CCQM Task Group on Intracellular Metrology for Gene Delivery: Terms of Reference

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Aims of the Task Group:

The task group will form between relevant working groups of the CCQM to understand multidisciplinary challenges and provide appropriate solutions that facilitate development of robust measurement infrastructure for entities that function as intracellular gene delivery systems. The group will foster collaboration between key stakeholders including regulators, product developers, analytical instrument providers and expert networks, as well as setting a strategy to establish traceability schemes including reference materials and reference procedure development to support emergent NMI capabilities in this area. Key measurands of interest include (i) gene encapsulating vehicles (e.g., virus-derived, and virus-like particles) per cell (count and mass fraction), (ii) delivered genetic material or genetic reaction per cell (copy numbers), (iii) viable cell count post-delivery (count) and (iv) gene loaded versus empty vehicles (gene loading capacity).

Specific Goals of the Task Group:

a) Engage with key stakeholders to explore needs and gaps for better measurement standards and best practice guidelines for the quantification of intracellular delivery mechanisms. These shall focus on the measurement of intracellular gene delivery at key stages of gene transfer in cells, i.e., uptake, expression (or silencing), maintenance of cell viability.

b) Support coordinating laboratories in the development of protocols for the comparison of measurement capabilities of intracellular delivery in cells with SI-traceable reference materials, envisaged to be implemented in the CCQM working groups, and to advise of current or future relevant studies within these groups that are of close relevance to an evolving work program on gene delivery mechanisms.

c) Propose a calibration hierarchy for measurements of gene transfer vehicles in the principal cells lines used for transfection and develop recommendations related to existing international standards that are the basis of traceability of Calibration and Measurement Capabilities (CMCs) NMIs and DIs are seeking to publish in the BIPM KCDB as well as recommendations to future international standards.

d) Review and publish where possible outputs from the CCQM workshops, such as “Metrology for viral systems as molecular tools” on-line workshop held in 01/2023, and any other workshop relevant to intracellular gene delivery.

e) Provide advice to CCQM CAWG/NAWG/PAWG and BIPM Headquarter laboratories on further development of comparisons and protocols for gene transfer in live and fixed cells and advice to CCQM SAWG and IAWG on comparisons and protocols for the measurement of gene loading capacity in commercial gene delivery vehicles and reference materials.

f) Propose and establish traceability schemes for the measurements of gene-loaded vehicles per cell, genetic material delivered per cell and gene loading capacity and develop a roadmap for achieving these including performance criteria to be met and technical challenges to be overcome.

Membership: Interested NMIs/DIs in CCQM and VAMAS

Chair: tbc

Time period for activities: 2023-2025

Background and Rationale:

Progress in biodesign creates high-value opportunities for economy. Advanced therapies and engineering biology emerge as particular beneficiaries as they continue to rely on effective intracellular gene delivery and compartmentalization. Biodesign enables increasingly effective synthetic tools and materials providing access to intracellular processes, their control and re-purpose. Virus-like particles (VLPs), synthetic or virus derived, constitute a major type of such tools. They meet the need for non-toxic and biologically differential activities and capitalise on the optimized structural uniformity of the virus architecture. The physical attributes of VLPs find use in related applications including as contrast agents for tissue imaging, internal standards for virus detection in biopsies or as in-process calibrators in medicines manufacturing to validate the efficacy of viral clearance in downstream processes. Traditional uses of VLPs such as vaccine platforms and gene delivery vehicles respond to emerging opportunities with the advent of RNA vaccines, exploring platforms capable of encapsulating RNA.

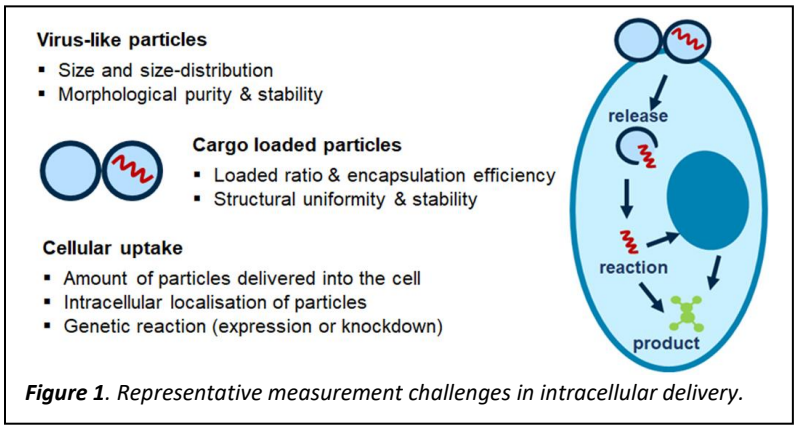

***Figure 1**. Representative measurement challenges in intracellular delivery.*

Virus-like and virus-derived gene delivery systems present an evolving area of gene therapy, with a steadily increasing number of clinical trials. Despite that there is a persistent lack of approved systems for systemic use. This re-emphasizes the need for suitable standards, reference materials and methods to benchmark the performance attributes of commercial gene delivery systems and ultimately help harmonize approaches for characterization and testing. Establishing correlative capabilities for intracellular measurements is critical to demonstrate reproducibility and traceability of the systems in correlation with their physicochemical and biological properties.

One relevant type of reference materials relies on high purity nucleic acids. These materials provide an effective means to evaluate the accuracy of next generation sequencing assays and can support the quantification of the total nucleic acid content, including in cells. However, they fall short of providing a quantitative insight into gene delivery systems themselves: their identity, purity, amount and loading capacities. Virus serotypes can be used to evaluate the particle, genome, and infectious titers of viral vectors but are very specialised, multi-component, not necessarily homogeneous and their constituents are challenging to fully characterize, which can lead to large uncertainties in measurement results.

As per regulatory directives for advanced therapy medicinal products (e.g., 2009/120/EC), such as gene therapy products, a reference material should be relevant and specific to products and substances it is used to benchmark, whilst its physicochemical properties shall be characterized and documented. Therefore, a candidate for a reference material for VLPs must be synthetically accessible and able to predictably assemble into VLPs, encapsulate and transfer nucleic acids into human cells and be devoid of the unwanted effects of replication-competent viruses. Complementary to this is the need to develop a

quantitative measurement procedure for intracellular gene transfer, which involves different measurands, requires more than one measurement approach, and above all depends on correlation between measurement results to address key challenges in intracellular delivery (Figure 1).

To focus global measurement capabilities on the persistent metrology challenges in intracellular delivery a CCQM task group is proposed. The task group will identify the capabilities and demonstrate their value through:

- Development of SI-traceable reference materials as calibrants for the traceable and quantitative measurement of intracellular gene delivery to benchmark the performance of commercial gene transfer and transfection reagents including, but not limited to, virus-derived and virus-like particles, polymeric and lipid nanoparticles, and macromolecular conjugates.
- Establishment of measurement uncertainties for all key stages of intracellular gene transfer and calibration uncertainties for reference materials as well as sources of variations in measurement results.
- Provision of SI-traceable reference materials and reference measurement procedures that meet the calibration uncertainties and requirements for the quantification of intracellular gene transfer measurements; and ensure global measurements are comparable, coherent and enabling industry to demonstrate compliance with existing policies (i.e. GxP for non-clinical gene and cell therapies).
- Provision of SI-traceable reference materials and reference measurement procedures as the underpinning metrology infrastructure, with a traceability hierarchy and quality system that allows the global provision of reference materials with long term stability and longevity of published data.
- Ensuring that the infrastructure, measurement capabilities and reference materials benefit from the quality assurance and recognition afforded by the CIPM MRA.
- Progress and comparison of non-commercial primary methodologies for the quantification of intracellular gene delivery, at any of key stages, for obtaining measurand specific data, including spatially and temporally resolved spectroscopy and microscopy, as well as machine learning algorithms and physics-based models to fit the obtained data with known quantum assignments wherever possible.