CAWG Autumn meetings

CAWG 23-02

7th-9th November 2023

KRISS. South Korea

Dr. Jonathan Campbell. Chair

Dr Boqiang Fu. Vice-Chair

Bureau International des Poids et Mesures

AGENDA
CCQM CELL ANALYSIS WORKING GROUP MEETING. Autumn 2023.
7th-8th November. KRISS. South Korea

Day 1 (7th November)

08:50 Shuttle bus from Lotte City Hotel to KRISS		
09:30 **(UTC+9)**	1) Welcome and register. 2) Appoint Rapporteur 3) Agree agenda 4) Review minutes and actions, Spring 2023 meeting	Jonathan Campbell, NML@LGC CAWG Chair
09:45	5) Project work. P205 "Enumeration of membrane intact E.coli" Results presentations (membrane filtration method)	NIM China Boqiang Fu & participants
10:30	Coffee Break. Lobby	
11:00	P205 continued Next steps	NIM China Boqiang Fu
11:30	6) Project work. P197 Proliferative stem cell number per unit area Sample distribution, reporting and next steps 7) New proposal. Cell counting in 3D hydrogel system	NPL Nilofar Faruqui NPL
12:20	8) Photo	
12:30-13:30	Lunch. Terrace Cafe	

12:30-13:30	Lunch. Terrace Cafe	
13:30–15:30	9) Afternoon session. CAWG Strategic planning Assessment of current work program. Transitioning to Key studies. Barriers / opportunities. Assessment/discussion of wider strategy. Horizon scanning activities: New technologies, New Stakeholders, Future measurement services	Chair led
15:30-16:00	Afternoon Tea	
16:00–17:00	10) CAWG procedural document. Update of development Update regarding NMI / DI capabilities. Update regarding region metrology activities	Chair led
18:00	Shuttle bus from KRISS to Lotte City Hotel	
Evening	**Welcome dinner 18:50. Lotte City Hotel. Crystal ballroom. 1st floor**	

Day 2 (8th November)

08:50 Shuttle bus from Lotte City Hotel to KRISS		
09:30 **(UTC +9)**	Short welcome	Chair
09:40	11) P217 Quantification of fixed mononuclear cells in suspension.	NIST Sumona Sarkar, David Newton **(Remote 20:40 EST)**
10:30-11:00	Coffee Break Lobby	
11:00	12) P222 "Particle enumeration for cellular analysis Project update. Next steps	NMIJ
12:00	13) CCQM task group on Foods. Jeremy Malenson and Ralf Josephs lead.	Dr Fu **(Remote 11:00 CST)**
12:30	Lunch. Terrace Cafe	
13:30– 14:30	KRISS lab tour	E. Lee
15:30-16:00	Afternoon Tea	
16:00 – 16:30	14) CCQM Task Group on Intracellular Metrology for Gene Delivery: Terms of Reference	Max Ryadnov NPL **(Remote 7:00** GMT**)**
16:30-17:30	15) CAWG current measurement services review 16) CAWG strategic planning	
17:30	Final remarks. Wrap up	Chair led
18:00	Shuttle bus from KRISS to Lotte City Hotel	
Evening	**Invitation to Gogimyungjak Korean BBQ** **Meet at hotel lobby 19:00**	

Day 3 (9th November). Joint Bioanalysis meeting

Thursday 9th November 2023 09:30-17:00 (UTC +9 Korea Standard Time)		
08:50 Shuttle bus from Lotte City Hotel to KRISS		
09:30-10:30		
1) Overview form each group.		
	Introductions ('tour de table' for those present, with online participants providing name and institution in the chat box). Agree agenda	Chairs
2) WG update		
	• CAWG	Chairs
10:30-11:00 Coffee		
11:00-12:30		
3) WG update		
	• NAWG • PAWG	Chairs
4) Wider stakeholder interactions		
	What service are out there? Stakeholder engagement Liaisons current, future (who should we be developing links with)	Chairs
5) Shared challenges		
	Measurand	Chairs
	Commutability	
	Traceability	
12:30- 13:30 Lunch		

13:30-15:30		
6) CMC database (Carla, Gavin, Liana [Alison])		
	Update on KCDB fields to make it more suitable for bio analytical measurands	Carla, Gavin, Alison/Liana
	WG support for RMOs, wider RMO interaction	All
7) Cross working group activities		
	Microbes	Mojca Milavec
	Fire-drill update	TBC
	SOGATS (WHO comparisons)	TBC
8) **Units and reporting**		
	The Mole. Future support for NMIs in the use of the Mole for Bioanalysis and macromolecules	Daniel Burke
	Orthogonal methods	
15:30-16:00 Afternoon tea		
16:00-17:30		
9) Task groups continued		
	CCU Focus Group on Counting and Number Quantities	Richard Brown
	CCQM Task Group on Food Measurement	Robert Wielgosz
	Update on wider Pandemic TG	Julian Braybrook
10) **Wrap up**		

Action items CAWG 23-01

CAWG/2023-1/1	CCQM-P222: Particle enumeration for cellular analysis Pilot study suspended. NMIJ and PTB to feedback any new routes to proceeding with study. Chair to compose letter of support to enable study to proceed.	Update of study status expected during the November meeting by Japan team
CAWG/2022-2/2	CCQM-P205: Quantification of membrane intact E.*Coli* in Drinking water Dr Fu to check and feedback to VNIIM members possible routes for sharing study materials.	**Dr Fu to update**
CAWG/2022-2/3	P217 Quantification of fixed mononuclear cells in suspension.	Drafting team to finalise analysis and write-up

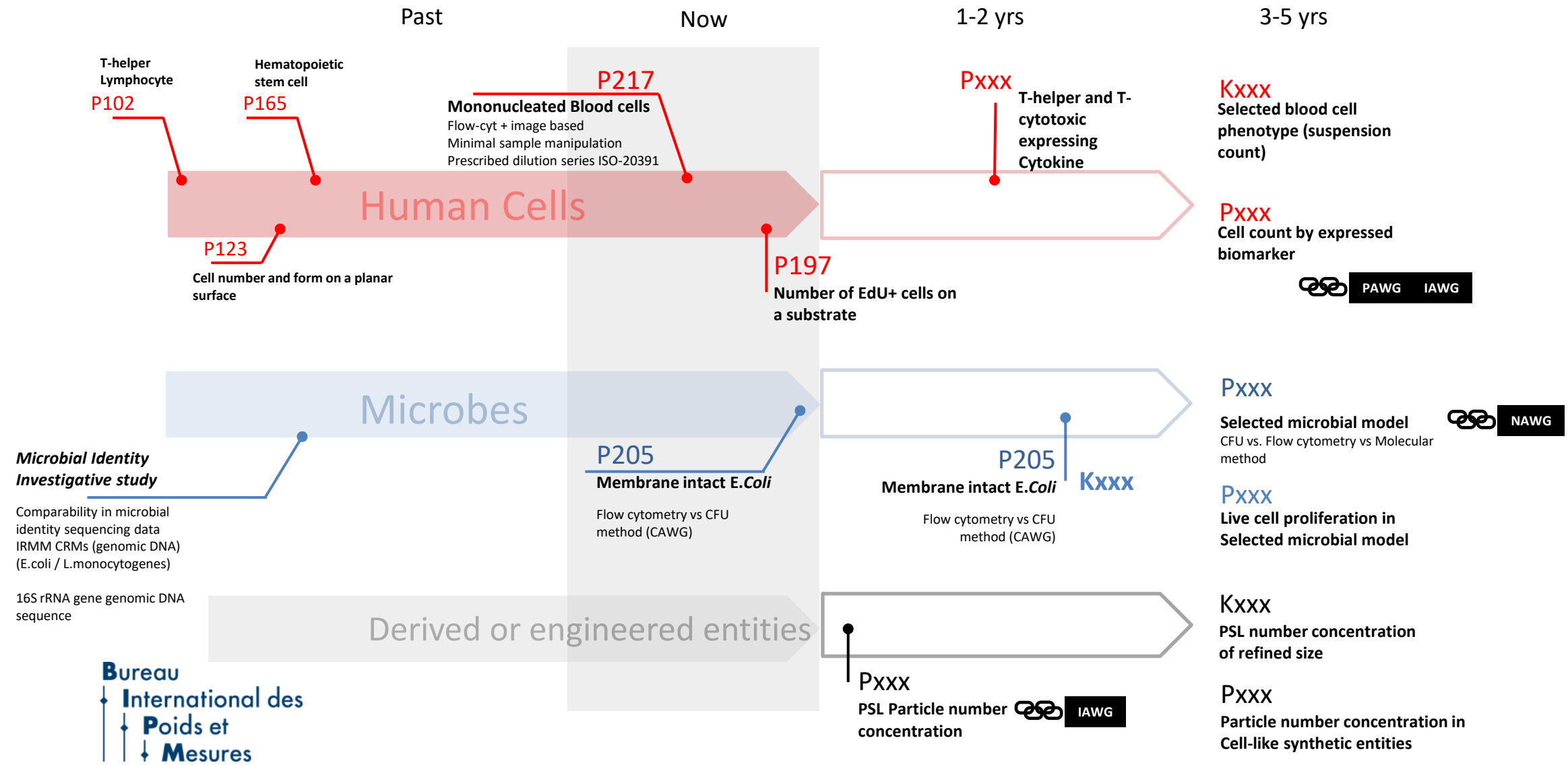
CAWG/2022-2/4	P-197. Proliferated stem cell number per unit area. Updated study timetable. Sample distribution by end April, return of data end of July, presentation of data by October, first draft of pilot by December. Study lead to update measurement claim language (as to MV suggestions).	**Dr Faruqui to update** Double-check change request for completion (KCWG rep please)
CAWG/2022-2/5	Review of Strategic document for the working group. WG to review the current strategic document and feedback areas that need revision, or new areas to incorporate. Chair to organise an online meeting to discuss this document in March.	Meeting pushed to November since face-to-face meeting

Our Charge

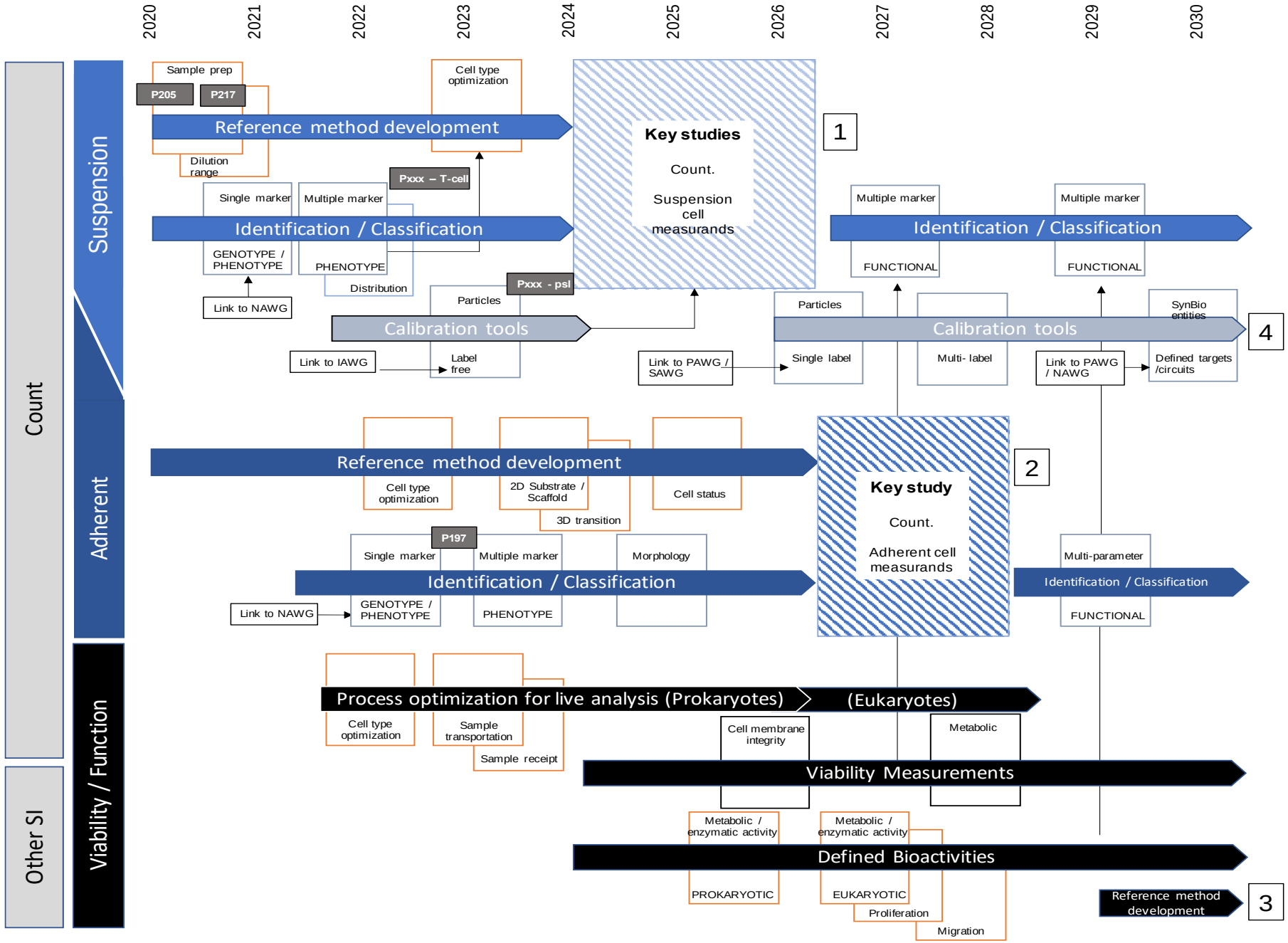
1) To carry out Key Comparisons and pilot studies, to critically evaluate and benchmark NMI/DI claimed competences for measurement services and capabilities including, but is not limited to **the identification and quantification of cells and cell properties indicative of function in complex** matrices and mixtures.

2) To identify and establish inter-laboratory work and pilot studies to enable global comparability of cell analytical measurement results through reference measurement systems of the **highest possible metrological order with traceability to the SI**, where appropriate and feasible, or to other internationally agreed units, **in response to the demands of NMI customers.**

Establishing cell counting metrology



Road Map



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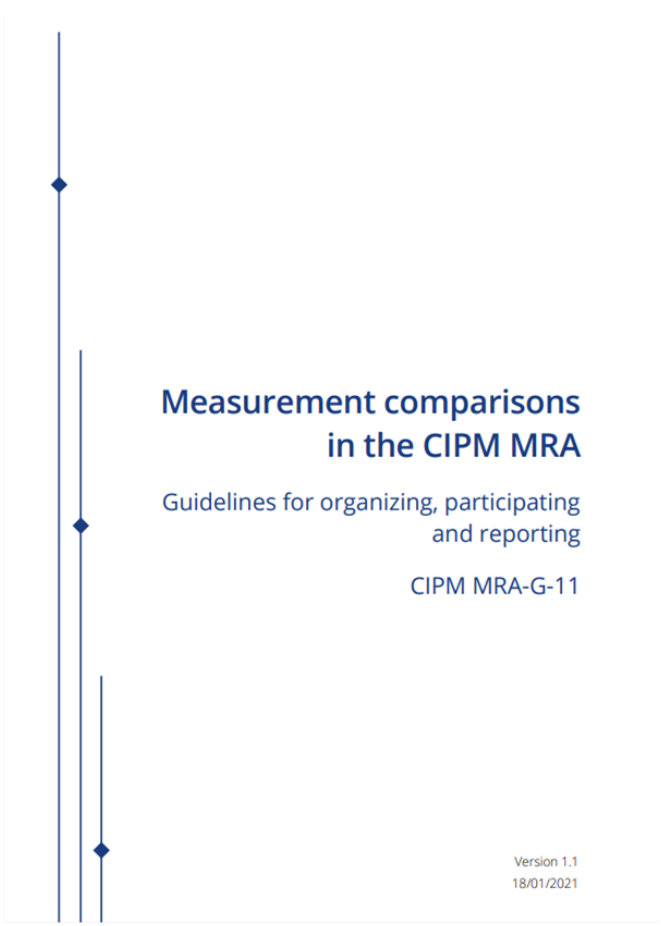
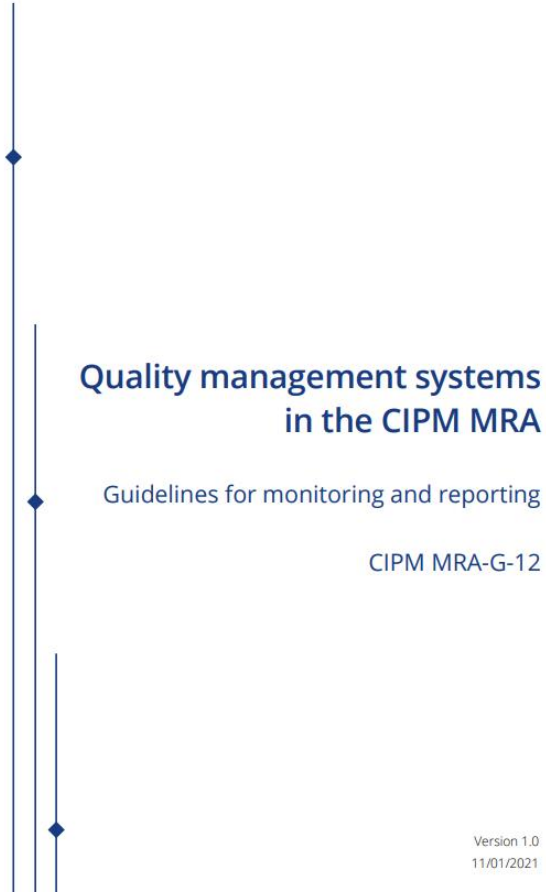
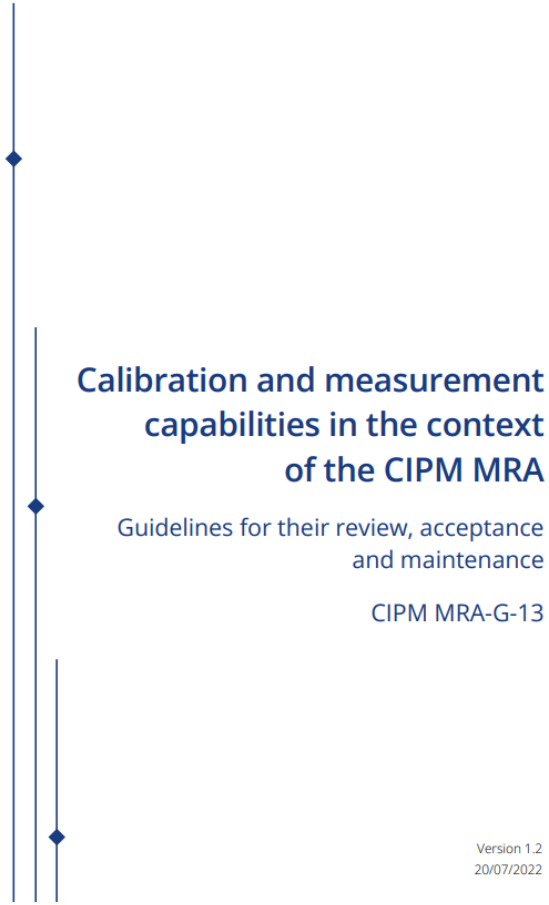
CAWG strategy discussions







Measurement comparisons in the CIPM MRA

Guidelines for organizing, participating and reporting

CIPM MRA-G-11

Version 1.1
18/01/2021

Table 1. An overview of the comparisons organized within the frame of the CIPM MRA.

Type / Activity	CIPM comparisons		RMO comparisons		Pilot studies
	Key	Supplementary	Key	Supplementary	
Objective (Section 2)	To test the principal techniques and methods in the field	To meet specific needs not covered by key comparisons	To extend the coverage of the CIPM key comparisons regionally	To meet specific needs not covered by RMO key comparisons	To establish measurement parameters for a "new" field or instrument, or as a training exercise
Organization (Section 3)	CCs and BIPM		RMO TCs/WGs		BIPM, CCs and RMOs
Technical protocol (Section 4)	Includes the proposal for the method of determination of the key comparison reference value	According to common requirements	Follows the CIPM key comparison and any relevant CC guidelines. Includes the way in which the results will be linked to the CIPM key comparison	According to common requirements	Depends on CCs and RMOs
Registration (Section 5)	Registered in the KCDB				Not registered in the KCDB.
Participation (Section 6)	Open to laboratories having the highest technical competence and experience (CC members). Participation may be restricted (see "2.Type of comparisons" for details) Associates may participate in special cases		Open to all RMO members and other institutes (including from other RMOs), subject to decision by the organizing RMO		CCs and RMOs
Outcomes (Section 7)	Measured values and measurement uncertainties				Measured values and measurement uncertainties
	Key comparison reference values and degrees of equivalence	May include degrees of equivalence	Degrees of equivalence	May include degrees of equivalence	
Approval of reports (Section 8)	Withdrawal is generally not allowed				According to practice of CCs and RMOs
	Approved by CCs	Approved by CCs	Approved by CCs	Approved by RMOs	
CMC support (Section 8.2)	Draft B may be used to underpin CMCs	Final report needed to underpin CMCs	Draft B may be used to underpin CMCs	Final report needed to underpin CMCs (overseen by CC)	Generally not used to support CMCs

Measurement comparisons in the CIPM MRA

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18/01/2021

2. Type of comparisons

A **key comparison** is selected by a Consultative Committee to test the principal techniques and methods in the field. Key comparisons may include comparisons of representations of multiples and sub-multiples of SI base and derived units as well as comparisons of artefacts. The key comparisons are essentially of two types:

- **CIPM key comparisons:** of international scope, are organized by Consultative Committees or the BIPM, and are restricted to laboratories of Member States and normally members of the corresponding Consultative Committees. CIPM key comparisons deliver a "reference value" for the key quantity chosen.
- **RMO key comparisons:** of regional scope, are organized at the scale of a region (though they may include additional participants from other regions) and are open to laboratories of Associates as well as Member States. RMO key comparisons are intended to provide RMO members with the means to link to the reference value established by the corresponding CIPM key comparison. The RMO key comparisons deliver complementary information without changing the reference value derived from the CIPM key comparison. A degree of equivalence derived from an RMO key comparison has the same status as one derived from a CIPM key comparison.

Key comparisons may be extended by **subsequent key comparisons**.

A **supplementary comparison** is intended to cover areas or techniques not addressed by key comparisons. These are complementary to key comparisons and are not intended as second-level comparisons. Their final reports are published in the KCDB, but degrees of equivalence are not necessarily computed.

Pilot studies are a third category of comparison normally undertaken to establish measurement parameters for a "new" field or instrument, or as a training exercise. The results of pilot studies alone are not normally considered sufficient support for calibration and measurement capabilities (CMCs) and the studies are not registered nor published in the KCDB.

CAWG study prioritization

Study	CCQM Objectives			Measurement service provision		Regulatory landscape	Fit with CAWG strategy	How-far the light shines?	Properties of the measurand
	1. Need for **Global** comparability	2. Progress State-of-the-art **in measurement**	3. Stakeholder needs and engagement		Reference value	Maturity of Regulations surrounding measurement need	Counting Differential Counting by key identity / viability		Stability Homogeneity etc.
Key comparison	x	x	x	x	x	x	x	x	x

Can we develop a tool for unbiased assessment of study fitness and for prioritization of studies in our work program?

This table might provide a template based on features and objectives of projects

We must consider a WG capacity for sequential ongoing studies. Suggest ≈2-3

CAWG study prioritization

Study	CCQM Objectives			Measurement service provision		Regulatory landscape	Fit with CAWG strategy	How-far the light shines?	Properties of the measurand
	1. Need for **Global** comparability	2. Progress State-of-the-art **in measurement**	3. Stakeholder needs and engagement		Reference value	Maturity of Regulations surrounding measurement need	Counting Differential Counting by key identity / viability		Stability Homogeneity etc.
Key comparison	X	X	X	X	X	X	X	X	X
P205 Membrane entact *E* coli counting	X	X	X	X	X	X	X	X	X
P217 PBMC number in suspension	X	X	X				X	X	
P222 *PsL bead count*	X	X	X				X	X	X
P197 *PsL bead count*		X	X				X	X	X

CAWG strategy discussions

- Consideration of work program.
  - New studies we should consider (ie. PFU/area – pandemic response)
  - Strategic studies with bioanalysis groups or wider? Joint meeting of Bio groups (ie. Cell counting and cell authentications support)
  - Transition to key comparison study. Which, Time scales
  - Road-map visualisations
- Stakeholder interaction
  - Improvement and bringing expertise into studies where necessary
  - Increasing our profile?
- Horizon scanning
  - New measurement methods of interest? How do we bring into new studies?
- CAWG structural framework
  - Handbook / policies

Pathways forward

- Reference materials. (New materials survey) (EuK)
  - Blood counting materials (Status / availability of synthetic blood (US)
  - Cells that survive lyophilisation
- Digital data send-rounds
  - explore uncertainty of measurement for subjective counting
  - Several different types of data could be distributed (automated image analysis, flow cytometry data, CFUs on membrane
- Transition to Key studies
  - P205 study (bacterial counting key)
  - Blood cell counting (Germany and UK to explore and develop?)
- New studies
  - Viability . Feasibility of a study exploring key uncertainties (ISO development)

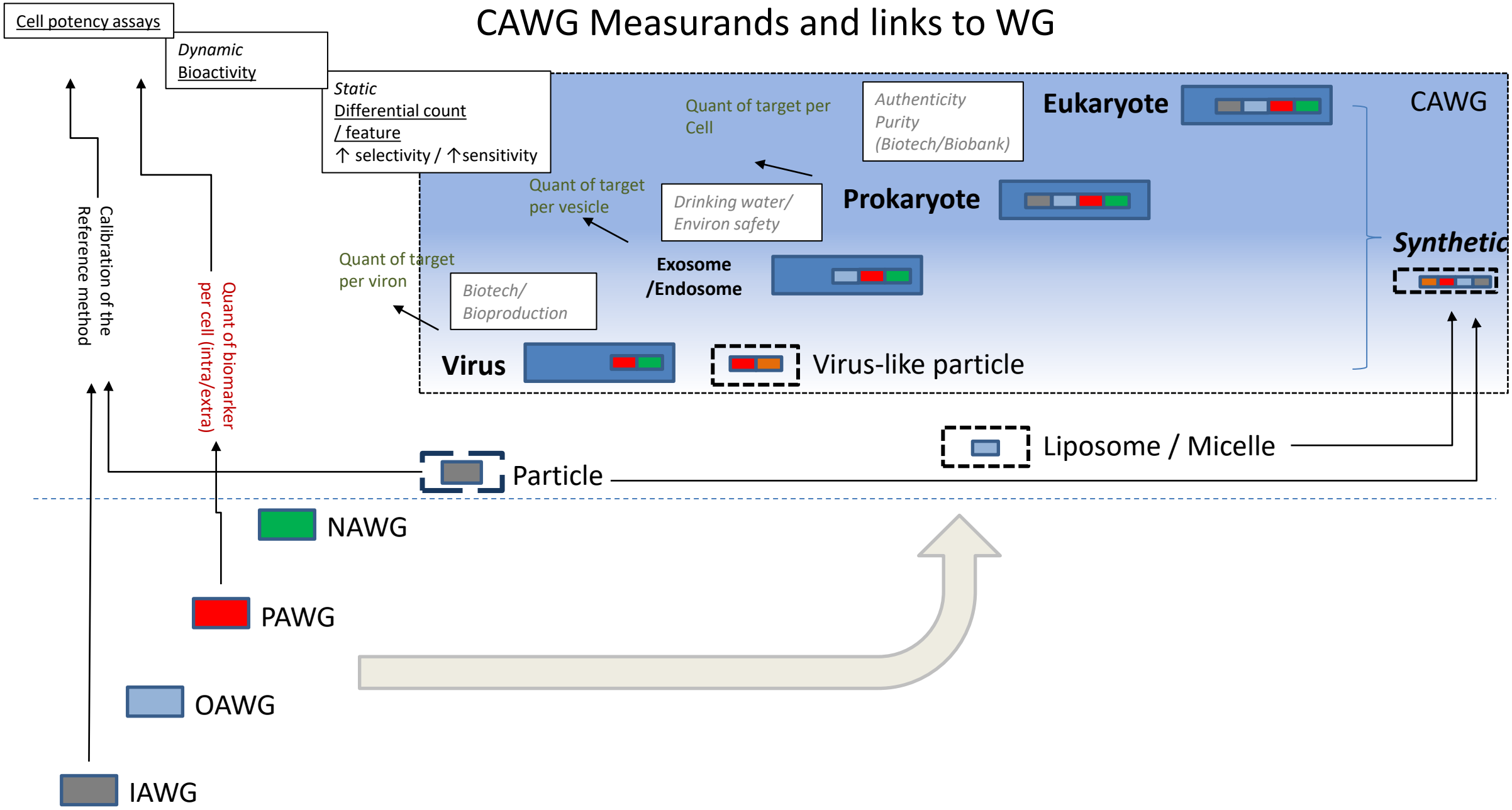
CAWG Task groups

- Key transition
- Horizon scan
- Stakeholders

- **Reference materials.** — Lead and team
  - Survey landscape (key stakeholders, key materials)
    - Blood counting materials (Status / availability of synthetic blood (US)
    - Cells that survive lyophilisation
  - Strategies for integration (into traceability chain)

- **Reference Methods** (Instruments and assays) — Lead and team
  - Survey landscape (key stakeholders, key materials)
  - Emergent methods
  - Development of Ref method candidates (ie. flow cytometry)
  - P205 study (bacterial counting key)
  - Blood cell counting (Germany and UK to explore and develop?)

CAWG Measurands and links to WG



Quantities relating to cell counting

Quantity	Other metric	Expression	Description	Application (example)
Total cell count		$number\ of\ cells$ [unit 1]	Count of cells Count of events	
Differential cell count (subset of cells of interest)		$number\ of x\ cells$ [unit 1]	Count of x cells Count of x events x = nominal property	
Cell (suspension) concentration		*number of cells / volume* *number of x cells / volume* *[unit count value/mL]*	Cell count per volume	Multiple / Diagnostic (viable cell concentration) (AIDS <200CD4+ cells/mL)
Differential cell index or fraction		*Number of x cells/Total number of cells*	Decimal Fraction	Multiple / Diagnostic % viability % mitotic index
Packed cell volume		*Height of PCV/Height of Plasma x 100.* [%]	Fraction	Rapid diagnostic (Estimate of RBC fraction in whole blood)
Cell area density		*number of cells / area* *[unit count value/mm²]*	Cell count per unit area	Discriminative Cell identity / morphology

Quantities relating to cell counting

Quantity	Other metric	Expression	Description	Application (example)
Average cell area		$\left(\frac{area\ occupied\ by\ cells}{number\ of\ cells}\right)$ [μm²]	2D area of cell	Discriminative Cell identity / morphology functional response
Cell confluency		$\left(\frac{area\ occupied\ by\ cells}{area}\right) x100$	Area of an adherent population of cells covering a substrate	Cell growth kinetics. Cell manufacture (Estimate of cell proliferation 2D substrate) (Optimized sub-culture)
	Colony Forming Unit / volume	$\frac{no.of\ colonies \times dilution\ factor}{Vol.culture\ plate}$	Group of growing cells visible at micro/macroscopic scale	Estimate proliferative viable cells *a. Bacterial contamination (ProK)* *b. Stem cell number (EuK)*
	Plaque forming units / volume	$\frac{no.of\ colonies}{Vol.infectious\ lysate \times dilution\ factor}$	Group of changed or absent cells in a confluent sheet of cells	Estimate number of infectious viral particles
	Turbidity	*OD600*	Absorbance measurement of a cell suspension at 600nm.	Correlate to Bacterial/Yeast cell number. (Proliferation in a bioreactor)

Pandemic awareness

Review of CAWG strategic document. 21-30. (Task to keep updated)

Health and life sciences.

Clinical Diagnostics.
(Morphological)
Complete blood count (CBC) , RBC, WBC, Reticulocyte, platelet count.
Packed cell volume (%, L/L)), Mean cell volume (fL), Red cell distribution width (%CV or fL) and mean cell hemoglobin (pg or fmol).
Exosomes

(Biomarker)
CD4+ Immunocompromisation
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.
CTCs early and less invasive detection of cancer, relapse monitoring, widens ongoing treatment/management options for patients in non-clinical settings.

Additional / Growth area

Digital pathology

Digitisation metrology.
AI metrology (choice of algorithm etc.)

Biotechnology

Total and differential cell counts for a range of industrially important eukaryotic cell lines CHO and HEK293T

Total and differential cell counts for Viable cell concentration (VCC), Viable cell volume (VCV) and wet cell weight (WCW)

Cell fragments
Exosomes (surrogate measurement of bioprocess)

Cell authentication. STR profiling *(NA method. What can Cells provide?)*

Additional / Growth areas

compatible analytics requiring calibration
RAMAN, NIR spectroscopy,
dielectric impedance spectroscopy.

Total and differential counts for CAR-T cells/ iPSC cells

Review of CAWG strategic document. 21-30. (Task to keep updated)

Drug discovery

Additonal / Growth area

Total and differential adherent cell number
Biomarker, cell viability or morphology indictors

Plaque forming unit per mL (PFU/mL)

Cytopathic

viral infectivity measurements (PFU/mL)

Standard scaffolds – 3D total and differential counts

Environmental monitoring and safety

Additonal / Growth area

Count of beneficial, pathogenic microorganisms in water, soil or other solid surfaces (biofilms)

- agriculture, production purposes, bioremediation and industrial sanitation.
- Initiatives to engineer the environment using microbial symbionts in plants and insects
- Environment risk/safety/security assessments - deliberate release of engineered micro-organisms
- Photovolaics

Combining NA measurement alongside count
Algal bloom / CO2 capture initiatives (Eukaryotics)

Food

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

Additonal / Growth area

Combining multiparametric measurements to aid speciation, viability etc.

Identified challenges

• Appropriate recognition of informative measurands for cells and how these relate to specific biological activities and attributes of cell.

• The realization of these measurands with sufficient stability and purity within candidate reference materials.

• Adopt novel or unconventional approaches in standards development. Ie. tiered system of increasingly stable or commutable materials that transition from synthetic to biological origin.

•Capturing the breadth of methods and instrumentation available , citing within group and expertise. Partner adoption outside of the NMI community.

• Changing historic practice in the community. Deal with subjective practices / opinion where necessary.

• Distribution and transportation challenges for biological materials. Customs /safety and environmental regulations.

• Regional availability and cost of ancillary reagents and consumables.

Grow a database of studies by Entity, containing stakeholder need, identified measurands, necessary links to other WGs and appraisal of the challenges.

Measurand	Entity		Allied WG	Needs (for CAWG / NAWG studies)			Considerations / Barriers	
	Natural	Man-made						
		Particle*	CAWG	Reference method calibration				
	Virus*	Virus-like particle*	NAWG/ PAWG	**determination of overall viral titre**	**characterisation of vector integrity (Empty, partial) etc)**	Transgene expression in Eukaryote	Integration/ transduction efficiency (single cell)	
	Exosome	Micelle / Liposome	OAWG / PAWG / NAWG	Count / Characterization of entity			Microflow cytometry takeup	
	Prokaryote	Future Synthetic cell	NAWG / PAWG OAWG / IAWG /SAWG	**Count of cell** (water, environmental safety)	Bioactivity of Cell Molecular Viability?		**Target species for 'standard'**	Extraction efficiency
	Eukaryote	Future Synthetic cell	NAWG / PAWG OAWG / IAWG /SAWG	**Count of cell CTC/rare cell detection. Increased selectivity / sensitivity of ref method.** **Cell Authentication**	Bioactivity of Cell? Cell proliferation Transgene expression	Engineered cells	**Key biomarkers?**	

Measurement Services Current and Near-Term (~2-5 yrs)

Reference Material Production

Eukaryotic Cell Systems

- Viable cell count 2D and 3D extracellular matrices (NPL, INRIM, NIBSC, KRISS, NIM, NRC, VNIIIM, INMETRO) [P197]
- Concentration of blood cells in a blood matrix (NIBSC, VNIIIM, UME, LGC, KRISS)
- Concentration of cell surface properties, CD4+, CD34+/CD45+ (NIST, VNIIIM, UME, LGC, NIBSC) [P102, P165]

Prokaryotic Cell Systems

- Biodegradation potential (BAM)
- Pathogens in water (NIM, JRC, KEBS, INMETRO, ISP?) [P205]
- Pathogens in food (NIM, ISP, JRC, KEBS INMETRO)

Cell analogs

- Peptide, lipid micro-shells (NPL)
- Cell mimic materials (VNIIM)
- Microspheres (NIST, NMIJ, NIM)

Reference Data

Calibration and measurement services

- Flow cytometry – biomarker expression (NIST, INRIM, LGC, NIM, NIBSC, INMETRO, KEBS) [P102, P165] [coming in October]
- FACS – internal standards (shell, sphere) for cell counting (NPL)

Reference value assignment

- Complete blood count (PTB, NIM, NMIJ)
- Reference Value – adherent cell on 2D support (INRIM) [P123, P197]
- Concentration of CD4+, CD34+/CD45+ (PTB, NIBSC)
- Antimicrobial susceptibility and biocidal properties (NPL, BAM)
- Cell viability measurements (NIM, NPL, INRIM)
- Cell count, yeast cell count (NIST, NMIJ, KEBS, NIM)

Proficiency test organizer and regional comparability coordination

- Blood cell counting (NIM)
- Pathogens in food (ISP, NIM, NMIA, KEBS)

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