Metrology of cellular analysis: problems and solutions

A L Runov1,2, N N Shevchenko3, T S Goryachaya1,4, E V Kurchakova1, M S Vonsky1,2

1 D.I. Mendeleyev Institute for Metrology, Moskovsky ave., 19, St.-Petersburg 190005, Russia
2 Almazov National Medical Research Centre, Akkuratova str., 2, St.-Petersburg 197341, Russia
3 Institute of Macromolecular Compounds of the Russian Academy of Sciences, Bolshoy pr., 31, St-Petersburg 199004, Russia
4 Institute of Cytology of the Russian Academy of Sciences, Tikhoretsky pr., 4, St-Petersburg 194064, Russia

a.l.runov@vniim.ru

Abstract. Cellular analysis is one of the perspective developing areas of metrology. The complexity of the measuring object requires novel approaches and correct metrological support for the quantitative methods of cellular analysis. Definition of the quantitative parameters and nominal properties of the cell, standardization of methodology – these are main tasks of the Cellular Analysis Working Group of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology. The results of a number of pilot studies carried out by Working Group can be used as a first step establishing metrological traceability of the cell measurements. Development of the stable reference materials for cell quantification is a challenging problem. One of the promising solutions seems to apply synthetic cell-mimicking particles. Currently we are in the process of developing candidate synthetic reference materials for cell measurements.

Introduction

The widespread use of cellular technologies demands measurements of biologically significant cell parameters. The lack of standardization and harmonization of the results in cell measurements leads to symptomatic irreproducibility of data in laboratory medicine and biotechnology [1], which demands development of metrological support for cellular analysis. Due to the complexity of a cell as an open living system, cell measurable parameters should be considered; a cell should be described using simplified models, and quantitative measurements require the development of adequate reference materials [2].

Cell measurements

Any cell-originated sample can be described as a complex multiparametric object. Such important parameters as cell types, geometric properties, biological activity, cell count, etc. play critical role in the definition of cellular functional activity. Some of this parameters represent quantitative values (cell count, shape, size), while the others can be described as nominal properties (cell type, viability, Gram staining color) [3]. The complexity of cells together with breadth of their applications leads to the
appearance of different cellular identification methods, often providing incomparable measurement results [4].

**International approaches to cellular metrology**

To ensure the reliability and international comparability of cell measurements, including measurements of cell number and determination of cell functional properties, a Working Group on Cellular Analysis of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CAWG) was founded in 2015 after the split of the Working Group on Bio Analysis [5]. CAWG sets the priority directions for the development of metrological support for cell measurements in the world, establishing global comparability of cell measurements based on the current needs of the biomedical stakeholders. One of these directions is the quantification of cells - a basic procedure, demanding standardization to provide metrological support for more complex methods. In this case, both a simple quantitation of cells of the same type without selective selection is possible (CCQM P214 pilot study for quantification leukocytes in suspension, CCQM P205 pilot study for counting E. coli cells in water), and selective quantification of cells expressing certain surface biomarkers (CCQM P165 for counting CD34 + cells).

Cells, due to their interaction with each other, reveal different behavior depending on their environment. Various bioanalytical tasks require cell quantification in suspension, on 2D surface monolayer or in 3D scaffolds.

Currently, in the field of cell measurements, there is no unified approach to the definition of measurable quantities. Even the basic procedure for determining the cell number, which requires standardization to provide metrological support for more complex methods, differs significantly from the common particle counting due to complex cell-shape, cell death and cell division processes. Cell counting can be performed both directly by the operator (under a microscope, in a Goryaev chamber or in a hemocytometer), and using automatic systems (automatic hemocytometer, impedance cell counter or flow cytometer) [6]. In this case, reference materials that can ensure the accuracy of cell counting are required. The question is raised about the comparability of measurement results obtained by different methods. Within the framework of international cooperation, CAWG carried out CCQM-P217 pilot study on the counting of blood cells in suspension by various methods, also CCQM-P205 on the count of bacterial cells (E. coli) in water are in progress.

**Reference materials for cell quantification**

The variety of methods for determining the number of cells forces to develop reference materials that could be used as calibrators when carrying out various cell measurements. There are several approaches for development of reference materials. One of the widespread methods is the production of reference materials from lyophilized stabilized cells [7, 8]. At the same time, the restoration of cells from the lyophilisate with a special buffer, the low stability of the resulting suspensions, and cell death and degradation during lyophilisation process have a great influence on the uncertainty of the metrological characteristics assigned to such reference materials.

One of alternative approaches involves the use of stabilized cells in suspensions, the characteristics of which are retained for a relatively long period of time (up to 3 months). This is, for example, GSO 10669-2015 – certified reference material of blood cell composition - hematological control (set of GK-VNIIM), produced by D.I. Mendeleyev Institute for Metrology (VNIIM) [9]. It is a suspension consisting of stabilized leukocytes, erythrocytes and haemoglobin of animals in the plasma of donated blood. This reference material is stable for 3 months and is used for verification and calibration of hematology analyzers in the field of public healthcare and veterinary medicine in Russian Federation. Unfortunately, development of such reference materials is possible only for certain types of cells; the stability is ensured in a narrow range of storage conditions. At the same time, the lifespan of such materials is not too long.

The CAWG is considering synthetic reference materials to solve the problem of instability of lyophilized cells based materials. Currently pilot comparisons on the counting of synthetic
microspheres with a diameter of 2-8 microns, which mimic spherical cells, are announced. Such reference material may be considered as the first step for cell-counters calibration. Nevertheless, it is not sufficient for determination of measurement capabilities for actual cells, as they represent different shapes and sizes.

**Cell-like artificial particles as a perspective reference material**

VNIIM together with the Institute of Macromolecular Compounds of the Russian Academy of Sciences, is currently developing reference materials for cell analysis based on polymeric substance. The developed method of synthesis makes it possible to obtain not only spheres of a certain diameter, but also particles imitating cells with complicated shapes. We have developed a technology for synthesis of latex particles of a given shape with given size. Figure 1 demonstrates toroidal erythrocyte-like and leukocyte-like synthetic particles. Particles were prepared through dispersion polymerization process. It is shown that the method of the dispersion polymerization allows creating strong crosslinked monodisperse microspheres of the diameter 1–10 μm based on polystyrene (or polymethacrylate) matrix crosslinked by ethylene glycol dimethacrylate (or divinylbenzene).

**Figure 1.** Erythrocyte-like (a) and leukocyte-like (b) synthetic particles

Samples are being studied for their further use in pilot comparisons of the CAWG. At present we are investigating the possibility of introduction of fluorescent dyes (Fluorescein) into the composition of latex particles (fig. 2). Mixture of fluorescent and non-fluorescent particles may imitate actual cell compound with fluorescently labelled surface biomarkers.

**Figure 2.** Fluorescent particles in transmitted light (a) and with fluorescence excitation (b)
The synthetic reference material we offer can be used as a starting point for the development of metrological support for cell measurements. Stable fluorescent labelled particles with preassigned size and shape may become adequate alternative to cell-based reference materials for cell quantification purposes.

**Conclusion**

Metrology of cell analysis is taking its first steps nowadays. Development of metrological support for cell number evaluation measurements is the major task for CCQM Cellular Analysis Working Group. Candidate reference materials for accurate cellular quantification are widely proposed. Development of perspective reference material on the basis of synthetic microparticles, may become a starting point for the establishing of metrological support for cell measurements.

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