New Trends for Radiation Applications in Medicine

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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 theses 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

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
blood is collected in a vacuum plastic tube (without anticoagulant or reagents) attached to the donor’s arm immediately after the collection, 100 μL of blood is transferred to ~2.5 cm² pieces of filter paper (Whatman, n°41) and dried using an infrared lamp.

The sample can be stored 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: $100 \pm 5\%\mu L$
- Irradiation time: 240s
- Thermal neutron flux: $\sim 5 \times 10^{12} \text{n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$
- 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 analyses using Ion Specific Electrode (Na and K) and Titrmetry (Cl)

The results are in agreement considering ±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 an EDXRF Spectrometer SHIMADZU Co., model Rany 720 (50kV, 100μA-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 Ka 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 (Kα lines) were measured with a Si Drift detector (25 mm² 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 Kα lines) was performed using “WINAXIL” software program.
Blood spectrum using portable spectrometer (PXRFS)

Si drift detector (US$9,900.00)

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

Blood spectrum using portable spectrometer (PXRFS)
Iron concentration on blood samples by NAA and XRF (using PXRFS)

![Graph showing iron concentration with confidence intervals and experimental data for NAA and XRF.]

**The portable apparatus can be used in clinical laboratories.**

Confidence Interval:
- 68%
- 95%

Experimental Data:
- ● : NAA
- □ : XRF

Using the same blood sample.
**Conventional Analyses**

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

**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 µL

**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

<table>
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<tr>
<th>Conventional Analysis/Technique</th>
<th>Urine/Quantities (ml)</th>
<th>Execution Time Days</th>
<th>(*) Cost US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcemia (Ca)/Colorimetry</td>
<td>20.0</td>
<td>1-2</td>
<td>22.78</td>
</tr>
<tr>
<td>Cloremia (Cl)/Colorimetry</td>
<td>5.0</td>
<td>1-2</td>
<td>27.82</td>
</tr>
<tr>
<td>Iron/Plasma Emission Spectroscopy</td>
<td>7.0</td>
<td>15</td>
<td>348.55</td>
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<tr>
<td>Magnesemia (Mg)/Colorimetry</td>
<td>5.0</td>
<td>2</td>
<td>39.62</td>
</tr>
<tr>
<td>Calemia (K)/Ion-Selective Electrode</td>
<td>5.0</td>
<td>1-2</td>
<td>25.20</td>
</tr>
<tr>
<td>Natremia (Na)/Ion-Selective Electrode</td>
<td>10.0</td>
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Actualized: 13/06/13

### Alternative techniques

**Quantity: 100 µL**

- 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.
Conventional Clinical Analysis

Serum and Plasma samples

2ml serum
4 ml blood
0.5m serum
0.5m serum
1 ml serum
3 ml plasma
2 ml serum

Colorimetry

Br*

Ion Specific Electrode

K, Na

Dry Chemistry

Mg

Conventional Clinical Analysis

Alternative Analytical techniques

Blood sample

0.1 mL

NAA and XRF

Simultaneous determination: Br, Cl, Ca, Fe*, I**, K, Mg, Mn, Na, P, S, Zn*

Execution Time
FRX: <30 minutes
NAA: 1 hour

**I determined by NAA

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

* execution time ~ 7 -10 days

I: not determined in serum, plasma or blood
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
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.

Whole saliva is the mixture of these glandular secretions, bacteria and epithelial cells

Ca and S in whole saliva are good biomarkers

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

Collaboration: IPEN, UNIBAN/Brazil
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 ~40µL of saliva
- ~30 tick were necessary for extraction of ~ 500µL
- 100 µL was fixed on filter paper

The elements concentrations determined in saliva samples

Saliva comparison of different *Amblyomma* species

Collaboration: IPEN/ Instituto Butuntan, Brazil
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 (100μL) 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

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

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

I and Cu can be used as biological markers for ARF
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MUCHAS GRACIAS!!!!!