# Interference of blood storage containing $K_2$EDTA and $K_3$EDTA anticoagulants in the automated analysis of the hemogram

## Interferência da estocagem de sangue contendo os anticoagulantes $K_2$EDTA e $K_3$EDTA na análise automatizada do hemograma

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## ABSTRACT

**Introduction:** The present work evaluated the possible alterations in different hemogram parameters as: total erythrocyte count, hematocrit, leukocyte, and platelet count, against different times of sample storage at refrigerated and room temperature towards the effects of anticoagulants that can affect the variation of laboratory results. **Objective:** Compare variations in automated hemogram analysis, with blood collected in tubes containing dipotassium ethylenediaminetetraacetic acid ($K_2$EDTA) and tripotassium ethylenediaminetetraacetic acid ($K_3$EDTA). **Material and methods:** A comparative study was carried out on differential leukocyte count and absolute leukocyte count, hemoglobin determination and red blood cell count and platelet count. Therefore, it researches the anticoagulant that brings less interferences in hemogram analysis according to storage, at temperatures from 2°C to 8°C and 25°C (room temperature), and the time window between collection and analysis (4, 6, and 8 hours). These determinations were carried out after homogenization from five to eight inversions using the automated device Sysmex XN-1000™. Eighteen biological samples of venous blood were collected, used as control, from patients aged 18 and over that did not have any hematologic disease. **Results:** The results were evaluated through descriptive statistical analysis, and comparison of mean values through the analysis of variance (Anova). In accordance with the results presented, there were not changes in the parameters by refrigerating the sample in the period of 8 hours, except for the platelet count, presenting oscillations when stored under refrigeration from 2°C to 8°C. **Conclusion:** At room temperature, the parameters mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and hematocrit presented significant statistical differences.

Key words: edetic acid; blood cell count; anticoagulants.

## RESUMO

*Introdução: O presente trabalho avaliou as possíveis alterações em diferentes parâmetros do hemograma, como contagem de eritrócitos totais, leucócitos, hematócrito e plaquetas, em relação a diferentes tempos de armazenamento da amostra em ambiente refrigerado e em temperatura ambiente quanto aos efeitos dos anticoagulantes, que podem afetar a variabilidade de seus resultados laboratoriais. Objetivo: Comparar as variações presentes na análise do hemograma automatizado, colhido nos tubos contendo ácido etilenodiamino tetra-acético dipotássico ($K_2$EDTA) e tripotássico ($K_3$EDTA). Material e métodos: Estudo comparativo realizado para contagem diferencial/absoluta dos leucócitos, determinação de hemoglobina e contagem de hemácias e plaquetas, com o intuito de investigar o anticoagulante que traz menos interferentes na análise do hemograma de acordo com sua estocagem – temperatura de 2°C a 8°C e 25°C (temperatura ambiente) e janela de tempo entre a coleta e a análise de 4, 6 e 8 horas. Essas determinações foram realizadas após homogeneização de cinco a oito inversões pelo aparelho automatizado Sysmex XN-1000™. Dezoito amostras biológicas de sangue venoso de pacientes com idade superior a 18 anos, sem doenças hematológicas, foram coletadas e utilizadas como controle. Resultados: Os resultados foram avaliados por meio de uma análise estatística descritiva, com comparação de médias através da análise de variância (Anova). De acordo com os resultados apresentados, não ocorreram*

*alterações nos parâmetros ao refrigerar a amostra no período de 8 horas, com exceção da contagem de plaquetas, que apresentou oscilações quando armazenadas sob refrigeração de 2°C a 8°C. **Conclusão:** Na temperatura ambiente, os parâmetros volume corpuscular médio (VCM), concentração de hemoglobina corpuscular média (CHCM) e hematócrito apresentaram diferença estatística significante.*

*Unitermos: ácido edético; contagem de células sanguíneas; anticoagulantes.*

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## RESUMEN

*Introducción: Este trabajo evaluó los posibles cambios en diferentes parámetros del hemograma, como conteo de eritrocitos totales, leucocitos, hematocrito y plaquetas, con respecto a diferentes tiempos de almacenamiento de la muestra en ambiente refrigerado y temperatura ambiente en cuanto a los efectos de los anticoagulantes, que pueden afectar los resultados del análisis de laboratorio. **Objetivo:** Comparar los cambios presentes en el análisis del hemograma automatizado, recogido en tubos que contienen ácido etilendiaminotetraacético dipotásico (K$_2$EDTA) y tripotásico (K$_3$EDTA). **Material y método:** Estudio comparativo realizado para conteo diferencial/absoluto de leucocitos, determinación de hemoglobina y conteo de eritrocitos y plaquetas, a fin de investigar el anticoagulante que trae menos interferencia en el análisis del hemograma según su almacenamiento – temperatura de 2°C a 8°C y 25°C (temperatura ambiente) y ventana de tiempo entre recuento y análisis de 4, 6, y 8 horas. Se realizaron las determinaciones luego de homogeneizar las muestras con cinco a ocho inversiones en el analizador automatizado Sysmex XN-1000$^{TM}$. Dieciocho muestras de sangre venosa de pacientes mayores de 18 años, sin enfermedades hematológicas, fueron recogidas y usadas como control. **Resultados:** Se evaluaron los resultados mediante un análisis descriptivo, con comparación de medias por análisis de la varianza (Anova). De acuerdo con los resultados, no hubo cambios en los parámetros enfriándose la muestra en el período de 8 horas, a excepción del recuento de plaquetas, que presentó oscilaciones bajo refrigeración (2°C-8°C). **Conclusión:** A temperatura ambiente, los parámetros volumen corpuscular medio (VCM), concentración de hemoglobina corpuscular media (CHCM) y hematocrito presentan diferencia estadísticamente significativa.*

*Palabras clave: ácido edético; recuento de células sanguíneas; anticoagulantes.*

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## INTRODUCTION

The hemogram is the most frequently ordered laboratory test in medical practice: it enables the assessment of the main components of peripheral blood, providing a basis of any hematological evaluation for disease diagnosis[1].

During the investigation of blood functions and dysfunctions, it is essential that, as much as possible, laboratories do not provide inaccurate results due to operational errors; therefore, care must be taken against errors in the method used to obtain this material. The method of collection, storage, and transportation of samples to laboratory directly influences results, compromising data reliability and impairing health professionals' conduct to reach a diagnosis[2].

Currently, many clinical laboratories are equipped with modern automated devices, able to process large amounts of hematological samples and in an efficient and timely manner. It is vital that samples are collected properly and that the adequate anticoagulant is used, to ensure reliability of results yielded by the equipment[3].

The anticoagulant commonly used is the ethylenediaminetetraacetic acid (EDTA) – an organic compound with a chelating function of metallic ions which, in blood, acts sequestering the available plasma calcium, inhibiting the coagulation cascade. It is the anticoagulant of choice because it does not alter erythrocyte morphology, becoming ideal for use in hematology[3, 4].

There are three available forms of EDTA: disodium (Na$_2$EDTA), dipotassium (K$_2$EDTA), and tripotassium EDTA (K$_3$EDTA)[4]. K$_3$EDTA is placed inside the tube in liquid form, what causes a slight dilution of the sample. In cases of prolonged storage or alteration in the ratio anticoagulant/blood using K$_3$EDTA, one can notice alteration in the average erythrocyte size. K$_2$EDTA is dispensed as powder, so it causes no variation in volume/dilution of the collected sample, becoming the recommended option for sample collection[3, 4].

Several pre-analytical factors alter the result obtained after analysis. Some studies affirm the excess of EDTA can decrease hematocrit value and mean corpuscular volume (MCV) due to plasma hypertonicity with increased ion concentration, causing an increase of mean corpuscular hemoglobin concentration (MCHC), without altering hemoglobin concentration; those changes are more pronounced with the use of $K_3$EDTA[4-7].

Goossens et al. (1991)[6] report that samples with high concentrations of $K_3$EDTA show a sharp decrease in leukocyte count (less than 50% of original value, 24 h after material collection).

Blood collection with a $K_3$EDTA volume smaller than the recommended (proportion of 0.1 ml of EDTA to 5 ml of blood) increases the incidence of artifacts; it is possible to observe crenation and sphering of erythrocytes[1-4].

Storage time and temperature are also important. Dalanhol et al. (2010)[1] claim that refrigerating samples delays changes, but it does not prevent them to happen, and that samples kept at room temperature or warmed present earlier changes. They also state that blood samples at room temperature for more than 12 hours or in the refrigerator for more than 24 hours go through undue increases of MCV and hematocrit, as well as a decrease of MCHC with normal levels of hemoglobin.

The objective of this work is to evaluate the possible alterations in different hemogram parameters in different storage times at refrigerated and room temperature, and how the effects of EDTA ($K_2$ and $K_3$) variations can affect the variability of their laboratory results. To that effect, a comparative study was carried out of differential/absolute leukocyte count, hemoglobin determination, and erythrocyte and platelet counts, to verify which of the used anticoagulant provides a lower rate of interference in the hemogram analysis results.

## MATERIAL AND METHODS

Experimental comparative study, for which 18 venous blood specimens were collected as control, from individuals aged 18 years or over without hematological diseases. Patients were informed about the study and consented to specimen collection. Collections were made in triplicate for each participant: 4 ml of venous blood according to standard procedure, using sterile needles with adapters designed for vacuum blood collection tubes containing $K_2$EDTA and $K_3$EDTA, by means of venipuncture. Blood samples were divided into two storage groups – refrigerated (2°C-8°C) and room temperature (25°C) –, with the following analyses:

- erythrocyte parameters – erythrocyte [red blood cell (RBC)] count, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), and MCHC;

- total leukocyte [white blood cell (WBC)] count, including neutrophils, monocytes, eosinophils, and basophils;

- platelet count. Analyses were carried out at different storage times: 4, 6, and 8 hours.

Those analyses were conducted after homogenization inverting samples eight times, using the automated device Sysmex XN-1000™ and the flow cytometry method for erythroblast [nucleated red blood cells (NRBC)] count; conduction of a 6-part differential count, which includes the parameter of immature granulocytes.

Among the 18 collected biological specimens, six were stored at room temperature (25°C); the other 12 were stored under refrigeration (2°C-8°C). The specimens were processed in the hematological counter in the times of 4, 6, and 8 hours after collection.

The results were assessed by means of a descriptive statistical analysis and with comparison of means by the analysis of variance (Anova).

## RESULTS

Erythrocyte count presented a profile with little variation, both for specimens refrigerated and at room temperature. However, refrigerated specimens with $K_2$EDTA presented increase from the mean in the fourth hour, varying, on average, 4.24 million erythrocytes/µl (**Table 1**).

$K_3$EDTA at room temperature achieved greater stability, from the first to the fourth hour; the mean was 12.6 g/dl, falling to 12.57 g/dl in the sixth hour; stability was observed up to the eighth hour. $K_2$EDTA refrigerated between 2°C and 8°C presented minimal elevation from the fourth to the sixth hour (12.42 to 12.47 g/dl). It was not possible to observe statistical variation for either of the temperatures (Table 1).

In the hematocrit analysis, for specimens at room temperature, the mean ranged from 36.77% to 38.5%. An increase beginning in the fourth hour of storage was observed, what resulted in statistical difference. In the refrigerated samples, this did not occur, causing small variation of the means: 37.15% to 37.55% (Table 1).

The results of analyses obtained for MCV mean levels were the same for both temperatures in the first hour of storage (85 fl to 87 fl). The mean profile at room temperature ranged from 85 fl to 89 fl, up to the fourth hour of storage.

TABLE 1 – Mean profile of erythrocyte parameters, according to temperature and storage time: erythrocyte count, hemoglobin and hematocrit

| Parameters | Storage time | RTK2 Mean ± SD | RTK3 Mean ± SD | REFK2 Mean ± SD | REFK3 Mean ± SD |
|---|---|---|---|---|---|
| Erythrocytes ($10^6$/mm³) | 1 h | 4.25 ± 0.44 | 4.43 ± 0.36 | 4.46 ± 0.18 | 4.5 ± 0.21 |
| | 4 h | 4.24 ± 0.41 | 4.41 ± 0.32 | 4.47 ± 0.2 | 4.51 ± 0.21 |
| | 6 h | 4.25 ± 0.42 | 4.43 ± 0.34 | 4.49 ± 0.22 | 4.5 ± 0.21 |
| | 8 h | 4.21 ± 0.4 | 4.41 ± 0.34 | 4.48 ± 0.19 | 4.5 ± 0.24 |
| Hemoglobin (g/dl) | 1 h | 12.13 ± 1.42 | 12.6 ± 1.15 | 12.97 ± 0.38 | 13.1 ± 0.44 |
| | 4 h | 12.17 ± 1.43 | 12.57 ± 1.14 | 13.03 ± 0.49 | 13.13 ± 0.49 |
| | 6 h | 12.23 ± 1.39 | 12.57 ± 1.21 | 13.1 ± 0.44 | 13.2 ± 0.36 |
| | 8 h | 12.23 ± 1.42 | 12.57 ± 1.12 | 13.07 ± 0.38 | 13.2 ± 0.53 |
| Hematocrit (%) | 1 h | 36.77 ± 4.07 | 37.7 ± 3.36 | 38.3 ± 2.26 | 38.13 ± 2.48 |
| | 4 h | 37.4 ± 4.03 | 38.17 ± 3.35 | 38.47 ± 2.55 | 38.33 ± 2.57 |
| | 6 h | 37.53 ± 4.29 | 38.4 ± 3.7 | 38.9 ± 3.3 | 38.23 ± 2.48 |
| | 8 h | 37.53 ± 4.11 | 38.5 ± 3.6 | 38.53 ± 2.55 | 38.23 ± 2.83 |

*RTK2: room temperature, K₂EDTA; RTK3: room temperature, K₃EDTA; REFK2: refrigerated temperature, K₂EDTA; REFK3: refrigerated temperature, K₃EDTA; SD: standard deviation.*

TABLE 2 – Mean profile of leukocyte count parameters, according to temperature and storage time

| Parameters | Storage time | RTK2 Mean ± SD | RTK3 Mean ± SD | REFK2 Mean ± SD | REFK3 Mean ± SD |
|---|---|---|---|---|---|
| Leukocyte ($10^3$/mm³) | 1 h | 8.51 ± 3.66 | 8.55 ± 3.72 | 6.8 ± 4.28 | 7.02 ± 4.66 |
| | 4 h | 8.61 ± 3.68 | 8.56 ± 3.69 | 6.92 ± 4.1 | 7.07 ± 4.57 |
| | 6 h | 8.48 ± 3.72 | 8.6 ± 3.7 | 6.82 ± 4.18 | 6.99 ± 4.54 |
| | 8 h | 8.54 ± 3.64 | 8.65 ± 3.75 | 6.79 ± 3.9 | 7.02 ± 4.42 |
| Neutrophil (/mm³) | 1 h | 4.79 ± 2.45 | 4.9 ± 2.5 | 5.82 ± 0.95 | 6.51 ± 0.98 |
| | 4 h | 4.92 ± 2.57 | 4.94 ± 2.51 | 5.96 ± 0.72 | 6.53 ± 0.88 |
| | 6 h | 4.82 ± 2.49 | 4.94 ± 2.53 | 5.86 ± 0.91 | 6.53 ± 1.06 |
| | 8 h | 4.83 ± 2.43 | 5 ± 2.53 | 5.84 ± 0.78 | 6.42 ± 1.01 |
| Lymphocyte (/mm³) | 1 h | 4.79 ± 2.45 | 2.92 ± 0.89 | 3.21 ± 0.49 | 3.52 ± 0.65 |
| | 4 h | 4.92 ± 2.57 | 2.9 ± 0.89 | 3.15 ± 0.53 | 3.54 ± 0.69 |
| | 6 h | 4.82 ± 2.49 | 2.92 ± 0.88 | 3.2 ± 0.6 | 3.5 ± 0.59 |
| | 8 h | 4.83 ± 2.43 | 2.94 ± 0.89 | 3.2 ± 0.58 | 3.47 ± 0.64 |
| Monocyte (/mm³) | 1 h | 0.57 ± 0.24 | 0.54 ± 0.25 | 0.61 ± 0.18 | 0.7 ± 0.2 |
| | 4 h | 0.63 ± 0.31 | 0.53 ± 0.23 | 0.63 ± 0.12 | 0.69 ± 0.16 |
| | 6 h | 0.58 ± 0.23 | 0.56 ± 0.23 | 0.59 ± 0.15 | 0.67 ± 0.2 |
| | 8 h | 0.58 ± 0.25 | 0.53 ± 0.25 | 0.62 ± 0.14 | 0.7 ± 0.16 |
| Eosinophil (/mm³) | 1 h | 0.14 ± 0.06 | 0.15 ± 0.09 | 0.19 ± 0.01 | 0.2 ± 0.02 |
| | 4 h | 0.14 ± 0.09 | 0.15 ± 0.08 | 0.18 ± 0.02 | 0.18 ± 0.02 |
| | 6 h | 0.14 ± 0.08 | 0.14 ± 0.07 | 0.19 ± 0 | 0.18 ± 0.02 |
| | 8 h | 0.15 ± 0.08 | 0.15 ± 0.08 | 0.18 ± 0.01 | 0.18 ± 0.02 |
| Basophil (/mm³) | 1 h | 0.04 ± 0.02 | 0.04 ± 0.02 | 0.06 ± 0.01 | 0.06 ± 0.01 |
| | 4 h | 0.05 ± 0.02 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.05 ± 0.02 |
| | 6 h | 0.04 ± 0.02 | 0.04 ± 0.03 | 0.05 ± 0.001 | 0.07 ± 0.03 |
| | 8 h | 0.05 ± 0.02 | 0.05 ± 0.03 | 0.05 ± 0.02 | 0.06 ± 0.02 |

*RTK2: room temperature, K₂EDTA; RTK3: room temperature, K₃EDTA; REFK2: refrigerated temperature, K₂EDTA; REFK3: refrigerated temperature, K₃EDTA; SD: standard deviation.*

In the refrigerated specimens, there was variation using K₂EDTA; decrease in the mean was observed from the fourth to the sixth hour, with an elevation occurring again in the eighth hour. The refrigerated specimens using K₃EDTA presented stability during the whole storage time (85 fl). Due to minimal variations, no statistical difference was observed (Table 1).

In the analysis of MCHC mean profile, a remarkable variation was observed in relation to room temperature. In the first hour, the obtained mean was 33%; in the fourth hour, it varied to 32%, stabilizing up to the sixth hour at 33%. With refrigerated specimens, variation was between 32% and 34%; K₂EDTA was stabilized from the fourth hour at 33%, up to the eighth hour of storage (Table 1).

In the result of MCH mean profile, the mean values were homogeneous, with minor variation for both temperatures, on average, 28-29 pg during the whole 8-hour period; there was significant statistical difference (Table 1).

Regarding the total leukocyte count, the samples kept at room temperature with K₂EDTA presented variation after 6 hours of storage, with mean of 8.48 leukocytes/mm³, suffering increase in the eighth hour, with 8.54 leukocytes/mm³. K₃EDTA did not suffer significant variation, remaining with 8.55-8.65 leukocytes/mm³ during the whole storage time. The refrigerated samples suffered variation of 8.38-8.33 leukocytes/mm³, with no statistical difference (**Table 2**).

In the analysis of neutrophils, variation in sample results was observed at room temperature, more specifically K₂EDTA; it was possible to observe increase in the number of neutrophils after 4 hours of storage, increasing from 4.79 to 4.92 neutrophils/mm³.

From the sixth hour to the eighth hour, the value was stable at 4.83 neutrophils/mm³. In the samples with K₃EDTA at room temperature, no significant variation was observed during the whole storage period (ranging from 4.9 to 5 neutrophils/mm³). In the refrigerated samples, significant oscillations were not observed among means; values ranged between 5.53 and 7.49 neutrophils/mm³ (Table 2).

In the mean lymphocyte count profile, there was variation between storage temperatures. In the refrigerated K₂EDTA, continuous decrease of lymphocytes was observed in the first hour of storage (2.98 lymphocytes/mm³) up to the sixth hour (2.9 lymphocytes/mm³); in the eighth hour, the means was 2.94 lymphocytes/mm³. Room temperature K₃EDTA proved homogeneous, not presenting mean variations (2.92 to 2.94 lymphocytes/mm³). The refrigerated samples presented greater stability, with no significant variations in their means (2.87 and 4.17 lymphocytes/mm³).

In the analysis of monocytes, a small variation in the samples kept in room temperature was observed, using, specifically,

$K_2$EDTA; there was increase of the mean after 4 hours of storage (0.57 to 0.63 monocytes/mm³). From the sixth hour, a slight decrease was observed; there was stability up to the eighth hour (0.58 monocytes/mm³). Room temperature $K_3$EDTA presented elevation in the sixth hour of storage (0.54 to 0.56 monocytes/mm³), returning to its initial means of 0.54 monocytes/mm³ in the eighth hour. The refrigerated samples did not present relevant variation, with no statistically significant results (Table 2).

In the eosinophil count, the variation of all temperatures was constant during the whole storage time, without presenting significant statistical difference (Table 2).

Analyzing the basophil count, the variation regarding room temperature and refrigeration was homogeneous during the whole storage period, not causing significant statistical difference (Table 2).

Significant variation was observed in platelet count. At room temperature, the mean ranged from 260,000 to 278,000/mm³. Under refrigeration, there was a decrease, ranging from 272,000 to 196,000/mm³. This decrease occurred between the first and the fourth hours, falling from 272,000 to 200,000/mm³ and reaching 196,000/mm³ up to the eighth hour of storage; this created a statistical difference for both temperatures (**Figure**).
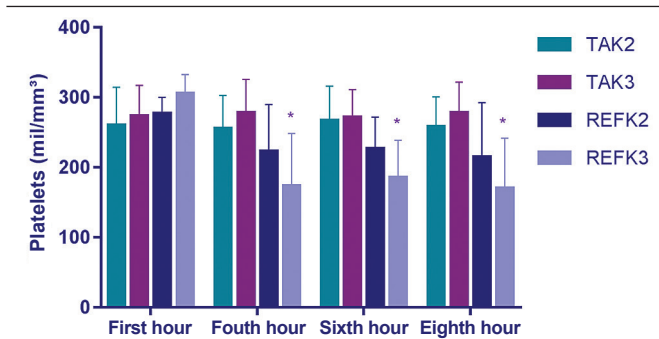


FIGURE – *Mean profile of platelet count parameters, according to temperature and storage time*

## DISCUSSION

In this study, there was no statistical significance in erythrocyte count between groups $K_2$EDTA and $K_3$EDTA, and, after the 8-hour period, it was possible to observe neither quantity nor morphological alterations in cells. In the cases in which EDTA concentration exceeds, it is possible to observe accentuated crenation and sphering in few hours[2, 4, 8].

When analyzing results obtained from samples at room temperature, we verified that hematocrit increased due to the increased volume of erythrocytes, demonstrating significant statistical variation. However, the samples under refrigeration kept their values stable. In different studies, hematocrit values and MCV suffered concomitant increase[1, 4, 9, 10]. In the MCHC values of samples at room temperature, there was a slight oscillation after the fourth hour, returning to stability after the sixth hour. According to De Baca *et al*. (2006)[3], MCHC decrease can happen when samples are stored during prolonged periods of time.

Although oscillation in the obtained values of MCHC was observed, this difference did not occur in the values of hemoglobin concentration; there was stability in both anticoagulants, in all temperatures.

As for MCH, there was stability in results, without creating statistical relevance for samples during the analyzed period.

The results of leukocyte count did not present significant statistical difference for either of the temperatures during the analyzed period. It is known that leukocyte count can be altered in excess of EDTA due to sample dilution, affecting the differential count. This problem is solved by performing the analysis within 24 hours of storage under refrigeration[2, 8, 11].

The results of lymphocyte, neutrophil, and monocyte counts did not present statistical difference, but greater stability in refrigerated samples was observed.

Studies show that neutrophils and monocytes are more sensitive, and lymphocytes are more stable in storage with EDTA[4, 12].

The results obtained in platelet count presented a progressive decrease at room temperature, but without statistical difference, while refrigerated samples presented a considerable decrease in platelet count, with important statistical difference, which can have been caused by spontaneous agglutination.

Samples collected with EDTA can suffer spontaneous agglutination or platelet satellitism – EDTA-dependent clustering by antibodies of immunoglobulin M (IgM) or G (IgG), which have higher aggregation power when kept at low temperatures –, decreasing their values, what precludes a reliable analysis[13].

## CONCLUSION

By assessing the obtained results, one was able to verify that most hemogram parameters remain stable when specimens are refrigerated from 2°C to 8°C, and, when stored at room temperature, variation is more pronounced. Refrigerated samples did not suffer significant change in the parameters when in the analysis within an 8-hour period, except platelet analyses, in which there were relevant alterations.

Factors of the pre-analytical phase, such as temperature and storage time, can interfere with variability of blood count parameters in a clinical laboratory routine, and can produce mistaken results.

The correct ratio anticoagulant/blood is a very important factor to obtain results with fewer oscillations for the analyzed hematological parameters.

According to the present study and several other results, we conclude that storing blood samples interferes with neither erythrocyte and leukocyte counts, nor hemoglobin concentration measurement. However, it becomes unfeasible to assess other parameters after the 8-hour period, regardless of the storage form.

One can also notice a marked decrease in platelet count, therefore we need to count and analyze coagulation parameters as soon as possible upon sample reception.

K$_2$EDTA choice is recommended, because it avoids chances of collection with incorrect blood quantity that interferes with the ratio anticoagulant/blood, as it commonly happens when K$_3$EDTA is used. We must always respect the correct ratio anticoagulant/blood on collection time and avoid conducting the blood count analysis after long periods of sample storage, aiming at keeping the integrity and the morphological features of blood, providing a reliable result for diagnosis.

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