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Comparison of Agarose Gel Electrophoresis and Capillary Zone Electrophoresis Methods Using Serum from Bottlenose Dolphins (*Tursiops truncatus*)

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Abstract

Serum protein electrophoresis is commonly used in veterinary medicine to obtain a broad picture of acute phase and humoral immune responses. Agarose gel electrophoresis (AGE) has been the most prevalent platform in laboratory settings; however, in human medicine, this method is becoming increasingly supplanted by capillary zone electrophoresis (CZE). CZE has been demonstrated to have superior accuracy in fraction quantitation as well as consistently resolved protein fractions that are generally not observed by AGE. The objectives of the current study were to compare these two methods using serum from bottlenose dolphins (*Tursiops truncatus*) and to generate preliminary reference intervals for this species. The methods were compared using Spearman's correlation, Passing–Bablok regression, and Bland Altman analysis. A varying but significant correlation was observed between the methods for all protein fractions and the albumin/globulin (A/G) ratio except for the beta globulin fraction. Comparable or better imprecision in fraction quantitation was observed for CZE. Constant and proportional error was detected for both alpha globulin fractions. Of note, CZE allowed for an easier definition of the beta globulin fraction into two fractions (beta 1 and beta 2 globulins), and other subfractions were also visible. In total, these statistical and visible differences indicate that the two methods are not equivalent which necessitates the generation of method-specific reference intervals. Additionally, as it becomes more widely available, CZE should be considered as the preferred method for electrophoresis in this species.

Key Words: agarose gel electrophoresis, capillary zone electrophoresis, bottlenose dolphin, *Tursiops truncatus*, reference intervals

Introduction

Protein electrophoresis (EPH) methods are widely applied in human medicine, especially to detect monoclonal gammopathies associated with neoplasia (Eckersall, 2008). In veterinary medicine, EPH has been employed for this reason as well as to accurately quantitate albumin and to view changes in globulin fractions which include immunoglobulins and over 200 acute phase proteins (APPs) that might indicate the presence of inflammation or infection (Cray & Tatum, 1998; Eckersall, 2008). While both fields have benefited from the validation of various assays for APP quantitation like C-reactive protein, reagents have not been found to be cross reactive with many species. Therefore, EPH has remained a mainstay, allowing for a broad view of changes that may be present during an acute phase response which has been estimated to reflect over 200 proteins (Eckersall, 2008; Cray, 2011). As with the use of APPs, the quantitation of protein fractions by EPH can be utilized in health assessments as well as for prognostic purposes (Eckersall, 2008; Cray, 2011).

The agarose gel electrophoresis (AGE) method has previously been described using dolphin serum samples through which differences were observed between free-ranging animals and dolphins under human care (Bossart et al., 2001). In addition, studies using AGE have indicated differences between AGE and chemistry analyzer-derived albumin levels (Goldstein et al., 2006). Changes were also reported in dolphins with an array of infections and diseases, including the presence of orogenital papillomas and morbillivirus infection (Bossart et al., 2001, 2006, 2008, 2011). AGE is often used as a routine test in cetacean species under human care (Bossart et al., 2001; McBain, 2001) and has also been used in health assessments

of free-ranging dolphins (Fair et al., 2013). Most recently, AGE results were reported in conjunction with a newly validated reagent to measure serum amyloid A which is a major APP in this species (Cray et al., 2013; Miller et al., 2017, 2020).

Currently in veterinary reference laboratories, EPH is conducted using semi-automated platforms and AGE. In human clinical pathology laboratories, a newer technology has been mostly adopted called capillary zone electrophoresis (CZE) (Bossuyt, 2006; Roudiere et al., 2006). Rather than the use of solid substrate like agarose, the sample is suspended in a capillary tube in which the exposure to high voltage allows for the separation of the proteins (Roudiere et al., 2006). Also, instead of using a protein stain as is done in AGE, the fractions are quantitated by a UV detector. In both human and in recent veterinary studies of CZE, the opportunity to define fractions and subfractions has been reported (Bossuyt, 2006; Roudiere et al., 2006; Crivellente et al., 2008; Giordano & Paltrinieri, 2010; Roman et al., 2013; Regeniter & Siede, 2018; Leineweber et al., 2019, 2020; Toonder et al., 2020). This has been observed in mammals, including the cat, dog, mouse, and marmoset (Crivellente et al., 2008; Giordano & Paltrinieri, 2010). In addition, various bird and reptile species have also been examined (Roman et al., 2013; Leineweber et al., 2019, 2020). To date, there has been a single implementation of CZE in bottlenose dolphins to study hereditary bisalbuminemia (Gili et al., 2016). The goals of the current study were to calculate new reference intervals for CZE and to compare AGE and CZE methods.

Methods

Samples

Fifty-four serum samples were obtained from free-ranging bottlenose dolphins in the Indian River Lagoon in Florida as part of the Dolphin Health and Environmental Risk Assessment (HERA) Project. These samples were collected from animals assessed with a normal physical examination and no bloodwork abnormalities (Reif et al., 2008). The sample set was composed of 31 males, 22 females, and one dolphin of unknown sex with an estimated age range of 3 to 16 y. Samples were frozen at -80ºC until transport to the University of Miami's Avian & Wildlife Laboratory in Miami, Florida, for analysis.

Agarose Gel Electrophoresis

Samples were analyzed using split beta gels and the SPIFE 3000 system (Helena Laboratories, Inc., Beaumont, TX, USA) as previously described (Bossart et al., 2012). Percentages of gel fractions were determined after scanning and then multiplied by total protein for the determination of absolute fraction values. The albumin/globulin (A/G) ratio was calculated by dividing the sum of prealbumin and albumin by the sum of the globulin fractions. The instrument was maintained according to manufacturer instructions, and normal and abnormal human control samples were utilized. Total protein was determined by the biuret method using a chemistry analyzer (Ortho Vitros 250; Ortho Vitros Diagnostics, Rochester, NY, USA).

Capillary Zone Electrophoresis

Samples were analyzed using the Capillarys Flex 2 piercing instrument following manufacturer procedures and a 1:4 dilution with urine protocol buffer (Sebia, Norcross, GA, USA). The Sebia analysis software was toggled to remove the automatic anodic and cathodic limits of the detected protein curve. The instrument was maintained according to manufacturer instructions, and normal and abnormal human control samples were utilized.

Method Comparison and Statistics

Statistical analyses were performed using *Graph Pad Prism*, Version 6.07 (La Jolla, CA, USA) and *MedCalc*, Version 19.2.1 (Ostend, Belgium). Method comparison was performed as previously described (Jensen & Kjelgaard-Hansen, 2006) and included Spearman's correlation, Passing–Bablok regression, and Bland Altman plots. Coefficient of variation (CV) analysis was performed to determine an intra-assay evaluation: two samples were run eight times in 1 d using both methods. Reference intervals were calculated and presented per American Society for Veterinary Clinical Pathology (ASVCP) guidelines; outliers (complete data for each animal for which substantial outliers were present) were removed after identification by Tukey's test as far out outliers (Friedrichs et al., 2012).

Results

Fraction Delimitation

Based on previous work with this species, six fractions—prealbumin, albumin, alpha 1, alpha 2, beta, and gamma globulins—were quantitated using AGE (Figure 1A). For CZE, more fractions could be defined (Figure 1B), including prealbumin, albumin, alpha 1, alpha 2, beta 1, beta 2, and gamma globulins. Of note, alpha 1, alpha 2, and beta globulin fractions often showed the presence of at least two protein bands that were not all consistently apparent in AGE. Fraction values may be affected by imprecision in the placement of fraction delimits which is done both by analysis software and technical staff. This imprecision was

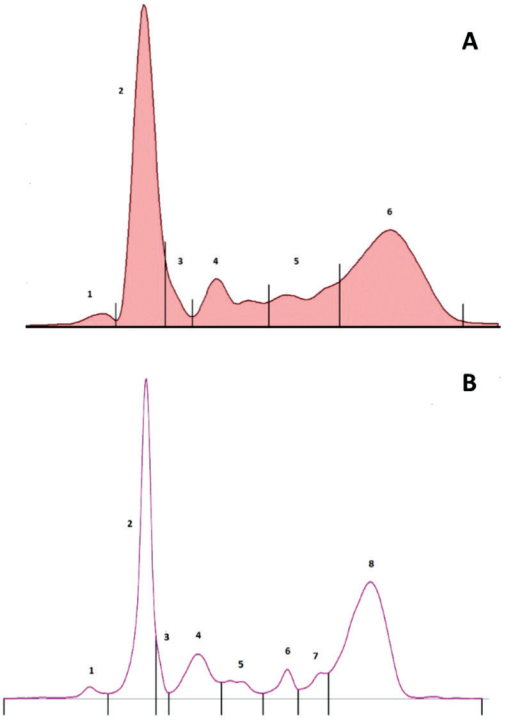

Figure 1. AGE (A) and CZE (B) derived electrophoretograms noting the number of fractions observed by each method. The same sample was analyzed by both methods. AGE fractions 1 to 6 correspond to prealbumin, albumin, alpha 1, alpha 2, beta, and gamma globulins. CZE fractions 1 to 8 correspond to prealbumin, albumin, alpha 1, alpha 2-1, alpha 2-2, beta 1, beta 2, and gamma globulins.

gauged by CV analysis. As samples were run in one batch, only intraday variation was assessed. The highest variation was observed for prealbumin which ranged between 7.8 to 14.9% by AGE but only 3.6 to 3.7% for CZE. The CV for the other fractions were lower or comparable by CZE. The largest globulin difference was observed in alpha 1 globulins for which the CV was 9.3 and 2.6% for AGE and CZE, respectively.

Reference Intervals

Reference intervals were generated per ASVCP guidelines (Table 1). All data were transformed before analysis using the robust method. Nine animals with repeated outliers were removed based on Tukey's test with notation as far out outliers.

Method Comparison

Passing–Bablok regression (Table 2) and Bland Altman analysis (Figure 2) were conducted, and the methods were found not to be equivalent. All fractions and A/G ratios were found to be significantly correlated between the methods to varying levels except for the beta globulin fraction. Constant and proportional error, based on the confidence intervals of y-intercept and the slope of the regression line, was found for both alpha globulin fractions. The difference between the methods is also evident in the Bland Altman plots (Figure 2).

Discussion

EPH has been used in veterinary medicine for many years (Eckersall, 2008). In addition, it has been well utilized in dolphins under human care and in health assessments of free-ranging dolphins with the goal of detecting underlying inflammatory processes and humoral immune responses (Bossart et al., 2001, 2006, 2008, 2011; Fair et al., 2013; Miller et al., 2020). In the current study, CZE analysis provided an increased level of detection of protein fractions with a clear definition of beta 1 and beta 2 fractions. In addition, other subfractions were evident, including an opportunity to subdivide alpha 2 into two fractions. These fractions should be a focus of future CZE studies, comparing samples obtained from clinically normal and abnormal dolphins to assess the clinical and research applications of this increased fraction resolution. Overall, as reported in studies involving other mammalian, reptile, and avian species, CZE was also found to have a higher precision than AGE, and these methods are not equivalent, necessitating the generation of method-specific reference intervals (Crivellente et al., 2008; Giordano & Paltrinieri, 2010; Roman et al., 2013; Leineweber et al., 2019, 2020; Toonder et al., 2020).

The reference intervals generated in the current study should be considered preliminary given the span of ages and low sample size. Compared to a previous study on AGE in free-ranging dolphins (n = 19), the sample set in the current study has generally resulted in overlapping and, in some cases, narrower intervals (Bossart et al., 2012). The observed differences in CZE include a higher overall level of alpha 2 globulins and a narrower range (as determined by mean ± SD) in alpha 2, beta, and gamma globulins. This may be related to the use of different sample sets obtained from the multi-year health assessment study and/or the increased precision of the CZE method.

Compared to a previous study to detect bisalbuminemia in dolphins under human care which utilized CZE, the albumin concentration was lower (Gili et al., 2016). However, this may be related to the previously described

Table 1. Reference intervals (RIs) for CZE fractions; sample size: $n = 45$. LRL = lower reference limit, URL = upper reference limit.

Analyte	Mean	SD	Median	Min	Max	RI	LRL (90% CI)	URL (90% CI)	Distribution[a]	Method[b]
Total protein (g/dL)	7.5	0.9	7.4	5.2	9.2	5.6-9.4	5.2-6.0	9.0-9.8	G	R, T
A/G ratio	0.77	0.09	0.76	0.61	1.03	0.57-0.95	0.53-0.62	0.91-1.00	G	R, T
Prealbumin (%)	1.5	0.8	1.3	0.3	4.5	0-2.9	-0.9-0.1	2.4-3.4	NG	R, T
Prealbumin (g/dL)	0.11	0.06	0.10	0.02	0.32	0-0.22	-0.06-0.01	0.18-0.25	NG	R, T
Albumin (%)	41.9	2.8	41.7	36.4	49.0	36.0-47.5	34.9-37.3	46.2-48.8	G	R, T
Albumin (g/dL)	3.12	0.41	3.06	2.33	4.30	2.24-3.93	2.06-2.46	3.73-4.12	G	R, T
Alpha 1 (%)	4.7	1.1	4.5	0.3	8.9	2.4-6.8	1.6-3.5	5.6-7.6	NG	R, T
Alpha 1 (g/dL)	0.35	0.08	0.35	0.02	0.62	0.18-0.51	0.13-0.25	0.44-0.57	NG	R, T
Alpha 2 (%)	12.4	1.2	12.2	10.3	15.6	9.8-14.9	9.3-10.3	14.3-15.4	G	R, T
Alpha 2 (g/dL)	0.92	0.14	0.91	0.63	1.24	0.64-1.20	0.59-0.69	1.14-1.26	G	R, T
Beta 1 (%)	7.1	1.4	7.0	0.7	9.4	4.4-10.1	3.3-5.3	9.0-11.1	NG	R, T
Beta 1 (g/dL)	0.24	0.06	0.23	0.13	0.39	0.11-0.35	0.09-0.14	0.32-0.38	G	R, T
Beta 2 (%)	4.0	0.7	4.0	2.8	5.2	2.6-5.4	2.3-2.9	5.2-5.6	G	R, T
Beta 2 (g/dL)	0.30	0.07	0.29	0.15	0.47	0.16-0.43	0.13-0.19	0.40-0.46	G	R, T
Total beta (g/dL)	11.2	1.3	11.1	8.7	13.9	8.4-13.9	8.0-8.9	13.2-14.5	G	R, T
Total beta (g/dL)	0.84	0.13	0.82	0.48	1.09	0.56-1.11	0.51-0.62	1.04-1.17	G	R, T
Gamma (%)	28.3	3.5	28.5	18.4	36.2	21.2-35.6	19.6-22.9	34.1-37.2	G	R, T
Gamma (g/dL)	2.12	0.42	2.15	1.24	2.98	1.25-2.98	1.08-1.44	2.79-3.15	G	R, T

[a]G = Gaussian, NG = non-Gaussian; [b]R = robust, T = transformed

Table 2. Passing-Bablok regression analysis, Bland Altman analysis, and Spearman's correlation of electrophoresis fractions quantitated by AGE and CZE in sera from bottlenose dolphins (*Tursiops truncatus*); sample size: $n = 54$.

Fraction	AGE, median (IQR[a])	CZE, median (IQR)	Spearman's correlation, r value (p value)	Passing-Bablok regression (y-intercept, slope)[b]	Bland Altman bias, mean (SD)
Prealbumin (%)	2.2 (1.8-2.8)	1.3 (1.0-1.6)	0.37 ($p = 0.0059$)	NE (0.69, 1.16)	-0.80 (0.93)
Albumin (%)	49.1 (47.3-51.8)	41.6 (39.4-44.0)	0.87 ($p < 0.0001$)	NE (6.05, 1.04)	-7.3 (2.2)
Alpha 1 (%)	3.3 (2.8-4.2)	4.5 (4.2-5.0)	0.34 ($p = 0.0116$)	CE, PE (-5.85, 2.00)	1.2 (1.2)
Alpha 2 (%)	10.7 (9.2-12.3)	12.5 (11.5-13.4)	0.53 ($p < 0.0001$)	CE, PE (-8.62, 1.57)	3.9 (15.2)
Beta (%)	9.3 (7.7-9.9)	11.1 (10.3-12.2)	0.03 ($p = 0.81$)	NE (-5.62, 1.31)	2.3 (2.0)
Gamma (%)	25.7 (23.0-28.7)	28.9 (25.0-31.8)	0.91 ($p < 0.0001$)	NE (0.88, 0.88)	2.8 (2.0)
A/G ratio	1.1 (0.94-1.17)	0.76 (0.68-0.84)	0.81 ($p < 0.0001$)	PE (-0.03, 1.44)	-0.31 (0.13)

[a]IQR = interquartile range; [b]NE = no error, CE = constant error, and PE = proportional error

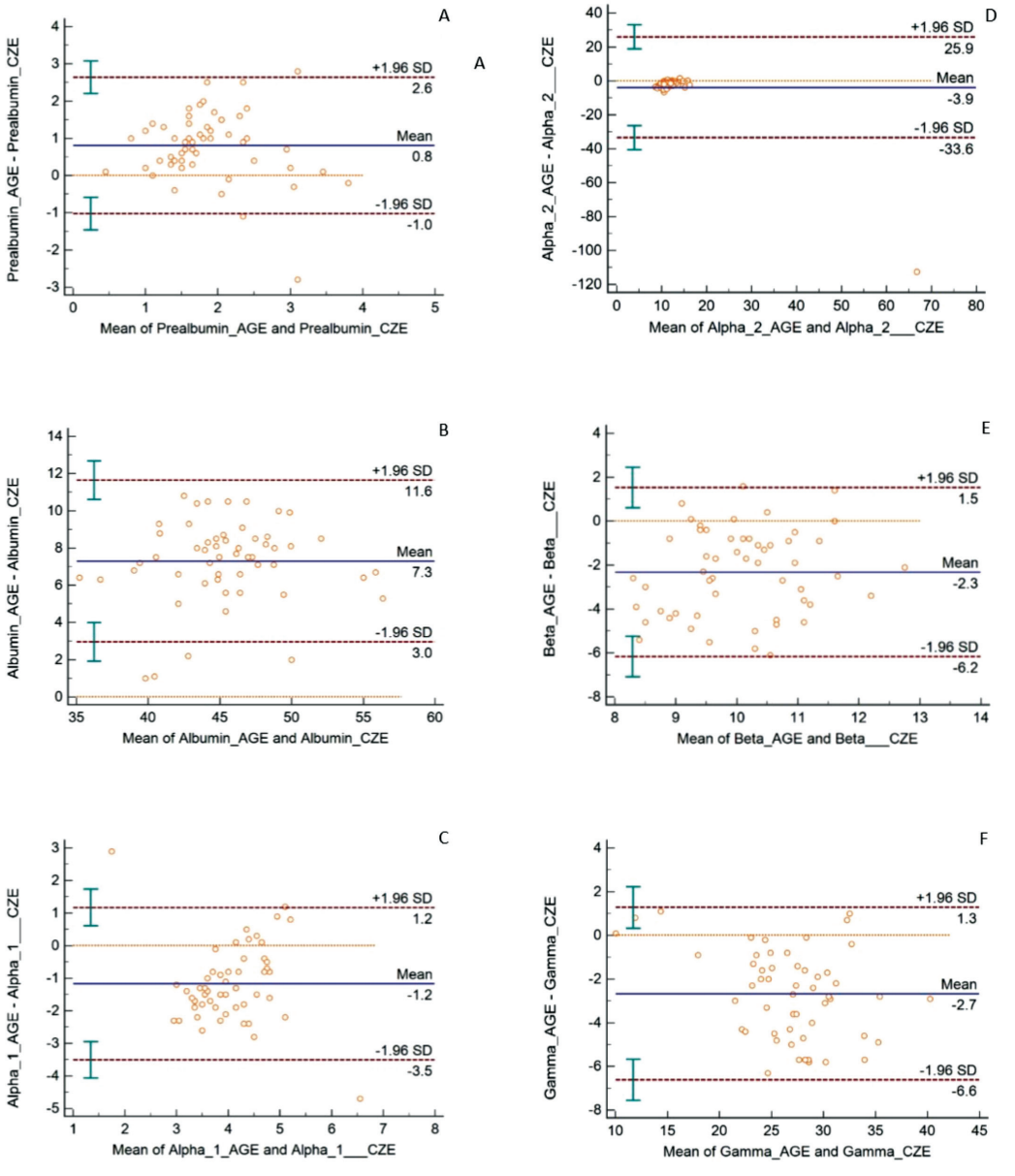

Figure 2. Bland Altman analysis of AGE and CZE data

differences observed in free-ranging vs managed care dolphins (Bossart et al., 2012). In the current study, none of the samples analyzed by CZE demonstrated bisalbuminemia, although this has been observed in samples from some bottlenose dolphins (C. Cray, pers. comm., 21 July 2020). Overall, CZE was noted to provide a lower quantitation of albumin levels. This was not observed in previous studies of humans and other mammals (Bossuyt, 2006; Crivellente et al., 2008; Giordano & Paltrinieri, 2010). Notably, CZE has been reported to overestimate human albumin concentrations when the levels are below 3 g/dL, which is within the range of values reported in the current study (Padelli et al., 2018). Further studies should be performed to address this difference when using dolphin sera.

With the advent of test options to quantitate serum amyloid A, which is a major APP in the dolphin, EPH may be used less often in routine health assessments in this species (Cray et al., 2013; Miller et al., 2017, 2020). While the magnitude of change is impressive in some clinically abnormal animals, this APP may not change in response to all stimuli and acute and chronic conditions (Cray, 2011). As CZE provides a clear resolution of fractions and subfractions not previously observed by AGE, the transition from AGE to CZE will ensure the use of an updated EPH method to view more changes that are present during the acute phase response. Future studies should examine the application of this new level of quantitation in samples from dolphins which are free ranging or under human care with a variety of health conditions. This may aid in the identification of new sensitive markers of disease or environmental stressors in the bottlenose dolphin.

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Literature Cited

Bossart, G. D., Reiderson, T. H., Dierauf, L. A., & Duffield, D. A. (2001). Clinical pathology. In L. A. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (2nd ed., pp. 383-436). CRC Press. https://doi.org/10.1201/9781420041637.sec4

Bossart, G. D., Goldstein, J. D., Murdoch, E. M., Fair, P. A., & McCulloch, S. (2006). *Health assessment of bottlenose dolphins in the Indian River Lagoon, Florida and Charleston, South Carolina* (Harbor Branch Oceanographic Technical Report 93). 36 pp.

Bossart, G. D., Romano, T. A., Peden-Adams, M. M., Rice, C. D., Fair, P. A., Goldstein, J. D., Kilpatrick, D., Cammen, K., & Reif, J. S. (2008). Hematological, biochemical, and immunological findings in Atlantic bottlenose dolphins (*Tursiops truncatus*) with orogenital papillomas. *Aquatic Mammals*, *34*(2), 166-177. https://doi.org/10.1578/AM.34.2.2008.166

Bossart, G. D., Romano, T. A., Peden-Adams, M. M., Schaefer, A., McCulloch, S., Goldstein, J. D., Rice, C. D., Saliki, J. T., Fair, P. A., & Reif, J. S. (2011). Clinicoimmunopathologic findings in Atlantic bottlenose dolphins *Tursiops truncatus* with positive cetacean morbillivirus antibody titers. *Diseases of Aquatic Organisms*, *97*(2), 103-112. https://doi.org/10.3354/dao02410

Bossart, G., Arheart, K., Hunt, M., Clauss, T., Leppert, L., Roberts, K., McCulloch, S., Goldstein, J. D., Gonzalez, C., Sweeney, J., Stone, R., Fair, P. A., & Cray, C. (2012). Protein electrophoresis of serum from healthy Atlantic bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals*, *38*(4), 412-417. https://doi.org/10.1578/AM.38.4.2012.412

Bossuyt, X. (2006). Advances in serum protein electrophoresis. *Advances in Clinical Chemistry*, *42*, 43-80. https://doi.org/10.1016/S0065-2423(06)42002-3

Cray, C. (2011). Acute phase proteins in animals. *Progress in Molecular Biology and Translational Science*, *105*, 113-150. https://doi.org/10.1016/B978-0-12-394596-9.00005-6

Cray, C., & Tatum, L. (1998). Application of protein electrophoresis in avian diagnostic testing. *Journal of Avian Medicine and Surgery*, *12*(1), 4-10.

Cray, C., Arheart, K. L., Hunt, M., Clauss, T., Leppert, L. L., Roberts, K., McCulloch, S. D., Goldstein, J. D., Gonzalez, C., Sweeney, J., Stone, R., Fair, P. A., & Bossart, G. D. (2013). Acute phase protein quantitation in serum samples from healthy Atlantic bottlenose dolphins (*Tursiops truncatus*). *Journal of Veterinary Diagnostic Investigation*, *25*(1), 107-111. https://doi.org/10.1177/1040638712467986

Crivellente, F., Bonato, M., & Cristofori, P. (2008). Analysis of mouse, rat, dog, marmoset, and human serum proteins by capillary electrophoresis: Comparison with agarose gel electrophoresis. *Veterinary Clinical Pathology*, *37*(1), 73-78. https://doi.org/10.1111/j.1939-165X.2008.00008.x

Eckersall, P. D. (2008). Proteins, proteomics, and the dysproteinemias. In J. J. Kaneko, J. W. Harvey, & M. L. Bruss (Eds.), *Clinical biochemistry of domestic animals* (6th ed., pp. 117-155). Academic Press. https://doi.org/10.1016/B978-0-12-370491-7.00005-2

Fair, P. A., Romano, T., Schaefer, A. M., Reif, J. S., Bossart, G. D., Houde, M., Muir, D., Adams, J., Rice, C., Hulsey, T. C., & Peden-Adams, M. (2013). Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (*Tursiops truncatus*). *Environmental Toxicology and Chemistry*, *32*(4), 736-746. https://doi.org/10.1002/etc.2122

Friedrichs, K., Harr, K., Freeman, K., Szladovits, B., Walton, R., Barnhart, K., & Blanco-Chavez, J. (2012). ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, *41*(4), 441-453. https://doi.org/10.1111/vcp.12006

Gili, C., Bonsembiante, F., Bonanni, R., Giordano, A., Ledda, S., Beffagna, G., Paltrinieri, S., Sommer, M., & Gelain, M. E. (2016). Detection of hereditary bisalbuminemia in bottlenose dolphins (*Tursiops truncatus*, Montagu 1821): Comparison between capillary zone and agarose gel electrophoresis. *BMC Veterinary Research*, *12*(1), 172. https://doi.org/10.1186/s12917-016-0801-x

Giordano, A., & Paltrinieri, S. (2010). Interpretation of capillary zone electrophoresis compared with cellulose acetate and agarose gel electrophoresis: Reference intervals and diagnostic efficiency in dogs and cats. *Veterinary Clinical Pathology*, *39*(4), 464-473. https://doi.org/10.1111/j.1939-165X.2010.00255.x

Goldstein, J. D., Reese, E., Reif, J. S., Varela, R. A., McCulloch, S. D., Defran, R. H., Fair, P. A., Bossart, G. D., & Hansen, L. (2006). Hematologic, biochemical, and cytologic findings from apparently healthy Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting the Indian River Lagoon, Florida, USA. *Journal of Wildlife Diseases*, *42*(2), 447-454. https://doi.org/10.7589/0090-3558-42.2.447

Jensen, A. L., & Kjelgaard-Hansen, M. (2006). Method comparison in the clinical laboratory. *Veterinary Clinical Pathology*, *35*(3), 276-286. https://doi.org/10.1111/j.1939-165X.2006.tb00131.x

Leineweber, C., Ofner, S., Stohr, A. C., Marschang, R. E., & Mathes, K. (2020). A comparison of thyroid hormone levels and plasma capillary zone electrophoresis in red-eared sliders (*Trachemys scripta elegans*) and map turtles (*Graptemys* spp.) depending on season and sex. *Veterinary Clinical Pathology*, *49*(1), 78-90. https://doi.org/10.1111/vcp.12838

Leineweber, C., Stohr, A. C., Ofner, S., Mathes, K., & Marschang, R. E. (2019). Reference intervals for plasma capillary zone electrophoresis in Hermann's tortoise (*Testudo hermanni*) depending on season and sex. *Journal of Zoo & Wildlife Medicine*, *50*(3), 611-618. https://doi.org/10.1638/2018-0155

McBain, J. F. (2001). Cetacean medicine. In L. A. Dierauf & F. M. D. Gulland (Eds.), *CRC handbook of marine mammal medicine* (2nd ed., pp. 895-906). CRC Press. https://doi.org/10.1201/9781420041637.sec8

Miller, S., Cray, C., Schaefer, A. M., Reif, J. S., Roberts, K., & Bossart, G. D. (2020). Assessment of serum amyloid A, haptoglobin, and protein electrophoresis in clinically healthy and abnormal bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals*, *46*(2), 131-136. https://doi.org/10.1578/AM.46.2.2020.131

Miller, S. N., Davis, M., Hernandez, J. A., St. Leger, J., Cray, C., Bishop, P., & Stacy, N. I. (2017). Serum amyloid A in healthy female bottlenose dolphins (*Tursiops truncatus*) during and after uncomplicated pregnancy. *Aquatic Mammals*, *43*(4), 417-420. https://doi.org/10.1578/AM.43.4.2017.417

Padelli, M., Leven, C., Le Reun, E., Labouret, T., Galakhoff, N., Labarre, M., Rouille, A., Kerspern, H., Lapegue, M., Chauvet, J., Plee-Gautier, E., & Carré, J-L. (2018). Is capillary zone electrophoresis a suitable method for estimating serum albumin: A comparison of four methods. *Clinica Chimica Acta*, *487*, 250-255. https://doi.org/10.1016/j.cca.2018.10.009

Regeniter, A., & Siede, W. H. (2018). Peaks and tails: Evaluation of irregularities in capillary serum protein electrophoresis. *Clinical Biochemistry*, *51*, 48-55. https://doi.org/10.1016/j.clinbiochem.2017.09.017

Reif, J. S., Fair, P. A., Adams, J., Joseph, B., Kilpatrick, D. S., Sanchez, R., Goldstein, J. D., Townsend, F. I., Jr., McCulloch, S. D., Mazzoil, M., Zolman, E. S., Hansen, L. J., & Bossart, G. D. (2008). Evaluation and comparison of the health status of Atlantic bottlenose dolphins from the Indian River Lagoon, Florida, and Charleston, South Carolina. *Journal of the American Veterinary Medical Association*, *233*(2), 299-307. https://doi.org/10.2460/javma.233.2.299

Roman, Y., Bomsel-Demontoy, M-C., Levrier, J., Chaste-Duvernoy, D., & Saint Jalme, M. (2013). Plasma protein electrophoresis in birds: Comparison of a semiautomated agarose gel system with an automated capillary system. *Journal of Avian Medicine and Surgery*, *27*(2), 99-108. https://doi.org/10.1647/2011-040

Roudiere, L., Boularan, A. M., Bonardet, A., Vallat, C., Cristol, J. P., & Dupuy, A. M. (2006). Evaluation of a capillary zone electrophoresis system versus a conventional agarose gel system for routine serum protein separation and monoclonal component typing. *Clinical Laboratory*, *52*(1-2), 19-27.

Toonder, M., Perrault, J. R., & Cray, C. (2020). Comparison of agarose gel and capillary zone electrophoresis methods using plasma from green turtles (*Chelonia mydas*). *Journal of Zoo & Wildlife Medicine*, *51*(1), 123-130. https://doi.org/10.1638/2019-0120