# Title: Metrology at the cellular and DNA level


## Abstract

In order to substantially reduce the need for animal-based testing and promote ‘3Rs’ methods (i.e. reduction, refinement and replacement of animal use in regulatory testing) metrological approaches for the characterisation of 2D and 3D cell cultures need to be developed. 2D and 3D cell culture models already provide physiologically relevant cell based systems which can be used for modelling complex diseases. However, by providing reliable measurements of cell function and quantification of clinically relevant biomarkers, as well as the effects of pharmacologically relevant drugs, the cultures could form the basis of standard tests and enhance the clinical acceptance of data obtained from *in vitro* tests.


## Conformity with the Work Programme

This Call for JRPs conforms to the EMRP Outline 2008, section on “Grand Challenges” related to Health on pages 7 and 8 and in the sections on pages 14, 21, 22, 37, 40 and 41.


## Keywords

3D cell cultures, 2D cell cultures, fluorescence, imaging, toxicity, *in vitro,* real-time quantitative PCR, flow cytometry


## Background to the Metrological Challenges

Technology advances and changes to European legislation over last 5-10 years have seen a dramatic increase in the development of *in vitro* cell based systems, particularly those used for disease modelling or the screening of compounds for cell therapy applications. The majority of these *in vitro* systems use cells in a traditional 2D culture where cells are grown in a monolayer on the surface of a cell culture flask. However, it is increasingly being recognised that cells in traditional 2D cultures often fail to adopt the phenotypes and biological responses characteristic of cell behaviour *in vivo*. For example, more than 96 % of drugs that pass 2D cell-based screening fail *in vivo*, which is unacceptable in terms of efficiency and leads to an increased reliance on follow-up animal testing.

In order to provide more *in vivo* relevant cell behaviour in cultures, 3D cell culture systems have been developed. These cultures allow cells to develop into tissue-like structures more similar to those formed in living organisms and can significantly improve the predictive capability of cell assays. However, without good measurement practices and standards the value of this data is weakened.

The development of improved 3D cell models has been prioritised by a number of EU organisations, such as the European Centre for the Validation of Alternative Methods (ECVAM), the Innovative Medicine Initiative (a joint undertaking between the European Union and the pharmaceutical industry association) and the European Partnership for Alternative Approaches (EPAA). The requirement for metrology to underpin cell based analysis is also supported by the CCQM BioAnalysis Working Group (BAWG) through two studies:

- Study P102 [1] - investigating the detection of a specific cell types using fluorescent labelling and flow cytometry
- Study P123 [2] - investigating the measurement uncertainty associated with cell counting and confluency measurement in 2D cultures.

Currently, the majority of drug discovery groups use fluorescence-based measurements with 2D and 3D cell cultures. However, the measurements taken are generally from relatively simple parameters such as cell viability. To provide validated *in vitro* measurements of biological responses, further parameters need to be included, such as changes in cell morphology, structure and cell-cell interactions. 3D cell models also require measurement techniques for ensuring cell quality to support their therapeutic application.

The challenge is to develop a robust metrological framework for assessing the quality and performance of cell cultures, whilst also supporting innovation in cell based measurements. Measurement improvements include the development of biomarkers and of quick, sensitive (i.e. µMolar) and high resolution (i.e. µm) detection systems and the characterisation of spatial distribution, flow dynamics, authentication (DNA profile) and genomic stability of cell cultures.

## 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 measurement and characterisation of cells in therapeutically relevant cell cultures.

The specific objectives are:

1. To develop reliable and repeatable reference methods for producing clinically relevant 2D and 3D cell cultures.
2. To accurately, characterise these clinically relevant 2D and 3D cell cultures, considering viability, morphology, proliferation, spatial distribution, flow dynamics, authentication (DNA profile) and the genomic stability of the cells.
3. To develop traceable methods for accurate quantification of cell number (e.g. flow cytometry) and DNA / RNA targets (e.g. real-time quantitative PCR).
4. To develop validated methodologies for accurately assessing the toxicity of clinically relevant compounds in 2D and 3D cell cultures. Methods could include both short and long term (e.g. days to weeks) assessment, non-invasive assessment and quantification of clinically relevant biomarkers.
5. To develop techniques for the quantitative, high resolution imaging of 2D and 3D cell cultures. Methods for 2D cell cultures should address the uncertainty associated with fluorescent measurements. Methods for 3D cell cultures should address the effects of scattering and refractive imaging aberrations.

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 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.

The total eligible cost of any proposal received for this SRT is expected to be significantly above the 2.7 M€ guideline for proposals in this call.

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

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 medical community.

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. 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.

## Additional information

The references were provided by PRT submitters; proposers should therefore establish the relevance of any references.

[1] CCQM/BAWG P102 “Quantification of CD4+ Cell Enumeration & Fluorescence Calibration”

[2] CCQM/BAWG P123 “Number and geometrical property of cells adhered to a solid substrate”