EMRP Call 2012 – Open Excellence, Industry and SI Broader Scope

Selected Research Topic number: **SRT-s17**
Version: 1.0

Title: Traceability for biologically relevant molecules and entities

Abstract

Bio-measurements need to be made traceable to the SI. To achieve this, the amount of substance of biologically relevant molecules and entities needs to be quantified via enumeration and with the help of calibration material. For selected targets, e.g. cells, DNA, haemoglobin variants, and other large bio-molecules such as prioritised by the JCTLM, this SRT calls for research to develop specific procedures, which are necessary to apply enumeration as a primary method for quantification of e.g. cells or viruses, namely to identify the respective target entities, to study influences of matrix effects and to derive corresponding systematic uncertainties. Traceability for manufacturers and end-users should be established by reference measurement procedures and reference materials. Internationally agreed measurement methods and associated protocols for the characterisation of purity for biological macromolecules intended for use as calibrators in SI traceable biological measurements should be developed.

Conformity with the Work Programme

This Call for JRPs conforms to the EMRP Outline 2008, section on “Grand Challenges” related to Industry & Fundamental Metrology on pages 8, 13 and 14.

Keywords

Traceability, enumeration, biological entities, DNA, haemoglobin, cell counting, biometrology, proteins, purity characterisation, reference measurement procedures, single molecule detection, reference material.

Background to the Metrological Challenges

Accurate counting of biological entities underpins many sectors including healthcare, security, environment, biotechnology, and food. Examples include viral load monitoring and host cell contamination in vaccines and biopharmaceuticals. Better agreement and accuracy of measurement data is needed. Non-reliable measurements can lead to economic burdens and fatalities (e.g. from allergenic ingredients in food), to false results for patient diagnostics and to false results for pathogen safety testing in blood donation.

The 2011 BIPM report “Study of Measurement Service and Comparison Needs for an International Measurement Infrastructure for the Biosciences and Biotechnology” clearly identified the measurement services, international comparisons and collaborative R&D needed to underpin the comparability of bio-measurement, based on identified needs for metrology support from industry and regulators. These needs included the key requirement of “Support for fundamental metrology, aimed at making bio-measurements traceable to the SI” in accordance with ISO17511.

A number of different measurement technologies are used for cell concentration measurements. These are frequently used to analyse various target cells in blood, cerebrospinal fluid, urine and in bone marrow biopsies. The results are used to decide patient treatments or to initiate further diagnosis. These technologies lack traceability to harmonised European standards and guidelines (e.g. higher order reference measurement procedures) are not available for these measurements and external quality assurance is based on national regulations. Generally, comparisons between laboratories are based on consensus values and do not necessarily reflect medical needs.

EURAMET, EMRP-MSU
National Physical Laboratory
Hampton Road, Teddington,
Middlesex, TW11 0LW, UK

Phone: +44 20 8943 6666
emrpA169@npl.co.uk
www.euramet.org

EMRP
European Metrology Research Programme
Programme of EURAMET

The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

Previous attempts to assign SI values to nucleic acid reference materials have proved difficult. However, recent developments in absolute molecular measurement approaches (see below) offer the potential for performing higher order measurements to which more precise values can be assigned.

Digital PCR (dPCR) transforms the exponential, analogue nature and single molecular sensitivity of classic PCR into a linear, digital signal. Single molecules are isolated by dilution and individually amplified by PCR; each product is then analysed separately. This is achieved by partitioning a sample prior to PCR amplification such that each reaction chamber contains 0 or ≥1 copies of target DNA. A Poisson correction can be factored into the result to account for chambers that contain more than one molecule, and an absolute target sequence quantity estimated. This partitioning of the sample also has the effect of diluting out the background signal and increasing the signal-to-noise ratio of low-abundance targets. dPCR has potential as a highly sensitive, precise and reproducible method for the molecular quantification of DNA targets; accuracy, however, relies on a number of assumptions which are hard to validate without independently measured reference standards with low uncertainty.

Next generation sequencing (NGS) relies on ultra high throughput sequencing of many millions of individual fragments of DNA, giving a count proportional to the numbers of copies of each sequence present. NGS technologies have, to date, mostly been applied to qualitative studies. Recently NGS has been applied to quantitative measurement and it may offer a solution to the dual issues of identification and counting of individual target sequences. However, NGS needs to be evaluated for its potential for absolute molecular counting. An accurate representation of the starting material is critical if NGS is to be considered for higher order measurement. The sample preparation steps, prior to sequencing, need to be evaluated for bias and variability. Also, the potential of NGS to quantify traces of nucleic acid contaminant in purified DNA solutions needs to be evaluated.

Enumeration technologies rely on two assumptions: i) That all the relevant entities present are counted; ii) That only the relevant entitles present are counted. Uncertainties arise, respectively, from selection of the population and the test group to be counted, and from the identification of the relevant entities. Counts are also subject to stochastic variation. Traceability to higher order references requires that these uncertainties are addressed. Model biological measurement systems need to be used to identify and develop strategies for addressing these uncertainties.

The lack of metrologically sound purity characterisation methods for large biological molecules forms one of the largest barriers to implementation of SI traceability for biological measurement. The following techniques are routinely used: For nucleic acids, routine verification relies on UV absorbance, which is informative, but not quantitative. For modest molecular masses (oligomers) liquid chromatography is applicable. For larger entities (plasmids, genomic DNA etc) sequencing information confirms identity of the majority components. Gel electrophoresis can provide semi-quantitative assessment of nucleic acids of similar molecular mass. For RNA, gel electrophoresis is also used to check semi-quantitatively for nucleic acid impurities. For proteins and peptides, liquid chromatography offers a separative method. Variants of electrophoresis, particularly 2-D gels, also provide sensitive, but only semiquantitative, indications of other proteinaceous material. In addition to the routine techniques, NMIs have more experience of applying multiple independent methods for purity certification, which can reduce the uncertainty available from individual techniques.

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 traceable quantification of the amount of substance of biologically relevant molecules and entities via enumeration and with the help of calibration material. Biological materials shall include cells, DNA, haemoglobin variants, and other large bio-molecules such as prioritised by the JCTLM. The project will exploit emerging technologies to provide enhanced structural information.

The specific objectives are

1. To establish traceability of biological measurements to the mole by enumeration of nucleic acid, proteins, and larger objects like cells, bacteria and viruses
2. To develop specific procedures, which are necessary to apply enumeration as a primary method for quantification of e.g. cells or viruses, namely to identify the respective target entities, to study influences of matrix effects and to derive corresponding systematic uncertainties.

- Procedures may be based on quantitative fluorescence techniques in microscopy, flow cytometry and real-time PCR, digital PCR, next generation sequencing and single molecule detection.
- Methods of uncertainty characterisation should be developed for each procedure, including the uncertainty of the count (stochastic variation), the uncertainty of the selection of the population for counting, and the uncertainty in the identification of the entities being counted (selectivity).

3. To develop reference measurement procedures and reference materials for the use by manufacturers and end-users.
4. To develop internationally agreed measurement methods and associated protocols for the characterisation of purity for biological macromolecules intended for use as calibrators in SI traceable biological measurements. These could include the measures necessary to establish traceability and measurement uncertainty for the purity values on higher order biologically relevant materials used as primary calibrators in biological measurements.

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes. To enhance the impact of the research work, the involvement of the larger community of metrology R&D resources outside Europe is recommended. A strong industry involvement is expected in order to align the project with their needs and guarantee an efficient knowledge transfer into industry.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this.

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. The available budget for integral Research Excellence Grants is 42 months of effort.

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 (e.g. letters of support) is encouraged.

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 healthcare, security, environment, biotechnology, and food sectors.

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

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 and includes the best available contributions from across the metrology community. 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.