin fractionation. This is why vendors of BSA may continue to refer to their method as "Cohn Fractionation", and their products as "Fraction V" -even through current manufacturing processes differ from the original multi-step process.

II. Defining & Overcoming *Limitations*

Later, there were many modifications and improvements to fractionation methods, and fractionation was also applied to the production of animal albumin. Additional changes came about as researchers found new applications for bovine albumin in diagnostics, cell culture, microbial culture and biochemical research.

Many of the new methods used heat, rather than ethanol as the main fractionation technique. Others used acetone or salts, such as ammonium sulfate, to separate the albumin from the other plasma components. Still other methods evolved to produce albumin on a small scale through column chromatography.

Figure B: BSA Residual Colorimetric Enzyme Activity: Response to Heat Treatment



For decades, ethanol and heat-fractionated BSA products have been widely accepted. But the reasons for sometimes inconsistent performance had been little studied or understood.

The manufacturing plants and equipment had never been modernized. Many fractionated BSA products are made by companies that inherited the original Cohn process and had little changed their procedures for 50 years. Compared to modern biochemical production, there were many lingering inefficient or inconsistent practices built into their procedures that undermined product quality and consistency.

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Moreover, older versions of fractionation are dangerous and environmentally unfriendly (using explosive, highly controlled organic solvents), and unsanitary (typically using open tanks and filter presses that increase the possibility of contamination). They may also be too gentle to inactivate enzymes (See Figure B) and kill certain kinds of viruses with heat.

Without heating, the BSA is sometimes contaminated with immunoglobulins. These can cause regulatory problems in cell culture-based pharmaceutical production and interfere with diagnostic assays.

III. Emergence of *Modern* BSA Manufacturing

Changes were finally driven by industry upheaval, as well as by basic research in albumin and fractionation chemistry. One of these changes was a shakeout among BSA manufacturers resulting from problems in the human blood fractionation industry.

Production of human blood plasma products came under increased attention from the Food and Drug Administration (FDA) in the 1980's with the incidents of HIV-transmission by human plasma and clotting factors. In August of 1996, an adverse event involving patient bacterial infection was traced to therapeutic human albumin manufactured by Centeon in Kankakee, Illinois.

The result was an even higher level of scrutiny of all human plasma fractionators. For Centeon, production was shut down, product recalls ensued and manufacturing only resumed many months later under a consent decree.

Little noticed outside the industry, Centeon was also an important manufacturing location for BSA produced for their former biochemical division (Armour Biochemicals), then called Intergen Company. Centeon's BSA production for Intergen was caught for a time in the middle of the plant shut down. Soon there was a worldwide shortage of BSA and a doubling in market price. The shortage continued when the industry learned that Centeon would cut back its bovine operations and eventually eliminating production of BSA in its Kankakee, Illinois facilities.

Until the Centeon shutdown, BSA manufacturing had been dominated Intergen and one other company, Pentex companies, Intergen and Pentex, owned by Bayer. Both were original offshoots of Armour Pharmaceuticals in Kankakee, one of the original Cohn collaborators. These shutdowns would prove to be costly to Intergen and disruptive to the BSA industry. Intergen lost credibility and market share.

Eventually both Intergen and Pentex were acquired by Serologicals and then by Millipore. Reduced BSA manufacturing continued after consolidation and closing several plants.

As manufacturers struggled to keep up with demand, the field was open for Proliant (known at the time as AMPC) to enter the market anew, unencumbered by existing manufacturing plants and 1950's-era production schemes. Proliant was and is the world's largest collector of animal blood products and producer of animal-derived proteins.

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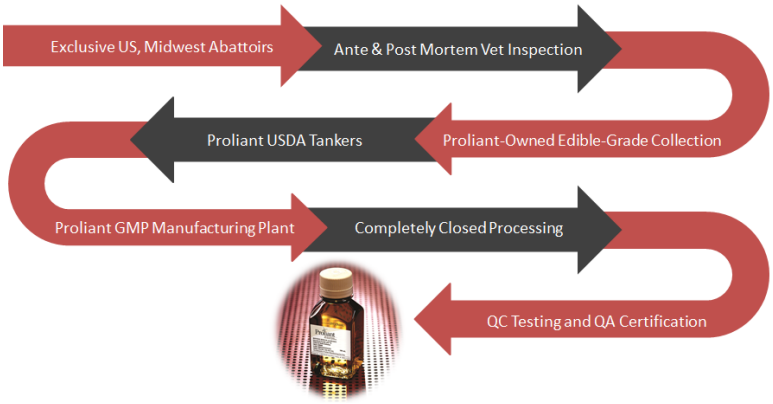
III. Con't.

The extension of the product line to BSA was a natural fit for AMPC/Proliant. For the first time, albumin processes could be developed from the ground up to specifically address inherent needs of the diagnostic and other non-therapeutic industries. A more consistent, reproducible and analyte-free product was required that would still be economical.

Proliant had begun research in the early 1990s on more economical, efficient and regulatory-friendly methods of producing a higher-purity BSA. Simultaneously with these developments, companies that had been forced by the shortage to re-evaluate and re-qualify their BSA vendors began applying some serious science to the question of albumin performance. In the past, there was often very little knowledge of what made one BSA vendor or lot superior to another.

Wide variations between vendors and among individual lots from the same vendor were apparent. Some material seemed to perform well in certain assays, and fail miserably in others. A flurry of applications research followed which identified some key properties of commercial albumin that were causing difficulties. Companies began to look for new manufacturers that had the flexibility to address these problems.



Among these common issues were residual enzymatic activity, protease-related degradation, the interaction between calcium and phosphate which were causing precipitation problems, residual IgG, and low molecular-weight contaminants.

With the BSA shortage in full swing, Proliant rolled out BSA products based on core fractionation technology but with many new manufacturing twists. Although we relied on the tried-and-true heat-shock manufacturing process, we avoided the open vats and manual filter presses that were common in historical animal product manufacturing.

These were replaced by a closed, computer-facilitated system with continuous-flow in-line centrifuges and modern filtration skids using the latest membrane technology. We also introduced several proprietary steps designed to efficiently pull albumin and/or its bound analytes out of the purification stream.

Complete control over raw material collection, Statistical Process Control, and large-scale continuous flow separation were some of the unique innovations we have introduced to bovine albumin manufacturing. These and other methods have allowed us to produce albumin with an unprecedented level of consistency. For example, albumin has historically been sold in many different grades, which reflects the pass/fail status of certain analytical requirements.

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In contrast, every lot of Proliant BSA would be engineered to be low protease, low endotoxin, and essentially fatty-acid and IgG free, in conformance to six Sigma statistical methods for determining compliance to specifications.

Gaining control over raw material was critical to this consistency. To minimize variability in an inherently heterogeneous biological material like plasma, Proliant introduced complete ownership and traceability of raw material and its collection. We minimized and standardized the time from collection to processing, and established a rapid, just-in-time manufacturing process in a closed, chilled environment. The result was an albumin with reproducibly low bioburden and endotoxin levels.

Not only is this an excellent overall measurement of biological production control, it is an important step in reducing the presence of bacterial enzymes. The final product also had outstanding solubility and filterability characteristics.

IV. Proliant *Closes* the *Loop*

Proliant Biologicals Dedicated Lyophilization and Packaging Clean Room

In early 2008, Proliant launched a new lyophilization and packaging suite. This completely "closed the loop" of our BSA manufacturing. Now, from raw material collection, transport, environmentally-closed fractionation though drying and packaging, one company finally has complete control over all aspects of production to ensure the highest level of consistency.

Again, not content with industry standards, Proliant sought to improve on the prevailing open-tray bulk lyophilization concept that was more characteristic of food manufacturing than critical biologicals. The new facility is a high-tech, dedicated drying and packaging suite for isolation of all BSA products. It incorporates many process innovations and includes one of the largest and most efficient biological lyophilizers (freeze-driers) in the world.

The innovations introduced by Proliant include a sterile bag-in-tray system and fully dedicated stainless-steel chamber that ensures the product remains contaminant-free during the drying cycle. The chamber is unloaded into an integrated, HEPA-filtered and monitored dry clean room for sifting and packaging.

Proliant's Lyophilization Chamber.

This process is a head-and-shoulders above industry standards for animal-derived products, where ancient cast-iron jet driers, food-grade contract toll driers and open-tray systems have predominated. Such conditions can lead to high bioburden or introduce particulate contamination that affects the safety, consistency and solubility/filterability of the product.

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V. Summary

Proliant BSA is available in both powder and solution form in a variety of package sizes (see last page for details)

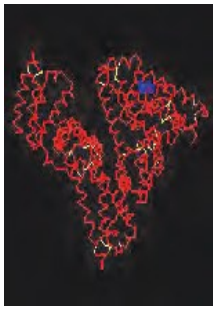
Proliant took a fresh approach to BSA production and perfected numerous manufacturing innovations that ensured the quality and consistency of BSA. Perhaps most critical was the development of "Closed Loop" production that ensured complete control over the entire purification process.

Today, Proliant is the unquestioned world leader in the production of quality BSA. Proliant's exclusive "Closed Loop" ensures the highest level of BSA consistency every time. We control everything from raw material collection and transport to environmentally-closed manufacturing. Plus, we've added dedicated lyophilization and packaging for absolute isolation of our products.

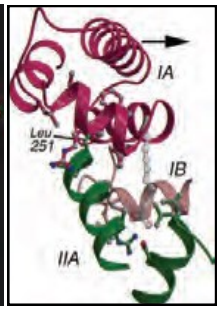
And, we're the only supplier that guarantees that every lot of every product is virtually free of protease and IgG, and low in endotoxin and bioburden without the need for a confusing array of "specialty" grades. It is no wonder we now provide over 50% of BSA products used in the world.

If it's from Proliant, you can be confident of getting the best in BSA.

Appendix of *Albumin* Technical Information



Primary Structure of BSA



HAS FA Ligand Binding

Albumin is the most abundant protein in blood plasma, circulating at a level of 35-50g/L. The protein is very similar across a variety of species of mammals. Properties of bovine albumin are nearly identical to that of human albumin, which has been studied extensively. Albumin is sometimes called the "Jack of All Trades" of proteins because it has so many functions.

The molecule operates as a transport protein, a detoxifier, a nutrient, and maintains osmotic balance. It is these many roles that make albumin so often used in laboratory reagents and medical diagnostics, particularly those involving blood chemistry and immunological reactions; as well as in cell, microbial and tissue culture.

Bovine Albumin has a molecular weight of 66kDa and contains 17 disulfide bonds that confer flexibility and stability to the molecule. A single free sulfhydryl group can result in disulfide exchange, so some albumin naturally exists in a dimeric or polymeric state.

Albumin is highly resistant to change in pH, and although will unfold at pH extremes, the molecule will spontaneously re-fold when returned to neutral pH. Although albumin can be heat-denatured at temperatures above 50 degrees C, the molecule can be stabilized by bound ligands and heated in excess of 60 degrees C for greater than 12 hours without damage.

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The ligand binding properties and sites of binding for albumin are well characterized. Albumin binds three moles of fatty acid per mole with high affinity, especially in the mid carbon chain lengths. Other less specific binding sites for fatty acids of up to 5 moles are present on the molecule. Constant association and disassociation of fatty acids allows albumin to function as a carrier of fatty acids to cells.

Other ligand binding sites exist for trace metals and ions, small hormones, drugs and metabolites. Strong affinity to such ligands can prove challenging in attempts to produce a high purity albumin fraction commercially.

Proliant *Bovine Serum Albumin*

Lyophilized Powder

68100 Standard Grade, pH 7.0

68300 Cohn Analog (Microbiological) Culture Grade

68500 Standard Grade, pH 5.2

68700 Reagent Grade, Fatty-Acid Free (< 0.01% NEFA)

All products are protease free, low endotoxin, low bioburden (micro) and virtually IgG-free to the ng range.

Available from 1 kg to 10kg bulk.

Solutions

Bovine Serum Albumin 22% and 30%

And custom manufactured for a variety of specifications and package sizes.

For Additional Information, *Contact*

Proliant Biologicals

2425 SE Oak Tree Court

Ankeny, Iowa 50021-7102 USA

Phone: 515-433-4740

Fax: 515-289-4369

CS.biologicals@proliantinc.com

www.proliantbiologicls.com

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