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Stability of blood baseline parameters under various storage conditions

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INTRODUCTION

Blood testing was introduced by the World Anti-Doping Agency (WADA) and some federations as pre-competition testing since the mid-1990's. In the latest list of banned substances, *The 2009 Prohibited List* [(1)], many substances are directly involved with the blood parameters, i.e. Erythropoiesis-Stimulating Agents, (*Prohibited Hormones and Related Substances (S2)*). Some methods such as blood doping and Hemoglobin Based Oxygen Carriers (M1) are also prohibited in the sport setting [(1)]. The hematological parameters indicate if an athlete has recently used a substance or method to alter erythropoiesis for blood manipulation purposes [(2)].

In South Africa the main concern for the laboratory is the stability of blood parameters during shipping of the samples from the events to the laboratory. The laboratory is geographically far from some events and time frame from of sample collection to receipt at the laboratory raises concern for blood analysis.

Blood samples are problematic as the mean cell volume (MCV) of the red blood cells is temperature dependant. Insufficient transport conditions might lead to false blood parameters and wrongful disqualification of athletes [(3)]. The MCV is used by blood analyzers to determine the hematocrit levels and poor storage conditions may lead to false hematocrit levels that may be above the cut-off level of 0.50 for males and 0.47 for females, set by the International Cycling Union (UCI) and the International Biathlon Federations[(4)].

AIM

The aim of the study was to determine the stability of the major blood constituents at room temperature and at 4ºC.

METHODOLOGY

Ethical committee approval was obtained for the study. The study group consisted of seven (7) volunteers (18 - 35 years, 3 males & 4 females). Two venous blood samples were collected at time 0 from each volunteer. The samples were divided into two groups, one for the RT-group (room temperature) and the other the 4ºC-group. Both groups of samples were analyzed using a Sysmex XT2000*i* Haematology analyzer directly after collection. One sample of each volunteer was kept at room temperature while the other sample was stored at 4^{o}C. The samples were re-analyzed after 8, 24, 48 and 72 hours.

RESULTS

The results showed that the hemoglobin values for all subjects were stable at both RT and 4^{o}C (Figure 1).

The Hematocrit values for the samples stored at 4^{o}C stayed very stable and were consistent over the 72 hour period. The Hematocrit of the samples kept at RT, changed drastically within the first 8 hours after sampling and this trend continued up to 48 hours after sample collection (Figure 2).The increase of the average hematocrit values was so significant that it increased as much as 15% over the 72 hour period of the study analysis. This changed the hematocrit values of almost all volunteers to high levels in the physiological range for healthy individuals (0.43 – 0.55 for males and 0.37 – 0.49 for females) and well above the cut-off levels of 0.50 for males and 0.47 for females [(4)].

During the study period the Red blood cell count (RBCs) for both samples from all individuals were very stable over time (Figure 3), however the mean cell volume of the RT samples changed drastically over time (Figure 4).The change correlated to the increase in the Hematocrit values and started within the first 8 hours after sample collection. The MCV parameter is directly influenced by the hematocrit value due to the calculations done by the Sysmex using this as a parameter for Hematocrit determination. The reticulocyte count, at room temperature , decreased initially, until about 24 hours after sample collection, but then started to increase to a value higher than the value at T = 0 (Figure 5). This result was also observed by previous authors [(5)]. The change in reticulocyte count can be attributed to the degeneration of the reticulocytes as to give false fluorescence staining patterns. This effect increases the immature reticulocyte population area and gives a wrong ratio of immature reticulocytes vs blood volume over this time period.

CONCLUSION

The transportation and storage conditions of blood samples are extremely important as temperature changes can influence the blood profile of an athlete. The influence of temperature can be seen from the reticulocyte and MCV parameters. The same pattern in parameter changes were seen by Robinson *et al.* 2004 [(3)] when they studied the hematological profile and concluded that it will be beneficial for federations to do on-site hematological testing.

As previously described by Lippi *et al.* (2005) [(4)], the MCV has a large effect on the Hematocrit value of the blood sample. If the seven volunteers in this study were competitive athletes and their samples were not stored correctly or transported under the correct conditions, they would not have been allowed to complete because of the Hematocrit values that were elevated above the UCI and IBF cut-off thresholds of 0.50 for males and 0.47 for female athletes.

The data clearly indicates that all blood samples must be transported cooled, or analyzed to limit samples degradation and false results. It is clear that the red blood cell content of the blood samples will not be influenced by temperature. This is favorable for the athlete, for it will not falsely indicate any EPO abuse or other RBC stimulating drug misuse. The new implemented *Biological Passport* for athletes has resulted in a long term monitoring of an individual athlete's blood parameters, therefore cases of false hematocrit levels, due to incorrect transport or handling, is omitted.

REFERENCES

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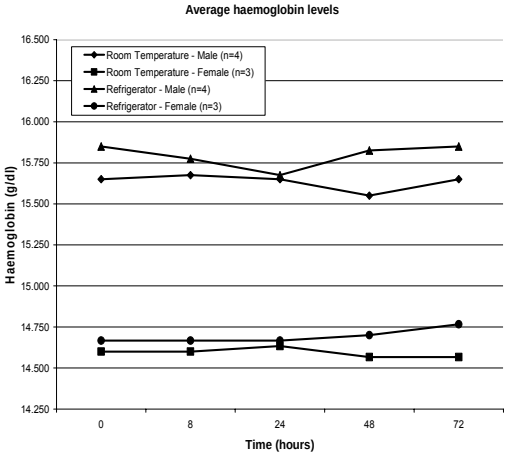


Figure 1: *Average Hemoglobin values for the 7 individuals for both RT and 4°C samples*:

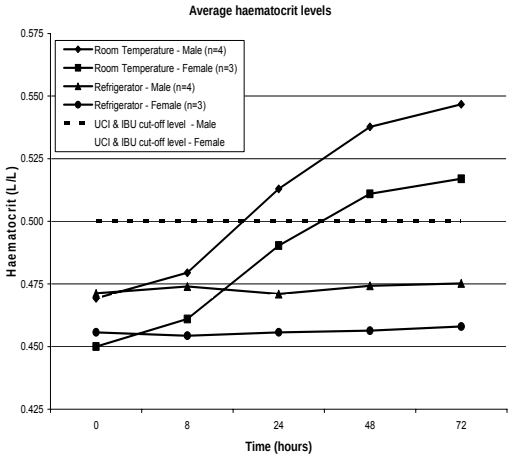


Figure 2: *Average Hematocrit values for the 7 individuals for both RT and 4°C samples*: *The dashed lines indicate the cut-off levels for male and female athletes of the UCI and IBU.*

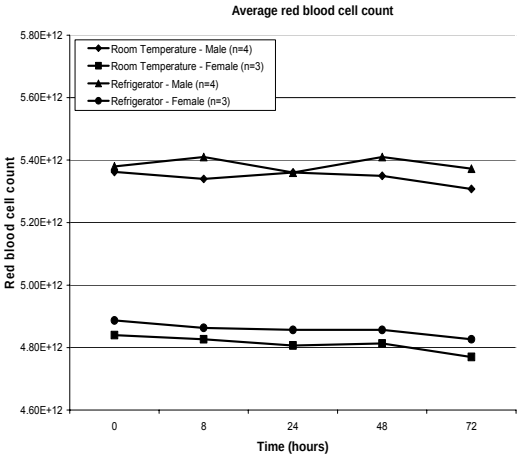
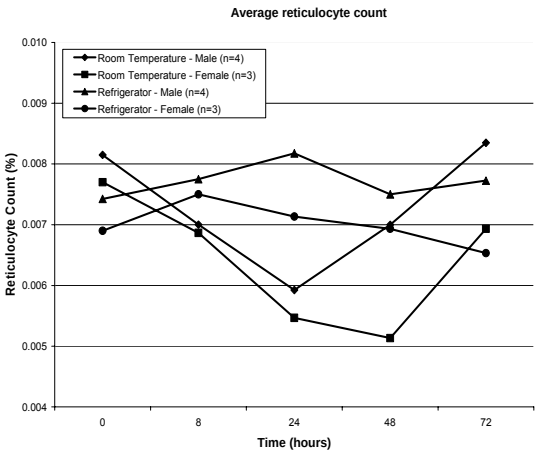


Figure 3: *Average RBC values of all volunteers for over the study period*



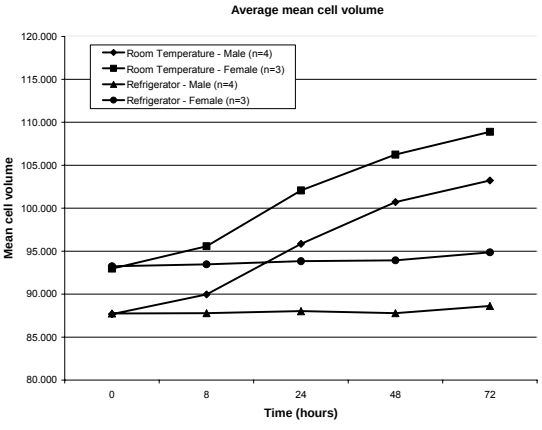


Figure 4: *Average MCV for the 7 volunteers recorded over the 72 hour period*

Figure 5: *Average Ret % for the volunteers for the two different temperature regimes*