# Diagnostic Application of Absolute Neutron Activation Analysis in Hematology

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The Absolute Neutron Activation Analysis (ANAA) technique was used to determine element concentrations of Cl and Na in blood of healthy group (male and female blood donators), select from Blood Banks at São Paulo city, to provide information which can help in diagnosis of patients. This study permitted to perform a discussion about the advantages and limitations of using this nuclear methodology in hematological examinations .

**Key Word:** clinical analysis, blood, serum, ANAA, gamma spectrometry.

## *Introduction*

The essential function of the hematology laboratory is to provide information which can help in diagnosis and clinical management of patients. This way, it is usual to perform several clinical examination, mainly on serum, some of them involving the elements concentrations to identify anomalies in human being body organs [1]. However, to perform these conventional analysis the usual procedure demands time and it is expensive [2] because different apparatus and reactants must be used in function of clinical examination to be performed as presented in Table 1.

In this paper we want to check the viability of using the ANAA technique to perform hematological analysis in human being using a simplified procedure .

Table 1. Some Clinical examinations to investigate the biological functions of human being organs.

| Conventional Analysis/ Technique [1-2] | Biological material/ Quantities (ml) | Execution time Days | (*) Cost US$ |
|---|---|---|---|
| Aluminum/ Atomic Absorption Spectrophotometry | Serum / 2.0 | 10 | 21.00 |
| Calcemia (Ca)/ Colorimetry | Serum / 0.5 | 1-2 | 15.00 |
| Cloremia (Cl)/ Titrymetry | Serum / 0.5 | 1-2 | 17.00 |
| Iron/ De Lauber | Serum / 1.0 | 1-2 | 16.00 |
| Magnesemia (Mg)/ Dry Chemistry | Serum / 0.5 | 1-2 | 16.00 |
| Manganese/ Atomic Absorption Spectrophotometry | Serum / 2.0 | 15 | 145.00 |
| Calemia (K)/ Ion Specific Electrode | Plasma / 1.0 | 1-2 | 15.00 |
| Natremia (Na)/ Ion Specific Electrode | Plasma / 3.0 | 1-2 | 14.00 |

(*) several laboratories have been investigated and these values are the medium cost (on May, 2004).

In the last years we have applied this nuclear methodology with success to investigate elements concentration in whole blood, urine, bone and also in several body organs of small and medium guinea-pigs resulting in an agile and economic way to perform clinical investigations in veterinary medicine [3-9]. Now, we intend to use this nuclear technique to perform hematological analysis in human being. But, for the development of this investigation the Chlorine concentration in serum must be first performed for comparing the nuclear results with the reference value established from the conventional methods [1] as well as to verify the accuracy and precision of the results. After that, the ANAA will be

applied to analyze Sodium in whole blood aiming to obtain an indicative interval for its reference value.

By definition, reference values reflect the findings in a select group of individuals [10]. In fact, it is expected that they are a reflection of natural concentrations influenced mainly by age, sex, lifestyle factors (as such smoking and drinking habits), medicine intake as well as the environmental exposure. Thus, reference values for trace elements in human clinical specimens can facilitate the interpretation of data deriving from clinical practice **.** This study is party of a big project : Determination of reference values for concentrations of trace elements in human whole blood using nuclear methodology, nowadays in development at IPEN (Instituto de Pesquisas Energéticas e Nucleares) in collaboration with Blood Banks and Hematological Laboratories from different regions of Brazil. The necessity to perform measurements in whole blood is related to the fact that most of conventional clinical analyses are performed using serum or plasma (see Table 1), so there are no reference value established in whole blood.

To perform the Sodium concentration determination in whole blood a select healthy group (male and female blood donators), age between 25 and 60 years at 50 and 85 kg were select from blood banks.

***Nuclear Analysis***

The basic principle of the ANAA technique is the irradiation of the biological material with neutrons followed by the measurement of the γ-ray activities induced in the biological sample, where the elements can be identified by the nuclear properties of γ-rays. To determine the concentration of the elements in whole blood or in serum each biological sample is sealed into individual polyethylene bag, together with the Au detector (small metallic foil ~1mg) used for measurement of the flux distribution [11], and irradiated in a pneumatic station in the nuclear reactor (IEA-R1, 2MW, pool type) at IPEN, allowing the simultaneous activation of these materials. Using this procedure the γ-ray activity induced in the Au detector as well as in the biological sample are obtained under the exact same irradiation conditions. After the irradiation , the activated materials (blood or serum and Au) are gamma-counted using a HPGe Spectrometer of High Energy Resolution [7] and the

areas of the peaks, corresponding to gamma transitions related to the nuclides of interest, are evaluated. The gamma spectra analysis evaluation is performed using the IDF computer software [12] and the calculation of the concentration for each element can be obtained from software developed by Medeiros [13]

***Collection and preparation of the biological samples***

In this study the biological samples came from Paulista Blood Bank at São Paulo city . The samples were collected following a procedure developed for the nuclear performance described as follows . About 2ml of whole blood was collected in a vacuum plastic tubing attached to the donator's arm and immediately after the collection exactly 100ul of blood was transferred to the filter paper. To perform this a calibrated pipette was used to draw up the correct amount of blood in it. After that, the sample was dried for few minutes using an infrared lamp. The biological material still in the plastic tubing was then centrifuged and the serum obtained was also transferred to the filter paper following the same procedure just described. Although anticoagulants, such as Sodium and Potassium salts EDTA or Trisodium Citrate are very suitable for routine hematological work, particularly to perform the nuclear analysis they cannot be used because, if they are added, they can interfere in the results mainly for Sodium concentration determination.

***Results***

For this investigation 26 samples of serum and whole blood were measured. The irradiation time of 2 minutes; counting time of 1 minutes for the Gold activation detector and 10 minutes for the biological sample and background radiation (BG) allowed us to conclude the analysis of each sample (whole blood or serum) in about half hour, making this nuclear procedure fast. The element concentrations of Cl and Na are shown in Table 2 and the gamma ray spectrum of whole blood in Figures 1 and 2.

According to these figures some other elements are also activated but with poor counting statistics so we decide to increase the irradiation time. The element concentrations of some whole blood samples irradiated until 10 minutes are shown in Table 3 and although few samples have been analyzed the results suggesting that these elements can also be

determined in this irradiation condition. As Fe was not activated until 10 minutes of irradiation time and its quantitative analysis is very important in hematology analyses we decide to check the irradiation time for its activation. According to our measurements it was activated only in a long irradiation time (~2hours). However in this irradiation condition the elements Al ($T_{1/2}$ = 2.2 min), Br ($T_{1/2}$= 16 min), Ca ($T_{1/2}$= 8.7 min), Cl ($T_{1/2}$=37 min) and Mg ($T_{1/2}$= 9.5 min), cannot be obtained because there are no activity related to them when the samples can be handled due their short half-life ($T_{1/2}$) [14].

Table 2. The Concentration of Cl and Na in blood by using the ANNA technique.

| Sample Code | Neutron flux x $10^{11}$ n $s^{-1}$ $cm^{-2}$ | Cl (μg/μl) serum<br>[a] 3.44 – 3.76<br>[b] 3.41 – 3.69 | Na (μg/μl) whole blood<br>[b] 1.51 – 1.67 |
|---|---|---|---|
| D1 | 1.16 | 3.36 ± 0.11 | 1.69 ± 0,05 |
| D2 | 6.51 | 3.52 ± 0.13 | 1.71 ± 0.09 |
| [ns]D3 | 1.27 | 2.99 ± 0.14 | 1.50 ± 0.08 |
| D4 | 6.08 | 3.77 ± 0.11 | 1.57 ± 0.09 |
| D5 | 4.85 | 3.42 ± 0.12 | 1.69 ± 0.09 |
| D6 | 4.99 | 3.96 ± 0.15 | 1.70 ± 0.09 |
| D7 | 1.38 | 3.37 ± 0.14 | 1.48 ± 0.08 |
| [ns]D8 | 1.49 | 2.99 ± 0.18 | 1.25 ± 0.07 |
| D9 | 1.35 | 3.26± 0.19 | 1.41 ± 0.08 |
| D10 | 4.90 | 3.32 ± 0.20 | 1.60 ± 0.08 |
| D11 | 3.34 | 3.76± 0.13 | 1.74 ± 0.06 |
| D12 | 3.34 | 3.77 ± 0.11 | 1.69 ± 0.06 |
| [ns]D13 | 1.17 | 2.89 ± 0.14 | 1.74 ± 0.05 |
| [ns]D14 | 1.21 | 2.99 ± 0.17 | 1.98 ± 0.07 |
| D15 | 1.18 | 3.44 ± 0.17 | 1.52 ± 0.06 |
| D16 | 5.99 | 3.76 ± 0.11 | 1.50± 0.05 |
| D17 | 6.11 | 3.65 ± 0.13 | 1.45± 0.08 |
| D18 | 4.80 | 3.32 ± 0.11 | 1.93 ± 0.09 |
| D19 | 3.15 | 3.44± 0.12 | 1.55 ± 0.08 |
| D20 | 4.23 | 3.76 ± 0.14 | 1.43 ±0.07 |
| D21 | 4.96 | 3.45 ± 0.12 | 1.41 ± 0.08 |
| D22 | 3.83 | 3.71 ± 0.13 | 1.51 ± 0.08 |
| D23 | 4.29 | 3.53 ± 0.13 | 1.65 ±0.07 |
| D24 | 4.24 | 3.49 ± 0.13 | 1.60 ± 0.08 |
| D25 | 6.02 | 3.52 ± 0.13 | 1.46 ±0.07 |
| D26 | 3.56 | 3.61 ± 0.13 | 1.57 ± 0.08 |

[a] reference value from [1]
[b] indicative interval proposed in this work
[ns] samples not select

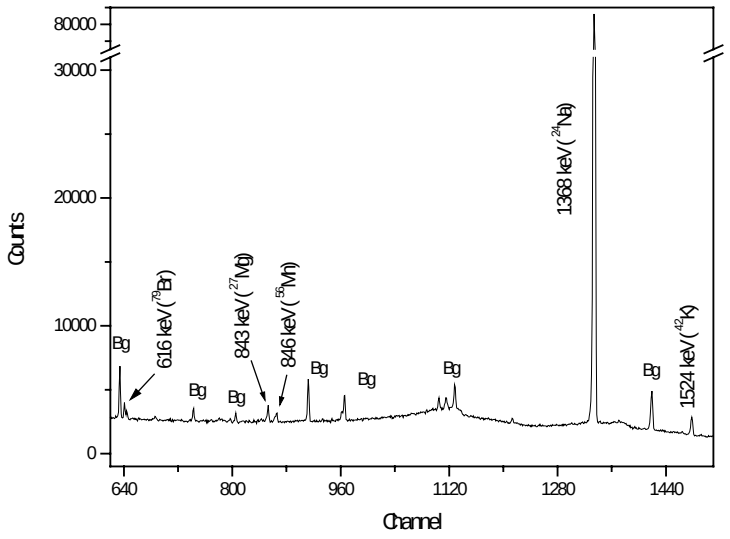



*Figure 1.*Partial γ - ray spectrum of blood sample, taken at two minutes of irradiation time. Values in peak are energies (in keV) and Bg indicates peaks occurring in natural background.

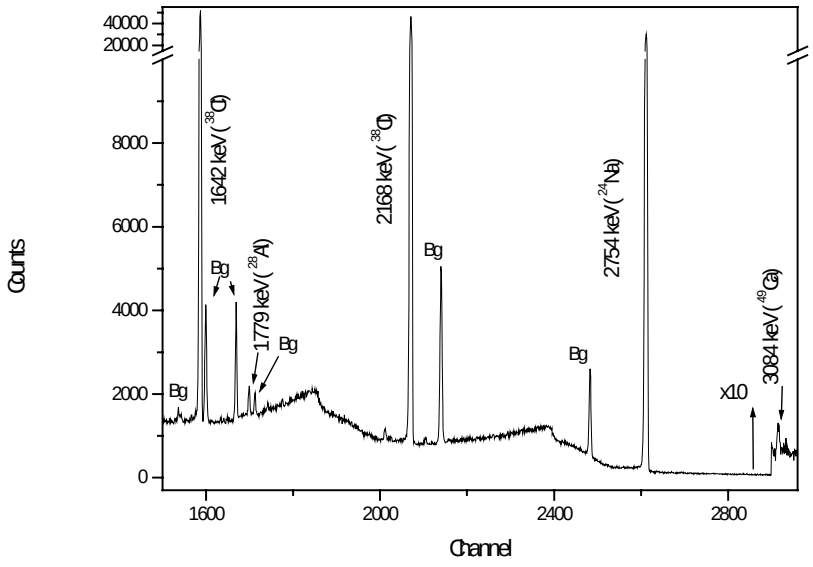


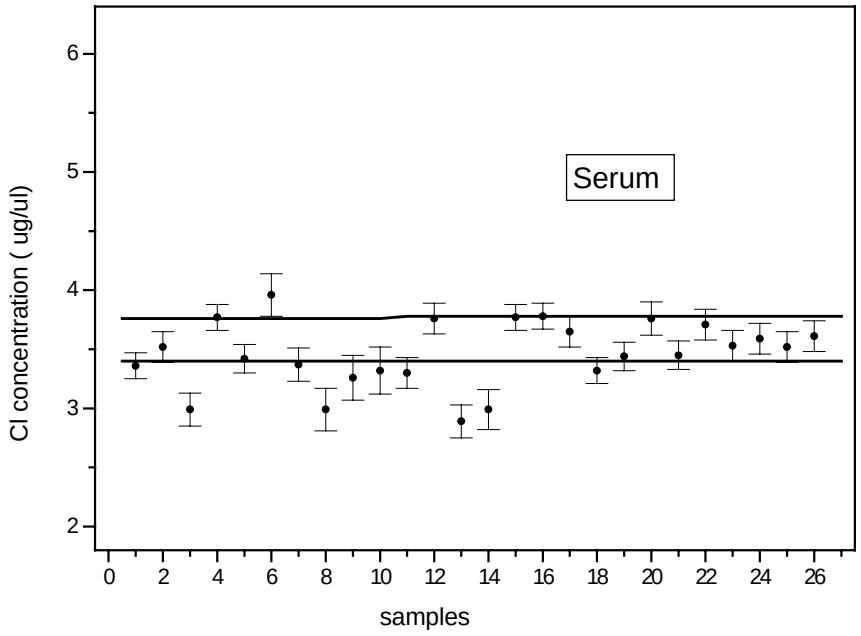

*Figure 2.* Partial γ - ray spectrum of blood sample, taken at two minutes time irradiation. Values in peak are energies (in keV) and Bg indicates peaks occurring in natural background.

All the results presented in Table 2 and 3 were obtained by analyzing replicate samples and the indicative interval for the reference values by using the median value considering one SD. Although the concentration of D3, D8, D13 and D14 samples have been determined these results were not considered in our calculations because according to the selection performed by the blood bank these donators are not completely healthy. For a illustrative visualization in the figure 3 the Chlorine concentration's results in serum is shown where the reference value [1] was included for comparison. According to this figure we can notice that the concentration's results of the samples not selected (D3, D8, D13 and D14) do not belong to the reference value showing the sensibility of the method.

Table 3. The Concentration of Al, Br, Ca, Cl, K, Mg and Mn on whole blood by samples using the ANNA technique.

| SC<br>Ti (min) | Al | Br<br>ng/µl | Ca<br>µg/µl | Cl<br>µg/µl | K<br>µg/µl | Mg<br>µl /µg | Mn<br>ng/µl |
|---|---|---|---|---|---|---|---|
| D1(3) | no | 6.5 ± 0.8 | nd | 2.87 ± 0.18 | 1.69 ± 0.14 | no | nd |
| D15(3) | no | 8.9 ± 3.1 | nd | 2.82 ± 0.17 | 1.64 ± 0.16 | no | nd |
| D16(4) | nd | 9.1 ± 2.8 | 0.22 ± 0.07 | 3.25 ± 0.20 | 1.71 ± 0.19 | no | nd |
| D17(5) | 0.022 ± 0.004 | 4.5 ±0.9 | 0.17 ± 0.05 | 2.74 ± 0.17 | 1.66± 0.10 | no | nd |
| D5(5) | 0.026 ± 0.004 | 6.4 ±0.9 | 0.16 ± 0.05 | 3.04 ± 0.18 | 1.64 ± 0.18 | no | nd |
| D20(6) | 0.021 ± 0.003 | 9.0 ± 1.0 | 0.25 ± 0.07 | 2.90 ± 0.18 | 1.46 ± 0.22 | nd | nd |
| D6(6) | 0.019 ± 0.002 | 7.1 ± 0.8 | 0.16 ± 0.04 | 2.94 ± 0.18 | 1.89 ± 0.18 | nd | nd |
| *D8(10) | 0.013 ± 0.003 | 3.5 ± 0.3 | 0.08 ± 0.02 | 2.32 ± 0.14 | 1.05 ± 0.09 | 0.06 ± 0.01 | 0.09 ± 0.01 |
| D21(10) | 0.020 ± 0.004 | 9.1 ± 0.3 | 0.18 ± 0.05 | 2.68 ± 0.10 | 1.52 ± 0.12 | 0.05± 0.01 | 0.45 ± 0.03 |
| D23(10) | 0.020 ± 0.004 | 8.9 ± 0.3 | 0.17 ± 0.04 | 2.56 ± 0.17 | 1.59 ± 0.12 | 0.04± 0.01 | 0.43 ± 0.03 |
| D10(10) | 0.018 ± 0.002 | 5.5 ± 0.4 | 0.20 ± 0.05 | 2.73 ± 0.17 | 1.48 ± 0.12 | 0.06 ± 0.01 | 0.30 ± 0.03 |

SC: Sample Code
Ti: Irradiation time
nd : observed but not determined (large uncertainly)
no: not observed
* sample not select




*Figures 3* . The concentration Chlorine results in serum samples.

***Discussions***

The Cl concentration was measured in serum and a good agreement was obtained when the indicative interval from the nuclear analysis (3.41 – 3.69μg/μl) is comparing with the reference value adopted (3.44 – 3.76 μg/μl) [1] suggesting this technique can be used as a sensitive and alternative method to perform some clinical blood examinations. Of course. more systematic [15] and large scale studies are needed to establish reference values using ANNA aiming its application, in the future, for studying in more details reference values of common deficiencies in Brazilian population helping their diagnostics as well as to transfer technology.

The time optimization established in this experiment to perform the whole blood analysis (irradiation time of 10 minutes; counting time of 1 minutes for Gold detector and 10 minutes for the biological sample and the background radiation) allowed us to conclude the analysis of several elements (Al, Br, Ca, Cl, K, Mg, Mn and Na) in each sample in about three hours or less making this nuclear procedure agile and fast .

Considering the short time irradiation and the use of small amounts of biological material (100µl) this absolute method induces low activity, reducing the radiation exposure during the handling process of the active material and allowing its storage for future reexamination without the need for any specific shielding after a few days .

Another important advantage is related to the low cost when this nuclear methodology is compared with the conventional analyses, because it is not necessary to use specifics reactants and different apparatus [2] and also when compared with another nuclear technique, the Instrumental Neutron Activation Analysis (INAA) [16], because the absolute method does not require standards which are imported and expensive. Still comparing these two nuclear techniques the comparative method (INAA) demand much more time, mainly when elements of short half-life are involved due to the necessity to analyze the standards and the sample separately and as some of them can decay before being gamma counted, several irradiation usually must be done.

Considering the advantages appointed it is possible to perform several clinical analysis simultaneously in whole blood in an agile, fast and economic way although Iron could not be obtained in a short irradiation time but, the main limitation of this method is, though, that it is necessary to have access to a nuclear reactor or other types of neutron sources to perform the activation in the samples. However a small size prototype of neutron irradiator is being developed [17], so we hope that in the future it can be used outside the reactor premises to perform these analyses.

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