Strategy 2021-2030+

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

CCQM Working Group on Protein Analysis (PAWG)

1. EXECUTIVE SUMMARY

PAWG's scope is:

- The development and validation of reference measurement procedures for purity assessment of high-purity peptide and protein materials suitable for calibration standards and (certified) reference materials.
- Qualitative and quantitative analysis of peptides and proteins in biopharmaceuticals and complex biological matrices, such as food and bodily fluids.
- Qualitative and quantitative analysis of post-translational modifications (PTMs) in proteins, such as phosphorylation, glycosylation and glycation.
- Functional analysis of peptide and proteins such as enzymatic and binding affinity activities, important to assess the performance attributes in diagnostics and therapy.
- Measurements of higher order protein structure, from secondary to quaternary structure including quantitative characterisation of protein assemblies, complexes or aggregates.

The PAWG is focussing on building the capacities required to enable NMIs/DIs to provide contemporary and fit-for-purpose measurement services necessary to meet the needs of the end-user both from industry and clinics. Good progress has been made in recent years towards this goal, demonstrated by the examples at the end of this document. Nine key comparison (KC) and twelve pilot studies (PS) with one additional KC currently in the process of drafting the report have been organised covering purity studies of peptides as well as the first studies of proteins in clinical matrices and enzymatic activity. The metrological basis for primary calibrator value assignments by different approaches (for example, amino acid analysis, qNMR and mass balance) has been established through the CCQM-K115 series of comparisons, thus providing the major route to SI traceability for peptide and protein quantification. As an answer to the SARS-CoV 2 pandemic, NIM, BIPM and NRC jointly organised a pilot study (CCQM-P216) concerned with the purity assessment of antibodies against SARS-CoV 2 spike and nucleocapsid proteins, respectively. In addition to the purity assessment, investigations regarding the structure of the antibodies were performed by the participants. In addition, a fire drill study to demonstrate pandemic preparedness of the metrology community is currently planned.

The long-term goal is to enable participants of Track A comparisons to claim broader scope calibration and measurement capabilities (CMCs) according to the section models developed for pure protein/peptides and peptides/proteins in matrix. Currently, only the section for purity determination of small synthetic peptides is deemed fit for such claims, but the scope of future studies will aim to enable broader scope claims in all areas of PAWG scope. To cover as many of the services provided by PAWG members as possible in future studies, three task groups have been established to plan the strategy of PAWG in the field of purity analysis of peptides and proteins (Task group I), determination of peptides and proteins in biological matrices (Task group II), and protein structure and activity (Task group III). Joint studies in the field of clinical analytes are discussed and organised also with other CCQM WGs, mainly OAWG, NAWG and CAWG, where appropriate.

The main stakeholders of the CCQM PAWG activities, besides the participating NMIs and DIs and the associated CCQM WGs OAWG, NAWG, and CAWG, are proficiency testing (PT) providers, clinicians, clinical laboratories, the *in-vitro* diagnostics (IVD), pharmaceutical, biotechnology and food industry as well as official parties involved in regulation and academics. To evaluate stakeholder needs and feedback the results obtained in PAWG, stakeholder workshops have been and will be organised. On the international level, the Joint Committee for Traceability in Laboratory Medicine (JCTLM), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the World Health Organisation (WHO) are

particularity important stakeholders and partners. As IFCC and BIPM have signed a memorandum of understanding (MoU), this cooperation is especially close.

2. SCIENTIFIC, ECONOMIC AND SOCIAL CHALLENGES

Peptide and protein analysis are mainly required by the clinical sector where the need for metrological traceability is driven by the legal requirements for clinical laboratory medicine. The sector uses a vast range of measurements, from highly automated, high throughput analysis to small scale specialised measurements. The sector is served by large multinational industrial corporations that provide entire *in-vitro diagnostic* (IVD) measurement services and solutions. As would be expected, in a sector where measurement results have an often immediate and important impact on the health of a person, the sector is highly regulated (e.g. Regulation (EU) 2017/ 746 of the European Parliament and of the Council on *in-vitro* diagnostic medical devices IVDR) and has internationally agreed quality standards for the metrological traceability of calibrators (ISO 17511), routine measurement services (ISO 15189), reference procedures (ISO 15193), reference materials (ISO 15194) and requirements for the competence of calibration laboratories (ISO 15195).

To evaluate the services currently provided or planned by the participating NMIs and DIs based on the needs of their stakeholders, a survey has been conducted. The outcome showed that PAWG members are mainly asked to provide reference materials and reference measurement procedures to their customers followed by calibration services and proficiency testing (PT) schemes. Besides pure materials and calibration solutions required to calibrate the methods, the main matrices handled are serum/plasma and pharmaceutical preparations followed by food and environmental samples, blood and tissue. Especially in the pharmaceutical area, measurements for structure and activity of proteins become increasingly important and have to be addressed by the metrology community. With the increasing use of novel and genetically modified food, the challenge of reliably identifying and quantifying potentially allergenic or otherwise harmful proteins in food is rising. Both challenges were discussed in detail with the relevant stakeholders during stakeholder workshops and will be considered in the planning of future studies.

The primary stakeholders of CCQM PAWG are the participating NMIs and DIs as well as the associated CCQM WGs OAWG, NAWG, and CAWG. PAWG is in close contact with those WGs and joint studies are organised where appropriate. Other main stakeholders are PT providers, clinicians and the IVD, pharmaceutical, biotechnology and food industries, official parties involved in regulation and academics. They are interested in and profit from PAWG's efforts. However, as PAWG is a relatively young WG within CCQM, more capacity building is required to enable the NMIs/DIs to provide the full set of services necessary to meet both the industrial, societal and clinical needs.

PAWG members are also members of relevant international committees. For example, MHRA in the UK provides about 95 % of all international biological standards (reference materials) under the World Health Organisation (WHO) as well as its own standards. There are also many members on ISO Technical committees, such as the TC276 (biotechnology) and TC334 (Reference Materials). Engagement is also maintained with IFCC, JCTLM, national FDAs and CODEX Alimentarius, whose needs continue to inform and shape this strategy.

Most recent efforts concern with the rapid growth in engineering biology (EngBio) and artificial intelligence (AI) sectors. Of special relevance is the experimental validation of predictions for protein function from structure and *de novo* protein design for the needs of EngBio industry developing applications for different sectors including plastic replacements, protein-based textiles, engineered proteins for therapeutics, diagnostics and food. PAGW members are actively engaged with industry and industry associations developing EngBio processes and biobased products, such as BioIndustry Association (BIA) and Bio-based and Biodegradable Industries Association (BBIA) in the UK, and global initiatives, such as AlignBio, developing open-source reference datasets and standards for designed proteins and structure to function predictions.

PAWG activities are:

- **Key Comparisons & Pilot Studies:** to benchmark and demonstrate NMI capabilities in peptide and protein measurements that lead to and help to maintain CMCs to underpin measurement services from NMIs and Dis.

- **Task Group Activities:** to identify and plan for attainment and evaluation of core measurement capabilities for protein metrology and future challenges (Task Group I for purity assessment; Task Group II for quantification of peptides and proteins in complex matrices, Task group III for protein structure and activity measurements)**.**
- **Technical Workshops:** to discuss and record metrology issues, requirements and challenges in protein measurement areas, proposed solutions and new methods, and interactions with stakeholders.

Status of activities and achievements up to and including 2025

KC / PS	Achievement	Status
CCQM-K115	Peptide purity determination - synthetic human C peptide (hCP)	completed
CCQM-K115.b	Peptide purity - synthetic oxytocin (OXT)	completed
CCQM-K115.c	Peptide purity - synthetic glycated hexapeptide of HbA1c (GE)	completed
CCQM-K115.d	Recombinant, cross-links-free protein in solution (PTH)	Samples distributed
CCQM-K115.2018	Peptide purity - synthetic hexapeptide of HbA0 (VE)	completed
CCQM-K151	Purity-assessed recombinant protein contents in buffer solution using insulin analogue	completed
CCQM-K177	The mass fraction of total human growth hormone in serum	completed
CCQM-K186	Quantification of Total Haemoglobin in Blood	Draft A
CCQM-P55.2	Peptide purity determination - synthetic human C peptide (HCP)	completed
CCQM-P58, CCQM-P58.1	Fluorescence in ELISA	completed
CCQM-P55.2.b	Peptide purity - synthetic oxytocin (OXT)	completed
CCQM-P55.2.c	Peptide purity - synthetic glycated hexapeptide of HbA1c (GE)	completed
CCQM-P55.2d	Recombinant, cross-links-free protein in solution (PTH)	Samples distributed
CCQM-P55.2.2018	Peptide purity - synthetic hexapeptide of HbA0 (VE)	completed
CCQM-P59, CCQM-P59.1	International comparability in spectroscopic measurements of protein structure by circular dichroism	completed
CCQM-P101	Glycan Species measurement in digested glycoprotein mixture	completed

CCQM-P137	Activity of alpha amylase in human serum	completed
CCQM-P191	Purity-assessed recombinant protein contents in buffer solution using insulin analogue	completed
CCQM-P164	Mass fraction of human growth hormone in serum	completed
CCQM-P201	Total haemoglobin concentration in human whole blood	completed
CCQM-P216	Quantification of SARS-CoV-2 monoclonal antibody in solution	completed
CCQM-P219	Determination of amount-of-substance fraction of hemoglobin A1c (HbA1c) in human hemolysate	completed

Key outcomes:

- Through the CCQM-K115/CCQM-P55 series of pilot studies and key comparisons, the metrological basis for peptide primary calibrator value assignments by different approaches (for example, peptide impurity corrected amino acid analysis, qNMR and mass balance) has been established, which provides the major route to SI traceability for protein quantification.
- Various aspects of metrological assessment of the purity of standard peptides have been identified thorough CCQM-K115 series. Some of the participants have been able to register CMCs based on the results of these comparisons.
- Furthermore, the CCQM-K115 series and K151 are first comparisons that can be used to extend the specific peptide claims to “broader scope” claims.
- A study to evaluate equivalence in the metrological assessment of catalytic enzyme activity was conducted (CCQM-P137). A follow up KC is planned.
- Studies to evaluate measurement capabilities in complex biological matrices addressing different challenges were organised: quantification of a low amount of protein in a complex matrix (CCQM-K177), quantification of a high abundant protein with complex structure in a complex matrix (CCQM-K186) as well as the quantification of amount-of-substance fraction of haemoglobin A1c in blood (CCQM-P219), the latter two in cooperation with RELA.

Challenges

One major challenge in protein measurements is the definition of the measurand. Often a protein specified by the stakeholders in reality consists of a multitude of similar molecules with slightly different modifications (such as different isoforms, conformational states or PTMs), which can be discriminated by high-order measurements such as qNMR and IDMS. To identify and define the relevant measurand, it is essential to work together with the stakeholders to understand the sample conditions and deconvolute the speciation of the protein sample before reference measurement procedures and materials can be developed. This will assure that the services ultimately provided by the NMIs/DIs will be fit-for-purpose.

The number of biomarkers used in clinical diagnostic and, thus requiring standardisation, is vast. There are national lists of important analytes used in diagnostics, such as the Guidelines of the German Medical Association (RiIlBÄK), that set the requirements a clinical laboratory must meet in interlaboratory tests to be allowed to offer their services. Also, for the necessary prioritisation of the various analytes worldwide, the International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR) has published a list of the most important biomarkers worldwide and their status of harmonisation across the clinical

laboratories. Despite its importance, harmonized results are often not traceable to the SI, potentially leading to problems when new analytical devices appear on the market.

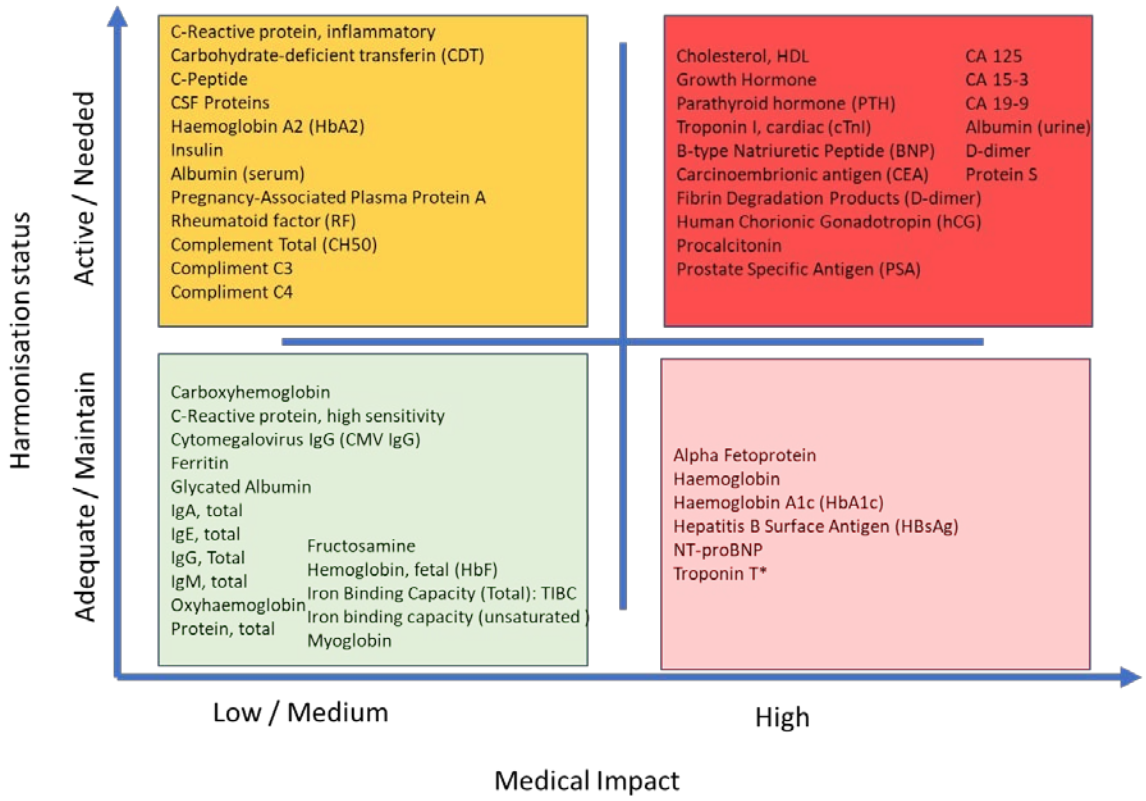

Figure 1: The analytes named in the list published by ICHCLR and the RiLiBÄK in Germany as to be the most important in laboratory medicine grouped by their medical impact and their harmonisation status. *The assessment of troponin T is based on the statement "Troponin T is available from a single IVD manufacturer, consequently harmonization is adequate". This statement is now outdated, and therefore, the needs are likely to be similar to troponin I.

The analytical challenges for these analytes are quite different regarding their size, complexity and concentration expected in human samples. Figure 2 gives an overview of the concentration range for the analytes listed in Figure 1.

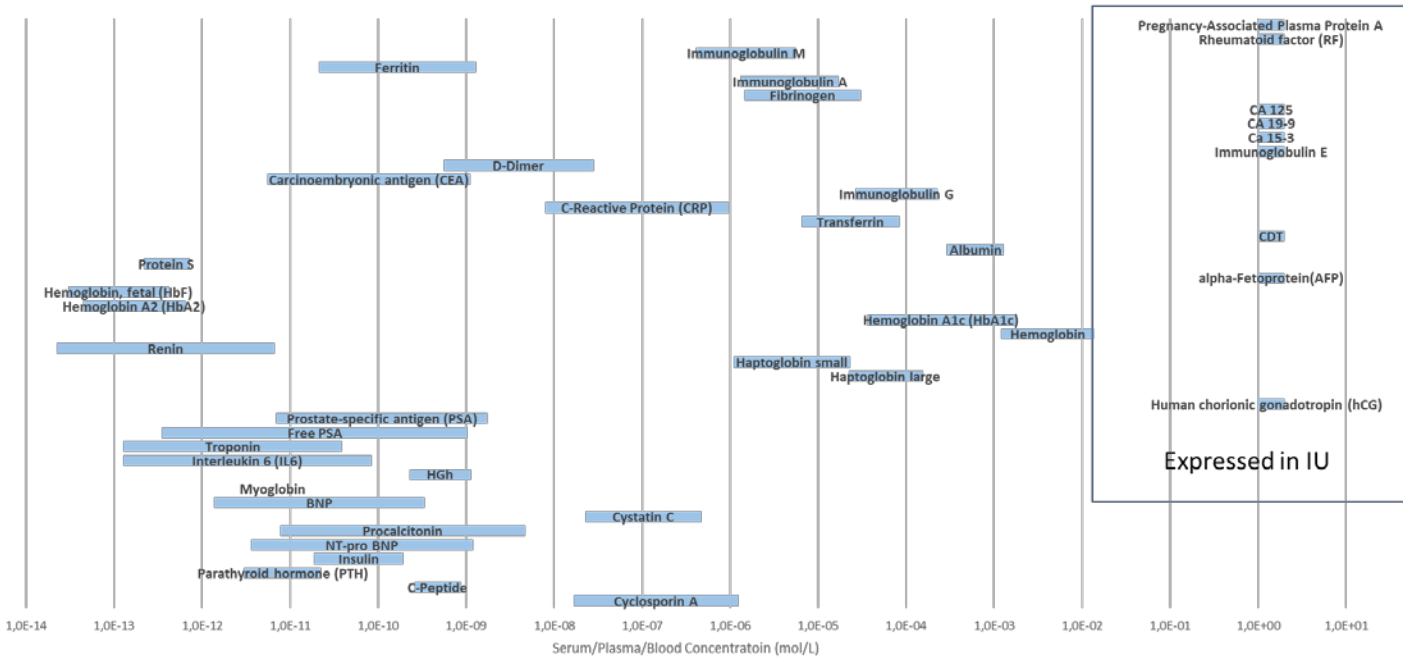

Figure 2: Priority protein measurands and their concentration in serum/plasma/whole blood according to the lists published by the German Medical Association (RiLiBÄK) and ICHCLR.

The analytes are often highly unstable and have complex structures, which presents unprecedented difficulties in preparation and purity assessment of standard materials. To resolve these issues, Task Group I of PAWG has established a roadmap which is updated continually.

Quantification of low abundance proteins in a complex matrix, such as serum or food, is challenging due to complex sample preparation as well as to achieving the necessary signal to noise ratios required to provide fit for purpose reference measurements. An additional challenge is how to deal with PTMs such as glycosylation, glycation and phosphorylation. Task Group II of PAWG works to resolve these issues and has established a roadmap which is updated continually.

Further challenges are the measurement of the activities and the link of the effect of the structure on, for example, protein drugs. The activity of proteins/enzymes, its link to the protein structure, affinity between different ligands, and how to establish traceability for these measurements as well as the quantification of the different isoforms is a challenge Task Group III of the PAWG is working to resolve. A roadmap has been established and will be updated continually.

Another challenge to the PAWG is how the metrology community can quickly respond to unexpected challenges such as the COVID-19 pandemic and its demands on reliable laboratory diagnostics. Currently a fire drill exercise is organised to show the state of the art.

3. VISION AND MISSION

The CCQM's vision is:

A world in which all chemical and biological measurements are made at the required level of accuracy 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.

The responsibilities of the CCQM are:

a. to demonstrate the global comparability of chemical and biological measurements, promoting traceability to the SI, and where traceability to the SI is not yet feasible, to other internationally agreed references.

b. to advise the CIPM on matters related to chemical and biological measurements including guiding international activities related to the definition and realisation of the mole and advising on the BIPM scientific programme.

c. to reach out to new and established stakeholders to promote the international measurement system and prioritize needs.

d. to progress the state of the art of chemical and biological measurement science and act as a forum for the exchange of information about measurement research, technical programmes and service delivery.

e. to contribute to the implementation and maintenance of the CIPM MRA with respect to chemical and biological measurements.

4. STRATEGY

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

To contribute to the resolution of global challenges especially in the field of healthcare including infectious disease pandemics, but also food safety 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, all uncertainty contributions, including those in converting from what is measured to what is intended to be measured are quantified. PAWG has taken first steps to achieve well-documented, open source and machine-readable formats for data submitted for comparison results and comparison reporting, thus supporting digitalisation activities of CCQM.

To promote the uptake of metrologically traceable chemical and biological measurements, 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 CCQM 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. This includes the progress from chemical purity to heterogeneous proteins and ultimately to structure and activity.

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 competencies.

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. This includes the application and review of our measurement capabilities to address and improve understanding of the issues in measurements of complex analytes such as cells or viruses also in cooperation with other WGs such as OAWG, CAWG or NAWG.

5. ACTIVITIES TO SUPPORT THE STRATEGY

5.1. PROGRESSING METROLOGY SCIENCE

The services offered by members of the PAWG to their customers are widespread. In order to coordinate and plan the studies required to support as many of these services as possible, task groups were created to develop a strategy that will provide evidence for broader scope claims in the future. The analytes will be chosen from the list of services the PAWG members offer to their stakeholders and reflect the needs of the stakeholders. The focus will be especially on studies from which multiple members will benefit and which will improve the overall metrology analytical capabilities of the PAWG members. Furthermore, the PAWG is in contact with international organisations such as IFCC and JCTLM as well as regional networks such as the European Network on Traceability in Laboratory Medicine (EMN-TLM) to identify international needs for standardisation and to support the work within e.g. IFCC WGs.

Studies planned to address the challenges described in Section 2

KC / PS	**Achievement**	**Status**
CCQM-K163	Activity of alpha amylase in human serum	planned
CCQM K115.e/P55.2e	Cyclosporin A	2026
K/P	Triskelion peptide	under discussion
K/P	IGF-1	under discussion
K/P	IgG	2030
K/P	Determination of amount-of-substance fraction of hemoglobin A1c (HbA1c) in human hemolysate	Planned for 2025/2026
P	Firedrill study for infectious diseases	Planned for 2025/2026
P	Binding affinity (KD or IC50) aligned with active concentration measurement?	To be discussed
P	Secondary conformation ratios and concentration measurement?	To be discussed

BIPM has greatly supported the PAWG by piloting various KCs of the CCQM-K115 series and the accompanying PS of the CCQM-P55 series on peptide purity. As this continues to be an important field for the PAWG members and their stakeholders, the PAWG members would appreciate future support for continuing these series. The relevant analytes for future studies are discussed within Task Group I, which BIPM is leading.

5.2. IMPROVING STAKEHOLDER INVOLVEMENT

The main stakeholders of PAWG are

- NMIs and DIs
- Laboratory Diagnostics: PT providers, clinicians, hospitals, reference material producers
- Public stakeholders: regulators, governmental labs, inspection agency for IVD and biopharmaceutical registration, third-party testing agency, veterinary diagnostic agency
- Industry: IVD industry, biopharmaceutical manufacturers, pharmaceutical industry, instrument manufacturers, veterinary diagnostic manufacture, food industry, biotechnology companies
- Academia: universities, metrological research institutes
- International Organisations: IFCC, JCTLM, ICSH, ICHCLR, WHO, IMEKO

International Organizations and Committees in Laboratory Medicine

The concept of reference measurements systems (reference methods, materials and measurements services) is well developed in the field of laboratory medicine, and the IFCC has been a member/liaison organisation of the CCQM since 2000.

Currently, the only BIPM sector-specific standing committee activity is within the field of laboratory medicine and IVDs, with the Joint Committee for Traceability in Laboratory Medicine (JCTLM) established in 2002. The majority of the reference materials are offered by the European commission's DG-JRC and most listed reference measurement procedures registered are IFCC methods. The majority of the analytes falls within the PAWG terms of reference.

Requirements for the properties and documentation for reference materials intended for use in the laboratory medicine field have been developed by ISO TC 212/WG2, used by the JCTLM, and cover both accuracy and commutability. The CCQM PAWG comparison programme addresses accuracy of measurement procedures but has no activity that covers commutability studies. The lack of appropriate commutability data for reference materials to support their intended use statements, can be a source of non-compliance with stated documentary requirements. Therefore, NMIs are encouraged to extend their activities not only to address metrological traceability but also commutability when assessing the "fitness for purpose" of any services being offered.

The IFCC, within its Scientific Division, has a Committee on Traceability in Laboratory Medicine, which coordinates and oversees the RELA schemes of comparisons for reference measurement services, and a Working Group on Commutability in Metrological Traceability. In order to strengthen liaison between the IFCC SD and the CCQM, the BIPM and IFCC have signed an MoU in 2020, which has facilitated cross representation between the organisations.

The ICHCLR was established following a 2010 workshop hosted by the AACC and NIST. Its mission is to provide a centralised process to organise global efforts to achieve harmonisation of clinical laboratory test results and strives to bring together interested parties to work on the standardisation of prioritized analytes.

Additional focus on involvement with IFCC and JCTLM would be expected to achieve the following:

- Streamlined JCTLM review process for reference materials covered by CMCs, including replacement batches
- Optimised CMC review processes so that both CIPM MRA and JCTLM requirements can be met when needed

- Improved synchronisation of CCQM and RELA inter-laboratory comparisons; a first step in this direction has been achieved for the CCQM-P219 on HbA_{1c} and CCQM-K186/P238 on total Hb in blood.

Mechanisms to achieve improvements in JCTLM and CCQM PAWG involvement will be to:

- Encourage NMIs active in the CCQM PAWG to further nominate experts for analyte specific JCTLM database review teams, when vacancies arise. Currently NIST is leading the protein review team and other CCQM PAWG members are members.
- CCQM PAWG members are involved in the JCTLM Quality Systems Development review team.
- CCQM PAWG to contribute and review outcomes of the JCTLM Task Force on Reference Measurement System Implementation (JCTLM-TF-RMSI).
- Encourage NMIs active in CCQM PAWG to participate in the biennial JCTLM Members and Stakeholders meetings.

All NMIs/DIs involved in peptide/protein measurements are members of the PAWG. PT providers and reference material producers are mainly linked to PAWG via their NMI/DI or at RMO level. For example, many European stakeholders are involved in metrology projects funded by EURAMET and the European Commission in the framework of the European Partnership on Metrology. The progress and results of these projects are presented to the PAWG by the participating NMIs/DIs. Additionally, a European Metrology Network on Traceability in Laboratory Medicine (EMN-TLM, led by LNE), has been set up in Europe to combine all in this field and provide metrological traceability as required by European regulation.

Furthermore, workshops were organised on progress in the relevant research fields and to evaluate currents stakeholder needs. Recent workshops organised were:

- Workshop "Towards standardisation of pathogen measurements" together with CAWG and NAWG (Oct 2024)
- Mini-workshop on IDMS for proteins and peptides (Oct 2024)
- Contribution to workshop on “Evolving needs in metrology: Metrology for food and food safety” organised by the CCQM task group on food (Feb 2025)
- Workshop on “Protein structure and activity” (Feb 2025)
- qNMR together with OAWG and NAWG (April 2025)

Future workshops to further this goal will be on:

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- Workshop on -omics and multi-omics together with NAWG and OAWG (planned 2025/2026)
- Protein analysis in cells together with CAWG

BIPM through its connection to JCTLM and associated organisations, such as ICHCLR and IFCC can facilitate the increased interlock of PAWG work with the efforts of those organisations.

5.3. PROMOTING GLOBAL COMPARABILITY

The services offered by members of PAWG to their customers are widespread. In order to coordinate and plan the studies required to support as many of these services as possible, task groups were created to develop a strategy that will provide evidence for broader scope claims in the future. The analytes will be

chosen according to the list of services the PAWG members offer to their stakeholders as identified in their answers to the regular surveys and with regard to the ICHCLR and similar national lists (figure 1).

Broader scope, analyte or method specific claims will all be required to demonstrate capabilities of the PAWG members to their stakeholders. Therefore, the PAWG agreed on the following strategy:

- **Track A** comparisons specifically designed to test the core competencies for measurement services delivered to the customers covering the range of the recognised measurement capabilities required to deliver reference measurement services and may be used to justify broader scope claims
- **Track C** specialised comparisons that would enable a “like for like” CMC and demonstrate the capability for services connected to specific peptides and proteins or forms of proteins, pure or in matrix, and method specific analytes such as enzyme and binding activities
- **Track D** all other studies which are not intended to lead to or support CMCs (e.g. pilot studies)

Each comparison type, regardless of the category, are further classified as either:

- **Model 1** the coordinating laboratory prepares a batch of samples which are established by the coordinating laboratory to be of suitable homogeneity and stability for the purpose of the comparison and an agreed number of sub-units from the batch are provided to each participant for value assignment.
- **Model 2** the participant value assigns a sample or set of samples which are forwarded to the coordinating laboratory. The coordinating laboratory analyses the ensemble of samples received from all participants under repeatability conditions. The agreement of the participant assigned values with those obtained by the coordinating laboratory is assessed.

In theory, all comparison types (A, C and D) could invoke either a Model 1 or a Model 2 design. However, a Model 1 design in which samples are dispatched from a coordinating laboratory is the typical practice for the PAWG.

Peptide and protein purity

As many NMIs/DIs are still developing their capabilities in the area of protein metrology, the PAWG is still evolving its expertise. To gain experience in this field, the participating NMIs/DIs started with studies in the area of peptide purity and are now advancing to pure proteins. The peptides were chosen according to the quadrant scheme developed in Task Group I "Peptide/Protein Purity" dividing the peptides and proteins according mainly to their molecular weight, but also to their structural complexity such as cross-linking or modifications such as glycation, phosphorylation and glycosylation.

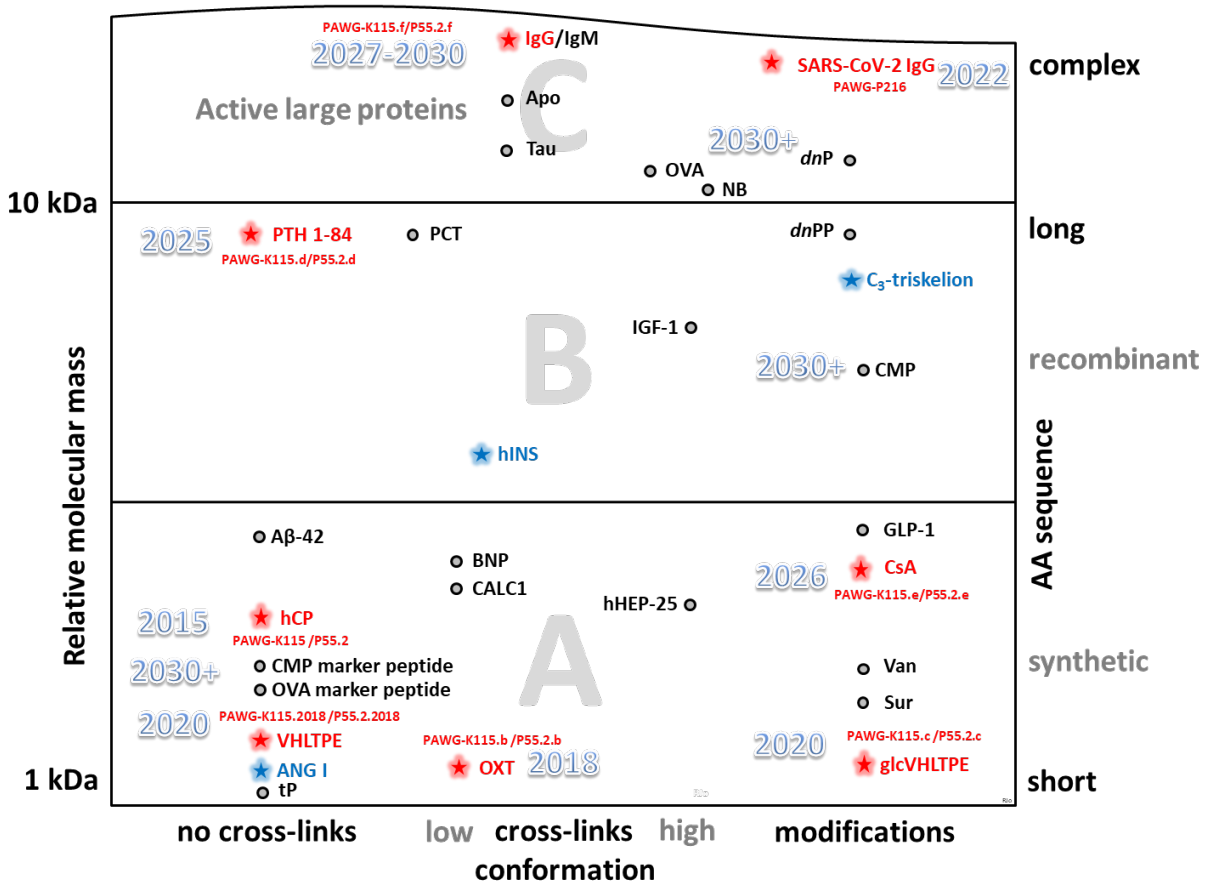

Figure 3: Section model for pure peptide/ protein and calibration solution studies to result in broader scope claims

The sections are defined as:

- **A**: 1 kDa ≤ peptide ≤ 5 kDa
- **B**: 5 kDa ≤ peptide ≤ 10 kDa
- **C**: intact proteins > 10 kDa

To support broader scope claims including the whole range of a section, the successful participation in at least 3 KCs in the relevant section is required including at least 1 KC for peptides with cross-linking and 1 KC for peptides with other modifications. For narrower claims, successful participation in KCs in the relevant areas are required. Currently only section A is deemed fit to support broader scope claims based on Track A studies for the whole section. For section C, the first study is in preparation and the future strategy will be to increase the numbers of studies in both section B and C to enable broader scope claims as soon as

possible. At the current stage, studies in section B and C will only support specific claims regardless of the studies being regarded as Track A or Track C study. As soon as there are enough Track A studies available, these studies may be used to support broader scope claims. The “How far the light shines” (HFTLS) statement in these study protocols will already include the range of such a broader scope claim.

To enable PAWG members to make broader scope claims the following studies are planned and proposed until and beyond 2030 to cover the various sections shown in Figure 3 with relevance to clinical chemistry, forensics, pharma, engineering biology and food analysis:

Section A

- Cyclosporin A (CycA), immune suppressant, 11 AAs (modified), 1.2 kDa, clinically relevant (CCQM approved K/P for 2026)
- GGL-QAR, ovalbumin signature peptide, 16 AAs, 1.6 kDa, food safety (allergen)
- MAI-INT, caseinomacropeptide (CMP) signature peptide, 19 AAs, 2.1 kDa, food safety (adulteration)
- Vancomycin (Van), glycopeptide antibiotic, 7 AAs (modified), 1.4 kDa, clinically relevant

Section B

- Caseinomacropeptide (CMP), whey marker, 64 AAs (modified), 6.7 kDa, food safety (adulteration)
- Semaglutide or glucagon like peptide-1 (GLP-1), lipid drugs, 30-31 AAs, 3-4 kDa, clinically and pharma relevant
- Procalcitonin (PCT), peptide precursor of the hormone calcitonin, 116 AA, 12 kDa, clinically relevant
- *De novo* polypeptide (dnPP), custom-engineered polypeptide, <50-100 AAs, 9 kDa, Engineering biology

Section C

- Immunoglobulin G (IgG), 180 kDa or Immunoglobulin M (IgM), 970 kDa or Nanobody (NB), 15 kDa (CCQM approved K/P for 2027)
- Ovalbumin (OVA), egg white protein, 42.7 kDa, food safety (allergen)
- Apolipoproteins (Apo), lipids, 10-550 kDa, cardiovascular biomarkers
- Tau, microtubule associated phosphoprotein, 6 isoforms, 45 kDa, Alzheimer marker
- *De novo* protein (dnP), custom-engineered protein, 100-500 AAs, 100 kDa, Engineering biology

The BIPM kindly volunteered to coordinate the ongoing series of comparisons to support and benchmark NMI technical capabilities for value assignment of peptide/protein pure material and solution calibrators. It has a dedicated laboratory facility to support these activities, which are essential for traceable and highly accurate biochemical measurements. The comparisons run by the BIPM are an integral part of the CCQM overall strategy to enable NMIs to demonstrate their measurement capabilities in the area of protein analysis and traceability to the SI.

Peptides and proteins in matrix

Quantification of peptides and proteins in biological matrices, such as clinical samples or food, is challenging. After experiences with the characterisation and quantification of pure peptides, first pilot studies (Track D) were conducted successfully, followed by first KCs. CCQM-K177 “mass fraction of total human growth hormone in serum” and CCQM-K186 “Quantification of Total Haemoglobin in Blood” have been completed. The long-term goal of PAWG is to also further the broader scope approach to peptide and protein quantification in biological matrices. Therefore, a model has been developed within Task Group II “Proteins in complex matrices” to plan future studies for potential broader scope claims according to the following scheme:

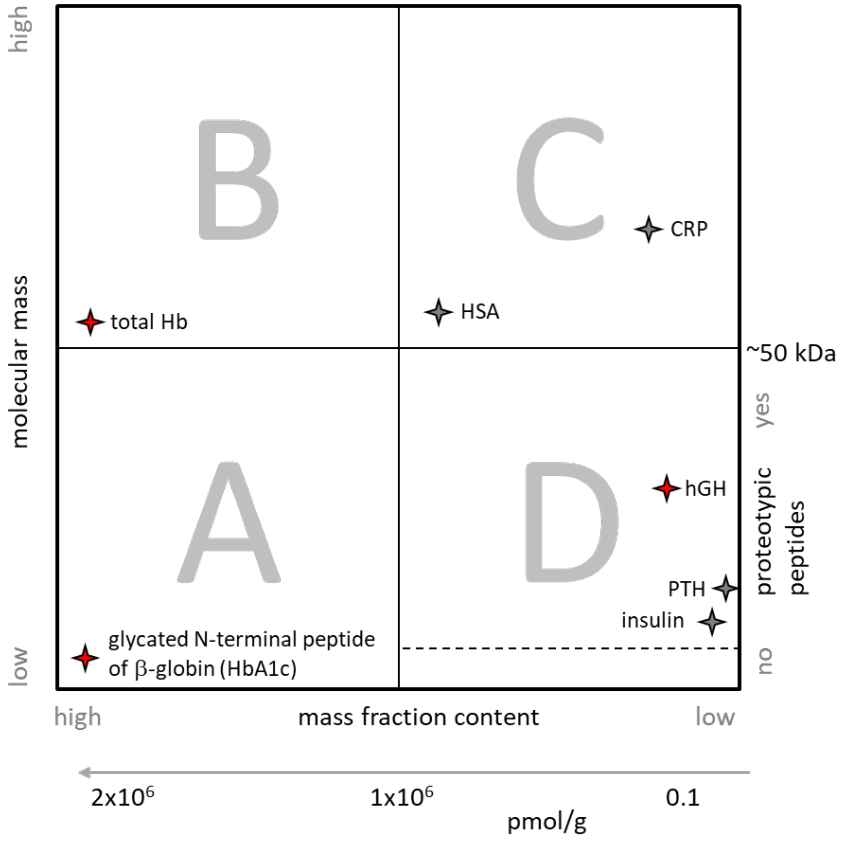

Figure 4: Section model for peptide and protein determination in matrix with the long-term goal to enable broader scope claims.

For peptides and proteins in matrix four sections in Figure 4 were identified by Task Group II:

- **A**: peptides/proteins ≤ 50 kDa, concentration range > $1 \cdot 10^6$ pmol/g
- **B**: proteins ≥ 50 kDa, concentration range > $1 \cdot 10^6$ pmol/g
- **C**: proteins ≥ 50 kDa, concentration range ≤ $1 \cdot 10^6$ pmol/g
- **D**: proteins ≤ 50 kDa, concentration range ≤ $1 \cdot 10^6$ pmol/g

As the experience in the quantification of peptides/proteins in matrix evolves, it might be necessary to refine the model further, possibly according to different types of matrices.

At the current stage, matrix studies will only support specific claims regardless of the studies being regarded as Track A or Track C study. As soon as there are enough Track A studies available, these studies may be

used to support broader scope claims. The “HFTLS” statement in these study protocols will already include the range of such a broader scope claim.

The following studies are planned:

- **Section A**
  GE/VE hexapeptide in blood

- **Section B**
  Still to be discussed

- **Section C**
  C-reactive protein (CRP) in serum
  human serum albumin (HSA) in serum/urine
  IgG in pharmaceutical preparations

- **Section D**
  PTH in serum
  Insulin

Structure and activity measurements

Another important field in laboratory diagnostics is the determination of enzyme activities, binding affinity, and identification and quantification of structural isoforms of the same protein including PTMs and the link between structure and function. Task Group III “Protein Structure and Activity” was created to define the most important analytes in this field. Based on the outcomes of the CCQM workshop on Protein Structure and Activity, the task group plans to organise international comparisons to support protein structure measurements including protein higher-order structure, and activity measurement capabilities, including catalytic concentration and binding affinity. Different models have been developed for both structure related analysis and activity.

For higher order structure measurements, the long-term goal is to determine the higher order structure and their ratios or concentrations, such as α-helix ratios or concentrations, or to determine protein complexes or aggregates with specific higher order structure, such as virus like particle (VLP) concentration. Higher order structure similarity measurements are also discussed.

The future studies in the field of structure related measurements will be planned to cover PTMs, determination of secondary, tertiary and quaternary structure, respectively, and protein assemblies.

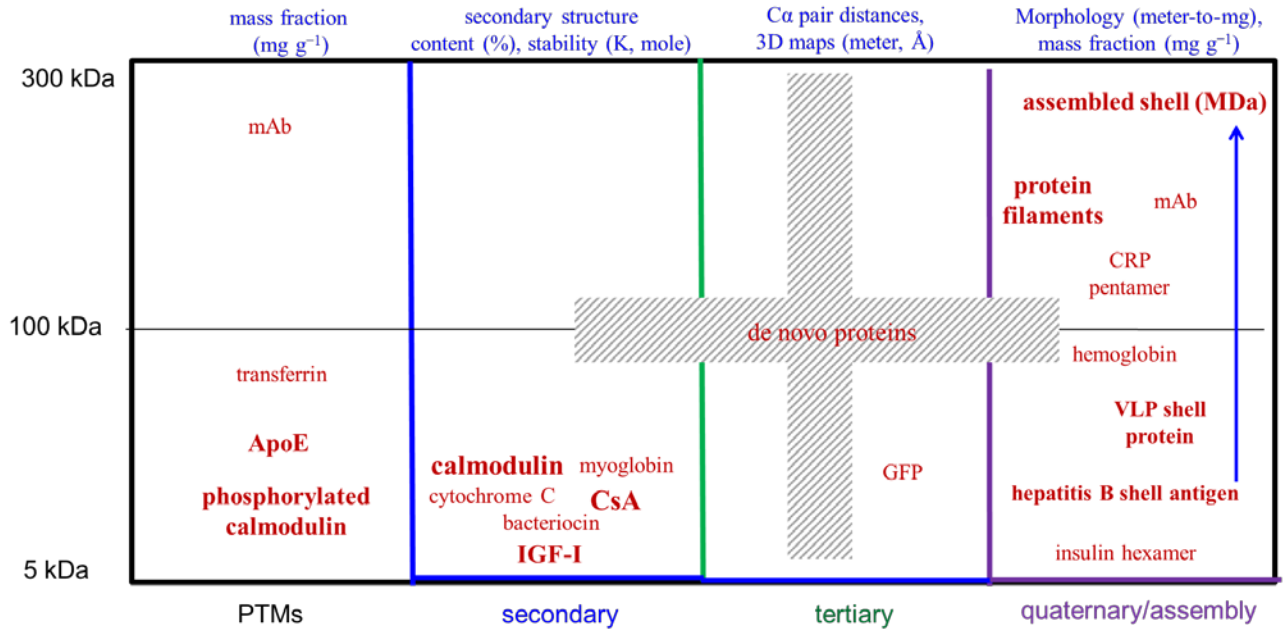

Figure 5: Section model for structure related measurement.

The following studies are planned:

→ PTMs

- Monoclonal antibody (mAb)
- Transferrin
- Calmodulin
- Apo A

→ secondary structure

- β-lactoglobulin
- Cytochrome C
- Myoglobin
- Calmodulin
- IGF 1

→ tertiary structure

- Green fluorescent protein (GFP)

→ quaternary structure

- mAb
- Haemoglobin

→ protein assemblies

- CRP
- Virus-like particles
- Insulin hexamere

For activity, the measurements can be separated in enzymatic activity and active concentrations as well as binding affinity. For the protein binding affinity measurement, the long-term goal is to be able to determine

accurate affinity constants between proteins or determine the activity via the protein affinity to its substrate caused by the different structural isoforms, thus quantifying an active concentration rather than a total amount-of-substance.

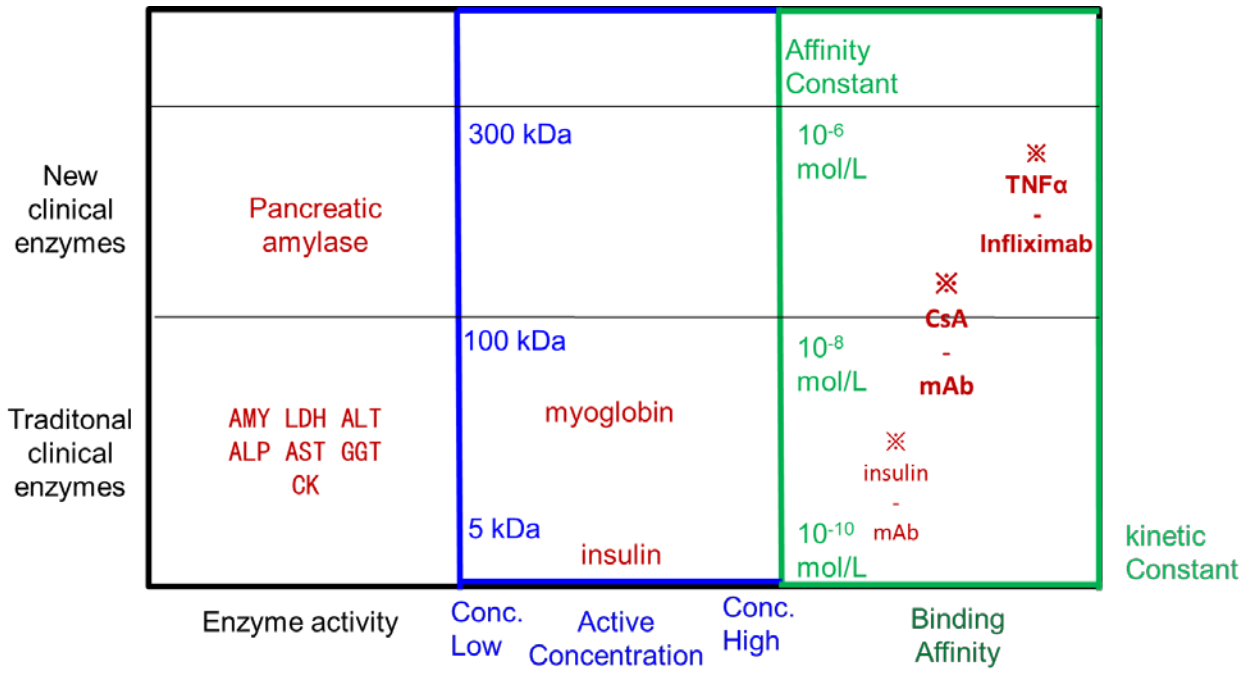

Figure 6: Section model for activity measurements.

Potential candidate analytes for enzymatic activity are the seven enzymes for which reference measurement methods have been developed and internationally agreed by the IFCC using kinetic spectrophotometry at 37 °C (e.g. alpha-amylase). The catalytic concentration ranges vary between the analytes.

Enzyme activity

- Alanine aminotransferase (ALT) 0.2-0.5 µkat/L
- Alkaline phosphatase (ALP) 0.2-10 µkat/L
- Alpha-amylase (AMY) 0.5-12 µkat/L
- Aspartate aminotransferase (AST) 0.5-4.5 µkat/L
- Creatine kinase (CK) 0.5-20 µkat/L
- Gamma-glutamyltransferase (GGT) 0.2-5.0 µkat/L
- Lactate dehydrogenase (LDH) 0.2-10 µkat/L

It is intended to devise the strategy in the field of enzyme activity in cooperation with the IFCC and in connection with the RELA scheme as far as possible. As enzymatic activity measurands are method defined measurands, all KCs in this field are deemed Track C studies. A first pilot study was finished successfully for α-amylase and a KC is currently in preparation. It is planned to organise a KC every three years for 2-3 clinical enzymes as defined by the IFCC. Thus, each enzyme will be covered every three comparisons.

Active concentration

- Myoglobin
- Insulin

Binding activity

- Insulin with different mAb
- CsA with mAb
- TNFα with mAb

To reduce the number of studies, it is intended to cover different aspects with the same analyte. Furthermore, binding affinity and higher order structure measurement studies will be designed and aligned with potential antibody purity comparisons.

Reaction to global challenges such as the SARS-Cov 2 pandemic

The global SARS-Cov-2 pandemic created a significant opportunity for the international metrology community, with measurement science directly implicated in societal impacting decision making, and the general public being exposed daily to terms such as “sensitivity”, “accuracy”, and “false negative or false positives”. The broad range of new diagnostic and serological tests that were developed internationally, and many gaining regulatory approval in certain countries in a very short time, showed the need for standardisation of these tests to gain acceptance as providing reliable measurements. Rapid antigen-based tests were deployed in various countries, which offer the potential for a rapid and cost-effective alternative to conventional RNA-based testing by polymerase chain reaction (PCR). With the availability of vaccines, antibody testing capable of determining COVID-19 immunity became increasingly important to monitor long-term efficacy of the vaccines, with clear economic implications including the resumption of international travel.

While the characterisation of large proteins such as antibodies has been in the long-term plan of the PAWG, the pandemic has drastically altered these timelines as the need for greater measurement science in this area has become immediate. Many NMIs/DIs made significant investments in battling the pandemic and developed services in the form of reference materials or reference measurements. In response to this need, the PAWG successfully performed a pilot study entitled “CCQM-P216: SARS-COV-2 Monoclonal Quantification”, coordinated by NIM, NRC, and BIPM. Building on existing competencies, the study has investigated the use of amino acid analysis and signature peptide quantification for such larger protein complexes. NMIs/DIs had also the opportunity to build capacity and demonstrate competency in the quantification of the intact monomeric antibody.

Upon the successful completion of this study, there are considerable opportunities for other studies related to solving pandemic-related measurement challenges. There are a handful of different antibodies that are relevant (i.e. IgG and IgM), so extension of CCQM-P216 to other antibody varieties followed by key comparisons to underpin measurement services has been included in the strategy. As NMIs become increasingly engaged in antibody epitope mapping using advanced structural characterisation techniques such as hydrogen-deuterium exchange mass spectrometry (HDX-MS), Task Group III has developed a strategy for developing and underpinning these capabilities.

Together with CAWG, NAWG and OAWG, a task group on response on future pandemics worked on a strategy for the metrology community to build capacities for a quick response in the case of future pandemics. One of the outcomes are so-called fire drill exercises which mimic a pandemic situation as closely as possible and demonstrate the capabilities and challenges the NMIs/DIs face in having to respond quickly to the needs of the IVD manufacturers and society. NRC is currently organising such a pilot study focused on the reliable and comparable preparation of calibrants for pathogen related proteins as a model 2 study.

Future challenges

An area of increased interest is characterisation, purity assessment and quantification of protein drugs and protein-drug conjugates including their structure analysis. To assess possible contributions of the NMIs/DIs to further this field, Task Group III organised a stakeholder workshop. The demand for standardisation of

biosimilar drugs, and here especially antibody-based drugs, was identified as high. It is currently discussed how this demand can best be met within the scope of PAWG and a first study has been proposed.

5.4. INTERACTION WITH RMO ACTIVITIES

The major protein measurement activities within the **Asia Pacific Metrology Programme (APMP)** are among KRISS, NMIJ and NIM under the framework of ACRM (Asian Collaboration on Reference Materials). The technical collaborations are carried out on the different levels of co-validation, co-characterization, and co-production. Co-validation means that the data provided by the participating labs are used only for reference to the result reported by the member who developed the CRM. Co-characterisation means that the data provided by all the participating labs are recommended to be used for the determination of the certified value of the CRM. At the stage of co-production, all members are sharing human resources, financial resources, and equipment. For this purpose, all members are encouraged to work more closely with each other for securing all the necessary human and financial resources required for this stage of collaboration. However, all the protein measurement activities are at the level of co-validation up to now. The co-validation collaboration has been finished on pure insulin (porcine) powder CRM (NIM), C-reactive protein in solution CRM (NMIJ), human growth hormone in solution CRM (KRISS), alpha-fetal protein in solution CRM (NIM), HbA1c CRM (NIM) since 2007. The project of human serum albumin solution CRMs (NIM, HSA) has been suggested to move to APMP comparison, and the project of cardiac troponin-ITC complex CRM (NIM) is still under discussion The biometrology working group was proposed and established in APMP in Nov. 2024 and more protein related comparisons will be organised in this group.

NMIs and DIs in Europe are working closely together and with the stakeholders within the framework of the European Partnership on Metrology jointly funded by the European Association of National Metrology Institutes (EURAMET) and the European Commission. Important projects on protein quantification as relevant clinical markers focused on neurodegenerative diseases such as Alzheimer's disease (ReMiND) and Parkinson's disease (NeuroMet 1 and 2), and cardiac markers (CardioMet). The results of these projects were presented to PAWG during the meetings. Current important projects are on fundamental protein metrology to support the definition of measurands, analytical targets, and their associated measurement uncertainty including the investigation of structure and modifications on quantitative results both from reference measurement procedures and routine assays (ProMET), and on manufacturing of commutable calibrators and quality control materials for standardisation and post-market surveillance of IVD tests (CoMET). Co-organisation of studies between the project consortia and PAWG is discussed.

Furthermore, the 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 European IVD regulation (Regulation (EU) 2017/745 and Regulation (EU) 2017/746). As the European PAWG members are also members of this network, they contribute to the strategy by reporting the stakeholder needs to the relevant task groups devising the PAWG strategy.

In the SIM region there is an ongoing project, partially supported by the Inter-American development bank (BID) for the development and further production of a reference material of Bovine Serum Albumin (BSA) in solution. It is a joint effort from CENAM, INMETRO and INTI to produce a protein reference material in the Latin America that may be used as a standard for protein quantification methods. Currently the viability and preliminary production and purification of BSA is being conducted by INTI.

ANNEX

1. GENERAL INFORMATION

CC Name: CCQM

CC Working Group: Protein Analysis Working Group (PAWG)

Date Established: 2015

Number of Members: ~27 participating institutes, ~ 90 members

Number of Participants at last meeting: 25 onsite and 31 online

Periodicity between Meetings: 6 months

Date of last meeting: Oct 2024 (hybrid meeting in Berlin)

CC WG Chair (Name, Institute, and years in post): Claudia Swart, 2 years

Number of KCs organized (from 2015 up to and including 2024): 9

Number of Pilot studies organized (from 2015 up to and including 2024): 12

Number of CMCs published in KCDB supported by CC body activities (up to and including 2024): 19

The agreed Terms of Reference (TOR) for the PAWG are:

- To carry out key comparisons and, when necessary, pilot studies to critically evaluate and benchmark NMI/DI claimed competence for measurement standards and capabilities for proteins and peptides of the highest metrological order and traceable to the SI, whenever possible:
- CMC registration
- To identify and establish inter-laboratory studies to enable the global comparability of protein and peptide measurement results through reference measurement systems of the highest possible metrological order with traceability to the SI, where feasible, or to other internationally agreed units
- To act as a forum for exchanging information and ideas for promoting implementation of metrology in protein/peptide measurement and to create opportunities for collaborations among stakeholders

The agreed scope of PAWG is:

- The development and validation of reference measurement procedures for purity assessment of high-purity peptide and protein materials suitable for calibration standards and (certified) reference materials.
- Qualitative and quantitative analysis of peptides and proteins in biopharmaceuticals and complex biological matrices, such as food and bodily fluids.
- Qualitative and quantitative analysis of post-translational modifications (PTMs) in proteins, such as phosphorylation, glycosylation and glycation.
- Functional analysis of peptide and proteins such as enzymatic and binding affinity activities, important to assess the performance attributes in diagnostics and therapy.
- Measurements of higher order protein structure, from secondary to quaternary structure including quantitative characterisation of protein assemblies, complexes or aggregates.

In cases where the scope overlaps with other working groups of the CCQM (for example, amino acids with OAWG, metalloproteins with IAWG, comparison of pathogen identification and quantification based on

protein, cell counts and nucleic acids in collaboration with CAWG and 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

https://www.bipm.org/en/committees/cc/ccqm/strategy.html

3. SUMMARY OF WORK ACCOMPLISHED AND IMPACT ACHIEVED (2020-2025)

As peptide and protein analysis is still quite new to CCQM, the members of PAWG are facing new challenges with every KC and PS, and in doing so, are continuously improving their capabilities. As the capacities of most NMIs/DIs are limited in this field, the members are supporting and learning from each other, thus improving the global comparability in this field. To address the needs of the stakeholders, the PAWG is in contact with the stakeholders listed in Section 5.2 either through collaboration of individual PAWG members or via mutual participation in workshops. RMO activities also feed back to the PAWG.

The following case studies demonstrate the activities the PAWG undertook to further metrology science and global comparability and involve the stakeholders in this process.

Mass fraction of human growth hormone in serum (CCQM-K177)

For the first study within PAWG for proteins in a complex biological matrix, human growth hormone (GH) was chosen. GH is a small protein that is produced and secreted into the circulatory system via the pituitary gland. The main isoform, containing 191 amino acids, is referred to as 22 kDa-GH. The determination of GH in serum is important for the diagnosis of growth hormone deficiency, which affects a child`s development and the health status of adults. Unfortunately, however, clinical intervention is hampered by the variability of measurement results, mainly attributed to differences between commercially available immunoassays.. A working group for GH standardisation was initiated by the IFCC, a major stakeholder of PAWG, in 2016 to resolve this issue and standardise GH measurements. This working group requested the support of laboratories using IDMS as a means to observe differences between the measurement techniques and provide a link between the SI-traceable antibody-independent MS measurement results and those obtained using immunoassays.

As a first step, a PS was organised by PTB in 2017. A spiked material containing recombinant 22 kDa-GH was prepared by standard addition to a blank serum and used as a first step to assess measurement capabilities among the NMIs. Regarding the participants using different protocols and signature peptides, the results were in good agreement. Therefore, it was decided to proceed with a KC on the determination of GH in human serum. However, the results of CCQM-K177 showed a much larger spread than the results from the PS. Currently the reasons for this are investigated. It seems that the behaviour of natural GH is different during digestion and detection than that of the recombinant protein used to prepare the study material of the previous PS.

Quantification of Total Haemoglobin in Blood (CCQM K186/P238)

For the second study of a protein in clinical matrix, haemoglobin (Hb) was chosen. It is a high-abundant protein which is only functional in its tetrameric structure. Hb is an important marker for anaemia. It is a Fe-containing protein in the red blood cells responsible for the transport and storage of oxygen. Low levels of Hb indicate anaemia, a condition in which a body is undersupplied with oxygen, causing fatigue and weakness. It can be caused by a loss of blood or a severe infection such as malaria. High levels of Hb usually are a sign for polycythaemia which can lead to heart failure, heart attacks or strokes. Hb abnormalities result in very serious hereditary diseases, such as sickle-cell anaemia and thalassemia. Therefore, blood preservations are all investigated regarding their Hb content. According to the French Health Insurance (Sécurité Sociale) refund statistics, haematology laboratory tests that include Hb analysis occupy the first place regarding the number of blood biomarker tests. It accounts for 32 million of tests per year with a total cost of 237 million Euros. Although the HiCN method, conversion of Hb to cyanmethaemoglobin (HiCN), recommended by the WHO has been used for routine determinations of Hb, it is often impractical for laboratories because of the toxicity of the potassium cyanide used for derivatisation and its restrictions on the use and disposal. Furthermore, as this method includes a conversion step which is usually not accounted for, SI traceability is difficult to achieve. Therefore, IDMS methods for the quantification of intact Hb were developed by the NMIs/DIs and applied in this study.

To compare these reference methods with the WHO reference method based on the conversion to HiCN, the study was organised in connection with the RELA scheme on total Hb in blood. All participants of the CCQM study were asked to also register at RELA to receive the samples. Pure human HbA_0 material was purchased by PTB and characterised and value assigned thoroughly using different approaches such as IDMS of Fe content, impurity corrected amino acid analysis and LC-ICP-MS. This material was provided to the participants to be used as calibrant or QC material at their choice. Only three participants really submitted results to RELA besides to the KC piloting laboratory and only two of them agreed with the results of the other RELA participants. As already seen with the results of CCQM-K177, this shows that the NMIs/DIs will have to improve their methods for protein quantification in matrix further to be able to provide SI traceability to the routine laboratories.

To support the digitalisation efforts of CCQM, PAWG has decided to use this study as a case-study for preparing a machine-readable report in addition to the traditional human readable version.

Recombinant, cross-links-free protein in solution: Mass fraction of parathyroid hormone 1-84 in aqueous solution (CCQM-K115.d/P55.2.d)

Parathyroid hormone (PTH) is an 84 amino acid peptide that plays a critical role in phosphate/calcium homeostasis. Measurements of PTH in serum or plasma are used in the assessment of hypo/hyper-parathyroidism and in the monitoring of bone disorders in patients with chronic kidney disease. The Committee on Bone Metabolism of the IFCC has defined the standardisation of PTH assays a priority and collaborates with metrology organisations, *in-vitro* diagnostic manufacturers and academia to achieve SI-traceability of PTH 1-84 measurements through the development of reference measurement procedures and certified reference materials.

The study material used will be a future primary reference material provided by NRC. This material was also further purified to be used by WHO to produce a secondary matrix reference material which will be value assigned using the primary reference material and the measurement capabilities demonstrated in CCQM-K115.d, thus ensuring SI traceability of the WHO reference material. The study is currently running and will be finished in July 2025.

Quantification of SARS-CoV-2 Monoclonal Antibody (CCQM-P216)

The global COVID-19 pandemic, which as of April 2025 had infected over 777 million people, as reported by WHO, has also led to increased focus on antibody quantitation methods. IgG are among the immunoglobulins produced by the immune system to provide protection against SARS-CoV-2. Anti-SARS-CoV-2 IgG can, therefore, be detected in samples from affected patients. Antibody tests can show whether a person has been exposed to the SARS-CoV-2, and whether or not they potentially show lasting immunity to the disease. With the constant spread of the virus and the high pressure of re-opening economies, antibody testing played a critical role in the fight against COVID-19 by helping healthcare professionals to identify individuals who had developed an immune response, either via vaccination or exposure to the virus. Therefore, many countries had launched large-scale antibody testing for COVID-19, for which the development of measurement standards for the antibody detection of SARS-CoV-2 was critically important. In this study, the SARS-CoV-2 monoclonal antibody was used as a model system to build capacity in methods that can be used in antibody quantification.

A final report on the first round of CCQM-P216 “Quantification of SARS-CoV-2 Monoclonal Antibody – Part 1” focusing on the assessment of both mass fraction determinations of different amino acids in the material and mass fraction determinations of proteotypic peptides belonging to the constant region of the mAb was published in Metrologia in 2022. The final report on the second round of CCQM-P216, Part 2 about the assessment of size heterogeneity purity determinations, results for mass fraction measurements of SARS-CoV-2 monoclonal antibody in the material by UV-VIS spectrophotometry, mass fraction measurements of proteotypic peptides belonging to the variable region of the mAb and mass fraction measurements of monomeric mAb in the material, were published in 2023.

Determination of the Amount-of-substance Fraction of [HbA1c/(HbA1c+HbA0)] in Human Hemolysate (CCQM-P219)

Haemoglobin A1c (HbA1c) is an important biomarker for the diagnosis of diabetes mellitus and for monitoring the long-term blood glucose level in diabetic patients to ensure proper treatment and management. In 2021, PAWG agreed that HSA, LNE, NIM and KRISS co-organise a Track D pilot study on HbA1c in human hemolysate (CCQM-P219) with the aim of evaluating the performance of IDMS methods for the determination of amount-of-substance fraction [HbA1c/(HbA1c +HbA0)] by different participating NMIs/DIs. As this study was organised in connection with RELA using the same samples, this pilot study also aimed to compare the IDMS method to the IFCC reference method with an alternative calibration hierarchy.

Eight NMIs/DIs reported their results of amount-of-substance of HbA1c using IDMS in this pilot study. The results from IDMS of all eight NMIs/DIs were found to be generally comparable with those from the IFCC reference method published on the RELA platform with DerSimonian and Laird (DSL) mean deviations of about 2 % (1.9 % for Sample A and 2.3 % for Sample B), which should be considered small when compared with the RELA limit of equivalence (± 4.5 %). However, the between laboratory variation of IDMS in this pilot study with CV of 5.4 % to 5.7 % was large compared to the between laboratory CV in the RELA study, which could be attributed to different quantification methods and proteolysis procedures. After intensive discussions and follow-up investigations, the issues have now been resolved within a PAWG internal task group and PAWG has decided to proceed to the organisation of a KC to allow the participants claiming the necessary capabilities to support their services.

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5. DOCUMENT REVISION SCHEDULE

PAWG Strategic Plan 2030+: V2, 28 April, 2025