EMPIR Call 2015 – Health, SI, Normative and Research Potential

Selected Research Topic number: **SRT-h03**
Version: 1.0

Title: Metrological standardisation of concentration and identification measurements of extracellular vesicles

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 could 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 diagnostics, cellular nanoparticles, clinical biomarkers, reference materials, flow cytometry, particle refractive index

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, cellular origin, composition, and function of EV are changed substantially when compared to healthy subjects.

Most clinical laboratories (>90 %) studying EV in body fluids use flow cytometry. To try and correct the different sensitivities of flow cytometers, polystyrene beads of 500 nm and 900 nm diameter are used to set light scattering thresholds. However, because the refractive index of polystyrene beads (>1.60) is higher than EV (<1.40), the polystyrene beads scatter more light than EV (of a similar diameter) and consequently lead to inaccurate measurements of EV. Further to this, new generations of flow cytometers are capable of detecting light scattering and multi-channel fluorescence of single EV of 180 to 200 nm in diameter, whilst older flow cytometers used in hospitals are far less sensitive, e.g. capable of only detection a single EV with a diameter of 1000 to 1200 nm. This results in huge differences in sensitivity, of up to 106-fold between measurements of EV concentration made by different instruments and institutes.

An alternative approach is Mie theory, which takes the particle refractive index into account. However, in order to validate this approach, the refractive index needs to be well-characterised and currently there are no reference materials available with well-defined size, concentration and refractive index within the size distribution range relevant for EV (50 to 1000 nm).

Another major hurdle in clinical EV measurements, is the determination of the cellular origin (or other biochemical properties) of EV. Identification of EV not normally present in body fluids, such as tumour-derived EV in blood, could diagnose the presence of a tumour in patients. But although fluorescence labelling is applied worldwide by laboratories to try and determine the cellular origin of EV, most results are inconsistent and incomparable due to numerous labelling and/or isolation artefacts. In addition, it is unknown whether smaller EV have enough binding sites for fluorescent antibodies to bind and/or a sufficiently high binding efficacy to enable fluorescence detection.

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

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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 EV in body fluids.

The specific objectives are

1. To develop clinically relevant EV reference materials, that contain stable spherical particles with: a defined concentration of less than 10^{12} particles mL^{-1}; a size range of 50 nm to 1000 nm; and a homogeneous refractive index between 1.38 and 1.63.
2. To develop traceable methods for the size distribution, concentration and refractive index of the developed EV reference materials. The uncertainty for each should be determined.
3. To develop accurate methods for the identifying the cellular origin of EV, using stained surface epitopes. The fluorescence of the EV should be compared to that of stained calibrated particles in order to quantify the surface expression of the epitopes and to determine the limits of detection.
4. To evaluate the performance of the developed EV reference materials via an inter-laboratory comparison. In addition, to disseminate methods for the size distribution, concentration, refractive index and identification of EV to medical laboratories and from the results establish EV reference ranges.
5. To facilitate the uptake of the technology and measurement infrastructure developed by the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers), standards developing organisations (ISO, CEN) and end users (medical practitioners, medical (academic) hospitals and industry).

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. 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 to the project. Any deviation from this must be justified.

Any industrial partners that will receive significant benefit from the results of the proposed project are expected to be unfunded 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 medical 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.