European Partnership on Metrology
Call 2023 –Fundamental Metrology, Industry, Normative and Research Potential

Selected Research Topic number: **SRT-f15**
Version: 1.0

Title: Fundamental metrological considerations when measuring number concentration of biological entities using the unit one

Abstract

Number concentration of biological entities are routinely measured using the unit 1. In health, food safety or biotechnology, such numbers are counted directly or estimated using indirect methods traceable to other units and converted to numbers. Yet these measurements often assume identity and overlook sources of bias. Because these fundamental metrological concepts are not addressed, reported numbers of biological entities cannot easily be realised within the SI system and are frequently inaccurate. This project will develop novel methods and strategies to improve traceability and better characterise uncertainty when measuring number and concentration of biological entities within the SI system.

Keywords

Cell counting; digital PCR (dPCR); flow cytometry (FCM); inductively coupled plasma mass spectrometry (ICP-MS); limit of detection (LOD), mass spectroscopy (MS); nucleic acid (NA) concentration; protein concentration.

Background to the Metrological Challenges

Accurate quantification of number concentration of biological entities underpins sectors including food/water safety, healthcare, biotechnology and environmental monitoring. Within these sectors, number-based measurements using the unit 1 are widely used by industry, regulators, research institutes and testing laboratories and correctly quantifying identified biological entities is essential.

Regulations exist for both *in-vitro* medical devices, environmental microbiological contamination and food safety. Improved traceability for number-based biological entity measurement will lead to iterative improvements enabling refinement of regulations, enhanced product specifications or better support the development of new technologies. Knowledge developed in (for example) the medical sector is not readily transferable to other sectors. This is an example where the 'sector agnostic' measurement science proposed by this project can have impact.

The CCQM strategy 2021-2030 identified a wide range of sectors in which life science measurements are made and reported, directly or indirectly, using numbers (and the unit 1). CCQM working groups are actively conducting studies and comparisons using numbers and identifying the wider challenges. This includes smaller proteins often reported using the mole, however protein-based measurements are increasingly focused on complex macromolecular entities such as viral particle count. This necessitates a link between mole and the unit 1 to be investigated with more rigor enabling traceability to SI such as that stipulated in the recently updated ISO17511:2020 and ISO15189:2022.

Current State of art.

While the numbers of biological entities are measured in a wide variety of sectors many of the methods are shared.

Bacterial numbers are measured for safety considerations. Directive EU 2020/2184 on water quality for human consumption details strict requirements of bacteria per 100 mL and the methods of analysis on foodstuffs sets limits for specific bacteria per g or per mL and analytical reference methods. However, the strict limits stated in the methods to conduct these measurements are known to be inaccurate.

Identity is challenging when counting bacteria as additional methods are needed to corroborate identity. When mixtures of bacteria are present, it makes test performance (e.g. antibiotic resistance testing) challenging to assess.

Eukaryotic Cell counting is typically recorded as number concentration, or per unit area for adherent cells. Cell counts requiring more detailed knowledge of **identity** are used to analyse various blood cells per unit volume. Requirements for cell-based counting indices have been defined by regional regulatory frameworks and supported by quality management systems, reference methodology and standards. External quality control across Europe generally conducts comparisons using consensus values which may not reflect medical needs..

Viruses: Viral titre is routinely reported but is challenging to measure due to small size (20 nm - 200 nm). This necessitates the development of high-resolution microscopic techniques (such as electron microscopy) for direct measurement and the correlation with orthogonal and indirect methods (such as nucleic acid analysis) outlined below. **Identity** will be explored by this project including whether a measurement is detecting a viral particle, if that particle is intact, contains cargo, or is capable of infection.

Indirect methods and/or methods that use different units to estimate copy concentration

When estimating cells or viral number (load) molecular assays used for **nucleic acid (NA) quantification** typically generate analogue signals based on population averages measuring the entities genomic material. Copies or international units (IU) are typically obtained through comparison to a reference standard. Assumptions are made, after correction for known factors (such as chromosome number), that a NA quantity corresponds directly to the entity in question, but the error associated with this is usually undefined. Developments in absolute molecular measurement approaches (digital PCR) offer higher order measurements for value assignment, yet the transition from NA measurement to estimation of associated biological entity requires more attention. **Proteins** in a biological entity can be measured using mass spectrometry and related to the number per biological entities with assumptions including the uniqueness of the peptide marker used to detect the protein. These assumptions have uncertainties associated to them, and whilst the initial protein quantification has established mechanisms to ensure traceability to the SI, the assumed conditions to relate this concentration and uncertainty to the biological entity do not.

The metrological deficit associated with different direct and indirect methods to estimate biological entity number was highlighted during the race to develop COVID-19 diagnostic tests where a variety of potential diagnostic solutions targeted NA and protein. As a result, the WHO published target product profiles stipulating required analytical performance criteria. These were described using number based unitage using the viral genome as the reference measurement. Yet there remains limited evidence to support such an assumption. The project will provide solutions to this and similar analytical questions using selected pan sector models for virus, bacteria and eukaryotic cell number measurements.

Per Unit Volume.

Most measurements of numbers of biological entities are requested per unit volume and/or refer to the entity within a matrix that is often reported in volume and the traceability of this important denominator is often overlooked. Tolerances of volumetric measuring devices are rarely included in uncertainty estimation and gravimetric measurements rarely performed. Consequently, the impact on the accuracy of such analytical steps is unknown. Furthermore, the errors associated serial dilutions or concentration steps prior to analysis is not typically included in the measurement uncertainty of biological entities.

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 protocol.

The JRP shall focus on routes to improve capturing the contribution of uncertainty from volume associated with routine biological entity measurement per unit volume.

The specific objectives are:

1. To improve traceability of biological measurements by developing, optimising and validating analytical techniques that use counting methodology (such as microscopy, FCM, dPCR, ICP-MS) to gain a high accuracy evaluation of biological entity number concentration (e.g. cells, bacteria or viruses). This project will reduce uncertainty to <10% and identify and define mathematically sources of reduced specificity.
2. To investigating assumptions applied to procedures for indirect estimation of the number of biological entities, such as utilising DNA copies as a surrogate for virus or cell numbers. This will include analytical approaches to characterise factors associated with interferences and stability that may lead to systematic error. The project will investigate the effect of differing LOD and dynamic range

on performance error and how molecular structure, fragment size, number per biological entity, etc. contribute to impurity when used to estimate the numbers of complex biological entities.

3. To explore how dimensional measurements contribute to error when determining concentration (per volume or mass) of biological entities by establishing traceable volume measurement for at least two measurands.
4. To develop analytical methods and statistical models for improved metrology solutions using outputs and methods from objectives 1-3.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (NMIs/DIs), research organisations (e.g. CCQM working groups CAWG, NAWG and PAWG), standards developing organisations (e.g. ISO TC 212, ISO TC276) and end users (e.g. academic and clinical research laboratories, test laboratories, diagnostic kit manufacturers, food/water safety, healthcare, biotechnology and environmental monitoring).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes. Proposers shall give priority to work that aims at excellent science exploring new techniques or methods for metrology and novel primary measurement standards, and brings together the best scientists in Europe and beyond, including other European Partnerships, whilst exploiting the unique capabilities of the National Metrology Institutes and Designated Institutes.

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

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 2.0 M€ and has defined an upper limit of 2.5 M€ for this project.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 40 % of the total EU Contribution across all selected projects in this TP.

Any industrial beneficiaries that will receive significant benefit from the results of the proposed project are expected to be beneficiaries without receiving funding or associated partners.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the 'end user' community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your JRP results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Facilitate improved industrial capability or improved quality of life for European citizens in terms of personal health, protection of the environment and the climate, or energy security,
- Transfer knowledge to the biological sector.

You should detail other impacts of your proposed JRP as specified in the document "Guide 4: Writing Joint Research Projects (JRPs)"

You should also detail how your approach to realising the objectives will further the aim of the Partnership to develop a coherent approach at the European level in the field of metrology and include 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 EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work.

Time-scale

The project should be of up to 3 years duration.