

## DETERMINATION OF REFERENCE VALUES OF ELEMENTS IN WHOLE BLOOD USING NUCLEAR METHODOLOGY

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### ABSTRACT

Reference values for some elements of clinical interest in whole blood of humans and also of animals, used in veterinary medicine for experimentation, have been determined through Neutron Activation Analysis, using Au as flux monitor. All these estimations were proposed considering 68% confidence interval for normal population. The comparison between reference values of Humans and the selected animals permits to check the similarities becoming possible to select the similar species as reference and for experiments.

### 1. INTRODUCTION

Several studies aim to elucidate the metabolism of living organisms, both in normal (healthy) and in dysfunctional (several pathologies) conditions. The elemental constituents in blood, especially metals Ca, Fe, K and Na, as well as Cl ions, even present in small amounts (< 2%) are of great importance for keeping the body's functions. Therefore, the quantitative knowledge of these elements in blood allows a clinical evaluation of the proper performance of different functions that regulate the organs. In this way, variations between excess and deficiency of these elements in blood are usually associated to some kind of ongoing pathology. On the other hand, the search for new medicines, vaccines and other products of interest in medical area, for any given disease, requires several *in vivo* tests for clinical

surveys of regulatory functions in organisms, focusing in the relations between these and the responses or reactions to their use, allowing or not their use in human beings. Usually these investigations are performed using serum, however some investigations, specially biochemistry analysis, can be performed using whole blood if the reference range (normality limits) is established [1]. Based on this, in recent years the Nuclear Structure Laboratory at IPEN has performed measurements relate to the interval reference for Cl, K and Na in whole blood in inhabitants from Brazil [2-4] as well as in several animals used for experimentation [5-7]. The success in these investigations has motivated us to continue these studies, extending this analysis to other elements, such as Ca and Fe.

For this investigation neutron activation analysis (NAA), the semi-parametric procedure [5], has been applied to determine the concentrations of elements in whole blood of human being. In addition, the results of some reference values established for animals of experimentation (Beagle, Mice, Hamster and Rabbits), some previously analyzed [6], were compared with human being whole blood estimation permitting n evaluation about the similarities.

## **2. MATERIAL AND METHODS**

The whole blood samples of Humans provided by several Brazilian Blood Banks. Ethical approval for this study was obtained from the Ethics Committee of the Blank Blood authority. The whole blood samples have been collected from healthy group (male and female blood donors), with ages varying from 25 to 60 years at 50 and 90 kg, were selected following the procedure conventionally established [8]. About 1.0ml of whole blood were collected in a vacuum plastic tubing attached to the donor's arm. Immediately after the collection an amount of 100  $\mu$ l of blood was transferred to the filter paper and dried for a few minutes using an infrared lamp. This procedure was applied for Ca, Cl, K and Na evaluation. An amount of 500  $\mu$ l of blood were transferred to a polyethylene tube (cylinder) for the determination of Fe.

For the selection of the donors the technical staff from blood banks perform screening tests in serum for transfusion transmitted diseases as Hepatitis B and C, AIDS, Sífilis and Chagas as well as for Iron deficiency, that have high prevalence in Brazilian population (60% of all Anemias) [9].

The blood samples of the animals provided by IPEN, Instituto Butantan and UNISA at São Paulo city and also from Centro de Pesquisas Aggeu Magalhães at Recife city and, they were prepared following the same procedure.

To determine the concentration of elements each sample was sealed into individual polyethylene bag, together with the flux monitor (small metallic Au foil), and irradiated in the nuclear reactor (IEA-R1, 2-4MW, pool type) at IPEN-CNEN/SP. Different irradiation times were performed for determination of Ca, Cl, Fe, K and Na. After the irradiation, the activated materials (blood and Au detector) were  $\gamma$ -counted using an HPGe detector coupled to a spectrometer and the concentrations of the elements were calculated using in-house software [10].

### 3. RESULTS AND DISCUSSION

The reference values for Ca and Fe and were evaluated for whole blood considering 68% of confidence interval for normal population. The mean values and the basic statistical treatment results are presented in Table 1.

A comparison between reference values for Cl, K and Na (actualized in the present study) involving Humans and the selected animals (Beagle, Mice, Hamster and Rabbits) are summarized in Tables 2, 3, 4 and 5.

**Table 1. Indicative interval for the reference values of the elements Ca and Fe in whole blood using NAA with Au as flux monitor.**

Elements	Mean <sup>[a]</sup>	SD	Minimum Value	Maximum Value
Ca, g l <sup>-1</sup> n = 22	0.233	0.083	0.090±0.004	0.390±0.019
Fe, g l <sup>-1</sup> n = 22	0.395	0.059	0.315±0.016	0.475± 0.021

n: number of samples analyzed in duplicate  
<sup>[a]</sup>the quoted uncertainties are the standard deviations

**Table 2. Mean values and the basic statistical treatment results for the element Chlorine in whole blood by using the NAA technique.**

Cl Concentration (g l <sup>-1</sup> )	Human n=184	Beagle n*=6	Mice n*=25	Rabbit [6] n=7	Hamster [6] n=10
Mean <sup>[a]</sup>	3.02	2.20	2.75	2.55	3.04
1SD (68%)	0.48	0.10	0.43	0.24	0.31

n: number of samples analyzed in duplicate  
n\*: number of samples analyzed in triplicate  
<sup>[a]</sup>the quoted uncertainties are the standard deviations

**Table 3. Mean values and the basic statistical treatment results for the element Potassium in whole blood by using the NAA technique.**

K Concentration (g l <sup>-1</sup> )	Human n=184	Mice n*=25	Rabbit [6] n=7	Hamster [6] n=10
Mean <sup>[a]</sup>	1.61	2.68	1.32	1.94
1SD (68%)	0.28	0.27	0.17	0.18

n: number of samples analyzed in duplicate  
n\*: number of samples analyzed in triplicate  
<sup>[a]</sup>the quoted uncertainties are the standard deviations

**Table 4. Mean values and the basic statistical treatment results for the element Sodium in whole blood by using the NAA technique.**

Na Concentration (g l <sup>-1</sup> )	Human n=184	Beagle n*=6	Mice n*=25	Rabbits [6] n=7	Hamster [6] n=10
Mean <sup>[a]</sup>	1.77	1.80	1.59	1.83	1.83
1SD (68%)	0.29	0.10	0.30	0.14	0.18

n: number of samples analyzed in duplicate  
n\*: number of samples analyzed in triplicate  
<sup>[a]</sup>the quoted uncertainties are the standard deviations

Although an indicative interval have been proposed for Ca and Fe more systematic and large scale studies are needed to establish reference values for better definition of the normal limits as well as for studying in more details reference values of common deficiencies in Brazilian population helping their diagnostics. Besides, the data obtained in this investigation will allow researchers to optimize their studies, both in terms of cost and time, selecting the species that fits to the experimental model as a clinical reference, and also the realization of biochemical analyses using whole blood. However, considering that reference values can be used for different applications, such as, medical diagnostic, for clinical practice and as limits of exposure for public protection and occupational health it is important a constant actualization of them.

## REFERENCES

1. C. B. Zamboni, L. Kovacs, M. R. Azevedo, P. S. Lins, N. F. Santos, "Avaliação bioquímica de ferro usando procedimento nuclear: Novo enfoque para o estudo de anemias," *Revista Brasileira de Hematologia e Hemoterapia*, **28**, pp. 3-3, (2006).
2. L. C. Oliveira, C. B. Zamboni, P. S. Lins, M. R. Azevedo, "The Chlorine reference value in whole blood using ANAA," *Brazilian Journal of Physics*, **35**, pp.793-796 (2005).
3. L. C. Oliveira, C. B. Zamboni, J. Mesa, " Quantitative estimation of Br, Cl, K and Na in sample blood by NAA," *Journal of Radioanalytical and Nuclear Chemistry*, **269**, pp.541-544, (2006).
4. L. C. Oliveira, C. B. Zamboni, J. Mesa, "Study of trace elements in blood from inhabitants of the Brazilian population using NAA," *Proceeding of XXVIII Reunião de Trabalho sobre Física Nuclear no Brasil*, Guarujá, São Paulo, Brasil, 07-11 setembro, (2005).
5. L. C. Oliveira, C. B. Zamboni, F. A. Genezini, A. M. G. Figueiredo, G. S. Zahn, "Use of Thermal Neutrons to Perform Clinical Analyses in Blood and Urine Samples," *Journal of Radioanalytical and Nuclear Chemistry*, **263**, pp. 783-786, (2005).
6. C. B. Zamboni, I. M. M. A. Medeiros, J. A. G. Medeiros, O. A. Sant'Anna, " Differences in trace element concentrations in whole blood of small sized animals using ANAA," *Proceeding of International Nuclear Atlantic Conference - INAC*, Santos, São Paulo, Brasil, 28 agosto a 02 setembro, (2005).
7. C. B. Zamboni, G. S. Zahn, O. A. Sant'Anna, "Trace elements at whole blood of distinct mouse lines by using NAA," *AIP*, 884, pp.507-509, (2007).
8. C. B. Zamboni, L. C. Oliveira, L. Dalaqua Jr., Diagnostic Application of Absolute Neutron Activation Analysis in Hematology, in: Proc. ANES, USA, CD DOM (2004).
9. S. S. Queiroz. Anemia ferropriva na infância. *Jornal de Pediatria* 76(2000), 298.
10. J. A. G. Medeiros, C. B. Zamboni, G. S. Zahn, L. C. Oliveira, L. Dalaqua Jr, "Software para realização de análises hematológicas utilizando processo radioanalítico," *Proceeding of 39° CBPC*, São Paulo, SP, Brasil, 19-22 outubro, CD ROM, (2005).