How Clean Is Your BSA?

Bovine serum albumin (BSA) is a critical component of many biotechnology and biochemistry systems. BSA is used in a variety of applications, including diagnostics, veterinary medical products, vaccine manufacturing, mammalian cell growth for biopharmaceutical production, and topical and ex vivo medical device applications. The impact of BSA on the performance of these systems is often overlooked and is identified as a possible root cause of variability only after extensive testing and elimination of other system components.

There are many different functions of BSA within the spectrum of biopharmaceutical applications. Diagnostic tests for both animal and human health industries use BSA as a protein carrier to stabilize low-abundance high-value proteins (e.g., monoclonal antibodies). BSA is used as an assay standard given the reproducible properties of a pure protein fraction and as a blocking agent to prevent nonspecific interactions with the functional components of the assay. BSA also is used as an inert surface coating to prevent nonspecific interactions on diagnostic devices and used extensively in enzyme-linked immunosorbent assays (ELISAs) (1, 2). For example, BSA coating the surface of a solid nanoparticle will improve circulating half-life by preventing opsonization and clearance through the renal system (3, 4). Finally, BSA is also a critical component in defined media formulations for the production of biopharmaceutical compounds. BSA supports the growth of both mammalian and bacterial organisms through the binding and transport of key nutrients, growth factors, and hormones as well as the binding of toxic compounds (e.g., excess iron) (5). BSA plays a role in those applications, and consistent BSA product ensures reproducible diagnostic assay performance and cell growth.

Endotoxins, proteases, immunoglobulins, and other plasma proteins can contaminate BSA and affect consistent performance in downstream biotechnology uses. Immunoglobulin and protease contamination can lead to variability in diagnostic performance and sensitivity, and endotoxin contamination can affect mammalian cell growth, protein production, and in vitro cell-based assay performance.

Objective

The purpose of this white paper is to present data comparing differences in endotoxin contamination of BSA from a number of manufacturers to highlight differences in manufacturing consistency and contaminant levels.

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Table 1: Standard grade — Fraction V BSA, pH 7.0

	Endotoxin Contamination (EU/mg)	Samples Tested
Proliant Biologicals standard 7.0	0.01 ± 0.02	3
Manufacturer A	12.7 ± 10.6	4
Manufacturer B	25.9	1
Manufacturer C	25.8 ± 26.6	6
Manufacturer D	0.71	1

Table 2: Reagent grade — fatty acid–free BSA

	Endotoxin Contamination (EU/mg)	Samples Tested
Proliant Biologicals reagent grade	0.008 ± 0.002	3
Manufacturer A	1.0 ± 1.2	3
Manufacturer B	0.2	1
Manufacturer C	1.6 ± 1.9	3
Manufacturer D	0.27	1

Endotoxin (lipopolysaccharide, LPS) is the primary component of the outer cell membrane of most gram-negative bacteria. Bacteria release varying amounts of LPS throughout their normal cell lifecycle. During normal proliferation, small amounts of LPS are released as the cells divide. However, bacteria disintegration following death or injury have the potential to release substantial amounts of LPS into the surrounding environment. Endotoxin contamination of final products is of primary concern for medical device and injectable drug manufacturers because it can induce pyrogenic

Table 3: Outline of the quality systems and characteristics of different BSA manufacturers

Traits	Proliant Biologicals	Manufacturer A	Manufacturer B	Manufacturer C	Manufacturer D
Manufacturer	X	X	X	X	X
Dedicated manufacturing facility	X				
"Closed Loop"	X				
ISIA traceability certified*	X				
Edible-grade products	X				
US and New Zealand Manufactured	X				

* Proliant is the only BSA manufacturer to receive an International Serum Industry Association (ISIA) Traceability certification

responses that include fever, chills, and septic shock (6). When considering any biotechnology application for BSA, it is important to limit the introduction of endotoxin to the process, rather than rely on removal of endotoxin as a final production step.

Methods for Endotoxin Quantification

BSA samples from various manufacturers were submitted for endotoxin testing at Associates of Cape Cod Inc. using the kinetic turbidimetric test method. The *Limulus* amebocyte lysate (LAL) test is performed by addition and mixing of the Pyrotell-T LAL reagent with samples prepared at various dilutions. The mixture is incubated at 37 °C. Samples with higher endotoxin concentrations will develop turbidity faster. Endotoxin is quantified in samples by comparison with a standard curve. The limit of detection for the assays used in this study was 0.001 EU/mg.

Test results quantifying endotoxin contamination in major manufacturers' standard grade BSA and reagent grade BSA (fatty-acid–free BSA) are shown in Table 1 and Table 2, respectively. Where multiple samples are analyzed, results are listed as average plus or minus one standard deviation. BSA manufacturers are indicated with letters A–D. Manufacturers are consistently labeled in both tables.

Results and Discussion

Endotoxin contamination has the potential to significantly affect the usefulness and consistency of BSA in biopharmaceutical applications, and particularly in cell-based applications. Results of this analysis show a wide range of endotoxin contamination in BSA products with varying results depending on manufacturer and grade of BSA (standard grade and reagent grade).

The first noticeable trend is the difference between standard grade and reagent grade BSA products. Across major manufacturers of BSA, the trend is for reagent grade BSA to have 10–100× less endotoxin contamination than standard grade BSA from the same manufacturer. It is not entirely surprising that reagent grade BSA has less endotoxin contamination than standard grade because of the additional processing involved in manufacturing of the fatty-acid–free BSA. The industry standard for the manufacturing of reagent grade BSA is exposure of a final BSA solution to activated carbon for extended periods of time. The activated carbon scavenges fatty acids bound to BSA with the potential to remove up to 99% of the endogenous fatty acids (7). The same activated carbon responsible for binding fatty acids also binds endotoxin (8). Therefore, the processing step using activated carbon in the production of reagent grade BSA aides in removal of endotoxin, resulting in a "cleaner" product as evidenced by results in Table 1 and Table 2.

Equally noticeable is the disparity in endotoxin contamination among manufacturers of either the standard grade or reagent grade BSA. Endotoxin levels in standard grade BSA range from ultralow 0.010 EU/mg (Proliant Biologicals) to contaminant levels that are 2,500× higher from other manufacturers. Similarly, reagent grade BSA manufactured by Proliant Biologicals contains only trace amounts of endotoxin (0.008 EU/mg), whereas reagent grade BSA from other manufacturers can be up to 200× higher. Endotoxin contamination can be extremely harmful in many different biopharmaceutical applications, making it critical to identify potential sources and develop strategies to mitigate endotoxin inclusion. Proliant Biologicals's manufacturing process yields BSA that is nearly endotoxin free and orders of magnitude cleaner than BSA produced by all other manufacturers.

Conclusion

BSA is a critical component to many different biotechnology applications, from supporting cell growth in biopharmaceutical production to controlling nonspecific protein interactions in various diagnostic assays. Although the role of BSA in biological products often goes unnoticed, BSA could be a major source of endotoxin. Endotoxin contamination has the potential to compromise the performance of cell-based therapeutic production and negatively affect assay reproducibility and consistency over time. Results of this study demonstrate that standard and reagent grade BSA produced by Proliant Biologicals contains at least 25× less endotoxin than its nearest competitor. Proliant's unique plasma collection methods, closed-loop processing, and robust quality systems enable the consistent production of BSA with only the trace contaminant levels as detailed in this report.

About Proliant Biologicals

With a focus on the life science industries, Proliant Biologicals manufactures high-purity plasma fractionations and animal extracts. Core markets include, but are not limited to in vitro

Table 4: Product listing

Product Description	Catalog Number
Standard grade pH 7.0	68100
Standard grade pH 5.2	68500
Cohn analog grade	68300
Reagent grade (fatty-acid free)	68700
New Zealand–sourced standard grade pH 7.0	69100
New Zealand-sourced reagent grade (fatty-acid) free	Coming soon

diagnostics (human and animal), vaccine, biopharmaceutical and medical device manufacturing, and the nutraceutical and life science research industries.

Since 2000, Proliant Biologicals has established the highest standards in the industry through innovation and continuous improvement in quality (traceability of raw material and final product consistency). Proliant has established the industry's only "closed loop" process that is vertically integrated from collection all the way through transport and production. Proliant is also the only BSA manufacturer with two dedicated facilities to serve all regions of the globe (Boone, Iowa, and Fielding, New Zealand).

Quality SysteMs

Proliant's "closed loop" process is designed to mitigate the introduction of any contaminants to the final BSA product during raw material collection, transport of plasma, or the manufacturing process. The bovine plasma used in the production of Proliant BSA is USDA certified "edible grade" and collected and transported in dedicated tankers under Proliant control. The Proliant manufacturing operations are CGMP compliant, and the facilities are registered with the US FDA (Boone, Iowa) and New Zealand Ministry for Primary Industries (MPI). Due to the strong level of control over collection and processing, the Proliant US facility has been granted a TSE Certificate of Suitability to the Monographs of the *European Pharmacopoeia*. Proliant BSA is USDA/APHIS inspected for EU regulations allowing export to Europe.

References

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