

Exploration of Methods of Fibrinogen Measurement in Avian Species

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Introduction

Fibrinogen is an acute phase protein found in all vertebrates. Levels tend to increase demonstrably in the first 5 days of an inflammatory response, but may remain elevated when chronic inflammation is occurring. Little is known about avian fibrinogen, but in preliminary studies of seabirds at rehabilitation centers, fibrinogen level has been inversely correlated with release readiness.

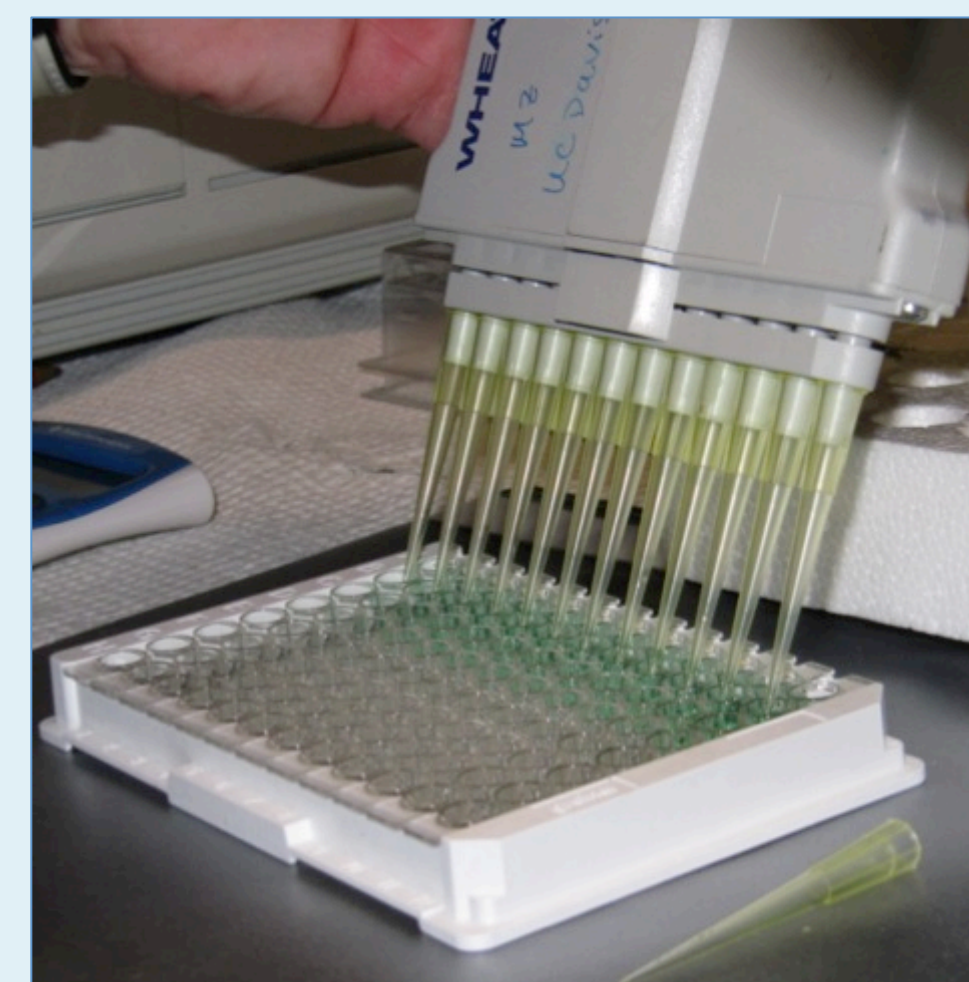


Fibrinogen can be quantitatively measured in birds, but a volume of ≥ 0.5 ml is needed, and samples must be frozen and transported on ice. In addition, a validated and optimized test for birds is not available. These constraints limit the utility of fibrinogen measurement, prompting this investigation of 3 different methodologies.

Methods

We used the Abaxis VSPRO (penguins & pelicans), the GenWay Human Fibrinogen Assay (penguins), and protein electrophoresis (EPH) (penguins) to measure fibrinogen.

- Birds: 8 rehabilitated wild brown pelicans (*Pelecanus occidentalis*), and 13 healthy captive African penguins (*Spheniscus demersus*)
- The VSPRO point-of-care analyzer performed fibrinogen analysis using 0.1 ml of citrated plasma
- The GenWay assay is a competitive ELISA kit marketed for use with human serum or plasma
- We subtracted EPH beta fractions in serum from those in plasma to indirectly measure fibrinogen
- The Abaxis Veterinary Reference Lab (AVRL) modified Clauss method performed on citrated plasma was used as the standard
- The UC Davis Clinical lab (UCD) was used as an independent comparison, as the AVRL fibrinogen test has not been validated on birds
- Fibrinogen values are reported here in mg/dl for all methodologies and in all figures



- Statistics
 - AVRL and UCD results were compared using Pearson correlation coefficients, linear regression, and Bland-Altman plots
 - VSPRO precision was evaluated using the coefficient of variation of repeated measures from each individual
 - Accuracy of the 3 methods was evaluated by visually examining plots of the data, Pearson's correlation coefficients, and linear regression

Results

- AVRL values were highly correlated with UC Davis results (Fig. 1)
 - This relationship remained in a Bland-Altman plot (not shown)

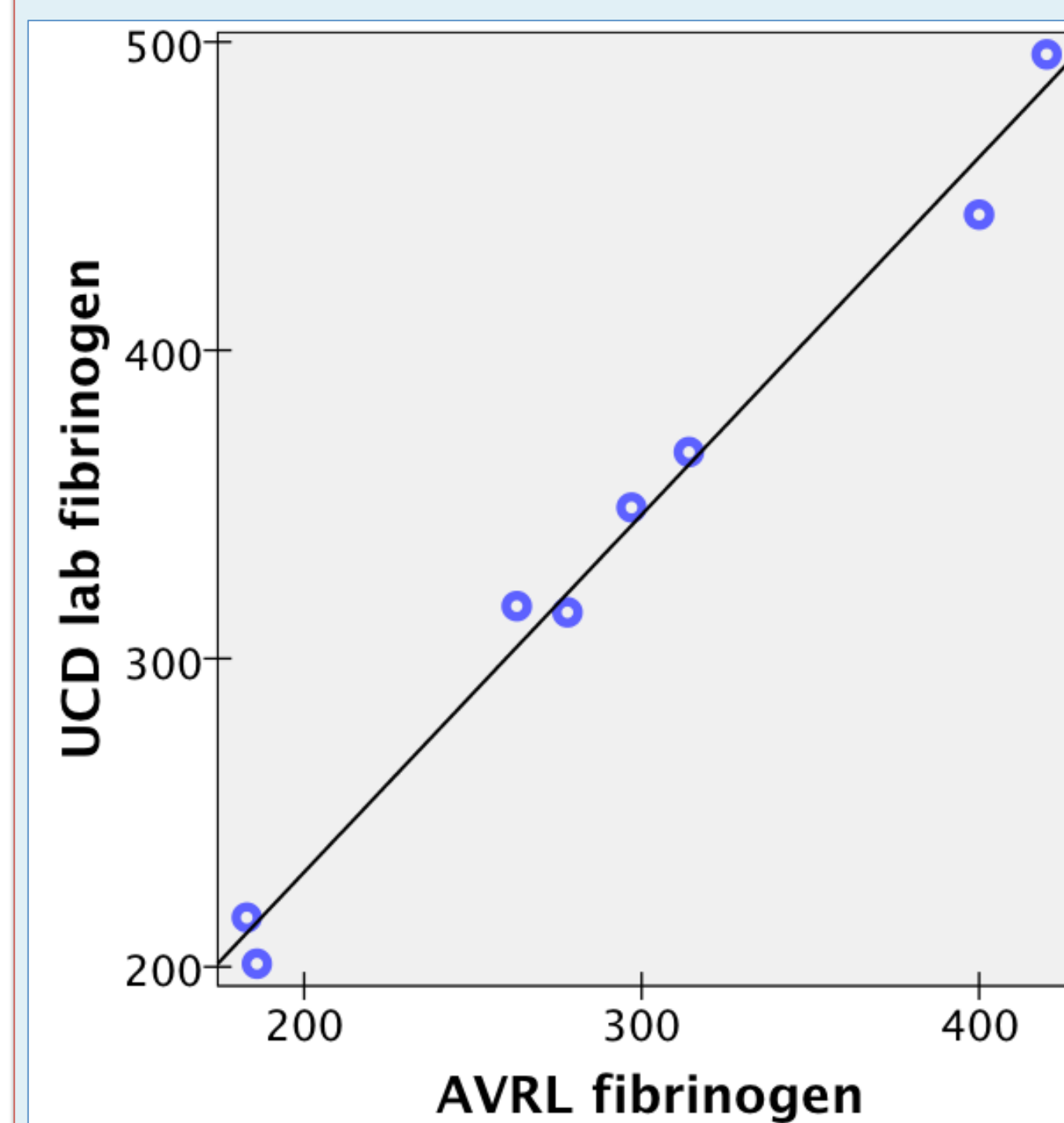
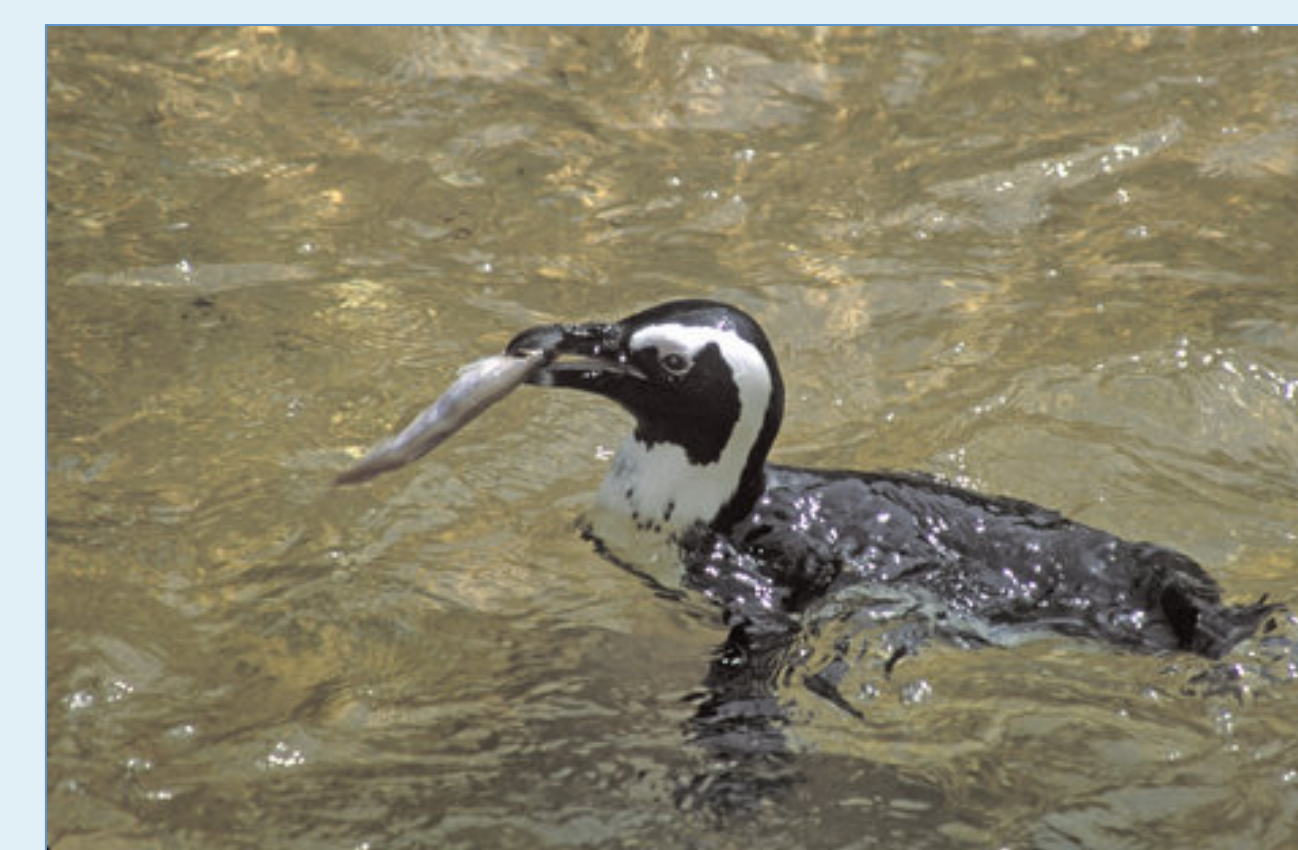


Figure 1. Fibrinogen values of pelicans measured by UC Davis lab and Abaxis Veterinary Reference Lab. Pearson correlation coefficient= 0.994; $r^2=0.987$



- The precision of the VSPRO for fibrinogen varied by species (Fig.2)

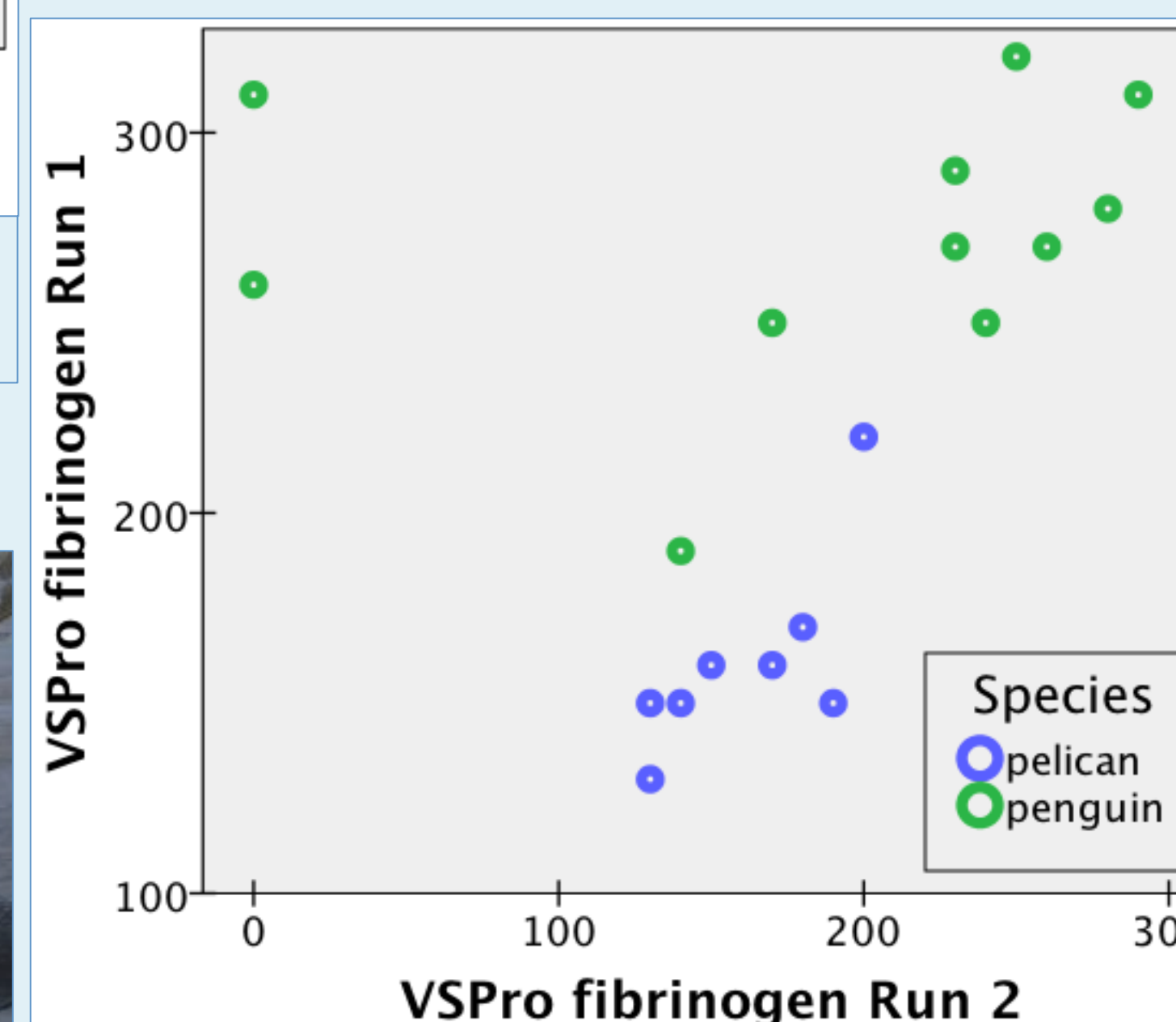
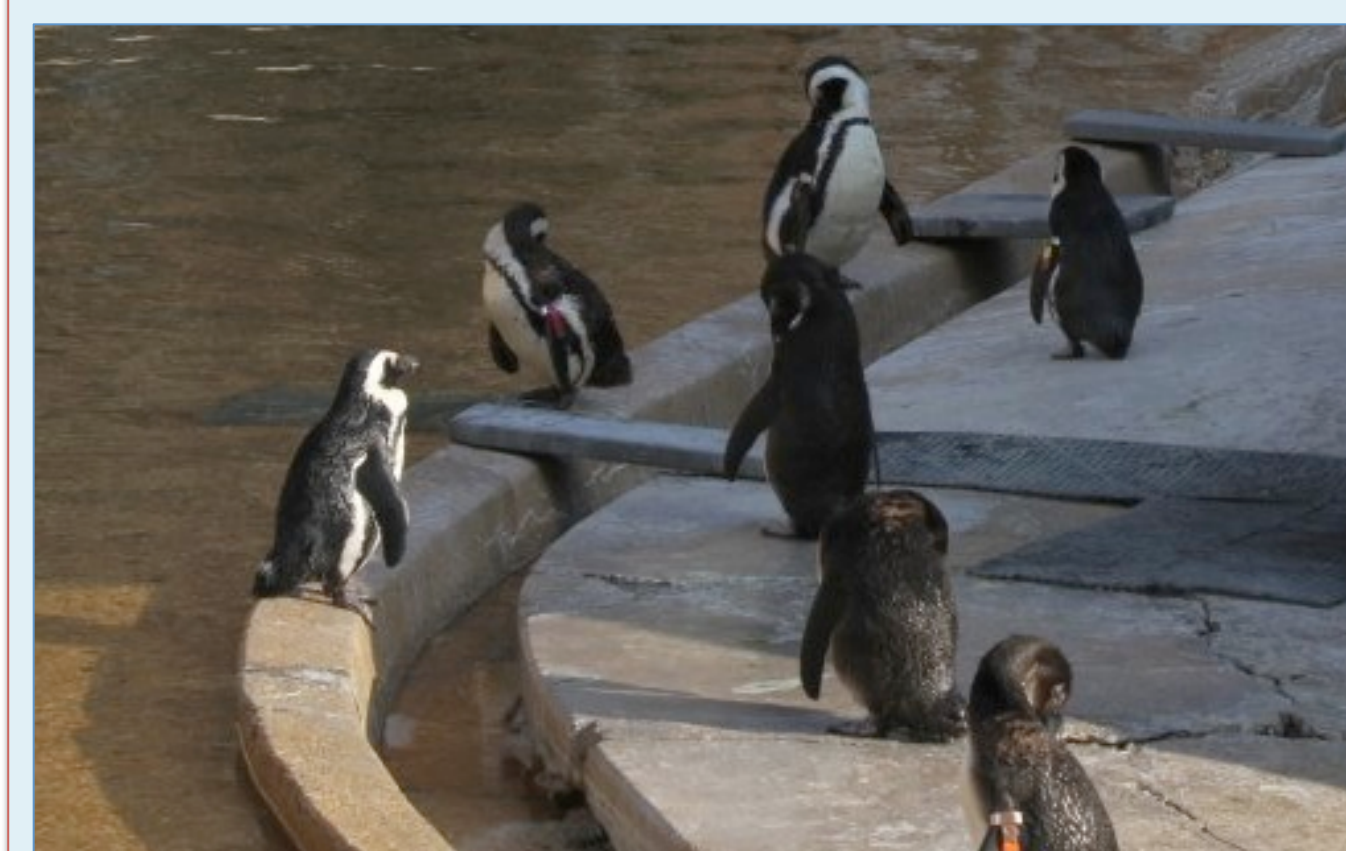


Figure 2. VSPRO precision: Mean coefficient of variation \pm SD: pelicans=8.8 \pm 3.3; penguins=13.0 \pm 9.1; both species combined=10.9 \pm 6.9



- The accuracy of the VSPRO varied by species (Fig 3)
 - Values of the VSPRO correlated poorly with those from AVRL
 - VSPRO accuracy was much lower for penguins than for pelicans
- Similarly, values from the EPH correlated poorly with AVRL values (Fig 4)

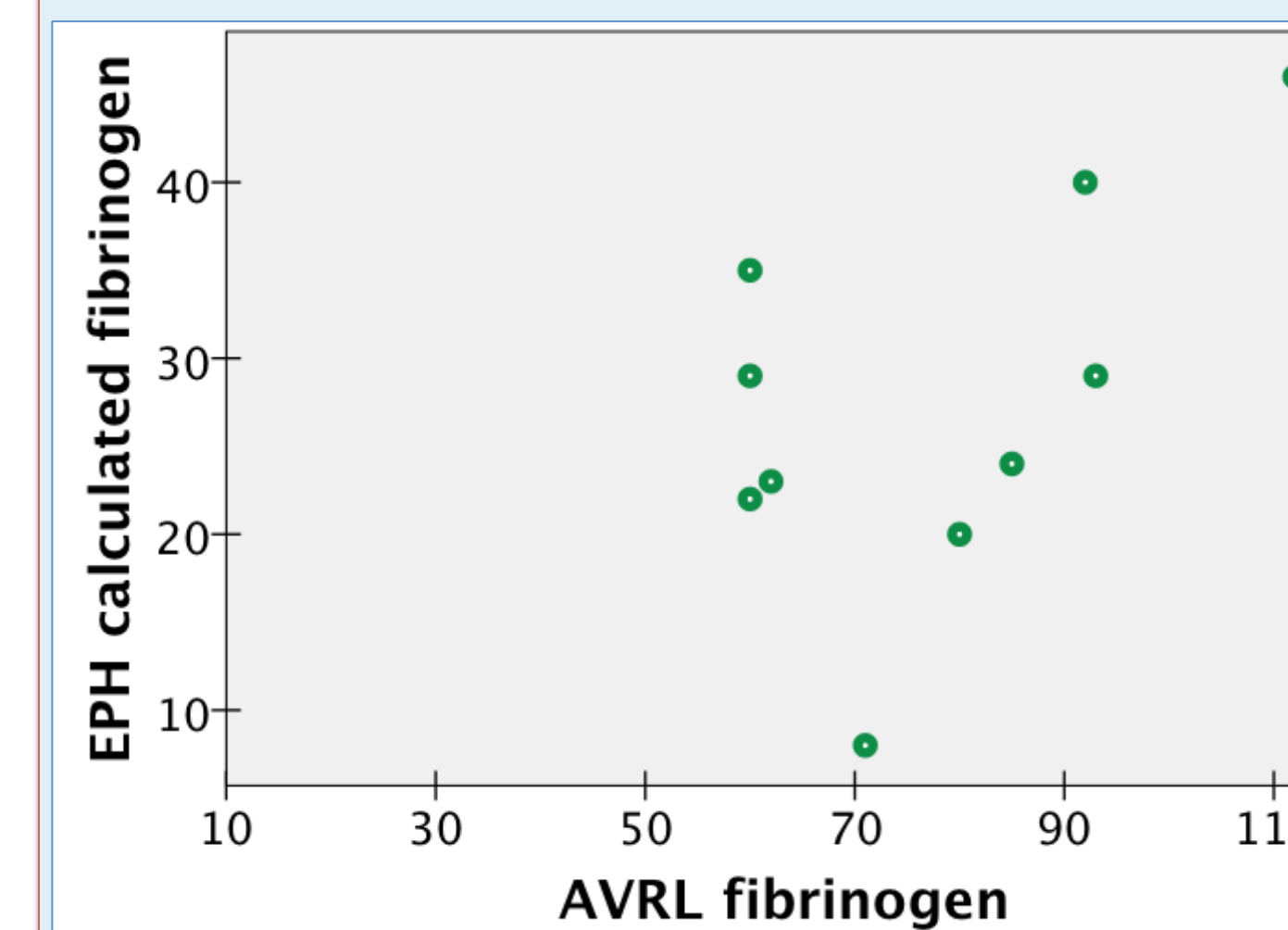


Figure 4. Fibrinogen values calculated from protein electrophoresis results using heparinized plasma and serum plotted against AVRL values (see text). Pearson correlation coefficient=0.535.

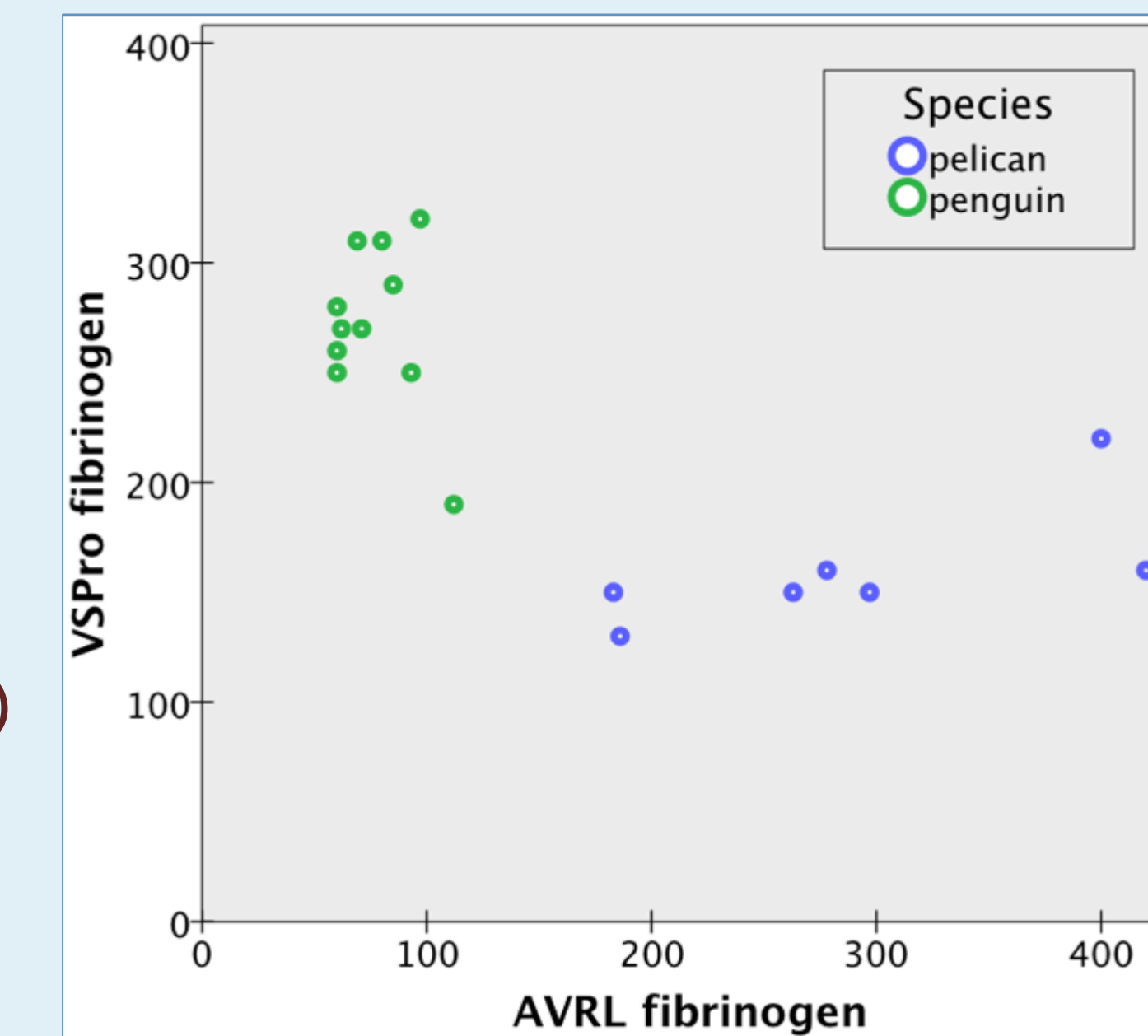


Figure 3. VSPRO values plotted against AVRL values. Pearson correlation coefficients: pelicans=0.580; penguins= 0.056; both species combined=0.695

- Values from the ELISA kit had even lower correlations with AVRL values, whether citrated plasma, heparinized plasma, or serum was used (Fig. 5)

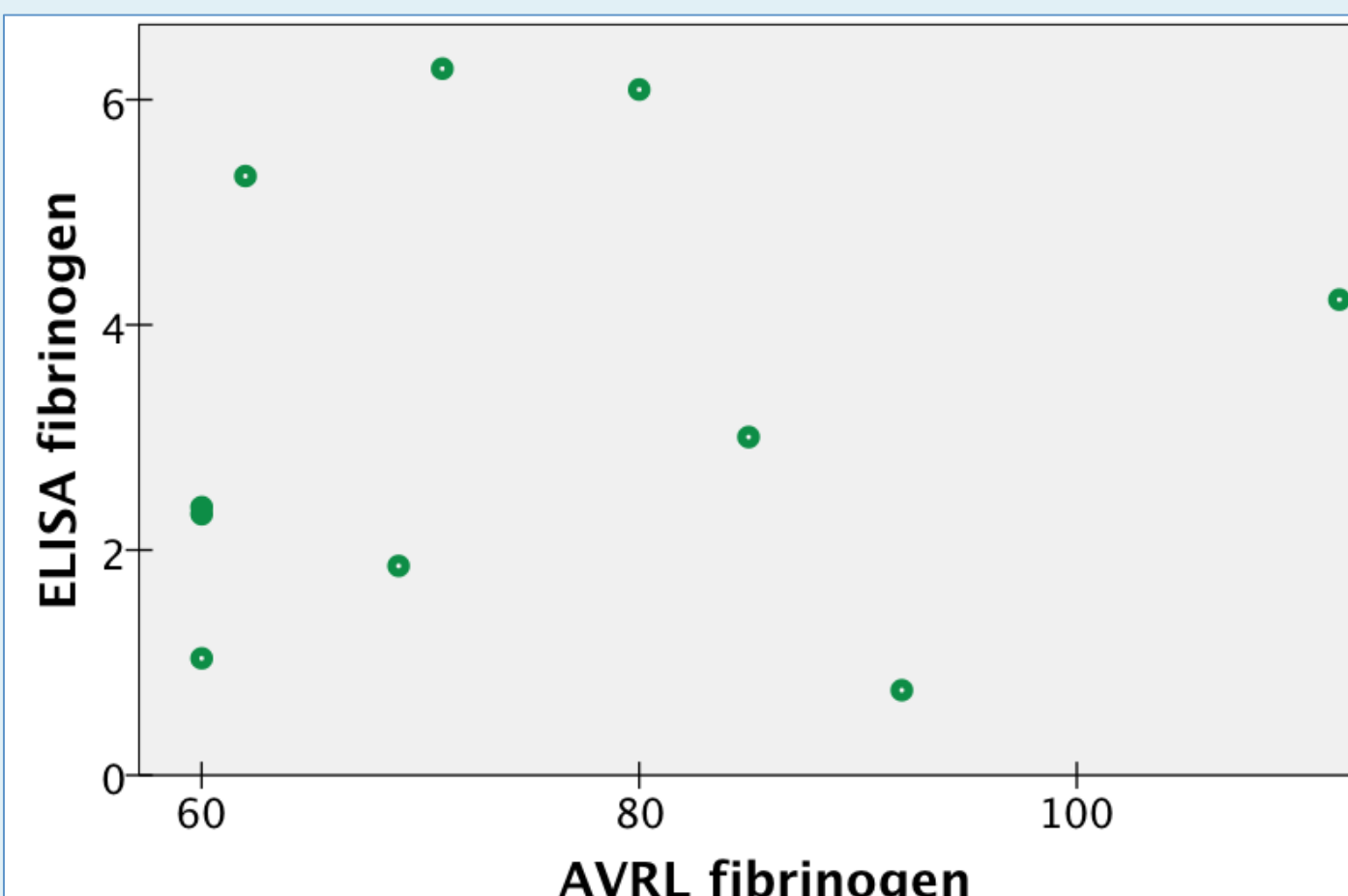


Figure 5. Fibrinogen values determined by ELISA kit on heparinized plasma plotted against AVRL values. Pearson correlation coefficient=0.532.

Discussion

- Despite the lack of validation for birds, the modified Clauss method produced consistent results when performed at 2 commercial labs
- Coefficient of variation (CV) ≤ 10 is considered acceptable precision for clinical diagnostic tests
 - The VSPRO had a CV of ~ 9 for pelicans, but >10 for penguins
 - The VSPRO was not accurate, based on low correlation coefficients
 - The relationship between the VSPRO results and the AVRL standard differed between the 2 avian species, illustrating the need for species-specific validation of diagnostic analyses
 - Neither the ELISA nor the EPH method produced accurate results
- **CONCLUSION: Despite its drawbacks, the modified Clauss method is currently the best option for quantitative avian fibrinogen determination**