Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(11)60038-8

Evaluation of preanalytical stability of the sample for complete blood count

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ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 27 June 2011
Accepted 25 July 2011
Available online 28 September 2011

Keywords:
Complete blood count
Automated
Stability
Lymphocytes
Eosinophils
Basophils
Neutrophils

ABSTRACT

Objective: To evaluate the effect of time of incubation on complete blood count (CBC) by using HmX analyzer. **Methods:** A cross sectional study was conducted at Manipal Acunova Limited, Bangalore. Ten blood samples which were stored at room temperature for CBC and differential count by using HmX analyzer were analyzed within one hour of draw and on 24, 48 and 72 hours of draw. **Results:** Differential count measured in an automated instrument changed over time. **Conclusions:** The finding of this study shows that some of CBC parameters can be changed with the incubation, therefore it is better to perform on a fresh sample.

1. Introduction

There has been a steady increase in the use of central lab for both diagnostic and clinical trial testing of routine hematology parameters like complete blood count (CBC), which is one of the most common and conventional blood test that physicians usually request. 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 indices of blood should be considered. However, the different subparameters in CBC panel may require different storage and transit temperatures. Subparameters like thrombocytes benefit from storage at ambient temperature, but subparameters like mean corpuscular volume (MCV) are more stable at refrigerated conditions. Other subparameters like erythrocytes and hemoglobin can tolerate both conditions.

Gulati and Hyland determined that the long-term incubation of blood in room temperature can change some blood indices[1]. WHO also released a document about the sample stability[2].

Since there are limited studies in this field, the aim of this study was to ascertain the preanalytical stability of samples on changes of CBC by using HmX hematology analyzer.

2. Materials and methods

This study was conducted at Manipal Acunova Limited, Bangalore, which is accredited by National Accreditation Board for Testing and Calibration of Laboratories as per ISO 15189 and College of American Pathologists. Ten venous blood samples were drawn into 4 mL K2 EDTA vacuum tubes of BD (CAT No. 367861) and were selected on a random basis from our routine work load. All samples were analyzed within one hour of draw and on 24, 48 and 72 hours of draw which were stored at room temperature for

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CBC and differential count by using HmX analyzer. HmX analyzer, manufactured and marketed by Beckman Coulter Inc. (Fullerton, CA, USA) is a bench top 5 part differential blood cell counter. The instrument in our setup is calibrated by using S cal calibrator and traceable to twice a year. The differential count is made using the VCS (volume, conductivity and scatter) principle, by the HmX instrument. As a routine practice we use 5C controls (manufactured by Beckman Coulter Inc) daily before any samples were analyzed. The CV of controls being used is in the range of 3%-5%[3].

3. Results

In this study, deviation in accuracy for each data pair was calculated by using the formula:

$$\frac{\text{value day 0} - \text{value day 1}}{\text{value day 0}} \times 100$$

It was noted that 9 (90%) of the samples showed a reduction in neutrophils and the same (9, 90%) showed an increase in lymphocytes. 1 (10%) showed a reduction of neutrophils of <10% and <20% each, 7 (70%) showed a deviation of 20%-42% from the day 0 values. Similarly lympocytes increased in 90% of cases on day 1 as compared with those observed on day 0. The difference ranged between 18.3% and 145.0%.

4. Discussion

It was noted by Caroline Kern that, On AcT Diff (instrument from Beckman coulter Inc, which works on a principle of impedence and cytochemistry principle) CBC parameters were very stable over the 72-hour period. The differential parameters showed acceptable stability. Neutrophil and lymphocyte values were stable up to 48 hours. A minor shift occurred in neutrophil and lymphocyte populations 9 hours after sampling with no clinical significance. The monocytes increased slightly after 24 hours[4]. Hedberg *et al* have indicated in their study that there was no substantial change in WBC parameters up to (24-48) h at (23±2) ℃ except for optical platelet count. CBC parameters were stable for 72 hours, except mean platelet volume, which slightly increased between 48 and 72 hour, at 4 ℃[5]. The results of the investigation carried out by Lippia *et al* suggest that, within certain limitations for parameters derived or calculated from cellular volumes, blood specimens stored for as long as 24 hours at 4 ℃ may be suitable for hematologic testing[6].

It is evident from this study that differential count measured in an automated instrument changes over time. Lymphocytes tend to increase (90% of cases) and neutrophil tend to reduce (90% of cases) from the baseline values. Monocytes also tend to reduce (90% of cases) and eosinophils reduced in 80% cases and increased in one instance. The granulocyte being measured by VCS principle, over time probably due to change in size of cell gets counted as lymphocyte. The 5C controls (manufactured and marketed by Beckman coulter Inc) used by the lab were in range as evidenced by small CV for differentials as the cells here are stabilized unlike the patient samples. However, the total white blood count (WBC) did not vary more than 5.7% and in 2 (20%) did not vary at all (accuracy 100%).

In conclusion, when differential counts are done on automated cell counter with a VCS principle, it may not be suitable for delayed processing of differential as happens in central lab model for clinical or diagnostic testing. Here the gold standard would be to use the fresh samples.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Mr. Girish, Mr. Arun Kumar G and Ms. Chetana, Scientists, Manipal Acunova Limited, Bangalore for having helped in publishing this article.

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