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Error in Hematocrit of EDTA-preserved Whole Blood from Black Bears Caused by Delayed Analysis

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ABSTRACT: Hematocrit values for K_3EDTA-preserved whole blood from black bears (*Ursus americanus*) were found to increase during refrigerated storage causing error in delayed laboratory analysis. This error was quantified using a regression type model based on repeated hematocrit testing of 66 blood samples over time. The model proved to fit quite well and provided corrected-to-day-zero hematocrit values for 42 samples from which day-zero values were not available.

Key words: Black bear, hematocrit, hematology, K_3EDTA, Minnesota, *Ursus americanus*.

Hematological assays are not always conducted the same day that samples are collected. Brittin et al. (1969) and Grenn et al. (1976) found that erythrocytes of various species collected in ethylenediaminetetra-acetate (EDTA) underwent changes in volume upon standing. This note quantifies the error in hematocrit values caused by delayed analysis of whole blood from the black bear collected in liquid K_3EDTA.

Samples of whole blood were collected from 66 chemically immobilized (11 mg/kg ketamine-HCl) black bears using Becton-Dickinson Vacutainer® brand holders, needle adapters with 20-ga needles and A3206QS collection tubes. The tubes were allowed to fill to capacity. Initial microhematocrits were determined either the day of collection (day-0) or the following day (day-1) using plain microhematocrit capillary tubes and centrifuged in an International Model MB Micro Hematocrit Centrifuge at 13,000 *g* for 4 min. An additional 259 microhematocrits were run on various subsequent days for up to 14 days after the collection date. Samples were refrigerated at 4 C between tests. Samples were allowed to come to room temperature and mixed on a rotary mixer for at least 15 min before hematocrits were taken. A minimum of two and a maximum of 11 tests were run on any individual blood sample.

Preliminary evidence indicated that we should expect to see an increase in hematocrit values over time when K_3EDTA-preserved whole blood from black bears was left standing. We did not expect changes to be linear with time. For lack of any applicable theoretical models and the general attractiveness of linearized parameters, we assumed that changes in hematocrit over time could be aptly described using a quadratic taking the form [1] ($\text{HEMATOCRIT} = ax^2 + bx + c$) where x = days since blood was sampled. Moreover, we assumed that the parameter influencing rate of change (a and b of [1]) would be common to all of our blood samples from bears, whereas the hematocrit value at day zero (c of [1]) would be unique to each bear. These assumptions allowed us to exploit the general methodology developed by Rosenberg (1973) in the following way. First, we randomly selected 21 of 42 cases (blood samples from bears) for which day-zero hematocrits were available. These were combined with all 24 cases for which no day-zero hematocrits were available so as to form a model training set. Given these data, we used maximum likelihood methods to fit the previously mentioned model. The model developed on the training set was used to

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TABLE 1. Estimated and observed day zero hematocrits for 66 whole blood samples taken from black bears in Minnesota and preserved in K_3EDTA.

Sample size	Observed hematocrit	Predicted hematocrit	Variance of pred.	Standard error
2	33.0	32.8	37.85728	4.35071
2	47.0	47.0	37.85728	4.35071
2	45.5	45.3	37.85728	4.35071
2	41.2	40.9	37.85728	4.35071
2	35.8	35.6	37.85728	4.35071
2	40.5	40.7	37.85728	4.35071
2	43.0	42.8	37.85728	4.35071
2	44.0	44.1	37.85728	4.35071
2	40.8	40.3	37.85728	4.35071
2	47.0	47.1	37.85728	4.35071
2	39.4	39.3	37.85728	4.35071
2	32.0	31.4	37.85728	4.35071
2	40.0	39.6	37.85728	4.35071
2	40.1	40.5	37.85728	4.35071
2	41.3	41.7	37.85728	4.35071
2	35.9	35.9	37.85728	4.35071
2	42.8	42.7	37.85728	4.35071
2	39.5	39.3	37.85728	4.35071
2	42.5	43.1	37.85728	4.35071
2	37.3	37.0	37.85728	4.35071
2	40.5	41.3	37.85728	4.35071
2	39.0	39.2	37.85728	4.35071
2		39.8	37.85728	4.35071
2		38.1	37.85728	4.35071
2	44.0	44.3	37.85728	4.35071
2	39.8	39.4	37.85728	4.35071
2		37.9	37.85728	4.35071
2		41.3	37.85728	4.35071
2		40.6	37.85728	4.35071
2		36.2	37.85728	4.35071
3	42.0	41.7	25.26846	2.90221
3	35.5	36.0	25.26846	2.90221
2	41.0	40.6	37.85728	4.35071
3	39.0	39.4	25.26846	2.90221
7	47.0	46.6	10.84421	1.24466
6		54.4	12.64940	1.45198
5		52.8	15.17564	1.74216
5		53.2	15.17564	1.74216
8		46.5	9.48990	1.08915
8		49.5	9.48990	1.08915
9	46.1	45.3	8.43631	0.96818
8		46.5	9.48990	1.08915
8		39.7	9.48990	1.08915
8		41.2	9.48990	1.08915
8		45.3	9.48990	1.08915
9	53.1	53.4	8.43631	0.96818
9	49.4	49.1	8.43631	0.96818
9	55.9	54.8	8.43631	0.96818
9	46.1	45.6	8.43631	0.96818
7		39.9	10.84421	1:24466

TABLE 1. Continued.

Sample size	Observed hematocrit	Predicted hematocrit	Variance of pred.	Standard error
8	46.2	46.9	9.48990	1.08915
7	50.1	49.0	10.84421	1.24466
10		49.7	7.59329	0.87139
9		49.5	8.43631	0.96818
9		58.8	8.43631	0.96818
11	47.2	46.3	6.90344	0.79220
11	55.9	55.5	6.90344	0.79220
9		47.9	8.43631	0.96818
9		47.5	8.43631	0.96818
9		49.4	8.43631	0.96818
9		48.0	8.43631	0.96818
9	44.0	43.9	8.43631	0.96818
7		50.5	10.84421	1.24466
6	44.4	43.9	12.64940	1.45198
5	47.3	47.3	15.17564	1.74216
3	49.2	48.9	25.26846	2.90221

predict day-zero hematocrit values for the validation set. Both average absolute errors and average squared errors calculated as measures of model predictive ability.

The mean daily cumulative increase in hematocrit value (volume %) over the 14-day trial demonstrated an early near linear increase which flattened out near the end of the trial (Fig. 1). The linear quadratic model fitted to the training set performed quite well. The average absolute error in predicting day zero hematocrit values for the 21 blood samples in the validation set was 0.65 with a maximum absolute error of 1.47. Good performance of the model using training and validation sets prodded us to duplicate the modelling effort over all blood samples combined. The resultant quadratic equation for predicting mean hematocrit for Minnesota black bears given the time delay to laboratory analysis was (MEAN HEMATOCRIT = -0.0343 (days stored)^2 + 1.04 (days stored) + 44.1) with an overall prediction variance of 0.234. The estimated day-zero hematocrit values for all 66 blood samples in the test are given in Table 1. Note that each blood sample would have its own predictive equation because we assumed that inter-

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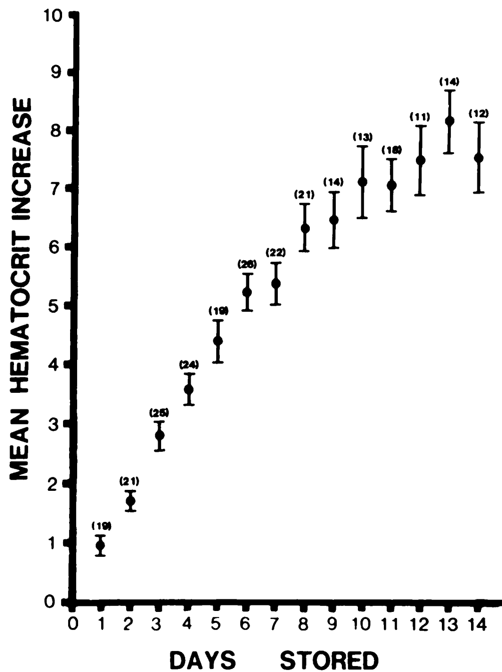

FIGURE 1. Mean daily cumulative increase (±2 SE) in hematocrit values (volume %) in blood from black bears drawn in K_3EDTA and stored at 4 C. The numbers in parentheses indicate the number of observations for that period.

cepts vary across bears. The adjusted r^2 for this set of predictive equations was 0.995, with the average absolute residuals being 0.324. The estimated parameters for this model apply only to the Minnesota black bear study, but the goodness of fit clearly demonstrates an association between hematocrit value and days stored.

The effect of increasing hematocrit over time in K_3EDTA-preserved black bear whole blood samples could cause considerable error in the resulting hematocrit and cell indices from samples in transit, held over weekends, or delayed in analysis for other reasons. The problem might be eliminated by using a balanced mixture of EDTA salts as demonstrated by Dubin et al. (1976) or the use of heparin as the anticoagulant. However, a balanced EDTA mixture is not commercially available, and heparin is more expensive and has an adverse effect on the staining of blood films.

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