New Strategy to Evaluate the Effectiveness of New Treatments using Animal Models

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Abstract

In this study we intend to present a new strategy to clinical practices that can help researchers in medical and correlated areas to evaluate the effectiveness of new treatments which has been tested in animal models. Furthermore, we also present a discussion about the economic viability of applying these alternative procedures in clinical laboratories.

Keywords

XRF; Whole Blood; Clinical Practices; Animal Models

Introduction

In health area animal models are used in medical investigation of new treatments, drugs, vaccines and many other biological products before being applied in human being. As a routine these animals are submitted to several biochemistry analyses (such as: Calemia, Calcemia, Natremia, etc.), usually performed in serum. However, when small size animal model is involved the biological material can be scarce and limit its collections. The viability to perform these clinical analyses using EDXRF (Energy Dispersy of X Ray fluorescency) technique can simplify several steps involved in checking the effectiveness of new treatments or drugs. The main advantage for using this technique is the viability to use small quantities of body fluids (10 times less, at least, compared with the conventional clinical tests). Recently the Spectroscopy and Spectrometry Radiation Laboratory at IPEN – CNEN/SP (Brazil) has applied this clinical alternative in body fluids analyses (mainly whole blood and urine) of animal models, mainly small size (such as mice, rats and rabbits). In this study we intend to present the details for implementation and execution of clinical practice using this alternative procedure.

Material and Methods

The biological samples from Wistar rats (n = 10, male), were collected according to the rules approved by Animal Research Ethics Committee (087/99). More details are presented in a previous study [Oliveira L., et al.]. Immediately after the collection 50μL of biological fluid (whole blood, serum and urine) was transferred to the filter paper (Whatman, nº 41) using a calibrated micropipette and each biological sample was dried for few minutes using an infrared lamp (see an example of whole blood dried in Figure 1a). The same procedure was applied for serum and urine. The XRF analysis was performed using two X-Ray spectrometers: an EDXRF Spectrometer SHIMADZU Co. model Rany 720 (Figure 1b), with: 50kV, 100 μA-variable, Rh target, Si(Li) detector and fixed time counting of 100s, and a MINI-X spectrometer (Amptek) (Figure 1c) with: 30kV, 5μL- variable, Ag X-ray Target and a Si Drift detector (25 mm2 x 500μm / 0.5 mil) with Be window (1.5") and variable range time (300s and 600s). The quantitative analyses used in the EDXRF and Mini X-Ray spectrometers were performed with Shimadzu and WINAXIL software's, respectively.

a) b) c)

FIG. 1 a) BLOOD SAMPLE; b) SHIMADZU EDXRF SPECTROMETER; c) AMPTEK MINI X-RAY SPECTROMETER

Results and Discussion

The adequacy of the measurment was evalute for Fe using the certified reference material IAEA-A-13 Animal blood. The evaluation of the Z-score test (-0.8) is satisfactory ($|Z| < 2$).

The Iron concentrations determined in serum, whole

blood and urine samples from boths spectrometers are in agreement. In Table 1 is presented a summary of the data obtained using MINI X-ray spectrometer. The results were expressed by: Time Counting (Tc), Median Value (MV), Standard Deviation (1SD), Detection Limit (DL), Quantification Limit (QL) and Reference Values (RV) (for a confidence interval of 95%).

TABLE 1. IRON CONCENTRATIONS RESULTS IN BIOLOGICAL MATERIAL

Serum (Tc = 600s)	**Fe, µg dL^{-1}**
MV ± 1SD	58 ± 18
DL	2.2
QL	6.7
RV	22 – 94
Urine (Tc = 600s)	**Fe, µg dL^{-1}**
MV ± 1SD	60 ± 13
DL	3.1
QL	9.4
RV	34 – 86
Whole Blood (Tc = 300s)	**Fe, mg dL^{-1}**
MV ± 1SD	388 ± 52
DL	7.5
QL	22.7
RV	284 – 492

To illustrate, in Fig. 2, 3, 4 and 5 are presented the results for detection limit and Iron concentration in whole blood and serum, respectively. In Fig. 6 the whole blood and serum spectra using MINI X-ray spectrometer are presented.

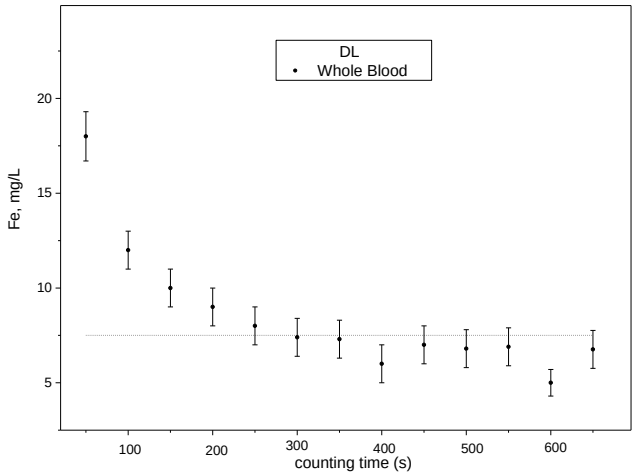

FIG. 2 WHOLE BLOOD DETECTION LIMIT USING PXRFS

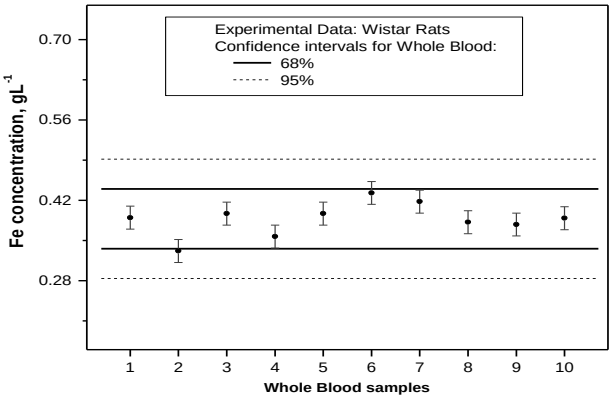

FIG. 3 WHOLE BLOOD IRON CONCENTRATIONS

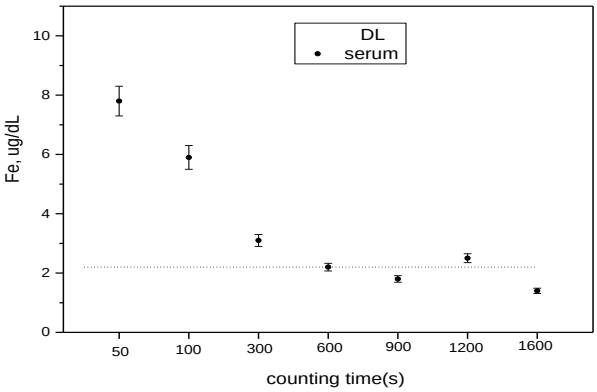

FIG. 4 SERUM DETECTION LIMIT USING PXRFS

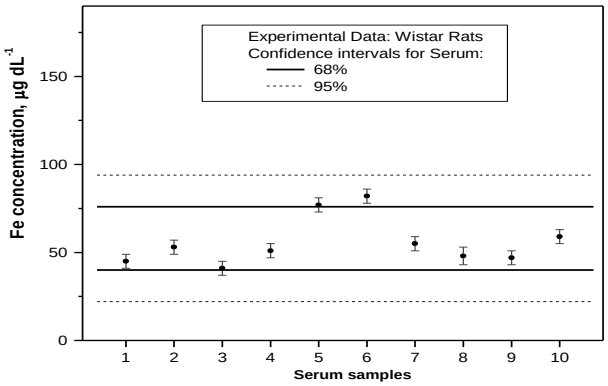

FIG. 5 SERUM IRON CONCENTRATIONS

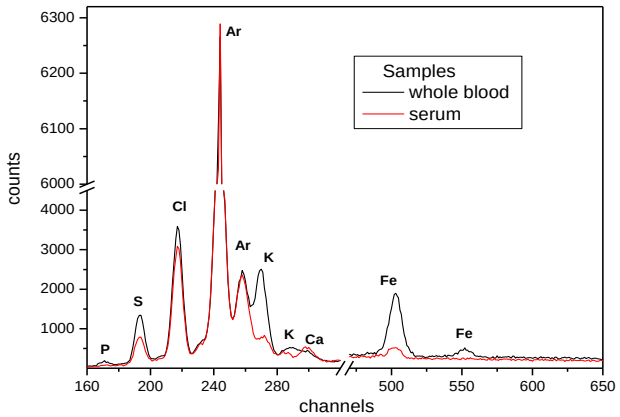

FIG. 6 WHOLE BLOOD AND SERUM SPECTRA USING MINI X-RAY SPECTROMETER

The XRF technique using the MINI-X Spectrometry showed to be adequate for Fe determination in whole blood, serum and urine samples from animal model. Its applicability requires a small amount of biological material, short time of analysis (minutes) and simple sample preparation. Moreover, the samples can be stored for a long period for future examination without refrigeration.

Recently, the MINI X-Ray Fluorescence Spectrometry performance was investigated to be used for clinical practices for Iron determination in whole blood and serum samples of Brazilian population showing to be appropriate for this clinical finalities. These results also emphasis its application in clinical laboratories.

Considering the viability of using MINI-X Ray Spectrometer for clinical investigations using whole blood, serum and urine, other elements also relevant in clinical practice, such as Ca, Cl, K,P and S, can also been evaluated simultaneously.

Finally, it is also relevant to discuss about the economic viability. According to the last Program External Quality Assessment of clinical laboratories (Brazil, 2012), 1.4 billion clinical tests have been done (~ 4% are biochemical test, mainly for Ca, Cl, Fe and K); moreover, it is estimated an increase of about 2% of clinical examinations in 2014. Considering these estimates, the MINI X-ray spectrometer can be an economical alternative for clinical practice, especially in underserved regions with poor medical care and hospitals.

Conclusions

This tool could help the researchers to evaluate the efficiency of new treatments as well as to compare the advantages of different treatment approaches, before performing tests in human being in fast and economic way. The principal audiences are researchers who work in health and related area using experimental animals, such as: Veterinary Toxicology, Immunology, Nutrition and Genetics. Additionally, this alternative procedure meets the needs of the Brazilian legislation that emphasizes the need to propose alternative methods for clinical practice that contribute to animal welfare.

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