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Fast screening for liposome encapsulated hemoglobin (LEH) using ADVIA 120 flow cytometry-based hematological analyzer

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Abstract

The artificial enhancing of the uptake, transport or delivery of oxygen, including the intake of modified hemoglobin products (e.g HBOCs) is a prohibited method included in the WADA List of Prohibited Substances (M1. Manipulation of blood and blood components). In this work we present a fast screening to detect liposome encapsulated hemoglobins (LEH) [1]. LEH can be detected in serum/plasma using flow cytometry-based techniques with a dual color staining procedure that requires several hours for sample preparation and analysis. The aim of this work is to test the potential of a cytometry-based hematological analyzer such as ADVIA 120 (Siemens) for a rapid screening of the presence of liposomes within a serum/plasma sample.

Introduction

Liposome encapsulated hemoglobins (LEH) are for all intents and purposes a potential form of blood doping and, in addition to class M1, they fall within class S0 as not yet being approved for their use in therapeutics. Exogenous hemoglobins can be easily detected as they cause miscoloration of plasma or by measuring the difference between the total hemoglobin (HGB total) and the intracellular hemoglobin (Hgb cell) content in a sample [2]. Hemoglobins encapsulated into liposomes become “invisible” to common screening methods. Liposomes can be stained and detected in dual-plot flow cytometry (Figure 1) with a procedure requiring 3-4 hours [3].

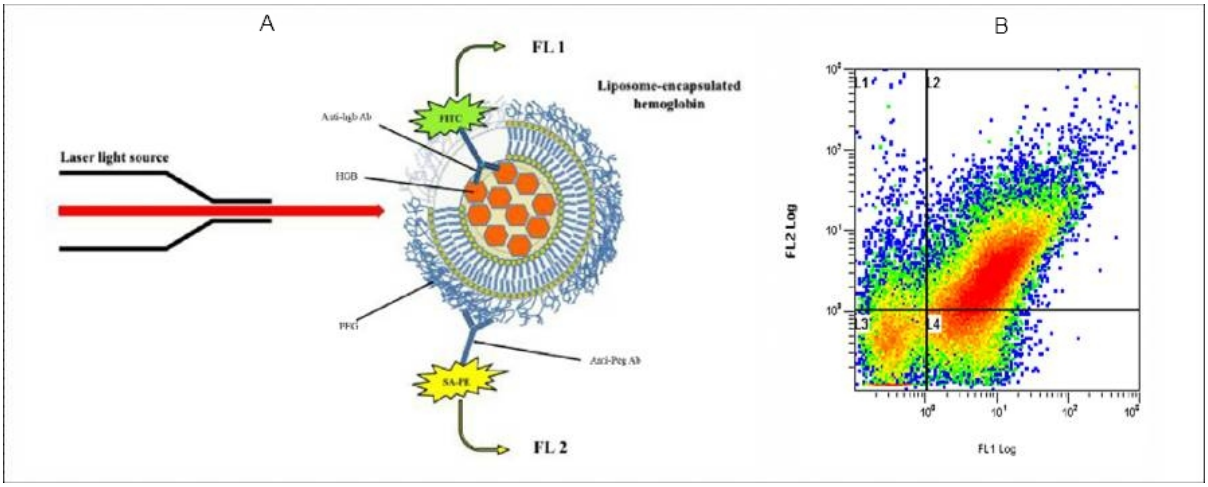

Figure 1: Detection strategy of pegylated-LEH by flow cytofluorimetry. Two antibodies are directed to different features of the LEH: FITC-conjugated antibody targets internal hemoglobin of the liposome and PE-conjugated antibody targets external pegylation of the liposome (A, left). Liposomes are so detected in a fluorescence based dual-plot of the cytometer as they fall into the L2 quadrant (B, right).

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Experimental

Two male and two famel pools of serum samples were spiked with liposomes of different cherges (cationic, non-ionic and anionic liposomes Coatsome EL series NOF America corporation, USA). Pools were spiked at 10, 5, 2.5 , 1, 0.5, 0.25, and 0.1 mg/mL and then analyzed by ADVIA120. In the automated method of analysis of basophils, the sample is mixed with a reagent composed by phthalic acid 20 mmol/L and hydrochloric acid 10 mmol/L as surfactant and preservative. This reagent triggers the lysis of red blood cells, platelets and white blood cells with the exception of basophils. The appearance of signals in the basophils cytogram represents the best way to detect liposomes within serum samples. The XY-axis show the expression of the high-angle scatter (related to the configuration of the nucleus) and the expression of the low-angle light scatter (related to the cell size). Analysis of samples show that liposomes signals fall mainly within the blast and noise region of the cytogram (Figure 2) but a certain degree of background also fall into the noise region. The presence of liposomes is also highlighted by flags alerts related to the blasts area.

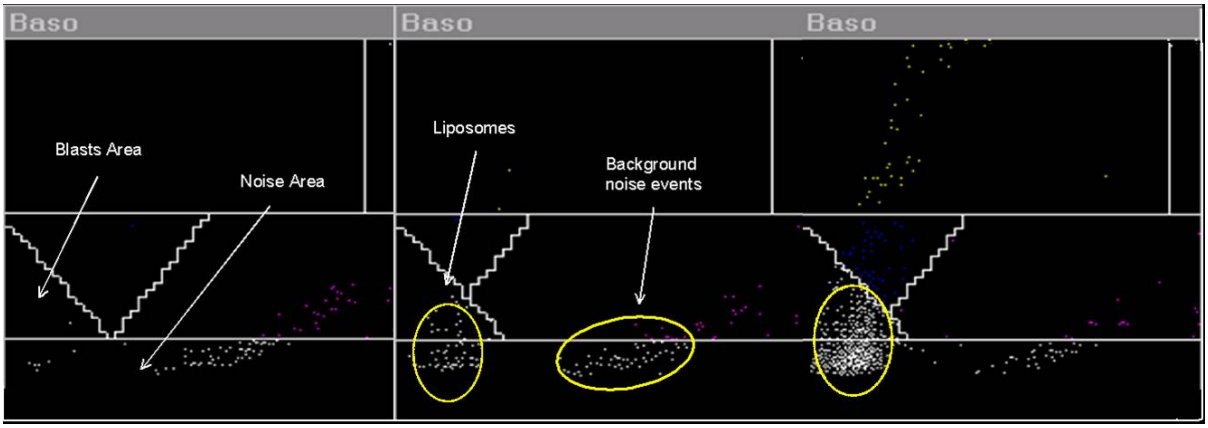

Figure 2 : Basophil cytograms obtained analyzing: a blank serum sample (left), a serum sample spiked with liposomes at 0.5 mg/mL (center) and a serum sample spiked with liposomes at 10 mg/mL (right). The presence suspect events within the blast area is alerted by ADVIA 120 using alert flags.

Results and Discussion

Figures 3 and 4 show ADVIA 120 to be able to detect liposomes (both cationic, anionic and neutral) within serum samples in different concentrations. Events falling into blasts and noise regions were counted as follow:

Blasts event count = valid_baso_cells * %blasts / 100
Noise event count = total_baso_cells – valid_baso_cells

We found a sensitivity up to 0.1 mg/mL of liposomal content counting events only within the blasts area. However the counting of events falling both within blasts and noise areas gived less sensitivity (0.25 mg/mL of liposome content). Moreover, we detected higher sensitivity for samples spiked with cationic liposomes rather than samples spiked with non-ionic or anionic liposomes. This can be due to the features of the basophil method of analysis. Cationic liposomes seems to be more resistant to the effects of the basophil reagent and yield higher event counts. This screening method for liposomes is also very rapid (less than one minute each sample), so by this approach, a reliable and rapid screening procedure for the analysis of a large scale of samples can be effectively set up.

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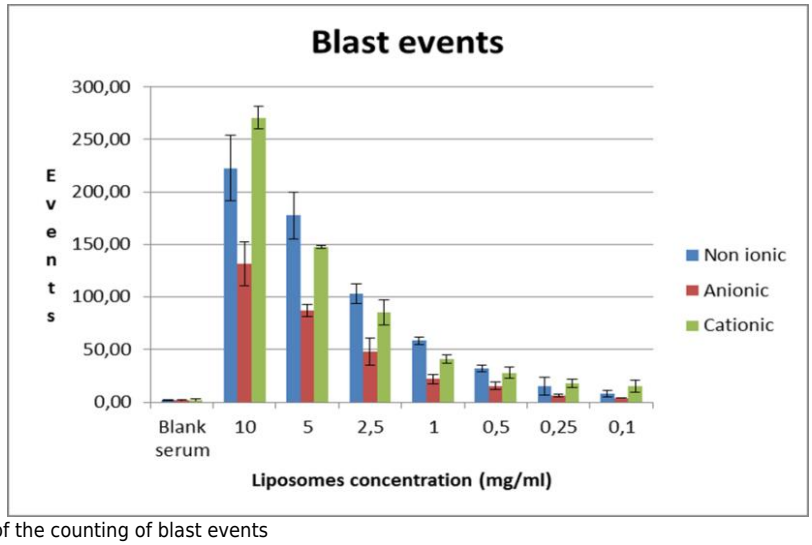

Figure 3: Histogram of the counting of blast events

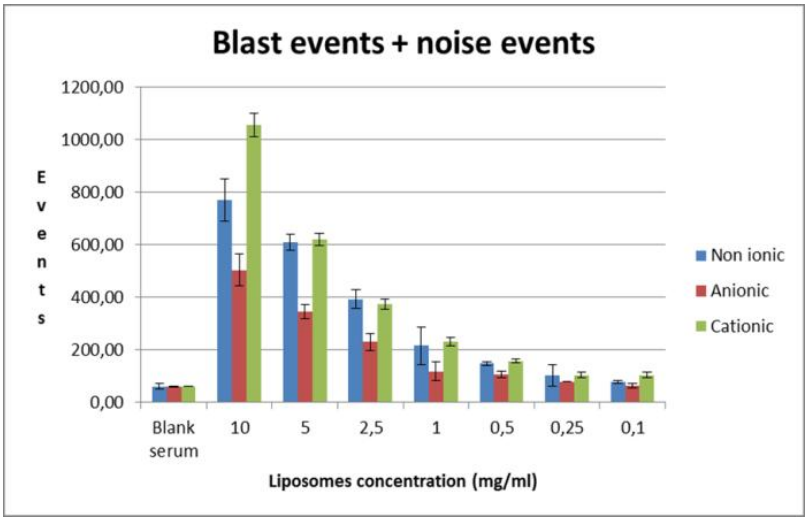

Figure 4: Histogram of the counting of blast and noise events

References

[1] Gaber BP, Yager P, Sheridan JP, Chang EL. Encapsulation of hemoglobin in phospholipid vesicles. FEBS Lett. 1983 Mar 21;153(2):285-8
[2] Donati F. Botrè F. A fast screening method for the detection of the abuse of hemoglobin-based oxygen carriers (HBOCs) in doping control. Talanta. 2010 Apr 15;81(1-2):252-4.
[3] Botrè F, Colicchia S, de la Torre X, Donati F, Esposito S, Mazzarino M. Liposomes and blood doping: from artificial hemoglobin to artificial erythrocytes? Presented at the 30th Manfred Donike Workshop on doping analysis, Cologne 2012

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