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FACULTY OF PHARMACY AND BIOCHEMISTRY
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COST ACTION CA24131
European Network for radiation-detection based Research and Innovation addressing increasing societal CHallenges (ENRICH)
ENRICH

The Role of X-Ray Fluorescence-Based Methods in the Biological and Biomedical Fields: Applications and Challenges

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

1st ENRICH COST Annual Meeting, 14 – 16 July 2026, Krakow

Outline of the presentation

- Why Elemental Analysis Matters in Biomedicine
- X-Ray Fluorescence-Based Methods: Principles and Opportunities
- Current Challenges and Opportunities
- Biomedical Applications Across Biological Matrices
- Future Perspectives and Emerging Trends
- Acknowledgements

The Critical Role of Elemental Analysis in Health

✓ Elemental analysis is fundamental to understanding biological systems and maintaining human health. Precise quantification of elements in biological fluids is essential for both research and clinical diagnostics.

Why Elemental Analysis Matters in Biomedicine ?

Physiological Processes

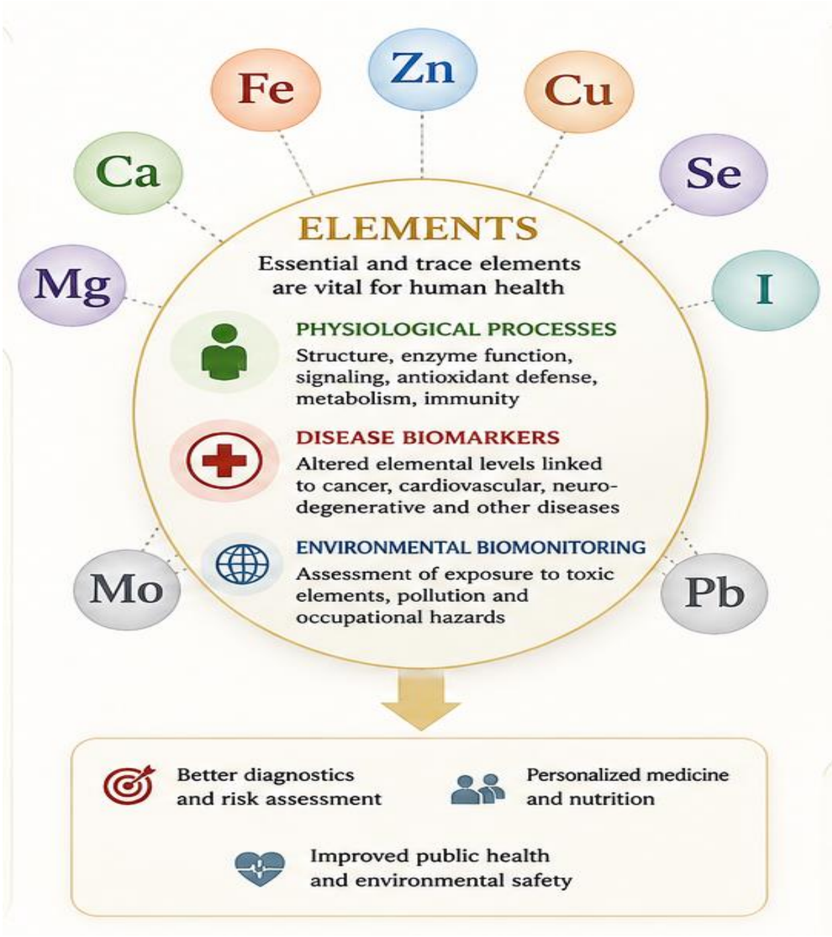
Elements are vital for numerous physiological functions.

Clinical Decision-Making

They serve as biomarkers for diagnosis, prognosis, and treatment

Environmental Biomonitoring

Essential for assessing exposure to environmental toxins.



Analytical Requirements for Biological Sample Analysis:

- ✓ Multielement capability
- ✓ High sensitivity for trace and ultra-trace elements
- ✓ Low limits of detection
- ✓ Rapid and simple sample preparation
- ✓ Minimal sample handling and contamination risk
- ✓ High accuracy, precision and reproducibility
- ✓ Compatibility with green analytical chemistry principles

Challenges in Elemental Quantification

- ✓ Quantifying elements in biological samples presents significant challenges due to their complex nature and the need for sensitive, accurate methods.

Matrix Complexity

Biological samples contain a diverse range of organic and inorganic compounds.

Limited Sample Amount

Often, only minute quantities of clinical samples are available for analysis.

Wide Concentration Range

Elements can exist from trace levels to major concentrations within the same sample.

Contamination Issue

The risk of contamination during sample collection and handling.

Instrumentation

Common methods for elemental analysis:

- ✓ Atomic absorption spectroscopy (AAS, FAAS, GFAAS)
- ✓ Inductively coupled plasma optical emission spectroscopy (ICP-OES)
- ✓ Inductively coupled plasma mass spectroscopy (ICP-MS)

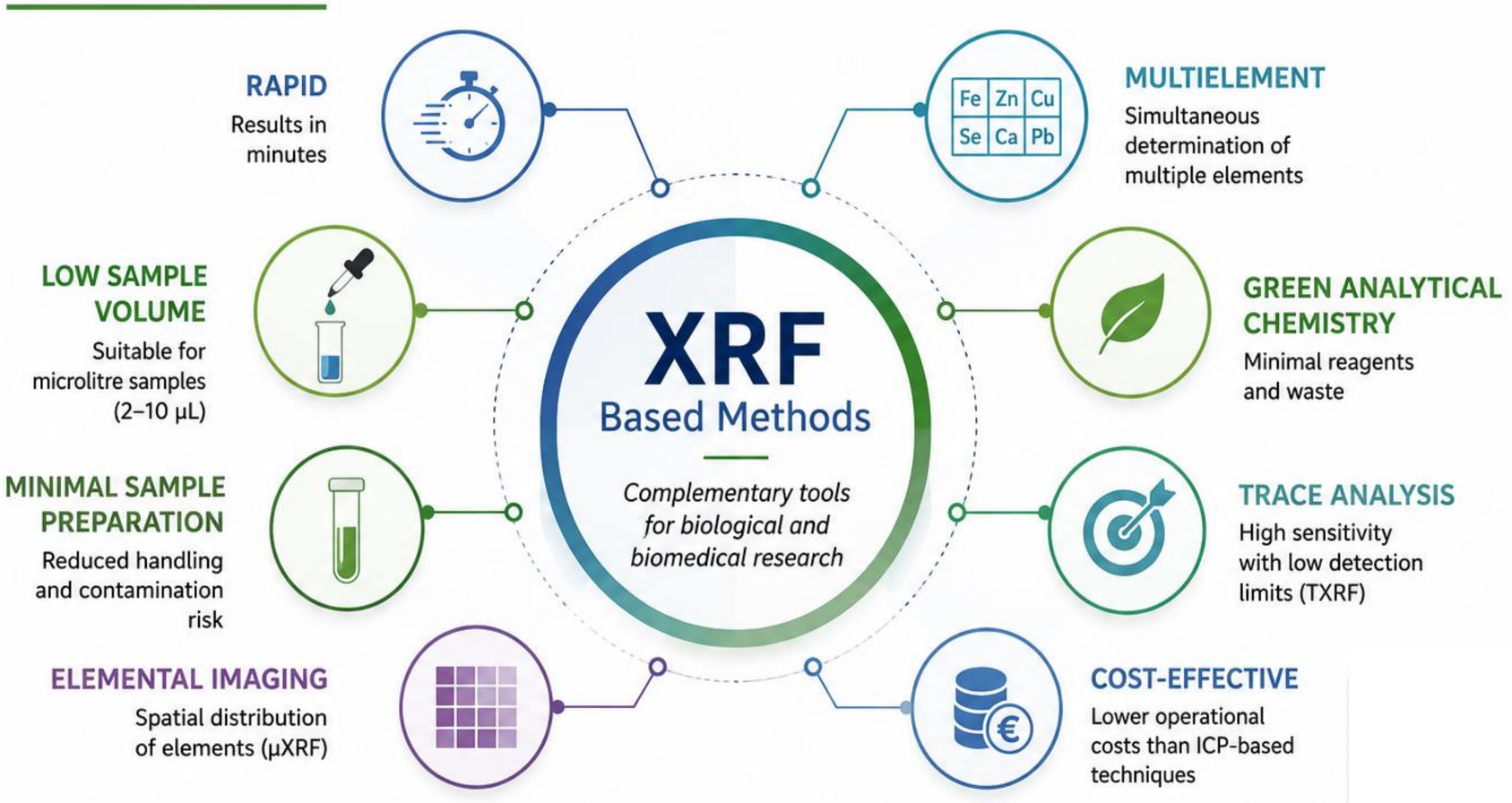
In recent years, efforts have been made to develop new screening or quantitative, low-cost analytical instruments that are more in line with the principles of environmentally friendly and sustainable analytical chemistry.

The requirements of such an analysis:

- **High demands** on laboratory environment and staff expertise.
- **Sample amounts** are often too high, unsuitable for limited clinical samples.
- **High dilution factors** or time-consuming sample digestion for organic matrix destruction, increasing error potential.
- **Restricted numbers** of analyzed samples per unit time.
- **High operational costs** for instrumentation and consumables.

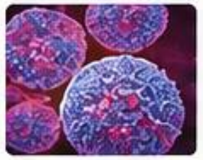
"There is a great interest in green analytical methods that offer multi-element capability with a minimum of sample treatment."

Why XRF-Based Methods for Biological Samples



XRF APPLICATIONS IN BIOMEDICINE

CLINICAL DIAGNOSTICS
- Nutritional status (Fe, Zn, Cu, Se, I)
- Metabolic and endocrine disorders
- Monitoring of therapy and prognosis



DISEASE RESEARCH
- Cancer biomarker discovery
- Cardiovascular & neurological diseases
- Inflammation and oxidative stress

ENVIRONMENTAL HEALTH
- Exposure to toxic metals (Pb, Cd, As, Hg)
- Pollution monitoring
- Vulnerable populations

FORENSIC & OCCUPATIONAL
- Toxicology and poisoning
- Occupational exposure assessment
- Forensic investigations

EMERGING APPLICATIONS
- Dried blood spots (POCT potential)
- Elemental imaging of tissues
- Multi-omics integration

Green Analytical Chemistry (GAC) Principles

GREEN REAGENTS

- Reduction of reagents
- Non-toxic, safe
- From renewable sources

MINIATURIZED & ENERGY EFFICIENT SYSTEMS

- Energy efficient
- Miniaturized
- Multiparametric

SUSTAINABLE SAMPLES

- Minimal number
- Minimal size
- Representative sampling

GAC
Green Analytical Chemistry

GREEN METHODS

- Automated, in-situ
- No sample treatment
- No derivatization
- Processes and operations integrated

OPERATOR SAFETY

- Safety ensured
- Reduced exposure
- User-friendly

WASTE MINIMIZATION

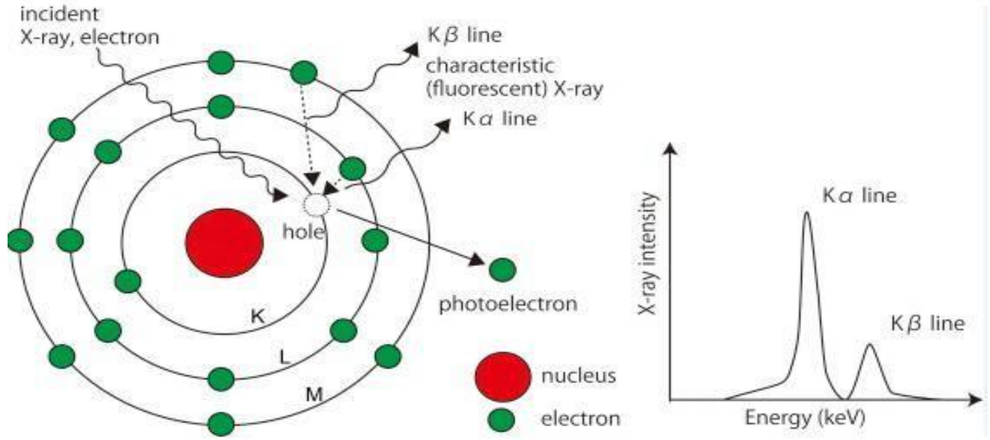
- Reduced volume
- Properly treated
- Recyclable / biodegradable materials

APPLICATION TO BIOLOGICAL SAMPLES

BENEFITS

- Environmentally friendly
- Cost-effective
- Sustainable
- Reliable results
- Better for health and society

XRF-Based methods: Principles and Opportunities

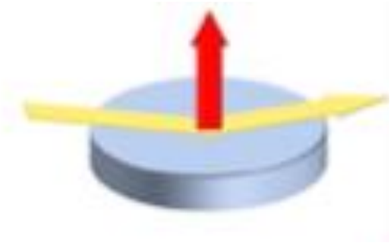


- ✓ In the X- ray Fluorescence (XRF) technique secondary (characteristic) fluorescence X-rays are excited from the elements present in any material using a primary X- ray source.

XRF
(EDXRF/WDXRF)

Bulk analysis
(macro)
e.g. Screening of solids

TXRF

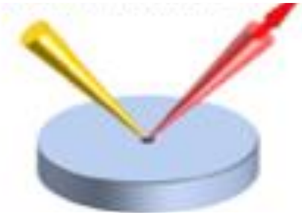


Bulk analysis (micro)
e.g. Trace analysis
of liquids

μ-XRF

Local analysis
Mappings (2D)
e.g. Spatial elemental
distribution

Confocal μ-XRF

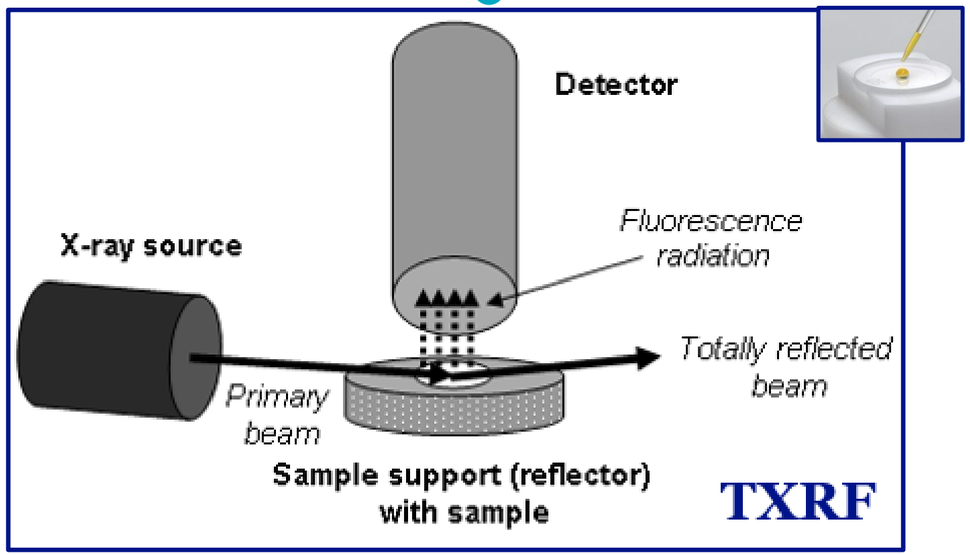


Local analysis
Mappings
(3D)

- **Lower sensitivity of EDXRF - non appropriate for the biological samples.**
- **A higher amount of sample is needed.**
- **A higher detection limit - due to the higher spectral background.**

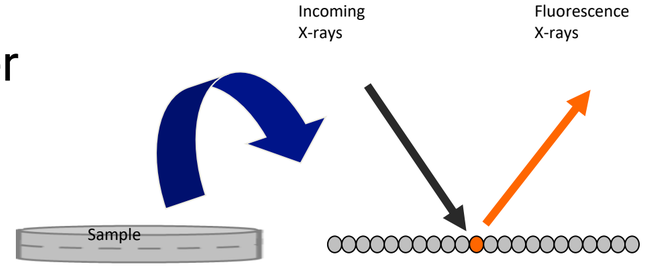
K. Nakano; Analytical Sciences 39(2023)1789-1790

The Attractive Features of TXRF for Biological Samples



- ✓General set-up of TXRF consisting of X-ray source, monochromator, second reflector with sample and detector
- ✓The primary beam hits the sample at a very small angle, $< 0.1°$
- ✓The detector is very close to the sample (0.5 mm) .

Sample as a thin layer

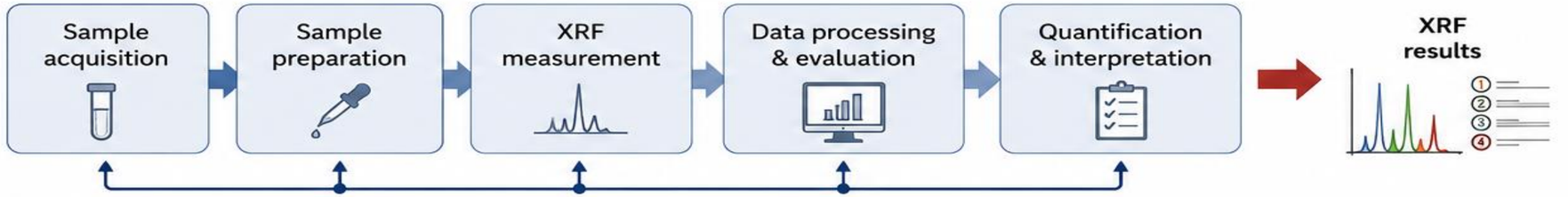


- Requirement of very small sample amounts e.g. a few μL *or* μg
- Samples must be prepared on a reflective media (polished quartz glass or polyacrylic glass disc).
- Dried to a thin layer, or as a thin film.
- Matrix effects are negligible.
- Simple quantification (Internal standardization).
- Detection limits comparable to other techniques, e.g. ICP- OES and ICP-MS.

The thickness and mass of the deposited sample are critical in order to avoid matrix effects

leads to an improvement in detection capability

Current Challenges and Oportunities



CHALLENGES

Sensitivity and detection limits
Need for lower LODs for trace and ultra-trace elements

Matrix complexity
High salt/protein content, interelement interferences, matrix effects

Sample preparation
Time-consuming, use of reagents, risk of contamination and analyte loss

Accuracy and reliability
Need for validated methods, CRM availability, quality control

Standardization and comparability
Lack of harmonized protocols and reference data

Data handling
Large datasets, multielement data interpretation

XRF

Unlocking the elemental information in biological samples

OPPORTUNITIES

Advances in instrumentation
Higher brilliance sources, detectors and optics → improved sensitivity

Miniaturization & automation
Smaller sample volumes, higher throughput, less user intervention

Green analytical approaches
Reduced reagents and waste, safer working conditions

New sample formats
Dried blood spots (DBS), microsampling, non/low-invasive sampling

Data science & chemometrics
Advanced data processing, AI/ML for pattern recognition and interpretation

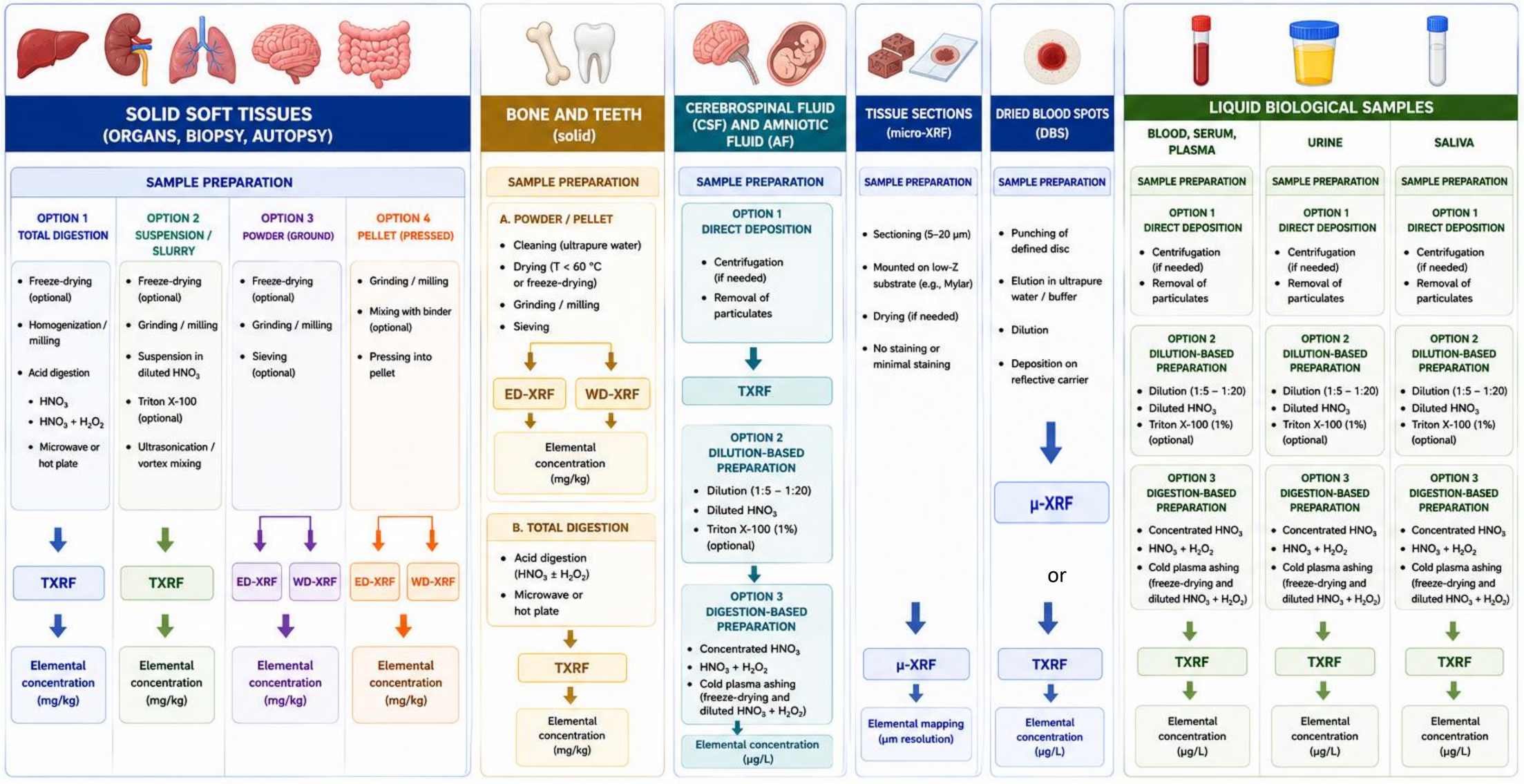
Broader applications
From clinical diagnostics to exposomics and precision medicine

OUR GOAL | Overcoming challenges while embracing innovation to deliver accurate, reliable and sustainable XRF-based solutions for biological and biomedical research.

Identified Issues in XRF-based analysis:

- Lack of reference materials
- No fit-for-purpose reference materials
- No standard operating procedures
- Little analytical rigor: from sampling to data analysis and quantification results

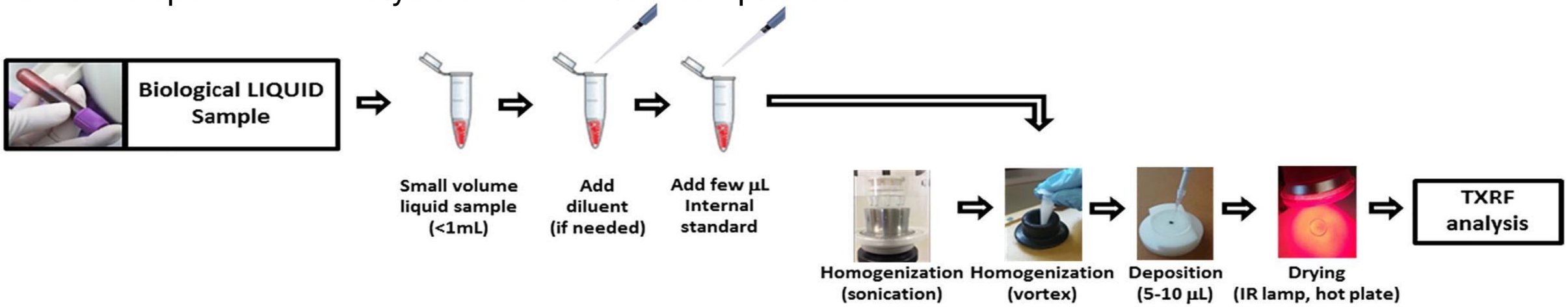
Biological Samples: From Fluids to Tissues



Examples: Human Biological Fluids

✓Human fluid samples may be directly analyzed (*for elements with an atomic number above 23)* or with a simple dilution step (*depending on the complexity and protein content of the biological matrix*).

The main steps in TXRF analysis of human fluid samples are:



Sample preparation pocedure will also depend on the type of sample and analyte to be determined.

Quantification:

$$c_i = \left(\frac{N_i C_{iS} S_{iS}}{N_{iS} S_i}\right)$$

where Ci: analyte concentration, Ni: analyte net peak area, Cis: IS concentration, Sis: instrumental sensitivity for the IS, Nis: IS net peak area, Si: instrumental sensitivity for the analyte.

Examples: Human Biological Fluids

Whole blood

- ✓ **Direct analysis of blood samples by TXRF**– **not possible** (residue was too thick and was detached from the surface of the reflector).
- ✓ Seronorm™ Trace Elements whole blood L-1

Sample Preparation strategy:

Dilution Method

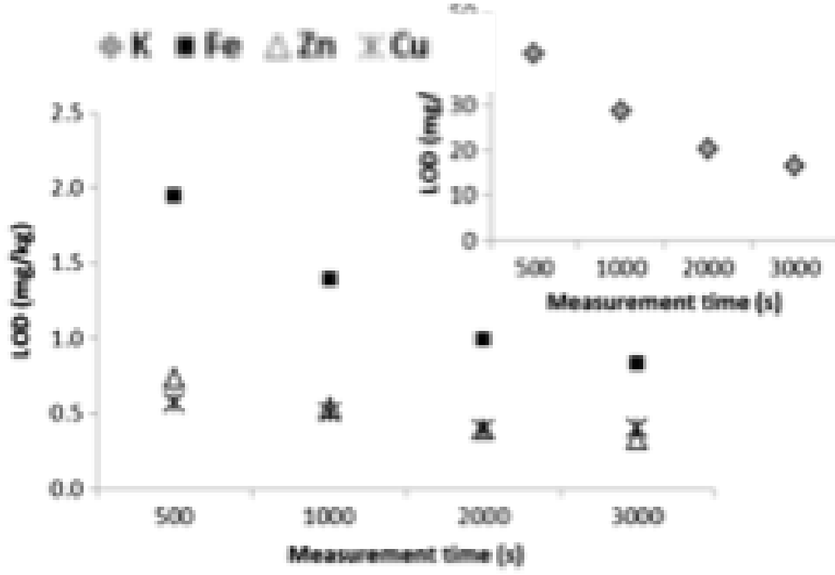
- **Sample weight:** 0.1 g of whole blood
- **Internal standard:** 0.05 g of Ga (100 mg/kg)
- **Dilution:** 1:5 with 1% Triton X-100
- **Homogenization:** Vortex mixing for 10 s
- **Deposition:** 10 µL onto quartz glass reflector
- **Drying:** Infrared (IR) lamp
- **Measurement time:** 2000 s
- **W-TXRF system**

Limits of detection (mg/kg) for the analysis of whole blood samples by TXRF after a dilution step (measurement time: 2000 s) :

- LOD: 0.2 – 1.1 mg/kg
- LOD for K; Ca: 24.9; 14.4 mg/kg (**absorption issues**)
- The LOD can be significantly reduced by using the TXRF system with **Mo X-ray tube**.
- Accuracy: 88.5-102.3% (R=80-120%)
- Precision: 5.7-8.1% (RSD≤20%; >4 mg/L)
- For Cu (< 1 mg/L): RSD=26 %

	Sample deposition volume	
Element	**5 µL/LOD**	**10 µL/LOD**
Cu	0.18 µg/L	0.06 µg/L
Zn	0.14 µg/L	0.05 µg/L

2. Measurement time

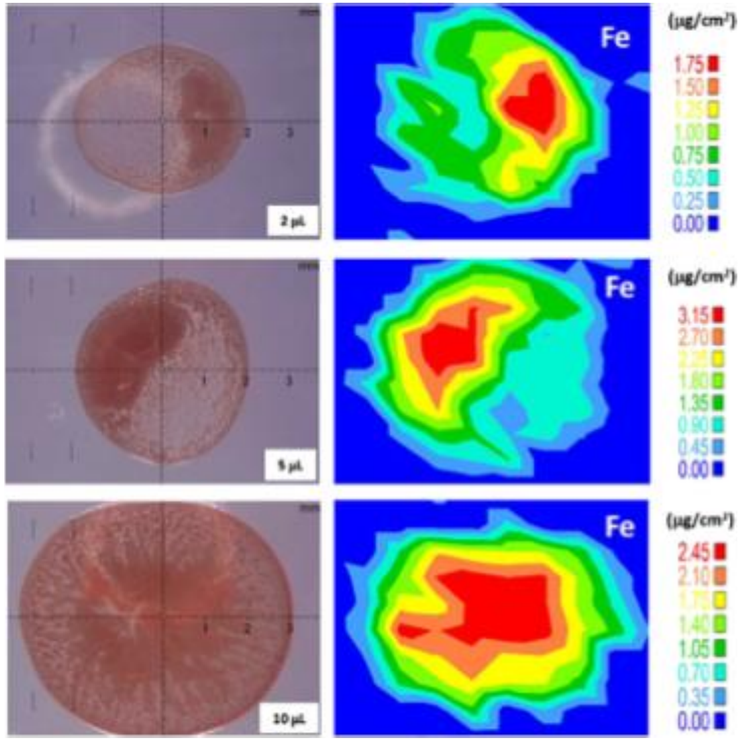


3. X-Ray tube (e.g. W or Mo)

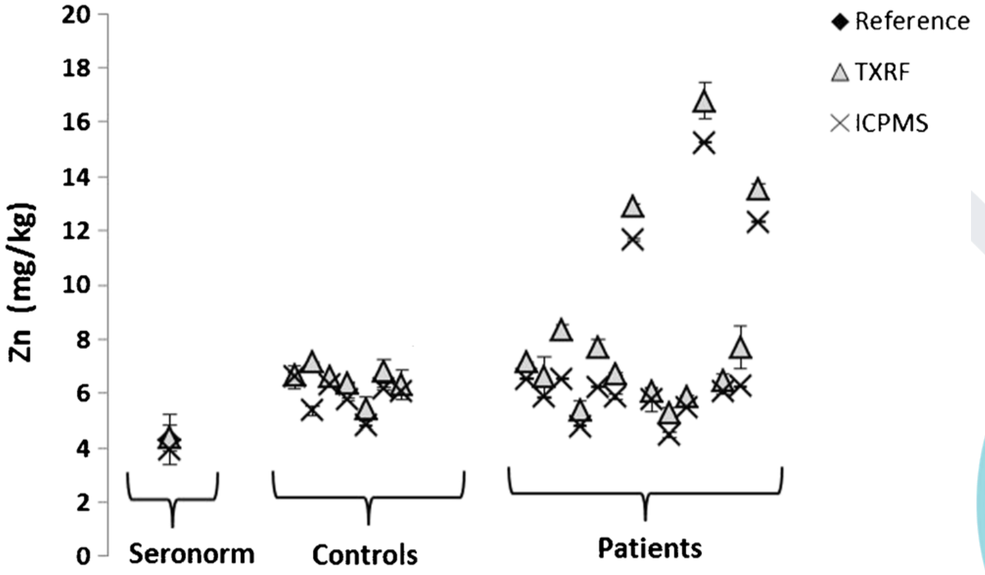
- *Marguí et al., ABC , 411 (2019)1659–1670*

Examples: Human Biological Fluids

μ-XRF



Effect of sample deposition volume on the shape and iron distribution (μg/cm2) of the solid residue on the reflector.



Although the TXRF method has lower sensitivity than ICP-MS, it offers several advantages for blood analysis, including simpler sample handling and quantification as well as lower measurement costs.

- *Marguí et al., ABC , 411 (2019)1659–1670*

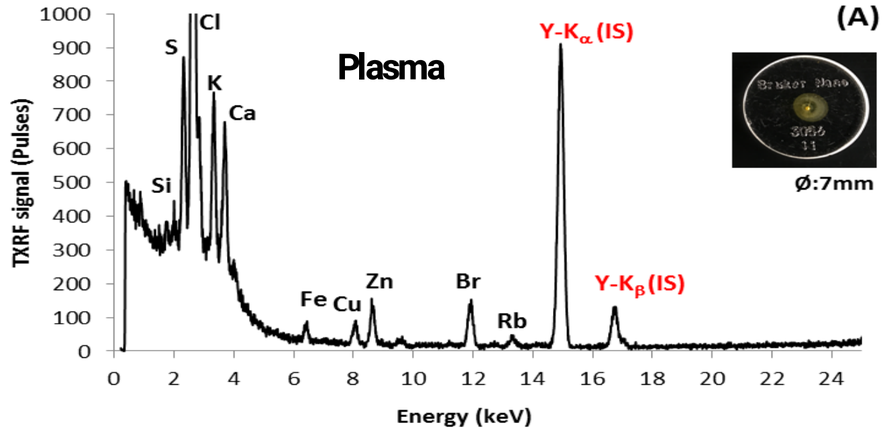
Examples: Human Biological Fluids

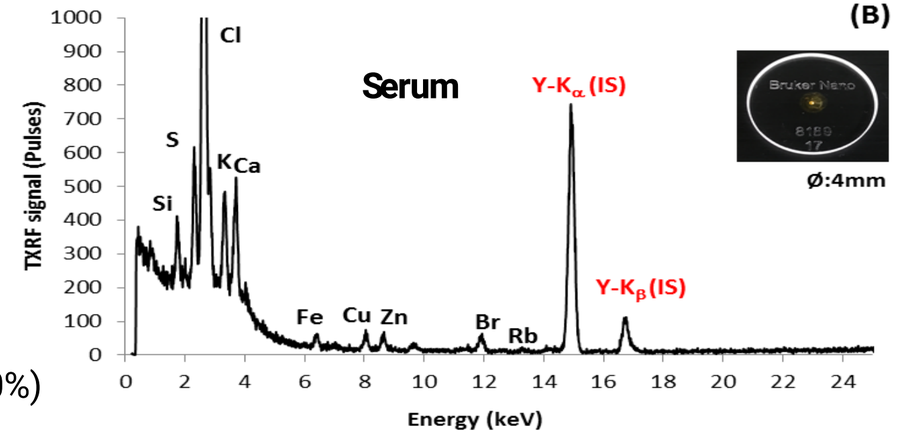
Serum & Plasma

Sample Preparation strategy:

Direct Method

- **Sample weight:** 0.25 g of serum or plasma
- **Internal standard:** 0.025 g of Y (100 mg/kg)
- **Homogenization:** Vortex mixing for 10 s
- **Deposition:** 12 µL of serum or 20 µL of plasma onto quartz glass reflector
- **Drying:** hot plate
- **Measurement time:** 2000 s
- **W-TXRF system**





- LOD: 0.03 – 0.20 mg/kg (plasma samples)
- LOD: 0.05 – 0.33 mg/kg (serum samples)
- Accuracy: 85-110 % and 85-115 %(R=80-120%)
- Precision: 3-11 % (RSD≤20 %; for TXRF)

- Fast "screening" - *advantage of TXRF over other atomic spectrometry techniques.*
- Possibility of easy determination of Br - *difficult to measure with other spectrometric methods.*
- In the case of light elements, the dilution of the sample is mandatory for obtaining quantitative results.
- The background at low energies (higher for plasma samples) - *due to a higher protein concentration.*
- *Mid Z-elements - negligible issue with background.*
- The element content in plasma and serum samples follows the same pattern. So it seems that both fluids can be used as biomarkers.

- *Jablan et al., Talanta 233 (2021)122553*

Examples: Human Biological Fluids

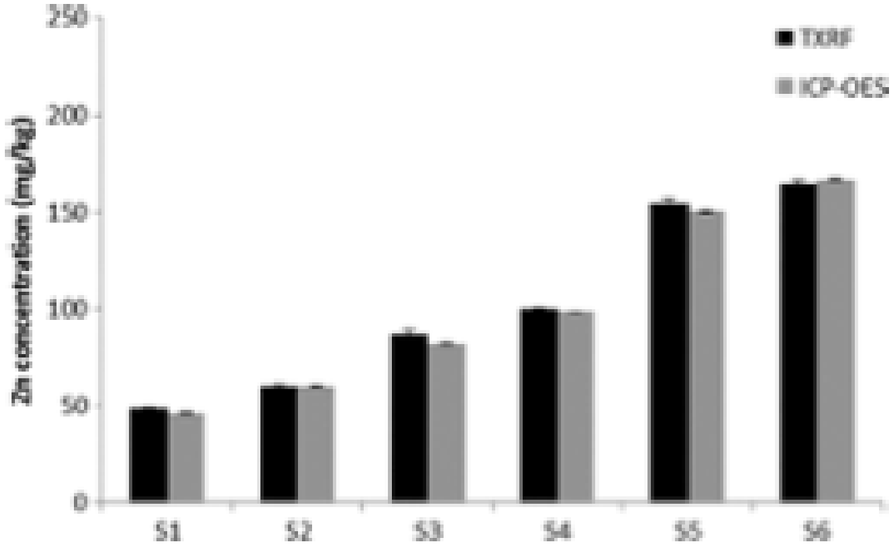
Seminal plasma

✓ **Direct analysis of seminal plasma samples by TXRF**– **not possible** (residue was too thick and was detached from the surface of the reflector).

Sample Preparation strategy:

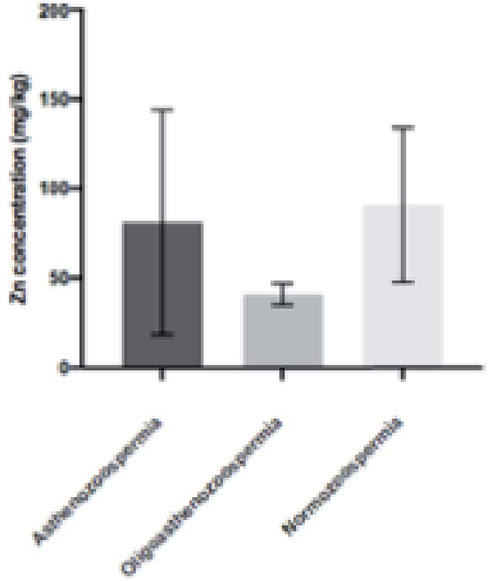
1. Dilution & TXRF

- **Sample**: 0.2 g of seminal plasma
- **Internal Standard (IS)**: 0.05 g of Y (10 mg/kg)
- **Dilution**: 1:1 with 1% Triton X-100 solution
- **Homogenization**: Vortex mixing
- **Deposition Volume**: 5 μL on quartz glass reflector
- **Drying:** Infrared (IR) lamp
- **Measurement time:** 2000 s



- LOD: 0.04 – 0.3 mg/kg
- LOD for K; Ca: 2.6; 1.9 mg/kg (> 50 mg/kg)
- Accuracy: 83-114% (R=80-120%)
- Precision: 2.7-12.3% (RSD≤20%)
- Only Zn could be quantified and some differences in Zn concentration were found between the groups studied .

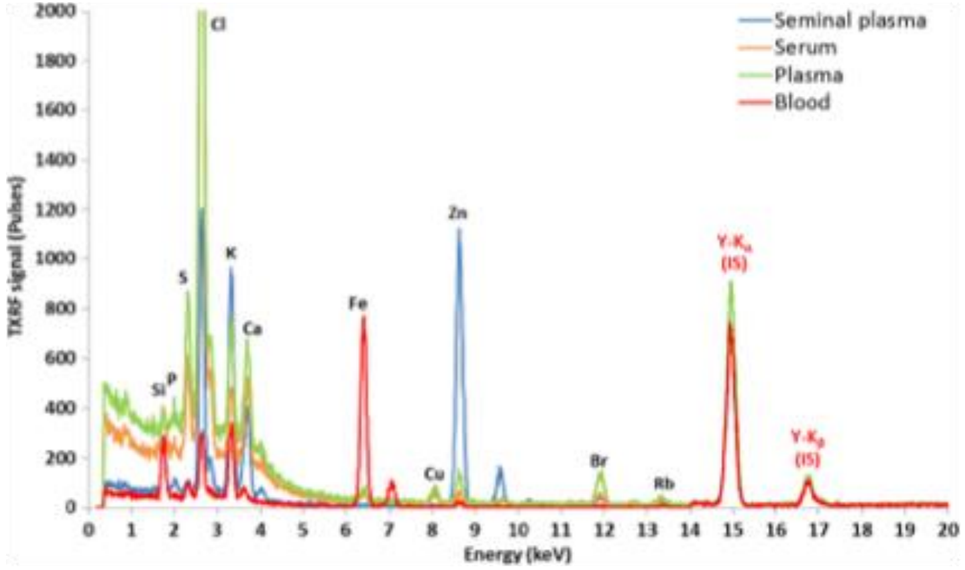
The Zn content in SP samples from patients with different diagnoses.



- *Jablan et al., Anal. Methods, 12 (2020)4899 - 4905*

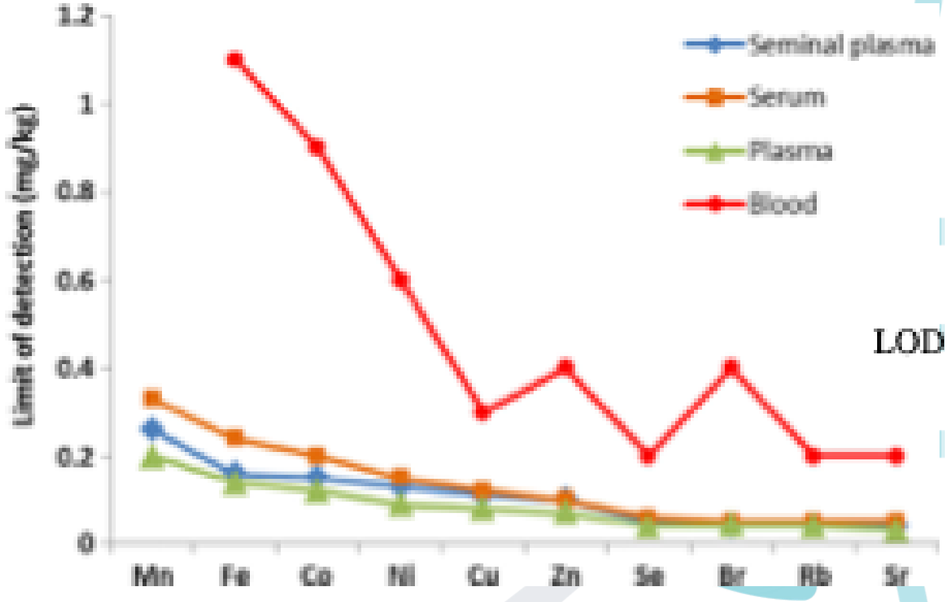
Examples: Human Biological Fluids

- ✓ Comparison of TXRF spectra obtained in the analysis of seminal plasma (dilution 1:1), serum (without dilution), plasma (without dilution) and whole blood (dilution 1:5).



- The analytical signals in the light elements region are decreased due to the dilution factor;
- The background is also decreased.

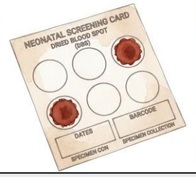
- ✓ Limits of detection estimated for seminal plasma, serum, plasma and blood sample analysis by TXRF.



$$LOD = \frac{3C_i\sqrt{N_{bkg}}}{N_i},$$

- The dilution factor greatly affects the determination of trace and ultratrace elements.

Examples: Dried Blood Spot



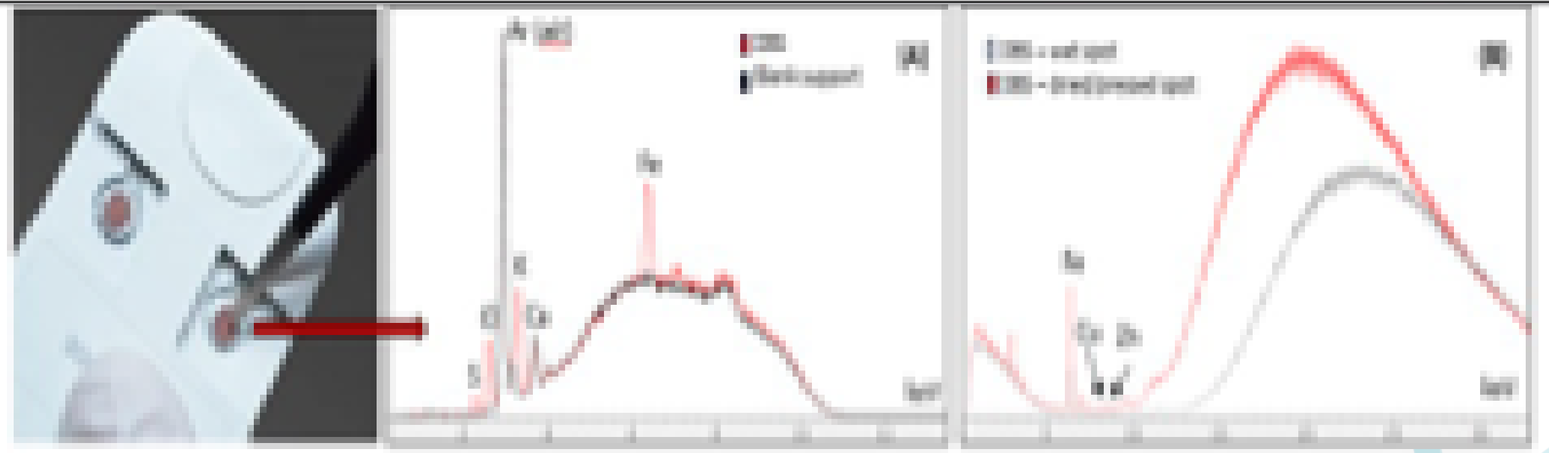
Clinical Potential

- Neonatal and pediatric screening programs.
- Point-of-care testing in remote or resource-limited settings.
- Retrospective analysis of archived dried blood spot (DBS) cards.

neonatal metabolic screening.

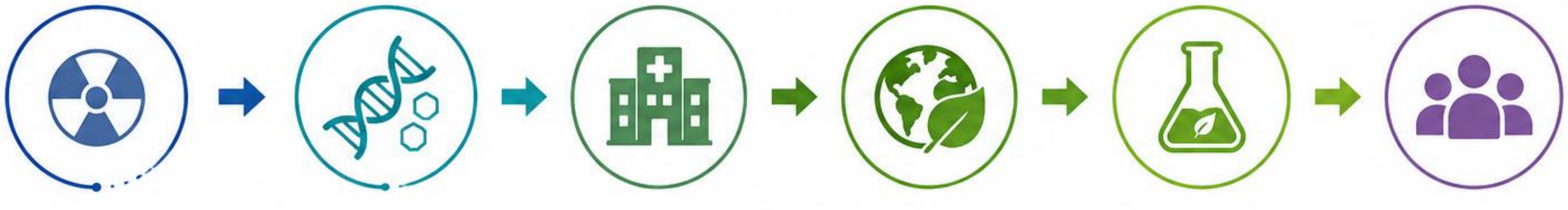
Preliminary Findings

✓ μXRF maps reveal spatial distribution of **Fe, Zn, and Cu** across the spot with quantifiable elemental signals and minimal sample preparation.



- **μXRF spectra of DBS (Seronorm, W tube, 300 s): (A) DBS vs. blank; (B) effect of sample preparation. Figure shows preliminary μXRF analysis of DBS, detecting several biomedically relevant elements. Signal enhancement by drying and pressing enables detection of additional elements (e.g., Cu, Zn), with further optimization expected to expand the detectable range.**

Why is this topic relevant for ENRICH CA?



RADIATION-BASED ANALYTICAL METHODS

XRF techniques (TXRF, μXRF) use radiation detection to obtain elemental information.

BIOMEDICAL RESEARCH

Elemental analysis of biological samples improves understanding of health, disease and biological processes.

MEDICAL DIAGNOSTICS

Elemental biomarkers support early diagnosis, disease monitoring and personalised medicine.

ENVIRONMENTAL BIOMONITORING

Human biomonitoring provides insight into exposure to essential and toxic elements.

SUSTAINABLE ANALYTICAL CHEMISTRY

Minimal sample preparation, low reagent consumption and reduced waste for greener and safer analytical workflows.

INTERDISCIPLINARY COLLABORATION

Bridging physics, chemistry, biology, medicine and data science to tackle societal challenges.

ENRICH

XRF-based methods bridge radiation detection, analytical chemistry and biomedical research, fully supporting the interdisciplinary vision of the ENRICH COST Action.

Challenges on the Road to Clinical Translation

Translating XRF methods from research into routine clinical practice requires addressing five interconnected challenges:

Challenge	Possible Solution
Matrix effects	Optimized sample preparation, matrix-matched standards
Detection limits	Synchrotron XRF, TXRF geometry
Standardization	Certified reference materials (CRMs)
Clinical validation	Harmonized protocols
Data interpretation	AI-assisted analysis, chemometrics

Acknowledgements

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COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

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Acknowledgements