Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology

Working Group on Cellular Analysis: Strategy 2021-2030

1. EXECUTIVE SUMMARY

The technical area covered by the CAWG includes, but is not limited to, identification and quantification of intact cells and cell properties indicative of function in complex matrices and mixtures. Whilst cellular measurement has underpinned the surveillance of pathogens for disease diagnosis for over 150 years, the application of metrological principles to cellular analysis remains challenging. In particular, the sufficient characterization of informative cellular measurands which can quantitatively describe commonly recognized cellular attributes including cell identity, viability and proliferation and their realization with sufficient purity and stability. For living cell systems, physiochemical characterization and biological activity or functional measurements are in many instances non-correlative and may require novel approaches to standards development, deployed instrumentation or study design. Indeed the majority of stakeholder measurement needs in this sector are still emergent. Alongside these challenges CAWG must develop and exploit external relationships were necessary, as well as develop inter-working group activities across the CCQM to establish rational metrological systems for cell quantification.

The CAWG covers cellular analysis in both prokaryotic and eukaryotic domains, each having particular measurement needs and challenges that continue to determine NMI participation and the prioritization of comparison studies. The CAWG is a young working group within the CCQM structure (founded in 2015) and currently does not provide measurement services across multiple NMIs. The focus of the ongoing work programme are Track D pilot studies (3 completed and 2 ongoing) that are helping to understand the limitations of NMI cell measurement capabilities and to identify where consistent measurement systems can be developed. The CAWG is working to outline follow-on studies, with immediate focus on comparisons that underpin existing measurement services where they are found, including the quantification of blood cells with specific phenotypes (ie. CD4+ and CD34+ cell surface properties), and quantification of bacteria in food and water matrices. Other developing areas of interest include underpinning reference value assignments for complete blood count, adherent cells on 2D supports, antimicrobial susceptibility, cell viability and the quantification of synthetic cell analogues.

The stakeholder landscape for CAWG is diverse including; NMIs and DIs, reference material or control material producers and PT providers, clinical laboratories or contract testing agencies, governmental laboratories and industry. Identified strategic sectors for the CAWG are fully aligned with several Grand Metrology Challenges identified by the CIPM including health & life sciences, food safety and for specific applications within advanced manufacturing (i.e. Advanced therapy development and manufacture) and digital transformation (i.e. Digital pathology). To realize these goals continued communication and cooperation between CAWG and international or regional organizations such as ISO, WHO, IFCC, JCTLM, ILAC, FAO, EMN-TraceLabMed is essential in order to identify key measurands, understand standardisation challenges and apply state-of-the-art metrology to facilitate public health and industry development. Many of these committees have active participation by CAWG members. In addition, joint studies with other CCQM WGs will be critical to the success of the CAWG, are actively being sought in specific areas, such as for cell enumeration (NAWG and PAWG) or more recently for particle counting (IAWG) where improved cellular comparison is the underlying objective.

2. SCIENTIFIC, ECONOMIC AND SOCIAL CHALLENGES

Summary of key sectors' reliance on chemical/biological measurement and how they are influencing the future strategy of CCQM WG (2021-2030)

Health and Life sciences.

The Health and life science sectors provide the majority of emerging stakeholder need for Eukaryotic cell measurement services in a range of related sub-sectors; clinical and *in vitro* diagnostics and drug screening, advanced therapeutics development, control and manufacture (cell therapy products) together with related biobanking applications.

Clinical Diagnostics

Key measurands for clinical diagnostics, particularly diagnostic hematology and blood counting are mature and associated in many cases with regional regulatory frameworks (i.e. EU Tissues and Cells Directives 2004/32/EC), quality management systems[1], reference methodology and standards (DIN 58934-1:1997-12 and DIN 58932-2:1998-06) and reference measurement procedures with regional utilization [2]. Examples include complete blood count (CBC) comprising red blood cell count, white blood count (WBC), reticulocyte and platelet count. Other important CBC measurands include hematocrit (packed cell volume (%, L/L)), Mean cell volume (fL), Red cell distribution width (%CV or fL) and mean cell hemoglobin (pg or fmol).

Other important clinical and biotechnology-relevant blood cell measurements with established or emerging international consensus[2] include of CD4+ and CD8+ T-cell count for diagnostics and monitoring of immunocompromised patients (i.e., in HIV infection), CD34+ cell count for apheresis products (i.e., bone marrow engraftment following ablation therapy), HLA-B27 antigen cell screening and the detection of circulating tumour cells within whole blood. Future CMCs for cell-derived entities such as cell fragments and exosomes promise enhanced disease surveillance and clinical management of patients, yet also underpin important emergent biotechnologies and advanced therapies, which will likely be facilitated further by recent published standards ASTM F2149–16, ISO 20391-2:2019 and ISO 21709:2020.

For AIDS diagnosis and treatment, defined by <200 CD4+ cells / mL of blood, the WHO and UNAIDS have targeted the provision of anti-retroviral treatment to 3 million within developing countries by 2005, citing the importance of standardized methods for monitoring CD4+ counts and percentages, for adults and children respectively, by improved laboratory based infrastructure and field-based rapid test methods in resource limited settings[3]. Additionally, the WHO have recommended accelerating access to effective and safe treatment by recommending instrument and reagent manufacturers submit clinical instrumentation for independent evaluation by external quality assessment, as well as to build local capacity for servicing and maintenance of CD4 equipment[31].

The detection of circulating tumour cells offers early and less invasive detection of cancer, relapse monitoring, and widens ongoing treatment/management options for patients in non-clinical settings. The detection of rare cells (consensus range 0.1 – 0.01%[2]) with features of neoplasia, challenges the state-of-the-art for metrology to improve sensitivity, specificity, accuracy, traceability and comparability of liquid biopsy assays. These will also support wider translation to the clinic in compliance with regional regulations (e.g., In Vitro Diagnostic Regulation EU 2017/746) and ISO clinical laboratory standards (e.g., ISO 15189:2012).

Digital pathology has recently undergone rapid advance, concomitant with, artificial intelligence and digital image processing and could be a key beneficiary of future measurement services. Digital Imaging and Communication in Medicine (DICOM) standards now detail material and processing specifications for whole slide image (WSI) capture and integration with existing clinical laboratory infrastructure[4]. Recent supplements

extend to digital 3D manufacturing (3D printing) for medical devices. Specific challenges highlighted with international consensus (ICSH) of relevance to CAWG include; mechanisms to objectively evaluate image quality [5], the development of morphological standards for red blood cell and white blood cell pre-classification using AI algorithms, the grading of abnormal cells [6] and the integration of cell counter results with digital imaging [7].

Biotechnology

Important measurands for cellular bioprocessing applications; viable cell concentration (VCC), Viable cell volume (VCV) and wet cell weight (WCW) are central to estimation of process efficiency and scalability of cellular production systems, accelerating product development to commercialization[8]. The use of process-analytical technology is now supported by regulators[9] and examples of compatible analytics requiring calibration include RAMAN, near-infrared spectroscopy and dielectric impedance spectroscopy. The value of reference cell line development for industrially relevant mammalian expression systems has been made to boost PAT and biologics production strategies [10]. The estimation of viability with traceable measurement is a considerable longer-range interdisciplinary challenge for the CAWG with potential wider CCQM WG involvement. The commercial advantage to stakeholders is obvious, with global monoclonal antibody production worth $100 billion per year by 2017[11]. In the near term, cell bioprocessing related measurands may include total cell counts and differential cell counts for a range of industrially important eukaryotic cell lines such as CHO and HEK293T. The identification of cell-quality related measurands including state-of-the art measurement approaches for estimating cell viability and biological activity standards, together with cell authentication beyond molecular methods also have important industrial potential. The surrogate measurement of cell quality is in many cases GMP compliant, and a growing area of interest for the CAWG where those indicators are cellular derivatives. The measurement of these entities will have cross-discipline relevance to clinical diagnostics, cell-free advanced therapy manufacture or for validating entities such as liposomes in synthetic biology applications.

Cell authentication is central to any definition of a measurand for cellular analysis and of importance to biobanking quality assurance. Unfortunately, even after the establishment of rigorous quality control processes in many cell repositories around the world, experimental results continue to be derived from misidentified cell lines with consequences for validity and reproducibility[12]. Initiatives have been established such as the International Cell Line Authentication Committee (ICLAC), which maintains an updated list of cell line identification errors and disseminates good practice guidance and validated methods for authenticity tests[13] as do ICH, WHO and the OECD. Additionally, cell authentication and biobanking guidance and documentary standards projects are in preparation or recently published (ISO/AWI 21709; ISO/ PWI 23511, ISO 20387:2018). Sequencing-based methods, especially short tandem repeat (STR) profile analysis are recommended due to high discrimination power[14], availability of commercial kits and established procedures and supporting data (ANSI/ATCC ASN-0002-2011), however there are limitations, such as a failure to discriminate cells derived from different tissues of the same donor, or sub-lines derived from the same parent line due to selective pressure. It may therefore be necessary to monitor any of; passage number, doubling time, growth curve, cell morphology, adventitious contamination, viability, tumorigenicity, detection of cell line gene mutations and specific cellular markers of differentiation considering uncertainties attributable to culture process and instrumentation[15].

Two leading classes of cell therapy product (CTP) will likely dominate advanced therapy and wider biotechnology provision during the next strategic period (2021-2030), albeit with a varied focus on cell types and differing manufacturing strategies and standardization requirements dependent on application. CAR-T cells, thanks to their ability to target previously incurable tumors are at present the subject of intense R&D focus. The growth in the CAR-T cell market is set to reach $7.4 billion by the end of 2028, with greater than 100 companies worldwide pursuing CAR-T development programs [16] together with 3 FDA/EHA licensed products

and a further 10 products in late stage (Phase 3<) trials (www.clinical.trials.gov). The autologous nature of CAR-T products creates further opportunities and challenges for calibration services aligned with distributed, low volume manufacturing networks and systems. In particular the industry will be challenged to validate their cell products against purity and authenticity measurands, ensuring starting material quality and CAR-T product viability and potency through tumor targeting surface receptors, cytotoxicity and ongoing immune surveillance.

Another class of CTP product involves the use of induced pluripotent stem cells (iPSCs). These hold promise for both allogenic and autologous cell therapy treatment strategies and promise the broadest range of applications and target tissues. A promising source for iPSC is bone marrow sourced CD34+ cells, and standardized protocols for their generation are now being recognized [17]. FACT-JACIE International Standards for Haematopoietic Cellular Therapy Product Collection (www.factwebsite.org) also play a role within clinical apheresis units to ensure the controlled collection of cells in a safe environment. Again, cell number measurands have important emergent applications for iPSC bioprocessing quality and safety, including optimal cell density per unit/area for efficient reprogramming or differentiation induction [18] and there are emergent regulatory considerations to limit residual (non-differentiated) iPSCs per inoculate given potential tumorigenic or other deleterious effects due to culture expansion *ex vivo* [19].[20]

Drug discovery

Drug development is a lengthy and costly process with lead candidate discovery and pre-clinical validation currently carried out in the majority of cases on 2D cell cultures. Higher order measurement services and reference material development and uncertainties are needed to support comparable measurement of adherent total and differential cell number by various properties including phenotype, cell viability or morphology indictors. Such measurement systems would have broad application for drug cytotoxicity measurement but are also linked to diagnostic fields (i.e., cytopathic / viral infectivity measurements such as TCID50) or bioprocessing fields (iPSC quality metrics). Recent standards to support the development of future metrology in this area include ASTM F2739 – 19 and ASTM F2998 – 14. Further stakeholder interest is developing in areas of organotypic tissue model development and related fields of tissue engineering and in vitro diagnostics.

CAWG members are active on committees that support interlaboratory comparison such as VAMAS (VAMAS.org) which supports world trade in product development on advanced materials, while providing the technical basis for harmonized standards and specifications. CAWG participating NMIs have active research programs developing materials with reproducible morphological or ultrastructural patterns that repeat over multiple length scales, offering cell substrates that are well placed to transition from planar to three dimensional formulations. Indeed, an increasingly common practice in drug screening is the use of organoid models (i.e., Labskin). These are commonly 3D cultures supported by hydrogel scaffolds that can be acellular extracellular matrix extracts such as Matrigel or increasingly more defined preparations. However, these materials tend to exert extracellular signalling cues to embedded cells, such as arresting cell proliferation rendering them unsuitable for most applications. There is therefore a need to replace these materials with "standard" scaffolds – minimalist, bottom-up synthetic matrices which exhibit no *a priori* biological bias. Much metrology is needed to establish and validate reference values for these scaffolds in relation to cellular responses, which is necessary to underpin any future standard for 3D cell culture.

Environmental monitoring and safety

Key drivers in this sector include the monitoring of beneficial, pathogenic or otherwise disruptive microorganisms in water, soil or other solid surfaces (biofilms), and the exploitation of microorganisms for environmental remediation covering in detail the use of micro-organisms for agriculture, production purposes,

bioremediation and industrial sanitation. Additional drivers include initiatives to engineer the environment using microbial symbionts in plants and insects, as well as environment risk/safety assessments for the deliberate release of engineered micro-organisms. Regulation in this area is regionally dependent, for example plant pest diagnostic services are set out in European guidance documentation (EPPO)[21], detailing target cell concentration ranges as upstream measurands for specific molecular, bioassay and serology-based assays dependent on target organism. Such guidance additionally sets management requirements through either ISO 17025 or ISO 9001. Future areas for cellular metrology in this sector are emergent, such as Algal biomass quantification and quality measurements for biofuel production or CO2 capture, and reference strains for algae have already been identified [22]

Biofilm growth on photovoltaic panels represents a crucial barrier to energy-conversion efficiency and panel long lifetimes[23]. Collectively, the combination of microbial and mineral accumulations on solar panels is referred to as soiling. Soiling is an important problem for the PV industry causing reductions of energy conversion efficiency greater than 10% [24],with substantial knock-on economic and environmental impact. Indeed, microbes are ubiquitous settlers on every solid surface [25]with microbial communities now recognized to develop on all solid-atmosphere interfaces[26]. Better knowledge of the contributions and causes of microbial-influenced soiling can lead to improvements in PV panel glass properties, and in strategies and materials for cleaning and maintaining panels supported by better quantitative methods and RMs.

Food safety

The safety of food and the microbiological quality of water for human consumption have been one of the many objectives of the WHO, however increasingly, informed consumers are demanding better sanitary and control conditions to guarantee the quality of products reflected in Codex Alimentarius guidelines [27] as well as the International Commission on Microbiological Specifications for Foods (ICMSF). There are also evolving regional regulations such as Regulation (EC) No 178/2002 (European Commission) and the Food Safety Modernization Act (FSMA, FDA, USA). Stakeholders for NMI services in this sector include materials producers, clinical labs, academic food and water analysis laboratories, quality control laboratories and public health laboratories.

Foodborne illnesses are usually infectious or toxic in nature and caused by bacteria, viruses, parasites or chemical substances entering the body through contaminated food or water. The most common foodborne pathogens are:

1.-Bacteria

- *Enterobacteriaceae*: *Salmonella* spp, *Campylobacter* spp*, Enterohaemorrhagic Escherichia coli, Shigella sp, Yersinia enterocolitica, Cronobacter sakazakii*
- Sporulated and toxigenic bacteria: *Bacillus cereus, Clostridium perfringens, Clostridium botulinum*
- Toxigenic bacteria: *Staphylococcus aureus*
- Intracellular bacteria: *Listeria monocytogenes*
- Epidemics bacteria: *Vibrio cholerae, Vibrio parahaemolyticus*
- Indicator bacteria: Mesophilic aerobes, Coliforms and *E. coli*

2.- Virus: Norovirus, Hepatitis A

3- Yeasts, Molds, and Mycotoxins – Genus: *Aspergillus, Penicillium, Fusarium*, *Cladosporium*

4.- Parasites in food: Giardia duodenalis, Cryptosporidium spp, Cyclospora cayetanensis, Toxoplasma gondii, Trichinella spiralis, Taenia saginata/Taenia solium

Decision-making in public health surveillance policies and risk assessments are reliant on a network of testing laboratories that will require essential higher order measurement services, including reference material provision, reference test methods and estimation of uncertainties. There is also need to support compliance with ISO / IEC 17025 testing and accreditation, ISO 17034 with peer evaluation for producers of reference

materials, and ISO 17043 for interlaboratory comparisons and proficiency testing. In response to these demands and challenges the CAWG envisage developing a strategy for prokaryotic measurement that supports:

- RM development that addresses analytical need with properties of interest established by orthogonal methods for extended characterization,
- Maintaining measurement services (Proficiency testing).
- Continues with pilot studies and intercomparisons for enumeration of prokaryotic cells with various properties including an intact membrane.
- Infrastructure for metrological traceability in cellular measurements along with increased knowledge transfer.

Summary of challenges for cellular measurement

Any overall strategy must recognize significant challenges for cellular metrology, some of which may include:

- Appropriate recognition of informative measurands for cells and how these relate to specific biological activities and attributes of cells and cell systems, responsive to stakeholders need.
- The realization of these measurands with sufficient stability and purity within candidate reference materials. Allied to this a recognition that products utilizing cells can be highly customized products, challenging the relevance of broad claim CMCs.
- For CAWG studies it may be necessary to adopt novel or unconventional approaches in standards development such as a tiered system of increasingly stable or commutable materials that transition from synthetic to biological origin as appropriate and recognition of where CAWG support/intervention is needed within such a framework.
- Adequately surveying the breadth of methods and instrumentation available for cellular analysis and to secure adoption of the most promising technologies within NMIs/Dis. Coupled to this gaining sufficient coverage of techniques and expertise within the NMI community to establish meaningful interlaboratory comparison and to recognise and adopt expertise outside of the NMI community where appropriate and possible.
- The task of the measurement community to change where necessary established measurement practices that rely to a large degree on subjectivity in measurement, or the opinion of established professionals.
- To meet the distribution and transportation challenges for cell materials capable of on-going biological activity and abiding by any regional and institutional safety and environmental regulations. Additionally, to overcome challenges associated with the regional availability and cost of ancillary reagents and consumables.

3. VISION AND MISSION

Vision and mission statements for the CCQM

The CCQM's vision is:
A world in which all chemical and biological measurements are made at the required level of accuracy with associated uncertainty to meet the needs of society.

The mission of the CCQM is:
To advance global comparability of chemical and biological measurement standards and capabilities, enabling member states and associates to make measurements with confidence.

4. STRATEGY

In line with the CCQM's vision and mission, the aims of the 2021 to 2030 strategy are:

To contribute to the resolution of global challenges by identifying and prioritising critical measurement issues and developing studies to compare relevant measurement methods and standards. A main focus will be on making sure the measurand is clearly understood and all uncertainty contributions, including those in converting from what is measured to what is intended to be measured, are quantified.

To promote the uptake of metrologically traceable biological measurement, through workshops and roundtable discussions with key stakeholder organisations, to facilitate interaction, liaison and cooperative agreements, and receive stakeholder advice on priorities to feed into CAWG work programmes.

To progress the state of the art of chemical and biological measurement science, by investigating new and evolving technologies, measurement methods and standards and coordinating programmes to assess them.

To improve efficiency and efficacy of the global system of comparisons for chemical and biological measurement standards conducted by the CCQM, by continuing the development of strategies for a manageable number of comparisons to cover core capabilities.

To continue the evolution of CMCs to meet stakeholders needs, incorporating the use of broad claim CMCs where applicable to cover a broader range of services and considering options to present these in a way that meets stakeholder needs and encourages greater engagement with the CMC database.

To support the development of capabilities at NMIs and DIs with emerging activities, by promoting a close working relationship with RMOs including mentoring and support for NMIs and DIs preparing to coordinate comparisons for the first time and promoting knowledge transfer activities including workshops, as well as secondments to other NMIs, DIs and the BIPM.

5. ACTIVITIES TO SUPPORT THE STRATEGY

5.1. PROGRESSING METROLOGY SCIENCE

A description of the activities to be undertaken to progress the state of the art of measurement science.

In order to meet a coherent strategy for comparison study development that meets the varied needs of stakeholders in an efficient manner, planned and future studies for CAWG can be organised by fundamental properties of a cellular measurement system; domain, cellular dimensions (size, structure/morphology), spatial presentation (fluid or substrate), expressed biomarkers and specific features of viable or biologically active cells (fig 1.). To meet the scientific challenges laid out in section 2, current pilot studies are planned using preserved specimens (lyophilised or chemical fixation). The measurement of live entities remains an aspirational goal and will be dependent on the development of both standards and state of the art metrology. Note, prokaryotic entities can survive preservation regimes, so dependent upon study design, methodology may incorporate actively growing entities. The present strategy is working to develop cell counting CMCs that develop in complexity to incorporate cellular properties of interest to stakeholders. It is envisaged that other SI traceable measures will emerge in time dependent upon need, technological advance and expertise. Studies will be selected that have broad benefit to NMI/DI WG members, particularly those that help to develop future, or improve existing measurement services.

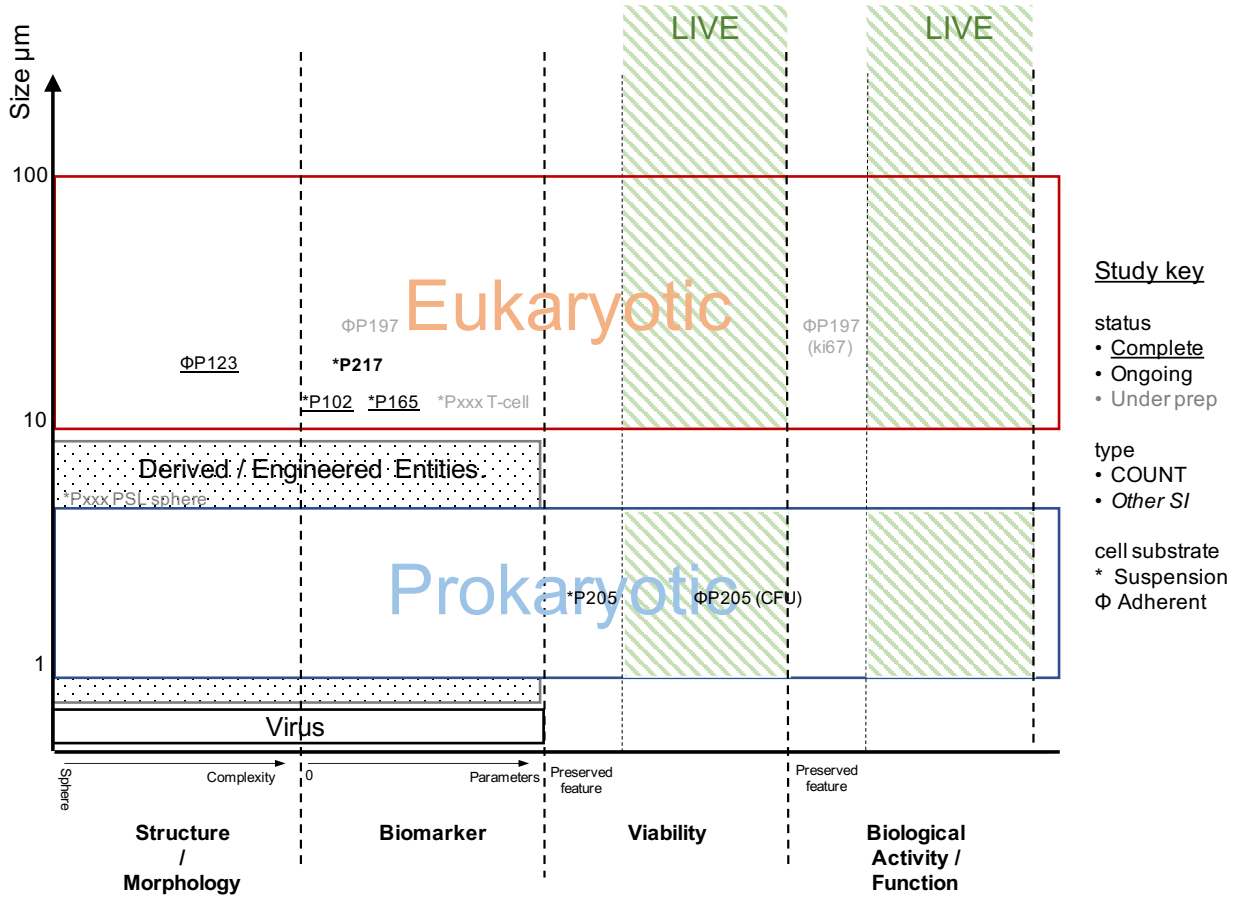

Figure 1. Conceptual framework of CAWG studies organised by entity, cellular attribute and matrix properties. Where necessary and relevant, CAWG studies may advance metrology for cell-derived measurands (ie cellular fragments, extracellular vesicles, liposomes, micelles) or engineered non-cellular particles of synthetic biomaterial

constructs. Note, the quantification of viruses is of relevance to a cellular metrology program dependent on stakeholder application (ie. Infectivity measurement) or by demonstrating state-of-the-art capabilities. Due to complexity, single studies can appear in more than one section of the diagram.

Amongst the most challenging considerations for cellular metrology is recognition of the measurand and how it relates to macromolecular or small molecular components of cells (i.e. small molecules, proteins, nucleic acids), recognised attributes of cells (ie. identity, viability, potency) and biological activities (i.e. cellular division, differentiation, programmed cell death, movement). The CAWG encourages discourse and the development of definitions appropriate to cellular analysis as the field progresses and recognises other standardisation efforts appropriate to these concepts (Section 5.2).

The present focus of the CAWG is Track D pilot studies, with a mixture of studies ongoing or under preparation (table 2) that demonstrate capabilities in novel areas or build technical expertise within NMIs for new or emergent techniques that will develop a system of measurement services underpinned by international comparisons (fig. 2). Both sample model distributions (1, or 2) are used, dependent upon specific application and study purpose. The present focus of pilot studies is to develop capabilities in cell counting metrology for a range of entities in both suspension and adherent cell formats (fig. 1). The strategy also recognising the continuing importance of flow cytometry and microscopy that underpin measurement services using flow cytometry and microscopy technologies respectively. The CAWG envisages that these services will ultimately sit at the apex of a measurement framework supported by traceable measurement systems for particles analysis, supporting instrument calibration, and the measurement of specific biological macromolecules of fundamental importance to cellular analysis. The measurement of these entities in insolation sits within the terms of reference of other working groups in the CCQM, but it is anticipated that inter-working group studies will develop with the common goal of improving measurement comparison for cellular analysis.

Table 1. Studies planned to meet challenges described in section 2.

WG	Reference No.	Description	Coordinating Laboratory	Start date	Status
CAWG	CCQM-P197	Proliferative stem cell number per unit area	NPL / INRIM	2021-2022	Under development
CAWG	CCQM-P205	Quantification of E. coli in drinking water	NIM	2021	Starting 2021
CAWG	CCQM-P217	Quantification of fixed peripheral blood mononuclear cells in suspension	NIBSC / PTB	2020	Ongoing
CAWG		Polysterene particle number concentration measurement for blood cell counting	NMIJ	2021-2022	Under discussion
CAWG		Quantification of T-cell therapeutic subsets	NIBSC / NIST / LGC	2021 - 2022	Under discussion

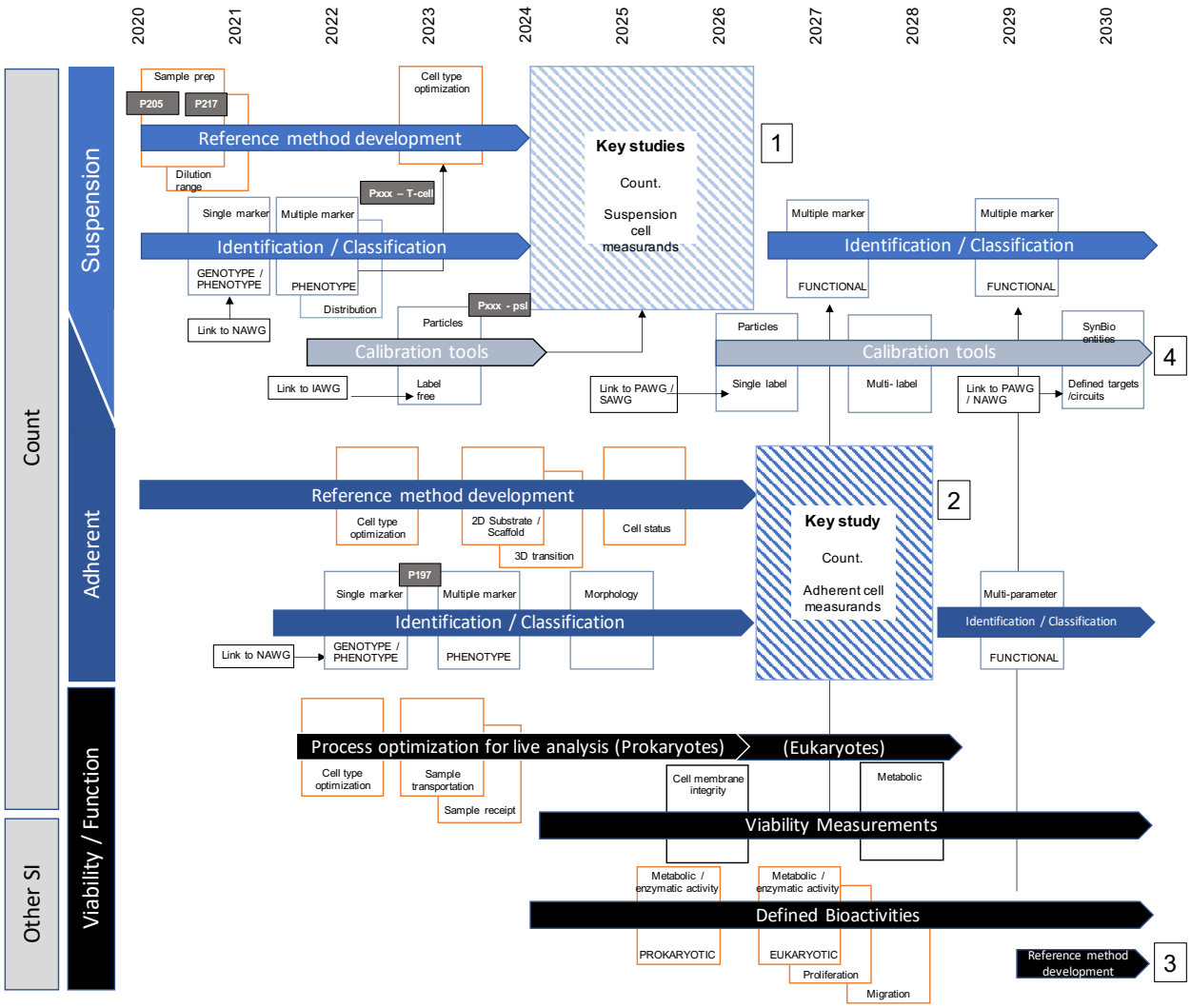

Figure 2. An outline roadmap for CAWG study development showing principle challenges and study features. Boxed numbers refer to sections in the text.

1. Cell counting metrology (Suspension)

Considering the drivers for cell metrology and the necessity for broad claims, a present focus for the CAWG is to establish CMCs that support cell counting services within liquid matrices. Flow cytometry is anticipated to provide a future primary measurement technique and benefits from a well-established metrological background:

- Traceable sample volume measurement for flow cytometry has been demonstrated either by weighing the (diluted) sample directly, or using gravimetrically calibrated syringe-motor combinations for direct sample injection [28]. Volume is determined from measured mass, including a traceable density measurement. It should be noted that traceable density measurement is already needed for a gravimetrically controlled sample dilution for both microscopic and flow cytometric measurement of cell concentration.

- Cytometer calibration beads of known fluorescence intensity are commonly used to calibrate, perform quality control, and standardize flow cytometers, as well as quantifying by phenotype expression using fluorescently labelled antibodies in units of equivalent reference fluorophores (ERF) or mean equivalent reference fluorophore (MESF) [29]. These cytometer-dependent units of expression, traceable to NIST SRM 1934 and Research Grade Reference Fluorophores can ultimately be converted to the number of antibodies bound per cells (ABC), an instrument independent unit that is enabled by the use of biological cell reference materials with well characterized antigen expression or established photon counting calibration rulers.

Cell counting by flow cytometry includes multiple procedural steps from sample collection, cell processing and staining, cytometer quality control, calibration and standardization, compensation, data analysis and reporting, with the potential for significant sources of variability to be introduced at each stage. Measurement assurance strategies, particularly the use of reference methods and materials, could effectively minimize these sources of variability.

The CAWG is actively exploring pilot studies analyzing key pre-analytical process and measurement uncertainties, selecting measurands of importance to healthcare or industrial relevance. CCQM-P102 aimed at cell concentration measurement of CD4 positive human cells. As a key result in establishing metrological traceability in the field of flow cytometric cell counting for in vitro diagnostic testing, the publications derived from this study are cited in ISO 17511:2020. CCQM-P165 expanded the approaches developed in CCQM-P102 to a more challenging diagnostic situation - the determination of stem cell concentration. Routine labs use the bead based one-platform approach for stem cell counting, which is regarded as a gold standard in this field. The CCQM-P165 study demonstrated that traceable volumetric counting could provide the same results without compromising usability of the method.

Two pilot activities utilizing flow cytometry are on-going:

CCQM-P217, Enumeration of fixed peripheral blood mononuclear cells in suspension, (led by NIBSC, UK) aims to examine participant ability to count single-nuclei white blood cells (T-cells, B-cell and NK cells) using a wide range of cell counting technologies together with a prescribed dilutions series design adapted from ISO 20391-2. The study is designed to utilize a minimal number of process manipulations beyond sample dilution adjustment, and also incorporates centralized analysis of data and accompanying control materials (synthetic beads). As well as evaluating lyophilized blood cell reference materials, the knowledge gained from this study will allow future blood cell counting pilot studies to build in complexity for specific key applications and technologies (i.e., specific T-cell subsets). This study sits within a family of cell counting pilots within liquid matrices (P102, P165) (Fig 2) that it is anticipated will lead to a future key comparison study in this area. Aspects to consider for future development include:

- Substituting primary cell materials for well characterized cell lines in a range of matrices.
- Targeting the focus of dilution range to specific applications, i.e., clinical diagnostics, cell therapy dosing or future standardized bioprocessing control material.
- Development of higher parameter flow cytometry studies, and the development of expertise and platform availability with participating NMIs.
- Cell identification by biomarker cell localization (i.e., cell surface, cytoplasmic or nuclear)

Flow cytometry is also anticipated to become a higher order method for Prokaryotic concentration determination. Routine prokaryotic cell counting still relies in many cases on cell culture to enable growth and enumeration of viable bacteria through various techniques including multiple-tube fermentation (MTF),

membrane filtration methods or standard bacterial culture techniques, however these methods are synonymous with a lack of specificity, false-positive counts. They can also fail to detect slow-growing microorganisms, so called "viable but nonculturable" cells (VBNC) considered to be an important issue in public health and food safety due to their non-detection by conventional food and water testing methods coupled to a propensity for bacteria to enter a VBNC state leading to false negative results. Direct microscopic counts of prokaryotic cells stained with fluorescent dyes (such as acridine orange (AO) and 4',6-diamidino-2-phenylindole (DAPI), can overcome biases arising from culture-based methods, However, these methodologies again suffer from limitations such as lack of reproducibility or time-consuming nature. Therefore, it is of utmost importance to develop and employ appropriate detection and quantification methods to distinguish the viability state of these cells and new technologies are allowing the analysis of biological function and activity in prokaryote cell measurement methods. **CCQM-P205 Enumeration of membrane intact E. coli (led by NIM China)** will support the development of higher order methods for assessment of the microbial contamination of drinking water, comparing the measurement performance of flow cytometry to the established compendial technique of CFU counting. Establishing a suitable reference material for this study is ongoing, noting particularly international transport regulations and laboratory safety requirements. A combined meeting of NAWG and CAWG (Turin 2019) expressed a provisional interest in developing future studies in this area utilizing molecular methods and this remains a longer-term strategic interest for the WG.

2. Cell counting metrology (Adherent cells)

Total and differential cell count is a fundamental measurement for cell growth or cell status determination. Cell morphology is consequent to various biological processes including cell cycle stage (including division), viability status, nutrient availability and diffusion, cell signaling (paracrine/juxtacrine) or cytopathic effect by challenge with biochemical or biological agent. Together with the measurement of dispersed single cells, the accumulation of packed cell populations (e.g. In CFU determination), or the fusion of multiple cells into single membrane entities (i.e., syncytia formation) presents a significant measurement challenges, alongside pre-analytical process-related uncertainties. The CCQM-P123 study has considered the basic parameters of microscopic cell counting demonstrating that cell-based reference materials can be produced, and that such materials can be used for more challenging measurands like cell size. A study under development, CCQM-P197 Proliferative stem cell number per unit area (led by NPL, UK) aims to expand on the complexity introduced in CCQM P123 Number and geometric property of cells adhered to a solid substrate, by testing participants ability to recognize features of dividing adherent cells with therapeutic potential. Discussions are ongoing to understand the best minimal set of cell identity and cell status features of interest to stakeholders. The study additionally utilizes a well characterized scaffold material (VAMAS) that can be formed in 2D and 3D architectures, allowing future cell quantification studies to transition towards relevant controlled 3D tissue architectures.

3. Cell viability and functional cell metrology

Measurement of viability or biological activities are highly challenging areas to quantify, due to their many modes of action and the distinction of these mechanisms from compound biological processes. There is a clear need to develop studies in these areas (section 2) for both Eukaryotic and Prokaryotic systems, provided sample stability and model distributions can yield meaningful results by interlaboratory comparison. CAWG members are working to develop future interlaboratory comparisons in this area and to better understand and move towards the adoption of SI traceable measurement for key cellular bioactivies. Key areas for consideration for studies in this area include:

- Candidate cell line phenotype

- selected biological signalling mechanisms and biological activities of interest
- The preservation of such biological activities in distributable materials
- Morphologies and how these relate to biological activities.
- Development of RMs indicative of cellular activity (i.e., particle uptake)
- The development of synthetic biology approaches to cellular measurement

4. Supporting metrology for cellular analysis

Alongside concentration measurements, several NMIs have presented the case for inter-laboratory comparison of particle number concentration measurement for application in cellular analysis. These particles are widely used for flow cytometry instrument calibration and experimental control and so far, are not value assigned by the measurement community. The development of studies of this kind not only needs to consider particle sizes and spatial conformation as they develop in complexity, but also appropriate matrix considerations and compatibility with available instrumentation. The measurement of microsphere particles analogous to cells is a potential CCQM inter-WG activity to establish calibrated services for cell quantification using a range of available technologies.

5.2. IMPROVING STAKEHOLDER INVOLVEMENT

A description of the activities to be undertaken to improve stakeholder involvement

The CAWG does not operate as a stakeholder-facing entity in its own right outside of the NMI/DI community, but its members are active on several supporting international committees that are fruitful for gauging the need for emerging cell measurement services as they evolve. The CAWG does recognize that a more active engagement strategy will be needed for 2021-2030 period.

The WG will encourage liaison and participation with aligned committees where necessary. Noted standardization efforts are underway of relevance to CAWG future measurement services in the following international committees and organizations:

- The JCTLM and International Council for Standardization in Hemocytometry (ICSH) has developed blood cell counting reference measurement methods with PTB for thrombocytes (C3RMMP34, C3RMMP35) and is developing platelet reference measurement procedures.
- THE CIPM signed a MoU with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 2020. Whilst the IFCC do not currently have working groups developing measurement procedures or reference materials for cellular measurands, several are of relevance including the scientific WG on Hemoglobin A2 standardization and the Education and Management (EMD) WG on flow cytometry.
- WHO. Recent standards proposal topics of interest include RBC transfusion-relevant bacterial reference strains, as well a reference reagent for mesenchymal stromal cells.
- ISO. Several participants of CAWG are active participants within ISO, including TC/276 Biotechnology and ISO TC/212 Clinical diagnostic testing and in vitro diagnostic test systems. The CAWG will encourage continued participation on these committees, adopting standards within comparisons where relevant to advance the strategic aims of the CCQM.
- The International Society for Cell and Gene Therapy (ISCT). Standards development is of increasing importance to cell and gene therapy. ISCT committee members continue to offer expert feedback on specific standardization efforts including quality standards for mesenchymal stromal cell biobanking.
- Foundation for Accreditation of Cellular Therapy (FACT) establishes standards for high quality medical and laboratory practice in cellular therapies. FACT is a non-profit corporation co-founded by the International Society for Cell and Gene Therapy (ISCT) and the American Society of Blood and

Marrow Transplantation (ASBMT) for the purposes of voluntary inspection and accreditation in the field of cellular therapy.

- The Joint Accreditation Committee-ISCT & EBMT (JACIE) is a not-for-profit entity established in 1998 for the purposes of assessment and accreditation in the field of haematopoietic stem cell (HSC) transplantation. JACIE's primary aim is to promote high quality patient care and laboratory performance in haematopoietic stem cell collection, processing and transplantation centres through the development of global standards and an internationally recognised system of accreditation.

Workshops will be organised which cover progress in relevant research fields with emergent measurement needs for stakeholders as well as to understand the need for comparability and NMI services. As many as three workshops will be held over the 2021-2030 period covering:

- Emergent need for cell standards in diagnostics, drug development and testing.
- Advancing the state-of the-art. Future primary measurement procedures for cellular analysis.
- Bacteria quantification and surveillance, development of primary measurement methods and standards.
- Viral vector measurement and standardisation for a biomanufacturing and advanced therapies.

5.3. PROMOTING GLOBAL COMPARABILITY

A description of the activities to be undertaken to promote global comparability including support of the CIPM MRA

At present the CAWG is working to identify measurement services requiring global comparability for cellular measurements in a range of sectors. It is anticipated that KC studies will develop and be required to underpin these services over the next decade in key areas including cell enumeration on a 2D substrates and cell enumeration in liquid suspension.

5.4. INTERACTION WITH RMO ACTIVITIES

A summary of RMO activities and their influence on CCQM WG Strategy and vice-versa

APMP: Major cell measurement activities focus on measurement of microorganisms, with participation from NIM China, KRISS, NIMT and IDTI. APMP QM-P35 Quantification of Escherichia coli. in drinking water is in progress. Quantification of other microorganisms in food matrix is also proposed and concerned by the members of APMP TCQM. The joint PT program covering microorganism quantification is also under consideration by APMP-APLAC.

EURAMET: NMIs and DIs in Europe are working closely together with stakeholders within the framework of EMRP and EMPIR. Important projects have examined cell quantification as relevant clinical markers or human bacterial pathogen detection such as Infect-Met and AntiMicroResist, as well as metrology for cell counting and immunophenotyping as part of BioSITrace and Septimet. Furthermore, the European network (EMN-TLM) brings stakeholders such as PT providers, IVD manufacturers, regulators and NMIs / DIs together to address stakeholder needs especially in the light of the new European IVD regulation (EU regulation 2017/745 and 2017/746). As the European CAWG members are also members of this network, they contribute to the strategy by reporting the stakeholder needs to the CAWG strategy.

ANNEX

1. GENERAL INFORMATION

General information and terms of reference in format used for 2017-2026 strategy
(Headings from previous template)

CC Name: CCQM

CC Working Group: Cell Analysis Working Group (CAWG)

Date Established: 2015

Number of Members: ~15 participating institutes, ~ 20 members

Number of Participants at last meeting: 29 via video conference

Periodicity between Meetings: 6 months

Date of last meeting: November 2020 (series of 3 video conferences due to Covid-19 pandemic)

CC WG Chair. Jonathan Campbell, National Measurement Laboratory, LGC limited, UK. 1.5 years

Number of KCs organized (from 2015 up to and including 2020): 0

Number of Pilot studies organized (from 1999 up to and including 2020): 5

Number of CMCs published in KCDB supported by CC body activities (up to and including 2020): 0

The agreed Terms of Reference (TOR) for the Cell Analysis WG are:

- To carry out Key comparisons & Pilot Study Comparisons to benchmark and demonstrate NMI capabilities in cell measurements that lead to CMC registration. KCs in the CAWG will focus on demonstrating competence and underpinning CMC claims in the areas of
    - Cell identification and purity analysis
    - Quantification of cells and cellular related pathogens in biological matrices.
    - Determination of cellular activity and function.
- To perform interlaboratory work and pilot studies where required to develop reference measurement systems for cellular analysis to support NMI/DI measurement service development, with the highest possible metrological order, with traceability to the SI where possible, or other internationally agreed units.
- To act as a forum for the exchange of measurement research: to discuss and record metrology issues, requirements and challenges in cell measurement areas, proposed solutions and new methods, and interactions with stakeholders

In cases where the scope overlaps with other working groups of the CCQM (for example, DNA with NAWG), collaborations ensuring the best efficiency will be pursued by co-organising studies and comparisons.

2. LIST OF PLANNED KEY AND SUPPLEMENTARY COMPARISONS AND PILOT STUDIES

Excel sheet link

WG	Reference No.	Description	Coordinating Laboratory	Start date	Status
CAWG	CCQM-P197	Proliferative stem cell number per unit area	NPL / INRIM	2021 - 2022	Under development
CAWG	CCQM-P205	Quantification of E. coli in drinking water	NIM	2021	Starting 2021
CAWG	CCQM-P217	Quantification of fixed peripheral blood mononuclear cells in suspension	NIBSC / PTB	2020	Ongoing
CAWG		Polystyrene particle number concentration measurement for blood cell counting	NMIJ	2021 - 2022	Future
CAWG		Quantification of T-cell phenotype subsets	NIBSC / NIST / LGC	2021 - 2022	Future

3. SUMMARY OF WORK ACCOMPLISHED AND IMPACT ACHIEVED (2017-2020)

A summary of the activities undertaken in CCQM/CCQM WGs 2017-2020 in the 4 activity areas (Section 5) – include 3 impact case studies as boxed examples in main text

Completed work program

KC / PS	Achievement	Status
CCQM- P102	Quantification of cells with specific phenotypic characteristics	completed
CCQM- P123	Cell quantification on solid substrate	completed
CCQM- P165	Quantification of CD34+ cell counts	completed

Key outcomes:

CCQM-P102: Quantification of cells with specific phenotypic characteristics

Reliable CD4 cell counts are required AIDS diagnosis (<200 CD4+ cells/mm^3 whole blood) and ongoing treatment and care decisions together with clinical findings[3,31]. P102 utilized a lyophilized cell standard (Labelled freeze dried PBMC with a fluorescein conjugated anti-CD4 antibody) to maximize sample stability and ensure commutable flow cytometry control measurement overcoming problems associated with commonly used lyophilized beads or cryopreserved PBMCs. The pilot study was organized to evaluate a candidate reference material and to demonstrate participant capability in flow cytometric measurements of CD4+ cell concentration, the prevalence of CD4 antigen expression on the PBMC surface using equivalent fluorescein

fluorophore values (EFFV) and to document the variability and measurement uncertainty of the measurement. Sixteen laboratories from eight countries participated using and evaluating a total of twelve different instruments (platforms) with 3 separate counting technologies .

The standard protocol used counting beads to obtain a quantitative measurement of CD4+ cell count, by relative measurement of the CD4+ cell number with respect to the number of calibration beads. In a separate experiment, the contribution of the calibration beads to the uncertainty of the CD4+ cell concentrations were determined by measuring the total number of beads per vial using impedance-based reference particle counters. The average number of CD4 antigens per cell was derived from measured equivalent fluorescein fluorophore value (EFFV). There was good overall cross-platform and counting method agreement between the reported results from 15 of the 16 participants. A major contribution to variation was tube to tube variation of the calibration beads, amounting to an uncertainty of 3.6%. Variation due to preparative steps equated to an uncertainty of 2.6%. There was no reduction in variability when data files were centrally re-analyzed. Remaining variation was attributed to instrument specific differences.

The PBMC are purported to have a fixed number of surface CD4 receptors. Based on microsphere calibration, the EFF value of the PBMC samples was measured to characterize the population average CD4 expression level of the PBMC preparations. Both the results of data analysis performed by each participant and the results of centralized analysis of all participants' raw data are reported. Centralized analysis gave a mean EFF value of 22,300 and an uncertainty of 750, corresponding to 3.3% (level of confidence 68%) of the mean EFF value. [30]

Significant findings from the study are:

- Major contribution to variation in CD4 counts was tube to tube variation in the internal reference counting beads (uncertainty of 3.6%)
- Variation due to sample preparation – uncertainty 2.6%
- No reduction in variability with central analysis of data
- Remaining variability attributed to instrument specific differences

CCQM-P165: Quantification of CD34+ cell counts

Accurate quantification of hematopoietic stem cells is needed to ensure transplantation success which is hindered by the low frequency of these cells in hematopoietic stem cell products.

The P165 study was set up to examine sources of measurement uncertainty in the enumeration of CD34 cells. The aim of the study was to demonstrate participant capability in flow cytometric measurements of hematopoietic stem cell concentrations, to document the interlaboratory variability and intra-laboratory measurement uncertainty of CD34 counts. The comparison between the well-established single-platform cytometry method using an internal reference bead standard with the increasingly available volumetric flow cytometry method was also performed. Twelve organizations from seven different countries participated in the CCQM-P165 study. Twelve different instrument platforms including seven different volumetric platforms were used and evaluated.

A CD34 reference standard composed of lyophilized G-CSF mobilized white blood cells was produced to serve as a sample for CD34 enumeration by flow cytometry (USP Catalog # 1084292). The CD34 cell enumeration was performed on six different vials of lyophilized G-CSF mobilized leukocytes reconstituted with two different

volumes of water. Participants followed a standard protocol for sample processing and data analysis and were provided with Trucount™ tubes of the same lot for the reference bead-based method.

Samples analyzed using either method met the expected range of CD34 cell concentrations. The results show that the two methods have similar uncertainties of measurement. The uncertainty of intra- and inter-laboratory vial to vial variation was combined with the uncertainty of the individual CD34+ cell concentration measurement by the error propagation law, including statistical uncertainties of the beads and CD34 cell enumeration and vial-to-vial variation within the reference beads provided. Systemic effects such as antibody clone, cell staining, and gating are not considered as they are not quantified.

The number of beads in Trucount™ tubes in the lot supplied by the manufacturer is 50650 beads/tube**.** The number of beads in Trucount™ tubes used in this study was determined with impedance-based reference particle counters and an optical reference flow cytometer. The weighted mean value and expanded uncertainties for the mean value and for repeat measurements of single tubes were calculated. The average value was determined to be 49171 beads/tube with a tube-to-tube variation of 2457 beads (4.8%).

The mean value and uncertainty of measurement estimated with the Trucount method was 26.2 +/- 0.9 CD34 cells per microliter and 8.6 +/- 0.4 CD34 cells per microliter for the high and low concentration samples, respectively. For the volumetric method, they were 26.0 +/- 1.5 for the high concentration sample and 8.1 +/- 0.5 for the low concentration sample. (*Saraiva et al., Comparison of Volumetric and Bead-Based Counting of CD34 Cells by Single-Platform Flow Cytometry. Cytometry Part B 2019; 96B: 508– 513.*).

Significant findings from the study are:

- The uncertainty of measurement found in this study was similar for volumetric and bead-based methods
- With the reference bead method, major contribution to variation in CD34 counts was tube to tube variation in the internal reference counting beads (uncertainty of 4.8%). The acquisition time and potential loss of beads to the tube could lead to further uncertainty although this was not quantified in this study.
- The volumetric method is an attractive alternative as the cell enumeration becomes independent of a bead count. However, the reliance on a bead count is replaced by the reliance on an accurate acquisition volume. Volume is a unit traceable to a higher order standard (mass) using the gravimetric method and therefore it seems plausible that the uncertainty of volume measurement could be calculated in different cytometer platforms. Such a method would be traceable to the International System of Units (SI) and would potentially allow for better agreement of absolute measurements of cell concentrations in different cytometers.

CCQM-P123: Number and geometric property of cells adhered to a solid substrate

The CCQM-P123 study was set up within the Cell Analysis Working Group to demonstrate capability of participants in the quantification of 4 measurands related to cells on a planar surface, using one reference material:

- number of adherent cells
- cell density,

- cell confluency
- average cell area.

The CCQM P123 pilot study has been the first study proposed on adherent cells within the EURAMET frame and had the unique feature to propose 4 measurands in one reference material and 3 measurement claims in one comparison.

The measurement claims for the CCQM-P123 study were:

1. number of cells in monolayer adhesion on a defined area[1], in the range 0 to 5000 cell/mm^2, defined as cell density and calculated as:

$$Cell\ density = \frac{number\ of\ cells}{area} \quad [\text{unit count value}/\text{mm}^2]$$

2. confluency fraction of cells in monolayer adhesion on a defined area[1], in the range 0 % to 100 %, defined as cell confluency and calculated as:

$$Cell\ confluency = \frac{area\ occupied\ by\ cells}{area} \times 100 \quad [\text{mm}^2/\text{mm}^2 \ \ \%]$$

3. average cell area of cells in monolayer adhesion on a defined area[1], derived from the previous measurements and calculated as:

$$Average\ Cell\ Area = \frac{area\ occupied\ by\ cells}{number\ of\ cells} \quad [\mu\text{m}^2]$$

[1] the defined area was intended on a planar (2D) surface.

In addition, the three measurements required several operations and different level of complexity. These made the study intrinsically ambitious.

Nine Institutions participated in the study. Metrology for cells provides confidence in both, scientific and applicative research. Several scientific fields, such as cytology, biochemistry, molecular biology and molecular genetics have cells as their direct topic of study. The cell is a fundamental unit of highly technological systems, employed, for example, in the development of new chemicals and drugs, in the evaluation of compound toxicity, in novel approaches to health, such as regenerative medicine. High content screening technologies are cellular imaging-based assays. In this framework, the cell density, the cell confluency and the cell size are measurands that can describe a biological effect of a molecule under examination. Reference materials for three levels of cell density and cell confluency were prepared for fluorescence microscopy and circulated among participants. Each reference material was a commercial imaging dish on which human cells were seeded, fixed and stained for nucleus and for whole cell. Each dish had a glass bottom divided in 400 squares; 4 squares for each dish has been selected for the analysis and these 12 squares are the measurement standards of the CCQM-P123 pilot study. In each square a defined area was selected as area of measurement. The use of a calibrated ruler to define the area of measurement assured the traceability to the International System of Units (SI).

The study protocol was particularly detailed and overall complex to be executed and the reference material was not enough resistant to survive several shipments and improper handlings. The first set of reference material was severely damaged after 5 shipments and relative handlings to perform the measurements. The

second set was damaged as well. This study allowed several considerations in terms of traceability, comparability of results and complexity of the comparison protocol. The traceability was assured by the use of the ruler. However, the instructions on the use of the ruler were not easily followed by all the participants. Comparability of results were compromised by failing to follow the protocol. However, the protocol itself should have given more precise and synthetic instructions.

Taken these considerations into account, in general the results for the four measurands showed a good agreement between the laboratories. In the 75 % of the measurement standards of set 2, the consistency between the laboratories' result and the proposed reference value was reached by all the laboratories for at least three measurands. For set 1, this consistency was obtained in the 50 % of the measurement standards. In conclusion, the study has been a preparatory exercise for a key comparison on at least one of the 3 measurements claims (cell density, cell confluency and cell area) related to adhered cells features such as cell proliferation, morphology and size. The study has been applied to the A549 cell line, selected for some properties (mainly adhesion and size). However, the measurement claims are not intended to be limited to this cell line, can be applied to any adherent cell type matching the measurands ranges of the measurement claims. In conclusion, the study has been a preparatory exercise for a key comparison on at least one of the 3 measurements claims (cell density, cell confluency and cell area) related to adhered cells features such as cell proliferation, morphology and size. In conclusion, the study has been a preparatory exercise for a key comparison on at least one of the 3 measurements claims (cell density, cell confluency and cell area) related to adhered cells features such as cell proliferation, morphology and size. The results from such a key comparison could be utilized by the NMIs to provide traceability to different methods of bio-analysis.

4. REFERENCES

Bibliography

1. Johnston, A. *et al.* Guidance for quality control practices and precision goals for CBCs based on IQMH patterns-of-practice survey. *International journal of laboratory hematology* 41, 15–22 (2019).
2. SUTHERLAND, D. R., ANDERSON, L., Keeney, M., NAYAR, R. & CHIN-YEE, I. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. *Journal of Hematotherapy* 5, 213–226 (1996).
3. World Health Organization - UNAIDS. Treating 3 Million By 2005: Making It Happen – The WHO Strategy. 1–61 (2003).
4. Committee, D. S. *Digital Imaging and Communications in Medicine (DICOM) Supplement 145: Whole Slide Microscopic Image IOD and SOP Classes*.
5. Yagi, Y. & Gilbertson, J. R. Digital imaging in pathology: the case for standardization. *J Telemed Telecare* 11, 109–116 (2005).
6. Palmer, L. *et al.* ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int. Jnl. Lab. Hem.* 37, 287–303 (2015).
7. Kratz, A. *et al.* Digital morphology analyzers in hematology: ICSH review and recommendations. *International journal of laboratory hematology* 353, 498–11 (2019).
8. Metze, S., Ruhl, S., Greller, G., Grimm, C. & Scholz, J. Monitoring online biomass with a capacitance sensor during scale-up of industrially relevant CHO cell culture fed-batch processes in single-use bioreactors. *Bioprocess and Biosystems Engineering* 43, 193–205 (2019).
9. Kourti, T. The Process Analytical Technology initiative and multivariate process analysis, monitoring and control. *Anal Bioanal Chem* 384, 1043–1048 (2006).
10. Kashi, L. *et al.* Heterologous recombinant expression of non- originator NISTmAb. *mAbs* 10, 922–933 (2018).
11. Global Monoclonal Antibodies Market Hit $100 Billion in 2017: Report. (2018).

12. Yu, M. *et al.* A resource for cell line authentication, annotation and quality control. *Nature* 520, 307–311 (2015).
13. Geraghty, R. J. *et al.* Guidelines for the use of cell lines in biomedical research. *British journal of cancer* 111, 1021–1046 (2014).
14. Capes-Davis, A. *et al.* Match criteria for human cell line authentication: where do we draw the line? *Int. J. Cancer* 132, 2510–2519 (2013).
15. Freedman, L. P. *et al.* Reproducibility: changing the policies and culture of cell line authentication. *Nat Meth* 12, 493–497 (2015).
16. CAR T Cell Therapy Market Global Forecast by Regions, Targeted Antigens, Clinical Trials/Study, Companies. (2020). Available at: (Accessed: 18 November 2020)
17. Sharma, R. *et al.* Clinical-grade stem cell-derived retinal pigment epithelium patch rescues retinal degeneration in rodents and pigs. *Science Translational Medicine* 11, eaat5580 (2019).
18. Marinho, P. A. Systematic optimization of human pluripotent stem cells media using Design of Experiments. *Nature Publishing Group* 1–13 (2015). doi:10.1038/srep09834
19. Baghbaderani, B. A. *et al.* cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications. *Stem Cell Reports* **5,** 647–659 (2015).
20. Sekine, K. *et al.* Robust detection of undifferentiated iPSC among differentiated cells. *Sci. Rep.* 1–9 (2020). doi:10.1038/s41598-020-66845-6
21. PM 7/98 (4) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *EPPO Bull* **49,** 530–563 (2019).
22. Bigelow, N. *et al.* Chrysochromulina sp.: A proposed lipid standard for the algal biofuel industry and its application to diverse taxa for screening lipid content. *ALGAL* **2,** 385–393 (2013).
23. Schmidt, F. *et al.* Biodeterioration Affecting Efficiency and Lifetime of Plastic-Based Photovoltaics. *Joule* **4,** 2088–2100 (2020).
24. Shirakawa, M. A. *et al.* Microbial colonization affects the efficiency of photovoltaic panels in a tropical environment. *J Environ Manage* **157,** 160–167 (2015).
25. Gilbert, J. A. & Stephens, B. Microbiology of the built environment. *Nat Rev Microbiol* **16,** 661–670 (2018).
26. Gorbushina, A. A. & Broughton, W. J. Microbiology of the Atmosphere-Rock Interface: How Biological Interactions and Physical Stresses Modulate a Sophisticated Microbial Ecosystem. *Annu. Rev. Microbiol.* **63,** 431–450 (2009).
27. CODEX ALIMENTARIUS International Food Standards. *CODEX Alimentarius* Available at: (Accessed: 18 November 2020)
28. Kammel, M., Kummrow, A. & Neukammer, J. Reference measurement procedures for the accurate determination of cell concentrations: present status and future developments/Referenzmessverfahren für die genaue Bestimmung von Zellkonzentrationen: Status und zukünftige Entwicklungen. *LaboratoriumsMedizin* **36,** 1–11 (2012).
29. Wang, L., DeRose, P. & Gaigalas, A. K. Assignment of the Number of Equivalent Reference Fluorophores to Dyed Microspheres. *J. RES. NATL. INST. STAN.* **121,** 269–18 (2016).
30. Wang, L. *et al.* Quantification of cells with specific phenotypes II: determination of CD4 expression level on reconstituted lyophilized human PBMC labelled with anti-CD4 FITC antibody. *Cytometry A* **87,** 254–261 (2015).
31. Supporting Information Document S1 – Open Letter to Manufacturers of CD4+ T-cell Enumerations Technologies from the World Health Organization. 24th March 2005.

5. DOCUMENT REVISION SCHEDULE

Document name; type of revisions; date

CAWG Strategic Plan 2021-2030: V1, 28 Dec. 2020