

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 complex biological matrices and biopharmaceuticals;
- Other more specialised measurements related to proteins such as catalytic enzymatic activities, as well as biotherapeutic and antibody characterisation.

The PAWG is still a relatively young WG within CCQM and is still working to build the capacity required to enable the NMIs/DIs to provide the full set of services necessary to meet both the industrial and clinical needs. Good progress has been made in recent years towards this goal, demonstrated by the examples at the end of this document. Three key comparison (KC) and seven pilot studies (PS) with two additional KCs and four PSs currently in the process of drafting reports 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 peptide 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 protein quantification. As an answer to the current SARS-CoV 2 pandemic, NIM, BIPM and NRC jointly organised CCQM-P216 concerned with the purity assessment of antibodies against SARS-CoV 2 spike and nucleocapsid proteins, respectively. Samples have been distributed and the measurements will be conducted at the beginning of 2021. Optionally, investigations regarding the structure of the antibodies will be performed by the participants.

The long-term goal is to enable participants of Track A comparisons to make broader scope claims 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 sections. To cover as much of the services provided by the PAWG members as possible in future studies, four focus groups have been established to plan the strategy of PAWG in the field of purity analysis of peptides and proteins (Focus group I), determination of peptides and proteins in biological matrices (Focus group II), determination of protein activity and characterisation (Focus group III) and quantification of complex structures such as protein drugs (Focus group IV).

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, the *in-vitro* diagnostics (IVD) and pharmaceutical 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 a special focus has been and continues to be on the cooperation with 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). An even closer future cooperation of PAWG with the IFCC is expected now that a memorandum of understanding (MoU) has been signed between IFCC and BIPM.

2. SCIENTIFIC, ECONOMIC AND SOCIAL CHALLENGES

Peptide and protein analysis are mainly needed in the clinical sector and its needs for metrological traceability are driven by the 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 (ISO17511), routine measurement services (ISO15189), reference procedures (ISO15193), reference materials (ISO15194) and requirements for the competence of calibration laboratories (ISO15195).

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 followed by blood and tissue.

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. Besides those the main stakeholders are PT providers, clinicians and the IVD and pharmaceutical industries, official parties involved in regulation and academics. They are interested in and profit from PAWG's efforts. However, as PAWG is relatively a 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 and clinical needs.

PAWG members are also members of relevant international committees. NIBSC, for example, provides about 95 % of World Health Organisation's (WHO) international biological standards as well as its own standards. Connections also exist and are intensifying with IFCC, JCTLM, national FDAs and CODEX Alimentarius. So PAWG is well aware of stakeholder needs and is increasing efforts to respond to them.

PAWG activities are:

- **Key & Pilot Study Comparisons:** to benchmark and demonstrate NMI capabilities in peptide and protein measurements that lead to CMC registration to underpin measurement services from NMIs and DIs
- **Focus Group Activities:** to identify and plan for attainment and evaluation of core measurement capabilities for protein metrology and future challenges (Focus Group I for purity assessment; Focus Group II for quantification of peptides and proteins in complex matrices, Focus group III for protein activity measurements and Focus Group IV for protein drugs)
- **Technical Forum/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 2020

KC / PS	Achievement	Status	Outcome
CCQM-K115	Peptide purity determination - synthetic human C peptide (hCP)	completed	3 CMCs
CCQM-K115.b	Peptide purity - synthetic oxytocin (OXT)	completed	
CCQM-K151	Purity-assessed recombinant protein contents in buffer solution using insulin analogue	completed	
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-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	Draft B	
CCQM-P201	Total haemoglobin concentration in human whole blood	Draft A	

Key outcomes:

- Through the CCQM-P55 series of studies and CCQM-K115 series of 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 through CCQM-K115 and CCQM-K115.b. 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 to being used to extend the peptide claims to “broader Scope” claims.
- A study to evaluate equivalence in the metrological assessment of catalytic enzyme activity has been conducted (CCQM-P137). A follow up KC is planned.

- Two studies to evaluate measurement capability in complex biological matrices addressing different challenges are currently in the reporting stage: quantification of a low amount of protein in a complex matrix (CCQM-P164) and quantification of a high abundant protein with complex structure in a complex matrix (P201).

Challenges

The major challenge is the definition of the measurand. Often a protein specified by the customers in reality consists of a multitude of similar molecules with slightly different modifications (such as different isoforms, structures or post translational modifications). To identify the relevant measurand it is essential to work together with the stakeholders before reference measurement procedures and materials can be developed.

The number of biomarkers used in clinical diagnostic and, thus requiring standardisation, is vast. For the necessary prioritisation of the various analytes, 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, harmonisation is not standardisation and the results are often not traceable to the SI, potentially leading to problems when new analytical devices appear on the market. Additionally, there are national lists of important analytes used in diagnostics, such as the Guidelines of the German Medical Association (RiLiBÄK) that set the requirements a clinical laboratory must meet in interlaboratory test to be allowed to offer their services.

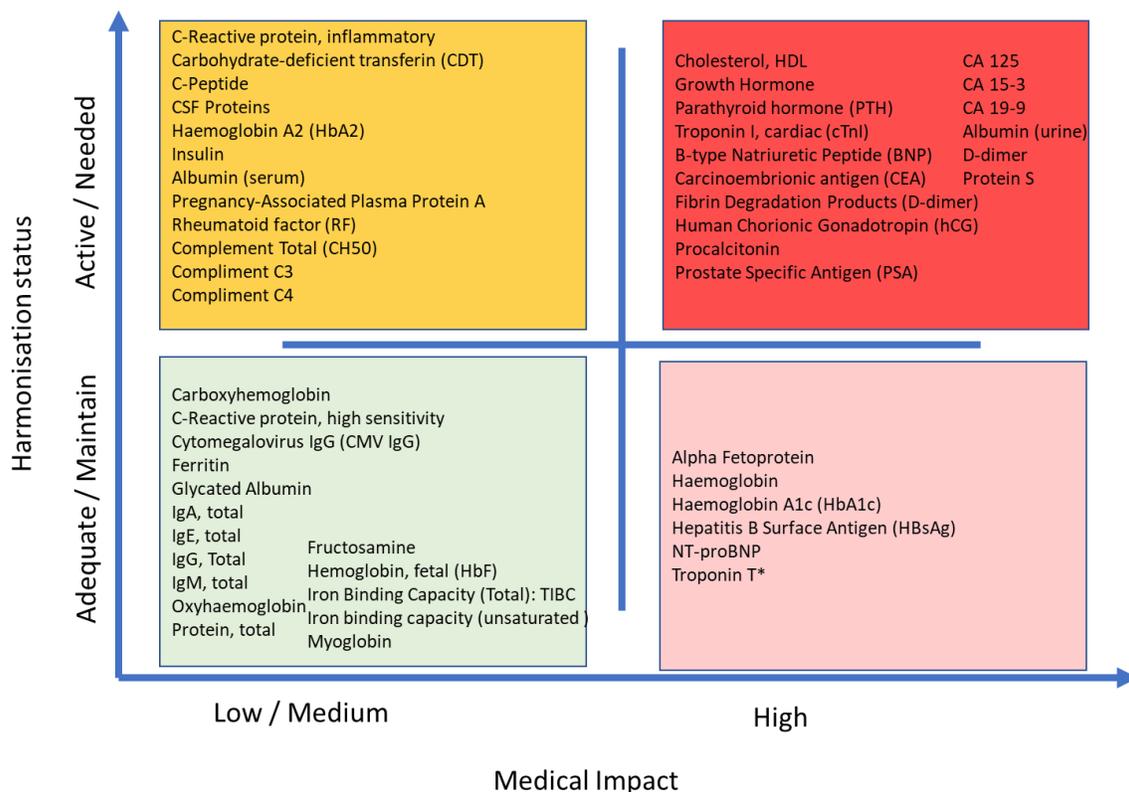


Figure 1: The analytes named in the list published by ICHCLR and the RiLiBÄK in Germany as to be the most important in laboratory medicine grouped by their medical impact and their harmonisation status. *The assessment of troponin T is based on the statement "Troponin T is available from a single IVD 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 analytes listed in Figure 1.

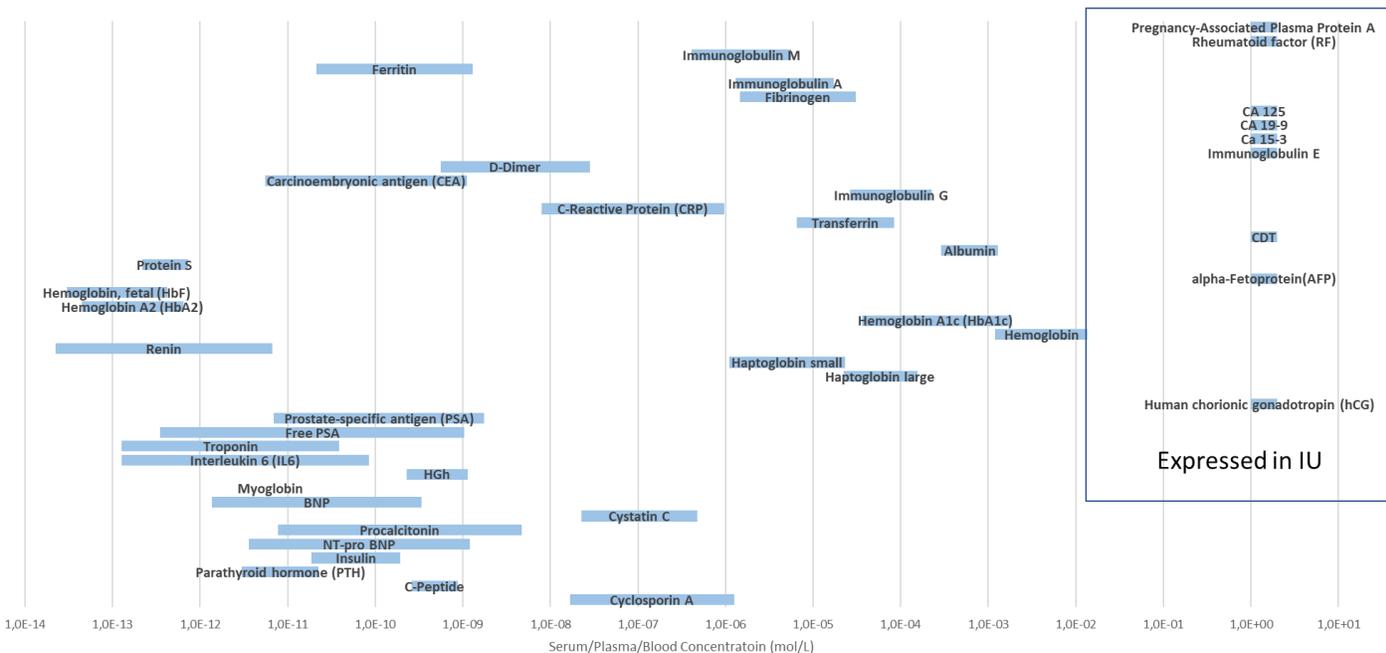


Figure 2: Priority protein measurands in serum/plasma/whole blood according to the lists published by the German Medical Association (RiLiBÄK) and ICHCLR.

The analytes are often highly unstable and of complex structures, which presents unprecedented difficulties in preparation and purity assessment of standard materials. These challenges are problems that needs to be solved. Focus group I of PAWG works on these challenges and has established a roadmap to resolve these issues which is continually updated.

Quantification of low abundance proteins in a complex matrix, such as serum, 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 post translational modifications such as glycation and phosphorylation. Focus Group II of PAWG works on these problems and has established a roadmap to resolve these issues which is continually updated.

Further challenges are the measurement of the activities and the link of the effect of the structure on e.g. proteins drugs. The activity of proteins/enzymes, its link to the structure of the protein and how to establish traceability for these measurements is a challenge Focus Group III of the PAWG is working to resolve. Whilst Focus Group IV is establishing a road map to resolve issues such as, how to measure highly complex analytes such as protein drugs qualitatively and quantitatively.

Another challenge to the PAWG is how to ensure commutability of reference materials from different sources and how the metrology community can quickly respond to unexpected challenges such as the current COVID-19 pandemic and its demands on reliable laboratory diagnostics.

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.

4. STRATEGY

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

To contribute to the resolution of global challenges 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.

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 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, focus 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 JTLCM 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-K115.c	Peptide purity - synthetic glycosylated hexapeptide of HbA _{1c} (GE)	ongoing
CCQM-K115.2018	Peptide purity - synthetic hexapeptide of HbA ₀ (VE)	ongoing
CCQM-K163	Activity of alpha amylase in human serum	planned
CCQM-P55.2.c	Peptide purity - synthetic glycosylated hexapeptide of HbA _{1c} (GE)	ongoing
CCQM-P55.2.2018	Peptide purity - synthetic hexapeptide of HbA ₀ (VE)	ongoing
CCQM-P216	Quantitation and characterization of SARS-CoV-2 antibody	ongoing
K/P	Peptide purity - parathyroid hormone (84)	agreed
K/P	Triskelion peptide	under discussion
KC/P	Quantification of HbA _{1c} in human hemolysate (in cooperation with RELA)	planned
KC	Follow-up of P164 Mass fraction of human growth hormone in serum	under discussion
KC	Follow-up of P201 Total haemoglobin concentration in human whole blood	under discussion

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 Focus 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
- Academia: universities, metrological research
- International Organisations: IFCC, JCTLM, ICSH, ICHCLR, WHO

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 JCTLM maintains a database of higher metrological order reference methods, materials and services. For proteins and peptides 27 reference materials, 23 reference measurement procedures and 8 measurement services are listed. The majority of the reference materials are offered by the European commission's DG-JRC, with only 5 coming from NMIs. The listed reference measurement procedures registered are mainly IFCC methods. Nevertheless, the majority of the analytes falls within the PAWG terms of reference and a closer cooperation should be pursued.

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 in Traceability in Laboratory Medicine, (this 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 will facilitate cross representation between the organisations.

The ICHCLR is a relatively new grouping that 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 together 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;
- Development and implementation of best practice procedures for demonstrating commutability of CRMs;
- Improved synchronization of CCQM and RELA inter-laboratory comparisons; a first step in this direction will be achieved for the KC on HbA_{1c}.

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

- Review with the CCQM President and other concerned WGs the requirement to establish a task group addressing commutability of reference materials in laboratory medicine, and other cross-cutting WG issues;
- Establish a CCQM liaison to the IFCC CTLM, and explore processes for best interlinking CCQM and RELA inter-laboratory comparisons;

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 LGC is leading the protein review group;
- Encourage CCQM PAWG to nominate an expert for 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 the CCQM PAWG to participate in the biennial JCTLM Members and Stakeholders meetings.

All NMIs and 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 or 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 Metrology Programme for Innovation and Research (EMPIR). The progress and results of these projects are presented to the PAWG by the participating NMIs / DIs. Additionally, a European Network EMN-TLM, led by PTB, has been set up in Europe to combine all European efforts 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 current stakeholder needs:

- Mini-Workshop: Recent Advances in Protein Analysis Techniques (Apr 2018)
- PAWG-IAWG Joint Workshop: Inorganic approaches for protein quantification (Apr 2018)
- Invited Lecture: Metrology for Biologics, S. Kim (KDDF) (Oct 2018)
- Special PAWG Workshop (Apr. 2019)
- Webinar on Covid-19 featuring international experts on SARS-Cov 2 testing (Jul 2020)

Future workshops to further this goal will be on:

- Protein analysis in food
- Protein analysis in cells together with CAWG
- Challenges and needs for SI traceability of protein measurement in tissues

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, focus 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 survey and with regard to the ICHCLR and similar national lists (figure 1). The survey will be repeated on a regular basis to ensure that the PAWG strategy still responds to the needs of its members.

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, pure or in matrix, and method specific analytes such as enzyme 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 purposes 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 of 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 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. The peptides were chosen according to the quadrant scheme developed in focus group I “Peptide/Protein Purity” dividing the peptides according mainly to their molecular weight, but also to their structural complexity such as cross-linking or modifications such as glycation and glycosylation.

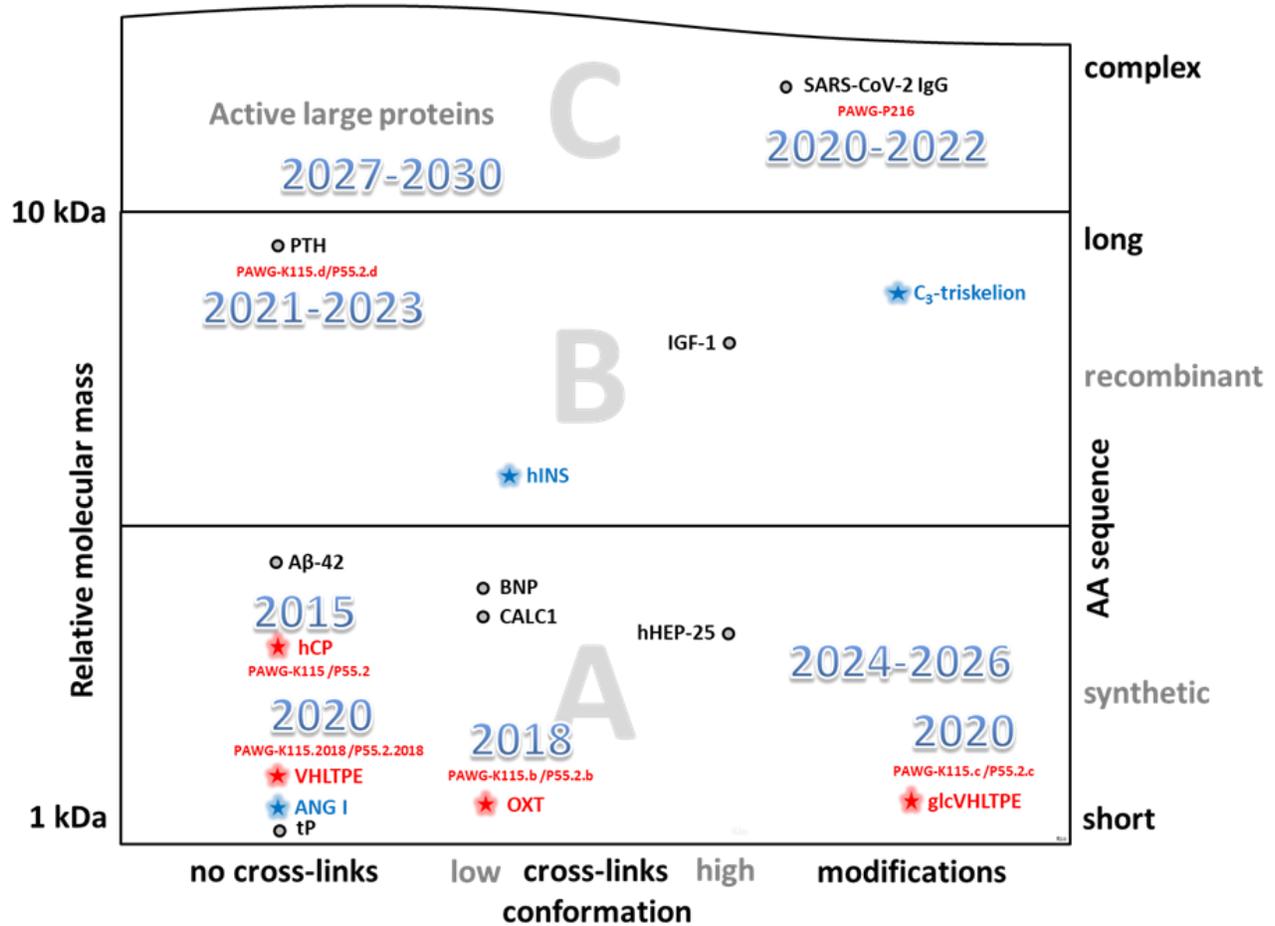


Figure 3: Section model for pure peptide and protein studies to result in broader scope claims

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 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 does the light shine” (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 to cover the various sections shown in Figure 3:

- **Section A**
GE/VE hexapeptide repeat?
CyclosporinA (CycA), immune suppressant, 11 AAs (modified), 1.2 kDa, clinically relevant
Vancomycin (Van), glycopeptide antibiotic, 7 AAs (modified), 1.4 kDa, clinically relevant
- **Section B**
Procalcitonin (PCT), peptide precursor of the hormone calcitonin, 116 AA, 12 kDa, clinically relevant
- **Section C**
Immunoglobulin G (IgG), 180 kDa or Immunoglobulin M (IgM), 970 kDa
Apolipoproteins (Apo), lipids, 10-550 kDa, cardiovascular biomarkers
Tau, microtubule associated phosphoprotein, 6 isoforms, 45 kDa, Alzheimer marker
Ricin (Ric), carbohydrate-binding protein, 267 AAs A chain and 262 AAs B chain SS-linked (glycosylated), 66 kDa, forensics

BIPM kindly volunteered to pilot 2-3 of these studies.

Peptides and proteins in matrix

The real challenges in peptide and protein analysis are their quantification in biological matrices. After experiences with the characterisation and quantification of pure peptides, first pilot studies (Track D) have been conducted successfully. The first KC will be organised in the near future. 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 focus group II “Proteins in complex matrices” to plan future studies for potential broader scope claims according to the following scheme:

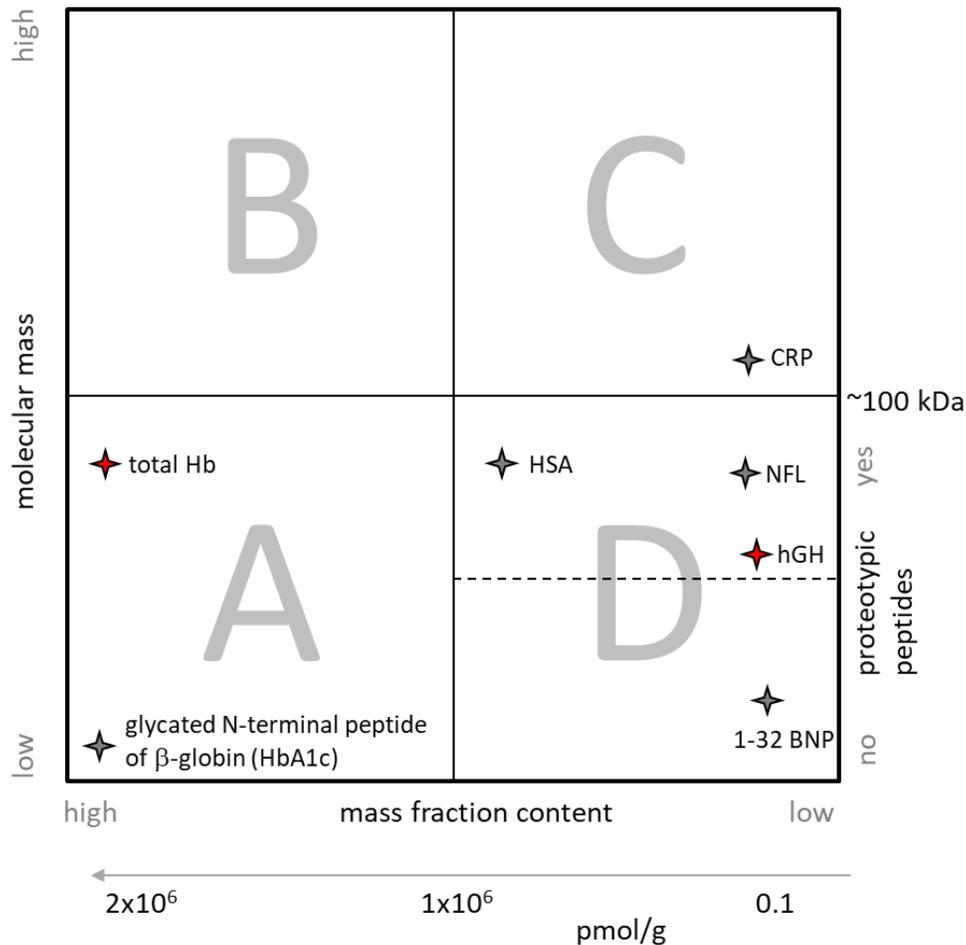


Figure 4: Section model for peptide and protein determination in matrix with the long-term goal to enable broader scope claims.

For peptides and proteins in matrix four sections in Figure 4 were identified by focus group II:

- **A:** peptides/proteins ≤ 100 kDa, concentration range $> 1 \cdot 10^6$ pmol/g
- **B:** proteins ≥ 100 kDa, concentration range $> 1 \cdot 10^6$ pmol/g
- **C:** proteins ≥ 100 kDa, concentration range $\leq 1 \cdot 10^6$ pmol/g
- **D:** proteins ≤ 100 kDa, concentration range $\leq 1 \cdot 10^6$ 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 matrix.

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
Total haemoglobin in blood
- **Section B**
Still to be discussed
- **Section C**
C-reactive protein (CRP)
- **Section D**
Human growth hormone (hGh)
Brain natriuretic peptide 1-32 (BNP 1-32)
Human serum albumin (HSA)
Neurofilament-light polypeptide (NFL)

Activity measurements

Another important field in laboratory diagnostics is the determination of enzyme activities. Focus group III “Protein activity measurements” was created to define the most important enzymes and a timeline for CCQM studies in this field.

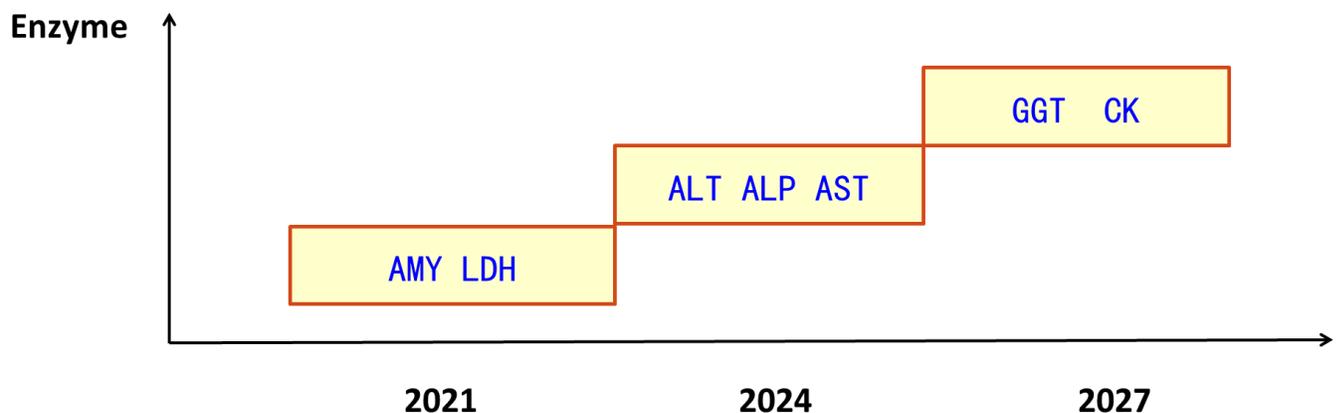


Figure 5: Timeline for planned KC in the area of clinical enzyme activity.

The enzymes listed in Figure 5 are the seven enzymes for which reference measurement methods for catalytic activity (e.g. alpha-amylase) have been developed and internationally agreed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) using kinematic spectrophotometry at 37 °C. The catalytic concentration ranges vary between the analytes:

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

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 enzyme activity measurands are method defined measurands, all KCs in this field are deemed Track C studies. A first pilot study has been 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.

The long-term goal is to be able to determine the activity via the protein affinity to its substrate, thus quantifying an active concentration rather than a total amount-of-substance.

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

The global SARS-Cov-2 pandemic has 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”. With the broad range of new diagnostic and serological tests in development internationally and many gaining regulatory approval in certain countries, standardisation of these tests will be essential in gaining acceptance as providing reliable measurements. Rapid antigen-based tests have been 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 prospect of vaccines on the horizon, antibody testing capable of determining COVID-19 immunity will become 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 have made significant investments in battling the pandemic and are developing services in the form of reference materials or reference measurements. In response to this need, the PAWG has launched a pilot study entitled “CCQM-P216: SARS-COV-2 Monoclonal Quantification”, coordinated by NIM, NRC, and BIPM. Building on existing competencies, the study will investigate the use of amino acid analysis and signature peptide quantification for such larger protein complexes. NMIs will also have 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 may be appropriate. In addition, NMIs could become increasingly engaged in antibody epitope mapping using advanced structural characterisation techniques such as hydrogen-deuterium exchange mass spectrometry, so capability building in this area may be required. Considerable opportunities also exist to study the SARS-COV-2 spike protein, which is considered the major antigen of SARs-CoV-2 so a prime candidate for matrix comparisons and is also implicated in some form with all leading vaccine candidates.

Finally, there could be unique measurement challenges associated with angiotensin-converting enzyme 2 (ACE2), which is what the spike protein binds to on the surface of host cells, initiating infection.

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, focus group IV “Protein drugs” was created. 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 on Trastuzumab (Herceptin®), one of the most widely used antibody-drugs, and also a popular candidate for biosimilars and biobetters, has been proposed.

5.4. INTERACTION WITH RMO ACTIVITIES

The major protein measurement activities within the **Asia Pacific Metrology Programme (APMP)** are among KRIS, 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-Characterization means that the data provided by all the participating labs are recommended to be used for the determination of 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 (KRIS), alpha-fetal protein in solution CRM (NIM) since 2007. The co-validation of HbA1c CRM (NIM) are still on-going and we also have a proposal from KRIS on co-validation of CRM for total hemoglobin.

NMIs and DIs in Europe are working closely together and with the stakeholders within the framework of EMPIR jointly funded by **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 are continuously presented to PAWG during the meetings.

Furthermore, the European network (EMN-TLM) brings stakeholders such as PT providers, IVD manufacturers, regulators and NMIs / DIs together to address stakeholder needs especially in the light of the new European IVD regulation (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 focus groups devising the PAWG strategy.

In the SIM region there is an ongoing project, partially supported by the Inter-American development bank (IDB) for the development and further production of a Reference material of Bovine Serum Albumin (BSA) in solution. It’s 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.

ANNEX

1. GENERAL INFORMATION

CC Name: CCQM

CC Working Group: Protein Analysis Working Group (PAWG)

Date Established: 2015

Number of Members: ~25 participating institutes, ~ 40 members

Number of Participants at last meeting: ~53

Periodicity between Meetings: 6 months

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

CC WG Chair (Name, Institute, and years in post): Jeremy Melanson, NRC, 1.5 years

Number of KCs organized (from 2015 up to and including 2020): 6

Number of Pilot studies organized (from 1999 up to and including 2020): 15

Number of CMCs published in KCDB supported by CC body activities (up to and including 2020): 5

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 purity assessment of high-purity certified reference materials (amino acids, peptides, and proteins), mass fraction/concentration of peptides and proteins in complex biological matrices and assessment of reference measurement procedures where the results and associated measurement uncertainties have a proven traceability to the SI.
- Qualitative protein and peptide analysis that may include the evaluation of chemical structure, protein folding, and the evaluation of protein-protein interactions and the associations of proteins with other biomolecules
- Measurement of catalytic enzymatic activities
- Appropriate preparation and application of protein/peptide CRMs and RMs

In cases where the scope overlaps with other working groups of the CCQM (for example, amino acids with OAWG, metalloproteins with IAWG or isotope ratio measurements with IRWG), collaborations ensuring the best efficiency will be pursued by co-organising studies and comparisons.

When proteins become important components of a complex subject such as a cell (e.g., cell sorting by the level of protein expression), the protein working group will consider participating in a multidisciplinary study as an expert protein working group.

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 (2017-2020)

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 and 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 current PS CCQM-P201 on the quantification of total haemoglobin results from the efforts of a former EMRP project, where potential reference measurement procedures could be developed and were compared using an existing reference material. For CCQM-P201 a complex protein (the tetrameric haemoglobin) in lyophilised whole blood was quantified for the first time at CCQM level. Although the concentrations of this protein are quite high and, thus, not challenging, the targeted expanded uncertainty of below 2 % is. As different measurement approaches were used, the advantages and difficulties of the various methods were revealed. In APMP, the technical collaborations were mainly focused on co-validation of potential reference materials, with joint characterisation and production planned in the future. The ability for the characterisation of human growth hormone was gained during the pilot study CCQM-P164, while the experience in the co-validation of C-reactive protein will lead to a CCQM comparison, which has been proposed to PAWG recently, as an analyte representative of section C of the model in focus group II.

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

Peptide purity - synthetic oxytocin (OXT) (CCQM-K115.b)

The provision of primary calibration reference services has been identified as a core technical competency for NMIs. NMIs providing measurement services in peptide/protein analysis are expected to participate in a limited number of comparisons that are intended to test and demonstrate their capabilities in this area. Primary calibration reference service refers to a technical capability for composition assignment, usually as the mass fraction content, of a peptide/protein in the form of high purity solids or standard solutions thereof.

The assignment of the mass fraction content of high purity materials is the subject of the CCQM-K115 comparison series. A model to classify peptides in terms of their relative molecular mass, the amount of cross-linking, and modifications has been developed. With the aim of leveraging the work required for the CCQM-K115 comparisons and thereby minimising the workload for NMIs and simultaneously focussing on a material directly relevant to existing CMC claims, human C-peptide (hCP) was the most appropriate choice for a study material for a first CCQM KC and parallel PS looking at competencies to perform peptide purity mass fraction assignment for linear peptides without cross-links and of up to 31 amino acids in length. The second cycle of peptide purity comparisons, CCQM-K115.b and -P55.2.b on oxytocin (OXT) covers short (1 kDa to 5 kDa), cross-linked and non-modified synthetic peptides such as OXT which is a cyclic peptide possessing nine amino acid residues and a disulfide bond. OXT is a chemically synthesized peptide hormone that is used to induce labour and as emergency medication for postpartum haemorrhage with no alternative medication being available. Additionally, nasal sprays containing OXT are sold in many countries as it reduces anxiety and depression.

Therefore, OXT was selected to be representative of chemically synthesised linear peptides of known sequence, without cross-links, up to 5 kDa. It was anticipated to provide an analytical measurement challenge representative for the value-assignment of compounds of broadly similar structural characteristics. For most participants the results for the mass fraction of OXT were in good agreement. However, results for the identification and quantification of related peptide impurities demonstrated that some participating laboratories might not fully understand their measurement protocols and may have to make improvements to existing procedures. As peptide and protein analysis at CCQM is still a very recent activity all participating laboratories benefit from these experiences and the subsequent discussions which are continually improving the level of expertise in metrological science for peptides and proteins.

Purity-assessed recombinant protein contents in buffer solution using insulin analogue (CCQM-K151)

Protein-related business and technology has developed significantly in various areas including in the clinical and pharmaceutical industries. This development has increased the demand for suitable protein analytical methods, since determining the content of a reagent can serve as a quality control measure and may help establish safety guidelines for human use. For this reason, NMIs are actively working on the establishment of measurement standards for protein quantification.

CCQM-K151 was designed to underpin and allow NMIs and DIs to assess participants' capabilities in assigning the mass fraction of a pure recombinant protein of a size below 10 kDa with several disulfide bonds in a buffer solution. The focus of this comparison was to demonstrate the capabilities of the participants to assign the mass fraction of a purified recombinant protein in an aqueous solution. This study was conducted as a special challenge not only to demonstrate the above capability but also to underpin the established primary measurement procedures based on AA analysis with another orthogonal method based on S analysis by collaboration with the IAWG.

Diabetes is one of the major challenges for healthcare systems world-wide with about 400 million patients. Therefore, the production of insulin and insulin analogues are a billion-dollar business (about 25 billion in 2018 for the major manufacturers). To ensure the safety of the patients, the purity of the insulin must be monitored. Because of its clinical importance a human insulin analogue was selected to be representative of purity-evaluated recombinant proteins up to 10 kDa containing up to 3 disulfide cross-links using amino acid-based liquid chromatography isotope dilution mass spectrometry (LC-ID-MS) and/or sulphur-based inductively coupled plasma mass spectrometry using isotope dilution (ID-ICP-MS).

With the exception of three laboratories, the results were in good agreement. Actions to improve the capabilities were successfully undertaken by these laboratories. The reference value from the parallel pilot study based on S analysis showed good agreement with the KCRV based on AA analysis, and this result underpins the complementary role both methods play in protein quantification.

Mass fraction of human growth hormone in serum (CCQM-P164)

In addition to providing primary calibration services and pure reference materials, the PAWG members are the source of traceability in the clinical context, by providing reference measurement procedures for clinical markers in biological matrices. ID-MS has been used successfully to achieve accurate SI traceable measurement results for the determination of small molecules in complex matrices for decades and can also be applied to peptides and proteins. The quantification is based on peptides specific for the analyte protein and calibration material such as those characterised in the K115 series are required.

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 (GHD), 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. The variability between immunoassays has been addressed by efforts for the standardisation of GH-measurements. In this regard, a working group for GH standardisation was initiated by the IFCC, a major stakeholder of PAWG, in 2016. This working group requested the support of laboratories using isotope-dilution mass spectrometry (ID-MS) 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.

With a broader objective of providing a suitable initial study for the PAWG, the Physikalisch-Technische Bundesanstalt (PTB) in Germany suggested GH in human serum as a simple protein measurand in a serum matrix for a CCQM-comparison. According to the proposal presented during the autumn meeting in 2014 in Tsukuba, Japan, the analysis of GH should serve as a model for the determination of low-abundant proteins in serum using ID-MS. During the PAWG-meeting of April 2017, in Paris, France, the group decided to start a pilot study on the determination of GH in serum. A spiked material containing only 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. Training samples were distributed to interested NMIs and DIs in preparation for the planned comparison. As the coordinating laboratory PTB, who along with LGC and NIST published the first IDMS based method for the quantification of GH in serum, provided the necessary guidance and training that enabled a successful outcome to the study.

Initially, a two-step study was intended to also include the measurement of the GH content in a clinical sample. Material-related difficulties led to the decision to repeat the first step of the comparison and to consider the determination of total GH and/or 22 kDa-GH in a clinical sample as a possible follow-up study in the form of a KC.

Regarding the participants using different protocols and signature peptides, the results are in good agreement. However, this PS shows that there is still room for improvements regarding also the measurement uncertainty for the IDMS methods developed by the participants to be considered as "fit for purpose" to provide an SI traceable reference point for routine testing methods. Currently, the participating laboratories are working on these improvements and intend to demonstrate their capabilities in the upcoming KC.

Measurement of the catalytic concentration of α -amylase (AMY) in serum (CCQM-P137)

Encoded by our genes, proteins form the basic living tissues and play important roles in all kinds of biological processes in our body. They exhibit all kinds of functions and regulate the metabolite process. These functions depend not only on the amount of primary structure, but also on their modifications and higher-order structures. Therefore, people concerns are more about the protein activity rather than the amount by its primary structure. There are various forms of protein activity, such as enzymatic activity, immuno-activity, proliferation activity, and so on. These activities can be obtained by various ways. In order to make all protein activity measurements accurate and comparable, NMIs are responsible to establish the traceability chain and carry out international comparisons to investigate the comparability for protein activity measurement.

Enzymes are usually protein molecules which act as biological catalysts, converting the substrate to a product or products. In order to make the clinical enzyme activity measurements comparable, IFCC proposed related reference methods for enzyme activity measurements. Numbers of international comparisons have been carried out for peptide/protein purity analysis or mass fraction determination.

However, there is a lack of international comparisons for protein activity measurement among NMIs. Therefore, the first pilot study of measurement of α -amylase activity in serum has been carried out.

CCQM-P137 was designed to investigate the comparability of enzyme activity measurement and lay the foundation for the next step comparisons supporting CMC claims. It is also a useful trial for a comparability study of reference method defined measurements. Most participants achieved successful measurement of the catalytic concentration of α -amylase; only one participant failed due to the corruption of the sample. For most participants, the measurement results agreed well with those from other NMIs and showed similar measurement performance, demonstrating good measurement capability for serum α -amylase activity.

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5. DOCUMENT REVISION SCHEDULE

PAWG Strategic Plan 2021-2030: V1, 22 December 2020