Differences in iron concentration in whole blood of animal models using NAA

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Differences in iron concentration in whole blood of animal models using NAA

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Abstract. In this study Neutron Activation Analysis technique (NAA) was applied to determine Fe concentration in whole blood samples of several animal models such as: mice (*Mus musculus*)**,** Golden Hamster (*Mesocricetus auratus*), Wistar rats, Albinic Rabbits of New Zealand, Golden Retriever dogs and Crioula breed horses. These results were compared with human whole blood estimation to check their similarities.

Introduction

In the health field several animal models are used for medical investigations, mainly those that involve testing of new medicines, vaccines, antibiotics, organ transplantation as well as medical diagnostic studies. While small-sized animal models such as: mice, hamsters, rats and rabbits are very convenient to perform medical investigation due to their low cost, easy handling and medico legal implications, medium and large sized animals are selected for medical diagnostic studies. The animal of choice is mainly Golden Retriever dogs, because of their physiological similarities with humans while horses (Crioula breed) are used for antivenom production in Brazil. In this study Neutron Activation Analysis technique (NAA) was applied for the determination of iron levels in the whole blood of these animal models. Iron plays important functions in blood; it acts as an indicator of a variety of anomalies and it is fundamental for the cardiac functioning and immune system [1,2]. An important aspect to be considered during these investigations (related to iron toxicity) is the need to check for similarities between the animal's and human's blood. For the development of this investigation, IAEA-A13 (Blood Animal) certified reference material was first analyzed for analytical quality control. After that, the analyses of whole blood samples of the selected animals were performed.

Experimental Procedure

The samples came from research centers in São Paulo city (Butantan and Bioscience Institute). The whole blood samples were collected from mice (*Mus musculus*)**,** Golden Hamster (*Mesocricetus auratus*), Wistar rats, Albinic Rabbits of New Zealand, Golden Retriever dogs and Crioula breed horses. About 1-2 mL of whole blood was collected from each animal model. Aliquots of 300 μL were collect from mice**,** hamsters and rats and 500μL from rabbits, dogs and horses, in duplicate when biological material was available. Each aliquot of blood was then transferred to a plastic cylinder and

irradiated together with the standard solution in the nuclear reactor IEA-R1 (4.0 - 4.5 MW, pool type) at IPEN (SP, Brazil). Samples of 300 μL were irradiated for 8 hours and samples of 500μL for 4 hours. Samples were gamma counted for 3 to 6 hours after a decay time of several days. A γ - spectrometer system with a semiconductor detector (HPGe) connected to an ADCAM multichannel analyzer were used to measure the induced gamma-ray activity. The Fe concentrations were obtained by using in- house software.

Results and Discussion

The results for the reference material are presented in table 1. The obtained Z-score values [3] indicated that the present results are satisfactory considering the range of certified data at the 95% ($|Z| \leq 2$) confidence level.

The Fe concentration results are summarized in table 2. To show in more detail the results in whole blood, figures 1 through 6 show all models investigated. For checking the similarities the mean values were compared to the human being estimation [4]. This comparison is shown in figure 7, where the indicative interval related to human being (in a confidence interval of 68% and 95%) was also included. The significance of differences between animal models and humans were assessed by Student's t-test. The level of statistical significance was taken as value of $p < 0.05$.

Table 1. Element concentrations obtained in the analysis of IAEA-A13 certified reference material

Element	Mean ±1 SD	Z-score
Ca, $mgkg^{-1}$	291 ± 29 286 ± 54^{CV}	0.09
Fe, $mg\ kg^{-1}$	2469 ± 138 2400 ± 144^{CV}	0.48
Mg, $mg\ kg^{-1}$	102 ± 33 99 ± 29^{CV}	0.11
K, $g\ kg^{-1}$	2.54 ± 0.24 2.50 ± 0.35^{CV}	0.10
Na, $g\ kg^{-1}$	12.90 ± 0.57 12.60 ± 1.01^{CV}	0.30
Zn, $mg\ kg^{-1}$	13.72 ± 1.21 13.00 ± 1.04^{CV}	0.21

^{cv}: certified values

Table 2. The Concentration of Fe (mgL^{-1}) in whole blood samples of animal models and Humans.

Animal model *(n)*	Mean	±1 SD	Minimum Value	Maximum Value	Range (95%)

Mice *(12)*	187	13	163	204	161 - 213
Rats *(15)*	393	42	297	492	309 - 477
Hamsters *(12)*	209	40	149	265	129 - 289
Rabbits *(14)*	363	29	163	380	305-421 301-530 [b]
Dogs *(9)*	585	17	441	605	551 - 619
Horses *(17)*	498	18	399	608	462 - 534 158-190 [c]
Humans [a]	385	28	199	474	329 – 441

[n] number of samples
[a] ref [4]
[b] ref [5]
[c] ref [6]

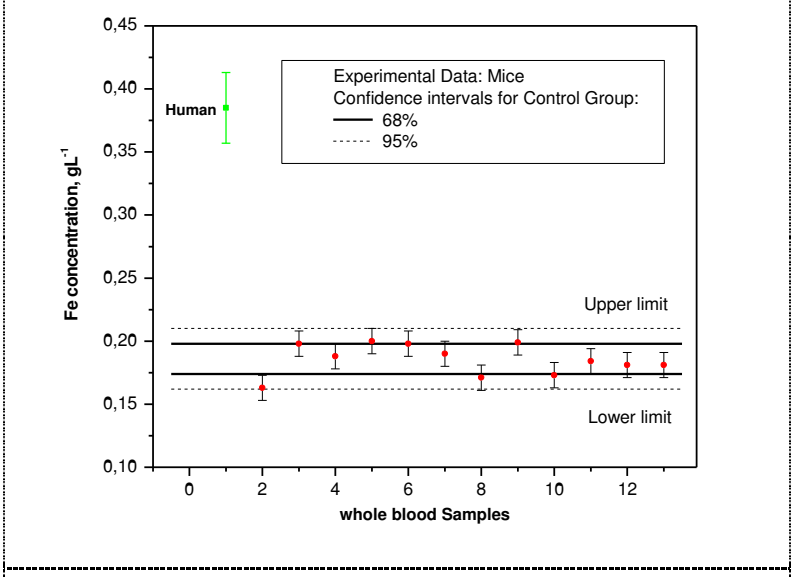

Figure 1. Fe concentration in whole blood samples of the Mice (*Mus musculus*). The human being estimation [4] was included for comparison.

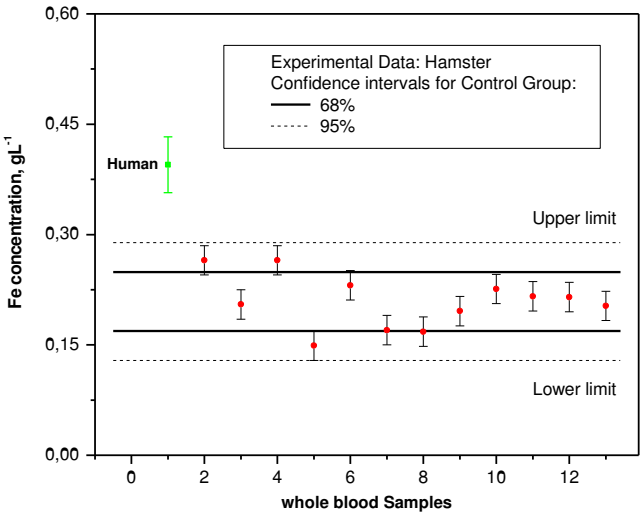

Figure 2. Fe concentration in whole blood samples of the Golden Hamster (*Mesocricetus auratus*). The human being estimation [4] was included for comparison.

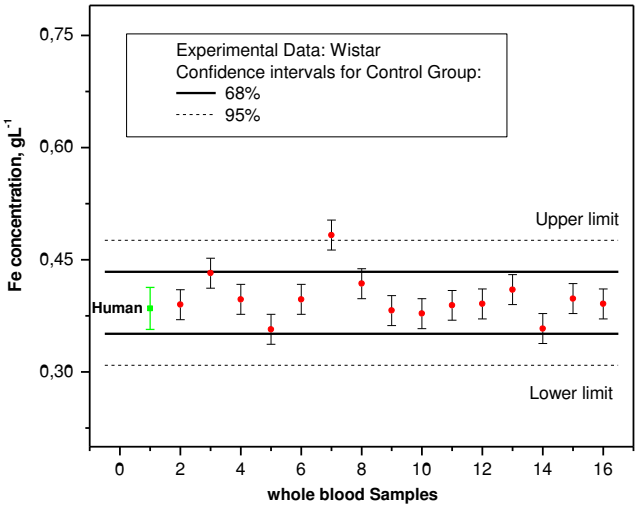

Figure 3. Fe concentration in whole blood samples of the Wistar rats. The human being estimation [4] was included for comparison.

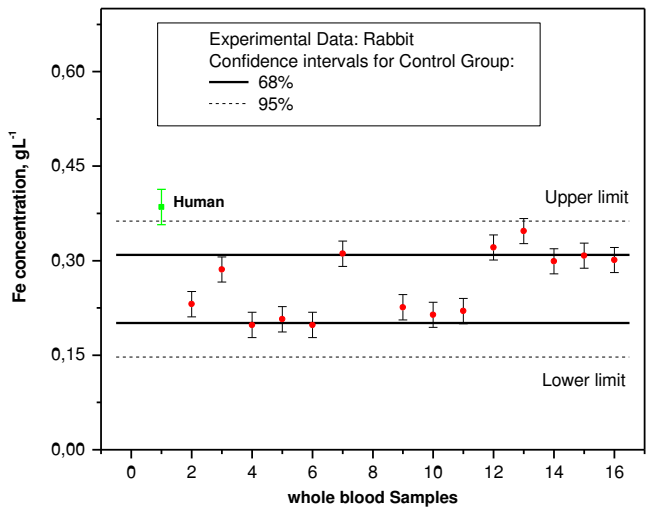

Figure 4. Fe concentration in whole blood samples of the Albinic Rabbits of New Zealand. The human being estimation [4] was included for comparison.

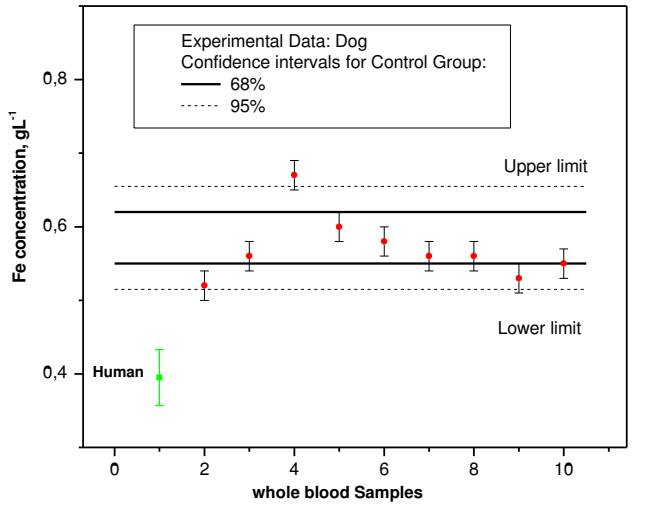

Figure 5. Fe concentration in whole blood samples of the Golden Retriever dogs. The human being estimation [4] was included for comparison.

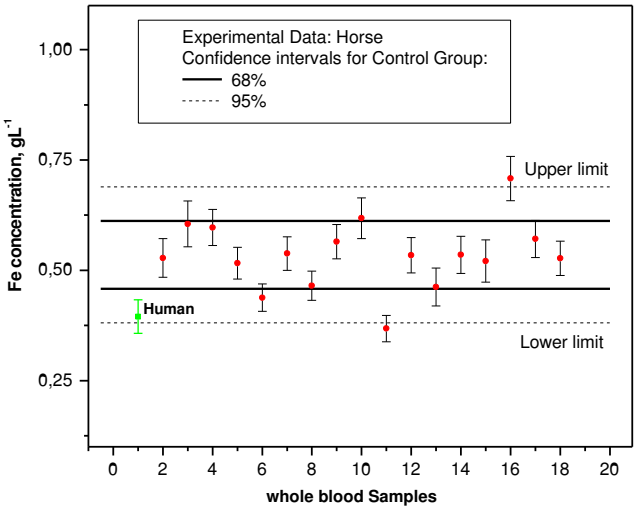

Figure 6. Fe concentration in whole blood samples of the Crioula breed horses. The human being estimation [4] was included for comparison.

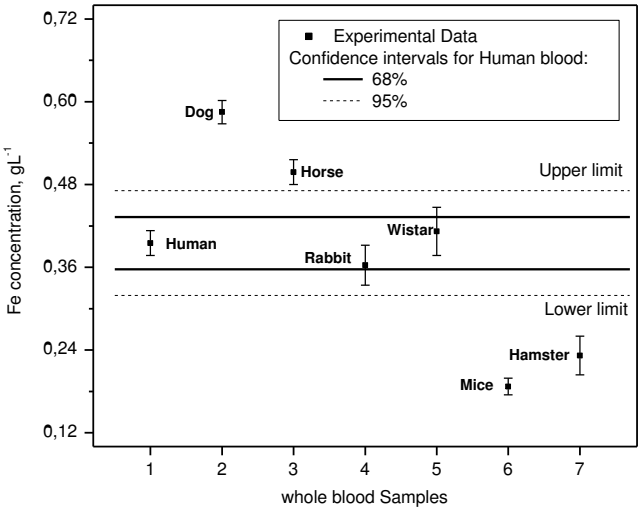

Figure 7. Fe concentration in whole blood samples of the animal models. The human being estimation [4] was included for comparison.

The results of Fe in whole blood of mice, hamster, rats and dogs are the first reference values. For rabbits there is in agreement with data from EDXRF analysis, but for horses the range determined by NAA is statistically different [6]. Regarding to the similarities with human being whole blood estimation there is a good agreement with the data from Wistar rats, Albinic Rabbits of New Zealand

and Crioula horses. However, the high Fe levels in Golden Retriever dogs, lower levels in mice (*Mus musculus*) and in Golden Hamster (*Mesocricetus auratus*) suggest that these experimental animal models are not convenient for researches even though the knowledge of Fe concentration in blood is necessary ($p < 0.05$).

Conclusion

In clinical trials where the knowledge of Fe concentration is necessary, this comparative study presents data that can help researches evaluate and conduct an appropriate medical investigation by choosing the convenient animal model. In addition, these data emphasis some differences in Fe concentration blood for mice (*Mus musculus*), Golden Hamster (*Mesocricetus auratus*), Golden Retriever dogs and Humans.

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