How Clean Is Your BSA: A Comparison of Endotoxin Contamination Across Manufacturers, Grades, and Lots of Bovine Serum Albumin

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Introduction

Bovine serum albumin (BSA) is a crucial component in many biopharma and biotechnology applications. The primary characteristics – structure, size, purity, and protein content – are the same across different manufacturers and grades of BSA. However, trace contaminants such as endotoxins, proteases, metals and immunoglobulins vary depending on lot, grade and manufacturer of BSA. Lot-to-lot differences in contaminants can cause variable performance in final applications such as diagnostics, vaccine manufacturing, and mammalian cell growth for biopharmaceutical manufacturing[1,2]. For this reason, it is recommended to assess lot-to-lot contaminant variability when evaluating BSA in a final application. The purpose of this study is to evaluate the consistency of BSA across multiple manufacturers as measured by residual endotoxin quantification and its effect on mammalian cell phenotype using expression of the inflammatory cytokine IL-8 in THP-1 cells.

Endotoxin contamination is of concern when BSA is included in cell culture media due to its ability to impact mammalian cell phenotypes and protein expression patterns[1,2]. Downstream purification to remove endotoxin contamination in biopharmaceuticals can be challenging, but is critical due to health risks associated with human exposure. To mitigate the potential impact of endotoxin contamination, the end user should consider differences in lot, grade and manufacturer of BSA due to the potential negative impact on mammalian cell based applications and downstream processing. The most common grades of BSA are Standard Grade pH 7.0 and Reagent Grade Fatty Acid Free. These grades were compared across samples from five manufacturers for both endotoxin content and its subsequent effect on production of the pro-inflammatory cytokine IL-8 in THP-1 cell cultures.

Methods and Materials

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, at 37 °C, with samples prepared at various dilutions. 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.

THP-1 monocytes were seeded at $3x10^5$ cells/ml in RPMI-1640/Glutamax, 10% FBS with penicillin and streptomycin at 1 ml per well in 24-well plates at 37°C and 5% CO_2. After 24 h the THP-1 cells were treated with 250 µl of BSA, prepared as 5% solids solutions, for a final concentration of 1% BSA. Cell supernatants were harvested 24 h after BSA treatment. IL-8 response was quantified in the supernatants using an ELISA for Human IL-8 (R&D Systems DY20805).

Table 1. Standard Grade – Fraction V BSA pH 7.0.

	Endotoxin Contamination (EU/mg)	Samples Tested
Proliant Biologicals	0.01 ± 0.02	3
Manufacturer A	12.07 ± 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	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

References

1. Epstein, J., et al. Effect of E. coli endotoxin on mammalian cell growth and recombinant protein production. In Viro Cell. Dev. Biol. 26:1121-1122; 1990
2. Schwarz, H., et al. Residual endotoxin contaminations in recombinant proteins are sufficient to activate human $CD1c^+$ dendritic cells. PLoS ONE 9(12):e113840; 2014

Results

- Endotoxin residuals in Standard grade BSA were up to 100-fold higher than in Reagent grade BSA from the same manufacturer.
  - The exceptions being Proliant and Manufacturer D where there was only a 1.25 and 2.6-fold difference between BSA grades, respectively.
- Endotoxin residuals varied dramatically between manufacturers
  - 0.002-58.1 EU/mg for standard grade BSA – 29,000-fold difference
  - 0.005-3.8 EU/mg for reagent grade BSA – 760-fold difference
- Endotoxin content of a particular grade of BSA varied across production lots from the same manufacturer up to 170-fold
- THP-1 cells treated with 1% BSA produced a strong does-response correlation between IL-8 response and BSA endotoxin residuals, $R^2 = 0.78$
- BSA samples with endotoxin less than `produced IL-8 responses below the limit of quantitation, 15 pg/ml, but above the limit of detection

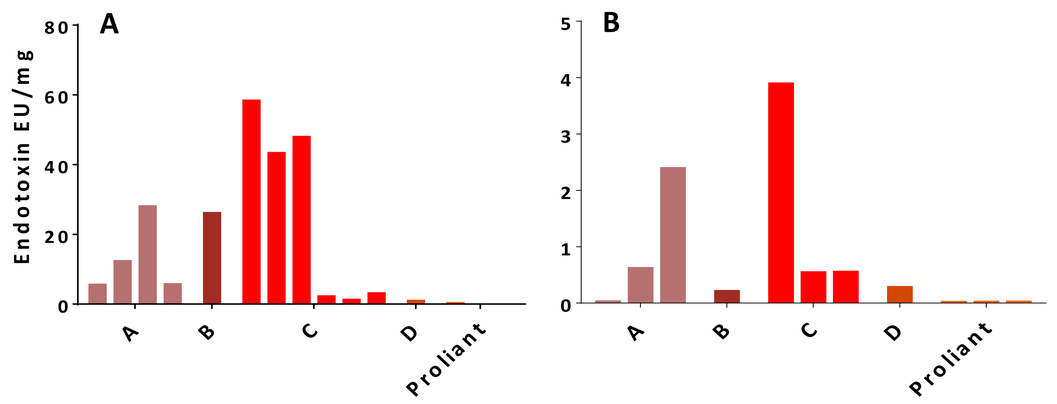

Figure 1. Endotoxin contamination versus manufacturer and lot. A) Standard Grade – Fraction V BSA pH 7.0. B) Reagent Grade – Fatty Acid Free BSA.

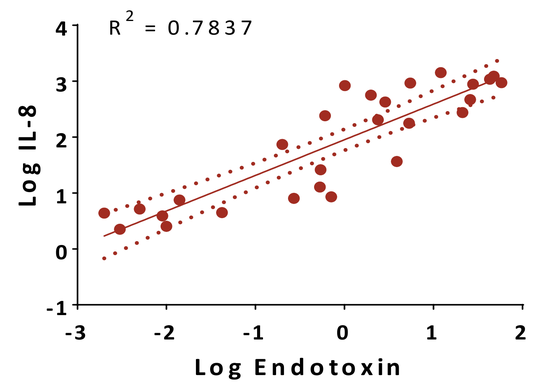

Figure 2. Relationship between endotoxin contamination in BSA and pro-inflammatory IL-8 response of THP-1 cells.

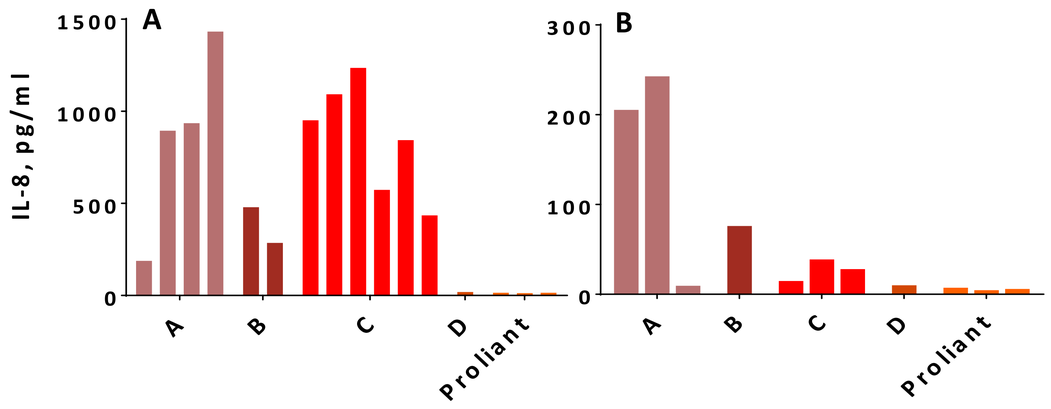

Figure 3. Pro-inflammatory IL-8 response of THP-1 cells treated with 1% BSA versus manufacturer and lot. A) Standard Grade – Fraction V BSA pH 7.0. B) Reagent Grade – Fatty Acid Free BSA.

Discussion & Conclusions

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. While the role of BSA in these products often goes unnoticed, BSA could be a major source of endotoxin. Endotoxin contamination has the potential to impact the performance of cell based therapeutic production and negatively impact assay reproducibility and consistency over time[1,2].

This study showed endotoxin contamination can vary widely across different grades of BSA, different lots from a single manufacturer and between manufacturer. The levels of endotoxin contamination were significant enough for immune reactive human cells to produce IL-8 responses ranging from below the limit of quantitation to saturation, over a 100-fold difference. To mitigate risk and variability in final applications end-users of BSA should evaluate manufacturers, grades, and lots of BSA for endotoxin contamination during product development.

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