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Comparison between Manual and Automated Methods for Determination of Canine and Feline Hematocrit and Hemoglobin Concentration

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ABSTRACT

A comparison of automated and manual methods to determine the hematocrit (Hct) and hemoglobin (Hb) concentrations was carried out using 50 canine and 30 feline blood samples. There were significant differences between manual and automated Hct for both the dog ($P<0.05$) and cat ($P<0.01$) samples, as well as between the manual cyanmethemoglobin and automated cyanide-free methods for both species (dog $P<0.01$, cat $P<0.05$). However, strong correlations using Pearson's correlation coefficient, R, between the two methods were observed (R for Hct of dog = 0.96, cat = 0.98 and R for Hb of dog = 0.96, cat = 0.87). The results indicated that the Hct and Hb values from the automated method could not be used to substitute for those of the manual method, though the values of the two methods were accurate and precise.

Key words: cat, cyanide-free hemoglobin, cyanmethemoglobin, dog, hematocrit

INTRODUCTION

The hematocrit (Hct) and hemoglobin (Hb) concentration are essential parameters of a complete blood count to determine the health status of an individual. Anemia is an unhealthy condition that develops in animals when the Hct and Hb levels in their blood are below certain specified limits (Jain, 1986).

Hct is the volume of the red blood cells compared to the volume of the whole blood sample (Lassen and Weiser, 2004), whereas Hb is a porphyrin iron protein compound that transports oxygen from the lungs to the body as well as carrying a major portion of carbon dioxide from the body to the lungs. Thus, a reduction in the Hct and Hb levels will result in an adverse effect on an animal's health status (Dessparis, 1999). The standard method for measuring the Hb concentration is the cyanmethemoglobin method as recommended by WHO (USAID, 1997). However, this method requires the handling of hazardous chemicals such as cyanide.

The gold standard for Hct estimation is the centrifugation method. Centrifuged Hct has a built-in bias due to the trapped plasma. When red cells had very abnormal shapes, this trapped plasma may be increased enough to cause a significant change in the Hct level (Kathleen, 2006). Hct in an automated measurement system is a calculated value. The volume of each cell is measured as it is counted and a mean cell volume

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is derived (Knoll, 2000). Hct can also be measured as the mean corpuscular red cell volume (MCV) multiplied by the red cell count (RBC). The two methods are not exactly the same. Hence, anything affecting the red cell count or volume measurement will affect the calculation of Hct (Kathleen, 2006).

Today, hematology analyzers based on Coulter's impedance principle are widely distributed in veterinary practices. These analyzers can generate a large number of novel parameters including the Hct and Hb concentration. Cell Dyn 3500® (CD 3500) is a fully-automated hematology system which is based on a combination of the impedance method and flow cytometry. Blood cells are counted by two separated channels; the electrical impedance and the optical flow channel. Hb concentration has been measured in the Hb flow cell on the basis of a cyanide-free procedure (Abbott, 1997). The objectives of this study were to assess the analytical performances between the gold standard method and the automated method by comparing centrifuged Hct to the calculated Hct and by comparing the cyanide and the cyanide-free Hb methods using canine and feline blood samples.

MATERIALS AND METHODS

Studied samples

Blood samples from the Veterinary Teaching Hospital, Kasetsart University, Thailand were randomly taken from 50 dogs and 30 cats regardless of age, sex and health status. All samples were anticoagulated with K_3-EDTA and processed within six hours of venupuncture. After proper mixing, the Hct and Hb concentration were estimated by Cell Dyn 3500®. Parallel estimations for the Hct and Hb concentration were done manually by microhematocrit centrifuge (Heraeus Sepatech microcentrifuge; Kalkberg, Germany) and spectrophotometer (Humalyzer 2000®; Wiesbaden, Germany), respectively.

Analytical procedures

Under the automated method, Hct was calculated from MCV and RBC, while Hb concentration was estimated by the cyanide-free method, using pre-installed software designed for veterinary applications. Red blood cells were lysed by a commercial reagent, Cell-Dyn CN-Free HGB/WIC lyse. The main components of this reagent were; quaternary ammonium salt 3.57%, hydroxylamine salt 1.68% and dodecyltrimethyl-ammonium chloride 3.57% (Abbott, 2006). The reagent converted Hb to a hemoglobin hydroxylamine (Hb_{HL}) complex which was a stable chromogen. The Hb concentration was directly proportional to the absorbance of the sample at 540 nm from the light source (Abbott, 1997).

With the manual method, the Hct and Hb concentration were determined by capillary centrifugation and the cyanmethemoglobin (Hb_{cy}) method, respectively. Twenty microlitres of blood sample reacted with Drabkin's reagent to form cyanmethemoglobin, which was assayed photometrically at 540 nm. The reagent used in this procedure contained 200 mg potassium ferricyanide, 52 mg potassium cyanide and 1,000 mg sodium bicarbonate in 1,000 ml of distilled water (Rodkey, 1967). The reagent was then standardized by an Hb calibrator (SmarTest Diagnostics; Cedex, France).

Statistical analysis

The results were expressed as a mean ± standard deviation (SD). Groups were compared by a paired *t*-test and Pearson's correlation coefficient (R) was calculated. Precision was determined using the coefficient of variation (CV). The determined variation included not only the within-run precision of the automated cyanide-free method, but also the variations due to the measured values over times that were made using the photometric cyanmethemoglobin method. The within-run precision for the automated cyanide-free method was also determined by analyzing five

individual dog samples five times and similarly for five cat samples. All tests were 2-tailed, and a *p*-value of less than 0.05 was considered significant.

RESULTS

A comparison of the Hct and Hb values determined by the manual and automated methods for the 50 canine and 30 feline blood samples is shown in Table 1. The mean ± SD of the observed values for each parameter, as well as the correlation coefficients for relationships between the manual and automated results are shown. The manual Hct and automated Hct were significantly different for both dog ($P<0.05$) and cat ($P<0.01$) samples. Likewise, manual Hb_{cy} and automated Hb_{HL} were significantly different in both species ($P<0.01$ for dog and $P<0.05$ for cat). Strong correlations for each parameter were found. (Table 1; Figures 1-4). Automated Hb_{HL} results from the within-run analysis of five randomly selected canine and feline samples were assayed. Each sample was run five times. The precision result was good and is summarized in Table 2. The repeatability of the photometric method for the Hb_{cy} estimation for 10 dogs and 10 cats at various times was good and is presented in Table 3.

DISCUSSION

This study found for both dogs and cats that the values determined by the automated measurement of Hct and Hb cannot replace the manual results obtained. However, the data indicated a strong correlation between the manual and automated methods. Most CVs for the reported parameters were greater than 0.95. The lower CV for Hb in cats (0.87) was most likely caused by Heinz bodies (refactile bodies). The incidence of Heinz bodies can differ greatly among cat populations, with a range from less than 1% to more than 50% being reported (Jain, 1986). Because Heinz bodies increased the optical density of the diluent containing the hemolyzed blood, a false-high Hb concentration was observed, when a spectrophotometer was used (Jain, 1986). This error was resolved by using the automated system, where the bodies floated on the top of the suspension and had no effect on the measurement (Abbott, 1997).

Table 1 Mean ± SD of hematocrit and hemoglobin concentration of dogs and cats evaluated using automated and manual methods.

Animals	Parameters	Automated	Manual	*P*-value
Dog	Hct (%)	32.47 ± 7.73	33.18 ± 7.27	0.03*
(n = 50)	Hb (mg/dL)	11.10 ± 3.53	11.96 ± 3.50	<0.01*
Cat	Hct (%)	32.19 ± 8.24	29.80 ± 8.83	<0.01*
(n = 30)	Hb (mg/dL)	10.33 ± 3.34	10.98 ± 2.94	0.04*

* $P<0.05$ = significant difference between methods

Table 2 Within-run precision of hemoglobin concentration of dogs and cats for the automated system.

Dog (D)	Observed			Cat (C)	Observed		
	Mean	SD	CV[1] (%)		mean	SD	CV (%)
D1	10.03	0.06	0.61	C1	6.50	0.16	2.4
D2	9.57	0.09	0.96	C2	9.94	0.09	0.9
D3	12.40	0.07	0.57	C3	10.18	0.04	0.4
D4	13.16	0.13	1.02	C4	10.84	0.09	0.8
D5	6.42	0.11	1.71	C5	12.64	0.30	2.1

[1] Coefficient of variation

Table 3 Repeatability of photometric method for cyanmethemoglobin estimation of dogs and cats at 10, 20, 30, 60, 90 and 120 minutes.

Dog	Observed			Cat	Observed		
	Mean	SD	CV[1] (%)		mean	SD	CV (%)
1	17.71	0.07	0.40	1	13.69	0.16	1.19
2	10.18	0.06	0.57	2	12.10	0.06	0.52
3	12.78	0.14	1.07	3	14.85	0.05	0.32
4	18.44	0.51	2.79	4	7.46	0.19	2.51
5	10.24	0.11	1.12	5	10.14	0.06	0.55
6	9.16	0.06	0.61	6	17.05	0.12	0.68
7	15.67	0.19	1.22	7	3.10	0.05	1.57
8	14.85	0.04	0.03	8	8.25	0.04	0.53
9	10.92	0.07	0.73	9	10.85	0.08	0.74
10	15.75	0.09	0.60	10	11.84	0.06	0.53

[1] Coefficient of variation

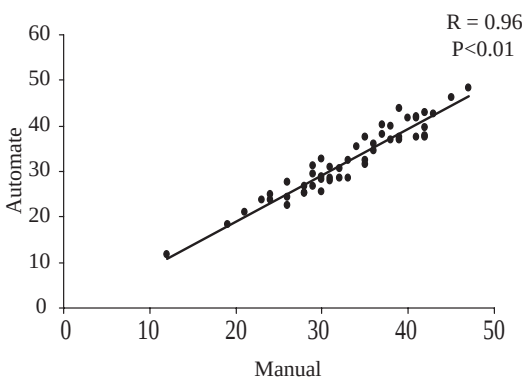

Figure 1 Correlation between manual and automated Hct (dog).

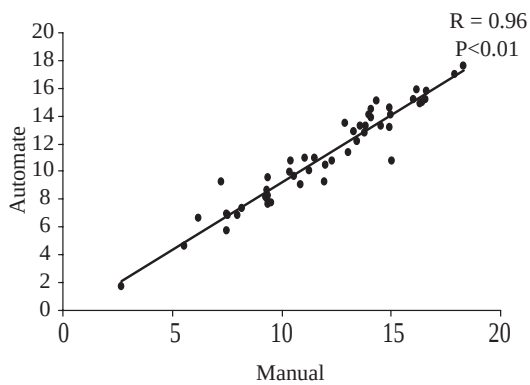

Figure 2 Correlation between manual Hb_{cy} and automated Hb_{HL} (dog).

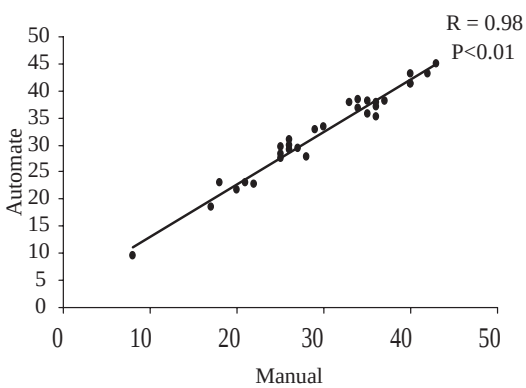

Figure 3 Correlation between manual and automated Hct (cat).

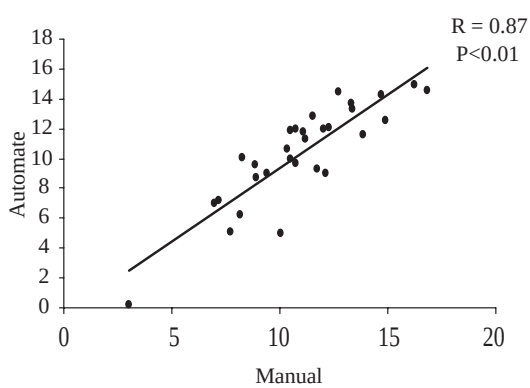

Figure 4 Correlation between manual Hb_{cy} and automated Hb_{HL} (cat).

Based upon the precision of the within–run of CD 3500, the Hb results from this equipment were considered reliable. Moreover, the good repeatability of the photometric method for the estimation of Hb_{cy} at various times indicated the stability of hemoglobin pigments.

In summary, the findings indicated that Hct and Hb results from the manual and automated methods were significantly different, but were correlated. These data indicated that the results from the automated method could not be substituted for the manual values, despite the accuracy and precision of the two methods. In addition, it implied that the results from each method should be compared to the reference values for that method.

CONCLUSION

Today, the automated hematology analyzer plays a more important role than manual procedures in small animal practices in Thailand. However, a proper feasibility analysis and evaluation should be done before adopting either method. In this study, Hct and Hb values determined by a manual process were compared to those determined using an automated process. The study found that the automated values of Hct and Hb were significantly different from those of the manual method, despite the correlated results in the data set. These findings indicated that automated Hct and Hb values could not be substituted for manual Hct and Hb values, even though these two methodologies proved to be accurate and precise. An economically-suitable method should be selected, according to the workload, staff and available equipment.

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