# Variables That Influence Bovine Serum Albumin Color and Their Importance To Diagnostic Applications #B-285

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## Introduction

- Bovine Serum Albumin (BSA) is a common stabilizer & blocker used in diagnostic applications whose appearance has been known to vary by grade, lot, and manufacturers[1].
- BSA color may range from colorless, yellow, green, or amber, Fig. 1.
- Both chemical and physical properties can influence color.
- Particle size is known to influence reflectivity and BSA particle sizes range from µm to mm.
- Albumin is known to bind chromogenic molecules such as bilirubin[2] (a yellow pigment) and biliverdin (a green pigment), byproducts of heme degradation.

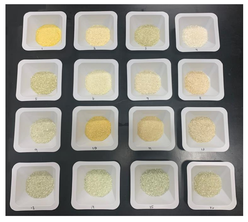

**Figure 1.** A display of 16 different lots of BSA.

- The objective of this work is to quantify BSA color and determine the effect of particle size, and heme degradation products on BSA color.

## Materials & Methods

**BSA Samples**

- BSA powders were obtained from Proliant Health and Biologicals (#68100, 68700, 69100, 69700, and 69760), Bovogen Biologicals (BSAM0.1), Sigma-Aldrich (#A1493), and Life Technologies (11020-021). Solutions were prepared to 20% w/v in DI water.

**BSA Color Measurements**

- CIE L*a*b* is a three-dimensional color space designed to match the human perception of color, Fig. 2[3].
- Color measurements on lyophilized powder (n=107) were made using a Hunter Labs Miniscan XE Plus. Data output in Hunter Labs was converted to CIE L*a*b* values[4].

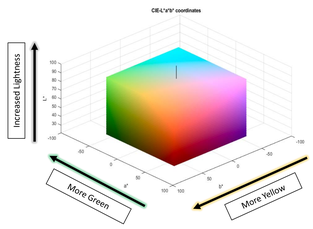


**Figure 2.** CIE L* a* b* 3D color space.

**Biliverdin Analysis**

- Biliverdin hydrochloride (Frontier Scientific #B655-9) stock solution was prepared in the dark in 100% DMSO to 1000 mg/L and serially diluted in DI water.
- A reagent blank containing 10 µl DI water, 10 µl buffer, 1 µl 100 µmol/L NADPH was used to zero the instrument at 450 nm. After blanking, the initial absorbance of the biliverdin standard or sample was read. The reaction was catalyzed by adding 10 µl of biliverdin reductase (Enzo Life Sciences #ADI-OSP-450-D). Change in absorbance was plotted and a best fit curve was fit using GraphPad Prism. Biliverdin concentrations were calculated from the standard curve.

**Bilirubin Analysis**

- Bilirubin was quantified using the ABX Pentra Total Bilirubin Assay (Horiba #1220001639).

## Materials & Methods

**Particle Size**

- Three different grades of BSA were sieved with three different mesh sizes (16, 30, and 45) to investigate how particle size affects color when chemical variance is constant.

## Results

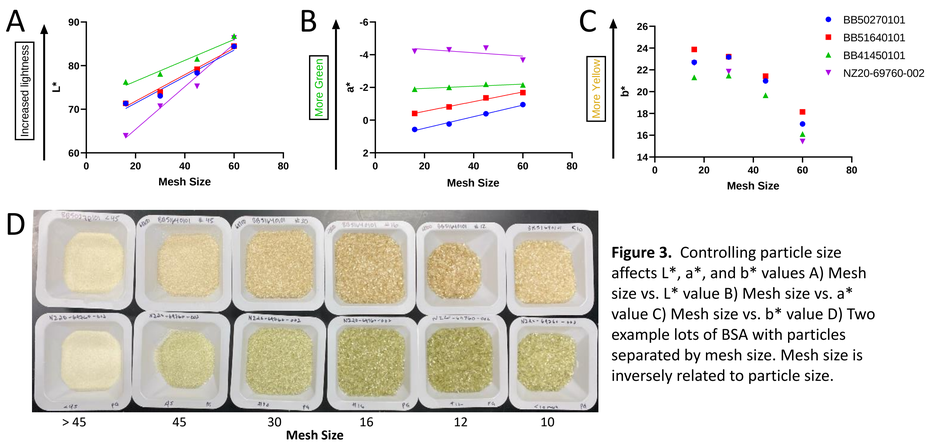


**Figure 3.** Controlling particle size affects L*, a*, and b* values A) Mesh size vs. L* value B) Mesh size vs. a* value C) Mesh size vs. b* value D) Two example lots of BSA with particles separated by mesh size. Mesh size is inversely related to particle size.

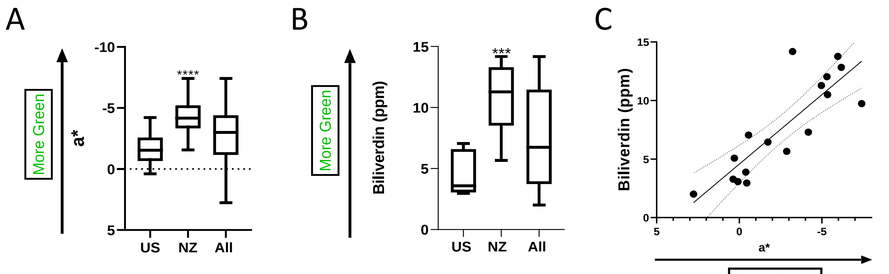


**Figure 4.** The color green, BSA, and Biliverdin A) a* values for BSA lots from different manufacturers B) biliverdin analysis of BSA samples from different manufacturers C) a* value vs. biliverdin concentration, dotted line represents 95% CI ($R^2$ = 0.7146). Box and whisker plots display max and min and *** indicates p<0.001 ****p<0.0001. US=Proliant USA origin, NZ=Proliant New Zealand origin, All=Proliant and BSAs from other manufacturers, ppm = Biliverdin (ug) : BSA (g).

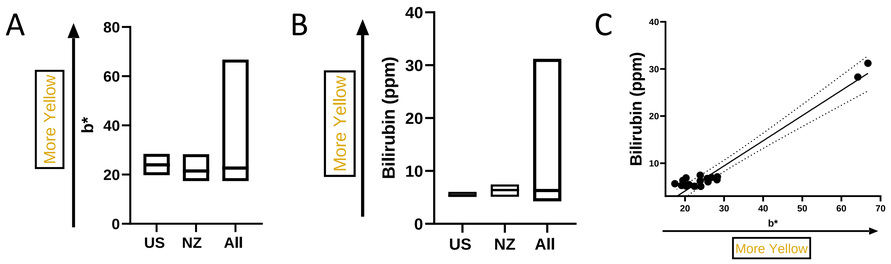


**Figure 5.** The color yellow, BSA, and Bilirubin A) b* values for BSA lots from different manufacturers B) bilirubin analysis of BSA samples from different manufacturers C) b* values vs. bilirubin concentration, dotted line represents 95% CI ($R^2$ = 0.8952). US=Proliant USA origin NZ=Proliant Zealand origin All = Proliant and BSAs from other manufacturers, ppm = Bilirubin (ug) : BSA (g).

## Results

- L* values significantly increase with decreased particle size for each BSA lot tested (p<0.05), Fig. 3A.
- Decreasing particle size can significantly influence a* values (to more green) for some lots of BSA – 2 out of 4 lots had slopes significantly different from zero, Fig. 3B.
- BSA lots trend to decrease in b* values (to less yellow) when particle size decreases Fig. 3C.
- An amber lot of BSA and a green lot of BSA look the same visually when particles < 45 mesh are compared to each other Fig. 3D.
- Green hues of BSA vary greatly across manufacturers, origin, and lots.
- Proliant USA and NZ origin BSAs have similar lot to lot variability and despite 50% overlap in a* values, NZ lots are greener on average (p<0.0001), Fig 4A.
- Proliant NZ origin lots contain significantly more biliverdin than US origin lots (p<0.001), Fig 4B.
- Differences in green color correlates strongly with biliverdin content of the BSA, Fig 4C.
- **The differences in green color between US and NZ Proliant lots is caused by < 10.0 ppm differences in biliverdin residuals.**
- Bilirubin content does correlate with yellow color across BSA from different manufacturers and grades – but this correlation is tenuous as it lacks data at mid-level b* values.
- Proliant BSAs from US and NZ showed no significant difference in b* values or bilirubin residuals.

## Conclusions

- BSA color is influenced by both physical and chemical variation.
- Smaller particles are associated with increased whiteness and, in some lots, increased green and decreased yellow.
- Differences in particle size distribution between lots or manufacturers can explain some color variation in BSA powders.
- BSA color is very sensitive to biliverdin and bilirubin residuals. Small changes in biliverdin and bilirubin levels result in substantial changes in BSA color.
- 10 ppm biliverdin causes BSA to appear green.
- 25 ppm bilirubin causes BSA to appear vibrant yellow.

## References

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4. EasyRGB. (2020). Color Math and Programming Code Examples. https://www.easyrgb.com/en/math.php