|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
PubMed (National Library of Medicine ID: 101738825)
Index Copernicus Value 2018: 75.71
Original Research Article
Volume 3, Issue 12; December: 2019; Page No. 51-58

VARIATION OF CBC PARAMETERS WITH STORAGE TIME AND TEMPERATURE

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Article Info: Received 10 November 2019; Accepted 8 December 2019
DOI: https://doi.org/10.32553/ijmbs.v3i12.792
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Conflict of interest: No conflict of interest.

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.*

Introduction

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. 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.

Laboratory staff needs 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 (DC) 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.

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).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.

STUDY DESIGN:

The study cohort consist of 88 patients (by **Sysmex XT-2000i**)

CASE SELECTION:

The study was conducted with fresh blood samples collected from patients attending Haematology Lab, Dept. of Pathology.

EXCLUSION CRITERIA:

- Blood sample collected indoor.

- Received from PHCs. (I.e. blood samples with questionable storage time and condition.)
- Received from peripheral labs for verification.

PROCEDURE:

88 randomly selected blood samples collected by venepuncture and anticoagulated with dipotassium EDTA were measured within 1 h of collection on**Sysmex XT-2000i**.This sample was taken as baseline (0 h) sample. The samples were taken from out-patients in 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 is 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.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 will not affect the clinical management of patients.

- **STATISTICAL ANALYSIS:**

The mean absolute difference and mean percent difference were calculated from delayed samples relative to measurements from samples within 1 hours of collection, together with respective 95% confidence intervals. The change in value (in %) obtained for each parameter was compared with 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.

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:

	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%

Table 2:

	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

Table 3:

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 4:

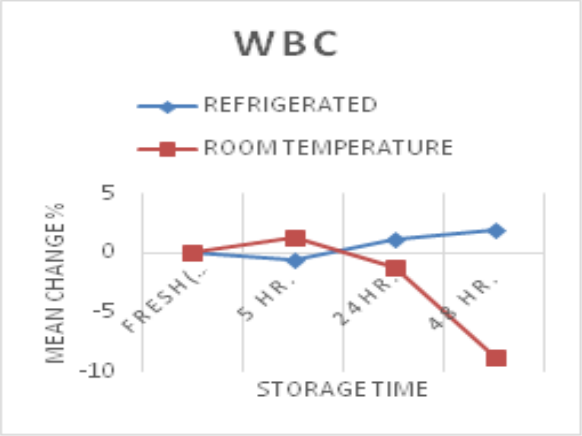
REFRIGERATED													
n=88	FRESH (<1hr)	5 hr.	Change%	CI (95%)		24hr.	change%	CI (95%)		48 hr.	change%	CI (95%)	
				Lower	Upper			Lower	Upper			Lower	Upper
WBC(x10^9 /L)	11.27	11.23	-0.56	9.76	12.70	11.37	1.16	9.89	12.85	11.39	1.93	9.91	12.87
RBC(x10^{12}/L)	4.48	4.55	1.38	4.35	4.75	4.48	-0.12	4.28	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 5:

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 6:

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



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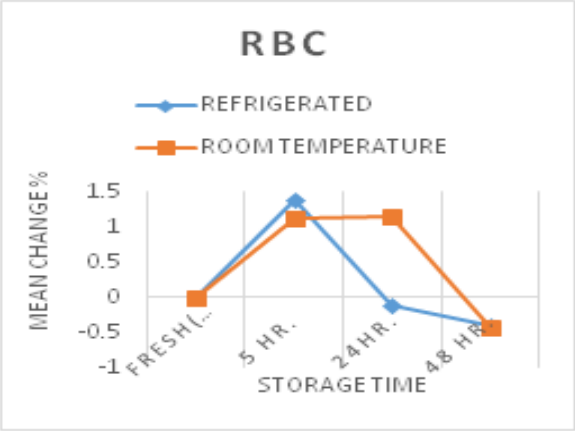

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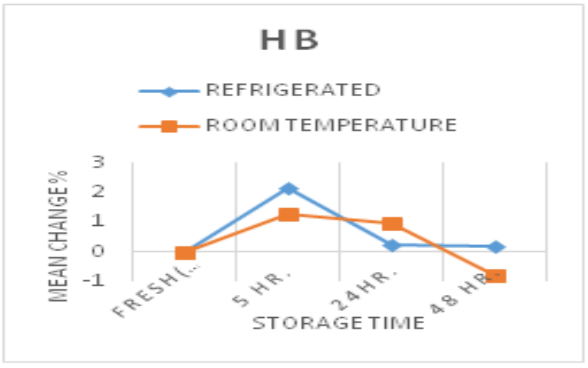

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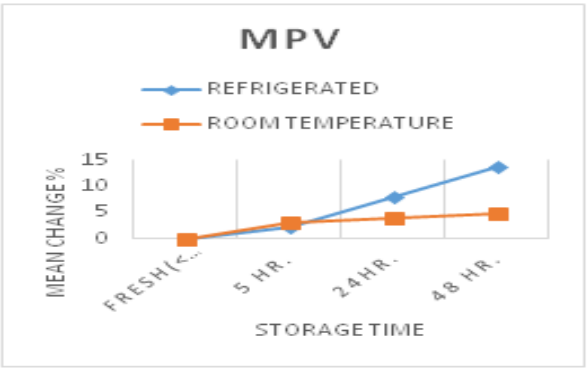

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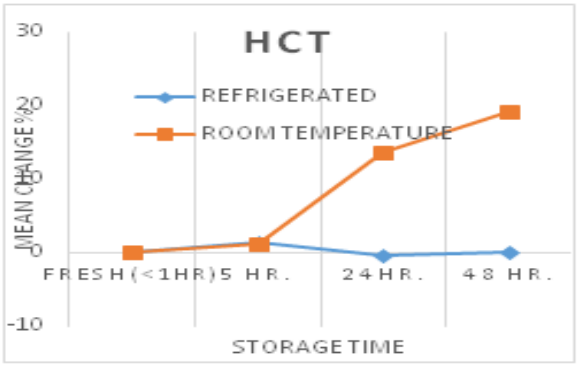

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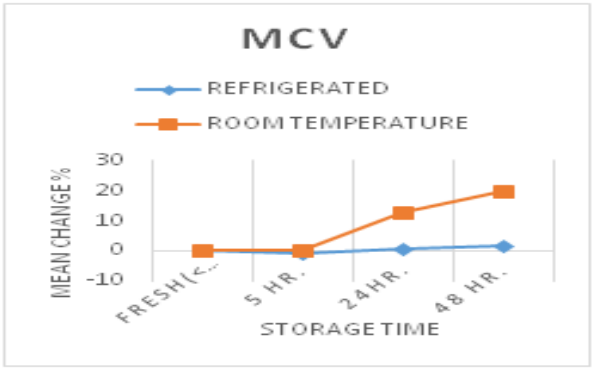

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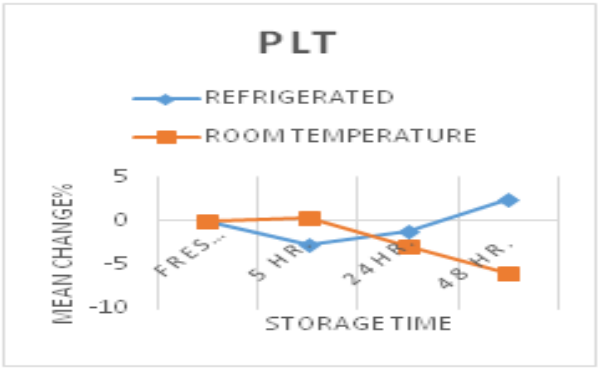

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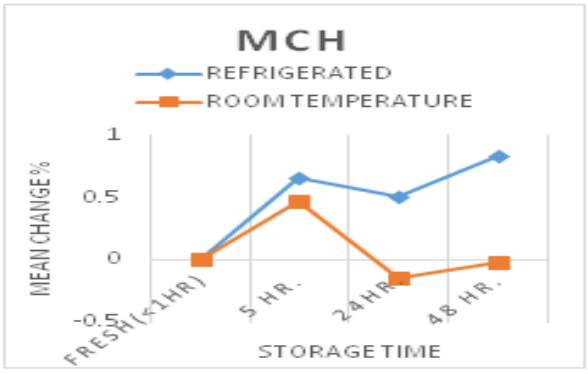

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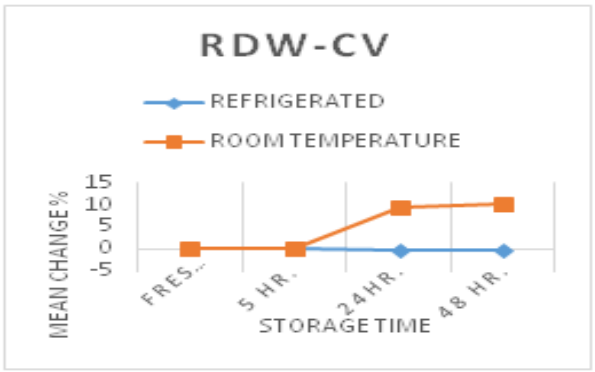

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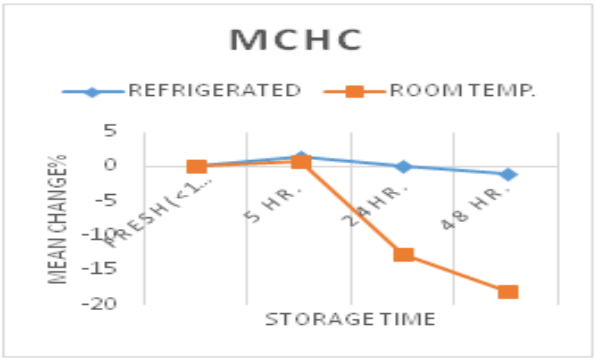

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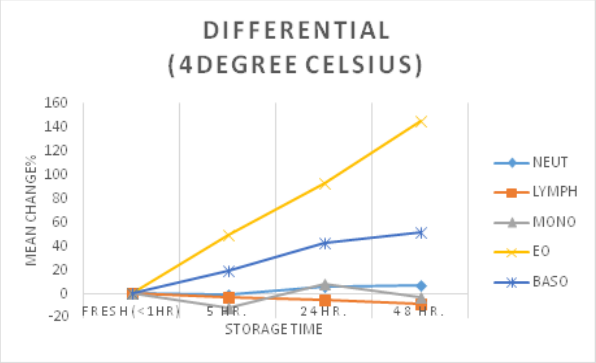

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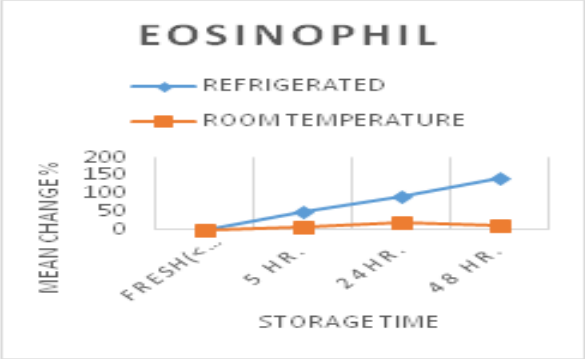

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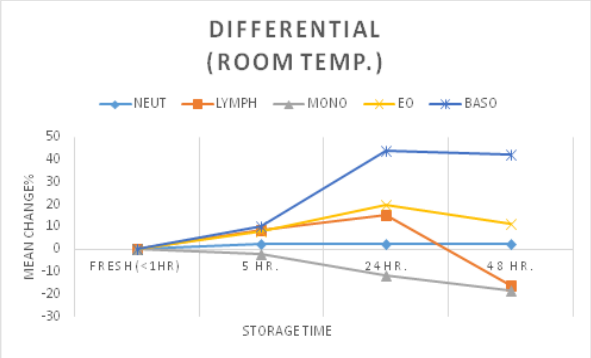

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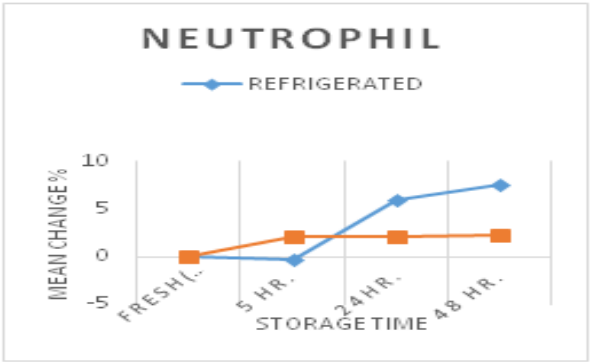

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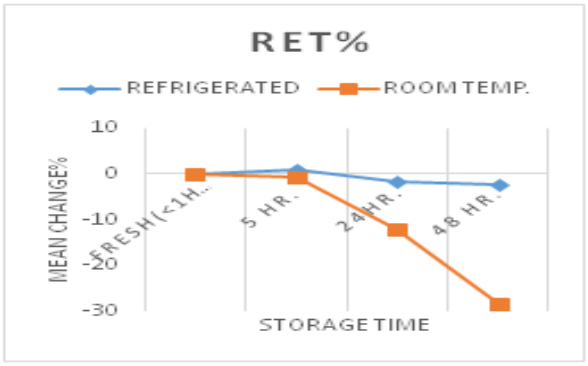

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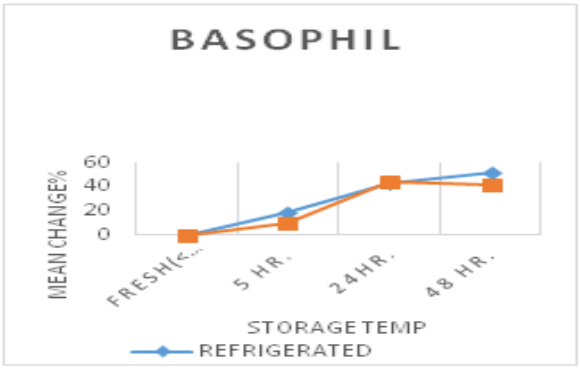

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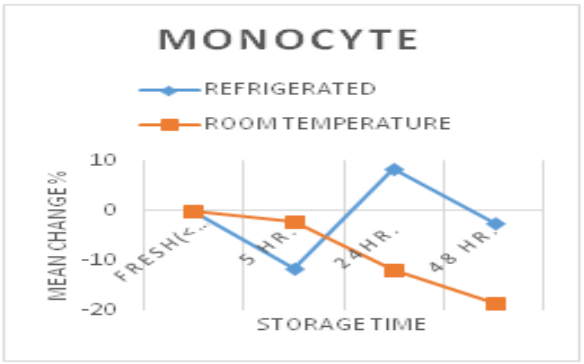

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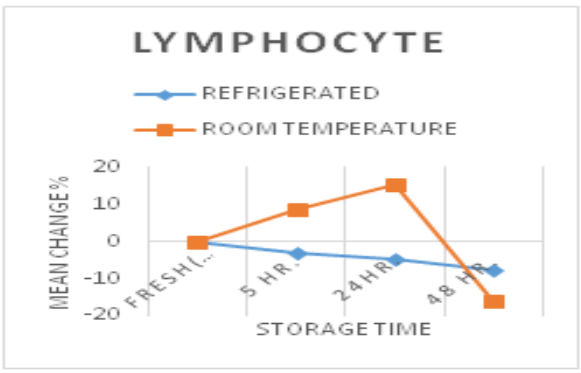

Figure 18:

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

Depending on the time the peripheral blood smear is prepared, morphological changes were seen in some but not all Cells. Some neutrophils 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 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:

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.).

Discussion

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. 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 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. WhereasLYMPHOCYTE dropped slightly, but BASOPHIL changed by 42% in 48 hours compared to 1 hour samples. 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.

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.

Conclusion

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.

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.

Acknowledgement

Special thanks to ICMR STS Programme, for this research exposure. Heart felt gratitude to prof. and HOD dr.Devi Prasad Mishrafor his constant guidance and support. And to the Lab Technicians, Department of Pathology MKCG, for the technical assistance.

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