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Short Communication

Evaluation of the effect of temperature and time of incubation on complete blood count (CBC) tests

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The complete blood count (CBC) is one of the most common tests requested by physicians. The results of this test are affected by different factors such as temperature and time of incubation. Therefore, the aim of this study was to evaluate changes in CBC results at room temperature (RT). In a cross-sectional study, 32 K_2EDTA (dipotassium ethylenediamine-tetraacetate)-anticoagulated blood specimens were processed for CBC testing after blood-taking and incubation for 24 h at RT. Specimens were selected from routine laboratory workload. Among the CBC parameters, there were no significant differences in WBC, Plt and Hb results before and after incubation at RT ($p>0.05$). However, there were significant differences in RBC, Hct, MCV, MCH and MCHC results before and after incubation ($p<0.001$). The findings of this study showed that some CBC parameters can change after incubation at RT. Testing should therefore be done on blood samples as soon as possible.

Key words: Complete blood count, white blood cell, red blood cell, platelet counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, incubation.

INTRODUCTION

All hematological tests begin with blood collection. To maintain good laboratory practice, it is essential to standardize pre-analytic procedures. These procedures consist of reception of test requests, registration of patient identification numbers, confirmation of test items, preparation of appropriate syringes and specimen containers, blood collection procedures, specimen storage and specimen transportation to the laboratory. Special attention must be paid to safe handling of blood (Tatsumi et al., 2002). Hematological testing must be performed on whole blood. As soon as a blood specimen is withdrawn from a patient, it is mixed with an anticoagulant to prevent coagulation.

Clinical laboratories equipped with modern automated analyzers are capable of processing large volumes of hematologic tests in an efficient and timely manner. These tests include complete blood count (CBC), differential leukocyte count (diff), reticulocyte count (retic), and more recently, the nucleated red blood cell count (NRBC). To ensure the reliability of the results generated by the instrument, it is imperative that the specimens are collected appropriately, mixed with a suitable anticoagulant and analyzed on a properly calibrated instrument within the time frame considered appropriate or recommended by the manufacturer. Dipotassium ethylenediamine-tetraacetate (K2-EDTA)-anticoagulated blood is the specimen of choice for all of these tests. Generally, specimens arrive in the laboratory and are analyzed within hours of collection from patients (de Baca et al., 2006).

The complete blood count (CBC) is one of the most common and routine laboratory tests, and is one of the

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Abbreviations: CBC, Complete blood count; WBC, white blood cell; RBC, red blood cell, Plt, platelet counts; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RT, room temperature.

Table 1. Range of automated CBC results*.

Parameter \ Time	Initial	24 h
WBC (10^3/µl)	4.0-11.80	4.0-11.70
RBC, (10^6/µl)	3.06-6.89	3.02-6.66
Hemoglobin(Hb) (g/dl)	8.60-19.10	8.70-18.90
Hematocrit (Hct) (%)	25.80-54.60	26.20-55.20
MCV (µm^3)	65.31-96.92	67.59-100.68
MCH (pg)	20.75-35.15	20.93-35.09
MCHC (g/dl)	20.75-35.15	31.17-37.50
Platelet (Plt) (10^3/µl)	226.41±7.70	226.75±7.45

*CBC, Complete blood cell count; WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; and MPV, mean platelet volume.

first steps in diagnosing an illness. The test is quick, easy and can give valuable information to the physicians. The results of the CBC can be affected by different factors such as the temperature and incubation period (Mahmoodi et al., 2006).

When such a specimen arrives at the laboratory, the staff needs to decide (Tatsumi et al., 2002) whether to accept or reject it. If accepted, whether to perform all the ordered tests or only those deemed appropriate based on the age of the specimen and (de Baca et al., 2006) what comments, if any, should be appended to the reported results regarding their reliability of the sample. Such decision making requires laboratory technicians and physicians to be familiar with changes known to occur in blood specimens during storage (Gulati et al., 2009; Peng et al., 2001a). Manufacturers of automated analyzers and published literature often cite that blood specimens, kept at either room temperature or at 48°C (refrigerated) for up to 24 h, generally yield reliable results for CBC testing and automated differential leukocyte count (differential) (Peng et al., 2001b; Tsuruda et al., 1999).

Occasionally, there is a period of time between sample collection and testing. In this situation, the satiability of the samples is very important. Since blood tests are more common than other biologic fluid testing using a standardized procedure for sample collection, incubation and control over various environmental factors that affect the blood's indices should be considered (Mahmoodi et al., 2006).

This study examined changes in various parameters of automated CBC resulting after storage of blood at room temperature for 24 h.

MATERIALS AND METHODS

In this cross-sectional study, 32 K_2-EDTA (dipotassium ethylenediamine-tetraacetate)-anticoagulated blood specimens were selected over several days from the routine laboratory workload at Fatemeh Zahra Hospital in Bushehr, Iran. CBC testing was carried out on blood samples by cell counter (Sysmex K800, USA) immediately after mixing. Each sample was then incubated at room temperature (RT) for 24 h, after which CBC testing was conducted again. The samples were mixed during the test. Paired-samples t tests were used to analyze the data.

RESULTS AND DISCUSSION

Specimens were selected from the routine laboratory workload for several days to represent a range of normal and abnormal CBC results, as indicated in Table 1. The findings of this study showed that differences in times of incubation and temperature lead to a number of RBC indices changing significantly. The changes in Hb (hemoglobin), WBC (white blood cell count) and Plt (platelet count) were not significant ($p>0.05$), but after incubating the samples at room temperature for 24 h, RBC (red blood cell count), hematocrite (Hct), MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration) increased significantly ($p<0.001$). MCH (mean corpuscular hemoglobin) was also significantly increased after 24-h incubation ($p<0.05$) (Table 2).

Storage of blood at room temperature for 24 h led to changes in some CBC parameters. These changes may be considered clinically insignificant. Hb, WBC and Plt were found to be stable for 24 h after collection of blood, while clinically significant changes were observed in the RBC, Hct, MCV MCH and MCHC after storage at room temperature.

Previous studies have provided both supporting and conflicting results. Hirase et al. (1992) demonstrated sample stability after one week of incubation. Similarly, 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) incubated samples for 24 h and found significantly increased WBC counts.

Hemoglobin concentration was found to be stable for

Table 2. Comparison of mean CBC parameters before and after storage of blood at room temperature.

Time / Parameter	Initial	24 h	P-value
WBC (10^3/µl)	6.45±1.94	6.41±1.93	p>0.05
RBC, (10^6/µl)	4.69±0.71	4.66±0.71	p<0.001
Hemoglobin(Hb) (g/dl)	14.08±2.15	14.07±2.16	p>0.05
Hematocrit (Hct) (%)	40.54±5.56	41.81±5.60	p<0.001
MCV (µm^3)	87.04±8.22	90.37±8.55	p<0.001
MCH (pg)	30.29±3.86	30.44±3.84	p<0.05
MCHC (g/dl)	30.27±3.86	34.69±1.70	p<0.001
Platelet (Plt) (10^3/µl)	226.41±7.70	226.75±7.45	p>0.05

the duration of the study. These findings are consistent with previous reports (Gulati et al., 2009; de Baca et al., 2006; Mahmoodi et al., 2006). Changes in Hct in this experiment were consistent with that of de Baca et al. (2006) study, while Mahmoodi et al. (2006) reported that Hct was constant in different temperature but increased after 48 h increasing temperatures.

In the present study, MCV, MCH and MCHC all increased after 24-h storage at room temperature. The increase in MCV is known to reflect red cell swelling at room temperature (de Baca et al., 2006). Our results of MCV and MCH were similar to previously reported results (Gulati et al., 2009; Mahmoodi et al., 2006), although, there was no change in MCH for up to 4 days after collection of blood (de Baca et al., 2006). Additionally, it was reported that MCV of fresh blood in acid citrate dextrose (ACD) and EDTA was unchanged after storage for one day at 4°C but increased when the storage temperature was 23°C (Lawrence et al., 1975).

In our study, platelets counts were 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). A study suggested that different temperatures and times of incubation can affect platelet counts and hemoglobin concentrations (Ho and Chan, 1995). 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).

It can therefore be concluded that in order to prevent variability of CBC results, the blood samples should not be left in the laboratory and testing should be conducted on blood samples as soon as possible, as delay in testing can lead to changes in certain CBC parameters.

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