

## SURVEY OF CHANGES IN COMPLETE BLOOD COUNT AND RED CELL INDICES OF WHOLE BLOOD INCUBATED *IN VITRO* AT DIFFERENT TEMPERATURES UP TO 48 HOURS

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**Background:** Complete blood count (CBC) is one of the most common and conventional blood test that physicians usually request. However the results of this test are affected by different factors such as, the temperature and duration of incubation, therefore the aim of this survey was to evaluate the effect of temperature and time of incubation on CBC, red blood cells (RBC) indices and white blood cells (WBC) differential count. **Methods:** In a cross-sectional study, blood samples were taken from 30 healthy medical students of Rafsanjan University (15 males and 15 females). The samples divided into three parts; CBC were done on the samples up to 48 hours incubation at temperature of 25, 30, and 37<sup>0</sup> C at the time of sampling, and after 2, 8, 24 and 48 hours. Data were statistically analyzed and the following results were obtained. **Results:** RBC count, hematocrit, MCH, percent of monocytes and eosinophils were constant in different temperatures, WBC count, MCHC, hemoglobin, 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 decreased with increasing temperature up to 8 hours and then significantly increased (from 83.89 to 87.50 fml/l, p<0.001). WBC, hematocrit, MCV, platelets count, and neutrophils' percent tend to increase by the time of incubation, but RBC count, MCHC, lymphocytes' percent decreased. Hemoglobin, MCH, and the percent of monocytes and eosinophils were constant. **Conclusion:** The finding of this survey showed that some of CBC parameters can be changed with the incubation, therefore it is better to do the CBC test after blood taking as soon as possible.

**Key Words:** CBC, RBC Indices, Temperature of storage, Time of storage

### INTRODUCTION

In clinical laboratories, the cycles of each test starts with the preparation of patient, and continues with biologic sample collection, preparation and incubation the sample in suitable conditions and finally finishes by reporting the result. It is sometimes necessary to redo a test to obtain a reliable result. Sometimes there is a period of time between sample collection and doing or redoing a test. In this situation the satiability of the samples is very important. Since blood tests are more common than the other biologic fluids, therefore using the standard methods for sample collection, incubation and the role of environmental factors that affect the blood's indices should be considered.

Complete blood count (CBC) is one of the most common and routine laboratory tests that is the first step to diagnose an illness and since this test is become easy and quick, it can give valuable information to the physicians.

The results of CBC can be affected by different factors such as the temperature and incubation period. Sometimes it takes time between blood samples collection and doing the test.

The study of Hogman and Knutson in 1999 showed that the level of 2,3- bisphosphoglycerate (2,3-BPG) could be changed by incubation the blood sample.<sup>1</sup> Gulati and Hyland in 2002 determined that the long-term incubation of blood in room temperature changes some blood indices.<sup>2</sup> In another study it has been demonstrated that incubation of blood samples in different temperature leads to considerable alteration in blood cells and red blood cells (RBCs) indices.<sup>3</sup> It has been also shown that RBCs are concentrated by incubation and in different times and temperatures changes will occur in blood cells.<sup>4</sup>

In Hogman and Knutson report has been mentioned that the studies in this field are not sufficient<sup>1</sup> therefore the aim of this study was to evaluate the simultaneous effect of different incubation times and temperatures on changes of CBC and RBC indices and also the differential count of white blood cells (WBC).

### MATERIAL AND METHODS

In this sectional study the blood samples of 30 (15 male and 15 female) of healthy students of Rafsanjan University of Medical Sciences, with age 18-26 years

old were collected. The volume of blood sample was 9ml for each subject that was collected in vials containing EDTA.

CBC was carried out on blood samples by cell counter (Coulter T890) immediately after mixing and then each sample divide into three parts of 3ml. The samples were incubated in three different temperatures (25, 30 and 37°C) and after 2, 4, 8, and 24 hours the CBC was done again on samples. The samples were mixing during the test and returned to the incubators immediately after CBC. The slides of blood smears for WBC differential count were prepared and stained by Gimsa staining solution for each temperature and time of incubation.

Repeated measure of ANOVA and Tukey's test were used to analyze the data.

## RESULTS

The finding of this study showed that in different times and temperatures, a number of RBC indices changed significantly. The changes in MCH, RBC and WBC counts were not significant, but with increasing the temperature and time MCV decreased significantly (tables 1 to 3).

MCHC after 8 hours decreased significantly. The change in Hct was not significant up to 24 hours incubation but after 48 hours increased with increasing the temperature. In WBC differential count lymphocytes and neutrophils counts after 48 hours of incubation decreased significantly. Hemoglobin concentration increased significantly with raising the temperature after 48 hours of incubation.

**Table 1: Comparison of the mean of MCV (fmol/l), in different times at 25°C**

Time	Initial	2 Hours	8 Hours	24 Hours	48 Hours
Mean	83.89	83.93	84.99	86.42	86.99
SD	5.07	4.95	5.12	5.28	5.34
SEM	0.93	0.90	0.94	0.96	0.97

The differences were significant (p<0.001) in different times.

**Table 2: Comparison of the mean of MCV (fmol/l), in different times at 30°C.**

Time	Initial	2 Hours	8 Hours	24 Hours	48 Hours
Mean	83.89	83.88	84.82	86.92	87.61
SD	5.07	5.07	5.23	5.35	5.01
SEM	0.93	0.91	0.95	0.98	0.91

The differences were significant (p<0.05) in different times.

**Table 3: Comparison of the mean of MCV (fmol/l), in different times at 37°C.**

Time	Initial	2 Hours	8 Hours	24 Hours	48 Hours
Mean	83.89	83.41	84.89	86.69	87.50
SD	5.07	5.09	4.88	5.34	5.36
SEM	0.93	0.93	0.89	0.97	0.99

The differences were significant (p<0.0001) in different times.

## DISCUSSION

In this study the effects of temperature and incubation time on blood indices in CBC test and differential counts of WBC was evaluated.

In WBC counts we found that after 2 and 8 hours of incubation there was an increase in the numbers of WBC but was not significant.

Vogelaar et al. that evaluated 304 blood samples of 17-70 years old individuals have reported that incubation blood samples in room temperature for 48 hours did not change the number of RBC, WBC and platelets<sup>5</sup>. According to the study of Hirase, the blood cells were stable after one week of incubation<sup>6</sup>, but in Wood's survey the incubation of samples for 24 hours resulted in the increase of WBC counts.<sup>7</sup>

The WBC counts at 30°C in our survey was similar to the research of Gulati who has reported that WBC counts would not change in first 3 days of incubation<sup>2</sup>, but the WBC counts at 25 and 37°C in our study was not in consistent with Gulati's study the difference between our results and the results of Gulati was due to the role of different temperatures, since Gulati studied the WBC counts in only one temperature.

The number of RBC at 37°C in our study decreased significantly (p=0.048) after 48 hours incubation that is against the results of Gulati who has reported that the RBC counts was constant up to 7 days in room temperature.<sup>2</sup>

The change in hemoglobin concentration in this experiment was in consistent with Gulati study that found that Hb concentration was constant up to 7 days at room temperature<sup>2</sup>. Our results in MCV and MCH were similar to the results of Gulati<sup>2</sup>.

Our findings showed that platelets counts after 48 hours incubation increased with raising temperature significantly (p=0.007), but Gulati has reported that platelets counts did not change up to 4 days incubation at room temperature<sup>2</sup>. In Zhang study the cause of platelets increasing explained as increase in whole blood viscosity.<sup>8</sup> Uchida in 2000, Shortland in 1997 and Qi in 2001 have reported that raising the temperature leads to changes in platelets' morphology and movement.<sup>9-11</sup> It has also been reported in a comprehensive study by Ho et al. that different temperature and time of incubation can affect in platelets counts and hemoglobin concentrations.<sup>12</sup>

## CONCLUSION

Overall the results of this study showed that delay in doing the CBC test can leads to changes in some parameters, therefore the blood samples should not be leaved in the laboratory and the test should be done on blood samples as soon as possible.

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