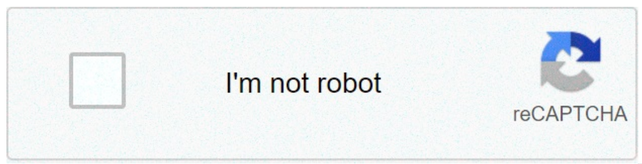

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Clsi guidelines for edta tube stability

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This is an Open Access article distributed under the Creative Commons Attribution license, which allows for unrestricted use, distribution and playback in any medium, provided that the original work is correctly mentioned. Extended the changes induced by storage in the parameters of hematology as shown in this original research. . • Introduction • Research and Design Method • Ethic considerations • Materials and setting • Design • Blood sampling and laboratory methods • Laboratory testing • Analysis •• Conservation at room temperature • Conservation at 4 °C – 8 °C • Discussion • Study restrictions • Conclusion • Recognition • Complementary interests • Contributions by authors • References Fund: Referral of samples for the work of hematologic disorders from remote laboratories can lead to a delay in analysis. Objective: The stability of complete blood count morphology (FBC), differential count (DIFF), reticulocyte and peripheral blood (PBS) during prolonged storage was assessed. Methods: Forty blood samples stored in ethylenediaminetetraacetic acid (EDTA) have been analysed on an ADVIA® hematology analyzer 120. Samples (25% abnormal; 75% normal) were stored at room temperature (RT) and at 4 °C - 8 °C. Sample analysis stored at RT was performed every 12 hours for two days. The analysis of samples stored at 4 °C – 8 °C was performed at 12 hours and then every 24 hours for seven days. Results: the FBC (red cell count, hemoglobin) and DIFF (percentages of low-philes, lymphocytes and monocytes) parameters were stable for at least 48 hours when they were stored at RT. The dishes were stable only for 12 hours and the number of white cells was stable for 36 hours when stored at RT. Memorization of samples at 4 °C – 8 °C significantly increased the stability of most parameters, in particular, the volume of medium cells and the percentage of reticulocytes. However, DIFF parameters were associated with a lower stability at 4 °C –°C. PBS morphology has been compromised before 12 hours is stored at RT or at 4 °C - 8 °C. Conclusion: This study provides proof that blood samples stored in EDTA at 4 °C – 8 °C for seven days are suitable for testing ADVIA® 120 for FBC and the percentage of reticulocyte parameters. However, storage at 4 °C - 8 °C is not a solution for the samples mentioned for DIFF and PBS morphology review. Preanalytical variables, such as storage time and temperature, affect the measurement of laboratory parameters collected in ethylenediaminetetraacetic acid (EDTA).1,2 The lab staff must be aware of the changes that occur during storage in their specific setting in order to decide whether to accept or refuse too old samples to obtain reliable results. Accurate measurement of full blood morphology (FBC), differential count (DIFF) and reticulocyte parameters, as well as peripheral blood morphology (PBS), are essential for the correct interpretation of the results of hematology. It is recommended that traditional parameters of the FBC such as red cell count (RCC), white cell count (WCC), hemoglobin and count of the platerin be analyzed 24 hours after the sample collection when they are stored at room temperature (RT).3,4,5 However, useful parameters for diagnosis and monitoring of hematologic disorders, such as the average volume of cells (MCV), reticulocytelic andand results in a misclassification of a microcytic anemia such as nomocytics and, in the same way, a nomocytic anemia as macrocytics.6 reticulocytes mature in red cells after 24 hours in circulation. The Institute of Clinical and Laboratory Standards (CLSI) recommends analyzing samples stored at RT for reticulocytes within six hours of collection.7 It is also recommended that PBS for morphological analysis be prepared within four hours, before the beginning of changes induced by EDTA in the morphology of red and white cells.8,9,10 With the centralization of laboratory services, it is not always possible to comply with these deadlines. Large academic laboratories are commonly faced with the scenario where a sample collected on Friday is not received in the laboratory for analysis until Monday morning. Recent studies indicate that longer retention periods are acceptable when samples are stored at 4 °C – 8 °C.3,5,6,11,12 However, stability information over 72 hours is limited. 6 Moreover, these studies are small, specific for the hematology analyzer, and the definition of stability used is not standardized. The purpose of this study was to assess the stability of the morphology FBC, DIFF, reticulocyte and PBS during prolonged storage at RT and at 4 °C - 8 °C in order to determine the laboratory criteria for the time of conservation and temperature for the samples mentioned for the work-up of hematologic disorders from remote laboratories. Method of research and design Ethical considerations The study wasof the Human Research Ethics Committee of the University of Witwatersrand (M090688.) All tests were carried out as part of routine diagnostics and no additional blood samples were taken from participants for this study. Materials and adjustment This study was conducted at the Main Haematology Laboratory of Charlotte Maxeke Johannesburg Academic Hospital (CMJAH,) National Health Laboratory Service Complex, Johannesburg, South Africa. Forty blood samples, patient population representative (25% abnormal and 75% normal samples,) which were left after routine tests were selected by the workload of hematology. Only samples collected in EDTA bottles (Becton-Dickinson, Oxford, United Kingdom) with adequate volume (> 4 mL) received within two hours of collection were included. Samples with results that indicated partial aspiration were excluded from final analysis. Blood samples for the evaluation of the parameters FBC, DIFF and reticulocytes were collected in the tubes K2EDTA (1.5-2.2 mg of potassium dihydrate EDTA per milliliter of blood). The parameters have been analyzed with the ADVIA® 120 emathology scanner (Siemens Healthcare Diagnostics, Inc, Tarrytown, New York, United States). The cells have been counted and sized by the light shedding technology with white light for white cells and laser light for red cells and platelets. Hemoglobin has been measured by the conventional cyanmetemoglobin method. The six-partthe analysis was performed in two channels. The cells in the peroxidase channel were measured by peroxidase color intensity and light spread. Low-filo/lobular channel cells have been measured with the spread of dual laser light, nuclear density and lobulation index. The reticulocytes were spotted with oxazine 750. The films were released on ADVIA® Autoslide slide maker and colored on the HEMA-TEK® 2000 sliding scale (Siemens Healthcare Diagnostics Inc, Tarrytown, New York, United States). Samples were analyzed within two hours of collection (zero time) to RT. The samples were rated in two sets; one was stored at RT (18 °C - 24 °C) and the other at 4 °C - 8 °C. Sample analysis stored at RT was performed after 12, 24, 36 and 48 hours of storage. Sample analysis stored at 4 °C – 8 °C was performed after 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours of conservation. A manual DIFF was executed on PBS of five samples stored at RT and 4 °C - 8 °C. Reviews were made at 12, 24, 36 and 48 hours. PBS was first examined for the presence of changes induced by EDTA, including red cell spherocytes, echinocytes, sphero-echinocytes, increased rouleaux formation, neutrophil degeneration and lobulation of lymphocytes, 10 because these changes preclude an accurate manual DIFF. The data was collected by the Scanner prints on ExcelTM spreadsheets (Microsoft Office ExcelTM 2007, Redmond, Washington, USA) and analyzed using Statistics 9.1(StatSoft, Tulsa, Oklahoma, United States). The average percentage difference from value to zero was calculated and tabulated. 13 The stability of a parameter has been defined in relation to the accuracy of the ADVIA® analytical method 120. Acceptable limitations were defined in accordance with the Royal College of Pathologists of Australasia guarantee of external quality annual review for 2013.14 The variable coefficients (% CV) for the parameters were as follows: WCC 3.4%, RCC 1.8%, hemoglobin 1.8%, hematocrite 2.4%, platelets count 2.4% and neutrophils percentages 0.9%, lymphocytes 4.9%, monocytes 5.6%, heosinophils 16.7%, 55% bassphiles and reticulocytes 8.1%. A parameter was considered stable, when its difference was less than 1% CV for the method evaluated.5 Conservation at room temperature The WCC was stable up to 36 hours after harvesting and showed a significant decrease to 48 hours after harvesting (Figure 1). A significant increase in neutrophils and heosinophils was observed in 24 hours and 36 hours respectively. Red cell parameters, including RCC, hemoglobin, media hemoglobin (MCH) and red cell distribution width (RDW) were stable for at least 48 hours after harvesting when they were stored at RT and were not significantly affected by storage temperature. On the contrary, other RCC measures, including hematocrite, MCV, average hemoglobin content (MCHC) and the percentage of reticulocytes, were not stable after storage at RT for 48 hours after storage.after storage rt for 24 hours, a significant increase in mcv was observed, as well as a decrease in the percentage of reticulocytes. platelet stability analysis showed platelets were stable for 12 hours and significantly decreased to 24 hours after harvesting. a rt storage, the stability of the average volume of platelets (mpv) was less than 12 hours due to swelling of artificial platelets. a rt storage, the stability of the manual diff was even less than 12 hours. neutrophils and monocytes showed significant increases, while the percentages of lymphocytes and heosinophils showed significant decreases to 12 hours from the collection. the slides examined contained too few basophiles to achieve reliable results for basophile stability. the modifications induced by eta were noticed 24 hours after the collection, which precluded a manual diff. Figure 1: Stability analysis (average percentage difference) at room temperature (18 °C – 24 °C) 4 °C preservation – 8 °C the wcc was stable at 4 °C – 8 °C up to 48 hours after harvesting (figure 2.) a significant decrease in neutrophil percentage was observed at 72 hours after harvesting. the percentages of heosinophiles, basophiles and monocytes were not stable when they were stored at 4 °C - 8 °C and showed significant increases respectively at 12, 24 and 48 hours. compared to rt storage, we observed better stability of rcc parameters when stored at 4 °C - 8 °C. haematocrit, mcv and mchc were stable up to 168 hours when stored4 °C – 8 °C. the percentage of reticulocytes was stable up to 120 hours after harvesting and showed significant decreases later. the dishes were stable until 96 hours after harvesting when stored at 4 °C - 8 °C. mpv showed a significant increase at 24 hours after harvesting. as a result of the presence of modifications induced by eta before 12 hours after the collection, a manual diff could not be performed. Figure 2: Stability analysis (average percentage difference) at 4 °C – 8 °C. routine tests such as fbc, diff, reticulocyte and pbs morphology are commonly referred to the cmjah emathology laboratory as part of the diagnostic work-up for hematologic disorders. in large academic laboratories, where age samples constitute a significant percentage of the workload, it is necessary to take into account the time of conservation and temperature of the samples. the results of this study performed on samples edta add to the tests that stability varies according to the time of storage and temperature. according to the results of this study, the parameters fbc, rcc, hemoglobin, mch and rdw and diff, i.e. the percentages of bassphiles, lymphocytes and monocytes, were less affected by temperature and storage time and can be analyzed up to 48 hours after the sample collection when stored at rt.5, it is recommended that the traditional parameters of the fbc are analyzed 24 hours after the collection onlywhen stored at RT. WCC stability was also found shorter than other studies, which recommended analysis up to 48 hours after harvesting when stored at RT5,11,15 In this study, WCC was stable only up to 36 hours after harvesting when stored in RT. In this study, the MCV was stable up to 12 hours after the collection. Imeri et al. has found that MCV has increased significantly after 4-10 hours, regardless of the hematology analyzer; 5 while other studies have indicated a longer stability up to 24 hours for MCV to RT.3,11,15 These discrete results can be attributed to the different statistical methods used in these stability assessment studies, which limits an accurate comparison. In this study, the percentage of reticulocytes was stable at RT up to 12 hours after the collection, which runs with current recommendations. 3.5 However, the stability of the parameters of the reticleocyte was found longer in RT on other hematology analyzers, such as Coulter® LH 750, Sysmex XE-2100TM and Cell-DYN SapphireTM.5,11 According to Wiegand et al., theoxazine 750 on ADVIA® hematology analyzers can not be enough to detect more maturized reticulocytes. 16 Imeri et al. conducted a three-way comparison study of Coulter® LH750, Sysmex XE-2100TM and ADVIA® hematology analyzers, which further illustrated how the stability of many hematology parameters depends on the used analytical method.5 Therefore, the results of this study are specific to ADVIA®Analyzers, which currently account for 60% of hematology analyzers in South Africa. Therefore, the results of this study have widespread local implications. However, CLSI recommends that 'laborators should assess the stability of the FBC in their specific settings'.17 The storage of samples at 4 °C – 8 °C for seven days has increased the stability of most parameters. The FBC parameters, i.e. WCC, count platelets, hematocrite, MCV and MCHC, as well as DIFF parameters, i.e. neutrophils and reticulocytes percentages, were more stable when stored at 4 °C - 8 °C. However, some DIFF parameters, i.e. the percentages of heosinophiles, basaophiles and monocytes, had a lower stability. The changes were present on the PBS morphology before 12 hours after the collection when they were stored at RT or at 4 °C - 8 °C. This prevented the evaluation of the morphological characteristics of displastics. It is recommended that PBS be prepared within a few hours for the evaluation of hematologic disorders, in particular for the presence of displastic characteristics. 9.10.18 Limitations of the study A limitation of this study is that PBS morphology was not assessed before 12 hours after the collection (i.e., four and six hours). As such, a manual differential could not be performed. In addition, this study was performed under optimal conditions on patient samples that were received in the laboratory within two hours of collection. Samples indicated for tests are often subject to temperature change during harvesting andIn conclusion, this study provides evidence about the feasibility of blood samples collected in EDTA bottles and stored at RT and 4 °C - 8 °C. The samples that were stored at 4 °C - 8 °C for seven days are suitable for testing the ADVIA® 120 analyzer for FBC and reticulocyte parameters. However, this is not a solution for the samples mentioned for the revision of DIFF or PBS morphology.We thank Celeste McPherson and the laboratory staff of the CMJAH National Health Laboratory Service Complex for their technical assistance. Additional interests Authors declare that they have no financial or personal relations that may have inappropriately influenced them in writing in this article. The contributions of the authors E.S. (Charlotte Maxeke Johannesburg Academic Hospital) was the author of the manuscript and also performed the input and analysis of data. D.P. (National Health Laboratory Services, University of KwaZulu-Natal) was responsible for data collection, data entry and manuscript revision. Queen E, Ifeanyi OE, Chinedum OK. The effect of accumulation on the blood count in different anticoagulants. IOSR JDMS. 2014;3(9):128–131. Guder WG. Preanalytic factors and their influence on analytical quality specifications. Scand J Clin Lab Invest. 1999;59(7):545–549. 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