European Partnership on Metrology
Call 2022 – Digital Transformation, Health, Integrated European Metrology, Normative and Research Potential

Selected Research Topic number: **SRT-h15**
Version: 1.0

Title: Standardisation of targeted and untargeted quantitative metabolomic methods for future use in routine clinical diagnosis

Abstract

Quantitative metabolomics methods have the potential to revolutionise routine clinical diagnosis and prognosis through the provision of complete metabolic profiles. However, the metabolomics data currently generated is not comparable between laboratories and across the large timeframe required to generate the data from large clinical studies. Therefore, standardisation is required to ensure that the results are traceable to a higher order reference and to meet current *in vitro* diagnostic regulatory standards. Full engagement with European and international metabolomics community driven harmonisation activities is required, as well as the provision of the reference standards and reference materials needed to assure patient data.

Keywords

Artificial intelligence, biomarkers, calibration, clinical diagnosis, internal standards, *in vitro* diagnostics, machine learning, metabolomics quantification, reference materials and standards, standardisation, traceability for precision medicine, uncertainty

Background to the Metrological Challenges

When operated in a targeted manner, mass spectrometry-based metabolomics can be used by clinical laboratories for routine monitoring and quantification of steroids, vitamins and therapeutic drugs. However, when operated in an untargeted approach, thousands of molecules can be simultaneously monitored. If properly calibrated, global quantitative biochemical information could be provided for a patient, making personalised precision medicine an affordable reality. However, the quality of these complex biomedical measurements is a key problem in medical diagnostics. This has been addressed in the *in vitro* diagnostic (IVD) medical devices regulation (EU) 2017/746, which requires all manufacturers of IVD devices to provide information on the safety, performance and metrological traceability of assigned values. Thus far, the metrological tools developed by NMIs have only been applied to single or small groups of metabolite measurands. In addition, the provision of fully characterised matrix calibrators for thousands of measurands is not currently possible, therefore an alternative approach would be to use machine learning and artificial intelligence (AI) to identify biomarkers that could act as anchor points or “beacon markers” for the relative quantification of larger subsets of molecules. Other computer-based approaches could be used to identify signatures of disease, to interpret the impact of therapeutic interventions, and to improve prognosis.

Reference measurement systems, which are used to support the use of targeted metabolomic data, need to be developed, including the automated preparation of calibration standards, to improve the comparability of metabolomic methods. Reference measurement procedures also need to be developed for the characterisation of specific multiplexed metabolite standard solutions. These need to be suitable for use as calibrators and/or reference materials within the traceability requirements of relevant ISO standards.

The most widely used Certified Reference Material (CRM) is human frozen plasma (NIST SRM-1950), which supports the highly regulated IVD and clinical laboratory medicine community as they rely on certified values to calibrate their methods. Those developing untargeted metabolomic methods also use this CRM as a common long term reference QC material to enable comparison of results. As it is used as a stable source of pooled serum, many of the metabolites used in the normalisation algorithms are not certified. Therefore, when the material expires there are issues with the translation of the results between old and new standards, which

often inflates the measurement uncertainty, as a conversion factor may be required. Standardisation and harmonisation of materials and practices, including SI-traceable reference values, would be beneficial.

Untargeted metabolomics methods have the potential be used as clinical tests that can identify isolated disease cases. However, the results have to be expressed in agreed units with standardisation to higher order references, thus ensuring the comparability of the measurement results over time and between labs. This has recently been assessed using a combination of CRMs and reference standards combined with targeted metabolite quantification. The expression of SI-traceable results, using metabolite specific CRMs, enables both the comparison of measurement results between large metabolomics studies and it also provides results that can be compared with reference ranges, which is a clinically accepted approach. Based on this, a route to standardisation needs to be developed for the untargeted methods, which are used to generate large-scale quantitative metabolomic measurement results. A set of calibration strategies, including “beacon molecules” needs to be developed and internal standards need to be selected and prepared for converting instrument signals to SI units. Suitable algorithms and processing protocols also need to be developed.

The metabolomics community have made great efforts to harmonise their data, largely through the Metabolomics Quality Assurance and Quality Control Consortium (mQACC), which aims to harmonise and disseminate best practice for untargeted metabolomics methods and to encourage the prioritisation and development of reference materials. mQACC’s dedicated Reference and Test Materials working group (WG) and the standards WG of the German Society for Metabolomic research have assessed current needs for reference and QC materials. The groups continue to promote and improve the quality of metabolomics results via the use of system blanks, experimental blanks, experimental pooled QC samples and through the use of a stable standard reference material measurement within every sample batch. A European contribution needs to be provided to mQACC and new matrix reference materials need to be prepared in collaboration with them. This will lead to the establishment and promotion of QA/QC best practice.

The huge potential, which multiparametric methods, like mass spectrometry-based metabolomics, offer for clinical diagnosis, has yet to be translated from research into the more regulated clinical environment. The reporting of some high-profile failures resulted in the publication of 37 criteria for the use of omics-based predictions in clinical trials. These include the validation, accuracy, and quality assurance procedures necessary for using these approaches. Nonetheless the use of mass spectrometry-based metabolomics data in clinical laboratory medicine is gaining widespread acceptance. Therefore, the following actions are needed to further improve the uptake and use of real patient metabolomics measurement results that are fit for purpose. Collaboration with EU Biobanks is needed to ensure that the developed calibration approaches help clinical labs meet the ISO15189 standard. Estimates of the measurement uncertainty, repeatability, reproducibility and the trueness of the measurement results generated need to be included. In addition, the suitability of machine learning and artificial intelligence protocols needs to be assessed for use in metabolomic profiling, as well as the influence of the measurement uncertainty.

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 the standardisation of targeted and untargeted quantitative metabolomic methods for future use in routine clinical diagnosis.

The specific objectives are

1. To improve the comparability of metabolomic methods by establishing the reference measurement systems that are needed to support the use of targeted metabolomic data. This should include the fully automated preparation of calibration standards. Reference measurement procedures should be developed for the characterisation of specific multiplexed (> 15 measurands) metabolite standard solutions. These should be suitable for use as calibrators and/or reference materials within the traceability requirements of relevant ISO standards (ISO17511, ISO15193, ISO15194, ISO17034).
2. To develop the route to standardisation for the untargeted methods, which are used to generate large-scale quantitative metabolomic measurement results. This should include the development of a set of calibration strategies, including “beacon molecules” and the selection of internal standards for converting instrument signals to SI units. In addition, to prepare internal standards (serum, plasma, dried blood spot, urine) for use in traceably reporting untargeted metabolomics measurement results. This should include the development of algorithms and processing protocols.

3. To prepare > 3 matrix reference materials, which should be regularly improved and updated, in collaboration with the Metabolomics Quality Assurance and Quality Control Consortium (mQACC). In addition, to ensure a European contribution to the mQACC and to establish and promote QA/QC best practice.
4. To improve the uptake and use of real patient metabolomics measurement results that are fit for purpose. This should be addressed in collaboration with a group of EU Biobanks to ensure that the developed calibration approaches help clinical labs meet the ISO15189 standard. Estimates of the measurement uncertainty, repeatability, reproducibility and the trueness of the measurement results generated should be included. To assess the suitability of machine learning and artificial intelligence protocols for use in metabolomic profiling as well as the influence of measurement uncertainty.
5. To establish a sustainable European infrastructure for the standardisation of metabolomics measurement results with support from the EMN for traceability in laboratory medicine and European and International metabolomics societies. To facilitate the take up of the metabolomics technology and measurement infrastructure developed in the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers, metabolomics standards providers), legislators, and end users (e.g. clinical laboratory medicine: to support personalised and precision medicine).

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, medical (academic) hospitals 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.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.9 M€ and has defined an upper limit of 2.6 M€ for this project.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 35 % 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,
- Transfer knowledge to the healthcare 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.