EMRP
European Metrology Research Programme
Programme of EURAMET

Publishable JRP Summary for Project T2J10/TRACEBIOACTIVITY. Traceable measurements for biospecies and ion activity in clinical chemistry

Project rationale

Traceable measurement results in clinical chemistry are mandatory in Europe. The legal requirement is a consequence of the EU in vitro diagnostic medical devices (IVD) directive (Directive 98/79/EC) which demands traceability to higher order standards. The need for the implementation of this requirement triggered the foundation of the Joint Committee for Traceability in Laboratory Medicine (JCTLM), a cooperation between the International Federation for Clinical Chemistry (IFCC) , the International Laboratory Accreditation Cooperation (ILAC) and the Metre Convention (CIPM). Within this framework, measurement methods and materials are evaluated which can serve as the required higher order reference points and which are linked with the SI as closely as possible. Reference and test laboratories through Europe directly profit from this newly developed system in a fast and efficient way.
The aim of the JRP is to develop and to provide the required reference points for biospecies and electrolytes, which are of key importance as health status markers for critical diseases like heart attack and depression. It has been shown recently that specific species are highly effective in cancer treatment and drugs
Current reference standards are restricted to the determination of the total amount of an element. It is well understood, however, that the clinical effect depends on identities and quantities of element species, or ion activities.
The increasing use of heteroatom-containing drugs (e.g. sulphur and selenium metabolites) to sensitise cancer cells to chemotherapy is an emerging area that requires analytical methods to study the distribution of sulphur and selenium at very low concentrations in biological fluids (e.g. human serum) and human cancer cells. The development of measurement methods for the reliable quantification of Selenium biospecies at concentrations close to the detection limits (e.g. at low parts-per-billion levels) in low micro-litres volume of biomedical matrices is a major challenge of this project. Calcium is one of the most frequently measured analytes in clinical chemistry (50.000.000 determination p.a., occasioning costs of about 100.000.000 Euro). Predominantly ion selective electrodes (ISE) are used for the measurement of ionised Ca in human serum and in whole blood. Currently used reference systems, however, cannot provide metrological traceability. Estimated deviations of ISE measurement results from the “true value” are of about ± 5% (relative) which is twice of the allowed uncertainty range. The project focuses on the development of activity reference standards and on on-line calibration procedures providing traceability to reference points.
The excellent scientific and metrological expertise of the project partners from various backgrounds will exploit a high degree of synergy.

Project Objectives

Traceable reference systems and biomedical matrix based reference materials for the selected health status markers will be developed as a basis for a substantial improvement in quality, safety and appropriateness of laboratory test results. The following specific tasks will be pursued

- Reference methodologies for the quantification and identification of toxic and essential heteroatom-containing species in bioclinical samples will be developed
- A system shall be established for measurement and calibration of chemical activity of ionic species in physiological matrices whose values are traceable to the SI

- The metrological basis of traceability for conductivity measurements of pure water, including steps toward establishing a practical standardised definition of electrolytic conductivity will be worked out.
- A Bayesian approach to modelling and data analysis will be applied to measurements in the field of laboratory medicine and clinical chemistry for the first time. The new methods and respective tools to compute reference values and their associated uncertainties will be demonstrated on practical examples.

Workplan

1. Development of methods for the quantification and identification of Selenium biospecies
   Advanced high-resolution separation techniques hyphenated with element-specific detection (ICP-MS) and molecule-specific detection (ESI or MALDI MS/MS) for species identification in combination with species-specific isotope dilution mass spectrometry (SS-IDMS) will be evaluated for this purpose.
2. Development of an interface for use of high-resolution micro and nano-LC systems and element-specific mass spectrometry detectors for the analysis of biological micro-samples.
3. Development and evaluation of activity scales for key electrolytes based on Pitzer's approach
4. Improvement of measurement systems and calibration procedures for the activities of mono- and divalent ions
5. Evaluation of a model for bulk transport and interaction in very dilute aqueous systems
6. Design and development of an facility to control low level contamination in circulating ultra-pure water
7. Evaluation of new strategies and practical procedures for systematically elaborating parametric models for analytical measurements in clinical chemistry
8. Development of methods to support quality assurance of measurement results in test laboratories

Progress to date

08-07-03
The project commenced on May 1 2008. Establishment of the infrastructure (instruments and personnel) has started. A study on efficient extraction methodologies for speciation analysis of heteroatom containing biomolecules is in progress. Work has commenced on selection and purity determination of reference material candidates. The design of a facility to control low-level contamination in ultra-pure water has been started. Investigations of requirements for improved mathematical tools in the European clinical sector are in progress.

08-10-31
The first period of the project comprised, to a large extend, start-up activities such as recruitment of staff and method developments. But first results are also available.
WP 2: Metallomic approaches to study element speciation in biosamples
The development of methodologies for the quantification and identification of selenomethionine (SeMet) and methyl-Se-cysteine (SeMC) was started. PTB, LNE and LGC have proven their ability to deliver accurate results for total Se in a complex biological matrix with 2% expanded uncertainty through participation in the international intercomparison CCQM-K60/P86.1 on the "Characterisation of selenised wheat flour for its total Se and selenomethionine content" coordinated by LGC.
WP3: Metrological aspects of chemical activity measurements of essential ionic species
Starting materials for candidate reference material for an automated flow through measuring system were selected, procured and portioned. An automated flow through measuring system has been established and is operative. Calibration experiments have been performed with

mixed electrolyte standards. Models on the consistency of the Pitzer activity scale for mixed electrolytes were developed and discussed.
WP4: Ionic contamination of ultra-pure water
Experimental set-ups for low range conductivity at a primary level will be designed and the design of primary cells will be improved in accordance. An equivalent circuit model was used to model the phenomena in low range conductivity measurements. Simulation results were compared to experimental data performed on a cell designed for pure water conductivity measurement. Also, a contamination setup has been designed and construction is in progress.
WP5: Data Analysis
Suitable data sets from interlaboratory comparison/proficiency testing schemes (PT) have been selected for the evaluation of the relevant statistical models under consideration. The studies comprise organic pollutants in water (BIPEA), creatinine in blood (AFSSAPS) and constituents in human serum (IRMM, IMEP17).

09-04-30
WP2: A ^{76}Se-enriched selenomethionine (SeMet) spike material has been produced by LGC. This material has been characterised *in house* for isotopic composition (99.81 ± 3.09% (k=2) ^{76}Se enrichment), homogeneity in terms of total Se and SeMet content and its stability assessment after storage of this material at different time and temperature is in progress. This material has been distributed to partners of WP2 to assist them in the development of HPLC-ICP-MS species-specific isotope dilution methods for the quantification of SeMet in biological fluids (e.g. serum).
WP3: SI-traceable assay values for chloride salts are based on coulometric chloride-determinations from SMU are used as the reference values. The sum of quantified trace impurities agrees with the chloride content.The salts can be used as starting materials to set up the activity base scale. METAS worked out a protocol to produce mixed activity standards. A new prototype flow system was realized at PTB.
WP4: A review article on charge transport in ultra-pure water has been drafted; the article is being extended with results based on a recent CCQM pilot study (P83).
Impedance spectroscopy data over wide frequency ranges were collected for various aqueous solutions over a wide range of conductivity values (0.05 mS/m – 1 S/m) and in different conductivity cells (Jones type, piston type, coaxial) by PTB, INRIM and DFM.
WP5: An investigation of the requirements of the European clinical sector for improved mathematical tools was carried out and a priority list was established

09-10-31
WP2: Production and characterisation of a ^{76}Se-enriched SeMet spike was completed. Isotopic composition, homogeneity and stability were investigated. This material has been made available to other partners for IDMS method development.
Method development for total determination of Se in serum by isotope dilution MS with collision reaction cell ICP-MS has been carried out at LGC.
A methodology for screening of Se species in food/supplements has been developed at PTB.
At UME, initial experiments on the determination of SeMet in real serum samples by ion-exchange HPLC-ICP-MS equipped with collision/reaction cell system have been carried out.
LNE and LGC have collaborated on the development of a systematic approach to the accurate quantification of serum Se-albumin (Se-Alb) *via* HPLC-ICP-IDMS quantitation of SeMet.
WP3: The characterisation of the high purity chloride salts is accomplished. The activity scale according to Pitzer's approach is established including the uncertainty evaluation. A flow system at PTB and METAS is operative and programs have been written to control the measurement systems. Data handling (archiving, storage and data evaluation) is automatised at METAS.
WP4: The review article on charge transport in ultra-pure water has been completed with contributions from the WP partners, including the results from analysis of a recent low-conductivity comparison (CCQM-P83). It was submitted 'Electrochemica Acta'.
Data evaluation and modelling of Impedance spectroscopy over wide frequency ranges is in progress.

WP5: *New tools (Bayesian probability theory) for interlaboratory comparison data: W*ork at different metrological levels was conducted. LNE works on a statistical model applied on PT among routine laboratories. PTB works on both, key comparisons performed by CCQM, and interlaboratory comparisons among reference and routine laboratories. An investigation was carried out of the Nordtest approach for calculation of measurement uncertainty of test results based on quality control and validation data.

10-04-30
WP2: Different sample preparation procedures followed by isotope dilution mass spectrometry as a reference methodology for the determination of the total selenium amount in human serum were developed and validated at LGC and LNE using the reference material BCR-637. Further progress was made by UME, PTB, LGC and UT on the development/validation of methodologies for the accurate quantification of selenium species (particularly, selenomethionine (SeMet)) in human serum.
WP3: The measurement flow systems at METAS and PTB are operational. The calibration procedures for the ion activities of the monovalent ion species Na^{+}, K^{+}, Cl^{-} were established according to schedule. A series of ionophores for the divalent ions Ca^{2+} and Mg^{2+} were tested for their suitability using mixed activity standards.
WP4: The assembly of an ultra-pure water (UPW) contamination set-up was finished. First conductivity measurements in ultra pure water were performed.
A comparison of conductivity measurements at UPW level with calculable cells (PTB, DFM) and a dilution system (SP) was performed. Initial results show an agreement in the order of 1 %.
WP5: The first application of statistical models for the evaluation of PT data among routine laboratories is on organic micro pollutants. The methodology has been applied to artificial survey data and accepted for presentation and publication.
A tabular “library” was developed that assigns to any kind of relevant influencing quantities and parameters in analytical measurement an appropriate PDF, the deduced expectation value and uncertainty. The “library” supports the proper evaluation of knowledge about input and influence quantities in analytical measurements.

Potential impact of project

Correct measurements in clinical chemistry are of fundamental importance in medicine as a whole and for every individual patient. Traceable measurements are legally required in Europe and are obligatory in many countries for this reason. The methods developed in this JRP shall be used as reference points for traceability for clinical test laboratories in Europe to the benefit of the patients and cost reduction in health care.
Currently, internationally accepted standards based on primary methods linking to the clinically active forms of elements are not available. The method developed in the project will go beyond this limit and provide such tools. This comprises reference methods for metal biospecies and electrolytes like Calcium2+ Such health status markers are essential in diagnosis of critical diseases like heart attack and depression. Further to that, it has been shown recently that specific species are highly effective in cancer treatment and drugs based on such species are increasingly applied in cancer therapy. This will be done in cooperation with European Pharmacopeia, CEN WG 332 and IFCC WG SEB and IUPAC.

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Publishable JRP Summary

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