Assessment of a point-of-care biochemical analyzer and comparison with a commercial laboratory for the measurement of total protein and albumin concentrations in psittacines

Matthew S. Johnston, VMD; Karen L. Rosenthal, DVM, MS; Frances S. Shofer, PhD

Objective—To determine agreement for total protein (TP) and albumin concentrations measured by a point-of-care biochemical analyzer in heparinized whole blood and plasma samples obtained from psittacines and compare results with those from a commercial laboratory.

Sample Population—Hematologic samples from 92 healthy birds.

Procedures—Duplicate samples of heparinized whole blood and plasma were obtained. A point-of-care biochemical analyzer was used to determine TP and albumin concentrations. To assess precision, intraclass correlation coefficient (ri) and Bland-Altman measures of agreement were used. These results were compared by use of Bland-Altman plots with those obtained from a commercial laboratory that used a biuret method for TP concentration and electrophoresis for albumin concentration.

Results—For the analyzer, there was excellent agreement (ri = 0.91) between heparinized whole blood and plasma samples for TP and albumin concentrations. Relative error was 0.9% for TP and 0.7% for albumin. Analyzer results correlated well with commercial laboratory results, with a downward bias of 0.6 for TP and 0.3 for albumin.

Conclusions and Clinical Relevance—The analyzer had excellent precision for analysis of heparinized whole blood or plasma samples for TP or albumin concentrations; analyzer values had good agreement with those from a commercial laboratory. The analyzer could be a valid method to measure plasma TP concentrations and provide point-of-care testing in apparently healthy parrots. Biochemical analyzer results for plasma albumin concentration were not validated by results from a commercial laboratory, so conclusions cannot be drawn regarding use of the analyzer in measurement of albumin concentrations in psittacines. (Am J Vet Res 2007;68:1348–1353)

The accurate determination of plasma protein concentrations has been more challenging in birds than in traditional domestic animals. The biuret method can be used to accurately determine serum TP concentrations in pigeons. Refractometry yields varying reliability and accuracy of serum and plasma TP concentrations in pigeons, ducks, chickens, and turkeys. To our knowledge, no studies have been conducted to evaluate the use of these methods for determining plasma TP concentration in psittacines. Biuret and refractometric methods have been discussed in detail elsewhere.

In most mammals, plasma or serum albumin concentrations can be accurately and precisely assayed with techniques that involve binding of BCG or brom cresol purple dye as well as immunohistochemical and electrophoretic methods. Laboratory determination of albumin concentrations in psittacines can be frustrating because of the relatively low plasma albumin concentrations in parrots, especially budgerigars (Melopsittacus undulatus) and cockatiels (Nymphicus hollandicus), which have low-end reference ranges for plasma albumin concentrations of approximately 0.7 g/ dL. Additionally, there is a lack of species-matched standards for calibration of dye-binding methods. Traditionally, biochemical analyzers that use these methods have been unable to resolve the albumin fraction at low concentrations.
In other orders of birds, specifically anseriformes, columbiformes, and galliformes, electrophoresis is superior to dye-binding methods for determination of serum or plasma albumin concentrations. It has been suggested that serum or plasma TP concentration should be measured by use of biuret method on the basis of its accuracy in determining serum or plasma TP concentrations in anseriformes, columbiformes, and galliformes, whereas serum or plasma albumin concentration can best be determined via electrophoresis. In psittacines, plasma protein electrophoresis has been suggested as the reference method for determination of plasma albumin concentration. Protein electrophoresis techniques for avian plasma have been described in detail elsewhere.

Point-of-care clinical biochemical analyzers have become more available to veterinarians. However, to our knowledge, studies in which samples from pet birds have been assessed by point-of-care analyzers have not been published. Potential benefits of point-of-care analyzers include immediate results, ease of use, small sample size, use of heparinized whole blood samples instead of plasma or serum (which thereby decreases the amount of technical time), direct control over a sample, direct quality control, and portability. A point-of-care biochemical analyzer would be potentially valuable to avian veterinarians for all of these reasons. The manufacturer of 1 point-of-care biochemical analyzer has made claims that only 0.1 mL of heparinized whole blood is necessary for analysis of 12 biochemical assays and that results are available in approximately 15 minutes. The objectives of the study reported here were to determine the agreement between values obtained for measurement of TP and albumin concentrations in heparinized whole blood and plasma samples when analyzed by use of a point-of-care biochemical analyzer and to compare TP and albumin concentrations determined by use of the analyzer with values determined at a commercial laboratory.

**Materials and Methods**

**Animals**—Only psittacines were eligible for inclusion in the study. Consent was obtained to use 92 birds owned by 2 commercial aviaries. The protocol for this study was approved by the client-owned animal protocol committee and the institutional animal care and use committee at the University of Pennsylvania.

**Collection of samples**—Each bird was manually restrained in a towel for jugular venipuncture by use of standard techniques. A 23-gauge needle on a 1-mL syringe or 22-gauge needle on a 3-mL syringe was used to collect 1 or 3 mL of blood, respectively, from the right or left jugular vein. The needle of the syringe was removed, and the blood was immediately placed into tubes that contained lithium heparin. Contents of the tubes were gently mixed. Samples were examined and discarded when clots were detected.

**Analysis of samples**—A commercially available point-of-care analyzer was used. Two reagent disks were used (1 was specific for mammalian biochemical analyses, and the other was specific for avian biochemical analyses). Although the technical aspects of the assays for TP and albumin were the same, they were optimized for variable dynamic ranges that exist between avians and mammals. The analyzer used an assay that binds BCG dye for determination of albumin concentrations. An endpoint reaction measured biuretically at 630 nm and 405 nm was used to quantitate the concentration of the albumin-BCG complex, with the concentration of bound albumin being proportional to the amount of albumin in each sample. By use of the mammalian rotor, this assay was calibrated with the standard for canine albumin, whereas for the avian-reptilian rotor, it was calibrated with a standard for turkey albumin because no albumin standards for the various psittacine species were commercially available.

The TP concentration was determined via the biuret method. Briefly, the sample was admixed with cupric ions in an alkaline medium; precipitation of copper hydroxide was prevented by the addition of sodium potassium tartate, and autoreduction of copper was prevented by the addition of potassium iodide. Peptide bonds within the protein reacted with the cupric ions and formed a colored copper-protein complex that was quantified by the difference in absorbance between 550 nm and 850 nm. The amount of protein in the sample was proportional to the absorbance of the copper-protein complexes.

To obtain results for whole blood and plasma samples on the analyzer, 0.1 mL of heparinized whole blood was aseptically removed from a tube by use of a pipette and placed onto a reagent disk; the sample was then tested in the analyzer. The remaining heparinized whole blood was then centrifuged at 2,000 × g for 5 minutes. Immediately after centrifugation, 0.1 mL of the resulting plasma was removed by use of a pipette and placed onto a reagent; the plasma sample was then tested in the analyzer.

The remaining plasma was manually decanted with a pipette and placed into nonheparinized plastic 0.7-mL tubes. A 0.3-mL aliquot of plasma was obtained from each bird. The plasma was stored at 4°C for 24 to 72 hours and then shipped to a commercial laboratory for measurement of TP and albumin concentrations. Samples were mailed overnight in polystyrene containers filled with multiple ice packs. These procedures were intended to mimic handling and shipping by typical veterinary practitioners.

Plasma samples were analyzed on arrival at the commercial laboratory. Laboratory personnel used temperature-controlled refractometry for measurement of plasma TP concentrations and agarose gel electrophoresis for measurement of plasma albumin concentrations.

**Data analysis**—Samples from birds with excessive lipemia were excluded from the analysis. Samples were excluded when they had a recorded value of 2+ on the analyzer, which represented a lipemic index for a total plasma triglycerides concentration ≥ 221 mg/dL.

To determine repeatability of the point-of-care biochemical analyzer, the r and statistical methods of Bland and Altman were used. These included bias and the limits of agreement. To measure agreement between results for the analyzer and the commercial laboratory, Bland-Altman statistical methods again were used. Bias
Results

Ninety-two birds were initially enrolled in the study. However, 22 were excluded because of lipemia (13 cockatiels, 3 sulfur-crested cockatoos, 2 Hyacinth macaw hybrids, 1 Moluccan cockatoo, 1 blue and gold macaw, 1 yellow-naped Amazon parrot [Amazona auropalliata], and 1 African grey parrot). Thus, samples from 70 birds were used in the study (Table 1). All birds were > 2 years old and appeared healthy.

Of the 70 samples analyzed with the point-of-care biochemical analyzer, 37 were analyzed by use of the mammalian rotor, and 33 were analyzed by use of the avian-reptilian rotor. Five samples were not of sufficient volume to allow for duplicate analysis; thus, there were 65 duplicate samples.

The point-of-care biochemical analyzer had excellent agreement between heparinized whole blood and plasma samples, with $r = 0.972$ for albumin concentrations and $r = 0.912$ for TP concentrations. Bland-Altman statistics revealed small relative errors (0.9% for TP and 0.7% for albumin) with bias = 0.01 for both (Table 2; Figures 1 and 2).

Values obtained by use of the mammalian rotor, although repeatable, were not accurate because of inappropriate optimization of the dynamic range of the rotor for avian protein values. Unfortunately, at the time of testing, the rotor with the appropriate dynamic range for birds was still in development. Therefore, results obtained by use of the biochemical analyzer for these samples could not be compared with results obtained at the commercial laboratory.

Table 1—Psittacines from which samples were obtained for determination of whole blood and plasma concentrations of TP and albumin.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species name</th>
<th>No. of birds</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cockatiel</td>
<td>Nymphicus hollandicus</td>
<td>18</td>
<td>25.7</td>
</tr>
<tr>
<td>Blue and gold macaw</td>
<td>Ara ararauna</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td>Umbrella cockatoo</td>
<td>Cacatua alba</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td>Catalina macaw</td>
<td>Ara macao $\times$ Ara ararauna</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>Moluccan cockatoo</td>
<td>Cacatua moluccensis</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Hyacinth macaw</td>
<td>Anodorhynchus hyacinthinus</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Solomon Island eclectus parrot</td>
<td>Eclectus roratus</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Harlequin macaw</td>
<td>Ara chloroptera $\times$ Ara ararauna</td>
<td>4</td>
<td>5.7</td>
</tr>
<tr>
<td>Sulfur-crested cockatoo</td>
<td>Cacatua galerita</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Hyacinth macaw hybrid</td>
<td>Anodorhynchus hyacinthinus $\times$ Ara ambiguа</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>African grey parrot</td>
<td>Pseitticus erithacus</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Green-winged macaw</td>
<td>Ara chloroptera</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Leadbeater’s cockatoo</td>
<td>Cacatua leadbeater</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Black palm cockatoo</td>
<td>Probosciger aterrimus</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Blue-headed pionus</td>
<td>Pionus menstruus</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>Blue-fronted Amazon parrot</td>
<td>Amazona aestiva</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 2—Results of Bland-Altman analysis to determine agreement between TP and albumin concentrations obtained by use of a point-of-care biochemical analyzer on samples of whole blood and plasma obtained from 70 psittacines.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TP</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference (ie, bias)</td>
<td>0.013</td>
<td>-0.013</td>
</tr>
<tr>
<td>Limits of agreement</td>
<td>-0.23 to 0.25</td>
<td>-0.31 to 0.29</td>
</tr>
<tr>
<td>Relative error</td>
<td>0.9%</td>
<td>0.7%</td>
</tr>
<tr>
<td>$r$</td>
<td>0.912</td>
<td>0.972</td>
</tr>
</tbody>
</table>

Results of 33 samples, as measured by use of the avian-reptilian rotor, were available for comparison with values determined at the commercial laboratory. Results from the biochemical analyzer correlated well with those from the commercial laboratory, with a downward bias of 0.6 (limits of agreement, –0.6 to 2.8) for TP and 0.3 (limits of agreement, –6.0 to 1.2) for albumin. The largest deviation from protein values determined at the commercial laboratory was evident for cockatiels (Figure 3). These values were outside the limits of agreement. When data for cockatiels were excluded, the bias decreased to 0.13.
with limits of agreement of –0.7 to 1.0. In contrast to TP values, albumin values measured by use of the biochemical analyzer differed from those measured at the commercial laboratory in a similar manner for all psittacine species (Figure 4).

**Discussion**

Agreement was excellent for the determination of TP and albumin concentrations between psittacine plasma and heparinized whole blood samples analyzed by use of the point-of-care biochemical analyzer. This substantiated the manufacturer’s claim that heparinized whole blood or plasma could be used to obtain a reliably repeatable result. This fact is critical in that use of heparinized whole blood instead of plasma would enable avian practitioners to use a smaller sample size and save time associated with preparation of plasma samples. This study only tested the agreement of the analyzer within the range of values obtained in apparently healthy birds because determination of health was not a part of the study. Additional studies of this biochemical analyzer to assess agreement over the entire dynamic range of values in sick and healthy birds would be ideal to further substantiate the recommendation for use of heparinized whole blood instead of plasma.

On the basis of the comparison of plasma TP concentrations determined with the biochemical analyzer by use of the avian-reptilian rotor and those determined at the commercial laboratory, we concluded that the biochemical analyzer could be used to accurately measure TP concentrations in apparently healthy birds. The small downward bias for plasma TP values revealed that the biochemical analyzer had a tendency to slightly underestimate the value, compared with results for the commercial laboratory method, when measuring values for the population as a whole. The reason for this downward bias was unknown, but several explanations were plausible. In pigeons, refractometric measurements of serum TP concentrations are consistently higher than those obtained on the same sample by use of a biuret method. Studies have not been conducted on psittacine plasma or for serum TP concentrations to determine whether a similar result could be expected in psittacines. One explanation put forth for this phenomenon has been that the high concentration of glucose in avian blood may affect the refractometer value. However, an in vitro study that used canine plasma to which various concentrations of d-glucose were added failed to confirm that glucose significantly affected TP refractometric analysis. Another explanation may be that the samples evaluated with the analyzer were assayed immediately after sample collection, whereas samples evaluated at the commercial laboratory were refrigerated and shipped overnight before analysis. Al-

![](image1)

Figure 2—Comparison of TP concentrations determined by use of a point-of-care biochemical analyzer on samples of whole blood and plasma obtained from 70 psittacines. Values correlated well (r, 0.912). See Figure 1 for remainder of key.

![](image2)

Figure 3—Bland-Altman plot of TP concentrations in plasma samples obtained from cockatiels (gray squares) and other psittacines (black circles) and measured by use of a point-of-care biochemical analyzer and at a commercial reference laboratory. The difference in TP plotted on the y-axis represents the concentration measured at the commercial laboratory minus the concentration measured by use of the biochemical analyzer. Results from the biochemical analyzer correlated well with results from the commercial laboratory, with a mean downward bias of 0.6 (dotted line) and limits of agreement of –1.6 to 2.8 (dashed lines). Notice that results for 3 cockatiels were outliers. When results for cockatiels were excluded from the analysis, the bias decreased to 0.13 with limits of agreement of –0.7 to 1.0.

![](image3)

Figure 4—Bland-Altman plot of albumin concentrations in plasma samples obtained from cockatiels (gray squares) and other psittacines (black circles) and measured by use of a point-of-care biochemical analyzer and at a commercial reference laboratory. Results from the biochemical analyzer correlated well with results for the commercial laboratory, with a mean downward bias of 0.3 (dotted line) and limits of agreement of –0.6 to 1.2 (dashed lines). See Figure 3 for remainder of key.
though the samples were in sealed plastic containers in the refrigerator, it is possible that there was some dehydration of the samples prior to analysis at the commercial laboratory. Dehydration of a sample will lead to concentrating the TP and hence a slightly higher result. Additional studies to evaluate species with low plasma TP concentrations (such as cockatiels and budgerigars) appear to be warranted because in the study reported here, cockatiels represented the species with the greatest deviation from the results for the commercial laboratory. When data for cockatiels were removed from the statistical analysis, the downward bias became relatively clinically negligible at 0.13.

The comparison of plasma albumin concentrations obtained with the biochemical analyzer by use of the avian-reptilian rotor with those obtained at the commercial laboratory by use of electrophoresis was less straightforward. In a concurrent study performed by our laboratory group that used the same samples obtained for the study reported here, we found that results for 2 commercial laboratories (1 of which was the commercial laboratory used for comparison in this study) had excellent agreement for measurement of plasma TP concentrations but only good agreement for measurement of plasma albumin concentrations in duplicate samples. We concluded in that concurrent study that although the measurement of plasma TP concentrations at a commercial laboratory can be used as a reference method, the measurement of plasma albumin concentration by electrophoresis may not be suitable for use as a reference method. In the study reported here, there was a downward bias for albumin, which again suggested that the biochemical analyzer tended to slightly underestimate plasma albumin concentrations in psittacines, compared with concentrations determined at the commercial laboratory. Furthermore, the limits of agreement (with 95% confidence) for albumin accounted for an actual albumin concentration that may have differed from the result for the reference method by as much as 0.9 g/dL. In parrots, in which the total plasma albumin concentrations are generally approximately 1.5 g/dL, a variability of 0.9 g/dL (ie, 60%) could lead to false clinical impressions. Therefore, on the basis of the study reported here and our concurrent study, the only conclusion that can be safely reached is that additional studies that use a more reliable reference method are warranted to assess the use of the point-of-care biochemical analyzer for measurement of plasma albumin concentrations in psittacines.

The decision to remove 22 birds from the study because of a high lipemic index was made on the basis of the biochemical analyzer’s inherent suppression of results with >10% interference attributable to lipemia. Additionally, lipemia adversely affects TP measurements when performed with a refractometer, as was performed at the commercial laboratory used for comparison.

The manufacturer’s claim that heparinized whole blood or plasma samples can be used for the measurement of plasma TP and albumin concentrations is upheld. Additional studies are necessary to assess the clinical use of the biochemical analyzer for parrots with abnormally high or low plasma TP or albumin concentrations. With the establishment of reference ranges specific to the method, this analyzer could become a clinically useful tool for the determination of plasma TP concentrations in psittacines.

Additional studies will be needed to establish the use of this analyzer for measurement of plasma albumin concentrations in psittacines because the commercial laboratory used for comparison could not provide a reliable reference method. However, because of the excellent precision of albumin measurements coupled with the good correlation with results for the only available reference method, it is likely that the point-of-care biochemical analyzer will become a useful tool for monitoring patterns of plasma albumin concentrations in psittacines. Reference ranges established for psittacine plasma albumin concentrations with the biochemical analyzer by use of the avian-reptilian rotor should be considered repeatable estimations as opposed to definitively accurate values.

References