Report from the CCQM Protein Analysis Working Group (April 2019 – July 2020)

Meetings

The PAWG met in Torino Italy on October 3-4, 2019 and held a productive meeting with 21 members. Virtual meetings were then held via Webex on April 30, May 7, and May 13, 2020, each attracting on the order of 50 – 75 participants.

Summary of Key Comparisons and Pilot Studies

Study number	Description	Coordinating Laboratory	Start date	Status
CCQM-K115.b/P55.2.b	Peptide purity - synthetic oxytocin (OXT)	BIPM/NIMC	2018	Published July 2020
CCQM-K151	Determination of the amount content of a purity-assessed recombinant protein in an aqueous calibration solution	KRISS	2017	Draft B
CCQM-P164	Mass fraction of human growth hormone in serum	PTB	2017	Draft A
CCQM-P201	;	PTB	2019	Draft A
CCQM-K115.c	Peptide purity - synthetic glycated hexapeptide of HbA1c (GE)	BIPM/NIMC/HSA	2019	Ongoing
CCQM-K115.2018	Peptide purity - synthetic hexapeptide of HbA0 (VE)	BIPM/NIMC/HSA	2019	Ongoing

Current study highlights

CCQM-K115.b – synthetic oxytocin

Seven NMI laboratories demonstrated equivalence for the purity determination of oxytocin, a model cyclic peptide with a single disulfide bond, using a range of different methods such as amino acid analysis, qNMR, and mass balance. This level of agreement was a significant improvement over that achieved in CCQM-K115 (C-peptide), highlighting a substantial improvement in capabilities in this area in only a few short years.

CCQM-K151 – insulin solution

Considered a specialized Track C comparison, CCQM-K151 coordinated by KRISS involved the "Determination of the amount content of a purity-assessed recombinant protein in an aqueous calibration solution". Using amino acid analysis, six NMIs demonstrated equivalence but three participants were biased low and their results were withdrawn. Additional experiments by these

NMIs proved that the bias we due to either incomplete hydrolysis, or using too small of a sample volume that yielded significant protein adsorption to vials. However, their revised results were excluded from the KCRV.

CCQM-P164 - human growth hormone (hGH) in serum

The seven participant NMIs used a range of different methods for this challenging study organized by PTB; some participants purified the intact hGH before tryptic digestion, while others purified signature peptides of hGH following whole serum digestion. Some participants reported results for a single peptide, while others reported on up to five. In regard to the different protocols used for analyte enrichment, the variability of reported results did not exceed 13 % in terms of CV from the reported results. In view of this variability, this suggests that all signature peptides selected by participants could be used as to calculate the concentration of the final hGH concentration.

CCQM-P201 - Total haemoglobin concentration in whole human blood

This pilot study coordinated by PTB attracted 11 NMI participants employing methods such as LC-ICP-MS of iron, LC-MS/MS on signature peptides, the cyanhaemiglobin (HiCN) method and the alkaline haematin D-575 (AHD) method. Surprisingly, the preliminary results suggested that three LC-ICP-MS results appeared to be mostly biased low, where one would expect LC-ICP-MS to generate higher values is anything due to the potential for unspecific iron detection. By contrast, LC-MS/MS yielded a majority of results near the mean value. Prior to proceeding to a KC, effort will be undertaken to elucidate the basis of this potential discrepancy.

New studies that require approval

Employing the two peptide materials currently being measured in CCQM-K115.c (HbA1c) and CCQM-K115.2018 (HbA0), a key comparison coordinated by HSA, LNE, and NIMC will involve measuring these two peptides as surrogates for their respective parent proteins in human hemolysate. These proteins are well-defined in the PAWG strategic plan and fall under Quadrant A (high amount contents and molecular mass < 70 kDa). In addition, a related but stand-alone pilot study will be completed to determine the amount-of-substance fraction of [HbA1c/(HbA1c+HbA0)] in human hemolysate, to assess the performance relative to the IFCC method for diabetes mellitus.

Given the global importance of the COVID-19 pandemic, NIMC is coordinating a COVID-19 antibody pilot study where approximately 10 NMIs will employ various characterization methods to determine the amount of antibody in solution, results of which could be used to standardize antibody assay kits.

Stakeholder engagement

Held in conjunction with the JCTLM workshop on 4 December 2019, the BIPM hosted a meeting on ‘Working together towards Standardization in Laboratory Medicine – co-ordination of international activities’. On July 7, 2020, a webinar was organized within CCQM entitled “Ensuring the reliability of measurements in response to the COVID-19 pandemic” to help inform NMIs as they build capacity in the area of diagnostics.