ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN
2019 International Nuclear Atlantic Conference - INAC 2019
Santos, SP, Brazil, October 21-25, 2019

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3

EVALUATION OF Cl IN BLOOD BY NAA AND XRF TECHNIQUES: AN ALTERNATIVE FOR PEDIATRIC PRACTICE

Juliana S. Melo[1], Cibele B. Zamboni[1], Maria R. Azevedo[2], Tulio Konstantyner[2]

[1]Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
juliana_melo10@yahoo.com.br
czamboni@ipen.br

[2] Universidade de Santo Amaro – UNISA
Rua Isabel Schmidt, 349
04743-030 Santo Amaro, SP, Brazil
mazevado@prof.unisa.br
tkmed@uol.com.br

ABSTRACT

The objective of this work is to analyze Cl in whole blood of newborns, concomitant with the traditional collection of "the heel prick test" (fourth drop). The dosage of Cl in whole blood samples of twenty newborns were determined by Energy Disperse X-Ray Fluorescence (EDXRF) and the Instrumental Neutron Activation Analyses (INAA) analytical techniques. Particularly, the alternative methodology based on EDXRF technology, using a portable XRF spectrometer, showed to be a fast and efficient procedure for Cl dosage in whole blood. We intend to introduce benefits to clinical practice in children, especially newborns and premature infants using this alternative procedure.

1. INTRODUCTION

The establishment of alternative methodology, focusing on the use of small amounts of whole blood (only a drop), contributes with actions to humanize health care with minimal suffering in the sense of adding another alternative for laboratory analysis in the pediatric practice [1] . The conventional clinical analysis for ions dosage usually requires the processing of the biological sample, i.e., serum-plasma separation (by centrifugation) and, at least 0.5 mL of serum or plasma for each ion analysis as well as the addition of anticoagulant and reagents [2,3]. Recently, two analytical techniques (INAA and EDXRF) were tested for K evaluation in whole blood [4]. Now, we intend to check the performance of these methodologies for Cl determination in whole blood.

Chlorine is found in the human body in the form of chloride. It predominates is in the extracellular compartment (main anion) but can move freely through the membranes, diffusing rapidly between intracellular and extracellular fluid. It combines with sodium in the extracellular fluid and with potassium in the intracellular fluid to maintain osmotic pressure and acid-base balance of the body. The chloride are absorbed in the gastrointestinal tract, filtered into the renal glomeruli and reabsorbed together with sodium in the proximal tubules and excreted mainly in the urine. In the blood it participates in the transport of oxygen and carbon dioxide. The excess of chloride in the blood deregulates sugar levels and the transport of oxygen, which can lead to dehydration, hyperventilation, and loss of renal function. Its

decrease is an indication of chronic renal failure, Addison's disease (when the adrenal or adrenal glands do not produce enough hormones), intestinal fistula and congestive heart failure. In newborns and premature infants is an efficient indicator of dehydration (for high levels) [5,6].

In this study, the use of the Energy Disperse X-Ray Fluorescence (EDXRF) and the Instrumental Neutron Activation Analyses (INAA) analytic techniques were optimized for Cl dosage in whole blood. These analytics alternatives for clinical practice allows the use of small amounts of whole blood (a drop), which can be extremely useful when the availability of the biological material is scarce. This is the case in the neonatal clinic. The dimension of this problem can be evaluated when a 3 kg newborn is considered to have between 280 mL and 300 mL of blood, while a preterm of ~ 1 kg is in the range of 180 - 200 mL. As a result, blood collections for laboratory tests (that includes ion dosage) in pediatric practice are the main causes of transfusions in infants, especially in premature babies [7,8]. The objective of this work is to analyze the whole blood of newborns, concomitant with the traditional collection of “the heel prick test” (using the fourth drop). The Cl dosage in whole blood samples of were determined using EDXRF and INAA techniques.

2. EXPERIMENTAL PROCEDURE

2.1 Sample Preparation

In this study, we analyzed whole blood samples of 20 newborns, obtained in the nursey of the General Hospital of Itapecerica da Serra. The collection was performed concomitantly with a conventional collection for "the heel prick test" without prejudice to the newborn with the approval of the Ethical Committee (CAAE: 69992117.7.0000.0081). The procedure consists in puncturing the foot sole on the heel side with a lancet and deposit a whole blood drop (50 μL) in filter paper (Whatman – nº41), and store in an appropriate receptacles, no need cooling.

2.2 Whole Blood analyses

The ED X-Ray Fluorescence analysis was performed using X-Ray Spectrometer (X-123 SDD model - Amptek®), with Silver (Ag) X-ray tube [9]. The characteristic fluorescent X-rays emitted from the samples ($Cl_{k\alpha}$ line, 2.62 keV) was measured with a Si Drift detector (25 mm^2 x 500 μm) with Be window (12.5 μm). The excitation conditions was optimized in 30 kV and 5 μA and counting time of 200 s. The spectra analysis was performed using WinQxas software program [10].

The INAA was performed using the nuclear reactor (IEA-R1, 3.5-4.5 MW, pool type), IPEN/CNEN-SP, Brazil [11]. Each sample was sealed into individual polyethylene bag, together with Standard Reference Material (SRM 1577c). Sample and Standard were irradiated for 120 s, in a pneumatic station at the nuclear reactor with a thermal neutron flux (ranging from 7.2 x 10^{11} to 8.6 x$\cdot 10^{11}$ n cm^{-2} s^{-1}). After the irradiation, the activated materials were gamma-counted for 300 s using a HPGe detector (Model GEM-6019), coupled to an MCA ORTEC (Model 919E). The gamma ray spectra analysis was performed using the ATIVAÇÃO software [12].

3. RESULTS AND DISCUSSION

The Cl concentrations determined in whole blood samples is presented in Table 1. The results were expressed by: Mean Value (MV), Standard Deviation (±1SD), Minimum (min) and Maximum (max) values and Reference Values (VR). To visualize, these results are presented in Figure 1.

Table 1. Cl concentrations results in whole blood of newborns

Cl, g L^{-1}	EDXRF	INNA
MV	1.69	1.78
± 1SD	0.30	0.36
min	0.96	1.10
max	2.32	2.52
*RV	1.09 - 2.29	1.06 - 2.50

*considering the confidence interval of 95% (adopted in clinical practice as a normal range)

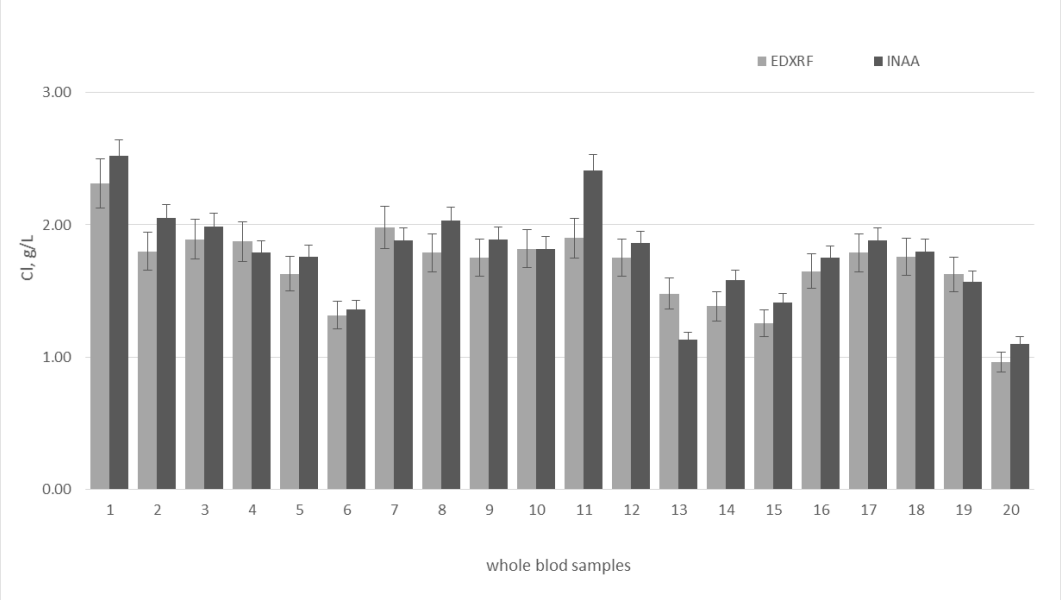

Figure 1. Cl concentration results in whole blood samples by EDXRF and INAA techniques

The Student's t-test was applied for results comparison and they are in good agreement. These procedures shown appropriate for whole blood Cl dosage of newborns, requiring a small amount of sample comparatively to the conventional tests [2,3], and short time analysis (minutes). In addition, these procedures offers a non-destructive alternative for clinical usage. Specifically, the EDXRF analyses using a portable XRF spectrometer can be an efficient alternative for clinical practice out of the nuclear reactor premises. However, more systematic and large-scale studies are needed to establish reference value for this ion in whole blood with high precision aiming its application in biochemistry tests.

4. CONCLUSIONS

These techniques showed to be appropriate offering a new contribution to the neonatal clinic. The simplicity involved in the sample collection as well as the reduced quantity contribute with actions to humanize health care, guaranteeing diagnostic accuracy with minimal suffering and exposure to risks in the pediatric practice. We intend to introduce benefits to clinical practice in children, especially newborns and premature infants using a portable XRF spectrometer as an alternative procedure.

ACKNOWLEDGMENTS

The authors thank the clinical staff at Hospital of Itapecerica da Serra (São Paulo city, Brazil) for technical assistance given during the blood collection. The CNPq (305373/17-0) and FAPESP (15/01750-9) supported this work.

REFERENCES

1. M. Carneiro-Sampaio, N. Slhessarenko. *Rev Paul Pediatr.* "Vamos reduzir o volume de sangue colhido para exames laboratoriais?", **32(2),** pp291-2 (2014).
2. W. K. Walker J *Principles and techniques of biochemistry and molecular biology,* Cambridge university press, Cambridge & United Kingdom (2010).
3. M. L. Bishop, E. P. Fody, L. E. Schoeff, *Clinical Chemistry: Principles, Techniques, and Correlations*, Wolters Kluwer Health, (2013).
4. C. B. Zamboni, G. O. Souza, A. M. Alvarenga, D. N. S. Giovanni, M. R. Azevedo, T. Konstantyner. "Detection of ions by XRF for use in the neonatal clinic," *XLI Brazilian Meeting on Nuclear Physics,* Maresias, São Sebastião, SP-Brazil, September 02 to 06, **Vol.1**, 1-4 (2018).
5. G. I. Sackheim, D. D. Lehman, "*Química e bioquímica para ciências biomédicas*". 8.ed. Manole, São Paulo & Brasil (2001).
6. W. J. Marshall, M. Lapsley, A. Day, R. Ayling *Clinical Biochemistry E-Book: Metabolic and Clinical Aspects* Elsevier Health Sciences, London & England (2014).
7. M. C. Leal, "Parto e nascimento no Brasil: um cenário em processo de mudança". *Cadernos de Saúde Pública*, **Vol. 34**, p. 1-3 (2018).
8. M. S. Carvalho, M. C. Leal, L. D. Lima, "Nascendo no Brasil". *Cadernos de Saúde Pública*, **v. 34**, p. 1-10 (2018).
9. C. B. Zamboni, M. R. Azevedo, S. Metairon, *Raios-X para dosagem de ferro em sangue*, Novas Edições Acadêmicas, Riga & Letônia (2018)
10. R. Capote, E. López, and E. Mainegra. "WinQxas Manual (Quantitative X-Ray Analysis System for Widows) Version 1.4." IAEA, Viena & Austria (2002).
11. C. B. Zamboni, *Fundamentos da Física de nêutrons,* Livraria da Física, São Paulo & Brasil (2007).
12. J. Medeiros, C. Zamboni, G. Zahn, L. Oliveira, L. Dalaqua Jr, "Software para realização de análises hematológicas utilizando processo radioanalítico", 39º Congresso Brasileiro de Patologia Clinica/Medicina Laboratorial, São Paulo & Brasil, de, 2005.