International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Variation of CBC Parameters with Storage Time and Temperature

Biswajita Rautaray

MKCG Medical College, Berhampur University, ICMR STS 2017

Abstract: ***This study demonstrates that it is possible to perform clinically valid CBC on K2-EDTA anti-coagulated blood 24 hours after collection on refrigerated sample, though not for differential count parameters. On 24-hour sample, MPV and RET%, MCHC should not be considered for diagnosis. HAEMATOCRIT, MCV and RDW consistently elevated with sample's age, even at 24 hours, not to be evaluated. After 24 hours WBC, RBC, HGB, PLATELET can be evaluated. Stability of WBC counts up to 24 hours is acceptable for all samples, except EOSINOPHIL (at 4 degree Celsius) counts not stable beyond 24 hours.Altered results obtained in manual differential count, cannot be considered a solution for differential count on aged samples. Most reliable haematological results are obtained from samples analysed same day within 5-8h, soon after collection. When immediate analysis is not possible, sample stability (only CBC parameters except MPV; not differential count) can be prolonged up to 48 hr. on refrigeration. A sample with borderline eosinophil or basophil count stored under refrigeration, still can give report of significant increase in parameters with prolonged storage. Thus refrigeration can't be considered as a solution to prolong storage time in case of bloodsamples, requiring differential count for clinical diagnosis.***

Keywords: CBC variation, Temperature, Time, Aged Sample, Refrigeration

1. Introduction

The complete blood count (CBC) is one of the most common and routine laboratory tests, used as a broad screening test, and is one of the first steps in diagnosing an illness. The test is quick, easy and can give valuable information to the physicians. It is a crucial test for the diagnosis and management of several haematological disturbances provided the quality throughout the testing process can be guaranteed.

Sample stability, a factor of the pre-analytical phase, is an important component of clinical laboratory results. Published values for total testing error range widely from 0.1% to 9.3% and this broad range includes both pre- and post-analytical errors.

Moreover, studies of factors within the pre-analytical phase including specimen collection, handling and storage indicate that 93% of errors are not related to the highly standardized analytical process. Delayed sample analysis could result in changes of measured parameters complicating the interpretation of results.

Pre-analytical variables, such as storage time and temperature affect the measurement of laboratory parameters collected in EDTA.Laboratory staff need to be aware of the changes that occur during storage in their specific setting in order to decide whether to accept or reject samples that are too old to obtain reliable results. Accurate measurement of Complete blood count (CBC) and differential count (DIFF) as well as peripheral blood smear (PBS) morphology are essential for the correct interpretation of haematology results.

Parameters useful for diagnosis and monitoring of haematological disorders, such as mean cell volume (MCV) and PBS morphology are unreliable after 12 hours.Osmotic swelling of red cells during storage at Room temperature affects volume-dependant variables and results in **misclassification of a microcytic anaemia as normocytic and similarly, a normocytic anaemia as macrocytic.**

2. Literature Survey

In a previous evaluation of the Sysmex XT 2000i modular system RBC count,*Mahmoodi M*[1], *Hajizadeh M, Rashidinejad Het al*[3]found that haematocrit, MCH, percent of monocytes and eosinophil were constant in different temperatures, WBC count, MCHC, haemoglobin, platelets count, the percent of lymphocytes and neutrophils were constant up to 24 hours and then tend to increase with increasing temperature except lymphocytes percent that tend to decrease. MCV (MEAN CELL VOLUME) decreased with increasing temperature up to 8 hours and then significantly increased (from 83.89 to 87.50 fmol/l, $p < 0.001$). WBC, haematocrit, MCV, platelets count, and neutrophils percent tend to increase with the time of incubation, but RBC count, MCHC, lymphocytes percent decreased. Haemoglobin, MCH, and the percent of monocytes and eosinophil were constant.

Interestingly, *Ashenden et al.*[4]recently demonstrated that haemoglobin was stable for at least 168 h using the Sysmex XT-2000i instrument, when maintained between 4 °C and 6 °C.

In a similar research conducted on performance of Sysmex XT 2000i[1],it was reported that after 24 hours, a limited report could legitimately include WBC, RBC, HGB, and I-PLT for all samples. Regarding the Diff count, the stability of Neutrophil, Lymphocyte, Eosinophil, and Basophil counts up to 72 hours is acceptable for all samples, while the Monocyte counts are not stable beyond 24 hours. Hb, WBC and Platelet were found to be stable for 24 h after collection of blood, while clinically significant changes were observed in the RBC, Haematocrit, MCV, MCH and MCHC after storage at room temperature[2].

Previous studies have provided both supporting and conflicting results. *Hirase et al. (1992)*[6] demonstrated sample stability after one week of incubation. Similar consistency was shown in RBC counts after 48 h and seven days of incubation at room temperature, respectively (*Vogelaar et al., 2002; Gulati et al.,* 2009). Contrastingly, Mahmoodi et al. (2006) reported that RBC count decreased after 48 h at 37°C, while *de Baca et al. (2006)* reported WBC count stability for up to 3 days after blood collection. *Wood et al. (1999)*[7] incubated samples for 24 h and found significantly increased WBC counts. Platelets counts remained unchanged after 24- h incubation at room temperature. A previous study has demonstrated similar findings for up to four days incubation (Gulati et al., 2009), while another contrastingly report showed increases in platelets counts after 48-h incubation and elevated temperature (Mahmoodi et al., 2006)[12].

Ho and Chan, (1996)[6] found that different temperatures and times of incubation can affect platelet counts and haemoglobin concentrations. A study showed that the mechanism for the laboratory effect is that raising the temperature leads to changes in platelets morphology and movement *(Qi et al., 2001).*

Further study[5] conducted on SYSMEX XE 2100 suggested, among the CBC parameters, HGB, RBC, and MCH were found to be stable for the duration of the study (i.e., up to4 days after collection of blood). The WBC and PLT were stable for up to 3 days after collection of blood. Clinically significant changes were observed in the MCV and its related parameters, Haematocrit, MCHC, and RDW beginning on day 1after blood collection. The MCV, Haematocrit, and RDW increasedwhile the MCHC decreased over time.

In a study conducted by *G. Zinietal (2013)*[8], it was reported that measurement of haemoglobin concentration and RBC count are stable up to 72 h after blood collection if blood is refrigerated at around 4 °C, while MCV and consequently, the haematocrit value tends to increase after 6–12 h in a predictable measure. Reticulocyte count are stable up to 72 h under refrigeration. The PLT count in specimens stored at 4 °C is considered stable for up to 24 or even 72 h.WBC count with automated differential count is stable at 4 °C for at least 24 h or even to 72 h, with significant differences depending on the type of automated blood cell analyser in particular, the monocyte count tends to increase, while eosinophil and lymphocyte counts tend to decrease over time; neutrophil count is stable up to 72 h with most new generation instruments**.**

A recent study, *by Daves, Zagleretal* (2015)[10] using SYSMEX XN, suggested no meaningful bias was observed after 3 h under different storage conditions, except for red blood cell distribution width (RDW) and platelet count (impedance technique, PLT-I) at 37 °C. After 6 h, meaningful bias was observed for mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) at room temperature, red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC), MCH, MCV and PLT-I at 4 °C, and RBC, RDW, MCHC, MCH and PLT-I at 37 °C. After 24 h, a meaningful bias was observed for MCHC, MCV, platelet count (fluorescent technique, PLT-F) and mean platelet volume (MPV) at room temperature, MCHC, MCV, PLT-I and MPV at 4 °C, and all parameters except RBC count and MPV at 37 °C.

3. Aims and Objective

Delayed sample analysis is a common situation, in clinical and laboratory practice especially when blood samples are shipped to centralised laboratories in tertiary care centres (like MKCG MCH), when the analysis can't be readily performed for organisational or technical reason. The definition of accurate and appropriate criteria for sample stability is necessary for clinical purpose but also for ancillary legal implication. This study is aimed to investigate the variation of CBC parameters with storage time and temperature, using the new generation of Sysmex haematological analysers (i.e. **Sysmex XT-2000i**).

For the most accurate and reproducible haematological results, whole blood specimens should be analysed as soon as possible after collection. With multi-centre studies, samples may be drawn off-site and then transported at ambient temperatures to another laboratory. Transportation times may vary depending on distance and location of the laboratories. Automated haematological analysis of specimens, particularly analysis of the differential count if delayed beyond 4 hours, has in the past yielded doubtful and often invalid results. Automated haematological analysers most commonly rely upon cell size or cell size together with scatter properties to differentiate white blood cell populations. Because the size and scatter properties of white cells change as a blood sample ages, automated analysis of the differential becomes more unreliable with time. The properties of red blood cells (RBCs) also change with time in stored samples, most notably cell size and the red cell indices related to size.

The 11 CBC parameters studied are: White Blood Cell Count (WBC), Haemoglobin (Hb) concentration, Red Blood Cell (RBC) count, Haematocrit (Haematocrit), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Mean Platelet Volume (MPV), Reticulocyte count (RET %), RBC distribution width (RDW) and Platelet Count (PLT).

Thus the **Aim and Objective** of this study is to obtain wide and cumulative knowledge concerning the behaviour of CBC Parameters over time under different storage conditions that will permit the identification of defined requirements and time thresholds for different applications in laboratory haematology according to specific diagnostic demands.

The results will be used to determine what haematological parameters can be reported as clinically valid results if a sample cannot be analysed in a timely manner. The stability over time of the manual Diff was also evaluated to demonstrate what use, if any, the preparation of a smear and the performance of a manual Diff count could have in a sample 5 hours old or older.

The Study can also suggest if refrigeration of specimen can help guarantee the quality of whole blood samples is preserved during extended periods of storage.

4. Materials and Method

Instrument Description:
The **Sysmex X-2000i** uses Impedance technology with hydro dynamic focusing to measure RBC, PLATELET, MPV, MCV write out on first reference, and HCT (Haematocrit).Fluorescence flow cytometry is used to measure WBC, Differential count and the optical PLATELET count. The system employs a 633nm semi-conductor laser for flow cytometry analysis. For the measurement by flow cytometry of the proportional count, expressed as % of total WBC, of neutrophils (NEUTROPHIL), lymphocytes (LYMPHOCYTE), monocytes (MONO) and eosinophil (ESO), white cells are stained with fluorescent dyes that bind to both DNA and RNA, Side scatter is employed to measure the internal complexity of the cell-the size, shape, and density of nucleus and granules of the cell .Fluorescence and scatter measurements are combined to characterize white cell population. Basophils are measured separately using cell size and scatter properties. Haemoglobin (HGB) is measured photocolorimetrically using SLS-HGB, a cyanide free method. The instrument provides an optional reticulocyte count in 1 of the flow analysis channels. RBC are stained, counted and measured for the size and fluorescence. Counts are expressed as %of RBC (RET %).

Study Design
The study cohort consist of 88 patients, who have their blood collected in the haematology lab for routine haematological testing (by **Sysmex XT-2000i**; installed in the section of haematology under Department of Pathology, MKCG Medical College, Berhampur.)

Case Selection
The study was conducted with the fresh blood samples collected from patients attending Haematology Lab, Dept. of Pathology MKCG MCH.

Exclusion Criteria:
- Blood sample collected indoor (MKCG MCH).
- Blood samples received from primary or secondary health care centres. (I.e. blood samples with questionable storage time and condition.)
- Blood samples received from peripheral labs for verification.

Procedure
88 randomly selected blood samples collected by venepuncture and anticoagulated with dipotassiumethylenediamine tetra-acetic acid (EDTA) were measured within 1 h of collection on**Sysmex XT-2000i**; (installed in the section of haematology under Department of Pathology, MKCG Medical College, Berhampur.)This sample was taken as the baseline (0 h) sample, and further measurements were made at 5, 24, and 48 h following the baseline measurement.

The samples were taken from out-patients in the morning as part of routine laboratory testing. After the base line CBC is performed, each patients' sample is divided into 2 identical aliquots (aliquots-1 and 2) without further addition of anticoagulant .Aliquots 1 are stored at room temperature (**20-25** degree Celsius) whereas aliquots 2 at **4** degree Celsius (Refrigeration). Additional CBC are then performed on all stored aliquots at three defined time points, i.e., 5, 24 and 48 h after initial storage.

All aliquots are tested on same analyser, (**Sysmex XT-2000i)** and with an identical lot of reagents and after an appropriate amount of time to stabilise testing temperature, as recommend by manufacturer. The effect of storage on CBC parameters was determined by comparing the results at 5,24and 48h to the 0 h (baseline) sample.

CBC is followed by Peripheral smear examination to look for any change in Morphology of RBC and WBC and manual differential count was performed in each sample.

This study is based on Left over samples of patients, and the result will not be reported .Hence it will not affect the clinical management of the patients.

Statistical Analysis
To evaluate the stability of the CBC and the Diff count measurements from delayed samples at 5 hours, 24 hours, and 48 hours post collection, the mean absolute difference and the mean percent difference were calculated from the delayed samples relative to the measurements from samples within 1 hours of collection, together with the respective 95% confidence intervals. In addition, linear regressions were performed for the measurements from delayed samples versus the measurements from samples within 1 hours of collection. The intercepts and the slopes, as well as Pearson's correlation coefficients, were reported with the respective 95% confidence intervals. The change in value (in %) obtained for each parameter was compared with the pre-determined imprecision value calculated for internal control of Sysmex XT 2000i. Between groups differences were evaluated with paired Student's T test. Values of p less than 0.05 were considered to be significant. (Software version used- MS Excel 2013)

5. Observations and Result

The results obtained in this study are compared withthe manufacturer's suggested percent limits for reproducibility for all parameters of the CBC (in table 1), to observe any significant variations. The limits of variation and correlation coefficients for the accuracy of white blood cell types in a Differential count as provided by the manufacturer are in table (Table 1) below.

Table 1: Limits for acceptance of CBC and differential counts as recommended by the manufacturer.

	Percent Limits
WBC	≤ 3%
RBC	≤ 1.5%
HGB	≤ 1.5%
HAEMATOCRIT	≤ 1.5%
MCV	≤ 1.5%

MCH	≤ 1.5%
RDW-CV	≤ 3%
PLT	≤ 4%
MPV	≤ 4%

	Percent Limits	Absolute Limits	Correlation Coefficient
NEUT	≤ 8%	± 3.0	≥ 0.90
LYMPH	≤ 8%	± 3.0	≥ 0.90
MONO	≤ 20%	± 2.0	≥ 0.75
EO	≤ 25%	± 1.0	≥ 0.80
BASO	≤ 40%	± 1.0	≥ 0.50

Storage at Room Temperature

- RBC PARAMETERS:

Red cell parameters including RBC, haemoglobin, mean cell haemoglobin (MCH) were stable for at least 24 hours after collection when stored at Room Temperature and were not significantly affected by storage temperature. In contrast, other RBC measurements, including haematocrit (*fig.4*), MCV (*fig.8*), and red cell distribution width (RDW) (*fig.6*), RET% (*fig.13*), MCHC (*fig.10*), were not stable at Room Temperature for 24 h Sample. After Room Temperature storage for 24 hours, a significant increase in MCV ($P<0.001$), as well as RDW-CV ($P<0.05$), and a significant decrease in MCHC ($P<0.001$) and Reticulocyte% ($P>0.05$) was observed.

- PLATELET :

Analysis of platelet stability showed platelets were stable for 24 hours and significantly decreased ($P>0.05$)in 48 hours roomsample. The stability of the mean platelet volume (MPV) was less than 24 hours (*fig.7*) as a result of artificial platelet swelling.

- WBC COUNT AND DIFERENTIAL:

The WBC was stable until 24 hours after collection and showed a significant decrease at 48 hours after collection*(fig.1)*. A significant increase in the percentages of Basophil and eosinophil was observed at 24 hours and 48 hours, respectively. The stability of the manual differential count was also less than 24 hours. The percentages of eosinophil*(fig.15)* showed significant increases, whereas percentages of lymphocytes*(fig.18)* and monocyte*(fig.14)* showed significant decreases ($P<0.05$) at 24 hours after collection.Neutrophil count was stable up to 24 h and increased slightly in 48h sample. The slides examined contained too few basophils to obtain reliable results for basophil stability. EDTA-induced changes were noted at 24 hours after collection, which precluded a manual differential count.

- Morphological Changes in Ps (Perepheral Smear):

Depending on the time the peripheral blood smear is prepared, morphological changes were seen in some but not all Cells. Some neutrophils showed nuclear swelling with changes in the chromatin, that is stained more homogenously compared to fresh nuclei, and loss of the structure of the lobes that may become separated; the cytoplasmic rim appeared ragged or less well defined and vacuolization and loss of granules may be observed. Nuclear shrinkage, chromatin condensation in dark masses, karyorrhexis, and degradation of cytoplasmic structure are early apoptotic changes.

Mononuclear cells are affected by similar morphological changes usually to a lesser extent than neutrophils; vacuoles and irregular nuclear lobulation up to partial nuclear disintegration may be observed too. Slight cytoplasmic vacuolization in monocytes can be found after 24 h, progressing to moderate after 48 h; vacuolization in neutrophil granulocytes appears after 5 h and progresses to moderate after 24 h. Some of the lymphocytes showed similar changes such as the presence of cytoplasmic vacuoles, nuclear budding, and homogenously stained chromatin. Increased numbers of smudge cells are seen on films prepared after 24–48 h. Normal RBCs are morphologically stable for up 5 h at room temperature: crenation, sphering, and fragmentation are observed after prolonged periods of time (>24 hr.).

Storage at low temperature (4 °C)

- RBC parameters:

Compared with Room Temperature storage, we observed improved stability of RBC. Parameters when stored at 4 °C. Haematocrit*(fig.4)*, MCV*(fig.8)*,RET%*(fig.13)* and MCH *(fig.9)*were stable until 48 hours when stored at 4 °C. No significant change observed in RDW *(fig.6)* and Hb (fig.3).

- Platelets:

Platelets *(fig.5)*were stable upto 48 hours after collection. MPV*(fig.7)* showed a significant increase ($P<0.001$)in 24h sample.

- WBC count and differential:

The WBC*(fig.1)* was stable at 4 °C until 48 hours after collection. A significant decrease in the percentage of lymphocyte ($P<0.001$) was observed at 48 hours*(fig.16)* after collection. The percentages of eosinophil*(fig.15)*, basophils*(fig.17)* and monocytes*(fig.14)* were not stable when stored at 4 °C and showed significant increases ($P<0.05$) at 24 and 48 hours, respectively.

- Morphological changes in PS (perepheral smear):

All of the changes in morphology of cells are retarded but not abolished in samples stored at 4 °C. Normal RBCs are morphologically stable for up 24 h at 4 °C: crenation, sphering, and fragmentation are observed after prolonged periods of time (>24 hr.).

6. Discussion

Routine tests such as the CBC, DIFF (Differential Count)and PBS (Peripheral Blood Smear) morphology are commonly referred to centralised laboratories as part of the diagnostic work-up for haematological disorders. In large academic laboratories, where aged samples make up a significant proportion of the workload, the storage time and temperature of samples must be taken into consideration. The findings of this study performed on EDTA samples add to the evidence that stability varies according to storage time and temperature.

According to the findings of this study, CBC parameters, namely TRBC, haemoglobin, MCH and Differential parameters, particularly percentages of Neutrophil were least affected by storage temperature and time and can be analysed until 48 hours after sample collection when stored at room temperature.

Though it is recommended that traditional CBC parameters can be analysed up to 24 hours of sample collection when stored at room temperature, but in our study only TRBC, haemoglobin, MCH were stable throughout the study (Irrespective of storage condition.) whereas there was a marked increase in Haematocrit value for 24h sample. The MCV was stable only until 5 hours after collection. There is a consistent and significant rise in red cell size with time, indicating these data should be interpreted with caution. We also observed changesin the results of the MCHC and RDW. The MCHC is calculated by dividing the HGB by the HAEMATOCRIT. As the HAEMATOCRIT rises, the MCHC is reduced; a relatively small rise in the HAEMATOCRIT create a significant drop in MCHC. The RDW is calculated with the standard deviation of the mean of the red cell volume (or the coefficient of variation of the mean of the red cell volume) divided by the MCV. The instability of the red cell size with time necessarily affects this parameter also. RDW CV rises progressivelywith storage time with a significant deviation from the control value.RET% showed significant decrease in 24h sample (may be as a consequence of possible *in vitro* maturation to RBCs).

Interestingly TRBC, Haematocrit and all the RBC indices did not show any significant variation from their respective control value, throughout the study period (48h) when stored at 4 degree Celsius.

The variations in red blood cell parameters observed in this study are mostly in accordance with the past studies conducted using various analysers.

The stability of the WBC was also found to be shorter than other studies, which have recommended analysis up to 48 hours after collection when stored at Room Temperature. In this study, the WBC was stable only until 24 hours after collection when stored at room temperature, after which a significant decrease in WBC count was observed (may be due to degenerative changes in cells).However no marked changes were observed for TWBC in refrigerated samples up to 48h.

Platelets were only stable up to 24 hours after collection when stored at Room temperature. Time-related and concentration-related changes in PLT shape from discoid to spherical and swelling do occur in specimens collected in EDTA; as a result of these changes, the mean PLT volume (MPV) is not a fully reliable value after 24 hours of storage at room temperature. Interestingly, MPV values in case of refrigerated sample showed increased deviation from the control value in 24h sample as compared to sample at room temp.

Storage of samples at 4 °C increased the stability of most parameters. CBC parameters, namely WBC, platelet count, haematocrit, MCV and MCHC, as well as DIFF parameters, namely percentages of neutrophils, were more stable when stored at 4 °C. However, some Differential parameters, namely percentages of eosinophil, basophils and monocytes, had lower stability. Thus though refrigeration can prolong the stability of many CBC parameters; but for the blood samples requiring Differential count for clinical diagnosis, refrigeration can't be considered as a solution.

The Differential results in **Table 4** and **Table 5** and show excellent stability up to 5 hours, and good stability to <24 hours, with the exception of the basophil count. The MONOCYTE count compares adequately to values on 1 hour samples only up to 24 hours. Beyond 24 hours, the monocyte count becomes progressively lower. *(Previous studies show conflicting results for monocyte count; study by joshietal, 2015 showed decrease where as that of G. Zinietal, 2013 suggested increase in monocyte count).*NEUTROPHILremained stable or raised slightly with time (*findings are similar to that of G. Zinietal, 2013),* whereasLYMPHOCYTE dropped slightly, but BASOPHIL changed by 42% in 48 hours compared to 1 hour samples. The absolute loss seems minimal, but the proportional loss is significant. The reason for the change is difficult to determine. The cell size, structure, or degree of granulation may change enough with time to make it no longer recognizable as a particular cell to the system's population analysis software.

All of the observed changes in the differential parameters most likely reflect the combined effects of cellular degeneration that is known to occur with cell aging and the loss of individual cell characteristics specifically measured by the analyser. Both or either of these features may lead to misidentification of one cell type for another by the analyser. Degeneration of monocytes and misidentification of degenerated monocytes as neutrophils by the analyser may account for the observed decrease in monocyte % and, with a concomitant increase in neutrophil %.

No statistically or clinically usable manual counts were obtained on samples 24 hours or more old.

7. Conclusion

This study was aimed at concluding the influence of storage time and temperature on CBC Parameters, which form a basic screening test in our setup.

On 88 random blood samples (those were collected by venepuncture in the morning from out patients, for routine test) baseline CBC study was performed (CONTROL).then each blood sample was divided equally in 2 different aliquots, without any further addition of anticoagulant. Aliquots 1 are stored at room temperature (**20-25** degree Celsius) whereas aliquots 2, at **4** degree Celsius (Refrigeration). Additional CBC are then performed on all stored aliquots at three defined time points, i.e., 5, 24 and 48 h after initial storage. The effect of storage on CBC parameters was determined by comparing the results at 5,24and 48h to the 0 h (baseline) sample. CBC is followed by Peripheral smear examination to look for any change in

Morphology of RBC and WBC and manual differential count was performed in each sample.

CBC parameters, namely RBC, haemoglobin, MCH and RDW, and Differential parameters, namely percentages of Neutrophil, were least affected by storage temperature and time and can be analysed until 48 hours after sample collection when stored at room temperature whereas Platelets were only stable until 24 hours. Storage of samples at 4 °C, increased the stability of most parameters. CBC parameters, namely WBC, platelet count, haematocrit, MCV, RET% and MCHC, as well as DIFF parameters, namely percentages of neutrophils, were more stable when stored at 4 °C. However, some DIFF parameters, namely percentages of eosinophil, basophils and monocytes, had lower stability. In PBS Normal cells are morphologically stable for up 5 h at room temperature: crenation, sphering, and fragmentation are observed after prolonged periods of time (>24 hr.).With refrigeration these changes were delayed but not deleted.

Thus, the most reliable haematological results are obtained from samples analysed the same day as they are collected (within 5-8, as soon after collection as possible. When immediate analysis is not possible, the sample stability (only CBC parameters except MPV, and not differential count) can be prolonged up to 48 hr. on refrigeration. If refrigeration is not available then valid results from samples that are 24 hours old and older can be reported within certain limitations discussed above. For the clinical diagnosis of hematologic disorders, on the other hand, the microscopic observation of cell morphology in peripheral blood smears, and in particular, the assessment of dysplastic changes, it is mandatory that smears be prepared within a few hours, irrespective of the storage temperature.

Table 2: Mean, mean percentage change and 95% confidence interval (CI) for the mean changes observed in CBC on storage of blood specimens at room temperature.

ROOM TEMPERATURE													
n=88	FRESH (<1hr)	5 hr.	Change%	CI (95%)		24hr.	change%	CI (95%)		48 hr.	change%	CI (95%)	
				Lower	Upper			Lower	Upper			Lower	Upper
WBC(x10^9 /L)	11.27	11.44	1.34	9.95	12.93	11.15	-1.23	9.69	12.61	10.42	-8.86	8.98	11.86
RBC(x10^{12}/L)	4.48	4.54	1.12	4.34	4.74	4.53	1.16	4.34	4.72	4.46	-0.42	4.27	4.65
HGB(g/Dl)	11.37	11.51	1.27	10.97	12.05	11.45	0.95	10.93	11.97	11.29	-0.78	10.75	11.83
HAEMATOCRIT (%)	34.98	35.38	1.13	33.78	36.98	39.62	13.67	37.94	41.30	41.61	19.17	39.82	43.40
MCV(FL)	78.22	78.31	0.12	76.53	80.09	88.31	12.84	86.00	90.62	93.62	19.70	91.42	95.82
MCH(pg.)	25.41	25.51	0.47	24.84	26.18	25.37	-0.15	24.68	26.06	25.41	-0.02	24.70	26.12
MCHC(g/dl)	32.42	32.65	0.72	31.99	33.30	28.29	-12.72	27.63	28.96	26.59	-17.99	25.94	27.24
RDW-CV (%)	15.11	15.10	-0.08	14.37	15.83	16.47	9.28	15.77	17.17	16.58	10.33	15.80	17.36
PLT(10^9/L)	258.30	257.20	0.28	228.82	285.58	247.06	-2.83	220.33	273.79	240.40	-5.89	213.98	266.82
MPV(Fl)	10.42	10.75	3.23	10.52	10.98	10.83	4.03	10.59	11.07	10.92	4.94	10.68	11.16
RET%	1.24	1.23	-0.81	1.07	1.43	1.09	-12.10	1.01	1.17	0.89	-28.23	0.13	1.65

Table 3: Mean, mean percentage change and 95% in confidence interval (CI) for the mean changes observed CBC on storage of blood specimens at 4 degree Celsius temperature

REFRIGERATED													
n=88	FRESH (<1hr)	5 hr.	Change%	CI (95%)		24hr.	change%	CI (95%)		48 hr.	change%	CI (95%)	
				Lower	Upper			Lower	Upper			Lower	Upper
WBC(x10^9 /L)	11.27	11.23	-0.56	9.76	12.70	11.37	1.16	9.89	12.85	11.39	1.93	9.91	12.87
RBC(x10^{12}/L)	4.48	4.55	1.38	4.35	4.75	4.48	-0.12	4.28	4.68	4.53	-0.41	4.33	4.73
HGB(g/Dl)	11.37	11.65	2.15	11.04	12.26	11.40	0.26	10.86	11.94	11.39	0.17	10.84	11.94
HAEMATOCRIT (%)	34.98	35.57	1.46	33.84	37.30	34.82	-0.46	33.09	36.55	35.07	0.03	33.28	36.86
MCV(FL)	78.22	77.77	-0.56	76.01	79.53	78.62	0.54	76.85	80.39	79.48	1.65	77.70	81.26
MCH(pg.)	25.41	25.55	0.66	24.89	26.21	25.52	0.51	24.84	26.20	25.59	0.84	24.91	26.27
MCHC(g/dl)	32.42	32.89	1.51	32.29	33.49	32.42	0.03	31.80	33.04	32.08	-0.97	31.37	32.79
RDW (%)	15.11	15.13	0.07	14.42	15.84	15.10	-0.24	14.39	15.81	15.09	-0.18	14.38	15.80
PLT(10^9/L)	258.30	248.96	-2.77	221.51	276.41	256.90	-1.10	228.44	285.36	263.19	2.52	233.18	293.20
MPV(Fl)	10.42	10.65	2.28	10.42	10.88	11.23	7.89	11.00	11.46	11.82	13.66	11.59	12.05
RET%	1.24	1.25	0.81	1.07	1.43	1.22	-1.61	1.01	1.71	1.21	-2.42	0.23	2.19

Table 4: Mean, mean percentage change and 95% confidence interval (CI) for the mean changes observed in Differential Count on storage of blood specimens at room temperature.

ROOM TEMPERATURE													
n=88	FRESH (<1hr)	5 hr.	Change%	CI (95%)		24hr.	change%	CI (95%)		48 hr.	change%	CI (95%)	
				Lower	Upper			Lower	Upper			Lower	Upper
NEUT	7.61	7.76	2.06	6.54	8.98	7.64	2.16	6.43	8.85	7.53	2.3	6.36	8.70
LYMPH	2.35	2.4	8.5	2.08	2.72	2.25	-15.26	1.97	2.53	1.94	-16.02	1.67	2.21
MONO	0.81	0.67	-2.28	0.58	0.76	0.258	-11.83	0.17	0.34	0.51	-18.5	0.40	0.62
EO	0.27	0.28	8.08	0.21	0.35	0.26	20	0.19	0.33	0.22	11.32	0.17	0.27
BASO	0.046	0.048	10.28	0.04	0.06	0.063	43.9	0.05	0.08	0.057	42.27	0.05	0.07

Table 5: Mean, mean percentage change and 95% confidence interval (CI) for the mean changes observed in Differential Count on storage of blood specimens at 4 degree Celsius temperature.

REFRIGERATED													
n=88	FRESH (<1hr)	5 hr.	Change%	CI (95%)		24hr.	change%	CI (95%)		48 hr.	change%	CI (95%)	
				Lower	Upper			Lower	Upper			Lower	Upper
NEUT	7.61	7.51	-0.34	6.41	8.61	7.8	5.9	6.58	9.02	7.84	7.51	6.62	9.06
LYMPH	2.35	2.23	-3.01	1.95	2.51	2.16	-4.69	1.92	2.40	2.1	-7.88	1.86	2.34
MONO	0.81	0.63	-11.47	0.55	0.71	0.66	-18.28	0.57	0.75	0.6	-25.92	0.50	0.70
EO	0.27	0.32	49.8	0.25	0.39	0.33	92.99	0.26	0.40	0.36	144.84	0.29	0.43
BASO	0.046	0.052	19.31	0.04	0.06	0.059	43.2	0.05	0.07	0.063	51.39	0.05	0.08

8. Graphical Interpretation

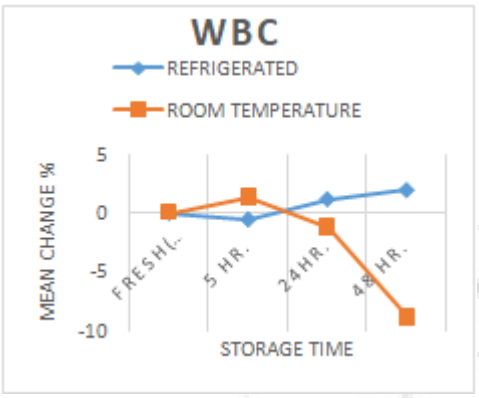

Figure 1: (variation of Total Leukocyte Count with prolonged storage time, in both incubated and refrigerated sample)

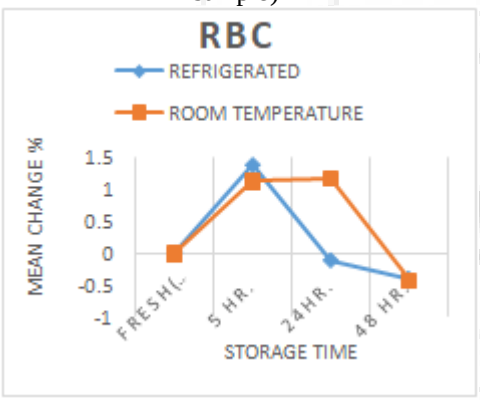

Figure 2: (variation of Total Red Blood Cell Count with prolonged storage time, in both incubated and refrigerated sample)

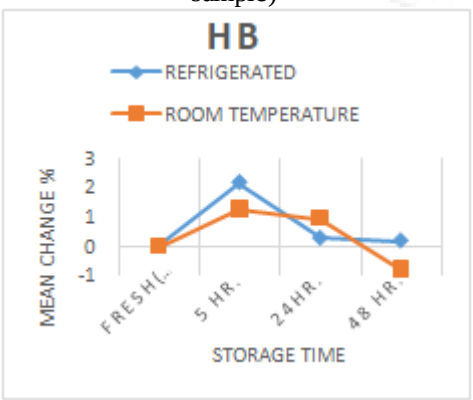

Figure 3: (variation of Haemoglobin% with prolonged storage time, in both incubated and refrigerated sample)

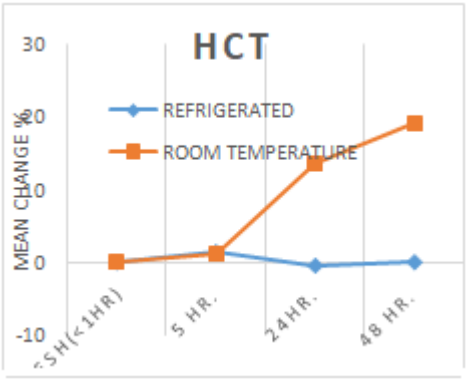

Figure 4: (variation of Haematocrit with prolonged storage time, in both incubated and refrigerated sample)

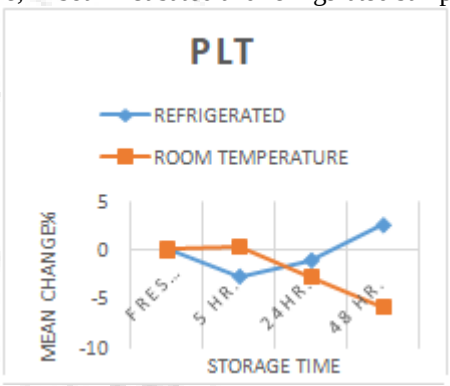

Figure 5: (variation of Total Platelet Count with prolonged storage time, in both incubated and refrigerated sample)

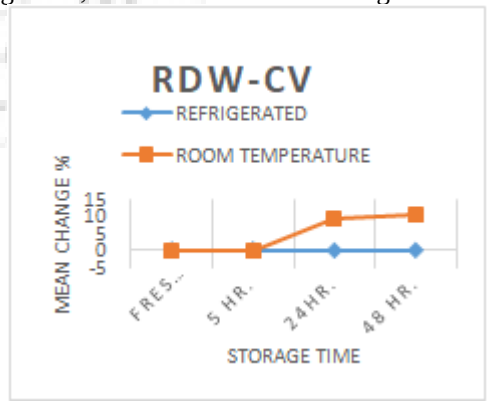

Figure 6: (variation of Red Cell Distribution Width with prolonged storage time, in both incubated and refrigerated sample)

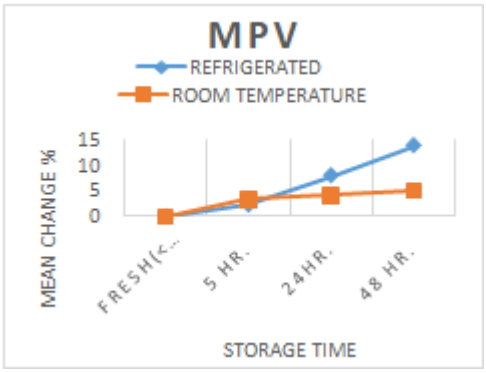

Figure 7: (variation of Mean Platelet Volume with prolonged storage time, in both incubated and refrigerated sample)

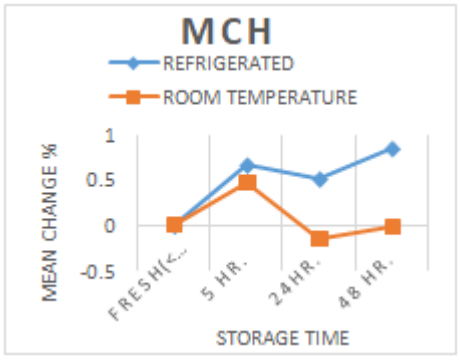

Figure 9: (variation of Mean corpuscular Haemoglobin with prolonged storage time, in both incubated and refrigerated sample)

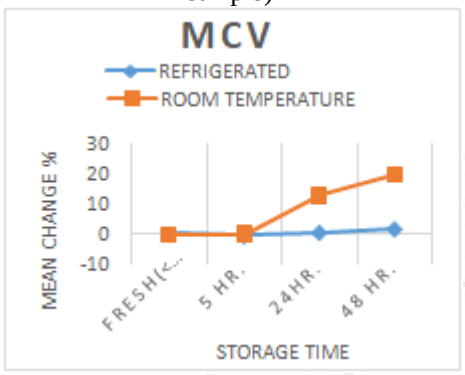

Figure 8: (variation of Mean Cell Volume with prolonged storage time, in both incubated and refrigerated sample)

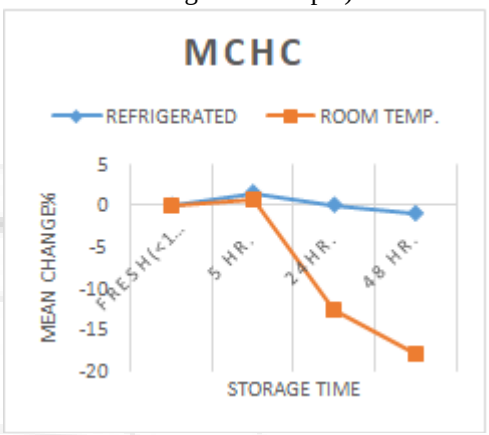

Figure 10: (variation of Mean Cell Haemoglobin concentration with prolonged storage time, in both incubated and refrigerated sample)

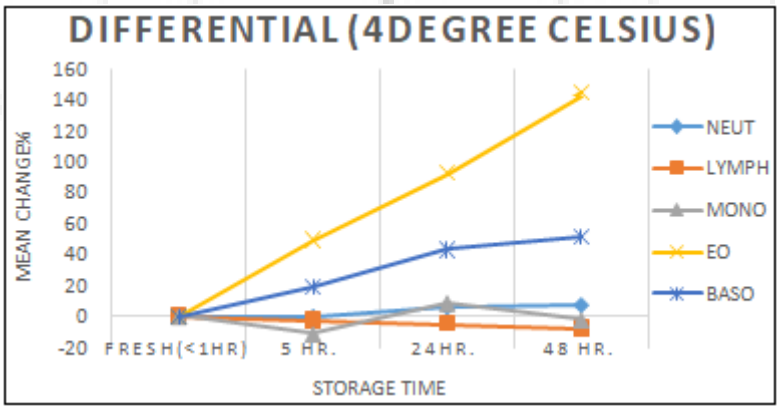

Figure 11: (variation of Differential Count Parameters with prolonged storage time, in refrigerated sample)

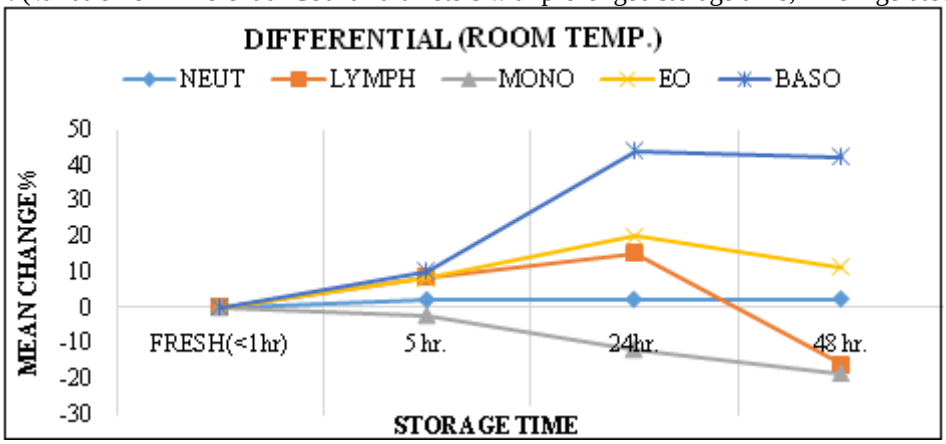

Figure 12: (variation of Differential Count Parameters with prolonged storage time, in room temperature sample)

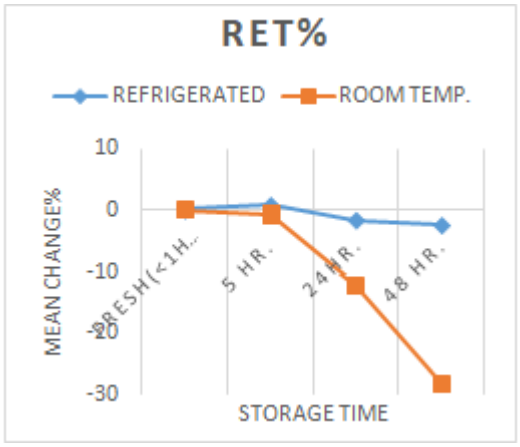

Figure 13: (variation of Total Reticulocyte Count (%) with prolonged storage time, in both incubated and refrigerated sample)

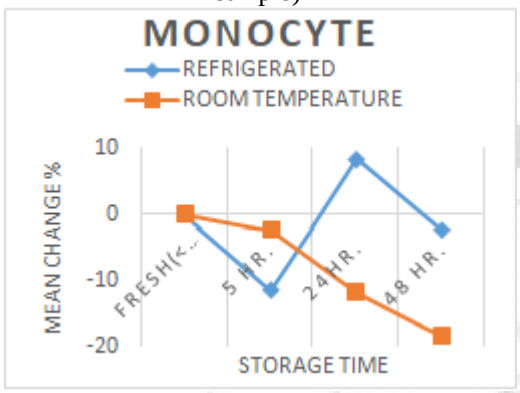

Figure 14: (variation of Total Monocyte Count with prolonged storage time, in both incubated and refrigerated sample)

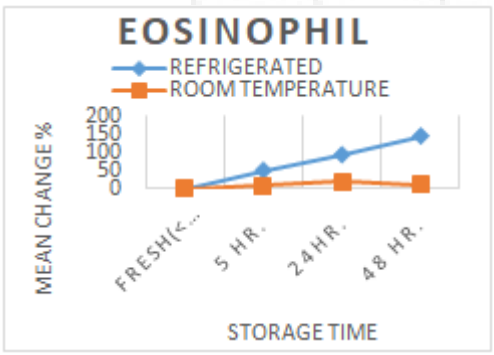

Figure 15: (variation of Total Eosinophil Count with prolonged storage time, in both incubated and refrigerated sample)

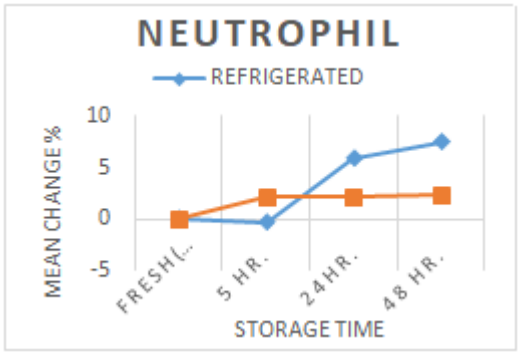

Figure 16: (variation of Total Neutrophil Count with prolonged storage time, in both incubated and refrigerated sample)

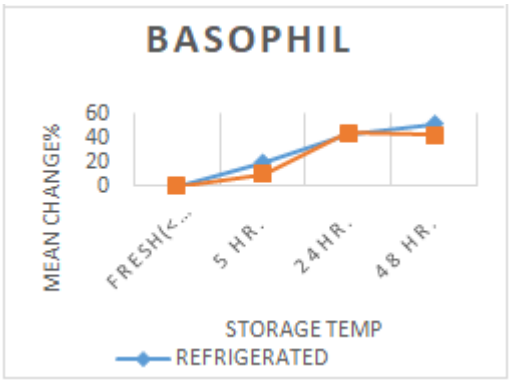

Figure 17: (variation of Total Basophil Count with prolonged storage time, in both incubated and refrigerated sample)

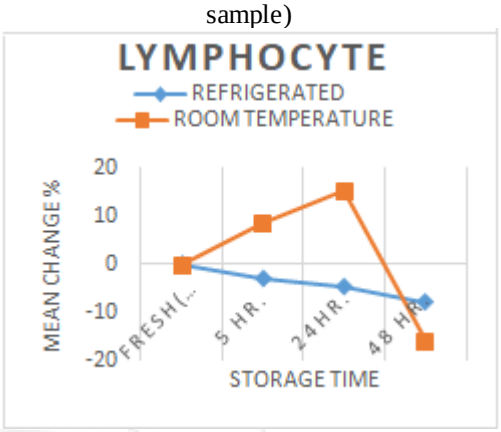

Figure 18: (variation of Total Lymphocyte Count with prolonged storage time, in both incubated and refrigerated sample)

9. Future Scope

Pre analytical errors involving Sample processing and storage, contribute majority of the erroneous haematological investigations. Thus study in this field could help guide the clinicians in interpretation of the CBC report with regards to blood sample storage conditions.

10. Acknowledgement

Special thanks to ICMR STS Programme, for this research exposure. Heart felt gratitude to prof. and HOD Dr.Devi Prasad Mishra and my mentor Dr. SumitaTripathy, for their constant guidance and support. And to the Lab Technicians, Department of Pathology MKCG, for the technical assistance.

References

[1] Evaluation of the Performance of the Sysmex XT-2000i Haematology Analyser with Whole Bloods Stored at Room Temperature.https://www.researchgate.net/publication/246759624_Evaluation_of_the_Performance_of_the_Sysmex_XT-2000i_Hematology_Analyzer_With_Whole_Bloods_Stored_at_Room_Temperature [accessed Aug 29, 2017].
[2] NargesObeidi, EisaSafavi and Habib Emami, (2011). Evaluation of the effect of temperature and time of incubation on complete blood count (CBC) tests.

[3] Mahmoodi M[1], Hajizadeh M, Rashidinejad H, Asadikaram G, Khaksari M, Mirzaee M, Seyedi N, Rahnema A, Sayadi A (2006). Survey of changes in complete blood count and red cell indices of whole blood incubated in vitro at different temperatures up to 48 hours. https://www.ncbi.nlm.nih.gov/pubmed/16773962.
[4] Effects of Storage of Blood at Room Temperature on Hematologic Parameters Measured on Sysmex XE2100 (PDF Download Available). Available from: https://www.researchgate.net/publication/246757989_Effects_of_Storage_of_Blood_at_Room_Temperature_on_Hematologic_Parameters_Measured_on_Sysmex_XE2100 [accessed Aug 29, 2017]
[5] Cohle SD, Saleem A, Makkaoui DE. Effects of storage of blood on stability of hematologic parameters. *Am J ClinPathol*. 1981; 76(1):67–69.
[6] Queen E, Ifeanyi OE, Chinedum OK. The effect of storage on full blood count in different anticoagulant. *IOSR JDMS*. 2014; 3(9):128–131. http://dx.doi.org/10.9790/0853-1392128131.

Author Profile

Biswajita Rautaray, 3rd year MBBS, MKCG Medical College, Berhampur.Odisha, 760004