

## DETERMINATION OF REFERENCE VALUES OF ELEMENTS IN KIDNEY OF THE WISTAR RATS USING NAA

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### ABSTRACT

The aim of the present study is to establish Reference Value for elements in kidney of Wistar rats using the Neutron Activation Analysis technique. The quantitative knowledge of these elements in this biological material allows 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, as well as the realization of biochemical investigation in kidney. In this study, the reference values (mg/kg) for kidney were determined for: Cr (0.0276 – 0.0384), Fe (110.23 - 212.87), Rb (2.8 - 6.8) and Zn (78.40 - 84.56).

### 1. INTRODUCTION

The search for new medicine and other products of interest in health area requires several *in vivo* tests using guinea-pig on experiments for clinical surveys of regulatory functions in organism, focusing in the relations between these and the responses or reactions to their use, allowing or not their use in human being. The Wistar rat is one of the most used worldwide in laboratory research.

Most mice strains of laboratory descended from a colony established at the Wistar Institute (Philadelphia) [1]. The Wistar rats are an albino strain of the species *Rattus norvegicus* and it is considered a general-purpose model. The breeding period is about 50-60 days, weight 250-300g (male) and 150-180 g (female) [2, 3]. The price may vary according to age: US\$13 (up to 23 days) until US\$50 (adult) [4]. The quantitative knowledge of elemental composition in body fluids and organs allows a clinical evaluation of the proper performance of the different functions that regulate the vital organs as well as metabolic and physiologic processes.

In the last years, the Nuclear Structure Laboratory (LEN/IPEN) has performed measurements related to the interval reference for elements of clinical interest of various biological materials (body fluids, bone and organs) in several animal models used for experimentation, such as, some mice strains, rabbits and Gold hamster [5-9]. These measurements were performed using Neutron Activation Analysis technique, which represents advantages especially when the availability of biological material is scarce [10]. Recently we investigated several elements of clinical relevance (Br, Ca, Cl, I, K, Na and S) in blood, urine, serum and kidney of the Wistar rats [11] and now we intend to extend this investigation for other elements in kidney. In this study Cr was investigated for evaluation of intoxications [12,

13] while Fe and Zn due their nutritional relevance [14-16]. Furthermore, considering that Fe acts in the transport and storage of oxygen, in the biosynthesis of several organic molecules, in the functioning of the cardiovascular system and cerebral function [17], among other biological functions, its evaluation can be extremely useful in animal experimentation, mainly in tests of new medicines, since it is directly affected by medicines intake. The concentration of Rb was also investigated; this element seems to be an electrolyte essential for the proper functioning of the cellular exchanges [18-20] but all their functions are not well known in the body.

## 2. MATERIAL AND METHODS

The biological samples were collected from adults rats Wistar (males) created in the bioterio of the UNIFESP (Federal University from São Paulo, Brasil). The clinical procedure for the removal of organs was performed in the Laboratório de Nefrologia (UNIFESP, SP, Brasil) and the kidney's samples were removed after the sacrifice. For irradiation each biological sample was weighed and sealed into a polyethylene capsule. Bovine Liver Powder (SRM 1557b and 1577c) was used as standards and for analytical quality control.

To determine the element concentrations each biological sample and standard were irradiated for 6hs under a thermal neutron flux of  $3 \cdot 10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  in the nuclear reactor IEA-R1 (4.5 MW, pool type) at IPEN. After a decay time of a week, a gamma counting of 4hs was used to determine  $^{51}\text{Cr}$  ( $T_{1/2} \sim 28\text{d}$ ,  $E_{\gamma} = 320\text{keV}$ ),  $^{58}\text{Fe}$  ( $T_{1/2} \sim 44\text{d}$ ,  $E_{\gamma} = 1099\text{keV}$ ),  $^{86}\text{Rb}$  ( $T_{1/2} \sim 18\text{d}$ ,  $E_{\gamma} = 1076\text{keV}$ ) and  $^{65}\text{Zn}$  ( $T_{1/2} \sim 243\text{d}$ ,  $E_{\gamma} = 1115\text{keV}$ ). The measurements were performed using an ORTEC Model GE1M-60195 and ORTEC 671 amplifier coupled to a MCA ORTEC Model 919E. The gamma spectra analysis was performed using the IDEFIX [21] computer software e the data was analyzed using in-house software [22].

## 3. RESULTS AND DISCUSSION

In Table 1 the certified values are compared with ours results indicating that they are satisfactory ( $|Z \text{ score}| < 2$ ). In Tables 2, 3, 4 and 5 the element concentrations for Cr, Fe, Rb and Zn in kidney of Wistar rats are presented as: the mean value, standard deviation ( $\pm 1\text{SD}$ ), minimum and maximum values, median and range for a confidence interval of 95% (adopted for clinical practice). Figure 1 presents all the concentration results.

**Table 1. Element Concentrations by NAA compared with certified values**

<b>Element</b>	<b>Present Study</b>	<b>Certified Values</b>	<b>  Z score  </b>
Cr, $\mu\text{g}\cdot\text{g}^{-1}$	$48 \pm 16$	$53 \pm 14^{\text{b}}$	0.36
Fe, $\text{mg}\cdot\text{kg}^{-1}$	$191 \pm 16$	$184 \pm 15^{\text{a}}$	0.47
Rb, $\mu\text{g}\cdot\text{g}^{-1}$	$13.5 \pm 1.2$	$13.7 \pm 1.1^{\text{a}}$	0.18
Zn, $\text{mg}\cdot\text{kg}^{-1}$	$182.3 \pm 1.2$	$181.1 \pm 1.0^{\text{b}}$	1.20

<sup>a</sup> NIST 1577b<sup>b</sup> NIST 1577c**Table 2. The Cr concentration in kidney of Wistar Rats**

<b>Element concentration</b>	<b>Cr (mgkg<sup>-1</sup>)</b>
Mean value	0.0312
$\pm 1\text{SD}$	0.0036
Minimum Value	0.0264
Maximum Value	0.0370
Median	0.0305
Range (95%)	0.0276 – 0.0384

**Table 3. The Fe concentration in kidney of Wistar Rats**

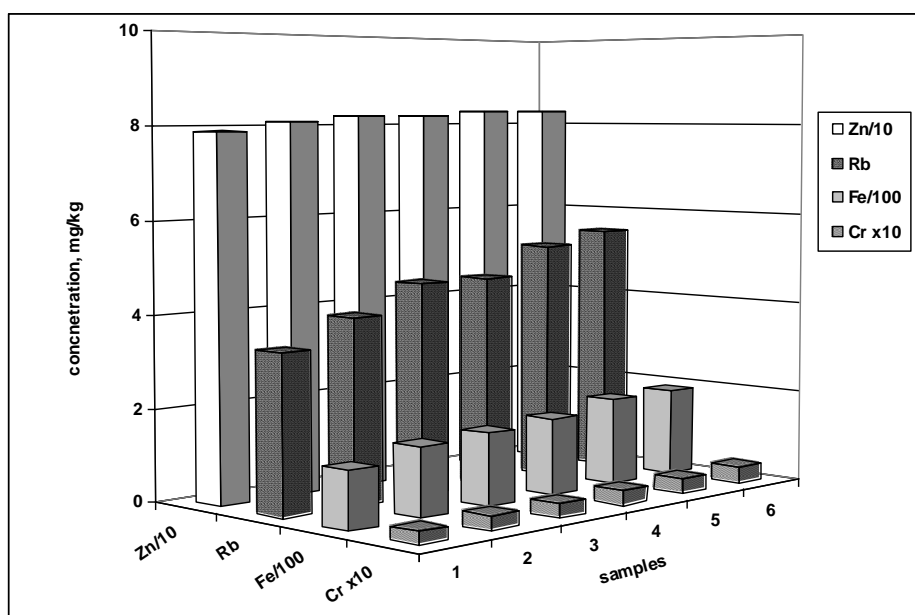
<b>Element concentration</b>	<b>Fe (mgkg<sup>-1</sup>)</b>
Mean value	161.55
±1SD	25.66
Minimum Value	122.50
Maximum Value	190.73
Median	160.07
Range (95%)	110.23 - 212.87

**Table 4. The Rb concentration in kidney of Wistar Rats**

<b>Element concentration</b>	<b>Rb (mgkg<sup>-1</sup>)</b>
Mean value	4.8
±1SD	1.0
Minimum Value	3.4
Maximum Value	5.9
Median	4.9
Range (95%)	2.8 - 6.8

**Table 5. The Zn concentration in kidney of Wistar Rats**

Element concentration	Zn (mgkg <sup>-1</sup> )
Mean value	81.48
±1SD	1.54
Minimum Value	79.01
Maximum Value	83.30
Median	81.67
Range (95%)	78.40 - 84.56



**Figure 1. The Cr, Fe, Rb and Zn concentrations in Kidney**

The determination of elements content in the kidney of the Wistar rats is important to many biological functions (cardiovascular, cerebral, nutrition and other), moreover these results can also be extremely useful, mainly in tests of new medicines, since the kidney is directly affected by medicines intake.

## 4. CONCLUSIONS

The determination of Cr, Fe, Rb and Zn concentration in kidney of the Wistar rats was evaluated by NAA technique for the proposition of an indicative interval. This technique (NAA) is an efficient alternative to perform clinical investigation when the biological sample is scarce.

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