Letter to the Editor

Questions conclusions in electrophoresis study

I recently read with interest an article titled "Assessment of the reliability of plasma electrophoresis in birds" by Rosenthal et al (*AJVR*, March 2005, pp 375–378). I believe there are important points that were not addressed. Additionally, I state I am a member of "laboratory B" and have studied electrophoresis (EPH) for 12 years.

Reliability studies are based on sound sample preparation. In the current study, samples were divided in nonsterile conditions and then refrigerated for a variable time. Shipping occurred without temperature control and analysis over an unknown period. This is completely contradictory to reliability studies as described by Tietz[1] (the only recognized standard of clinical chemistry analysis). Samples should be aseptically divided in dehydration-proof containers, frozen, and shipped on dry ice. Related to this issue are findings of Pudlak et al[2] revealing that refrigeration alters globulin migration. The authors did not note that control avian plasma used by laboratory B (stored and handled properly) produces fractions with a variance within strict limits of manufacturer's standards. In light of this internal gel control, the results with the mishandled specimens are questionable. It is key in any reliability study to minimize or eliminate as many sources of bias as possible. Such discrepancies are direct threats to the validity of the findings.

A second preanalytical variable is ensuring the samples span the clinical range of analysis. That is, normal samples and abnormal samples should be analyzed. This is important as it has been shown that the alpha globulins in psittacines are poorly defined.[3] With high values, the bands are more visible, aiding increased precision. With equal numbers of abnormal EPH under review, these comparative measurements are different. This is briefly discussed in the *AJVR* article, and although the data are completely supportive, they are discarded in favor of questioning the overall reliability of EPH. Also notable is that precision with samples from raptors or waterfowl with resting values for alpha 1 of 20% to 30% (vs 2% to 5% for psittacines) would be quite different.

Last, rather than erroneously question the value of EPH, it is better to understand the finding that sample handling is important. Also, EPH does have a manual component, which is well discussed in the literature.[4] Interpretation of EPH results is based on a visual inspection of the gel, not just values. Knowledge of the species and major fractions aids in interpreting substantial changes. With an understanding of the technique, EPH can be found valuable as a prognostic indicator in general examinations and in dissecting test results for underlying disease.

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1. Koch DD, Peters T. Evaluation of methods—with an introduction to statistical techniques. In: Burtis CA, Ashwood ER, eds. *Tietz fundamentals of clinical chemistry*. 5th ed. Philadelphia: WB Saunders Co, 2001;234–250.

2. Pudlak KA, Donahue TM, Vladutiu AO. Alteration of electrophoretic mobility of serum globulins after storage. *Arch Pathol Lab Med* 1989;113:808–809.

3. Cray C, Tatum LM. Application of protein electrophoresis in avian diagnostic testing. *J Avian Med Surg* 1998;12:4–10.

4. Kahn SN, Strony LP. Imprecision of quantification of serum protein fractions by electrophoresis on cellulose acetate. *Clin Chem* 1986;32:356–357.

The authors respond:

Thank you for the opportunity to respond to Dr. Cray's letter. We appreciate her comments on laboratory reliability studies and wish to point out that our article was not intended to test that hypothesis. Clinicians perform their jobs in real time under a variety of conditions, not in an "ideal" laboratory setting. Our intent was to merely point out that there is considerable variability in electrophoresis (EPH) testing (regardless of laboratory) and clinicians should use caution in interpreting these results.

Although preparation and handling of samples with regard to sterility was not discussed in our paper, all samples were aseptically divided and sterility was never breached. We acknowledge that our samples were not sent in dehydration-proof containers, were not frozen, and were not shipped on dry ice. But the samples were all kept cold by refrigeration and sent by overnight courier on ice. This is how clinicians send in their samples. Few, if any, send in samples in dehydration-proof containers on dry ice. According to Dr. Cray, unless the clinician can send in samples that are sterile and on dry ice in dehydration-proof containers, the results that Dr. Cray's laboratory provides to clinicians would be questionable. It is of interest to note that Dr. Cray's laboratory (laboratory B in our study) processed all of our samples without any comment to us that the samples were improperly received, warm, or in improper containers.

We agree with Dr. Cray that more variability in the EPH results might improve the precision, but we clearly state this in our paper. She also states, "With high values, the bands are more visible, aiding increased precision… Also, EPH does have a manual component." We are delighted to see that Dr. Cray agrees with us, as we clearly discuss these two important points in the discussion. In fact, those two issues may be the crux of the unreliability of avian EPH results.

Finally, it should be noted that EPH testing provides income for Dr. Cray's laboratory, and this article may not be in that laboratory's best interest. It was not our intention to denigrate EPH testing or the work performed by either laboratory but to give avian veterinarians a better understanding of the test and its limitations. In fact, laboratory A has used our results to help improve clinicians' understanding of the EPH results with regard to the health status of their patients.

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