EMPIR Call 2018 – Health, SI Broader Scope, Normative and Research Potential

Selected Research Topic number: **SRT-h11**
Version: 1.0

Title: Standardisation of concentration measurements of extracellular vesicles for medical diagnoses

Abstract

Medical diagnosis requires reliable information, of which a major part comes from the routine analysis of body fluids. Such body fluids contain extracellular vesicles (EV) which can be used to provide information for medical diagnosis. However, currently this information is not clinically relevant as there are no standardised methods for establishing reference values of EV in healthy humans. In order to determine reference values of EV traceable reference materials and standardised procedures are required for the identification and measurement of the concentration of EV. Once established, EV reference values will enable the comparison of results between hospitals, and will support the use of EV measurements for the diagnosis of diseases such as cancer, diabetes, and cardiovascular disease.

Keywords

Extracellular vesicles, medical diagnosis, clinical biomarkers, standard operating procedures, reference materials, concentration, nanoparticles, optical characterisation

Background to the Metrological Challenges

EV are small, membrane-enclosed vesicles, which can be considered as biological (cellular) nanoparticles. EV are released by cells into body fluids, and in all diseases studied so far, the concentration and composition of EV are changed substantially when compared to healthy subjects. EVs provide real time information about disease and offer an alternative to tissue biopsies. In contrast to biomarkers based on analytes, EVs also provide specific information on cellular origin and function.

Most clinical laboratories use flow cytometers to determine the concentration of EVs in body fluids. Flow cytometers work by detecting scattered light and the fluorescence of EVs labelled with fluorescent antibodies in a fluid stream. However, due to differences in optical sensitivity, flow cytometers have been shown to measure concentrations of EVs with more than two orders of magnitude difference for the same samples. Further to this, new generations of flow cytometers are capable of detecting light scattering and multi-channel fluorescence of single EVs with diameters >180 nm whereas older flow cytometers used in hospitals are far less sensitive, e.g. capable of only detecting a single EV with a diameter of >1000 nm. Other methods such as atomic force microscopy, electron microscopy, nanoparticle tracking analysis, resistive pulse sensing, and small-angle X-ray scattering, could provide more reliable and accurate EV measurements. However, they are not clinically applicable as the time required to measure a given number of EVs is 100 fold greater compared to flow cytometry.

Currently, to try and correct the different sensitivities of flow cytometers, polystyrene or silica beads are used to set light scattering thresholds. However, because the refractive index of such beads is higher than that of EVs with a similar diameter, the beads scatter 10 to 100 fold more light and consequently lead to inaccurate measurements. Therefore, there is a need for accurate EV reference materials (RM) for flow cytometer calibration.

Another major hurdle in clinical EV measurements, is the determination of their cellular origin. In order to establish the cellular origin of EVs, they are labelled with fluorescent antibodies that bind to cell-specific proteins on EVs and the fluorescence intensity determined. Calibration of the fluorescence intensity is done using RMs with assigned MESF (Molecules of Equivalent Soluble Fluorochrome) values and currently RMs with known MESF values are available within typical cell diameters (5-9 µm). However, these RMs are too bright to be used in the flow cytometers used for EV measurements and therefore fluorescent beads with assigned MESF values between 100 and 100,000 MESF and within the size range of EVs are needed.

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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 traceable measurement and characterisation of extracellular vesicles (EV) in body fluids.

The specific objectives are

1. To develop clinically relevant synthetic EV reference materials, that contain stable spherical particles with (i) concentrations in the range 10^9 - 10^{12} particles mL^{-1}, (ii) discrete diameters between 50 nm and 1000 nm, (iii) a refractive index in the range 1.37 - 1.42, and (iv) a visible fluorescence intensity between 100 and 100,000 molecules of equivalent soluble fluorochromes (MESF).
2. To develop traceable measurement methods for the number concentration, size distribution, fluorescence intensity and refractive index of the reference materials from objective 1. The uncertainty for each method should be determined.
3. To develop traceable methods to characterise the number concentration, size distribution, refractive index distribution, and fluorescence intensity of biological test samples containing EVs from human body fluids. The uncertainty for each method should be determined.
4. To evaluate and validate the performance of the clinically relevant synthetic EV reference materials from objective 1 via an inter-laboratory comparison with an adequate number of clinical end users. This should include an assessment of the reproducibility of measurements of the concentration of EV in biological test samples, across a range (≥ 20) of clinical instruments.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers), standards developing organisations and end users (medical practitioners, clinical and academic laboratories and hospitals).

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, 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. In particular, proposers should outline the achievements of the EMRP project HLT02 MetVes and how their proposal will build on those.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.8 M€, and has defined an upper limit of 2.1 M€ for this project.

EURAMET also expects the EU Contribution to the external funded partners to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

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 medical and health 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 EMPIR 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.