Evaluation and Comparison of Stability and Reliability of CBC Parameters Determined by Using Automatic Celltac G MEK-9100 Hematology Analyzer during Extended Storage at 4°C

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Abstract

Introduction: Sample stability is necessary for the maintenance of the quality for the final results obtained at different storage time intervals during CBC analysis. In our present study, we have evaluated the stability of various CBC parameters of blood samples stored in k2-EDTA (BD) vials at 4°C (extended storage time: 10 days).

Materials and methods: Blood sample (2.5 ml) was drawn directly in K2-EDTA vials. Measurements were done by using MEK-9100 hematology analyzer at regular intervals over an extended period up to 246 h (10 days). Significant differences were analyzed by Paired student's t-test. Mean percent differences of the all intervals were compared with baseline means.

Results: Among CBC parameters, WBC count was stable for up to 126 h, RBC and HGB levels were statistically stable for up to 186 h and 90 h. No significant changes were observed in NE, LY, MO, EO and BA for up to 42 h, 42 h, 66 h, 66 h, and 6 h respectively. PLT counts were stable for 6 h. Furthermore, results of HCT, MCV, MCH, MCHC, RDW-CV, RDW-S, PCT and MPV were statistically stable for up to 54 h, 42 h, 18 h, 30 h, 42 h, 30 h, 6 h and 6 h respectively.

Conclusion: Estimation of RBC, WBC and HGB were qualitatively reliable ~186 h, 126 h and 90 h respectively. However, most parameters of CBC were unchanged ~48 h except for the PLT (6 h). To avoid changes in few parameters, such as MPV, basophiles, it is best to store the sample at 4°C if any delay is anticipated.

Keywords: CBC; Stability; Hematology; Reliability; Extended storage

Introduction

Hematological parameters are one of the most important and fundamental diagnostic tools to investigate the abnormalities of organic diseases, parasitic diseases and evaluation of the metabolic conditions [1]. Stability of blood sample, if long storage is required, is a basic and significant part of clinical laboratory practice to achieve a good quality final result. However, error aspect for studies in the pre analytical phase requires considerable attention on procedures like blood sample collection, identification of patients, selection of proper blood collection vials, accurate blood suction, specimen labeling, clerical error and storage. Work by many others shows that 93% of errors does not relate to the high standard analytical method [2-4]. Central laboratories have been established in many countries in addition to local initiative hospital laboratories. These labs cannot arrange all tests required by their patients. These labs therefore have to send their samples to some central labs for investigation. In addition, specimen collection units have been established that collect the blood samples and then send to central laboratories for processing the samples for final tests [5]. The samples suffer long delays until they reach the central lab. During transportation, the samples need long refrigeration to deterioration due to high environmental temperature. The desiccation of sample has also a serious effect on sample quality due to high rate of evaporation. The condition is worst in tropical countries like Pakistan where humidity and high temperature are at the peak. It is therefore very important to know to save the stability of samples in conditions existing in tropical areas [5,6]. Running pre-stored blood samples is very frequent in our laboratories. It is therefore imperative to define storage conditions for these samples. This study presents a quantitative data for sample storage as regards storage time using a fixed temperature (4°C). This may be very helpful in increasing the reliability of haematological investigations.

Material and Methods

Aim and objectives: Heamatology laboratory, MINAR, Multan, is a very busy clinical laboratory in our oncology department, where hundreds of blood samples are analyzed daily. The blood samples are often kept/stored for very long periods before analysis. There is a risk that the samples may be damaged when they are run for analysis. There is therefore a need to establish the effect of storage time on the quality of measurement. In our present study, our main objective was to evaluate the stability of various CBC parameters of blood samples stored in k2-EDTA (BD) vials at 4°C.

Subjects: 16 normal volunteers (all males) from employees of MINAR were selected for the study. The age of the subjects ranged from 20-30 yrs. Every individual was clinically examined for any pathology before including in study group.

Study area: This study was conducted in Multan Institute of Nuclear Medicine and Oncology (MINAR) located in the city of Multan, Pakistan, it provides clinical facility to south districts in Punjab of Pakistan over the population of 22,374,844 in Cense 2017 [7]. Its geographical location is latitude 30° 9' 26.8488" N, longitude 71° 31' 29.694" E and 129 meters high from sea level [8].

The choice of coagulants: K2-EDTA plastic vial, size 3 ml was chosen for the study. K2-EDTA having a less diluents effect and slighter influence on MCV, haematocrit, and effects on red blood cell size to increase the concentration, had been selected in our study over K3-EDTA [7,9-13].

Storage condition and temperature: In this study, the selected temperature was $4 \pm 1°C$ for storage, which is ideal for storage by WADA current guidelines [14,15]. Blood stored at <2°C can cause freezing injury to RBCs leading to homolysis while storage at >6°C can cause overgrowth of unspecified bacteria during blood sampling [10,12].

Sampling collection: A single, expert Phlebotomist took blood sample from the each of the employee with appropriate safety precautions to avoid the contamination, following the guideline International Standard by Clinical Laboratory Standard Institute and WADA [5]. Blood was drawn by venipunture with BD syringes precision Glide Needle 23 G 1 TW (0.5 mm × 25 mm), (lot No. 6130882, Becton, Dickinson Company, Made in Singapore) directly to Lavender BD, K2-EDTA vacutaiter® (5.4 mg, 3.0 ml, Becton Drive, Franklin Lakes, NJ 07417,USA, lot no. 7067941) with protective glove [5].

Laboratory testing: The baseline measurement (before 20 min) were carried out after collection of samples on automatic MEK-9100 (Nihon Kohden Corporation), which works laser scatter+flow cytometry technology (Dyna Helix flow technology) [7,14,15]. After that, they were stored in refrigerator at 4°C. The interior storage temperature was measured with the double sensor digital standard indoor and outdoor thermometer and follow up chart was filled daily as quality control procedure. Before each measurement, the batch of blood samples were mixed on roller mixer for a proper amount of time to become the stable of testing temperature as recommended by the manufacturer. After measurements, samples were transferred immediately to the refrigerator after analyzing. All samples were re-tested on same analyzer with identical lot of reagent diluents. WBC with differential neutrophils, lymphocytes, monocytes, esnophils, and basophiles, RBC and HGB with their indices HCT, MCV, MCHC, RDW-CV, RDW-SD, PLT, PCT and MPV parameters were included in our study. Calibration of the analyzer was performed on daily basis with appropriate reference standard material and verified with the use of proprietary control with different concentrations (high, Normal and low) by instructed manufacturer [14,16].

Statistical analysis

Statistical analysis was conducted in excel (Microsoft office). P-value and variation between baseline measurements and other time interval points were evaluated by the Paired t-test after confirmation of normal distribution by the D'Agostino–Pearson test. Wilcoxon's test was applied for those parameters that had non-normal distribution pattern. P<0.05 was considered for significant result. Baseline values were taken as 100%, and were compared with the remaining hours interval values. Mean percent change difference was calculated by the formula (mean difference=(test tube mean-reference tube mean)/reference tube mean × 100] of baseline with other hours interval are shown in graph.

Results

All result of our study and mean percentage change difference are shown in Table 1 and graph for each parameters. During the whole study, WBC showed satiability (P=0.27) up to 126 h (5.25 days) in within run precision of 2% , after 138 h to 246 h, the mean percent change difference was fluctuated ranging between -6.5 to 1.9 with statistically significant of P<0.01. In sub-population of WBC, stability were found (P=0.08) in NE up to 42 h, in LY up to 42 h (P=0.07), in MO up to 66 h (P=0.061), in EO up to 66 h (P=0.23), and in BA up to 6 h (P=0.4) in within the run precession of the analyzer value 5%, 5%, 12%, 20% and 30% respectively. After stability hours of WBC differential, NE and MO were observed decreasing with mean percent change difference ranging between -7.08 to -67.0 and -7.25 to -41.0. LY, MO and BA were assessed progressively increased with regular interval hours after collection of blood. In RBC, up to 186 h, there was no significant result (P=0.89) assessed within analyzer reproducibility value 1.5%, and after its, mean percent change difference was observed ranging between 0.9 to -1.61 up to 246 h. Statistically stability were found in HGB up to 90 h (P=0.13) within run precision of 1.5%, HCT up to 54 h (P=0.68) within 1.5%, MCV up to 42 h (P=0.3) within 1%, MCH up to 18 h (P=0.31) within 2%, MCHC up to 30 h (P=0.065) within 2%, RDW-CV up to 42 h (P=0.081) within 3%, RDW-SD up to 30 h (P=0.083) within 3% as well as for PLT up to 6 h (P=0.08) within 4% run precision, PCT up to 6 h (P=0.06) within 6% and MPV up to 06 h (P=0.08) within run precision of 4%.

N:15	Precision of MEK-9100 (Reproducibility)	<20 minutes mean	No Significant changes up to Hours Mean (CV %)/Hrs. P-Value	Significant change at Hours Mean (CV %)/Hrs P-Value
*WBC x (10^3/μL)	2.0%	10.165	10.273 (101.06)/126 h P=0.27	10.563 (103.91)138 h P<0.01
†NE x (10^3/μL)	5.0%	6.470	6.381 (98.62)/42 h P=0.08	4.542 (70.20)/54 h P<0.001
*LY x (10^3/μL)	5.0%	2.884	3.650 (126.58)/42 h P=0.07	4.785 (165.92)/54 h P<0.001
†MO x (10^3/μL)	12.0%	0.532	0.954 (195.21) 66 h P=0.061	1.097 (206.32)/78 h P=0.01
†EO x (10^3/μL)	20.0%	0.237	0.283 (119.16)/66 h P=0.23	0.385 (162.45)/78 h p<0.02

*BA x (10^3/µL)	30.0%	0.075	0.079 (104.82) 6 h P=0.4	0.178 (236.14)/18 h P<0.01
*RBC	1.5%	5.061	5.056 (99.91) 186 h P=0.89	4.975 (98.31)/198 h P=0.038
*HGB (g/dl)	1.5%	13.369	13.451 (100.61)/90 h P=0.13	13.475 (100.79)/102 h P=0.021
*HCT %	1.5%	41.4	42.017 (101.49)/54 h P=0.68	42.882 (103.45)/66 h P<0.001
†MCV (fL)	1.0%	82.436	83.17 (100.89)/42 h P=0.20	85.918 (104.42)/54 h P=0.004
†MCH (pg)	2.0%	26.645	26.80 (100.58)/18 h P=0.31	27.082 (101.64)/30 h P=0.002
*MCHC (g/dl)	2.0%	32.227	32.482 (100.79)/30 h P=0.065	31.664 (98.25)/42 h P<0.001
*RDW-CV (%)	3.0%	13.473	13.770 (102.21)/42 h, P=0.081	14.182 (105.26)/54 h P<0.001
*RDW-SD (fL)	3.0%	43.909	44.627 (101.64)/30 h P=0.083	47.855 (108.99)/42 h P<0.001
*PLT x (10^3/µL)	4.0%	335.591	348.127 (103.74)/6 h P=0.08	365.0 (117.79)/18 h P=0.001
*PCT (%)	6.0%	0.255	0.272 (106.41)/6 h P=0.06	0.301 (117.79)/18 h P<0.001
*MPV (fL)	4.0%	7.645	8.026 (104.98)/6 h P=0.08	8.436 (110.34)/18 h P<0.001
*Normal distribution, †Non Normal distribution, The data is showed as means of parameter values, changes (% from original value, which is 100%) at time interval point where statistically stable and significant change. Statistically significant at 0.05 level.				

Table 1: Change of parameters induced by storage of blood at 4 ± 1 °C.

Discussion

According to recommendations of International Committee of Hematology Standardization and Formal Assessments, the maximum storage interval for good stability at 4°C was at least 24 h to 72 h depending on the type of CBC analyzer. Improved stability was observed in samples stored as K2-EDTA-anticogulated blood sample at refrigeration [5,9,10,17-19]. Significant time and temperature related morphological alterations could happen with the long extended storage of blood on refrigeration (Figure 1) [17,20,21].

Many other studies conducted in past conflicting results regarding findings of WBC, RBC and HGB at room temperature [5,9,12]. However, some studies, which were conducted using refrigerated temperature show that WBC, RBC, and HGB parameters, were constant up to 72 h [21,22]. Only Ashenden et al. recently confirmed that haemoglobin was stable for at least 168 h using the Sysmex XT-2000i instrument, when maintained temperature between 4°C and 6°C [18]. Where our study finds that these parameters like WBC counts, RBC counts and HGB level were statistically stable up to 126 h, 186 h and 90 h respectively. The difference of stability observed between our HGB levels and those observed by some others may be due to different experimental conditions and analyzers type.

Our results of differential WBC counts (Ne, Ly, Mo and Eo) show stability up to 42 h to 66 h. Basophiles were relatively much less stable (6 h). Studies of Baca et al. [21] Muller et al. [23] and A. Joshi et al. [15] show a stability time of 48 h to 72 h which is not much different from our findings. Demonstrated by Hedberg et al. [20] and Muller et al. [23] slight decrease in neutrophils at 4°C during 48 h to 72 h is identical to our result.

Our result of HCT was stable up to 54 h that is similar to other studies at 4°C [6,15,17,19,20]. Result of MCV was found to be stable up to 42 h, regarding result for MCV at 4°C, previous studies are also show supporting with our findings [5,6,15,20,21]. Studies by Turhan et al. [5] and Lippi et al. [2] demonstrated increment was observed on the second day due to reflect the swelling of RBC at room temperature. Indicated by Wood et al. increment is prevented by refrigerated temperature, which is supporting to our study [24].

The results of MCH up to 18 h, MCHC and RDW-SD up to 30 h as well as RDW-CV up to 42 h. The MCV, HCT, RDW-CV, and RDW-SD, increased while the MCHC decreased over the time interval. The conclusion of these parameters is identical investigated by Monica Ed Baca et al. [21] room temperature using Sysmex X-2100 and Sven christen Voss et al. [25] at 4°C.

One of the main significant findings in our study was the statistically significant decline in PLT, PCT and MPV from 6 h to onwards. Some studies have revealed that platelet were stable even up to 24 h and 48 h at 4°C [12], which conflicts with our result. Scott Murphy et al. and Richard M. Kaufmam et al. proved life span of platelet is less at 4°C than room temperature. Standard refrigerated storage (at 4°C) resulted in a marked shortening of the life span .i.e. the lifespan (t1/2) of cold- and RT-stored platelets are 1.3 and 3.9 days, respectively [26-28]. Concluded by Massimo Daves et al. Platelet count using (impedance & fluorescent technique) is stable at 4°C up to 6 h and Dammika Gunawardena et al. is also concluded PLT and MPV was stable up to 6 h [6,4]. Increment in MPV over time reveal by many studies [5,17,20,21,29]. Above discussion shows, PLT and MPV may be analyzed within 6 h of collection.

Many studies have showed selected pre-analytical storage depending on required purpose of diagnosis for the effects of storage condition on CBC parameters. Influence of storage and variability on the majority of the blood count parameters can be minimized and if the analysis takes place before 24 h at 4°C [6,9,12,18].

Figure 1: Each data point represents the mean percent change difference with baseline.
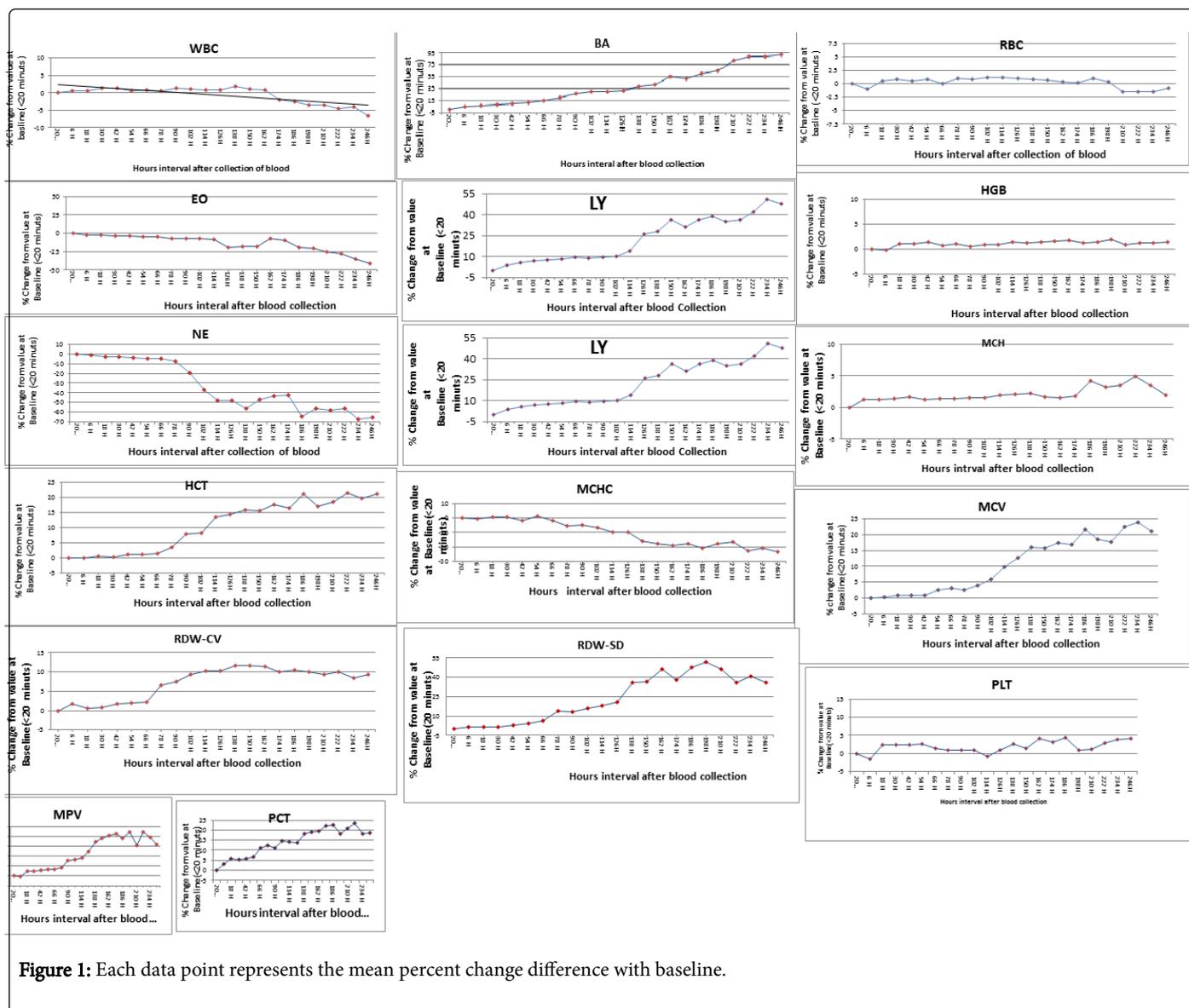
Conclusions

Our results and review of literature, it is favorable to analyzed CBC and differential count on blood sample as soon possible after the blood collection. The only parameters, which were assessed clinically qualitatively reliable during the extended storage up to 7 days, 5 days and 3.75 days, were RBC, WBC and HGB. According to result of our study, CBC blood samples can be stored at 4°C when delay is anticipated up to 2 days except PLT. It is therefore, there is no guarantee for the quality and reliability of CBC parameters for refrigerated blood samples during extended periods of storage at 4°C after 2 days except that of RBC, HGB and WBC.

Conflicts of Interest

The Authors declare no conflict of interest.

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