CCQM Cell Analysis Working Group
KRISS, Daejeon South Korea

7-10 Nov 2023

Meeting Report

Participants: J Campbell (LGC, UK) (Chair), N Faruqui (NPL, UK) (Rapporteur), S Fujii (NMIJ, Japan), J Choi (KRISS, Korea), Sook-Kyong Kim (KRISS, Korea), A Runov (VNIIM, Russia), Andreas Kummrow (PTB, Germany), Hee Min Yoo (KRISS, Korea) Ji Youn Lee (KRISS, Korea), J Riveria (LGC, UK), Ana (BAM, Germany)

F Boqiang (NIM, China)[ǂ], J Cavalcante (INMETRO, Brazil), L Pierce (NIST, USA)[ǂ],
Sang-Ryoul Park (KRISS, Korea), Robert Wielgosz (BIPM, France) Julian Braybrook (LGC, UK) , Sumona Sarkar (NIST, USA)[ǂ], David Newton (NIST, LGC)[ǂ]
[ǂ] Attended via teleconference for part of the meeting; [+] attended in person for part of the meeting

1. Introductions

J Campbell welcomed delegates to the meeting and proceeded to brief introductions. Members introduced themselves accordingly.

2. Administrative

2.1 Agree agenda

J Campbell introduced the proposed agenda and summarised the minutes points from the May 2023 virtual-meeting.

2.2 Appointment of rapporteur

N Faruqui (NPL, UK) was appointed as a rapporteur.

2.3 Minutes of the CAWG meeting of May 2023

Minutes had been circulated for comment and correction after the previous meeting. The corrected minutes were agreed without further change.

2.4 Matters arising from the minutes

The actions from the previous meeting were reviewed:

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

3. **P205 – study update**

Boqiang Fu gave an update on P205, describing the study, rationale and design. Transportation issues have proved challenging due to carrier company and import rules. He went on to describe the membrane filtration method that the participants had to adopt and described in detail the uncertainty calculations that will be employed in the study. Boqiang presented the low level membrane filtration results from all participant NMIs/Dis. Both High level membrane filtration results and flow cytometry method (high level) results are outstanding. Reported values and uncertainties were shown from KRISS, Chile, INMETRO, NMIA Australia, KEBS Kenya. Dr. Fu discussed apparent differences in some of the submitted results, but they are still within the limits within the expected range of the material. He acknowledged various members for facilitating the study and for submitting their results. Outstanding flow cytometry method results are to be **completed by 30th Nov (NIST, NPL, VNIM).** A **Draft A report is due in April 2024**.

The Chair thanked him for the presentation. Andreas asked if the participants use the same volume method (Trucount beads or volume method) because if different methods is used then that can introduce some error. Jeanne Rivera asked if different types of Flow-cytometers are used then that can also give different results based on the threshold differences. Julian suggested that follow up reminders can be issued for outstanding Flow method results and could include request for further methods data to aid evaluation. Ongoing stability study data can be used to aid with late data

submission analysis. Although study participants received samples at different times, Boqiang confirmed that it should not pose any issues. It was decided on this basis to proceed with the study with no modification to the study design to accommodate late data submission. Sample stability was also monitored during the transportation. All the samples were received within 7 days Jun Choi asked if Boqiang has received the results from NIST. He confirmed that they (NIST) have received the sample in the middle of September and will need some time to do the study. NPL has also not sent the results. VNIM received the sample only a month ago.

Actions:

- **NIST / VNIIM / NPL are still to submit results. Flow results to be completed by 30th Nov (NIST, NPL, VNIIM).**
- **NIM to consider if a further request for information is needed if it supports the analysis of outstanding data submissions.**
- **Final results are to be completed (NIM)**
- **To proceed to the draft report A preparation in 2024**

4. **P197- Study update**

Nilofar provided the update on the project and informed the members that the samples have been prepared for the distribution in December. All the contact persons will be individually contacted before the shipment. An extensive discussion of the rationale for the study as well as the study design followed. Matters discussed included the necessity to include the description of the cell type (mesenchymal stromal cell) within the title of the study and the measurement claim. The term Mesenchymal stromal cell will be used instead of mesenchymal stem cell. There was an extensive discussion around the necessity of NMIs to measure the same slide field (ie. A4, B5 etc), since different NMIs will receive separate slides with a centralised analysis performed by NML. Representatives from LGC and KRISS pointed out that the study simply tested participants ability to measure green labelled nuclei from blue labelled Hoechst nuclei, but that measuring this ability could still yield useful insights. It was decided to proceed with the study with no modification to the study, considering the many aspects the project will test for recognising and counting cells on a 2D surface.

Actions:

- **Leader (N. Faruqui) to contact all listed contact persons from the interested NMI.**
- **Sample distribution to be organised by Dec 2023.**

5. **P217- Study update**

David Newton (NIST) gave an update on the quantification of fixed mononuclear cells in suspension. He provided the data which was included for analysis including consensus estimation at the highest concentration level and analysis of the full dilution series. The median of all the data from the flow, manual and automatic methods analysis was also shown. Different labs show different amounts of variability with each method. The replicate observation, replicate sample, stock solution and lab were considered as different parameters to see the highest variability. The Flow-method seems to have the least variability. Lab to lab variability is the greatest element to uncertainty in the dilutions series design. The study also evaluates quality metrics from the ISO cell counting standard(ref). The Proportionality constant shows that manual method gives higher count estimates than other methods. A Proportionality index shows higher deviation to the manual method. A little more analysis is needed by NIST, but Sumona and David are confident this will be complete before Christmas.

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Jeanne asked if the differences/variability that they observe is based on one concentration or all dilution series or higher concentrations. Sumona added that in all method there is no loss of the proportionality. Andreas asked that they selected the method. Would it be on the participant to select the data? Sumona answered that they selected data blinded and shows the one that they determined was the most suitable (for e.g., clinically relevant counting method). Jonathan commented about the

quality metric analysis based on ISO guidance. Based on the analysis, do we have enough data to get the best method? David answered that it can provide some suggestions in terms of which methods has the best proportionality. Sumona added that looking through dilution series data there could be some insights into proportionality. Jun choi asked if the outlier data can be excluded. Andreas said that no data can be excluded except perhaps mistyped data. Sumona added that they checked all of the data for transcription errors. They did not go back to the participants but at their end did a check to make sure that they haven't mistyped. Sumona added that in the automatic method the outlier could be a real artifact due to the method and hence could represent a real scenario. Jeanne added that it could be software related overcount a cluster as they also observe it in their analysis. Jonathan added that if we are discussing about transitioning to a key comparison we will need a decision about a reference value. If so is there a target value we should aim for or what is useful? Sumona answered that there is promise of the flow method showing a systematically lower value bias in comparison to the manual method. Therefore a consensus value is method dependent. Jeanne asked about the flow rate that the participant had used in the study. As different instruments were used for the study so the rates might not have been same between all the participants. Andreas said that with dilution series would cover for the coincidence loss. Jonathan asked about the draft report. Sumona answered that the data presented is the way evaluation will be conducted and if the members are confident about the direction of the analysis. The work is needed for the dilution series. She added that they can share the data and people can do their own analysis or recommend any analysis suggestions. In few weeks they will be completing the analysis in terms of figure generation.

Action:

- **NIST to share the presentation/data and the feedback from the members can be given in couple of weeks.**
- **Outstanding figure generation to be complete by the end of this week (17/11/23)**

6. P222- Study update

Yuki Kuruma (NMIJ) gave an update of P222 Particle enumeration for cellular analysis. NMIJ are not able to distribute the samples, and thus not able to start the study due to transportation restrictions between Japan and Russia at this time. NMIJ are performing long term stability testing of the sample which shows that sample is stable. PTB are co-leading the study and Andreas (PTB) suggested that if we ask Russia to withdraw from the study, then it might be possible to go ahead. He asked the members about their opinion. Jonathan said that it is was perhaps the only option at this time if we want to study to go ahead now. Andre (VNIIM) said that it not ideal but very graciously said that the study can go ahead without them if it makes it easier. The CCQM president reminded the group of the spirit of the metre convention. Under this convention no member state can be excluded, but there may be outstanding political situations that make participation of states impossible. He suggested a bilateral study in parallel could be a way for Russia to continue with participation if another participating member could be found that would be willing to distribute samples.

Action:

- **NMIJ to confirm as the study organiser if they are happy to go ahead with the study without Russian participation.**
- **NMIJ (Japan) to confirm if they would be happy for the study to go ahead with Russia in a parallel bilateral arrangement with another organisation that is willing to supply materials to Russia.** Note Tubitak has been able to perform such comparisons in the past.
- **Shin-Ichiro Fujii to feedback to the CAWG about the NMIJ decision by END Dec 2023, and a decision will be made about the path forward at this time.**

7. Review

7.1 CCQM strategy discussions

Chair initiated a strategy discussion for the group. Points of consideration include;

- Review of the current work program. New studies/areas/applications that the group should consider and strategies that may be adopted to meet this developing program.
- The need to move to Key comparisons studies where possible in a timely manner.
- Identification of strategic areas of overlap with other Bio groups for certain projects (i.e. cell counting and cell authentications support), or wider groups if necessary within the CCQM.
- Need to review the Road map should be updated.
- Increasing stakeholder interactions, and drawing in expertise in an appropriate manner to maintain the state-of-the-art for the group. Also the need to increase the groups public profile if this is suitable.
- Updated horizon scanning exercise covering new measurement instruments, assays, new reference materials, or materials that could be elevated to this status.
- Outline a working group handbook/policy document that sets the agreed strategy for the group, mechanisms to identify and prioritize new studies that meet the requirements for a CCQM comparison study and the strategy, and outlines agreed responsibilities, process/time-line for presenting studies to the group, conducting and reporting studies.

The following discussion covered: Sample format/quality that is easy/hard to distribute (ie. samples on slide versus lyophilised materials). Andreas suggested that with lyophilized blood, biosafety is an issue for these kinds of materials. The Chair asked if we need a strategy to bring material from other groups. Do we need to start making new linkages/relationships to bring such materials into a study? Andreas mentioned our lacking of a key comparison study. Julian mentioned that key comparison around blood cell counting would be ideal. There are different ways of stabilising cells, we should understand the variability that it will introduce. There are lyophilised CD34 cells blood cells and it could be good to do a viability assessment of these cells. Julian outline, it is one thing to count cells and second is to do viability. The definition of viability is to be thought about. Viability is very complex, and it is difficult to ascertain the threshold for it. Distributing samples for viability will be tricky as it must be done on the same day. Sample distribution is difficult then standardised data that can be sent around. If there is enough variation in data interpretation. This we can get around the problem of the stability. Stakeholders can be onboard with data and can get good coverage and engagement. Jun suggested that P205 can be taken into a key comparison study. As the sample is relatively more stable than mammalian cells to be distributed around more effectively.

The Chair continued the discussion on identifying pathways forward in terms of reference materials, for e.g. blood counting materials, cells that survive lyophilisation, key measurement process uncertainties that can be evaluated without a physical samples, ie. Data that can be evaluated by subjectivity (ie. Flow plots, captured images etc.). Julian added that post-COVID viral studies are very topical. The production of a PFU/area study would be good to consider. Studies that support counting viral particles, cell infectivity, dosing.

The Chair flagged the documents that WG members should be familiarise themselves with, particularly as we transition to key comparisons. Of note; CPM-MRA-G11 CPM-MRA-G12, CPM-MRA-G13. The secretariat confirmed to the chair that a CMC claim should not be made on the basis of a pilot study. These claims are made on the basis of a reference value and degrees of equivalence, provided by Key comparisons (and not a requirement for Pilots). He went through the type of comparisons listed in the guidance document. He went on to look at tools to capture our progress and study prioritization. We need to also consider the groups capacity for ongoing studies, since we are a small group, and this is important to manage expectation for the CCQM. We should develop an unbiased assessment prioritization of studies. The Chair gave his assessment of the current studies and prioritization

assessment. Sang-Ryoul stated that the goal is to provide the measurement service and emphasised that manageable studies should be conducted that build towards a common goal. More complex challenges could be addressed by combining completed building blocks.

The Chair went on to propose some task group within the working group with regards to how we can transition to key comparisons. He talked about reference materials and reference methods, that the group would need to focus on. Jeanne said that blood cell counting is an area of focus, and for that the material is important, with stringent biosafety level requirements etc. Chair suggested that a well-defined survey questionnaire could be a way forward. Robert added that it is important to think about the reference material which is required for the study, this can be a huge investment and its important to carefully consider it.

There may be an opportunity to reassess some previous pilot proposals, now some time has pass and key industries have developed. For example, a discussion the T-cell study, proposed by LGC/NIBSC/NIST could be updated and reevaluated for a pilot. Could it be combined with activities in other working groups (ie. PAWG activity if a biomarkers). There are also new possible areas to consider? Anna mentioned about Biofilm which can be linked to PAWG.

Action:

- **Review survey done in 2017 by early January and issue updated survey to the working group by February.**
- **Evaluate data for a constructive discussion as a group during the April meeting, that could identify further activities to build knowledge of useful studies for stakeholders. Such activities could be future CAWG level workshops.**
- **Creation of a CAWG knowledge base (Chair proposal) and a less formal idea database (Jun Choi proposal) so that we can keep track of the areas we are considering for studies and prioritise future studies.**

7.2 Review of CAWG strategic document

Chair reviewed the document and went through the list of areas like clinical diagnostics etc. and suggested that he will send the document by email for comments from the working group members. There was discussion related to various new technologies that are emerging in cellular analysis, single cell analysis, spectral combined analyses. He went through the challenges that faces cellular analysis.

The Chair introduced a prioritization framework, that could be further developed as tool by the WG. This should link current pilots to a history of capability building and link to identified needs from the strategy document. Future proposed studies should address some of CCQM objectives, measurement service provision, regulatory needs, fits with CAWG strategy, how far the light shines statements and the properties of the measurand.

8. CCQM task group discussion

8.1: Task group on Foods (Jeremy Melanson and Ralf Josephs lead)

Boqiang gave an overview of the importance of quantification of bacteria in food and for the health of human beings and for probiotics. Quantification of microorganism is important. We can consider other pathogenic bacterial quantification in food for e.g., probiotics in yogurt or other types of food. The count and activity of microorganism in food is important. There is a requirement for stakeholder engagement to know which type of material they require with priority and to discuss also which method can be compared and have a high order method for the study.

Robert gave an update regarding the CIPM grand challenges. Food safety has been identified as a key area, and the CCQM would like to be able to say what measurement services for the food safety sector the BIPM currently offers, or are being developed within the stated strategy for CCQM 2021-2031. It is important to emphasise that if there are no food safety services under development by the group, that this is ok.

Any food safety measurement services should be led by a clear stakeholder need identified by participant NMIs/Dis to the CAWG. There is a CCQM level task group under development, led by Jeremy Melanson (NRC) and Ralf Josephs (BIPM) which are currently drafting the terms of reference. The CAWG representative to this group would be expected to help the chair of the TG to develop this document. For information, NAWG is looking at GMO, PAWG is doing allergen which they haven't done before.

We had some discussion of the matrices for bacterial cell counting that we are considering. Boqiang added that quantification of target bacterial species in water is a simpler matrix to food matrix. He talked about identification or quantification of fungi etc in food industry and this is something to discuss with NAWG to see if we could do a joint study as appropriate. Dr. Park suggested Milk, as a development in complexity of water, that could satisfy the requirements for 'food safety'. Robert added that any proposals in this area should be realistic about what the group can achieve in terms of time scales. Sook-Kyong added that in terms of milk there is a requirement for microorganism screening and dairy industry is heavily regulated. Andreas mentioned that the in ISO committee there is a group working on food safety and it is a huge area. Chair added that we need to look at the current study and see if they can be applicable to food for e.g., quantification of *E. coli*, how the claim can be broadened to investigate alternative matrices of interest to the food sector.
Chair asked Boqiang if he would be happy to be the representative for the task force from the group and Boqiang confirmed that he is happy to represent

Action:
Boqiang to feedback regarding the quantification of microorganism in food matrix for e.g. milk etc.

8.2: Task group on intracellular metrology for gene delivery: terms of reference (Max Ryadnov leads)

Max Ryadnov gave a presentation about this working group level task group activity that would sit within the CAWG in the first instance. He listed the workshops that was organised in intracellular measurements. He talked about the major challenges related to intracellular gene delivery. There are various modular virus-like particles which have been developed at NPL. With respect to CAWG relevance would be the measurement of cellular uptake of these virus- like particles. He outlined the aim of the task group and goals for the task group.

Action:

- **Chair to share the terms of the reference for this task group with CAWG members and to ask for WG comments before Xmas break**

9. New study proposals

9.1 Cell counting in 3d hydrogel – cell counting per unit volume

Nilofar proposed a new project idea to the committee members for their consideration. The synthetic hydrogel developed at NPL is based on the well characterised 2d coating material. An interlaboratory

study of the hydrogel property is being proposed under VAMAS for the characterisation of the material and can be a suitable standard candidate for a future 3d cell-hydrogel.

Action:

- **Chair to share the slide deck for this proposal for onward consideration by CAWG members and further discussion at the next meeting . Paris . April.**

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10. **Future meetings**

10.1 2024 meetings: 20th-26th April. Paris. <u>CAWG meeting 23rd April Myria L-2 with hybrid facility.</u> CCQM Plenary (25th-26th)

Please note that meetings at the BIPM are on a tighter meeting schedule for 2024, and thus the single day meeting.

10.2 2024 Fall meeting: 7th-10th October Berlin.

The NMI hosting the autumn meeting is PTB and the city finalised is Berlin in Germany. The dates for the meeting will be finalised as soon as possible alongside a possible workshop.

11. **Any other business**

The outgoing chair Dr. Jonathan Campbell thanked colleagues for their support and friendship during his tenure as the chair. He feels sure the group will make excellent progress in the field of cell measurement and wishes them well in this important endeavour.

There was no other remaining business.

12. **Close**

The CAWG meeting concluded at approximately at 16.34 pm on 10th Nov 2024

Annex 1: List of actions for CAWG 2023-11-10

ID	Action	Comment
CAWG/2023-2/1	CCQM-P205: Quantification of membrane intact E.*Coli* in Drinking water • NIST / VNIIM / NPL are still to submit results. Flow results to be completed by **end of Dec 23** (NIST, NPL, VNIIM). • NIM to consider if a further request for information is needed if it supports the analysis of outstanding data submissions. • Final results are to be completed (NIM) • To proceed to the draft report A preparation in 2024	
CAWG/2023-2/2	P-197. Proliferated stem cell number per unit area.	

	• Leader (N.Faruqui) to contact all listed contact persons from the interested NMI. • Sample distribution to be organised by **Dec 2023.**	
CAWG/2023-2/3	P-217. Quantification of fixed mononuclear cells in suspension. • NIST to share the presentation/data and the feedback from the members can be given in couple of weeks. • **Outstanding figure generation to be complete by the end of this week (17/11/23)**	
CAWG/2023-2/4	P-222. Particle enumeration for cellular analysis • NMIJ to confirm as the study organiser if they are happy to go ahead with the study without Russian participation. • NMIJ (Japan) to confirm if they would be happy for the study to go ahead with Russia in a parallel bilateral arrangement with another organisation that is willing to supply materials to Russia. Note Tubitak has been able to perform such comparisons in the past. • Shin-Ichiro Fujii to feedback to the CAWG about the NMIJ decision **by <u>end Dec 2023</u>**, and a decision will be made about the path forward at this time.	
CAWG/2023-2/5	Review of CAWG strategic document • Review CAWG survey done in 2017 (CAWG strategy preparation for 2021-2030 period) by early January and issue updated survey to the working group by February. • Evaluate data for a constructive discussion as a group during the April meeting, that could identify further activities to build knowledge of useful studies for stakeholders. Such activities could be future CAWG level workshops. • Creation of a CAWG knowledge base (Chair proposal) and a less formal idea database (Jun Choi proposal) so that we can keep track of the areas we are considering for studies and prioritise future studies.	
	CCQM task group on foods.	

	• Boqiang to feedback regarding the quantification of microorganism in food matrix for e.g. milk etc.	
	CAWG task group on intracellular metrology for gene delivery. • Chair to share the terms of the reference for this task group with CAWG members and to ask for WG comments **before Xmas break**	Max Ryadnov shared terms of reference 14/11/23
	New project proposal from NPL. Cell counting in 3d hydrogel – cell counting per unit volume • Chair to share the slide deck for this proposal for onward consideration by CAWG members and further discussion at the next meeting . Paris . April.	