F.Donati, F. Botrè

Using Advia 120 hematological analyzer as a fast screening method for HBOCs detection in human whole blood

Antidoping Laboratory of Rome – FMSI , Largo Giulio Onesti 1, 00197 Rome, Italy

Introduction

A recent development of doping practices is the use of artificial oxygen carriers (AOCs) by athletes. AOCs are chemical substances, mimicking the action of human hemoglobin (HGB), administered to improve the blood capacity to deliver oxygen to muscles. AOCs include perfluorocarbons and hemoglobin-based oxygen carriers (HBOCs). Recently HBOCs have been included by WADA in the list of prohibited substances in sports. Common screening tests to reveal HBOCs misuse by athletes are based on colorimetric detection as HBOCs use causes discoloration of the plasma. In this communication we are presenting a different approach for the screening detection using an ADVIA 120 hematological analyzer (Bayer Diagnostics, Tarrytown, NY, USA). ADVIA 120 evaluates haemoglobin by two methods: a standard cyanmethemoglobin colorimetric method to calculate the amount of total haemoglobin (HGBtot) and a flow cytometric optical method to calculate the amount of haemoglobin within the red blood cells (HGBcell). Thanks to this dual contemporary hemoglobin measurement, the HGBdelta value (corresponding to free HGB), is automatically calculated by subtraction of HGBcell from HGBtot and can be used as fast screening index of HBOCs abuse. We tested the effectiveness of this approach using normal blood samples with different basal HGB values spiked with three different HBOCs at different concentrations. We firstly evaluated ADVIA 120 performances calculating the correlation degree between HGBcell and HGBtot values in normal samples. Then we used a simple statistical approach to calculate a reliable HGBdelta cut-off (or Limit of Decision) value to discriminate between a clear negative sample and a suspect sample to submit to a confirmation analysis.

Materials and Methods

68 healthy athletes' whole blood samples coming from routine analysis in our lab were

analysed to search correlation between HGBtot and HGBcell measurements. Samples were first analysed with colorimetric method to be sure they were negative for HBOCs and then analysed using ADVIA 120. Results are showed in fig.1

To the aim to get whole blood sample spiked with different amounts of HBOCs, to the final concentrations indicated in Table 1. Three Fresh K3-EDTA–anticoagulated blood samples have been spiked with the following HBOCs (Oxyglobin and Hemopure from Biopure Corp, Cambridge, MA, USA, and Polyheme kindly provided by Northfield Laboratories Inc.Sherman Avenue Evanston, Illinois). Varying proportions of HBOCs solutions and whole blood were mixed to the aim to observe linearity of this method in a wide range of HBOC concentration spiked as showed in tables 1,2 and 3. Three hematologic parameters (HGBtot, HGBcell and Delta HGB) were determined in triplicate with ADVIA 120. For each parameter we calculated mean value and standard deviation. Statistical analysis of data has been performed using SPSS and Microsoft Excel softwares.

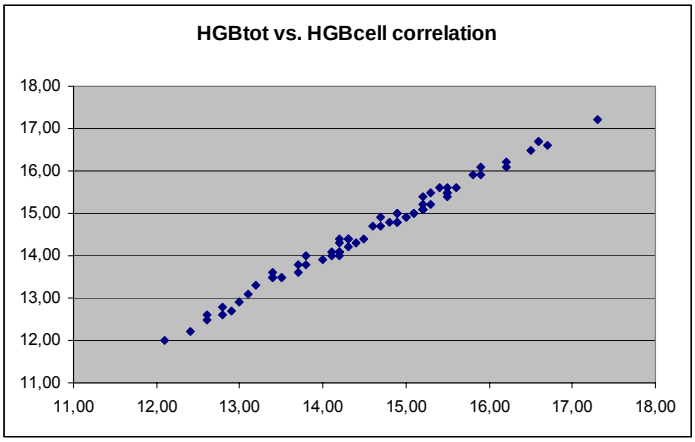

Fig.1: Correlation between HGBtot and HGBcell in 68 healthy athletes' whole blood samples

Results and Discussion

Correlation between HGBcell vs. HGBtot values provided by Advia 120. Figure 1 shows the comparison of HGB values obtained with optical method (HGBcell) and standard colorimetric method (HGBtot) on 68 healthy athletes' whole blood samples. Both parameters are strongly correlated with $R^2 = 0.9905$. Results obtained shows flow cytometry is a viable method of Hgb measurement.

Samples spiked with HBOCs at different concentrations. Table 1 shows mean values and standard deviations (enclosed in parenthesis) obtained for HGBtot, HGBcell and the Delta

HGB. Advia 120 is able to spotlight the exogenous contribution of an HBOC within a sample and the results are agreeing for each HBOC considered in this study. Delta HGB values are accurate and precise in all range of linearity we looked upon. This results gain importance if we consider the different origins of the HBOCs used. While both Hemopure and Oxyglobin are made from bovine hemoglobin (with Hemopure used on humans and Oxyglobin better intended for veterinary use) , Polyheme is instead a product from human hemoglobin, that is a solution of human hemoglobin extracted from human red blood cells and modified using polymerization process. It is interesting to remark the reproducibility of results with these HBOCs matrix-based differences.

polyheme	Sample 1 (basal HGB 12,80 g/dL)			Sample 2 (basal HGB 14,30 g/dL)			Sample 3 (basal HGB 17,00 g/dL)		
g/dL	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB
0	12,80 (0,10)	12,80 (0,15)	0,00 (0,06)	14,30 (0,06)	14,30 (0,10)	0,00 (0,06)	17,00 (0,06)	17,00 (0,06)	0,00 (0,06)
0,5	12,80 (0,10)	12,30 (0,12)	0,50 (0,06)	14,20 (0,06)	13,60 (0,10)	0,60 (0,12)	16,70 (0,12)	16,20 (0,16)	0,50 (0,17)
1	12,50 (0,00)	11,50 (0,17)	1,00 (0,17)	14,00 (0,06)	13,00 (0,12)	1,00 (0,15)	16,30 (0,12)	15,30 (0,06)	1,00 (0,06)
2,5	12,20 (0,12)	9,70 (0,06)	2,50 (0,15)	13,40 (0,06)	11,00 (0,06)	2,40 (0,06)	15,20 (0,06)	12,70 (0,12)	2,50 (0,06)
5	11,50 (0,12)	6,50 (0,15)	5,00 (0,21)	12,20 (0,12)	7,40 (0,15)	4,80 (0,06)	13,40 (0,17)	8,50 (0,06)	4,90 (0,06)
10	10,00 (0,10)	0,00 (0,00)	10,00 (0,10)	9,90 (0,06)	0,00 (0,00)	9,90 (0,06)	10,00 (0,10)	0,00 (0,00)	10,00 (0,10)
hemopure	Sample 1 (basal HGB 13,00 g/dL)			Sample 2 (basal HGB 14,60 g/dL)			Sample 3 (basal HGB 17,70 g/dL)		
g/dL	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB
0	13,00 (0,06)	13,00 (0,06)	0,00 (0,06)	14,60 (0,05)	14,60 (0,05)	0,00 (0,06)	17,70 (0,05)	17,70 (0,10)	0,00 (0,15)
0,5	13,00 (0,06)	12,50 (0,10)	0,50 (0,10)	14,50 (0,00)	14,00 (0,05)	0,50 (0,11)	17,40 (0,11)	16,90 (0,11)	0,50 (0,00)
1	12,90 (0,06)	11,90 (0,17)	1,00 (0,20)	14,30 (0,06)	13,30 (0,06)	1,00 (0,00)	17,30 (0,11)	16,30 (0,11)	1,00 (0,10)
2,5	12,90 (0,17)	10,20 (0,15)	2,70 (0,10)	14,30 (0,10)	11,50 (0,23)	2,80 (0,15)	16,70 (0,11)	14,00 (0,06)	2,70 (0,11)
5	12,90 (0,00)	7,40 (0,06)	5,50 (0,06)	13,90 (0,00)	8,30 (0,06)	5,60 (0,00)	15,70 (0,00)	10,00 (0,10)	5,70 (0,10)
10	10,00 (0,11)	0,00 (0,00)	10,00 (0,11)	10,00 (0,06)	0,00 (0,00)	10,00 (0,06)	10,00 (0,05)	0,00 (0,00)	10,00 (0,05)
oxyglobine	Sample 1 (basal HGB 13,00 g/dL)			Sample 2 (basal HGB 14,30 g/dL)			Sample 3 (basal HGB 17,60 g/dL)		
g/dL	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB	HGBtot	HGBcell	deltaHGB
0	13,00 (0,00)	13,00 (0,08)	0,00 (0,10)	14,30 (0,06)	14,30 (0,06)	0,00 (0,06)	17,60 (0,10)	17,60 (0,17)	0,00 (0,06)
0,5	13,00 (0,00)	12,40 (0,17)	0,60 (0,17)	14,20 (0,06)	13,70 (0,06)	0,50 (0,00)	17,30 (0,10)	16,80 (0,10)	0,50 (0,20)
1	12,90 (0,12)	11,80 (0,15)	1,10 (0,00)	14,10 (0,06)	13,10 (0,06)	1,00 (0,06)	17,20 (0,06)	16,10 (0,00)	1,10 (0,00)
2,5	13,00 (0,00)	10,40 (0,10)	2,60 (0,06)	14,00 (0,06)	11,50 (0,10)	2,50 (0,06)	16,30 (0,12)	13,70 (0,06)	2,60 (0,10)
5	13,00 (0,00)	7,50 (0,05)	5,50 (0,06)	13,90 (0,06)	8,90 (0,06)	5,00 (0,10)	15,50 (0,10)	10,10 (0,12)	5,40 (0,06)
10	10,00 (0,00)	0,00 (0,00)	10,00 (0,00)	9,90 (0,06)	0,00 (0,00)	9,90 (0,06)	10,0 (0,06)	0,00 (0,00)	10,0 (0,06)

Table 1 – Summary of data obtained on whole blood samples spiked with different amounts of HBOCs

The method is fast and precise. The screen of a sample can be achieved in less than one minute. The analysis performed is a true haematological analysis. It is not only a visual, colorimetric determination but it allows to determine more parameters at the time. So, apart from screen the sample for HBOCs, the analysis allows to monitor the whole haematological status of the athlete. Also, the analysis can be performed without the need to open the tube. There is no need to separate serum from the rest so that the operator does not get in contact with the blood.

The data show a strong correlation between colorimetric and optical method accomplished by Advia 120 in HGB determination. This means that in the condition of correct calibration, a normal blood sample has a delta value approximately equal of zero, taking as unimportant the small contribution of free natural HGB. So, in a screening stage, the developing of a threshold value to discriminate against a negative and a suspect sample for HBOCs is necessary.

Table 2 shows mean and standard deviation of 68 whole blood samples negative for HBOCs. We applied a 3ds rounded up criterion at the mean of the negative samples in order to get a threshold value to use as Limit of Decision. In this case a sample with delta value below the cutoff 0,35 g/dL (3,5 mg/mL) can be considered as negative and a sample with a delta higher or equal at 0,35 g/dL is considered suspecious and needs confirmation analysis. The adding of 3ds at the mean grants to a get a plausibile threshold value so as to avoid the chance of false positive and like so to send suspect samples in confirmation with a reliable confidence.

	HGBtot	HGBcell	Delta
mean	14,57	14,56	0,01
ds	1,15	1,18	0,11
mean + 3ds			**0,35**

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