

# New Trends for Radiation Applications in Medicine



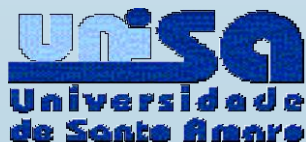
C.B. Zamboni, S. Metaiorn  
Instituto de Pesquisas Energéticas e Nucleares,  
IPEN/CNEN, Brasil



Ministério da Ciência  
e Tecnologia



M.A. Rizzutto, S. Bernardes  
Universidade de São Paulo, IFUSP, Brasil



M.R.A. Azevedo  
Universidade de Santo Amaro, UNISA, Brasil

## Focus

To show the advantages to use alternatives analytical techniques (NAA and XRF) for clinical practices

## Why use these techniques?

The main advantage is the viability to use small quantities of **blood (0.1mL)** comparatively to conventional analyses performed in serum or plasma (using at least 0.5 to 10mL)

using conventional procedure:

- anticoagulant and reagents
- To wait for coagulation
- To perform the centrifugation

using BLOOD

all these steps are eliminated

Since 2000 **NAA** technique has been applied for clinical finality at IPEN/CNEN-SP, and more recently **XRF** ("good results")

(2004) in collaboration with Blood Banks and Research Centers from Brazil we started a Project: "Determination of REFERENCE VALUES (RV) for elements of clinical relevance in human blood using alternatives analytical techniques (NAA and XRF) " ... it is still in progress

Why to determine Reference Values of inorganic elements in blood?

Conventional analyses are performed in serum or plasma, so the limits (normal range) for clinical practice are well known for Brazilian population

However, for blood these limits are not established

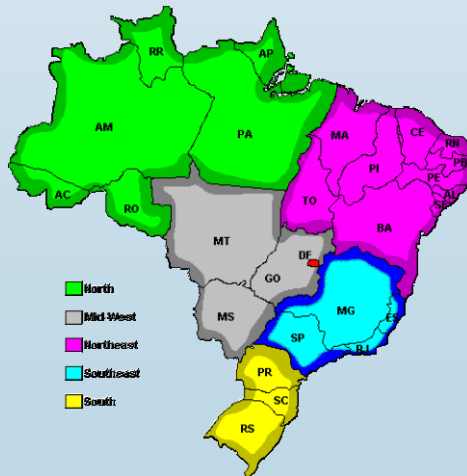
**Partners:** UNISA, IFUSP, Banco de Sangue Paulista, UNIFESP, Instituto Butantan (SP), HEMOPE, UFPE, LABEX/UNICAMP, UFF, UNIBAN

**Financial Support:** CNPq, FAPESP, CAPES, IPEN/CNEN

# Reference Values in blood for Brazilian population

Environment

Geographic localization



Physiological

Gender

Age: 16 - 65 years

Weight: above 50kg

Life style Factors:

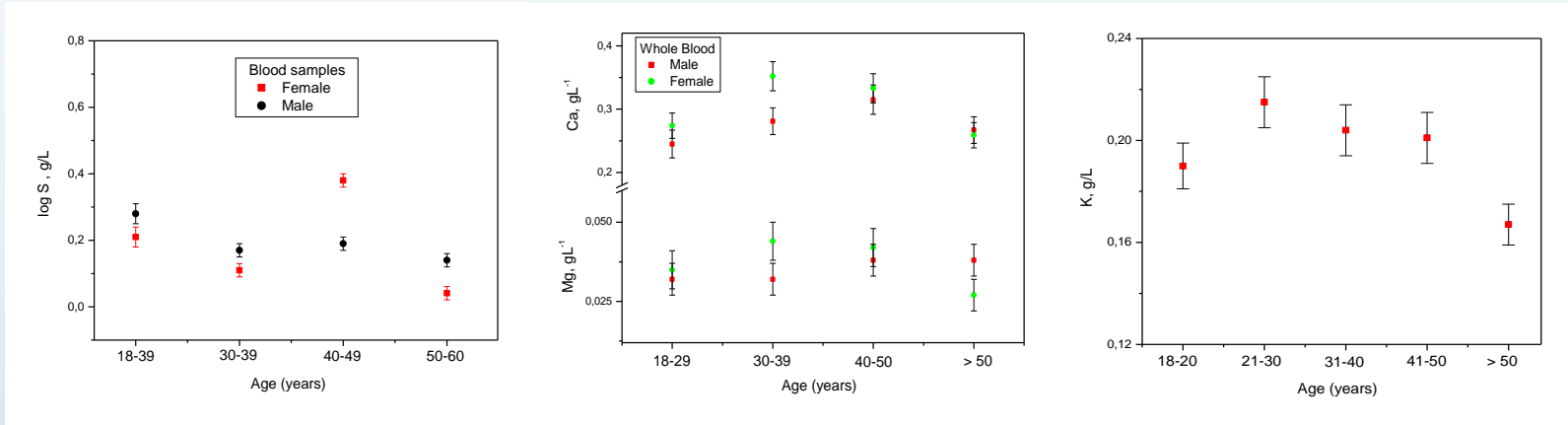
- smoking and/or
- drinking habits
- medicine intake
- physical exercise

Pathological

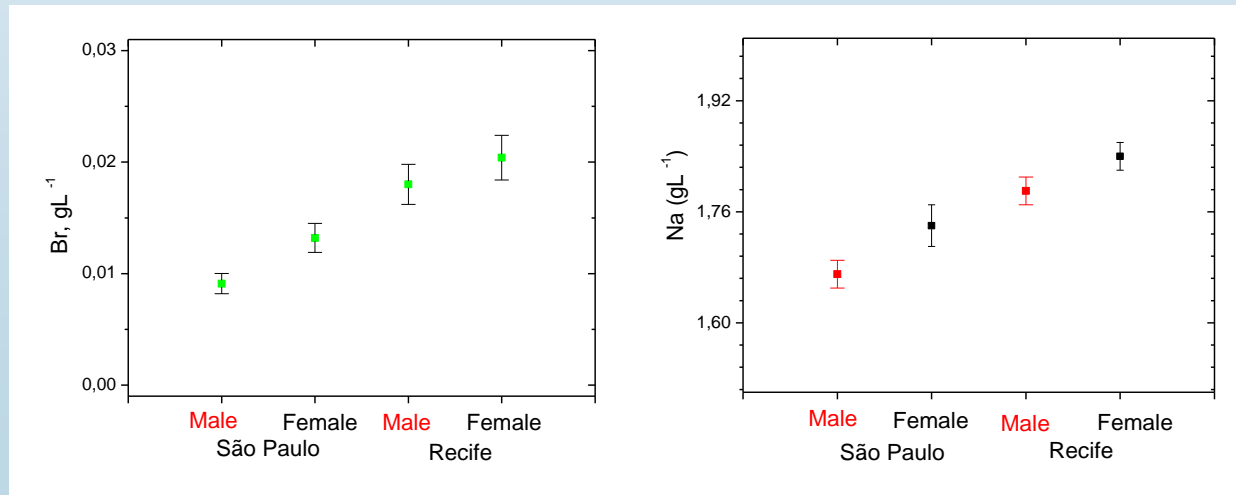
Blood banks perform screening tests:  
HIV, Sífilis, Hepatitis B and C and Chagas

Health Donors

# Blood concentrations of Ca, K, Mg and S for inhabitants of Brazil by age and gender

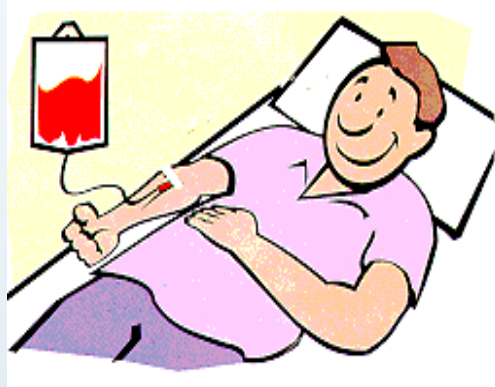


# Blood concentrations of Br and Na for inhabitants of Brazil by gender and geographic localization

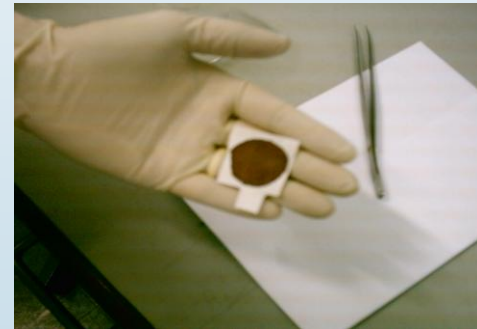


# Sample Preparation

blood is collected in a vacuum plastic tube (without anticoagulant or reagents) attached to the donor's arm



immediately after the collection **100 $\mu$ L of blood** is transferred to  $\sim 2.5$  cm<sup>2</sup> pieces of filter paper (Whatman, n°41) and dried using an infrared lamp



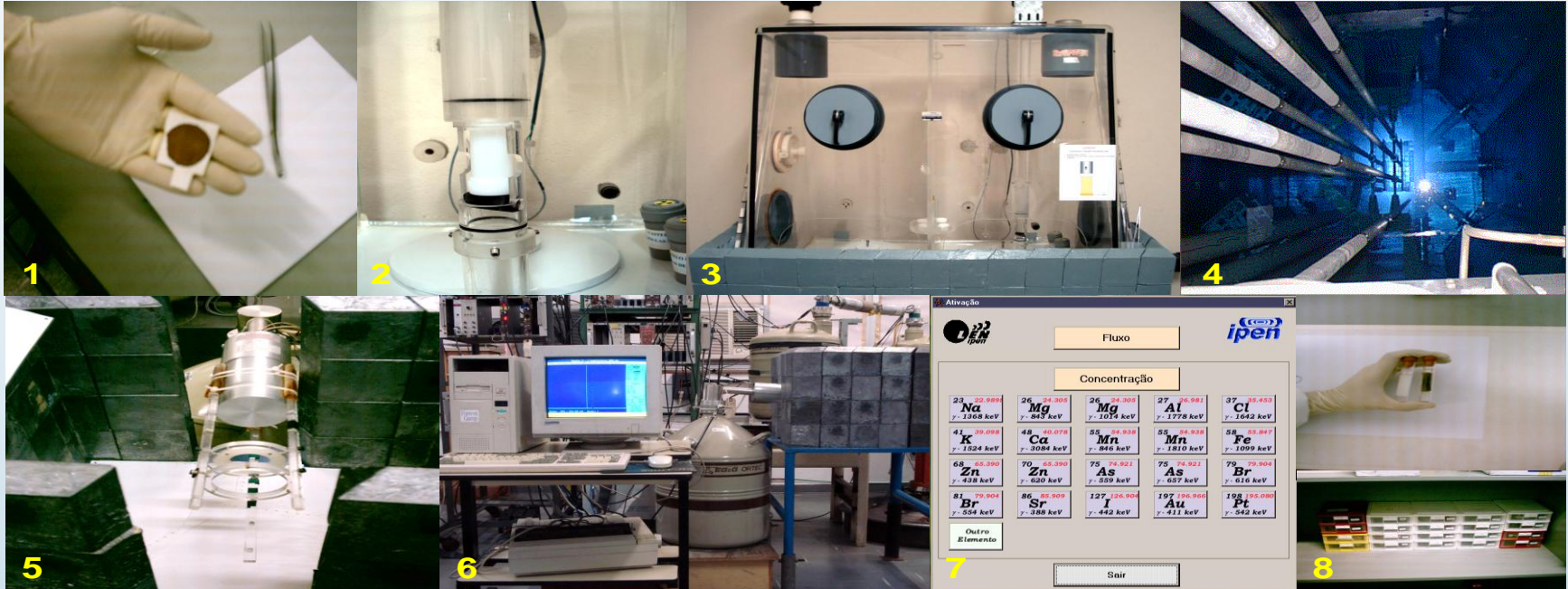
The sample can be storage without refrigeration (non destructive method)





# Experimental Procedure using NAA

The samples are collected and prepared at blood bank and after, they are irradiated at the IEA-R1 nuclear reactor (3- 4.5 MW, pool type) at IPEN/CNEN-SP

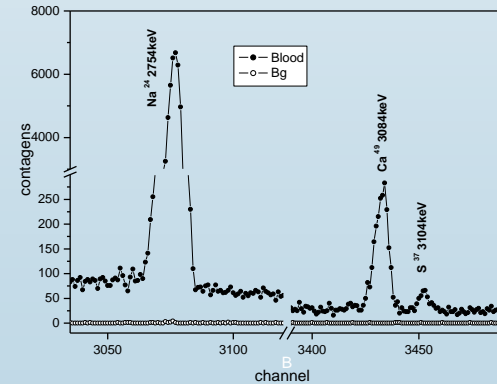
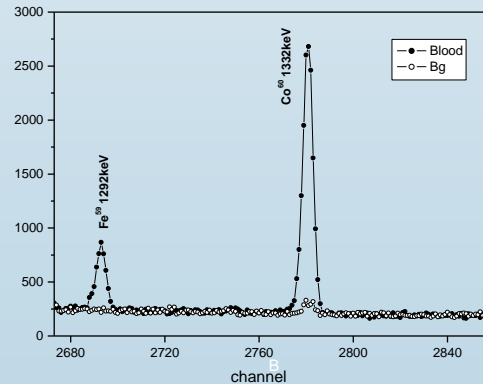
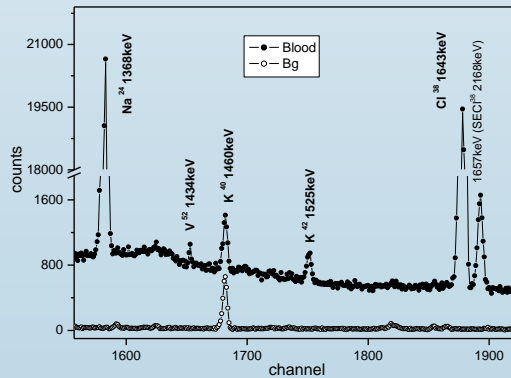
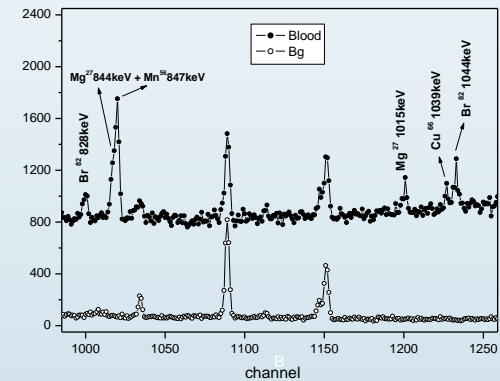
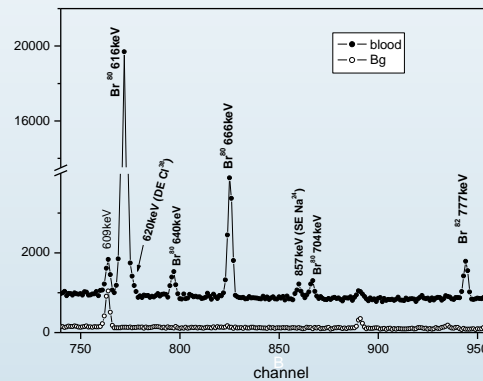
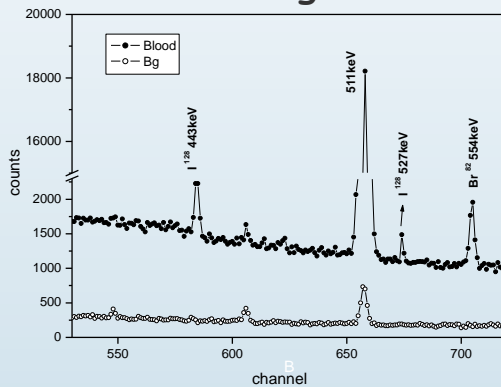


1. Blood sample
2. Detail of sample irradiation at the Automatic Pneumatic Station
3. Automatic Pneumatic station
4. Nuclear Reactor
5. HPGe detector (GEM -60195 ORTEC)
6. Nuclear instrumentation and data system (Amplifier - Ortec 671 and MCA - Ortec 919E)
7. Quantitative analysis using (in-house) *Ativação* software
8. Storage samples

# Gamma ray spectrum of blood using NAA

Considering the optimized conditions:

- Blood samples :  $100 \pm 5\% \mu\text{L}$
- Irradiation time : 240s
- Thermal neutron flux:  $\sim 5.10^{12} \text{ n}\cdot\text{cm}^{-2} \cdot \text{s}^{-1}$
- Counting Time : 900s

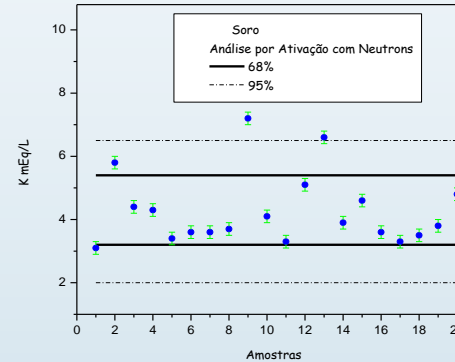
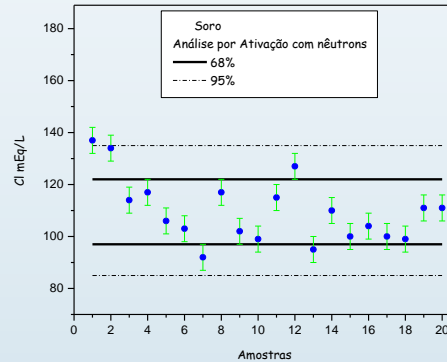
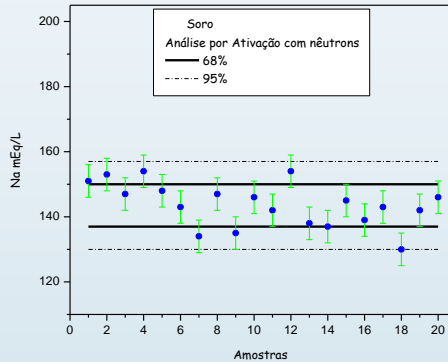


Simultaneous determination of elements of clinical relevance (such as: Cl, Ca, I, K, Mg, Na,...)



# Comparison (serum): NAA & conventional techniques

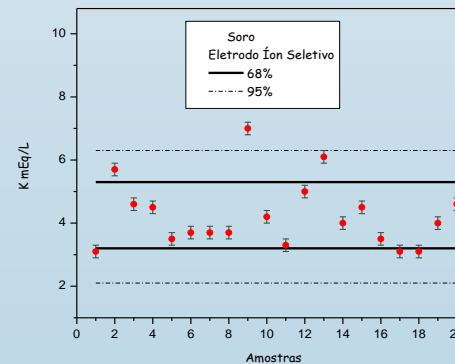
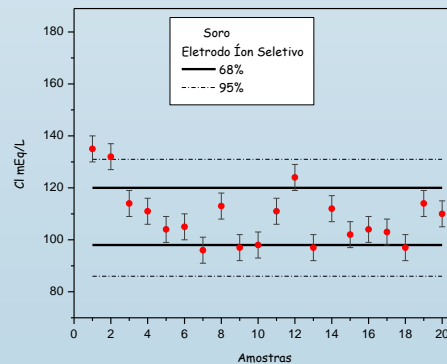
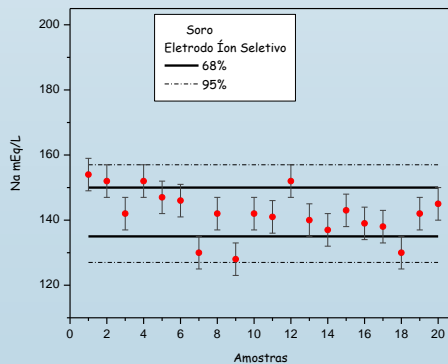
## Serum analyses using NAA



## Serum sample



## Serum analyses using Ion Specific Electrode (Na and K) and Titrimetry (Cl)



The results are in agreement considering  $\pm 1SD$

**Limitation of using NAA:** The necessity of nuclear reactor or accelerator



We decided to check the viability of using XRF technique

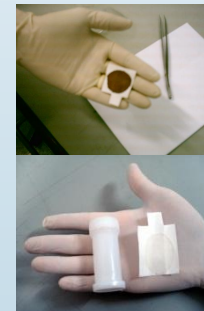
### Experimental Procedure

The measurements were carried out on an EDXRF Spectrometer SHIMADZU Co., model Rany 720 (50kV, 100 $\mu$ A-variable, Rh target) at Centro de Quimica e Meio Ambiente/IPEN



Blood and Serum samples fixed on filter paper were analyzed using:

- Si(Li) detector
- Fixed time: 100s
- Line emission  $K\alpha$  for all elements: Ca, Cl, Cu, Fe, K, Mg, Na, P, S, Zn
- Quantitative Analysis was performed using Fundamental Parameters method software from Shimadzu



The results (NAA, XRF and Conventional) are in a good agreement



This alternative procedure can be used for clinical finalities

## In progress

To optimize the performance of the **Portable X-Ray Fluorescence Spectrometry (PXRFs)** for clinical analyses

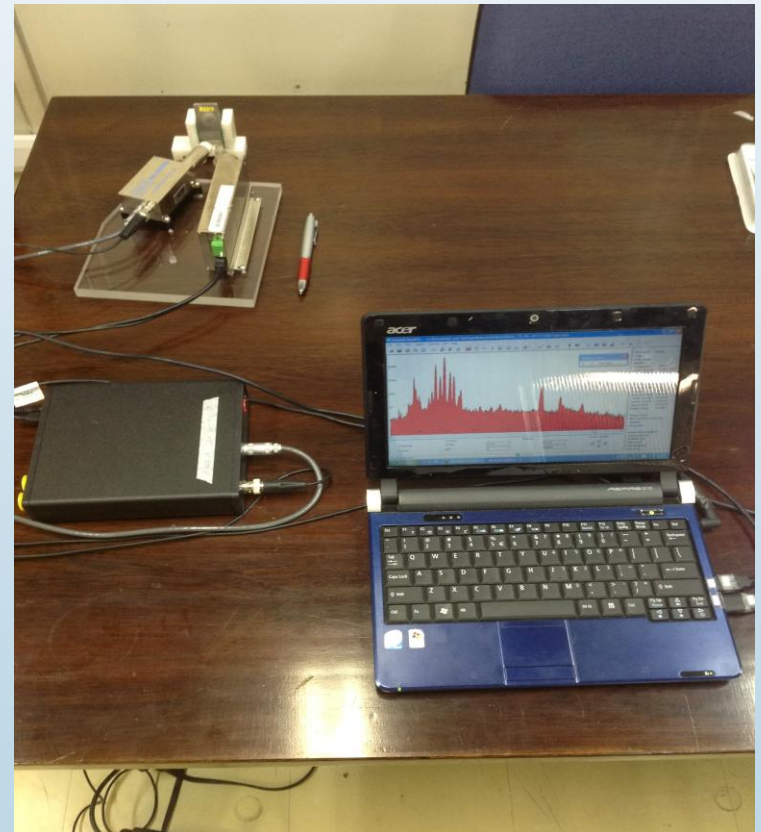
### Experimental Conditions using PXRFs

The XRF analysis was performed using MINI X spectrometer from Amptek, with Ag X-Ray tube at Universidade de São Paulo / IFUSP

The characteristics X-Ray fluorescent intensities ( $K\alpha$  lines) were measured with a Si Drift detector (25 mm<sup>2</sup> x 500µm / 0.5 mil) with Be window (1.5")

The blood samples (100µL) were irradiated for 300s (fixed time) using 30kV and 5µA excitation)

The quantitative analysis (using  $K\alpha$  lines) was performed using "WINAXIL" software program.

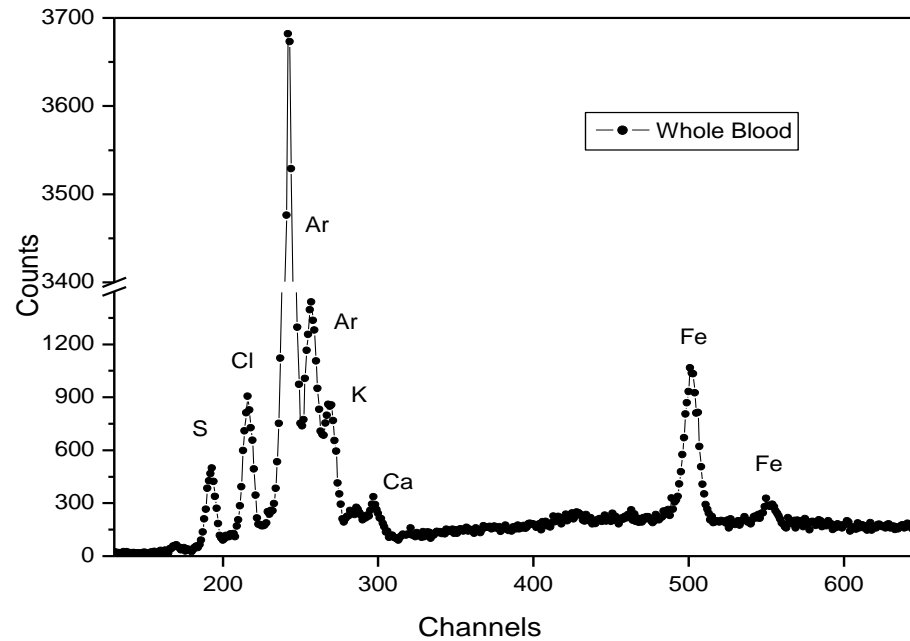




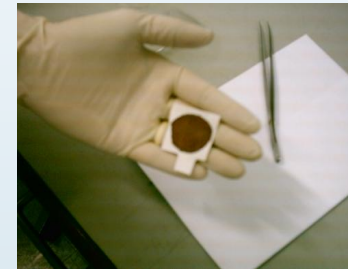
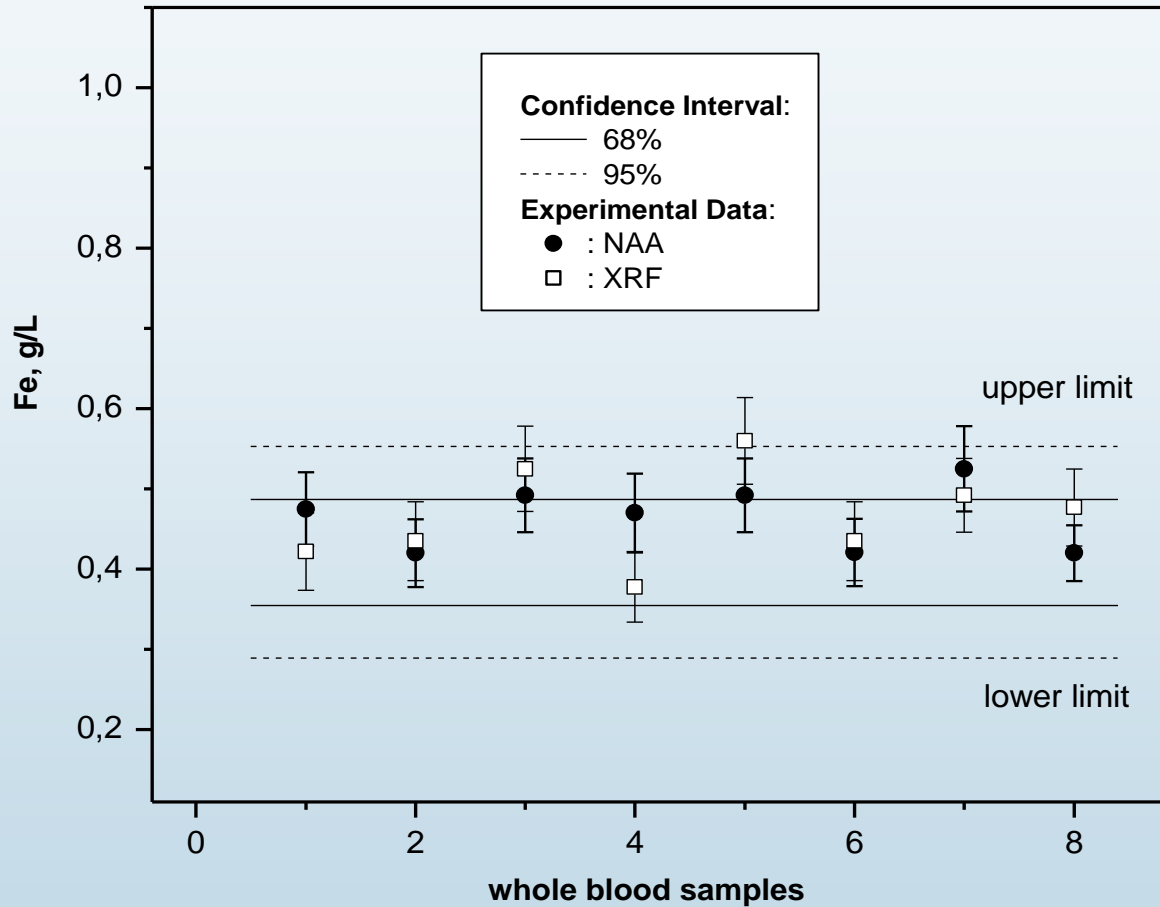
Si drift detector (US\$9,900.00)

Mini X-Ray tube with Silver target (US\$6,500.00)

## Blood spectrum using portable spectrometer (PXRF)



# Iron concentration on blood samples by NAA and XRF (using PXRFs)



using the same  
blood sample

The portable apparatus can be used in clinical laboratories

## Conventional Analyses

Conventional Analysis/ Technique	Biological material/ Quantities (ml)	Execution time Days	(*) Cost US\$
<b>Brometo (Br<sup>-</sup>) / Colorimetry</b>	<b>Blood / 4.0</b>	<b>15</b>	<b>211.62</b>
Calcemia (Ca)/ Colorimetry	Serum / 0.5	1-2	22.78
Cloremia (Cl)/ Dry Chemistry	Serum / 0.5	1-2	26.25
Iron/ Dry Chemistry	Serum / 1.0	1-2	22.78
Magnesemia (Mg)/ Colorimetry	Serum / 0.5	1-2	37.38
Calemia (K)/ Dry Chemistry	Serum / 1.0	1-2	25.20
Natremia (Na)/ Dry Chemistry	Serum / 3.0	1-2	25.20
Zinc (Zn)/ Atomic Absorption Spectrophotometry	Serum / 2.0	10	43.05

## Conventional Analyses

Serum: Ca, Cl, Fe, K, Mg, K, Na, Zn

Blood: Br

Execution Time: 1-15days

Cost: **US\$ 394,26**

## Alternative techniques

Quantity: 100  $\mu$ L

## NAA (IEA-R1 nuclear reactor)

Blood: Br, Ca, Cl, Fe, I, K, Mg, Mn,  
Na, Rb, S and Zn

Cost (irradiation) : **US\$ 23,00**

## EDXRF (Rh X-Ray tube)

Blood: Al, Br, Ca, Cl, Fe, K, Mg, Mn,  
Na, P, S and Zn

Cost: **US\$ 25,00**

## PSXRF (Ag X-Ray tube)

Blood: Ca, Cl, Fe, K and S

Cost: **US\$ 25,00**

\* Laboratório Fleury S/C Ltda. São Paulo

Actualized: 13061



## Conventional Analyses

Conventional Analysis/ Technique	Urine/ Quantities (ml)	Execution Time Days	(*) Cost US\$
Calcemia (Ca)/ Colorimetry	20.0	1-2	22.78
Cloremia (Cl)/ Colorimetry	5.0	1-2	27.82
<b>Iron/ Plasma Emission Spectroscopy</b>	<b>7.0</b>	<b>15</b>	<b>348.55</b>
Magnesemia (Mg)/ Colorimetry	5.0	2	39.62
Caemia (K)/ Ion-Selective Electrode	5.0	1-2	25.20
Natremia (Na)/ Ion-Selective Electrode	10.0	1-2	25.20
Zinc (Zn)/ Plasma Emission Spectroscopy	10.0	10	43.05

## Conventional Analyses

Urine: Ca, Cl, Mg, Fe, K, Na  
and Zn

Cost: **US\$ 532.22**

## Alternative techniques

Quantity: 100  $\mu$ L

Urine: Br, Ca, Cl, Cu, Fe, I, K,  
Mg, Na, S and Zn

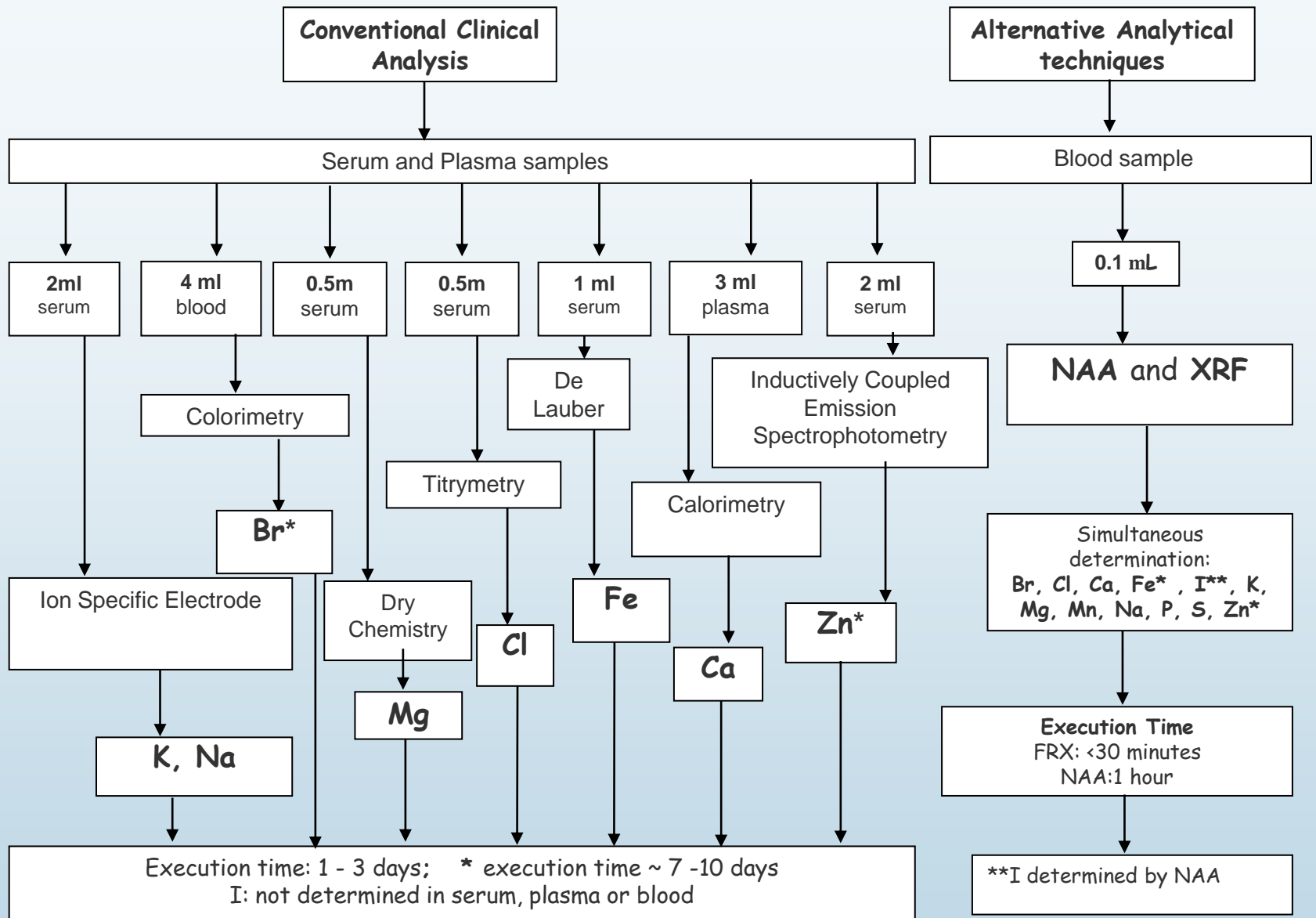
Cost (irradiation): **US\$ 23.00**

## Perspective

the cost of Iron analysis can  
be reduced using these  
alternative techniques

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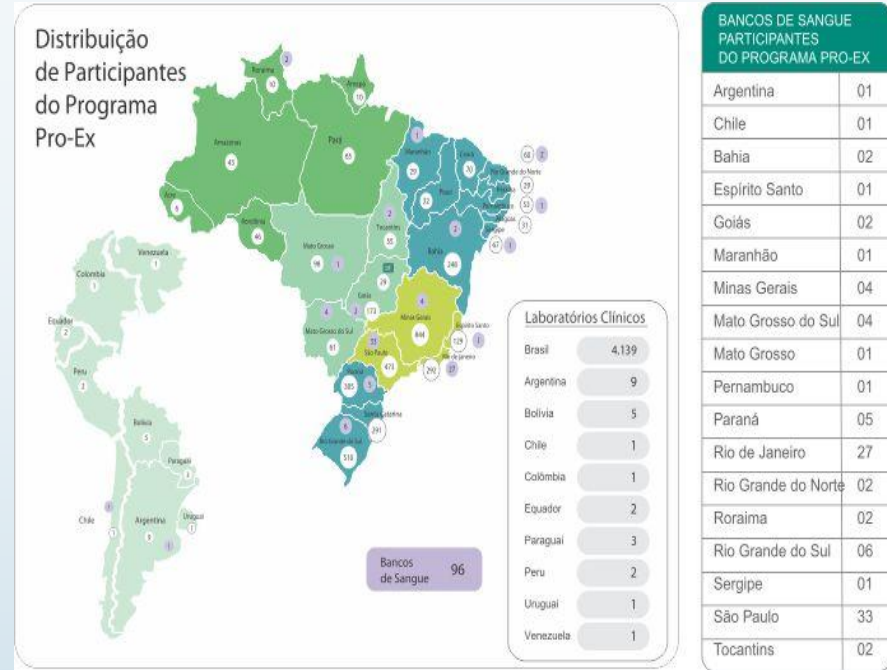


small quantity of blood, simplicity in the sample preparation, simultaneous determination and reduced cost

# Economic Viability

According to the last ProEx (Programme External Quality Assessment of clinical laboratories in Brazil) carried out in 2012

**1.4 billion clinical examinations were performed:**



- ✓ average of 8 exams per inhabitant/Brazil
- ✓ average cost of R \$ 13,00/exam
- ✓ estimated an increase ~2% clinical examinations in 2013
- ✓ average of 3% of biochemistry exams (Na, Cl, K, Fe)
- ✓ average of ~1% other (Mg, Ca, Br and Zn)

~56 million of clinical examinations can be done using these alternatives techniques

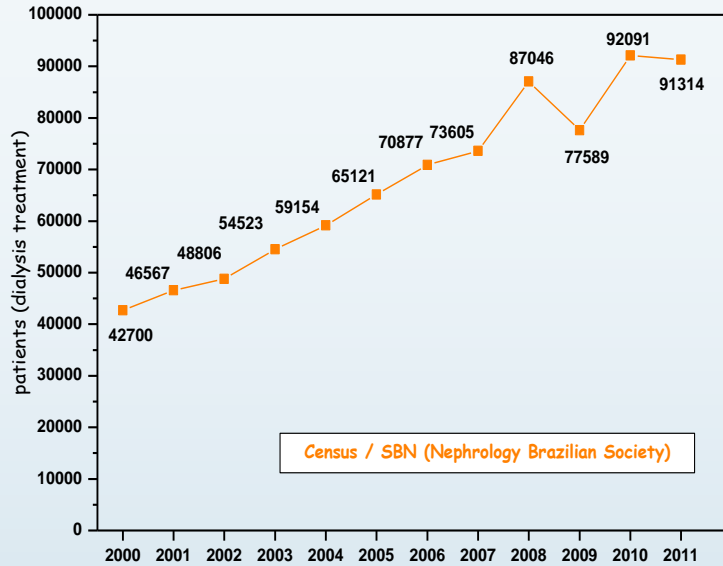
Others applications:

The use of these alternatives procedures (NAA and XRF) are very useful

- ✓ when the biological material is scarce
- ✓ in veterinary medicine to perform clinical analyses in **small size animal** (mice, rabbits, hamsters,...)
- ✓ For analyses of other body fluids (**saliva , urine, serum...**)
- ✓ Quality control of biological products developed for applications in public health area (antivenom, new reagents, ...)

**To give an idea I will shown some applications**

# PATIENTS WITH CHRONIC KIDNEY DISEASE



Situation in Brazil:

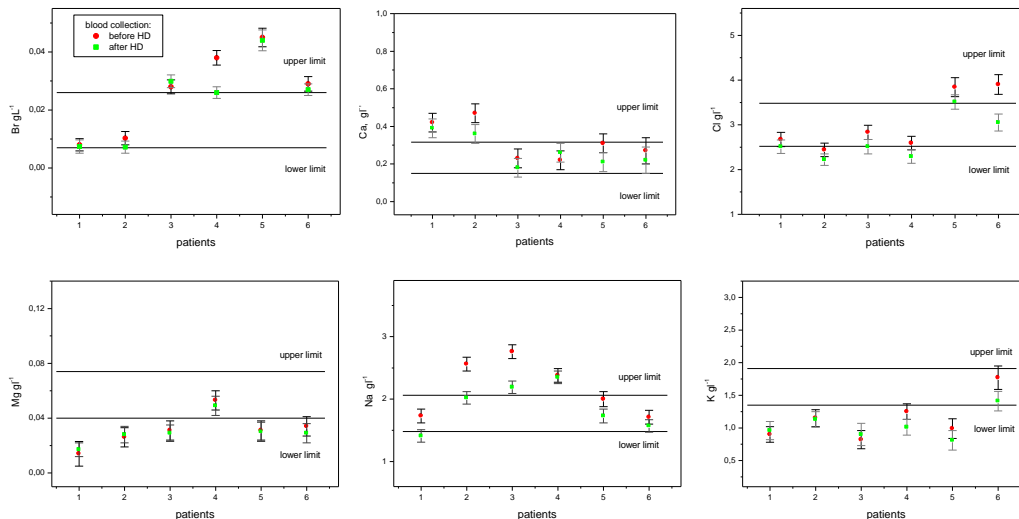
Percentile variation (2000/11) increase of 114%

## PUBLIC HEALTH PROBLEM

Nowadays there are **687** units of Hemodialysis

According to ANVISA the treatment evaluation is performed by clinical analysis of: **Na**, **Cl**, **K** and **Mg** in serum (before and after dialysis) and **Ca** and **Fe** monthly

During 2004/2006 NAA was applied to investigate blood of patients submitted a dialysis (before and after) and the results were compared with conventional (good agreement)



These alternative procedures can be used in the units dialysis treatment

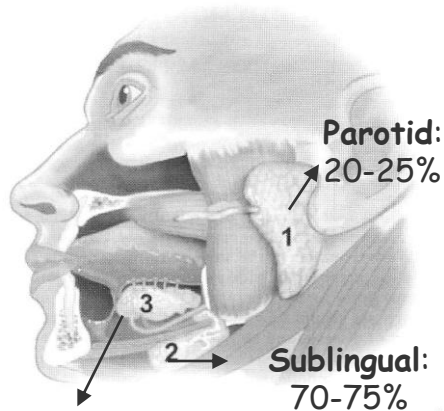
Collaboration: IPEN, HEMOPE, UNISA, UFPe

# Diagnostic: Saliva of patients with periodontal disease

## PERIODONTAL DISEASE

It is an infectious inflammatory disease that affects the gum tissue and support the teeth (bone loss).

According to Public Health Service (ANVISA, 2009) **severe forms** affects between **5 to 20%** of the **Brazilian population**.

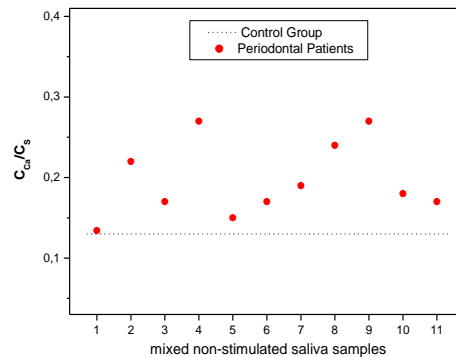
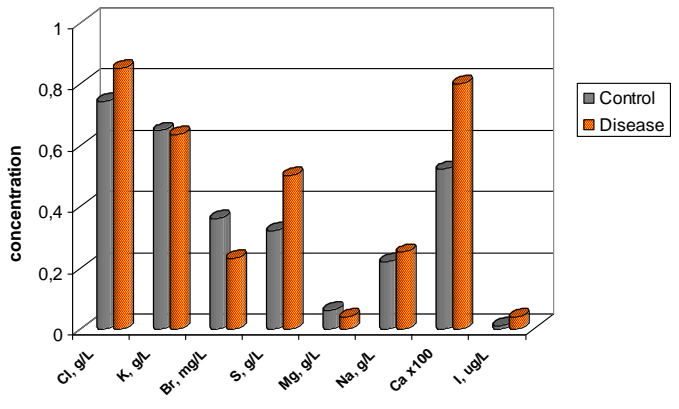


Parotid:  
20-25%

Sublingual:  
70-75%

Submandibular and  
small glands: 1-5%

Whole saliva is the mixture of these glandular secretions, bacterias and epithelias cells



Ca and S in whole saliva  
are good biomarkers

The use saliva (100µL)  
and the PXRFS can be  
used in ontological office



# Analysis of saliva tick (*Amblyomma cajennense* species) from Brazil

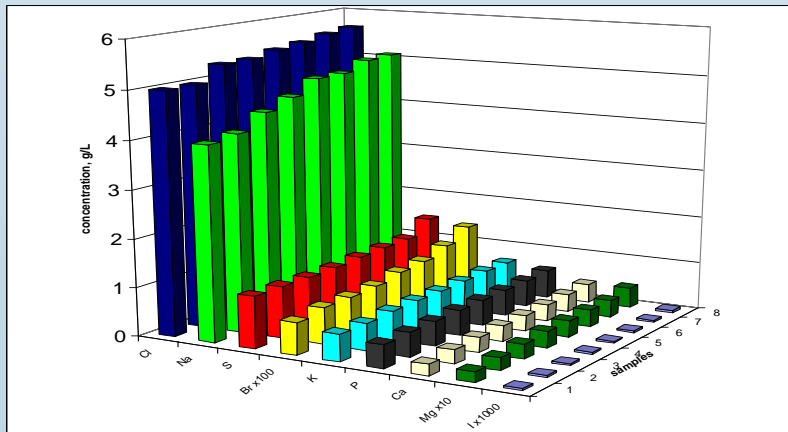
It has medical applications



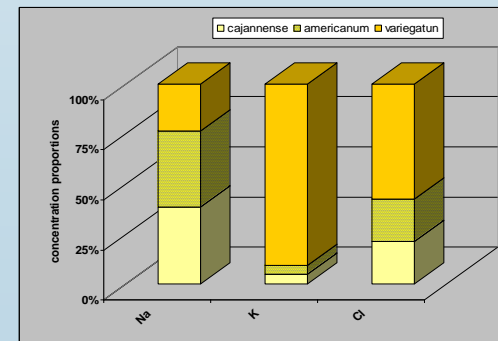
## Saliva collection

- The saliva was collected into capillary tubes (75 length x 1.5 mm diameter) attached to the hypostome
- Each tick produces ~40 $\mu$ L of saliva
- ~30 ticks were necessary for extraction of ~ 500 $\mu$ L
- 100  $\mu$ L was fixed on filter paper

The elements concentrations determined in saliva samples



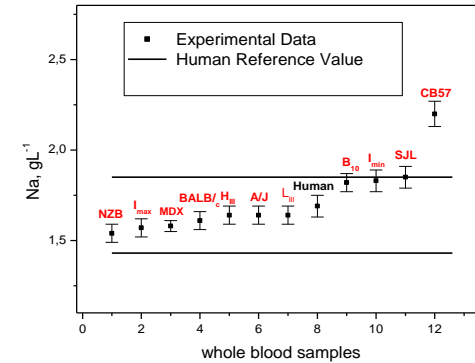
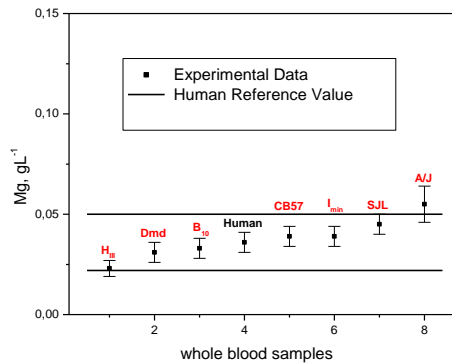
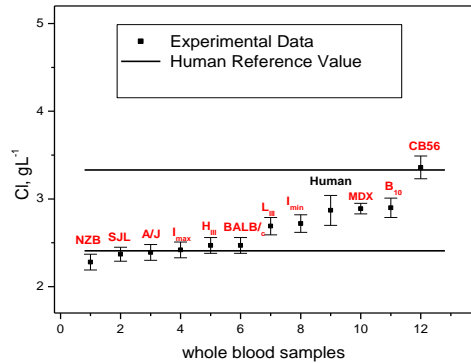
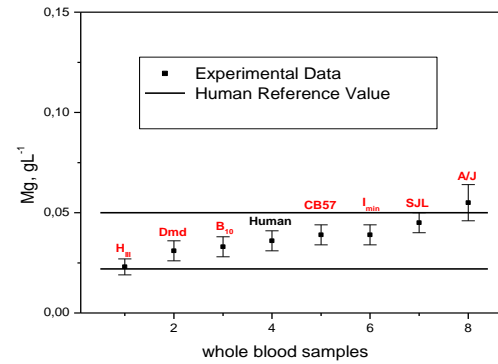
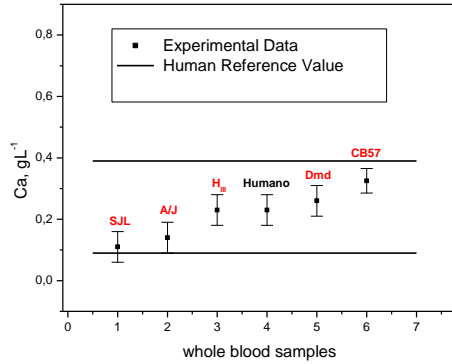
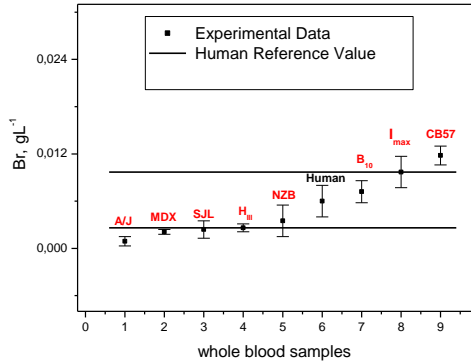
Saliva comparison of different *Amblyomma* species



Collaboration: IPEN/ Instituto Butantan, Brazil

# Veterinary Medicine: to perform clinical analyses in small size animal

## Test of new vaccines and medicines



**Potential for Future applications**

## Clinical practice performed in premature infants

A premature baby (~1kg) has ~100mL of blood circulating in the body and depending on the dysfunction/treatment the child may need **two to three blood samples/day**.

Consequently, blood samples for laboratory tests in pediatric practice are the main causes of transfusions in premature infants

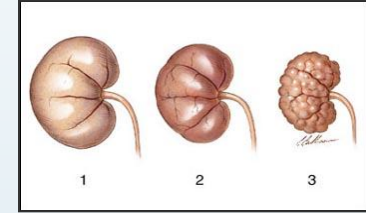
According to the Children's Institute at Brazil are performed ~18.000 blood collections/month.



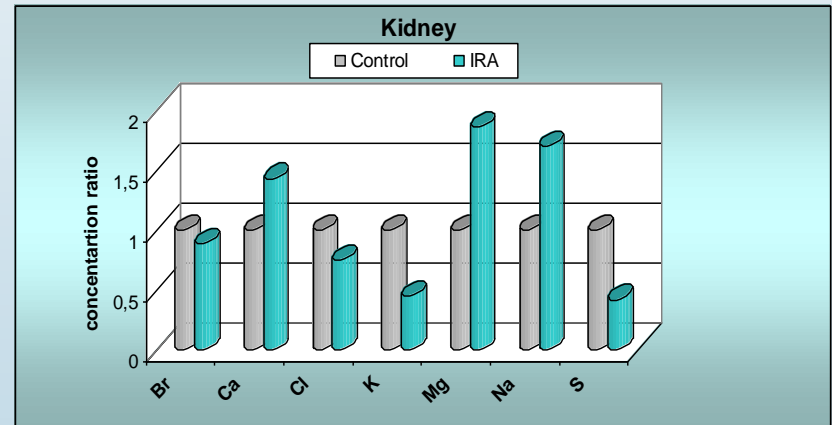
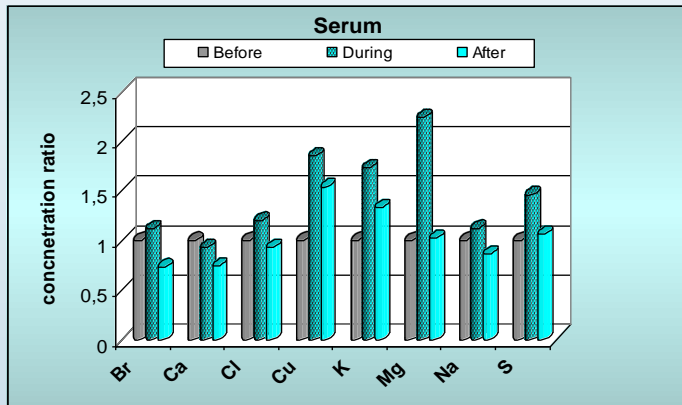
The use **Blood sample** (100 $\mu$ L) fixed on paper and the **Portable Spectrometer** can be a promissory alternative procedure

# Pre diagnostic of Acute Renal Failure

## Analysis of elements in serum, urine and kidney in Wistar rats with Acute Renal Insufficiency using NAA

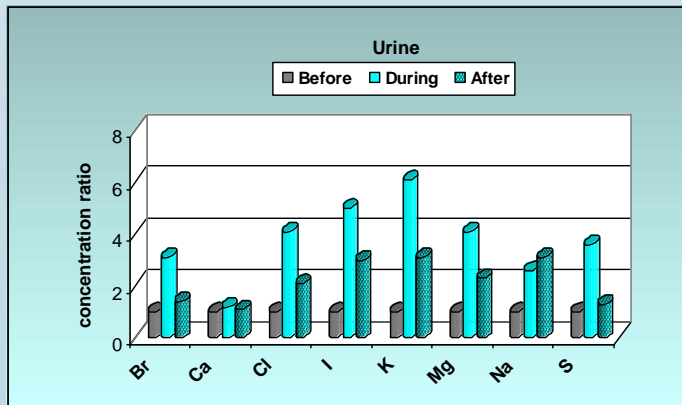


- 1 - kidney with acute renal failure
- 2 - normal kidney
- 3 - kidney with chronic renal failure



The correlation analyses between kidney & urine and kidney & serum suggest that:

**I and Cu** can be used as biological markers for ARF



Laboratório de Espectroscopia e Espectrometria das Radiações- LEER  
IPEN/CNEN-SP

C. B. Zamboni; S. Metairon; J. A. G. de Medeiros; L. C. Oliveira;  
L. Kovacs and I. M. M. A. Medeiros

czamboni@ipen.br

and collaborators:

Maria Regina A. Azevedo (UNISA)

Márcia A. Rizzutto (IFUSP)

Suene Bernardes (IFUSP)

Ivone Mulato Sato (CQMA/IPEN)

Denise Vaz de Macedo (UNICAMP)

Lázaro Alessandro Soares Nunes (UNICAMP)

Thiago F. Lourenço (UNICAMP)

Hugo R. Lewgoy (UNIBAN)

Osvaldo Augusto Sant'Anna (Instituto Butantan)

Simone. M. Simons (Instituto Butantan)

Daniella G. L. Oliveira (Instituto Butantan)

Roberto Meigikos dos Anjos (UFF)

Fernanda T. Borges (UNIFESP)

Miriam F. Suzuki (CBT/IPEN)

Carlos R. Bueno Junior (Instituto de Biociências/USP)

**MUCHS GRACIAS!!!!**