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HEMOLYSIS INFLUENCE ON THE ANALYTICAL PERFORMANCES OF THE AUTOMATONBS 300 IN THE DOSAGE OF 5 PARAMETERS

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ABSTRACT

The aim of this study was to determinehemolysis influence on the analytical performances of the automaton BS300 (Mindray®) on the dosage of biochemical parameters. The hemolysis additions methodwas run to create an increasing hemoglobin range of concentration varying from 0 to 12g/dl. To determine an influence of the hemolysis on the measure, the variation limit of 10 % waschosen. The studied parameters were creatinine, albumin, total protein, calcium and total cholesterol. Total cholesterol and total proteinwere the positively impacted parameters leading to an overestimate of the result. As for the rest, there was not any interference of the hemolysis.

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INTRODUCTION

The interference is defined as a source of bias in the concentration measure of ananalyte caused by another component or by one property of the sample (Wayne, 2005). In fact, it is the capacity of a substance contained in the sample to modify the right value of an analyte dosage result expressed in concentration or in activity (Kroll, 1994). Hemolysis consists in the rupture of the erythrocyte plasma membrane which leads to the liberation of its intercellular contents and one of which is an important quantity of hemoglobin. After the centrifugation of the whole blood sample, hemolysis is characterized by pink to redstaining of the plasma or the serum (Kroll, 1987). The staining intensity varies according to the free hemoglobin concentration (A. Damien. University of Angers, France. thesis)

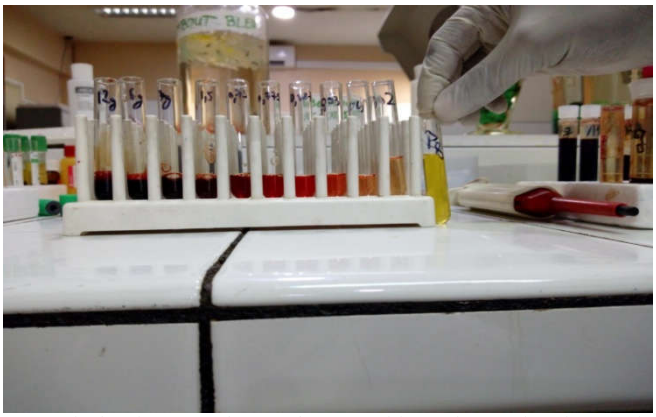
The effect of the hemolysis on the results of classical biochemical exams from biological samples has been taken in account since a long time by biologists. The aim of this study is to determine the hemolysis influence on analytical performances of the automaton BS 300 (Mindray®) on the dosage of 5 parameters, when performing with various concentrations.

MATERIALS AND METHODS

Constitution of plasma pools: Plasma pools were constituted from the remainders of patients samples collected from laboratory. The plasma was collected after analysis realization. Visibly hemolysed, icterical or lactescentsamples including one or several biological results outside the normal range were excluded. The constitution of plasma pools was realized from April 8th to April 12th 2019.

Overloading of plasma samples

Preparation of hemolysat: Plasma pools consisted of a preparation of the hemolysat from full blood pool obtained on sodium heparinate. These blood samples were centrifuged at 2 500 g during 10minutes at 25°C on a centrifuge GR4i (Jouan®). Supernatants were expelled and red blood cells collected after plasma removal and the Buffy coat were pooled. Three washes volume to volume of these collected cells were thereafter hemolysed by addition of distilled water volume to volume. A centrifugation at 4000 g enabled to remove cells debris. Therefore we got hemolysat. Then hemoglobin on the hemolysat was measured on semi-automatic spectrophotometer Secomam (BASIC®). The hemoglobin concentration within the hemolys at was adjusted at 12g/dL, then a series of 8 dilutions was realized in order to obtain a concentration range of H1 to H9 (H1=12g/dL H2=6g/dL H3=3g/dL H4=1.5g/dL H5=0.75g/dL H6=0.37g/dL H7= 0.187g/dL H8=0.093g/dL H9=0.046g/dL) (Figure 1).



Source: Biochemistry Joseph Ravoahangy Andrianavalona University Hospital

Figure 1. Hemoglobin concentration range

Plasma samples hemoglobin overloading: From the hemolysat concentration range, hemoglobin overloading was realized by mixing volume to volume the hemolysat of each point of range with plasmas for each studied parameter. And then each parameter was dosed on BS300 (Mindray®). The performing automaton of clinical biochemistry was the automatonBS300 (Mindray®) by Shenzhen Mindray Bio-medical Electronics.

It is an open system including:

- A specimen processing system and the reactive plateau
- A management system of the reaction mixture with disposal optical bowls
- A photometric measure system (UV-Visible photometry and an ISE module)

An information processing system (patients data, calibration and control data) which consists of a computer with the Chemistry Analyzer Software BS300 (Mindray®). The automaton BS300 allows the dosage of routine biochemical parameters such as substrates, electrolytes and enzymatic activities. The validity of measures was guaranteed by the passage of the calibrator and the control before the parameters dosage. The multi-calibrator was a freeze-dried human serum (Multi-calibrator CC/H Chromatest®REF1975005). It was diluted with distilled water according to the provider instructions. The control solutions were freeze-dried. They were diluted with distilled water according to the provider instructions. Only qualified technicians were able to reconstitute them. Two control levels were used, an abnormal one and a pathological one. The choice was made about the 10% threshold of the variation coefficient compared to real value defining a perturbation. The data entry and processing were performed on Excel.

Instrumentation: Parameters for which the influence of hemolysis has been studied on BS 300(Mindray®) (Table 1)

Seric indexes measure.

Table 1. Parameters for which the influence of hemolysis has been studied on BS 300®

Parameters	Principlesof dosage	Dosage methodes	Automate
Creatinine	Colorimetric method	Jaffé method	
Albuminemia	Colorimetric method	Bromocresol green reagent	BS 300®
Calcium	Colorimetric method	Complexonortho cresolphtalein (OCC)	
Total cholesterolemia	Enzymatic colorimetric method	cholesterol oxidase/peroxidase	
Total protein	Colorimetric method	Biuret reagent	

Automaton BS300 (Mindray®) was not able to identify the seric index. The correspondence between hemolysis index and hemoglobin concentration was visually determined. This correspondence was carried out by 4 qualified interns in medical biology, two of them were second year interns and another one was a third-year interns, by three laboratory technicians respectively with 10 years, 6 years and 1-year experiences. The achieved results were collected and were as follows:

The hemolysis index[++++] corresponds to a rate of hemoglobin concentration from H1 to H3.
The hemolysis index [+++] corresponds to H4
The hemolysis index [++] corresponds to H5 and H6
The hemolysis index [+] corresponds to H7, H8 and H9.
Its degrees of hemolysis were obtained by the averages of visual evaluations made by all the people involved in the research and then confirmed by a 7 years experienced biologist.

RESULTS

Each overload pool was dosed 10 times. The average of the 10 dosages was taken (Table 2). Then, hemoglobin overloading was realized for each parameter. Creatinine, albuminemia and calcium showed no significant variations. (Figure2,3,4). The total cholesterol and the rate of proteins were positively influenced by the hemolysis (Figure 5,6).

DISCUSSION

This study showed that interference of hemolysis was positive and significant on the determination of the total cholesterol from 6g/dl hemoglobin. Another study had shown a positive interference from a low concentration at 0.04g/dl

Table 2. Assay result of each parameter repeated 10 times without hemoglobin overload

	1st dosage	2nd dosage	3d dosage	4th dosage	5th dosage	6th dosage	7th dosage	8th dosage	9th dosage	10th dosage	Average
Créatinine in µmol/l	58	56	55	57	59	58	58	58	56	55	57
Albuminemia in g/l	35	36	35	36	35	35	35	36	35	35	35.3
Calcium in mmol/l	2.30	2.22	2.32	2.29	2.32	2.28	2.28	2.30	2.28	2.31	2.29
Total cholesterolemia in mmol/l	3.7	3.2	3.4	3.5	3.5	3.5	3.6	3.5	3.5	3.6	3.5
Total protein in g/l	75	76	74	72	73	75	74	75	76	75	74.5

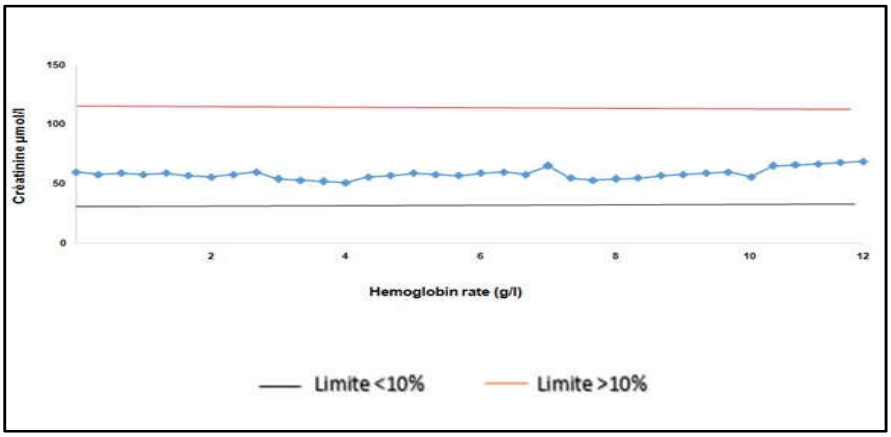


Figure 2.Creatinine dosage experimental result

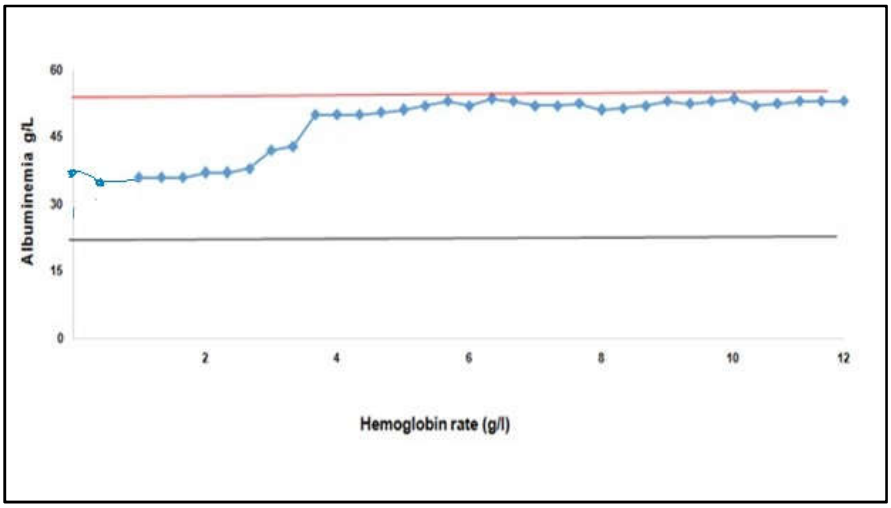


Figure 3. Albuminemia dosage experimental result

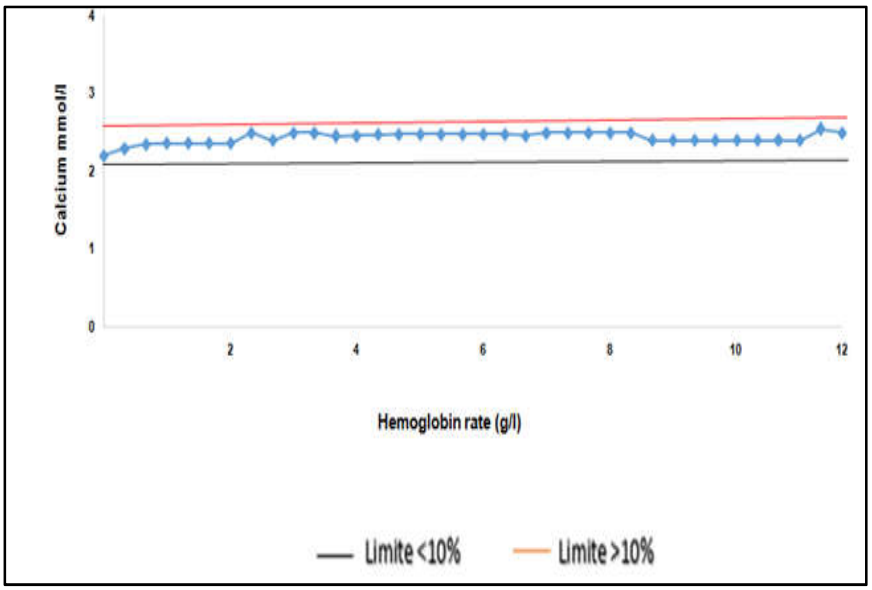


Figure 4. Calcium dosage experimental result

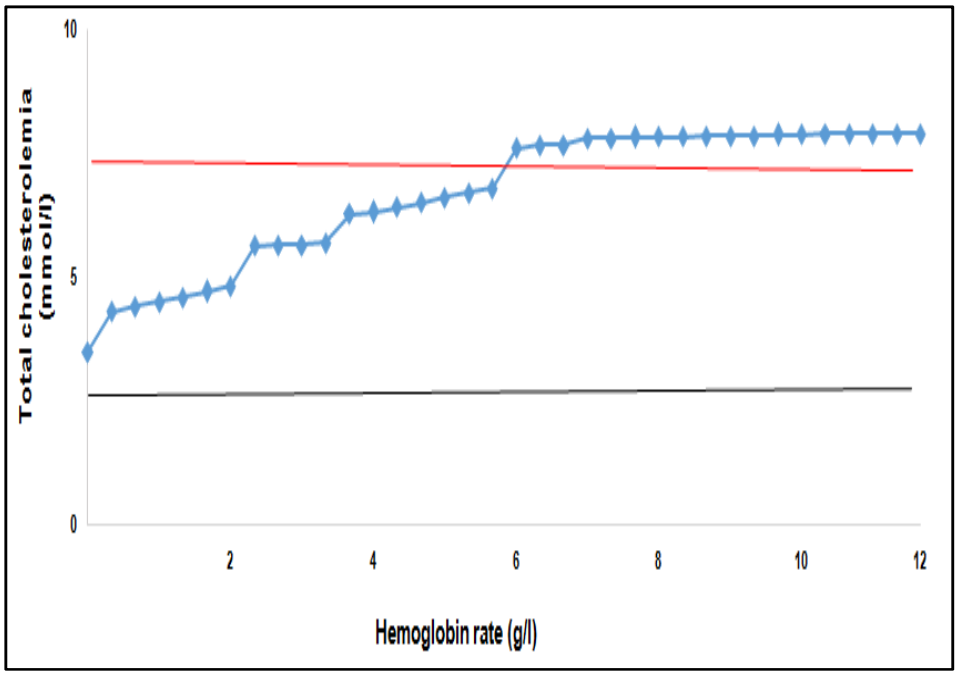


Figure 5. Total cholesterolemia dosage experimental result

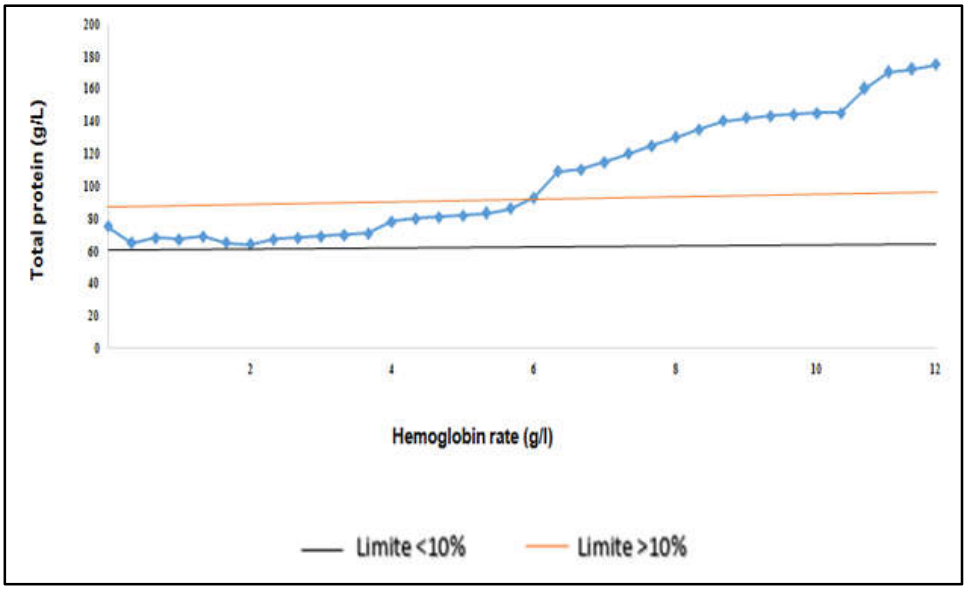


Figure 6. Total protein dosage experimental result

(Ben Mohammed, 2003). In fact the dosage of this parameter was based in the final reaction of Trinder, which was significantly influenced by hemoglobin chromogenic and peroxydasicproperties (Benchekroun, 2007). Hemolysis did not interfere in the calcium dosage, this result matched with all these authors even at high concentrationsof hemoglobin (Kroll, 1987; Benchekroun, 2007 and Damien, 2014). For total protein, this study had defined a positive interference from 6.4g/dl of hemoglobin, which is in opposition to a study where there was practically not any interference of hemolysis on the determination of the total protein with a lower rate of variation: 2% (Ben Mohammed, 2003). Other studies showed a positive interference but particularly in the case of a very intensive hemolysis (Glick, 1986). This interference was directly related to the proteic overload induced by the hemoglobin, besides it was a parameter with intra erythocytar values much higher than plasmatic values and consequently where the hemolysis impacted positively and rationally on the result (Damien, 2014).

This positive interference could be qualified as a supplyinterference (Kroll, 1987). No hemolysis interference was observed on the measure of the albumin. This result matched with other studies [6]. In accordance with the context, this dosage should be an alternative to the dosage of the total proteins, and it was widely impacted by hemolysis. For the creatininemia, none interference was observed until 12g/dl. Our results did not match with those of Benchekroun L et al. [5] which had checked a negative interference. But on the other hand, another study confirmed a lack of this interference on the dosage of creatininemia [4].

Conclusion

The study of the impact of the hemolysis on the biochemical parameters led us to dwell on the control of the preanalyticsampling. Otherwise it allowed us to extend our conditions of delivery of the results for some parameters, particularly in the case of pathological values. 2 paramaters

(total cholesterol and total protein), among the 6 studied parameters, were positively impacted by the hemolysis.

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Conflict interest: None

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