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Influence of storage time and temperature on the stability of reticulocyte parameters

Influência do tempo e da temperatura de armazenamento na estabilidade dos parâmetros reticulocitários

Influencia del tiempo y de la temperatura de almacenamiento en la estabilidad de los parámetros reticulocitarios

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Abstract: Reticulocytes are markers of erythropoietic activity, and their assessment may be influenced by preanalytical conditions. Objective: To evaluate the influence of storage time and temperature on the stability of the reticulocyte percentage and immature reticulocyte fraction (IRF). Materials and Methods: This experimental, prospective, quantitative study included 20 peripheral blood samples from newborns and umbilical cord blood samples collected in EDTA at a private laboratory. After excluding one sample, 19 samples were analyzed using the Beckman Coulter DxH 900 hematology analyzer at 0, 6, 12, 18, and 24 hours under room temperature (22–25 °C) and refrigerated (2–8 °C) conditions. Results: The reticulocyte percentage decreased significantly between 0 and 18 hours and between 0 and 24 hours in samples stored at room temperature, whereas no significant differences over time were observed under refrigeration. Storage temperature had a significant effect on the reticulocyte percentage ($p = 0.0036$). No significant changes in IRF were observed under either storage condition. Conclusion: Refrigeration was associated with the maintenance of reticulocyte percentages throughout the 24-hour evaluation period, whereas room-temperature storage was associated with significant changes after 18 hours. IRF remained statistically unchanged under both conditions, highlighting the importance of controlling storage conditions during the preanalytical phase.

Keywords: Clinical Analysis, Pre-analytical phase, Reticulocytes, Quality Control, Hematology.

Resumo: Os reticulócitos são marcadores da atividade eritropoiética, cuja avaliação pode ser influenciada pelas condições pré-analíticas. Objetivo: avaliar a influência do tempo e da temperatura de armazenamento sobre a estabilidade da contagem relativa de reticulócitos e da fração de reticulócitos imaturos (IRF). Materiais e métodos: estudo experimental, prospectivo e quantitativo, realizado com 20 amostras de sangue periférico de recém-nascidos e de cordão umbilical, coletadas em EDTA, provenientes de um laboratório privado. Após a exclusão de uma amostra, 19 foram analisadas no equipamento Beckman Coulter DxH 900, nos tempos de 0, 6, 12, 18 e 24 horas, sob temperatura ambiente (22–25 °C) e refrigeração (2–8 °C). Resultados: a contagem relativa de reticulócitos apresentou redução significativa entre 0 e 18 horas e entre 0 e 24 horas nas amostras mantidas à temperatura ambiente, enquanto não foram observadas diferenças significativas ao longo do tempo sob refrigeração. A temperatura apresentou efeito significativo sobre a contagem ($p = 0,0036$). O IRF não apresentou alterações significativas em nenhuma das condições. Conclusão: a refrigeração esteve associada à manutenção da contagem relativa de reticulócitos durante as 24 horas avaliadas, enquanto o armazenamento à temperatura ambiente apresentou alterações após 18 horas. O IRF permaneceu sem variações estatisticamente significativas, reforçando a importância do controle das condições de armazenamento na fase pré-analítica.

Palavras-chave: Análises Clínicas, Fase pré-analítica, Reticulócitos, Controle de Qualidade, Hematologia.

Resumen: Los reticulocitos son marcadores de la actividad eritropoyética, cuya evaluación puede verse influida por las condiciones preanalíticas. Objetivo: Evaluar la influencia del tiempo y de la temperatura de almacenamiento sobre la estabilidad del recuento relativo de reticulocitos y de la fracción de reticulocitos inmaduros (IRF). Materiales y métodos: Estudio experimental, prospectivo y cuantitativo, realizado con 20 muestras de sangre periférica de recién nacidos y de cordón umbilical, recogidas en EDTA, procedentes de un laboratorio privado. Tras excluir una muestra, se analizaron 19 en el equipo Beckman Coulter DxH 900, a las 0, 6, 12, 18 y 24 horas, a temperatura ambiente (22–25 °C) y bajo refrigeración (2–8 °C). Resultados: El recuento relativo de reticulocitos presentó una reducción significativa entre las 0 y las 18 horas y entre las 0 y las 24 horas en las muestras mantenidas a temperatura ambiente, mientras que bajo refrigeración no se observaron diferencias significativas a lo largo del tiempo. La temperatura tuvo un efecto significativo sobre el recuento ($p = 0,0036$). La IRF no presentó cambios significativos en ninguna de las condiciones. Conclusión: La refrigeración se asoció al mantenimiento del recuento relativo de reticulocitos durante las 24 horas evaluadas, mientras que el almacenamiento a temperatura ambiente presentó cambios después de 18 horas. La IRF permaneció sin variaciones estadísticamente significativas, reforzando la importancia del control de las condiciones de almacenamiento en la fase preanalítica.

Palabras clave: Análisis Clínicos, Fase preanalítica, Reticulocitos, Control de Calidad, Hematología.

1. Introduction

Hematopoiesis is the process of blood cell formation from hematopoietic stem cells, whereas erythropoiesis refers to the production and maturation of erythrocytes, regulated primarily by erythropoietin (EPO) (Paiva; Rego, 2014; Hall; Hall, 2021). During this process, extrusion of the nucleus from erythroid precursors gives rise to reticulocytes, immature cells containing residual RNA that complete their maturation in the peripheral circulation in approximately one to two days (Zago; Falcão; Pasquini, 2014).

The reticulocyte count is an important indicator of bone marrow erythropoietic activity, assisting in the investigation and monitoring of anemia and in the assessment of treatment response. Automated hematology analyzers now allow these cells to be quantified and complementary parameters, such as the immature reticulocyte fraction (IRF), to be assessed, contributing to the evaluation of erythrocyte production and the standardization of laboratory results (Gois et al., 2019; João; Pinto; Costa, 2008).

However, the reliability of results depends on preanalytical conditions, particularly the time elapsed between collection and analysis and the storage temperature. Because reticulocytes continue to mature after collection, cellular changes during storage may alter the

amount of residual RNA and interfere with their identification and quantification. Thus, processing delays may compromise the representativeness of results and their clinical interpretation (Briggs et al., 2014; Schapkaitz; Pillay, 2015; Urbina; Palomino, 2018).

Assessing the stability of reticulocyte parameters under different storage conditions is relevant to routine laboratory practice, especially when immediate sample processing is not possible. Investigating the effects of time and temperature may contribute to improving preanalytical procedures and obtaining more reliable results. However, preservation conditions must be assessed in light of the sample type and analytical method used.

In this context, this study aimed to assess the influence of storage time and temperature on the stability of reticulocyte parameters in peripheral blood samples from newborns and umbilical cord blood samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA). To this end, reticulocyte percentage and IRF results obtained immediately after collection and after 6, 12, 18, and 24 hours of storage at room temperature and under refrigeration were compared using an automated hematology analyzer.

2. Theoretical framework

2.1. Hematopoiesis and hematopoietic stem cells

Hematopoiesis is the process responsible for the formation, renewal, and maintenance of blood cells throughout life. It occurs mainly in the bone marrow and depends on hematopoietic stem cells (HSCs), which have the capacity for self-renewal and differentiation into the various blood cell lineages. Self-renewal maintains the pool of these cells, whereas differentiation ensures the production of erythrocytes, leukocytes, and platelets according to the body's physiological needs and in situations of increased demand, such as hemorrhage (Paiva e Rego, 2014; Silva Junior; Odongo; Dulley, 2009).

The regulation of hematopoiesis involves growth factors and cytokines that control cell proliferation and differentiation. Erythropoiesis is notably regulated by erythropoietin (EPO), a hormone produced predominantly by the kidneys in response to reduced oxygen availability in tissues. Its main function is to stimulate the survival and maturation of erythroid precursors, promoting red blood cell production (Paiva; Rego, 2014).

During embryonic and fetal development, hematopoiesis occurs at different sites, including the placenta and fetal liver. Subsequently, the bone marrow progressively assumes

this function and becomes the main site of blood cell production, a role it maintains after birth (Paiva; Rego, 2014).

2.1.1. Erythropoiesis

Erythropoiesis refers to the formation and maturation of erythrocytes, a process occurring predominantly in the red bone marrow. Its function is to maintain an adequate number of red blood cells for oxygen transport to tissues. Because these cells have an average lifespan of approximately 120 days, their production occurs continuously to compensate for the removal of aged cells (Zago; Falcão; Pasquini, 2014).

The process begins with HSCs, which give rise to erythroid progenitors undergoing successive stages of differentiation: proerythroblast, basophilic erythroblast, polychromatic erythroblast, orthochromatic erythroblast, reticulocyte, and mature erythrocyte. Maturation involves a reduction in cell size, chromatin condensation, hemoglobin synthesis, and nuclear extrusion. After release into the circulation, the reticulocyte completes its maturation and becomes an erythrocyte (Zago; Falcão; Pasquini, 2014).

Erythropoiesis is regulated primarily by EPO, which acts on erythroid precursors in the bone marrow, promoting their survival, proliferation, and differentiation. In addition to this hormonal stimulus, adequate red blood cell production depends on the availability of nutrients such as iron, vitamin B12, folic acid, vitamin B6, and amino acids (Zago; Falcão; Pasquini, 2014).

Iron is essential for the synthesis of the heme group and, consequently, hemoglobin. Approximately 60% to 70% of body iron is estimated to be incorporated into erythrocyte hemoglobin, highlighting its importance for erythropoiesis and oxygen transport (Negri, 2017).

2.2. Reticulocytes and clinical applications

Reticulocytes are immature erythroid lineage cells released by the bone marrow into the peripheral circulation before completing their maturation. They are characterized by residual RNA and remaining cellular components, which are gradually eliminated during their transformation into mature erythrocytes (Zago; Falcão; Pasquini, 2014; João; Pinto; Costa, 2008).

After release into the circulation, reticulocytes undergo maturation for approximately one to two days, during which residual RNA is degraded, organelles are eliminated, and the

membrane and cytoskeleton are remodeled. These changes result in mature cells specialized in oxygen transport (Urbina; Palomino, 2018; Parodi; Romano; Ramenghi, 2020).

The reticulocyte count is an indicator of bone marrow erythropoietic activity because it reflects recent red blood cell production. Elevated values may occur in compensatory responses, such as hemolysis and hemorrhage, whereas reduced values may indicate insufficient erythroid production associated, for example, with bone marrow impairment, erythropoietin deficiency, or nutritional deficiencies (Zago; Falcão; Pasquini, 2014; Parodi; Romano; Ramenghi, 2020).

This parameter is also used to monitor treatment response in anemia and assess bone marrow recovery after hematological treatments. Counting can be performed manually, using supravital staining, or by automated hematology analyzers, which identify reticulocytes based on their residual RNA content. Automated methods favor standardization, speed, and reproducibility of results (Zago; Falcão; Pasquini, 2014; Parodi; Romano; Ramenghi, 2020).

2.3. Analytical methods and reticulocyte parameters

Automated hematology analyzers allow the assessment of different reticulocyte parameters. Among these, the relative and absolute reticulocyte counts provide information on erythroid production, whereas the immature reticulocyte fraction (IRF) helps assess recent erythropoietic activity.

In systems using fluorescence, reticulocytes can be classified according to signal intensity into low- (LFR), medium- (MFR), and high-fluorescence (HFR) fractions. This classification is related to the amount of residual RNA: more immature cells exhibit greater fluorescence, whereas intensity decreases during maturation. IRF corresponds to the sum of the medium- and high-fluorescence fractions (MFR + HFR) (João; Pinto; Costa, 2008).

The absolute reticulocyte count complements the percentage by expressing the number of cells per unit of blood volume. Reticulocyte hemoglobin, reported as CHr or Ret-He depending on the instrument, estimates the hemoglobin content of reticulocytes and helps assess the functional availability of iron for recent erythropoiesis (Mast; Blinder; Dietzen, 2008; Parodi; Romano; Ramenghi, 2020).

Interpreting these parameters requires attention to age. During the neonatal period, erythropoietic activity and reticulocyte counts are physiologically elevated at birth and change after the transition to extrauterine life. Results must therefore be interpreted using age-appropriate reference intervals, avoiding the direct application of adult values to newborns (Al-

Ghananim et al., 2016; Parodi; Romano; Ramenghi, 2020; Ringoringo et al., 2023; Cerri; Dionisio, 2024).

2.4. Preanalytical factors and sample stability

The preanalytical phase comprises the stages preceding laboratory analysis, including sample collection, identification, transport, storage, and processing. In hematology, controlling these stages is essential because preservation conditions may alter cellular characteristics and compromise the reliability of results (Silva et al., 2016; Mankar et al., 2024).

Reticulocyte stability warrants special attention because these cells contain residual RNA that progressively decreases during maturation. The interval between collection and analysis, as well as storage temperature, may influence results. As described in the original reference, Zhou et al. (2026) recommend analysis preferably within six hours at room temperature or refrigerated storage when delays occur. However, stability conditions must be considered according to the method and analyzer used.

Studies cited in the literature report different results depending on the conditions assessed. George, Basu and Kar (2022) observed stability for up to six hours, with changes in reticulocyte maturation after 24 hours. Khalid et al. (2025) reported a decrease in manual counts after 24 hours under refrigeration, whereas Rocha et al. (2024) found refrigeration beneficial for preserving reticulocyte parameters in automated systems. These differences reinforce the need to assess storage time and temperature jointly, considering the particularities of each analytical method.

Hematological analysis commonly uses whole blood collected in tubes containing ethylenediaminetetraacetic acid (EDTA), an anticoagulant that acts by chelating calcium and preventing coagulation. An appropriate blood-to-anticoagulant ratio, along with proper mixing after collection, helps preserve sample quality (Briggs et al., 2014; Silva et al., 2016).

Accordingly, assessing the stability of reticulocyte parameters at different storage intervals and temperatures is relevant to establishing processing conditions compatible with reliable laboratory results.

2.5. Importance of sample quality control in reticulocyte analysis

Reticulocyte counting is used to assess erythropoietic activity, assist in monitoring anemia, and monitor treatment response and hematopoietic recovery. The reliability of these results depends on control of the preanalytical, analytical, and postanalytical stages, since

failures in any of them may compromise clinical interpretation (Leonart, 2007; Urbina; Palomino, 2018).

The preanalytical phase warrants special attention because the time between collection and analysis, temperature, and storage conditions may influence reticulocyte stability. During sample preservation, these cells continue to mature, with a progressive reduction in residual RNA. These changes may alter the reticulocyte count and the distribution of maturation fractions, compromising how well results represent the patient's condition at collection (Urbina; Palomino, 2018).

Analytical quality also depends on standardized procedures, equipment maintenance and calibration, and the use of internal and external controls. Although automated hematology analyzers offer greater standardization and reproducibility, their performance must be monitored continuously. Manual counting, in turn, requires well-defined protocols and trained professionals, given its greater dependence on the observer's assessment (Leonart, 2007).

In this context, quality control must cover all stages of the laboratory process, including standard operating procedures, staff training, equipment monitoring, and compliance with recommended collection, transport, and storage conditions. Defining appropriate limits for processing time and preservation temperature helps reduce preanalytical changes and increase the reliability of reticulocyte parameters (Leonart, 2009; Urbina; Palomino, 2018).

3. Materials and methods

3.1. Study design and sample selection

This experimental, prospective, quantitative study was conducted in a private clinical analysis laboratory using peripheral blood samples from newborns and umbilical cord blood samples. Neonatal samples were selected because they physiologically contain more reticulocytes than adult samples, facilitating the assessment of changes in this parameter during storage.

The study was conducted after approval by the Research Ethics Committee Involving Human Subjects (CEPEH), under CAAE No. 00322926.0.0000.0109 and opinion No. 8.669.259, respecting the ethical principles applicable to research involving human subjects.

Initially, 20 samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA), with a minimum volume of 3 mL, were included. Intact samples without macroscopic evidence of coagulation or hemolysis and whose initial analysis could be performed

immediately after collection were eligible. Samples with insufficient volume, coagulation, hemolysis, an inappropriate anticoagulant, or analytical failures preventing results from being obtained were excluded. After one sample was excluded, 19 were included in the final analysis.

3.2. Storage conditions and analysis times

Each sample was assessed immediately after collection (time zero) and subjected to two storage conditions: room temperature (22–25 °C) and refrigeration (2–8 °C). Subsequent analyses were performed after 6, 12, 18, and 24 hours. Results obtained at the different intervals were compared to assess changes in reticulocyte parameters over time and between the two storage conditions.

3.3. Laboratory assessment

Analyses were performed using a Beckman Coulter® DxH 900® automated hematology analyzer, subjected to internal quality control according to routine laboratory procedures. Two parameters available from the instrument were assessed: reticulocyte percentage (%) and immature reticulocyte fraction (IRF).

3.4. Statistical analysis

Data were organized in an electronic spreadsheet and analyzed using GraphPad Prism® software, version 10.1. Descriptive analysis was initially performed, with results presented as mean and standard deviation (SD), and normality was assessed using the Shapiro–Wilk test.

Two-way repeated-measures analysis of variance (ANOVA) was used to assess the effects of storage time and temperature on reticulocyte percentage, with the Greenhouse–Geisser correction for effects involving time. Storage conditions at each interval were compared using the paired t-test or the Wilcoxon signed-rank test, according to the distribution of differences, with p-values adjusted using the Holm–Bonferroni method. The Friedman test and, when applicable, Dunn's multiple-comparisons test were used to compare different storage times within each condition. The significance level adopted was 5% ($p < 0.05$).

4. Results and discussion

Reticulocyte parameters were analyzed in 19 samples. As shown in Figure 1, two-way repeated-measures ANOVA identified neither a significant effect of storage time ($p = 0.1226$)

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nor a significant interaction between time and temperature (p = 0.0561). However, a significant main effect of storage temperature was observed (p = 0.0036).

Comparisons between storage conditions at each interval showed no statistically significant differences at 6 hours (p = 0.0536) or 12 hours (p = 0.3747). However, differences were identified at 18 hours (p = 0.0092) and 24 hours (p = 0.0327), after adjustment for multiple comparisons using the Holm–Bonferroni method.

Figure 1 – Variation in reticulocyte percentage (%) according to storage time and temperature.

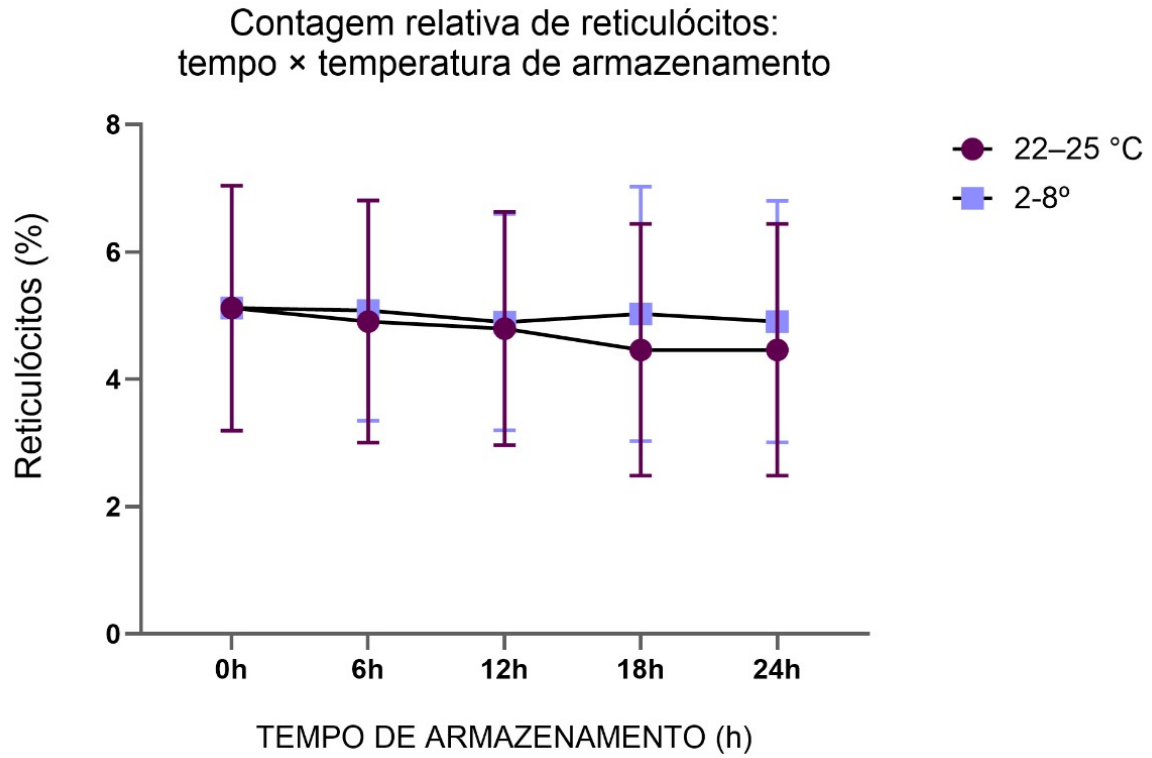


Figure labels: Reticulócitos = Reticulocytes; tempo × temperatura de armazenamento = storage time × temperature; tempo de armazenamento (h) = storage time (h).
Note: Data are presented as mean ± SD for 19 samples assessed at 0, 6, 12, 18, and 24 hours at room temperature (22–25 °C) and under refrigeration (2–8 °C). Analysis was performed using two-way repeated-measures ANOVA, with the Geisser–Greenhouse correction for effects involving time. No significant effects of time (p = 0.1226) or interaction between time and temperature (p = 0.0561) were observed. A significant main effect of storage temperature was observed (p = 0.0036). Source: Prepared by the authors (2026).

Comparisons between storage conditions at each analysis time identified no statistically significant differences at 6 hours (p = 0.0536) or 12 hours (p = 0.3747). However, significant differences were observed at 18 hours (p = 0.0092) and 24 hours (p = 0.0327). These results indicate differences between storage conditions at the later intervals assessed, although the overall ANOVA did not identify a significant interaction between time and temperature.

In samples stored at room temperature (Fig. 2A), a significant difference in reticulocyte values was observed among the times assessed (Friedman test, p = 0.0003). Dunn's multiple

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comparisons identified significant differences between the initial time (0 h) and 18 hours (p = 0.0059), as well as between 0 h and 24 hours (p = 0.0010). The other comparisons showed no statistically significant differences.

In samples stored under refrigeration (Fig. 2B), no statistically significant differences in reticulocyte values were observed among the times assessed (Friedman test, p = 0.6225). Mean values showed little variation over the 24 hours of storage, with no significant differences between assessments.

Figure 2 – Variation in reticulocyte percentage during storage at room temperature (22–25 °C) and under refrigeration (2–8 °C).

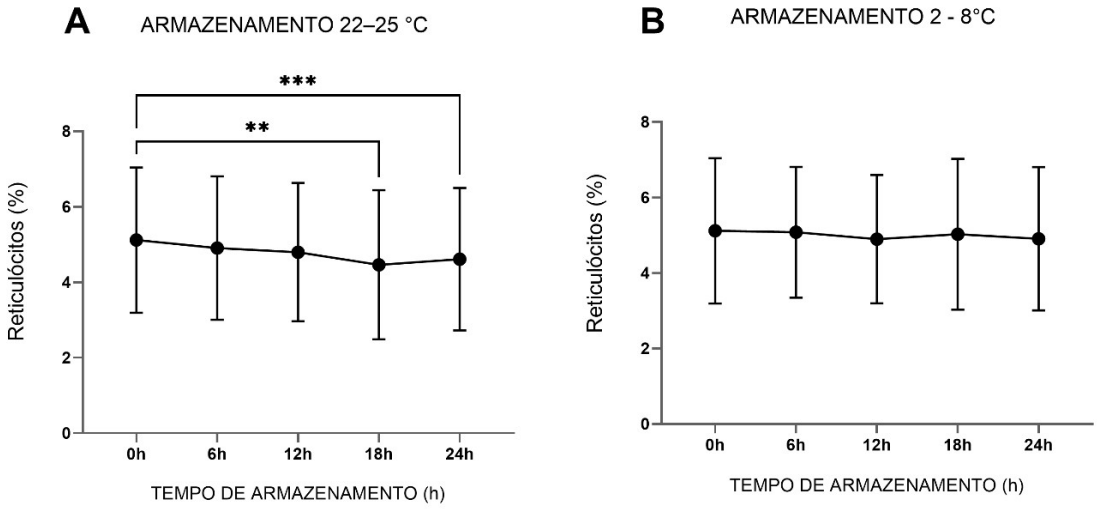


Figure labels: Armazenamento = Storage; Reticulócitos (%) = Reticulocytes (%); tempo de armazenamento (h) = storage time (h).
Note: (A) Data are presented as mean ± SD for 19 samples. The overall comparison among times was performed using the Friedman test (p = 0.0003), followed by Dunn's multiple-comparisons test. Significant differences were identified between 0 and 18 hours (p = 0.0059) and between 0 and 24 hours (p = 0.0010). **p < 0.01; ***p < 0.001. (B) No statistically significant differences among storage times were identified using the Friedman test (p = 0.6225). Source: Prepared by the authors (2026).

The immature reticulocyte fraction (IRF) showed no statistically significant differences among storage times in samples kept at room temperature (Friedman test, p = 0.6240) or under refrigeration (p = 0.7581). Although small fluctuations in mean values were observed over the 24 hours, no significant temporal change was identified under either condition assessed (Figure 3).

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Figure 3 – Variation in immature reticulocyte fraction (IRF) according to storage time and temperature.

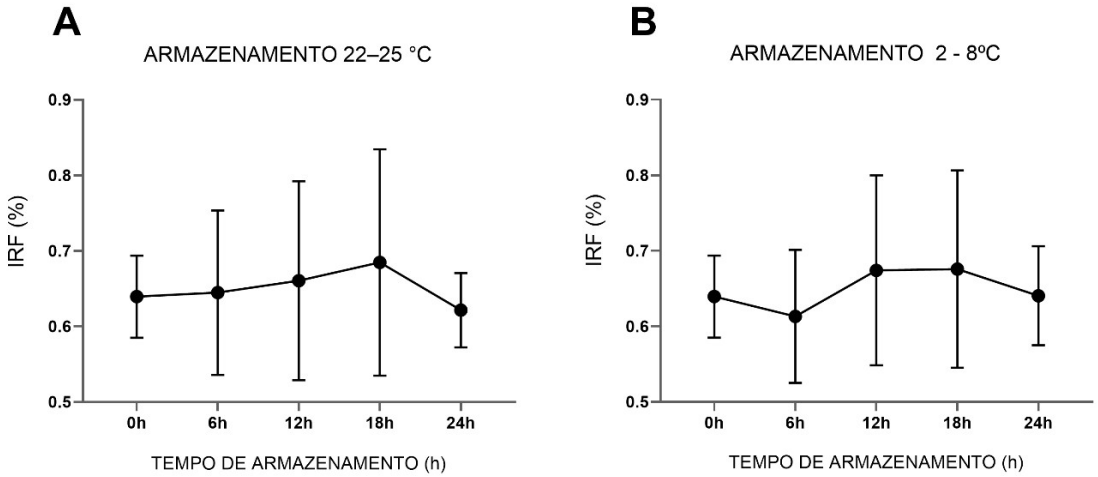


Figure labels: Armazenamento = Storage; tempo de armazenamento (h) = storage time (h); IRF = immature reticulocyte fraction.
Note: Data are presented as mean ± SD for 19 samples assessed at 0, 6, 12, 18, and 24 hours. (A) Samples stored at room temperature; (B) samples stored under refrigeration. The Friedman test identified no statistically significant differences among times in samples kept at room temperature ($p = 0.6240$) or under refrigeration ($p = 0.7581$).

Our results show that reticulocyte percentage decreased significantly after 18 hours of storage at room temperature, whereas under refrigeration (2–8 °C), no significant differences were observed over 24 hours. This finding partially agrees with Rocha et al. (2024), who reported stability of reticulocytes and the immature reticulocyte fraction (IRF) in refrigerated samples for up to 72 hours. Although the observed stability periods differ, the results reinforce the importance of storage temperature for preserving reticulocyte parameters.

The influence of temperature on the stability of hematological parameters was also observed by Dalanhol et al. (2010), who identified significant changes in hematocrit, mean corpuscular volume (MCV), and red cell distribution width (RDW) in samples kept at room temperature, with discrepant results after 12 hours of storage. These changes were not observed under the same conditions with refrigeration. The authors considered the release of results between 12 and 24 hours after collection satisfactory. Although the study assessed hematological parameters other than reticulocytes, its findings reinforce the relevance of storage conditions to the reliability of laboratory tests. In this study, the significant main effect of temperature in two-way ANOVA ($p = 0.0036$) also demonstrates the influence of this variable on reticulocyte percentage.

Additionally, Ferreira et al. (2023) also investigated the effects of storage time and temperature on cell morphology and observed artifactual echinocytosis in 35% of samples after 8 hours at room temperature, reaching 100% after 24 hours. Under refrigeration, this change

was observed in all samples only after 48 hours, whereas leukocytes remained stable for up to 12 hours. The authors concluded that refrigeration delays morphological changes and recommended processing samples within 12 hours at room temperature and 24 hours under refrigeration.

Although these findings concern cell morphology rather than reticulocyte parameters specifically, they reinforce the importance of controlling storage temperature and time. In the present study, a significant reduction in reticulocyte percentage was identified from 18 hours at room temperature, highlighting the need to consider the specific stability of each laboratory parameter.

Regarding IRF, no significant differences were observed over 24 hours of storage, either at room temperature or under refrigeration. This result partially agrees with Rocha et al. (2024), who reported only a slight reduction in correlation over 72 hours in refrigerated samples.

However, Khalid et al. (2025) observed a significant reduction in the manual reticulocyte count after 24 hours under refrigeration (2–8 °C), from 2.99% to 2.41%, differing from the results obtained for reticulocyte percentage in the present study. This difference should be interpreted in light of methodological particularities, since the authors highlighted the observer dependence and low reproducibility of manual counting, as well as the absence of a comparison with automated methods.

Taken together, this evidence suggests that the stability of reticulocyte parameters may vary according to the analytical method used, reinforcing the importance of considering the characteristics of the procedure when comparing studies and establishing storage conditions.

5. Conclusion

The results demonstrated that storage temperature significantly influenced reticulocyte percentage. In samples kept at room temperature, significant reductions were observed after 18 and 24 hours, whereas under refrigeration (2–8 °C), no significant differences were identified over the 24 hours assessed. No significant changes in IRF were observed under either storage condition.

These findings reinforce the importance of controlling time and temperature during the preanalytical phase for the reliability of reticulocyte results. Refrigeration proved to be an alternative associated with maintenance of reticulocyte values during the period assessed, although further studies are needed to confirm these findings in different populations, storage conditions, and analytical systems.

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