Assessment of the reliability of plasma electrophoresis in birds

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Objective—To determine the reliability of plasma electrophoresis (EPH) in psittacine birds.

Animals—93 psittacine birds.

Procedure—Jugular venipuncture was performed on 93 awake psittacine birds. The plasma was centrifuged, separated, aliquoted into duplicate samples, frozen, and sent to 2 commercial laboratories that routinely perform avian EPH. Samples from 51 birds were sent to laboratory A, and samples from 42 birds were sent to laboratory B. The reliability of EPH results within each laboratory was assessed, but not between laboratories. To determine the reliability (agreement between duplicate samples) of total protein, albumin, prealbumin, α1-, α2-, β-, and γ-globulin concentrations, the intraclass correlation coefficient (rI) was calculated.

Results—Both laboratories had excellent agreement between samples for measurement of total protein concentration and only good agreement for albumin concentration. Except for the prealbumin concentration measured at laboratory B, both laboratories had poor agreement for all other values of the EPH.

Conclusions and Clinical Relevance—These data indicate that plasma EPH for measuring prealbumin, α1-, α2-, β-, and γ-globulin concentrations may not be a reliable tool for assessing avian health. Small amounts of these proteins in birds plus human variation in reading the EPH curves may lead to variable results. Avian veterinarians should cautiously interpret results from plasma EPH assays for these protein fractions. (Am J Vet Res 2005;66:375–378)

The plasma protein electrophoresis (EPH) assay has been used for decades in human medicine to investigate individual proteins and find abnormalities related to acute and chronic inflammation.1 This investigative assay has been applied by veterinarians to avian patients, and the plasma EPH assay is recommended for both the well-bird examination and the sick-bird visit.2,4 Plasma EPH complements the findings of the CBC and serum or plasma biochemical analyses.5 Used in conjunction with the WBC count, plasma EPH is another laboratory measure of inflammation or infection.2,3 Reportedly, a second important function of plasma EPH is to accurately and precisely measure plasma albumin concentration.6 An accurate measurement of avian albumin is likely not possible on most biochemical analyzers because of the relatively small amount of albumin in avian plasma, compared with mammalian plasma, and the lack of species-specific dye binding.5,6

Clinical judgments, including diagnoses and treatments, are based on the interpretation of the results of plasma EPH. Therefore, plasma EPH is an important decision-making tool in avian medicine.7 Despite the importance given to this assay, there are little or no data regarding the reference range, reliability, accuracy, sensitivity, and specificity of plasma EPH as applied to avian patients. In the pet psittacine literature, there are no peer-reviewed studies on the reliability of plasma EPH; only case reports and literature reviews exist.1,10-15

Therefore, to improve the confidence level of the clinician in plasma EPH as a descriptive test of avian health, the accuracy, reliability, and reference range of each constituent of this test should be known. As a first step, the purpose of the study reported here was to determine the reliability of plasma EPH in samples from psittacine birds.

Materials and Methods

Birds—Jugular venipuncture was performed on 93 awake, manually restrained psittacine birds, including various psittacine species (Table 1). All birds were > 2 years of age, and sex was not known for most of the birds. Determination of health status of the birds was not necessary for this study, although for all birds, a comprehensive plasma biochemical panel was performed at the same time that blood was obtained for plasma EPH. A full physical examination was performed by a board-certified (American Board of Veterinary Practitioners) avian veterinarian, and all birds appeared healthy. Birds were reported to be acting and eating normally for the 3 months prior to and after the study period. This protocol was approved by the University of Pennsylvania Animal Care and Use Committee and the Client Owned Animal Protocol Committee.

Blood collection—By use of either a 22- or 25-gauge needle on a 1- or 3-mL syringe, 1 to 3 mL of blood was withdrawn from either the right or left jugular vein. The needle of the syringe was removed, and the blood was immediately placed into lithium heparin microtainers6 and gently mixed. Samples were examined for clots and discarded if any were found. The heparinized blood was centrifuged, and the plasma was immediately decanted and aliquoted into plastic
Eppendorf bullet tubes. At least 0.2 mL of plasma from each bird was placed in each tube. Two duplicate samples were stored at 4°C for 24 to 72 hours, packed on ice, and sent overnight to each commercial laboratory for plasma EPH. Fifty-one duplicate samples were sent to laboratory A, and 42 duplicate samples were sent to another laboratory (laboratory B). Samples were labeled such that duplicates could not be identified by each laboratory. These 2 commercial laboratories were chosen because each advertises that it performs plasma EPH for avian samples. The study was designed to compare reliability within each laboratory, not between laboratories. Methodology for laboratory reliability studies of plasma EPH has been described.16

Blood variables—Laboratory A used biuret methodology for determination of plasma total protein concentration, and laboratory B used temperature-controlled refractometry for plasma total protein concentration. Both laboratories used agarose gel electrophoresis for determination of plasma prealbumin, albumin, α₁-, α₂-, β-, and γ-globulin concentrations. The methodology for determination of the components of the plasma EPH has been described elsewhere.2

Statistical analysis—To assess agreement of the total protein, albumin, prealbumin, α₁-, α₂-, β-, and γ-globulin concentration results from each laboratory, the intraclass correlation coefficient of reliability (rï) was calculated. The

![Figure 1](https://example.com/figure1.png)  
*Figure 1—Agreement among measurements of total protein concentration (as measured by the intraclass correlation coefficient rï) in duplicate avian samples analyzed via plasma electrophoresis (EPH) at 2 commercial laboratories.*

![Figure 2](https://example.com/figure2.png)  
*Figure 2—Agreement among measurements of albumin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.*

![Figure 3](https://example.com/figure3.png)  
*Figure 3—Agreement among measurements of prealbumin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.*

Table 1—Distribution of species among 93 psittacine birds used in a study of the reliability of plasma electrophoresis.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species name</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cockatiel</td>
<td>Nymphicus hollandicus</td>
<td>32 (34)</td>
</tr>
<tr>
<td>Blue and gold macaw</td>
<td>Ara ararauna</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Umbrella cockatoo</td>
<td>Cacatua alba</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Sulfur-crested cockatoo</td>
<td>Cacatua galeraita</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Catalina macaw</td>
<td>Ara macao X Ara ararauna</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Moluccan cockatoo</td>
<td>Cacatua moluccensis</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Hyacinth macaw</td>
<td>Anodorhynchus hyacinthinus</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Hyacinth macaw hybrid</td>
<td>Anodorhynchus hyacinthinus X Ara ambigua</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Solomon Island eclectus parrot</td>
<td>Eelectus rotatus</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Harlequin macaw</td>
<td>Ara chloroptera X Ara ararauna</td>
<td>4 (4)</td>
</tr>
<tr>
<td>African grey parrot</td>
<td>Psittacus eritracus</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Green-winged macaw</td>
<td>Ara chloroptera</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Leadbeater's cockatoo</td>
<td>Cacatua leadbeateri</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Black palm cockatoo</td>
<td>Probosciger aterimus</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Blue-headed pionus</td>
<td>Pionus mensratus</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Blue-fronted Amazon parrot</td>
<td>Amazona aestiva</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Yellow-naped Amazon parrot</td>
<td>Amazona auropalliata</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>
considered to be in excellent agreement (precise) when the \( r_i \) was > 0.90, in good agreement when the \( r_i \) was from 0.80 to 0.90, in fair agreement when \( r_i \) was from 0.70 to 0.80, and in poor agreement when \( r_i \) was < 0.70. Coefficients were only calculated within each of the laboratories because the methodology differed between the laboratories (ie, measurement of the total protein concentration). All analyses were performed by use of statistical software.b

Results

Of the 51 duplicate samples sent to laboratory A and 42 sent to laboratory B, only 37 and 33 samples, respectively, were used to measure reliability of plasma EPH. Samples were discarded if the plasma sample was insufficient to perform duplicate plasma EPH assays or if the sample was lipemic and an interpretable result could not be obtained.

Both laboratories had excellent agreement for measurement of the total protein concentration (Figure 1). Albumin concentration had good agreement in both laboratories (Figure 2). Laboratory B had excellent agreement between measurements of the prealbumin concentration but there was poor agreement for measurement of the prealbumin concentration at laboratory A (Figure 3). Both laboratories had poor agreement for measurements of \( \alpha_1 \)-globulin concentration (Figure 4), and \( \alpha_2 \)-globulin concentration (Figure 5). Similarly, the \( \beta \)-globulin concentration did not yield good agreement at either commercial laboratory (Figure 6). Measurements of \( \gamma \)-globulin concentrations had fair and good agreement (Figure 7).

Discussion

There may be a number of explanations why many of the results found in this study were not reliable. Although care was taken to handle duplicate samples in a similar manner, it is possible that there was sample degradation that affected each duplicate sample differently. If this were true, then one might expect all analytes to not agree; yet the total protein concentration yielded reliable results within the duplicate samples. It would be difficult to describe a degradation process that would affect all analytes of plasma EPH except for total protein.

One possible explanation why total protein concentration measurement had excellent reliability yet the other values (except for prealbumin at

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Figure 4—Agreement among measurements of \( \alpha_1 \)-globulin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.

Figure 5—Agreement among measurements of \( \alpha_2 \)-globulin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.

Figure 6—Agreement among measurements of \( \beta \)-globulin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.

Figure 7—Agreement among measurements of \( \gamma \)-globulin concentration in duplicate avian samples measured via plasma EPH at 2 commercial laboratories.
laboratory B) did not could be related to the methodology. Total protein concentration is measured by a machine or refractometer, whereas plasma EPH values are determined by both machine and human measurement. Technicians subjectively place lines where there appears to be normal transition between the 2 curves to define the various protein components. The lines separating the peaks can be drawn in more than 1 location because there is no definite transition between some of the peaks.

A second explanation for lack of good agreement between duplicate samples pertains to the amounts being measured. It appears the agreement is better for larger fractions. Fractions with smaller concentrations may be more susceptible to small changes in values; that is, the lines separating the components are drawn slightly differently for each duplicate sample, magnifying the error of measurement. This may explain why the albumin concentration, the largest component of the plasma EPH, had good agreement between measurements but why smaller concentrations (ie, α-, β-, and γ-globulins) had mostly poor agreement between measurements. This may also explain why these variables in other species with higher concentrations of plasma EPH constituents have excellent reliability.17

Another explanation for variation of the plasma EPH values between duplicate samples is discussed by Fernandez et al.1 When proteins are stained, as is done with the plasma EPH assay, variables include staining time and temperature, stain concentration and age, destaining time, and room temperature. These all influence dye binding and lead to errors in quantitation.5

A third explanation of the poor agreement in most of the plasma EPH fractions is a limitation of the statistical method used. Because there was a small range of individual bird plasma EPH values in this study, the r value may have been spuriously low. Had the range of plasma EPH values among birds been larger and the range of differences within birds remained the same, the r value would have been greater. This is illustrated with the prealbumin fraction for which laboratory B had excellent agreement (r = 0.922) and laboratory A had poor agreement (r = 0.611). The range of differences within birds was about the same for both (0.94 and 1.12 for laboratories A and B, respectively). However, the range of prealbumin values between birds was more than twice as large for laboratory B, compared with laboratory A (2.76 vs 1.11, respectively).

Birds are well known for concealing their illness, and there is much reliance on objective testing to determine the health status of birds. Plasma EPH, like all diagnostic assays performed in birds, gives the avian veterinarian insight into the health of the patient. Unfortunately, compared with other species, the small amounts measured in avian samples create greater room for error. Despite the fact that the results of this study revealed aspects of plasma EPH to be unreliable, it is still possible that plasma EPH is a tool that is useful to the avian veterinarian. It may be that a finding of a value within the reference range and cautious interpretation of values above the reference range are more useful than an absolute cutoff value for clinical normality.

References