EMRP Call 2011 - Health, SI Broader Scope & New Technologies

Selected Research Topic number: **SRT-h17**
Version: 1.0

Title: Metrology for metalloproteins

Abstract

Metallomics; the science of metallobiomolecules, is a relatively new and rapidly growing research field. It includes the study of metalloproteins, (i.e. proteins containing a metal cofactor) such as platinum-containing proteins, (iron-)transferrin, selenoproteins, C-reactive protein, superoxide dismutase and ceruloplasmin, which are used in the clinical diagnosis and treatment of diseases. Although metalloproteins are used in the treatment and diagnosis of disease, there are currently no reference values or standard methodologies for metalloprotein identification. Therefore, traceable methods and reference standards to accurately quantify and characterise metalloproteins urgently need to be developed.

Conformity with the Work Programme

This Call for JRPs conforms to the EMRP Outline 2008, section on “Grand Challenges” related to Health on pages 7 and 8 and in the sections on pages 21 and 22.

Keywords

metalloproteins, traceability, quantitative diagnostic, multimodal approach, treatment,

Background to the Metrological Challenges

Metalloproteins such as platinum-containing proteins, (iron-)transferrin, selenoproteins, C-reactive protein, superoxide dismutase and ceruloplasmin are currently used in the treatment and diagnosis of many diseases including cancer, diabetes, Down’s syndrome and dementia (due to Alzheimers or Parksinson’s disease). However, despite their wide spread clinical use, there are currently no reference values or standard methodologies to accurately quantify and identify metalloproteins. This lack of basic metrology is decreasing the clinical efficacy of metalloproteins as there is no reliable quality control of diagnostic assays or scope for accurate inter-laboratory comparisons.

The lack of metrology for metalloproteins also conflicts with current legislation and standards. The *in vitro* devices (IVD) Directive 98/79/EC [1] states that ‘the traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order’. Whilst standards ISO 17511:2003 [2] and ISO 18153:2003 [3] require the provision of reference measurement systems, including reference measurement procedures for the determination of analytes in samples of human origin.

For the quantitative determination of metalloproteins, new methods need to be developed with increased sensitivity and selectivity compared to those currently used (i.e. immunoassays, immunoturbidimetry, immunonephelometry or fluorometry). For example, methods based on mass spectrometry such as inductively coupled plasma mass spectrometry (ICP-MS) or isotope dilution mass spectrometry (IDMS) are highly sensitive and could be used to identify metalloproteins at low concentrations (e.g. (iron-)transferrin which is found in human serum at concentrations between 15 to 300 ng/mL). In addition, Surface-Enhanced Raman Scattering (SERS) could be explored as a new method for protein quantification. SERS allows the identification of molecules from specific vibrational bands and can provide highly accurate quantification when combined with isotope-dilution. SERS could also be used for the identification of metalloproteins with sample amounts in the nanogram-picogram range, as could nano-HPLC with micro-nanosample ranges.

The use of complementary methods is also required, for example mass spectrometry (e.g. ICP-MS, MALDI-TOF-MS and ESI-Q-TOF-MS) integrated with an appropriate separation method. The separation of target metalloproteins from body fluids will be challenging, however methods such as size exclusion

chromatography, capillary electrophoresis for specific isoforms, reversed phase HPLC, affinity chromatography, field flow fractionation should be investigated as well as methods for the determination of metalloprotein distribution in tissues.

Scientific and Technological Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the JRP-Protocol.

The JRP shall focus on the measurement and characterisation of metalloproteins for diagnosis and treatment of e.g. cancer.

The specific objectives are:

1. To develop accurate methodologies for metalloprotein identification and quantification. Methods may include:
   - mass spectrometry coupled to separation methods (chromatography or field flow fractionation),
   - new methods such as Surface-Enhanced Raman Scattering (SERS), which can be combined with isotope dilution mass spectrometry (IDMS).
   - methods for micro- and nanosample detection
   - methods for multiple metallospecies detection.
2. To develop new and reliable methodologies for the separation of metalloproteins in body fluids and for the determination of metalloprotein distribution in tissues.
3. To prepare and characterise isotopically labelled spike materials and stable, validated reference materials.
4. To develop reliable and traceable primary measurement methods using isotopically labelled metalloproteins.

The materials under investigation and the applied methods need to be carefully selected and prioritised along the stakeholder needs, such as by refering to recommendations of the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

For all objectives, proposers should consider current standards and legislation (e.g. the European *in vitro* device (IVD) Directive 98/79**/**EC).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners and industry is strongly recommended, both prior to and during methodology development.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this, including the currently funded EMRP project T2 J10 Tracebioactivity; ‘Traceable measurements for biospecies and ion activity in clinical chemistry'.

The total eligible cost of any proposal received for this SRT is expected to be around the 2.7 M€ guideline for proposals in this call.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the “end user” community. This may be through the inclusion of unfunded JRP partners or collaborators, or by including links to industrial/policy advisory committees, standards committees or other bodies. Evidence of support from the “end user” community (eg letters of support) is encouraged.

You should detail other impacts of your proposed JRP as detailed in the document “Guide 4: Writing a Joint Research Project”

You should detail how your JRP results are going to:

- feed into the development of urgent documentary standards through appropriate standards bodies
- transfer knowledge to the medical community.

You should also detail how your approach to realising the objectives will further the aim of the EMRP to develop a coherent approach at the European level in the field of metrology. Specifically the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of Member States and countries associated with the Seventh Framework Programme whose metrology programmes are at an early stage of development to be increased
- outside researchers & research organisations other than NMIs and DIs to be involved in the work

Time-scale

The project should be of up to 3 years duration.

Additional information

The references were provided by PRT submitters; proposers should therefore establish the relevance of any references.

[1] European *in vitro* device (IVD) Directive 98/79**/**EC

[2] ISO 17511:2003 In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials.

[3] ISO 18153:2003 In vitro diagnostic medical devices -- Measurement of quantities in biological samples -- Metrological traceability of values for catalytic concentration of enzymes assigned calibrators and control materials