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Effects of Storage at Room and Refrigerator Temperatures on Dogs Blood Parameters

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Abstract:

Blood should be analyzed immediately after collection. However the delay in its analysis may be unavoidable if the samples are taken from farms away from labs or, when samples are collected at the end of week. The purpose of this study consists in comparison of the CBC results in dogs and morphological changes observed in blood smears, stored at room temperature (22° C) and in refrigerator (4°C). The blood was collected with EDTA from 11 clinically healthy dogs, and was analyzed, immediately after collection and then after 6, 24, 48 and 72 hours by using hematologic analyzer BC -2800 Vet. The number of erythrocyte cells and the percentage of neutrophils and lymphocytes were stable for 24-48 hours at their respective storages temperatures. While the number of platelets and MCV were increased after 6 hours at both temperatures of storing, it also falls after 48 hours, while the concentration of hemoglobin was stable during the period of study. The morphological changes observed in blood were: smudge cells (unspecified forms), pyknotic leukocyte, ecinocite and sferoecinocyte.

Keywords: blood, CBC, dog, artifactual changes, delayed analysis

1. Introduction

Hematological and biochemical parameters are one of the most important diagnostic tool in veterinary medicine, as are the core component for the clinical diagnosis of organic diseases, infections and parasitic diseases, and evaluation of the metabolic conditions. Several of biological and environmental factors, such as physiological condition, age, sex, race, body weight, activity of different living, climate, seasonal oscillations, stressful situations, nutrition, etc. they influence on hematological and biochemical parameters of clinically healthy for the animals [2]. They can have also a significant impact on the results of laboratory tests, which should be taken into account during the interpretation, especially when values were obtained near the limits of them. Another factor also important that need to be taken in considerate is the time of evaluation of samples in the laboratory. There are recommended to do the analyses directly after the sampling. Practically, it is not possible, especially for the blood samples collected in the farms far away from laboratories. Also, when used the manual procedures instead of automatic hematology analyzer, especially for a large number of samples cannot be analyzed immediately upon arrival in laboratory and need more time for testing. The time from sampling to analyzing of them could be delayed even when the blood sample must be sent to a reference laboratory when retesting is needed or when the analysis cannot be performed easily [18]. Unnecessary delays in testing blood samples for hematological indicators can lead aging the sample and they can compromise the credibility of the results [4]. The sample aging means a process that determine the change of the actual values of parameters, depending on the time of transport, storage and its conservation. It does not come as a normal process for all indicators of sample. Some of them can change faster than other indicators. The aging of samples can come from lack of sample transport and by delays in carrying out the analysis. So the vets and labs specialist should not allow aging of samples. For that reason is necessary to be known the hematological alterations during the blood samples aging [13]. Many authors have performed studies in different animals and they have concluded that different storage conditions can affect the results of hematological indicators. While other studies (4, 11) concluded that maintaining blood in refrigerated conditions minimizes the changes of blood indicators.

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Until now has no any specific study related to the effect of temperature and duration of storage of blood sample in hematological parameters in animal breeding in our country. Therefore the aim of this study consists in identification of the changes of hematological values of dog's blood, stored up to 72 hours after sampling, in room and refrigerator temperature

2. Materials and methods

The study was carried out in Diagnosis Laboratory of Faculty of Veterinary Medicine, Agricultural University of Tirana. This study was performed in 11 clinically healthy dogs. Blood was collected from the cephalica vein in tubes with anticoagulant K3 - EDTA. To study the influence of sample age, and their preserve conditions, the hematological indicators, were analyzed within one hour, and 6, 24, 48 and 72 hours after sampling. For each dog were taken two samples, one sample was preserved in a refrigerator at a temperature of 4^{o}C, and another sample was maintained at room temperature at 22^{o}C. The hematological parameters were evaluated for the number of erythrocytes (RBC) for 1µl blood, hematocrit (Hct), hemoglobin concentration (Hb), erythrocytes indicators (MCV, MCH, MCHC), the number of leukocytes (WBC) for 1µl blood, the number of platelets (PLT). Evaluation of these parameters was made with automatic method Analyzer Hematologic BC-2800 Vet. (Mindray).

The artifactuale morphological blood changes were observed through the same preserving time and method. The smears were colored with May-Grunwald-Giemsa and they were observed with immersion objective 1000x [17].

The statistical analysis, for all the obtained data was calculated for the average value, standard deviation and minimum and maximum values. The results were statistically evaluated using the Student t-test with P ≤ 0:01 test taken as significant.

3. Results and Discussion

The changes observed by evaluation of blood samples stored at room temperature and in the refrigerator are summarized in Table 1. The data are presented as averages values obtained for each sample and their standard deviations.

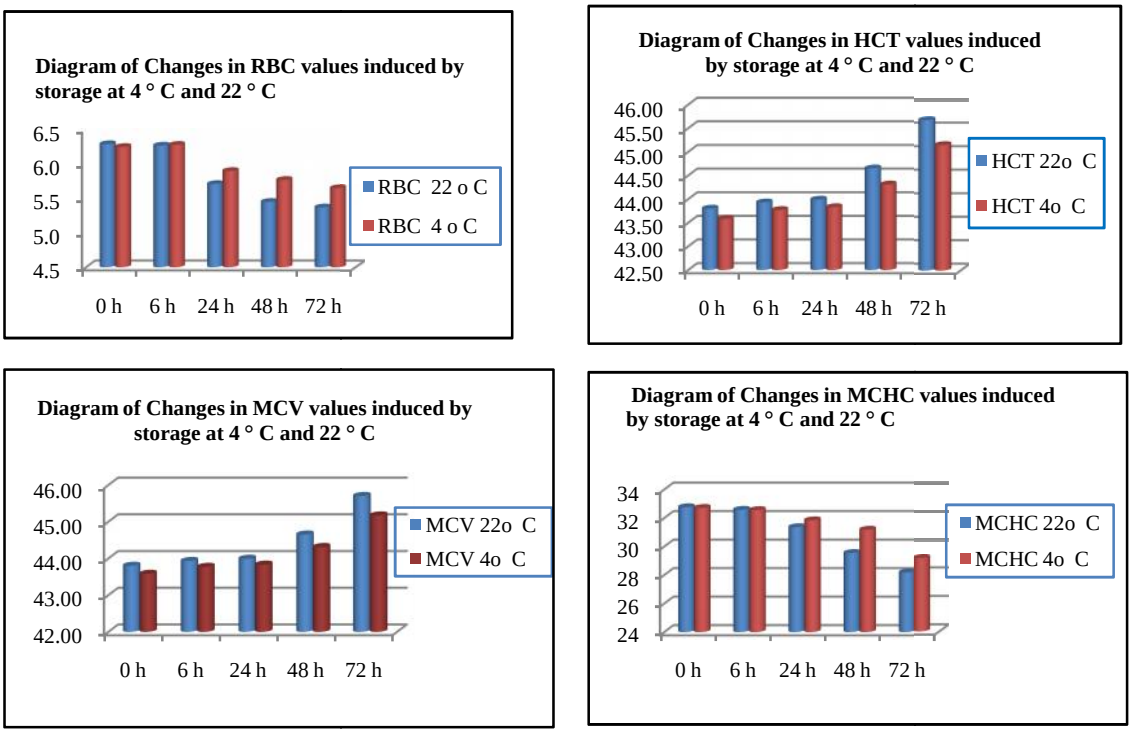
Table 1. Changes in CBC Parameters Induced by Storage at 4 °C and 22 °C[*]

Treguesit	**1 h**		**6 h**		**24 h**		**48 h**		**72 h**	
	22^{o}	**4 ^{o}**	**22^{o}**	**4 ^{o}**	**22^{o}**	**4 ^{o}**	**22^{o}**	**4 ^{o}**	**22^{o}**	**4 ^{o}**
RBCx10 ^{12}/L	6.29 (0.50)	6.25 (0.48)	6.27 (0.42)	6.28 (0.44)	5.71 (0.29)	5.9 (0.40)	5.45 (0.3)[a]	5.8 (0.5)[b]	5.37 (0.35)[a]	5.65 (0.37)[a]
Hgb gr/dl	14.23 (2.27)	14.17 (2.18)	14.23 (2.27)	14.17 (2.18)	13.75 (1.59)	13.9 (1.75)	13.11 (1.48)	13.72 (1.52)	12.79 (0.81)	13.11 (1.19)
Hct %	43.80 (3.63)	43.58 (3.63)	43.93 (3.64)	43.77 (3.62)	43.99 (2.51)	43.83 (2.5)	44.65 (3.15)	44.31 (3.95)	45.70 (3.25)	45.17 (3.39)
WBC x0^{9}/L	6.07 (0.92)	6.06 (0.92)	5.88 (0.96)	5.96 (0.96)	6.24 (0.98)	6.23 (0.97)	5.88 (0.97)	5.96 (0.96)	6.7 (0.94)	6.88 (0.68)
MCV(fL)	70.07 (6.8)	69.10 (6.40)	70.37 (7.15)	69.94 (7.01)	77.28 (6.5)[b]	74.69 (7.91)	82.04 (6.4)[a]	77.2 (6.8)[b]	85.59 (11.1)[a]	80.12 (6.7)[a]
MCH (pg)	22.7 (4.28)	22.81 (4.06)	23.14 (4.24)	22.67 (3.80)	22.79 (3.43)	23.58 (2.61)	22.02 (3.35)	23.99 (3.6)	23.90 (1.93)	23.34 (3.01)
MCHC (gr/L)	32.67 (5.74)	32.71 (5.33)	32.58 (5.46)	32.56 (5.32)	31.36 (4.12)	31.84 (4.42)	29.55 (4.41)	31.18 (4.5)	28.18 (3.11)[a]	29.16 (3.14)
PLT (×10^{9}/L)	320.69 (88.06)	320.7 (88.06)	481.07 (59.8)[a]	440.1 (63.5)[b]	544.6 (53.6)[a]	514.2 (76.9)[b]	575.6 (60.1)[a]	537.9 (60.6)[a]	615.6 (51.5)[a]	582.7 (53.9)[a]

[a]Significantly ($P \leq 0.001$) different from value at time 1 h; [b]Significantly ($P \leq 0.01$) different from value at time 1 h

[*]Data are presented as mean (SD)

As showed from the data presented in graphic 1 and table 1 the values of red blood cells (RBC) present no significant changes in the first hour of their assessment, their numbers remain relatively stable throughout the 24 hours. After 24, 48, and 72 hours, RBC number was decreased in significant values (p <0.001). These changes are thought to be related to the fact that erythrocytes in vitro destructed over time and blood hemolysed [28].



Graphic 1: Diagram of changes in RBC, HCT, MCV, MCHC values induced by storage at 4 ° C and 22 ° C

Regarding blood storage mode there are no significant changes in the erythrocyte values. The values in the first hours are the same as for samples stored at 4°C as well as those stored in the room temperature. After 24 hours, the value of samples stored at 4°C are more stable than those stored at room temperatures, although these differences were not significant. In accordance with other studies [20, 29], the parameter of the direct measurement of the concentration of hemoglobin (HGB) was stable in both forms of storage, refrigerated and ambient temperature during 72 hours.

Referring to the values of the hematocrit (HCT) presented in table 1 and graphic 1 were observed a growing trend of its values with increasing of samples storing time. The increasing Hct values begin after 24 hours of storage and they are also evident in samples stored at ambient temperature. Different authors (9, 19, 21), have presented the same results as they are in our study.

The erythrocytes indicator as MCV, MCHC, MCH, are an integral part of blood indicators and their test provide an objective and quantitative assessment of the volume of red blood cells and hemoglobin content in red blood cells.

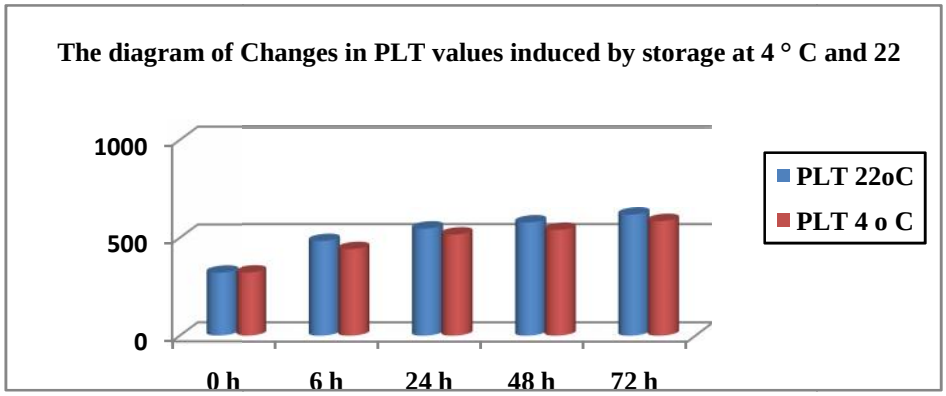
As many other earlier studies [10, 13, 21, 9] and in our study MCH values remain almost constant throughout the period of the study, in both forms of storage. The changes that are observed are insignificant.

Regarding evaluation of MCHC was found a clear downward trend in its values ($P \leq 0.01$), which is more emphasized after 48 and 72 hours of blood sampling. Among the erythrocyte's indicators there were a significant change in average of blood corpuscular volume (MCV). The values of this indicator in our study remain stable for up to 6 hours after sampling, while after 24, 48 and 72 hours the MCV values were increased ($P \leq 0.001$). This change of MCV values was described in previous studies similar to our study. [1, 12, 16 , 20, 22].

And as it is shown in graphic 1, the MCV values during the first hours of sampling were stable at both storage temperatures, while after 24, 48 and 72 hours these values have increased. The MCV increasing values were evident in stored samples at room temperature compared with samples stored at refrigerated conditions. The different authors (20, 22, 9) have noted in their work that in blood samples stored at room temperature increasing of MCV were evident. The increase of MCV values according to the extension of conserving time for this parameter as well as many authors have concluded [6, 15], it is related to red cells swell with storage. The red cells

swell occurs very rapidly, within 24 hours of collection, especially if blood samples that are not kept in temperature 4°C. Another factor, which is thought to be related with increasing of MCV is the agglutinin of erythrocytes. Agglutination of red blood cells is closely related with glucose metabolism during storage of blood cell [24.8].

In this study, the averages values of thrombocytes were increased considerably during the entire time of evaluation ($P \leq 0.001$). As shown in the graph 2 and table No1 the increase of their values were started after 6 hours of evaluation and they were evident during all the time of this study.



Graphic 2: The diagram of changes in PLT values induced by storage at 4 ° C and 22 ° C

The increasing of the number of the platelet is thought to be related to the incorrect counting by the hematologic analyzer of the fragments of damaged erythrocytes or the microcytes erythrocytes as red blood cells. The same conclusions is reported and by the other authors in different animals [6, 7, 27].

While the other authors [20, 25], are against to this version, based on the fact that in blood smears, is evident the presence of platelets cluster while their total number does not change or dos not growth, and there is not detected the presence of shizociteve (erythrocyte fragments) in it. Regardless of the reason, the platelet should be measured immediately after blood collection because, as it was observed, the number of platelet were significant even after 6 hours of blood sampling.

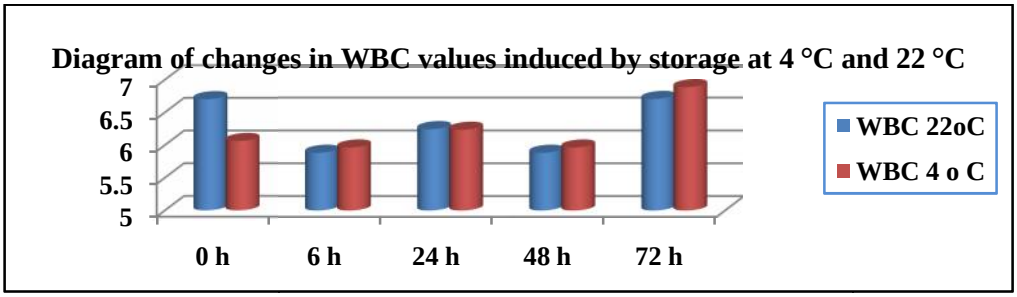
The white blood cells (WBC) has an important role in organism. Their diagnostic evaluation is very important. According to the samples preserving time and method WBC does not significantly change in the first hours and increased after 48 hours.(graphic n 3)

According Médaille. C, 2006, the increase of leukocytes number after 48 hours is thought to be associated with the counting of aggregates platelets as leucocytes identified by automatic analyzer.

Table 2. Mean percent change at leucograma induced by storage of blood at 4 °C and 22 °C*

Time storage	1 h		6 h		24 h		48 h		72 h	
	22°	**4 °**	**22°**	**4 °**	**22°**	**4 °**	**22°**	**4 °**	**22°**	**4 °**
Neutrophils (%)	22.8 (4.3)	22.6 (4.1)	23.3 (3.9)	23.1 (3.73)	23.9 (2.9)	24.7 (4.05)	24.7 (3.9)	24.3 (3.3)	24.2 (3.7)	23.2 (2.9)
Lymphocytes (%)	4.3 (2.3)	4.1 (1.9)	3.9 (1.9)	3.9 (1.5)	3.8 (1.8)	3.9 (1.5)	3.8 (1.7)	3.6 (1.5)	3.1 (1.4)	3.2 (1.1)
Monocytes (%)	4.2 (1.9)	4.7 (1.2)	4.7 (1.6)	4.7 (1.1)	4.6 (1.5)	4.8 (0.6)	3.9 (1.1)	3.92 (1.03)	3.7 (1.4)	3.7 (1.4)
Eosinophils (%)	68.2 (6.5)	68.6 (6.4)	67.9 (6.1)	67.6 (4.5)	67.7 (4.01)	66.6 (4.1)	66.5 (5.02)	66.5 (5.1)	65.5 (4.9)	65.5 (6.1)

*Data are presented as mean (SD)



Graphic 3: Diagram of changes in WBC values induced by storage at 4 °C and 22 °C

Findings in blood smears

Compared with the values within the first hour of leukocytes measuring, number of destroyed cells (smudge) accounted for 100 leukocytes was higher ($P \leq 0.01$) after 24 and 48 hours in both storage temperatures. As well as various authors have noted [14, 22,23, 30], it can come as a result of mechanical damage of leukocytes during the opening blood smears and because of the increase of leukocytes fragility during the prolonged time of blood conservation. We also observed the increased number of pyknotic leukocytes ($P \leq 0.001$) after 24 hours. This increase is more significant in stored blood samples in temperature 20^{o}C.

The evident changes that were observed at morphologic of erythrocytes were related with the presence of echinocytes and spheroechinocytes. These changes were evident after 24-48 hours in both blood storage temperatures. During this time the number of echinocytes were decreased and the number of spheroechinocytes were increased. Several factors seems to cause this morphological changes, including erythrocyte ATP depletion and disturbances of RBC calcium homeostasis [3, 5]. The reasons for non-pathologic echinocytes formation include exposure to alkaline pH, such as may occur with exposure to glass slides or tubes [14]. Our study indicates that blood smears should be prepared within 1 h of specimen collection for accurate evaluation of red blood cell morphology

4. Conclusions

The delaying of analysis of dogs blood samples caused artificial changes in CBC results, mainly in RBC morphology, MCV and platelets parameters, that are readily using the BC -2800 DETECTED own. The best practice is to measure the hematological parameters immediately after blood sampling collection. In the event of delayed analysis, blood samples should be stored in the refrigerator and the artifactual changes of the smears must not be misinterpreted.

5. References

1. Ameri M, Schnaars HA, Sibley JR, Honor DJ: **Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4°C in EDTA using the ADVIA 120 hematology analyzer**. *Veterynary Clinical Pathology* 2011,40(2):188-193
2. Awah and Nottidge, H.O: **Serum biochemical Parameters in Clinically Healthy Dogs in Ibadan**. *Tropical veterynary Medicine* 1998, 16: 123-129
3. Brecher G, Bessis, M: **Present status of spiculated red cells and their relationship to the discocyte–echinocyte transformation**: *a critical review. Blood* 1972.,40:333–344.
4. Buttarello M: **Quality specification in haematology: the automated blood cell count**. Clinica Chimica Acta 2004, 346(1):45–54
5. Chin-Yee I, Arya N, d'Almeida MS: **The red cell storage lesion and its implication for transfusion**. *Transfus Sciense* 1997, 18:447–458
6. Cora MC, King D, Betz LJ, Wilson R, Travlos GS: **Artifactual changes in sprague-dawley rat hematologic parameters after storage of samples at 3 °C and 21 °C**. *Journal American Association Laboratory Animal Science* 2012, 51(5):616-21.
7. Crabbe G, Van Poucke M, Cantinieaux B: **Artifactually-normal automated platelet counts due to malaria-infected RBC.** Clinical Laboratory Haematology 2002, 24:179–182.

8. file:///F:/Mean%20Cell%20Volume%20(MCV).htm: Mean Cell Volume (MCV)
9. Furlanello T, Tasca S, Caldin M, Carli E, Patron C, Tranquillo M, Lubas G, Solano-Gallego L: **Artifactual changes in canine blood following storage, detected using the ADVIA 120 hematology analyzer**. *American Society for Veterinary Clinical Pathology* 2006, 35:1 pg 42-6
10. Giordano A, Rossi G, Pieralisi C, Paltrinieri S: **Evaluation of equine hemograms using the ADVIA 120 as compared with an impedance counter and manual differential coun**t. *American Society for Veterinary Clinical Pathology* 2008, 37:1 pg 21-30
11. Goosens W: **Pre-analytical effects on automated differential blood cell counts**. *Nouvuelle Revue Francaise d' Haematoogiel* 1994, 36 pg 114–117.
12. Gulati GL, Hyland LI., Kocher W, Schwarting R: **Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature**. *Archives Pathology Medicine*. 2002, 126(3):336-42
13. Hedberg P, Lehto T: **Aging stability of complete blood count and white blood cell differential parameters analyzed by Abbott CELL-DYN Sapphire hematology analyzer**. *International journal of Laboratory Hematology*. 2009, *31(1):87-96*
14. Houwen B: **Blood film preparation and staining procedures.** *Clinical Laboratory Medicine* 2002, 22:1–14.
15. Ihedioha JI, Onwubuche RC: **Artifactual changes in PCV, hemoglobin concentration, and cell counts in bovine, caprine, and porcine blood stored at room and refrigerator temperatures**. *American Society for Veterinary Clinical Pathology* 2007, 36:1. pg 60-3
16. Imeri F, Herklotz R, Risch L, Arbetsleitner C, Zerlauth M, Risch GM, Huber AR. 2008. **Stability of hematological analytes depends on the hematology analyzer used: a stability study with Bayer Advia 120, Beckman Coulter LH 750, and Sysmex XE 2100.** *Clinical Chimistry Acta* 2008, 397:68–
17. Latimer KS: **Leukocytes in health and disease**. In textbook of Veterinary Internal Medicine 4th ed. Vol.2 1995: 1892-1929
18. Lippi G, Salvagno GL, Solero GP, Franchini M, Guidi GC: **Stability of blood cell counts, hematologic parameters and reticulocytes indexes on the Advia A120 hematologic analyzer**. *Journal of Laboratory and Clinical Medicine* 2005,146:333–340
19. Maysam Tehrani Sh, Mehrdad A, Sogand M, Hamid Sh, Seyed Mohammad H, Seyed Mohsen A: **Artifactual changes in hematological variables in equine blood samples stored at different temperatures and various anticoagulants**. *Comperative Clinical Pathology* 2010, Vol. 21(4): 449 - 452
20. Médaille C, Briend-Marchal A, Braun JP: **Stability of selected hematology variables in canine blood kept at room temperature in EDTA for 24 and 48 hours**. *Veterynary Clinical Pathology* 2006, 35(1):18 23
21. Mehrdad A, Henry A S, John R S, David J H: **Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4°C in EDTA using the ADVIA 120 hematology analyzer.** *Veterynary Clinical Pathology* 2011, 140 (2):188-93
22. Norbert N, Oguz K. B, Herbert J. M, Ferenc K, Mehmet U, Timea H, Erika S, Peter K, Kalman T, Istvan F, Iren M: **Storage of laboratory animal blood samples causes hemorheological alterations :Inter-species differences and the effects of duration and temperature**. *Korea-Australia Rheology Journal* 2009, Vol 21, No. 2, pp. 127-133
23. Nowakowski GS, Hoyer JD, Shanafelt TD, Geyer SM, LaPlant BR, Call TG, Jelinek DF, Zent CS, Kay NE**.. Percentage of smudge cells on routine blood smears predicts survival in chronic lymphocytic leukemia**. *Journal Clinical Oncology* 2009 27:1844–1849.
24. Ozer F L, Chaplin H J R: **Agglutination of stored erythrocytes by a human serum. characterization of the serum factor and erythrocyte changes**. *Journal of Clinical Investigation* 1963, Vol. 42, No. 11
25. Pastor J, Cuenca R, Velarde R, Viñas L, Lavin S: **Evaluation of a hematology**

analyzer with canine and feline blood. *Veterynary Clinical Pathology* 1997, 26:138–147

26. Pierre RV: **Red cell morphology and the peripheral blood film**. *Clinical Laboratory Medicine* 2002, 22:25–61.
27. Piknowski R: **Difference between impedance and optical platelet count methods in patients with microcytosis of red blood cells**. *Laboratory Hematology* 1999, 5:22–27.
28. Sawant R. B, Jathar S K. , Rajadhyaksha S B, Kadam P. T**: Red cell hemolysis during processing and storage**. *Asian Journal of Transfusion Science* 2007, (2): 47–51.
29. Silvia T, Marco C, Erika C, Carlo P, Massimo T, George L, Laia S G: **Artifactual changes in canine blood following storage, detected using the ADVIA 120 hematology analyzer**. *Veterinary Clinical Pathology* 2006, 35(1) pp 42, 46.
30. Valenciano AC, Decker LS, Cowell RL: **Interpretation of feline leukocyte responses** 2010, p 335–344. In: Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology, 6th ed. Ames (IA): Wiley–Blackwell.