Traceable measurements for biospecies and ion activity in clinical chemistry

EURAMET
European Association of National Metrology Institutes

The need for the project

Traceable and comparable measurements in clinical chemistry are a mandatory requirement of EU legislation (In Vitro Diagnostic Medical Devices 'IVD' - Directive 98/79/EC). However, the measurement of biospecies in blood serum is currently restricted to the determination of their total amount, although it is well understood that a 'clinical' effect often depends on the identity and quantity of biospecies or ion activities rather than the total amount.

What is required is the ability to identify and quantify biospecies and measure ion activity in clinical samples. This is particularly important for calcium, one of the most frequently measured analytes in clinical chemistry, and heteroatom-containing drugs (e.g. sulphur and selenium metabolites), which are used to sensitise cancer cells to chemotherapy.

This project aimed to provide internationally accepted reference points to calibrate new and existing measurement systems in the medical diagnostic field.

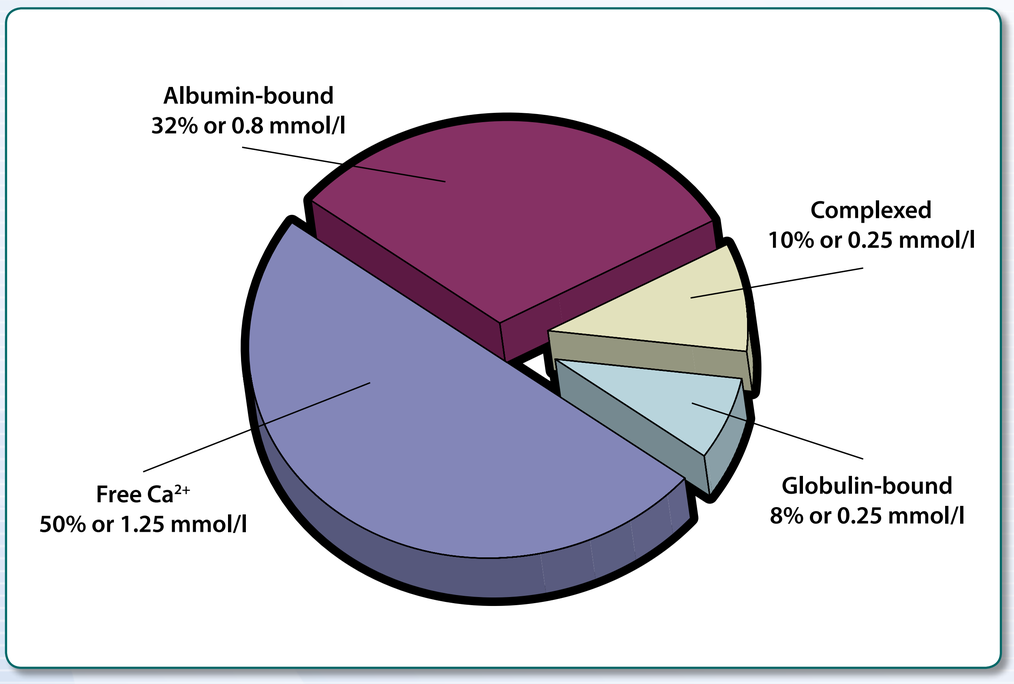
Photo of the closed loop measurement setup for conductivity measurements in purified water with controlled electrolytic contamination: the temperature control box can be seen on the right hand side, containing the conductivity measurement cell, the pump and the contamination vessel; the right hand side shows the purification system for highly purified water, the dosage unit for contamination and the ion chromatograph.

Technical achievements

Reference methodology for the accurate trace measurement and identification of toxic and essential heteroatom-containing species in human serum: selenomethionine (SeMet) and methyl-Se-cysteine were selected as target analytes. ^{76}Se-enriched SeMet was produced and characterised as a labelled spike, which was then used for species-specific isotope-dilution mass spectrometry method development. Enzymatic and acid hydrolysis procedures were also developed to extract and quantify SeMet from albumin. A largely accelerated extraction procedure was achieved by microwave-assisted extraction without compromising SeMet extraction efficiency.

A system for the SI traceable measurement of the ionic species activity in physiological matrices: the ions focused on were sodium, potassium, chlorine, magnesium and calcium. High purity materials were characterised on a primary level and subsequently used to prepare gravimetrical mixtures as calibration standards. The unknown activities, the free biological active part of the compounds in these mixtures, were calculated based on established model calculations. Measurement systems as well as measurement methods including defined procedures for signal acquisitions and data evaluation, were developed in order to quantify the ion activity and measurement uncertainty.

An experimental set-up for low range conductivity of highly purified water at a primary level: the conductivity of highly purified water is an indispensable reference point for activity measurements and quality control parameters for pharmaceutical purposes.



Pie chart indicating different calcium species in serum. Only the ionised free calcium (Ca^{2+}) is physiologically relevant.

Input to clinical trials

Developed methods that have been used in an ongoing European Clinical Trial *Selenium and Prostate Cancer: Clinical Trial on Availability to Prostate Tissue and Effects on Gene Expression*. The study is run by Wageningen University, the Netherlands, and is a double-blind, randomised, placebo-controlled intervention trial aimed at examining the effects of selenium supplementation. The trial examines the relationship between dietary selenium intake and changes in gene expression profiles that might be responsible for selenium-induced chemoprevention.

Intercomparison of SeMet measurements

Measurement methods for SeMet in human serum have been validated by an international intercomparison. The intercomparison (EURAMET TC-MC project 1165) included National Metrology Institutes and expert laboratories (e.g. NIMT Thailand, CCS Switzerland, DIMCI/DQUIM Brasil).

New measurement procedures

Submitted new measurement procedures for ion activity to the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

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