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Alterations in Some Hematological Parameters of Feline Blood Samples Preserved at Different Temperatures for a Duration of up to 48 hours

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ABSTRACT

Hematological parameters serve as vital diagnostic tools in veterinary medicine, aiding in the identification of various diseases and metabolic conditions in animals. However, their reliability can be influenced by a multitude of factors, including storage conditions and duration. Pre-analytical errors, which account for up to 70% of laboratory diagnostic errors, primarily stem from issues such as sample collection, transportation, and storage. To address this, the European Federation of Clinical Chemistry and Laboratory Medicine-Working Group for the Preanalytical Phase conducted a survey to understand pre-analytical practices among human laboratories. Despite significant interest, diversity in monitoring approaches was noted among European laboratories. The complete blood count (CBC) stands as a frequently requested test by veterinarians, but its accuracy can be affected by pre-analytical variables like storage temperature and delay in analysis. This study aimed to assess the stability of hematological parameters in feline blood samples stored at different temperatures (4°C and 20°C) for up to 48 hours. Ten clinically healthy cats were included in the study, with blood samples collected and analyzed immediately and at various time points over 48 hours. Results showed stability in hemoglobin concentration for 48 hours at both temperatures. However, RBC and PCV values increased significantly after 24 hours at 20°C. MCV, MCH, and MCHC values remained relatively stable over time, with minor fluctuations. Notably, WBC counts declined significantly after 48 hours at 20°C, while no significant changes were observed at 4°C. These findings underscore the importance of prompt analysis and appropriate storage conditions in maintaining the integrity of hematological parameters in veterinary practice. In conclusion, blood samples from cats stored at 4°C remain reliable for up to 48 hours for most hematological parameters, while samples stored at 20°C are suitable for up to 24 hours. These findings provide valuable insights for veterinarians and laboratory professionals, emphasizing the need for standardized pre-analytical practices to ensure accurate diagnostic results in veterinary hematology.

1. INTRODUCTION

Hematological parameters represent a fundamental diagnostic tool in veterinary medicine, serving as crucial components for the clinical identification of organic diseases, infections, parasitic diseases, and assessment of metabolic conditions. These parameters are influenced by various biological and environmental factors including physiological status, age, gender, breed, body weight, activity level, climate, seasonal variations, stressful conditions, nutrition, and others. These factors collectively impact the hematological profile of clinically healthy animals. Moreover, hematology results are often influenced by the time between blood sampling and measurement, as well storage conditions. Up to 70% of errors in laboratory diagnostics stem from pre-analytical factors, primarily attributed to issues in patient preparation, sample collection, transportation, and preparation for analysis and storage, rather than

the standardized analytical process (Unalli and Ozarda, 2021). In contrast to the standardized procedures observed during the testing process, the pre-analytical phase lacks a high level of standardization, making it more prone to errors. To address this issue and provide guidance, the European Federation of Clinical Chemistry and Laboratory Medicine-Working Group for the Preanalytical Phase (EFLM WG-PRE) conducted a survey among human laboratories to understand pre-analytical practices. Cadamuro et al. (2019) conducted a survey to assess human laboratories' preparedness for engaging in pre-analytical analyses. Among 1265 respondents, 94% expressed their intention to monitor errors. Despite significant interest in pre-analytical matters, considerable diversity was observed among European laboratories in terms of how they monitor the pre-analytical phase.

The complete blood count (CBC) stands as one of the most frequently requested and routine hematological laboratory tests by veterinarians. Pre-analytical variables such as temperature and incubation period can influence the results of the CBC. Although a blood sample should be analyzed shortly upon collection, a delay is sometimes unavoidable. Delayed analysis of blood samples may be caused if specimens are collected or couriered from remote locations to the laboratory at the end of the work-week. Delays may occur from the sampling stage to the analysis, particularly if the blood sample needs to be sent to a reference laboratory for retesting or when the analysis cannot be easily executed. Moreover, employing manual procedures instead of an automatic hematology analyzer, particularly with a high volume of samples, may result in delays as these samples cannot be promptly analyzed upon arrival at the laboratory and require additional testing time. Consequently, testing is frequently postponed for 12 to 24 hours or even longer following venipuncture. Prolonged delays in processing, however, could potentially undermine the credibility of the results, nullifying the efforts made to ensure accurate and precise analysis. Numerous studies have been conducted regarding the stability of whole blood specimens for CBC testing, yet findings from these studies vary considerably and are heavily influenced by the analyzer being used (Unalli and Ozarda, 2021). Given the criticality of sample stability in producing dependable results for CBC analysis and the absence of consistent data regarding the ideal sample storage temperature and duration, this study was formulated to assess the stability of samples concerning some hematology parameters. The aim of this study was to investigate changes that occur in the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration and packed cell volume (PCV), due to storage conditions (e.g., temperature and time). For this purpose, blood samples were stored at refrigerator (4°C) and water bath (20°C) temperatures during a storage period of 48 hours.

2. MATERIALS AND METHODS

2.1 Animals and laboratory methods:

Ten clinically healthy cats were used in this study. Blood samples were obtained from adult cats of both sexes from the jugular vein into tubes containing EDTA as anticoagulant (Becton Dickinson). Hematological analysis was performed by automated hematology analyzer on the blood samples immediately upon collection to obtain base values (BV) and after 4, 24 and 48 hours of storage at different storage conditions (+4°C and 20°C of water bath temperatures).

Hematological parameters included red blood cells count (RBC), hemoglobin concentration (HGB), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH) and white blood cells count (WBC) were assessed using an automated multi-parameter hematology analyzer.

2.2. Statistical Analysis:

Changes in values of examined hematological parameters from the BV were determined. Data were analyzed using the IBM Statistical Package (SPSS Version 20.0). To assess whether the hematological parameters differed from their base values (at 0 h, BV) after storage of blood samples at 4°C, or 20°C for 4 h, 24 h, and 48 h, repeated measures one-way ANOVA was employed. In cases where significant changes were identified by the repeated measures one-way ANOVA, the Tukey test was utilized to determine the corresponding p-values. Furthermore, statistical comparisons among values observed at identical time points in blood samples stored at distinct temperatures were conducted using one-way ANOVA. A significance level of $p < 0.05$ was adopted for statistical significance. The data were presented as the mean ± standard deviation (SD).

3. RESULTS

The values for RBC and WBC are displayed in the graphs (Fig. 1 and Fig. 2). The values for other investigated hematological parameters depending on duration of storage during different conditions are presented in Table 1.

Table (1): Values of HGB, PCV, MCV, MCH and MCHC obtained from feline blood samples during 48 hours of different storage conditions (4°C and 20°C).

4°C	BV	4h	24h	48h
HGB (g/L)	89,6±8,29	108±14,37	97+11,57	110±7,64
PCV (%)	35,2±4,46	37,4±1,12	37,6±1,43	35±1,73
MCV (fL)	46,9±1,12	49,7±1,18	48,6±1,05	43,5±1,24
MCH (pg)	11,93 ±1,98	14,36±1,14	12,55±1,69	13,68±1,25
MCHC (g/dL)	254,5±24,6	288,8±12,7	258±10	314,3±16,97
20°C	BV	4h	24h	48h
HGB (g/L)	89,6±8,29	90±12,65	100±17,56	115±20,15
PCV (%)	35,2±4,46a	39,5±1,55b	40,25±1,95b	42±2,01b
MCV (fL)	46,9±1,12	50,2±1,79	41,6±1,97	41,2±1,37
MCH (pg)	11,93±1,98	11,44±0,47	10,34±0,96	11,29±0,79
MCHC (g/dL)	254,5±24,6	227,8±11,9	248,4±12,4	273,8±24,8

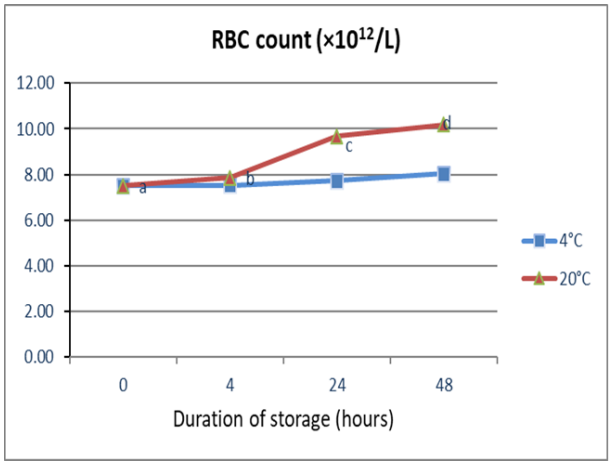

Fig. 1. Values of RBC over 48 hours depending on storage conditions. Lowercase letters (a, b, c) indicate significant differences in samples stored at 20°C.

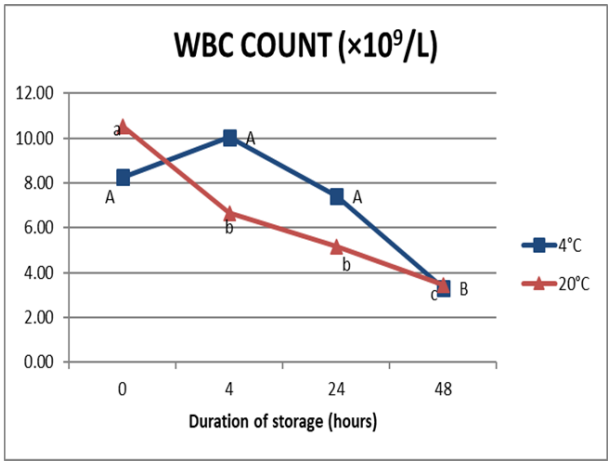

Fig. 2. Values of WBC over 48 hours depending on storage conditions. Lowercase letters (a, b, c) indicate significant differences in samples stored at 20°C. Uppercase letters (A, B, C) indicate significant differences in samples stored at 4°C.

4. DISCUSSION

Results for RBC count, showed no significant difference ($p<0,05$) from the BV of samples stored at 4°C, while an increase was determined at 20°C after 24, as well as 48 hours of storage. At 4°C, the metabolic activities of cells are reduced, which helps preserve the integrity of the cells and their counts for up to 48 hours. At 20°C, increased metabolic rates lead to more rapid degradation and changes in cell morphology, impacting the reliability of hematological parameters after 24 hours. Furthermore, blood storage at room temperature can lead to morphological changes in RBCs, such as swelling or shrinking, depending on the osmotic conditions within the storage environment.

Our study showed that HGB concentration remained stable for 48 hours, when blood samples were stored at either 4°C or 20°C. More recent research by various authors has also verified that measurements of HGB concentrations and RBC counts remain stable for up to 72 hours after blood collection when refrigerated at approximately 4°C (Unalli and

Ozarda, 2021; Pintér et al., 2016). The findings of this study corroborate these observations. The alterations noted through the assessment of blood samples stored in refrigerator and at ambient temperature are outlined in Table 1.

The RBC indices such as MCV, MCHC, and MCH constitute essential components of blood parameters, offering an objective and quantitative evaluation of red blood cell volume and hemoglobin content within them. Consistent with numerous prior studies (Jaya et al., 2022; Unalli and Ozarda, 2021; Rautaray and Tripathy, 2018) and our own investigation, MCH values exhibit minimal variation throughout the study duration, irrespective of the storage conditions. The observed variations were found statistically insignificant.

In the assessment of MCHC, a noticeable change in its values was observed ($P < 0.05$) after 4h at in blood samples stored at 4°C, with this trend becoming more pronounced after 48 post-blood sampling. The stability of hemoglobin concentration and other red cell indices (MCH and MCHC) despite varying storage conditions is indicative of the inherent stability of hemoglobin molecules and RBC structural integrity under refrigerated conditions. However, at higher temperatures, subtle changes in hemoglobin structure could occur due to denaturation, leading to variations in hemoglobin readings. Furthermore, the alterations in blood sample quality over time are compounded by erythrocyte agglutination and glucose metabolism, which directly impacts measurements like MCV. The swelling of RBCs, particularly noted in samples stored at room temperature, suggests an osmotically driven fluid shift into the cells, artificially raising MCV values.

As depicted in Table 1, MCV values remained stable during examined period of sampling at both storage temperatures. In contrary to our findings, study conducted by Unalli and Ozarda (2021) reported more pronounced rise in MCV values in samples stored at room temperature compared to those stored under refrigerated conditions, which is believed to be associated with the swelling of red blood cells, particularly within the initial 24 hours post-collection. Rise in MCV values in feline blood samples stored at room temperature and under refrigerated conditions was also found in our study, however changes were not statistically significant. Another contributing factor to the increased MCV is thought to be erythrocyte agglutination, which is closely linked to glucose metabolism during blood cell storage (Unalli and Ozarda, 2021). As previously discussed, storing blood at room temperature can cause RBCs to either swell or shrink, which is influenced by the osmotic conditions present in the storage environment. This osmotic imbalance can lead to altered cell volumes, affecting MCV and PCV values.

No significant difference was found for WBC count in blood samples stored at refrigeration conditions during examined period – up to 24h (Fig. 2). However, a decline in WBC counts was determined after 48h of storage. Decline in WBC count noted particularly at room temperature (20°C) can be linked to the increased metabolic activity and consequent faster degradation of white blood cells compared to refrigeration at 4°C. At higher temperatures, enzymatic activities remain high, accelerating the breakdown of cells. This is exacerbated in white blood cells due to their relatively short lifespan and higher metabolic rates compared to red blood cells.

Statistically significant decline in WBC count and an increase in RBC and PCV were observed already after 4 hours when blood samples were stored at 20°C (Fig. 2, Table 1).

White blood cells play a crucial role in the body, and their diagnostic assessment holds significant importance. When stored at refrigerator temperature, WBC counts remain relatively stable over 48 hours. However, when stored at 20°C, their values significantly decrease as early as 4 hours into storage, with a tendency to further decline throughout the 48-hour examined period. Similar decreases in WBC counts and increases in PCV have been documented in previous studies when blood samples were stored at room temperature (Imeri et al., 2008).

No significant difference was found for examined hematological parameters in blood samples stored at 4°C, except for MCHC, which exhibited a significant increase after 48 hours of storage. However, at 20°C, red blood cell counts and PCV values showed increase already after 4 hours. Increase of hemoglobin concentration was determined after 48hours of storage, however without significant difference. Decrease was observed in white blood cells count at 24 hours of storage, while MCH and MCHC did not exhibit significant changes regardless of the storage time throughout the studied period. Further time of storage at 20°C did not affect PCV values obtained after four hours (24h and 48h, respectively).

5. CONCLUSIONS

Based on our research results we conclude following: blood samples obtained from cats stored up to 48 hours at 4°C are reliable for RBC count, WBC count, hemoglobin concentration, as well as for values of PCV, MCV and MCH were reliable for 24hours 4°C.

Blood samples provide legitimate results for MCV, MCH and MCHC stored up to 48 hours at 20°C. The hemoglobin concentration remains reliable for 24 hours after sampling at room temperature, whereas the values of RBC, WBC, and PCV significantly increase after just 4 hours of storage.

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Authors' declarations

Publication consent

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All data of this study is provided.

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N.H.: Conceptualization, Formal Analysis, Investigation, Supervision, Resources, Writing – original draft

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A.L.: Conceptualization, Data curation, Formal Analysis, Resources, Supervision, , Writing – review and editing.

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