CCQM-NAWG STRATEGY DOCUMENT 2021-2030

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1. EXECUTIVE SUMMARY

The focus of NAWG is to support global comparability and metrological traceability of measurement results for the analysis of nucleic acid polymer sequences, their modifications and their abundance. Nucleic acid analysis is used for a wide range of global measurements which underpin research and practical applications in most areas of life science. The various sectors in which molecular methods can be applied to identify and characterize nucleic acids and their modifications, along with the concomitant development of techniques allowing genetic sequences to be stored, modified and harnessed, has meant that nucleic acid analysis is not only used for genetic applications, but also in the application of molecular techniques supporting broader areas of bioanalysis such as protein and cellular analysis.

Since its initiation in 2015, the NAWG has primarily served the foods and feed and health sectors, although nucleic acid analysis is also important in the environmental (e.g. species/microbial surveillance) and biotechnology (spanning industrial, agricultural and pharmaceutical) sectors. These areas are also likely to benefit from NAWG activities in the future. The food authentication area is the most metrologically advanced (with respect to traceability and understanding of sources of uncertainty) in terms of routinely applied measurements and it is in this sector that NMIs have the most NAWG supported CMCs. While molecular testing is used in clinical diagnostics, metrology is not as widely applied in this sector as it is for clinical chemistry; however, this is changing due to the desire of stakeholders to apply increasingly advanced high throughput traceable and quantitative measurements to assist in medical decisions and patient management.

In addition to NMIs, DIs and associated CCQM WGs (CAWG, PAWG and OAWG), key NAWG stakeholders include other reference material producers and proficiency test/external quality assurance providers and the industries they serve. As metrology is a fairly new concept in the field of nucleic acid measurements, considerable efforts are ongoing to inform potential stakeholders how measurement science can support their applications. This is achieved via (locally, regionally and NAWG led) workshops, publications, regional interactions and communication and cooperation between NAWG and international or regional organizations such as ISO, CEN (The European Committee for Standardization), WHO, JCTLM, IFCC, SOGAT (Standardisation of Genome Amplification Techniques), ESCMID (European Society for Clinical Microbiology and Infectious Disease) and EMN-TraceLabMed to better understand standardization challenges and to apply state-of-the-art metrology to support existing and future stakeholders. NAWG members actively participate in many of these groups and committees. In addition, joint studies with other CCQM WGs are actively being sought in specific areas, such as microbial measurement (CAWG).

To date all NAWG Key Comparisons have been in the foods analysis space. NAWG activities in the healthcare sector are increasing; molecular diagnostic tools are applied to genotype patients, to quantify pathogenic burden, to measure transcriptional surrogate biomarkers of disease and to measure disease predictors using changes at the epigenetic level. To support NMI measurement capabilities in these areas, a series of medically relevant pilot studies have been conducted which focused on the identification and quantification of nucleic acid sequences and key comparisons are planned. The ubiquity of the nucleic acid analysis methods, the molecules they target and challenges associated with their measurement are typically agnostic to sectors. Consequently the NMIs are in a unique position to advance the measurements in one specific subject area (such as *in vitro* diagnostics) using knowledge and techniques from another (such as food and feed testing). This was exemplified by the response of the NAWG to the COVID-19 pandemic in the rapid implementation of the SARS-CoV-2 pilot study (P199b) with the participation of 18 NMI/DIs, many of whom had not previously measured viral genomic RNA material. The

NAWG will need to explore how this pan sector characteristic can be capitalized on to support broader scope measurement capabilities.

As we progress into the next decade, NAWG activities will likely continue to support food and feed associated testing with Key Comparisons reflecting additional unmet needs, such as different matrices, species and molecular challenges (such as discriminating edited genes edited from natural sequences). It is anticipated that food and feed associated measurements will expand to the agriculture/biotechnology sector; pilot studies to monitor crop disease are planned. The NAWG strategy heading for 2030 will considerably strengthen activities associated with reference measurement procedures (likely to include the measurement of sequence structure and composition) and materials to support clinical nucleic acid analysis. These are likely to build on established capabilities for underpinning SI traceable quantitative measurements of nucleic acids copy number concentration in aqueous solutions and explore routes to apply such capacity to assist in matrix-based reference materials and/or reference measurement procedures on real samples; such capacity will also be of value to the other sectors such as industrial biotechnology where novel methods, such as CRISPR CAS-9 based tools, are being applied. The increasing worldwide application of genomic, transcriptomic and epigenomic analyses means that the NAWG will need to explore the development of strategies to support advanced sequencing capabilities, including for purity analysis. The measurement challenges associated with these types of 'non-targeted' methods are likely to increase with the development of newer, simplified sequencing technologies more suitable to less specialized settings.

2. SCIENTIFIC, ECONOMIC AND SOCIAL CHALLENGES

While nucleic acids were discovered in the late 19th century, it was not until almost a century later that their role in heredity was confirmed. Nucleic acids are used by living organisms to write the instructions for reproducing, building and maintaining a cell/multicellular organism. In this analogy, the letters of the genetic sequence spell out genes. A key mechanism by which nucleic acids enable biological function is through the Central Dogma whereby protein-coding genes are transcribed from DNA into a complementary RNA copy which is then translated into an amino acid sequence. It is within the sequence of nucleic acids monomers that the code for all the protein amino acids sequences required for cellular life are written. Nucleic acid sequence analysis is used to measure genetic changes, identify the presence of species of organisms or even to determine the relatedness of organisms within a species.

A number of high accuracy chemical methods, used by NAWG members, are able to measure the presence of nucleic acids and, in some cases, to identify the individual nucleotide monomers. However, as all living organisms essentially use the same set of nucleotides to function, it is necessary to 'read' the sequence and determine the arrangement of these monomers to determine (for example) whether the sequence in question as a nominal property originates from a genetically modified organism, a human gene (that may contain a clinically important mutation) or that of an infecting pathogen. Molecular methods allow nucleic acid sequences to be determined, and where necessary, quantified and further evaluated for epigenetic differences. The development of molecular methods, including complementary hybridization, nucleic acid amplification technologies (such as the polymerase chain reaction) and sequencing coupled with the ubiquity of nucleic acids in life sciences, has meant these same methods are used for nucleic acid analysis across different specialties.

Despite this, metrology and metrological considerations (such as routes to traceability and sources of uncertainty) are not commonly investigated, with those applying nucleic acid analysis typically assuming

their systems are fit for purpose in this respect. Certainly many breakthroughs in sequence discovery, genome evaluation and routine monitoring have succeeded without the assistance of the measurement science community. Yet as these fields have pushed to offer ever more high-throughput ('omic') and precise quantitative solutions they have been met with challenges associated with standardization and traceability. Many of those working with these methods neither realize that measurement science is a field in its own right, nor that a global infrastructure exists (of which the CCQM NAWG is a part) to support them in this respect. Given the wide variety of applications in the nucleic acid analysis space the challenges associated with supporting in terms of measurement science are vast. The NAWG will continue to support the various sectors that are more metrologically established while also championing advancements in the other sectors.

In September of 2020, a questionnaire was sent to all NAWG members to determine the current and future priorities of the NAWG. Eighteen institutes responded to the survey: BVL (Germany), EXHM/GCSL-EIM (Greece), GLHK (Hong Kong), HSA (Singapore), INMC (Colombia), INMETRO (Brazil), INRIM (Italy), JRC (EU), KRISS (Korea), NIB (Slovenia), NIMC (China), NIMT (Thailand), NMIA (Australia), NMIJ (Japan), NML at LGC (UK), TUBITAK UME (Turkey), NIST (USA) and VNIIM (Russia).

The questionnaire covered following topics:

- current and potential future subject areas in which NMIs/DIs wish to claim CMCs (for both absolute measurements and fractional abundance measurements)
- types of nucleic acids measurements (with relation to matrix)
- nucleic acids measurement procedures (specific/non-specific)
- identification of measurement capabilities which require NAWG attention and support
- questions, related to NMI participation in Key/Pilot studies and leading/co-leading a study

For fractional abundance nucleic acid measurements (Figure 1), more than half of respondents currently hold CMCs or intend to hold CMCs in the future in food analysis (72%), *in vitro* diagnostics for infectious diseases (67%), *in vitro* diagnostics for human genetics and non-communicable diseases (67%), regenerative medicine and gene therapy (61%), and biotechnology (56%). For absolute nucleic acid measurements (Figure 2), more than half of respondents currently hold CMCs or intend to hold CMCs in the future in *in vitro* diagnostics for infectious diseases (78%), regenerative medicine and gene therapy (67%), *in vitro* diagnostics for human genetics and non-communicable diseases (61%), biotechnology (56%), and food analysis (56%).

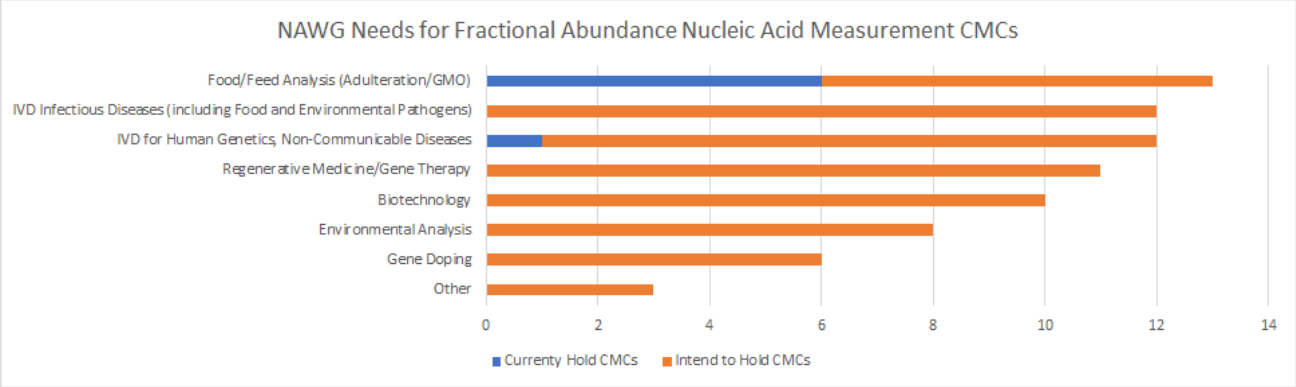

Figure 1. Subject areas in which NAWG members currently hold CMCs or intend to hold CMCs within the next 5 years for fractional abundance nucleic acid measurements.

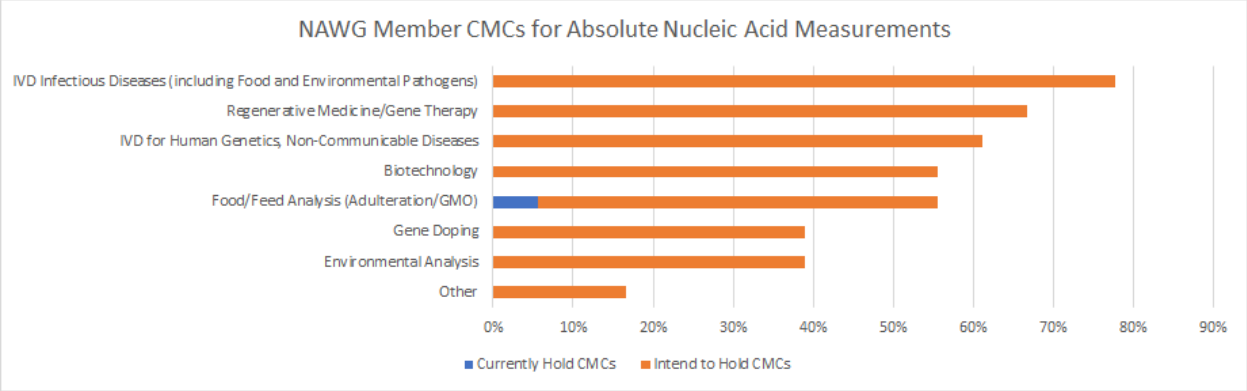

Figure 2. Subject areas in which NAWG members currently hold CMCs or intend to hold CMCs within the next 5 years for absolute quantification of nucleic acid measurements.

Member NMIs and DIs offer a wide range of products and services to their stakeholders including certified and non-certified reference materials, calibration services, and regulatory functions. The NAWG engages with its members and stakeholders through key comparisons and pilot studies, workshops with academia and industry, liaisons with other relevant working groups and committees.

As evidenced by the COVID-19 pandemic, new pathogens and clinically important variants of established pathogens are constantly being discovered; it may not always be possible to create certified reference materials for each new, important nucleic acid sequence in a timely fashion. The work of the NAWG has demonstrated the potential use for candidate SI traceable reference measurement procedures for nucleic acid measurement (such as IDMS, optical counting and digital PCR) and value-assignment of reference materials that enables a dynamic means of supporting the established reference material routes for enabling traceability for laboratory medicine.

In parallel with recognizing current and future needs in standardization and traceability, NAWG is also addressing some of the fundamental scientific challenges associated with nucleic acid analysis. Nucleic acids vary in their stability which can impact both reference materials and biobanks as well as routine testing during sample handling. DNA is generally considered stable; however, low mass concentrations (less than 5 ng/µL) of both DNA and RNA require the addition of stabilizing molecules (such as yeast tRNA or other background heterologous RNA/DNA) to prevent decreases in concentration over time. RNA can be less stable than DNA; both due to inherent chemical reasons and because RNases, which can quickly degrade RNA, can be both ubiquitous and stable in the environment. Reduced stability of an analyte (be that in reference material or clinical samples) may impact on the quantity of a given sequence or the composition of its matrix, both of which may impact the measurement result. Further, information on the wider target sequence composition may also be useful with storage as factors like fragment size may also be affected by stability resulting in potential discrepancies between methods.

Scientific challenges associated with the measurement of a given nucleic acid can vary due to the complexity of the nucleic acid in question (e.g. sequence, size, secondary structure, etc.) as well as the matrix in which it is being measured, with nucleic acids almost exclusively being measured in solution. Consequently, considerations around sources of uncertainty and routes to traceability must frequently

include volume as an additional consideration. Nucleic acid analyses are typically multi step processes requiring prior manipulation of the sample to isolate and purify the nucleic acids for analysis ('pre-analytical' steps).

When conducting nucleic acid analysis, a key distinction is whether the measurand is a part of a larger molecule (typically the case with genes associated with genetic modification) or whether it is contained within the sequence being measured (such as when measuring actionable genetic variants during cancer treatment). From an analytical point of view, these two categories can present distinct challenges when considering sources of uncertainty. Furthermore, the analytical sources of uncertainty can differ when the same nucleic acid sequence is measured in different matrices (whether pure nucleic acids in aqueous solution, matrix materials or biological samples).

When considering a given measurand, the type of analysis (Figure 3) can be categorized by whether the nucleic acid analysis is a method that is 'targeted' (such as PCR or hybridization) to identify a defined sequence(s) (and potentially quantity and/or any modifications) where the intended target is specified, or whether the nucleic acid sequence(s) (and potentially quantity and/or any modifications) is unknown and the 'non targeted' (such as sequencing) examination intends to determine this. The type of analysis also needs to consider the type of measurement (Figure 3) which can be broadly categorized into presence/absence 'nominal' examination (e.g. sequence is present or not), relative quantification/fractional abundance (e.g. percentage of sequence) or absolute quantification (e.g. copy number of sequence per unit volume).

When discussing a given measurement/examination and associated challenges, the type of sample (including properties of the nucleic acid and its matrix) also need to be considered (Figure 3). At their simplest level of complexity nucleic acids can vary from a few tens to billions of bases (term used to describe a nucleotide monomer which is approximately 300 Daltons, consequently large DNA molecules can be >100 million kilo Daltons) and this may be comprised of mixtures of different genomes, further complicating the measurement with certain techniques. Further sources of uncertainty will be added when additional steps are required to prepare the nucleic acids for analysis such as purification and nucleic acid digestion (e.g. restriction enzyme treatment), adaptation (e.g. adapter ligation), modification (bisulfite conversion) or generation of complementary nucleic acids (reverse transcription). Purification (or extraction) is often required when performing nucleic acid measurements on real samples, due to the fact the matrix is typically unsuitable for the final molecular analysis steps. For example, formalin fixation of biological tissue, for preparation of formalin-fixed paraffin-embedded (FFPE) samples is widely used in pathological laboratories to preserve patient's biopsies from degradation for further investigations. These materials can be stored for decades and are often considered as a valuable source of well characterized human samples although are not ideal for storage of nucleic acids. Isolation of nucleic acids from FFPE samples is challenging due to formalin-induced crosslinking of proteins and nucleic acids, and the fragmentation of high molecular weight genomic DNA and mRNA molecules. All of these steps contribute uncertainty and must be considered when exploring routes to traceability and the most accurate measurements.

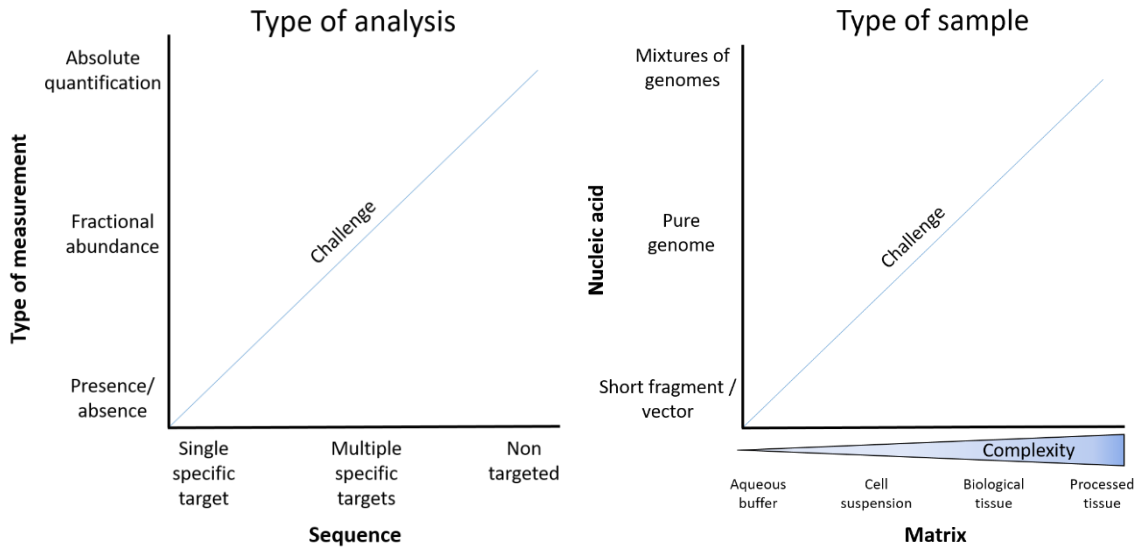

Figure 3. Type of sample and Type of analysis NAWG studies vary in the complexity of the analysis and the type of sample. The simplest type of study would be the detection of the presence or absence of a plasmid in a buffered solution. A much more complex study would be the identification of all nucleic acid species in a processed food matrix, along with the abundance of each.

3. VISION AND MISSION

The CCQM's vision is:

A world in which all chemical and biological measurements are performed 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 NAWG strategy are

To contribute to the resolution of global challenges by improving the accuracy of nucleic acid measurements, associated with areas such as food safety and healthcare including infectious disease pandemics, by identifying and prioritizing critical measurement issues and developing studies to compare relevant measurement methods and standards. The aim is to ensure the measurand is clearly understood, how it is relevant to what is intended to be measured and that all sources of uncertainty are considered and, where possible, quantified.

To promote the uptake of metrologically traceable chemical and biological measurements, through workshops and roundtable discussions with key stakeholder organizations, 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 progressing from fractional abundance measurements to application of absolute SI traceable methods for nucleic acid analysis.

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 capabilities while also supporting capacity development in the fast advancing field of nucleic acid analysis.

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.

To maintain organizational vitality, regularly review and, if required, update the CCQM structure for it to be able to undertake its mission and best respond to the evolution of global measurement needs, by prioritizing where new areas or issues should be addressed within the structure and evolving working group remits as required.

5. ACTIVITIES TO SUPPORT THE STRATEGY

5.1. PROGRESSING METROLOGY SCIENCE

In order to achieve its strategic goals in addressing global scientific challenges within nucleic acid analysis area, the NAWG works to support members in nucleic acid RM development and calibration services, in areas where the NAWG support is desired (Figure 4). In addition, the NAWG provides an active forum to discuss investigative research, which underpins nucleic acid measurement expertise.

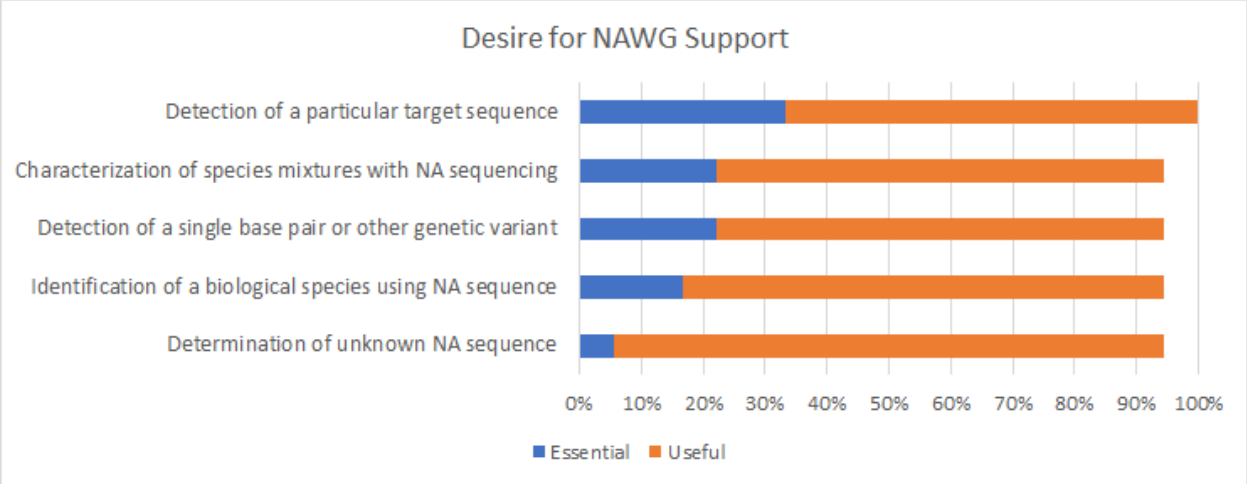

Figure 4. Areas in which NAWG's support is desired according to results from the questionnaire (2020)

A major impact that the NAWG (and its predecessor the Bioanalysis Working Group) has achieved is in supporting the application of wider capabilities that have been developed by individual, or groups of NAWG members (Bhat et al. 2010; Bhat et al. 2009; Burns et al. 2005; Corbisier et al. 2010; Dong et al. 2015; Haynes et al. 2013; Holden et al. 2007; Pavsic et al. 2017; Redshaw et al. 2014; Sanders et al. 2011; Sanders et al. 2013). A good example is in the measurement of genomic DNA from plant tissues with series of key comparisons supporting CMCs from a variety of different biological matrices (Corbisier et al. 2012; Dong et al. 2018; Mester et al. 2020); this series is continuing with the high protein matrices in K86.d. Another considerable major technical goal of the NAWG has been to demonstrate routes for SI-traceable nucleic acid measurements with NAWG members working as a group to demonstrate the first examples of this (Whale et al. 2018; Yoo et al. 2014; Yoo et al. 2016).

In order to realize the strategic plan, the NAWG intends to pursue key comparisons and pilot studies to support nucleic acid measurements in sectors including, but not limited to, food and feed, health, environment and biotechnology (Figure 5).

Future Planned Studies:

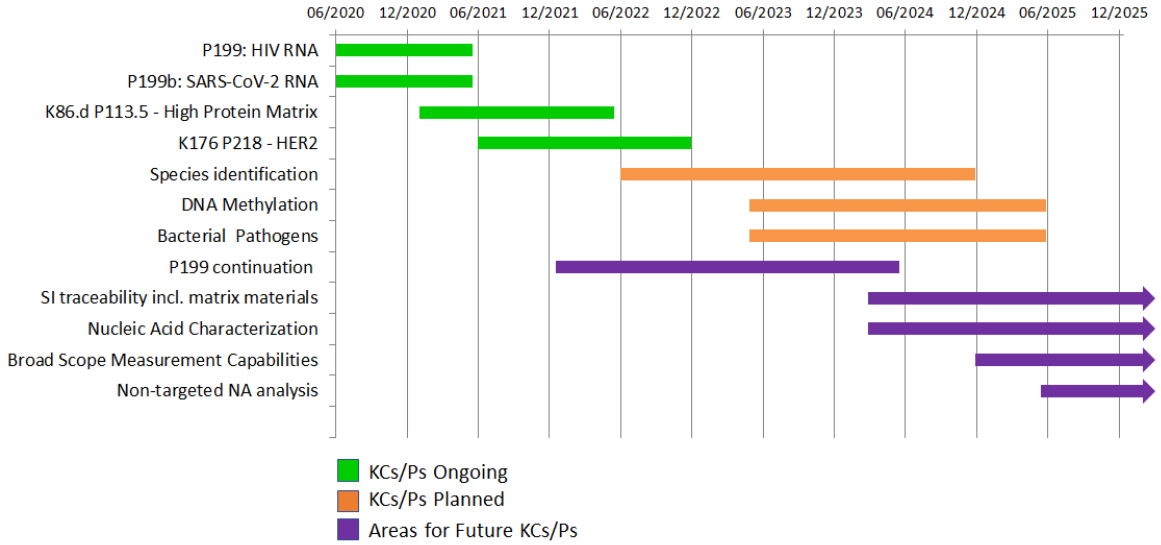

Figure 5. List and proposed timeline for planned studies (2020-2025)

These studies include sequence-specific RNA measurements (molecular diagnosis of RNA viruses), DNA measurements (high protein matrix, cancer biomarkers, plant pathogens, etc.), qualitative DNA identification (biological species determination) and DNA modification measurement (DNA methylation in biological matrix). By completing these planned studies, the NAWG will broaden its scope within its measurement space in terms of type of analysis (Figures 6) and type of sample (Figure 7).

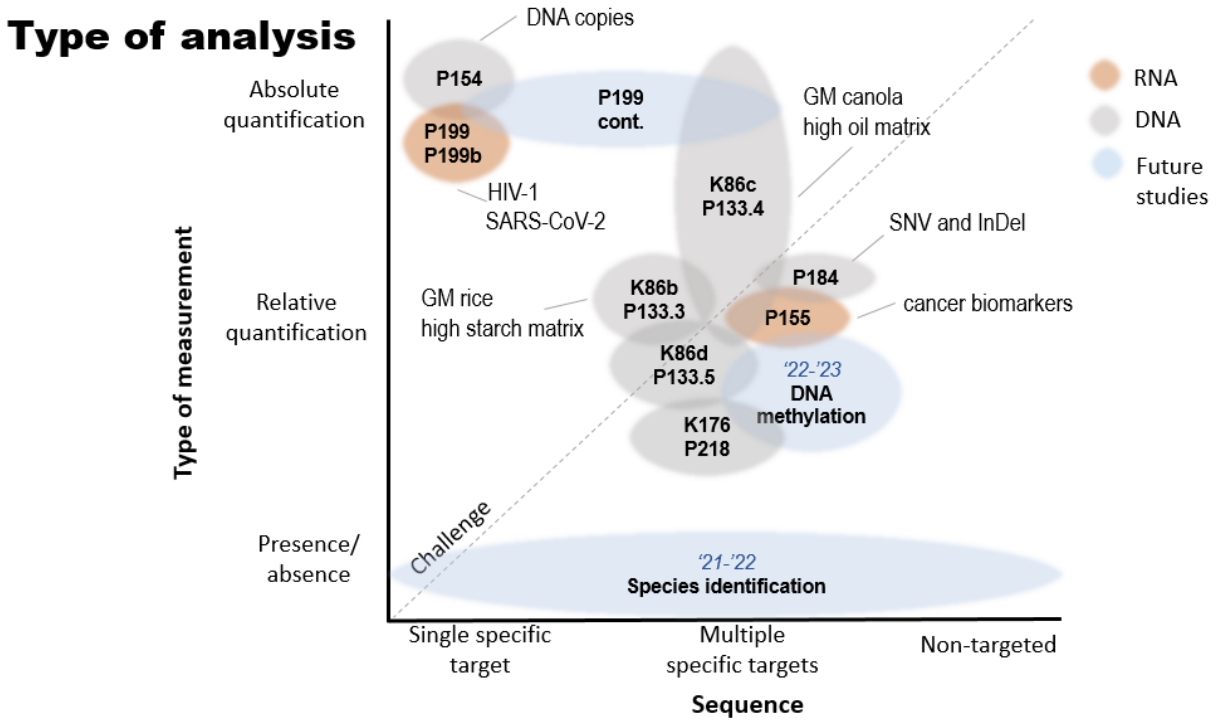

Figure 6. NAWG measurement space in type of analysis

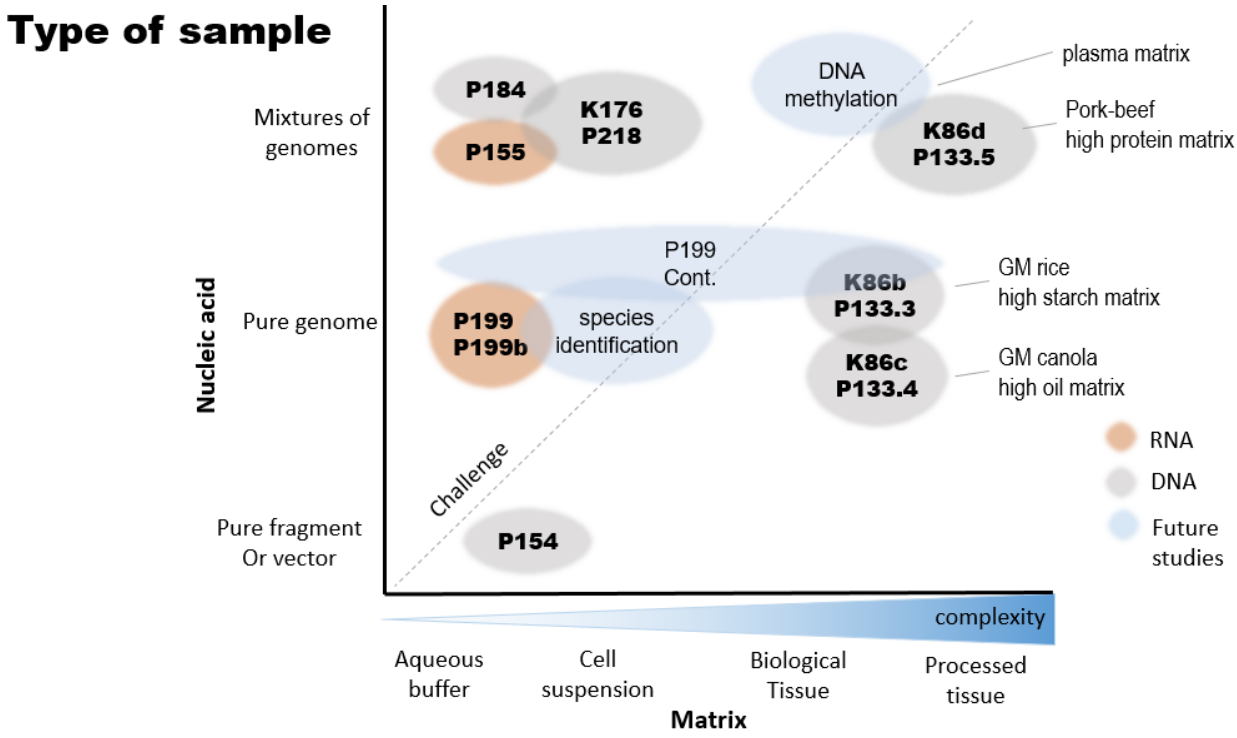

Figure 7. NAWG measurement space according to type of sample

Current and near Future NAWG Studies

<u>The P199 series (CCQM P199 and P199b Studies: HIV and SARS-CoV-2 RNA Quantification) and ongoing</u>

The NAWG is currently analyzing data from the CCQM P199b study (SARS-CoV-2 RNA copy number quantification) which follows on from the 2019 CCQM P199 study (HIV-1 RNA copy number quantification). CCQM P199 was the first CCQM study to explore absolute traceable RNA quantification by enabling comparison to orthogonal methods; participants were tasked with quantifying the *gag* gene in samples containing HIV-1 RNA. In P199b, participants measured several different gene targets from the SARS-CoV-2 viral genome; this reflects the variety of sequences measured in different diagnostic tests. In addition, by measuring two distinct RNA viruses in these studies, NAWG is establishing that these measurement approaches are broadly applicable to a wide range of RNA sequence templates. P199b included four materials; three of these materials were in a concentration range (10^1-10^4 copies/µL) which is suitable for digital PCR measurement. The fourth study material was more concentrated (10^9-10^{10} copies/µL) in buffer alone (with no background RNA or DNA), which is suitable for measurement by methods orthogonal to digital PCR (as well as measurement by digital PCR after appropriate dilution). Three of the study materials were *in vitro* transcripts (synthetically made RNA), but one of the materials was isolated from lentiviral particles (which had been genetically modified to produce large fragments of SARS-CoV-2 RNA), representing a more complex sample. Twenty-one NMIs, DIs and expert guest laboratories participated in the study and demonstrated that it was possible to measure the amount of the SARS-CoV-2 viral RNA with high accuracy using reverse transcription-digital PCR (RT-dPCR).

Due to the relevance of viral detection in the context of the COVID-19 pandemic, other pathogenic viruses and the use of viruses as vectors (e.g. for vaccines, gene therapy, synthetic biology, etc.), it is likely that Key Comparisons will be performed (P199 continuation) to further demonstrate and broaden capabilities in viral genome measurement in aqueous solutions. Additionally, NAWG will perform Pilot Studies to develop capacity with whole virus materials that require sample processing (potentially within a biological matrix). These studies will likely be planned in the next five years.

K86d P113.5 (High Protein Matrix)

This study aims to measure the percent of pork in beef, both in a matrix and in a solution, which is relevant for processed food measurements. The study will have both a KC portion and a P portion. In the KC portion, participants will be required to measure two types of materials: unknown percentage pork/beef mixture and unknown percentage solution of pork/beef. In the P portion, participants will measure unknown percentage pork/beef mixture, but this will be a much lower percentage than in the KC portion. This study builds on previous work by the NAWG in K86b (high starch matrix) and K86c (high fat matrix).

K176 P218 HER2 Copy Number Variant

HER2 overexpression, caused by an amplification of the HER2 gene within cells, is important in breast cancer; while treatments for HER2+ cancer are available, they can have serious cardiac contraindications, so they should only be used when necessary. Consequently, accurate measurement of HER2 copy number in the genome is important to ensure those at most risk are correctly managed. The two main methods of detection HER2 overexpression are IHC (immunohistochemistry) and FISH (fluorescence in situ hybridization), which can have equivocal results. This NAWG KC will investigate candidate reference measurement procedures for the quantification of HER2 gene copies. This study builds on previous NAWG studies (P154, P184) and expands participants' capabilities from SNV (single nucleotide variant) and INDEL (small insertions and deletions) to large structural variants.

Species Identification

The proposed species identification study builds on previous food studies performed by the NAWG. The aim of this study is to promote species authenticity analysis and identification of food and feed samples. The measurand is the mitochondrial DNA sequence and the suggested measurement methods are NGS (next generation sequencing), Sanger Sequencing, and digital PCR. The matrix will be whole meat samples (from a finite list of species with a single species per sample, without pre-processing) that have been lyophilized and sieved.

Epigenetic analysis

A study is planned that will be a continuation of the P94 series (P94 in 2006, P94.1 in 2008, P94.2 in 2012). The aim of the study is to support SI-traceable measurement of DNA methylation levels in a sample which resembles a liquid biopsy. The study will allow NMIs to demonstrate competency in analyzing DNA methylation levels and in producing relevant methylation reference materials. The measurand will be the methylated DNA copy number ratio [mC/(mC+C)]. The suggested measurement methods are high resolution melting (HRM), dPCR, and NGS following bisulfite treatment.

Bacterial identification and quantification

Bacterial plant pathogens are commercially relevant, as they can have devastating socioeconomic consequences and also negatively influence biodiversity. Most jurisdictions have import restrictions to prevent the spread of plant pathogens, but there is a lack of reference materials and reference methods

for bacterial plant pathogens. Additionally, another type of bacteria, Mycoplasma, is a common cell culture contaminant which can have a negative impact on biomedical research, biotechnology and biopharmaceutical production. Additional clinically important bacterial targets may also be investigated as part of this study series. NAWG participants will measure genomic copy number concentrations of bacteria. As this study builds on the previous DNA copy number quantification studies P154, P184, and the K86 series, it will expand NAWG measurement capabilities to bacterial gene quantification. These activities will likely include cross working group comparisons with the CAWG, in which quantification of whole bacteria cells are included.

Future challenges, longer term plans (2025-2030):

The nucleic acid analysis field is fast moving in terms of technological development and its application. The last decade has seen a transformation in massively parallel sequencing and PCR-based nucleic acid quantification, with an increasing application of these approaches in applied settings. Longer term NAWG priorities will need to reflect this dynamic analytical space - although the similarity in the associated analytical challenges between sectors will enable the NMI community to be well positioned to respond to this. It is likely that the NAWG will need to expand its understanding of matrix effects on analysis, moving from the leading work in the foods space (K86 series) to more complex genomic examinations (such as sequencing) as well as other biological matrices (such as clinical and environmental samples).

To ensure the NMI community is ready for the next priority, pilot studies that allow NAWG members to develop capacity in new areas will be important. This may be the case whether there is an increase in the use of sequencing in foods and feed analysis, epigenetic IVD solutions, the need to identify CRISPR modifications, etc. A wider application of CRISPR, and other gene editing technologies, may require NMIs and DIs to develop analytical tools from capabilities that enable reading the genome to understanding how to write it.

Five broad areas predicted to form the subject of key comparisons and/or pilot studies (Figure 5) over the next ten years include:

1) Continuation of the P199 series

Viruses represent the simplest biological entity capable of replication (albeit dependent on a host cell) and the NAWG had initiated studies investigating viral genome measurements prior to the COVID-19 pandemic. As a result, the NAWG was able to provide members with the ability to develop capacity and demonstrate their capabilities for measuring SARS-CoV-2 quickly, with several NAWG members supporting the value assignment of SARS-CoV-2 external quality control materials and the WHO international standard. It is likely that more key comparisons and pilot studies will be needed in this space to support capabilities, both in terms of different viruses/gene target measurements of nucleic acids in aqueous solution, as well as for whole viral materials (in cases in biological matrices). This will be needed for IVD associated measurements as well as potentially for the characterization of virus and virus like particles for other uses (such as gene delivery of vaccines).

2) SI traceability, including matrix materials

The last decade has seen the NMI community demonstrate SI traceable DNA measurement is possible using chemical analysis of pure materials in high concentration or, when using digital PCR, more complex mixed samples capable at very low concentrations using enumeration. The P199 series is addressing this this for RNA and the NAWG will continue to explore this space with key comparisons to broaden the scope of measurement, both in terms of sequence type and complexity. Reference measurement systems that

include methods, as well materials, to support traceability may become increasingly important to support matrix materials, or direct clinical measurements, as more nucleic acid targets are measured. How reference measurement systems can support precise and multiple quantitative nucleic acid measurements will need to be explored for this to become a reality.

3) Nucleic Acid Characterization

Nucleic acid reference materials can differ in their composition and homogeneity in terms of sequence, fragment size and, where appropriate, matrix. This can impact on how a material may perform and characterization of these will metrics enable a better understanding of a given measurement. NAWG will explore the measurement of these characteristics using different sizing and sequencing methods to develop and compare capacity amongst NAWG members.

4) Broad scope measurement capabilities

An important aspect of the NAWG activities will be to ensure comparisons enable capabilities with broad application. While the ubiquity of essentially the same nucleic acids across life sciences lend themselves to broader scope claims, the NAWG will need to demonstrate they are appropriate. Examples might be how capabilities demonstrated in the K86d participation, which will deal with high protein food matrices, might be applied to a clinical biopsy or how many different viral pathogen genomes will need to be evaluated to claim broader capabilities in viral measurement.

5) Non-targeted NA analysis

With the development of increasingly advance sequencing technologies a new paradigm in nucleic analysis has emerged. Instead of applied analytical approaches measuring a specific target, or targets, nucleic acid analysis is increasingly capitalizing on the ability of methods to examine what is present in an untargeted approach; such as when exploring the presence of a potential undefined change that may confer resistance to a cancer therapy or antimicrobial or unique genetic modification. As these approaches become increasingly applied it is likely the NMI community will need to consider supporting their accuracy and the NAWG will commit time to considering this challenge as needed.

5.2. IMPROVING STAKEHOLDER INVOLVEMENT

The main stakeholders of NAWG are:

- NMIs and DIs
- The food and feed industry and regulators
- Laboratory medicine sector
- Industry: IVD industry, biopharmaceutical manufacturers, pharmaceutical/advanced therapy medicinal products (including gene therapy), IVD instrument manufacturers, veterinary diagnostic manufacturers
- Academia: universities, regional and international (EMBL) research institutes
- International Organizations: ISO, IFCC, JCTLM, WHO, SOGAT, ESCMID
- Public stakeholders: regulators, governmental laboratories, reference laboratories, inspection agency for IVD and biopharmaceutical registration, third-party testing agency

Due to the fact that the application of metrological concepts is relatively new to nucleic acid analysis, the NAWG is not as well established amongst stakeholders as other CCQM working groups or those from other CCs. However, NAWG members have made considerable efforts to disseminate NAWG's work, to transfer knowledge of NMI/DI activities, and to solicit feedback, both independently, regionally and as part of NAWG.

Activities include

Workshops

> The NAWG held a workshop in April 2018 on "Digital PCR as a Reference Measurement Procedure". This workshop brought together NAWG members, technology manufacturers and clinicians.
>
> A series of workshops are planned for the 2021-2030 period with initial workshop ideas to explore the use of sequencing technologies to improve the characterization of molecular reference materials as well as how molecular reference measurement systems can be better applied to support specific sectors.

Webinars

> NAWG members frequently give webinars to disseminate NMI activities. With molecular methods offering the main testing method for SARS-CoV-2 infection the NAWG is a contributor to the CCQM webinar series on 'Ensuring the Reliability of Measurements in Response to the Covid-19 Pandemic.'

As well as the usual routes of scientific interaction (such as conference presentations, peer reviewed publications, etc.) NAWG members are also active participants in numerous committees and working groups involved in standardizing and applying nucleic acid analysis. Examples include:

The leader of the Nucleic Acid Review Team of the Joint Committee for Traceability in Laboratory Medicine (JCTLM) is also a member of the NAWG. The JCTLM maintains a database of higher metrological order reference methods, materials and services. The cited nucleic acid reference measurement procedures have all built on regional and international work from NAWG members some of which benefited from NAWG led activities. With the successes of NMIs and DIs over the last ten years in developing capacity/knowledge and the resultant outputs it is likely that activities will continue to feed into the JCTLM. JCTLM Working Group on Traceability: Education and Promotion (JCTLM-TEPWG) considers nucleic acids measurements to be a rare area in laboratory medicine, where routes to explore how SI units can be better applied to afford standardization of clinical tests results.

NAWG members are active participants in several ISO working groups including TC/276 Biotechnology and ISO TC/212 Clinical diagnostic testing and in vitro diagnostic test systems. Through these activities NAWG members have been instrumental in ensuring nucleic acid metrology is considered in several recent documents including ISO 20395:2019 (Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR) and ISO 17511:2020 (In vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples). NAWG members are also part of the drafting committee of the 276/212 joint working group 6 "Quality Practice for detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by nucleic acid amplification methods."

NAWG members participate in SoGAT (The international working group on Standardization of Genomic Amplification Techniques) which supports the development and implementation of WHO reference materials. NAWG members are also increasing activities to drive knowledge transfer around nucleic acid analysis metrology with additional stakeholder committees/group such as IFCC (Committee for molecular

diagnosis) and the European Society for Clinical Microbiology and Infectious Diseases (Study Group on Genomic and Molecular Diagnostics).

5.3. PROMOTING GLOBAL COMPARABILITY

The strategic approach for selecting the NAWG comparisons continues to evolve due to the necessity of accommodating the full range and complexity of nucleic acid measurement challenges encountered by CCQM NMI/DIs in delivering services to their customers. The NAWG priorities in the latest member survey focus on medical diagnostic tests and food and feed analysis, followed by biotechnology, environmental analysis and gene doping.

NMI and DIs provide services, such as:

- CRM production and value assignment (ranging from nucleic acid variant and mutation detection, pathogen quantification to microbial metagenome sequencing);
- Digital PCR calibration services for proficiency testing, nucleic acid reference materials and external quality assessment (EQA) material value assignment;
- GMO detection, identification and quantification.

The NAWG provides support to member NMIs and DIs in performing these services.

The activities of the NAWG have followed on from those initiated in the Bioanalysis WG (BAWG). Within NAWG, two Track A and seven Track D (pilot) studies were organized (Table 1 and 2). All studies were Model 1.

Table 1. Description of the types of studies that the NAWG performs.

	Objectives	**NAWG examples**	**Misc.**
Track A **(Key Comparison)**	To test the core competencies for measurement services delivered to customers, and cover the range of recognized measurement capabilities required to deliver reference measurement services in nucleic acid analysis	Fractional abundance of sequences associated with genetic modification	
Track C **(Key Comparison)**	To assess emerging areas of global interest and importance	None conducted in NAWG	

Track D **(Pilot Study)**	To assess capability for new techniques and areas	targeted gene expression, absolute quantification of DNA/RNA	Number of P studies relatively is high in NAWG, these studies are used to support CMCs

The NAWG Track A comparisons test the core competencies for measurement services delivered to customers and cover the range of recognized measurement capabilities which are required to deliver reference measurement services in nucleic acid analysis. Under a Track A comparison, successful demonstration of a competency in the comparison (e.g., relative quantification of DNA/RNA fragments copy number, fractional abundance of genetic variants) is considered to be broadly applicable to the measurement of a range of analytes in a variety of matrices, as described in the "How Far The Light Shines" (HFTLS) associated with the comparison, provided the same general measurement approach is used.

Table 2. NAWG Key Comparisons and Pilot Studies

	Description	Coordinating Laboratory	Start Date	Status (as of 03/2021)	Comments
CCQM-K86.b	Relative quantification of Bt63 in GM rice matrix sample	NIMC/GLHK	2015	Study complete. Report Draft A discussed & KCRV agreed October 2016. Draft B report agreed April 2017 submitted to KCDB following minor amendments requested on CCQM WG Chair review. Available on KCDB. Will be published in Metrologia.	Run in parallel to CCQM-P113.3
CCQM-K86.c	Relative quantification of genomic DNA fragments extracted from high oil matrix (OSR/canola)	NRC/IRMM	2016	Study complete. Preliminary results discussed April 2017.KCRV determined & Draft B report agreed. Approval for CMC review April 2018. Final report minor amendments in progress - prior to submission to KCDB.	Run in parallel to CCQM-P113.4
CCQM-	Relative	NIMC/GLHK	2015	Study complete	Run in parallel with

P113.3	quantification of Bt63 in GM rice matrix sample				CCQM-K86.b
CCQM-P113.4	Relative quantification of genomic DNA fragments extracted from high oil matrix (OSR/canola)	NRC/IRMM	2016	Study complete	Run in parallel with CCQM-K86.c
CCQM-P154	Absolute quantification of DNA	KRISS(NMIA, IRMM, NML at LGC)	2013	Study complete. Report submitted to KCDB. Published Anal Chem.	Clearly defined scope to measurement claim - supporting studies may be required for some CMCs
CCQM-P155	Multiple cancer cell biomarker measurement	NML at LGC	2014	Study complete. Final report agreed and published on CCQM NAWG website. Scientific publication in preparation.	Reporting delays due to CIM participation issues discussed and resolved April 2017. Final report approved Oct 2017 (WIKI available). Publication in progress
CCQM-P184	Copy number concentration and fractional abundance of a mutation (SNV or INDEL) mixed with WT DNA	NMIA/NML at LGC	2017	Study complete. Core competence in nucleic acid mutation measurement.	Preliminary report discussed NAWG April 2018, final report will be circulated for discussion at meeting Apr 2019
CCQM-P199	Copy number concentration of HIV-1 RNA genomic sequences	NML at LGC/(NIBSC)	2018	Draft report compiled due for circulation	While additional analysis has been applied to investigate findings the following study P199.b has delayed drafting of P199

CCQM-P199b	Copy number concentration of SARS-CoV-2 RNA genomic sequences	NML at LGC/NIMC/NIBSC/NIST	2020	Draft report circulated amongst NAWG	BIPM press release of findings circulated in December 2020.

The NAWG's strategic plan for future Key comparisons and pilot studies is based on types of analyses provided in services by NMIs/DIs and more specifically informed by a participant survey to prioritize NA measurement study areas required to support services. The NAWG strategy prioritizes:

- Quantification of nucleic acids, including DNA and RNA. Specific measurands include absolute copy number concentrations and fraction abundances, within and between different samples. Applications include GMO determination, gene expression and quantification of genes: including single nucleotide variants.
- Nucleic acid sequencing (massively paralleled generation sequencing and Sanger sequencing). There is a need for standardization in this area. Sequencing is increasingly used for identity examinations, metagenomics and sequence purity analysis, including purity determination for reference materials.
- Identification and quantification of epigenetic differences, particularly methylation.

In the short to medium term it is expected that these services will be extended, with an emerging trend towards the use of nucleic acid sequencing.

5.4. INTERACTION WITH RMO ACTIVITIES

APMP: The bioanalysis area is part of the APMP-TCQM in which there have not been any studies that are directly relevant to NAWG activities. The most relevant APMP study is APMP QM-P35 Quantification of *E. coli* in drinking water (in progress). In addition, quantification of other microorganisms in food has been considered and proposed to the APMP members. However, the methodology considered in these studies are culture-based, which is not a nucleic acid measurement.

COOMET: The Bioanalysis subcommittee was established in the COOMET Technical Committee 1.8 "Physic-Chemistry'' in 2019. In this meeting, the importance of genetic technologies and reference material development to ensure the traceability of bioanalytical measurements was noted. The first TC 1.8/SC 6 meeting is expected to be held in 2021 and its activity will be coordinated with the NAWG. One new COOMET topic is relevant to nucleic acids: a pilot comparison on quantitative determination of human DNA, coordinated by VNIIM.

EURAMET: European NMIs and DIs work closely together with stakeholders through EMRP (European Metrology Research Programme) and EMPIR (European Metrology Programme for Innovation and Research). Notable projects with relevance to nucleic acid analysis include Infect-Met, AntiMicroResist, BioSITrace and SEPTIMET. Furthermore, EURAMET's Metrology Network for Traceability in Laboratory Medicine (EMN-TLM) brings stakeholders such as proficiency testing providers, *in vitro* diagnostics

manufacturers, regulators, and NMIs/DIs together to address stakeholder needs, which is especially important in the light of the new European IVD regulation (EU regulation 2017/745 and 2017/746). Since European NAWG members are also members of this network, they contribute to the strategy by reporting the stakeholder needs to the NAWG strategy.

- ANNEX

1. GENERAL INFORMATION

General information and terms of reference in format used for 2017-2026 strategy
(Headings from previous template)

CC Name: CCQM
CC Working Group: Nucleic Acid Analysis Working group (NAWG)
Date Established: October 2015
Number of Members: 25 NMIs and DIs
Number of Participants at last meeting: 53
Periodicity between Meetings: 6 months
Date of last meeting: November 2020
CC WG Chair (Name, Institute, and years in post): Jim Huggett, LGC UK, 1.5 years
Number of Nucleic Acid (NA) measurement KCs organized (from 1999 up to and including 2020): 4 (2 NAWG, 2 BAWG)
Number of Pilot studies organized (from 1999 up to and including 2020): 19 (6 NAWG, 13 BAWG)
Number of CMCs published in KCDB supported by CC body activities (up to and including 2020): 21 NIMC (3), GLHK (3), CENAM (1), VNIIM (1), NIB (3), TUBITAK UME (4), NML at LGC (6)

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

To carry out Key Comparisons and pilot studies, to critically evaluate and benchmark NMI/DI claimed competences for measurement standards and capabilities for nucleic acid (NA) analysis. This includes, but is not limited to, the analysis of chromosomes, DNA, nucleotides, oligonucleotides, modified DNA (e.g. DNA methylation and other epigenetic modifications), mRNA, miRNA (and other non-coding RNAs) in a biological measurement context. The measurements in the studies include, but are not limited to, the identification and quantification of nucleic acids in complex matrices (such as those derived from plant, animal and microbial origins).

2. LIST OF PLANNED KEY AND SUPPLEMENTARY COMPARISONS AND PILOT STUDIES

Excel sheet link

3. SUMMARY OF WORK ACCOMPLISHED AND IMPACT ACHIEVED (2017-2020)

PROGRESSING METROLOGY SCIENCE

Case studies:

I. Absolute SI –Traceable Quantification of DNA

In plants, animals, bacteria and some viruses, DNA carries most of the basic inherited information that shapes the organism. Since the discovery of chemical and physical characteristics of DNA, the precise and accurate quantification of a specific DNA molecule has been intensely sought. Since the early 2000s, the CCQM NAWG (and BAWG prior to 2015) has worked to develop the SI-traceable methodologies for absolute DNA quantification.

The CCQM P154 study demonstrated NAWG members' competency in calibration-free enumeration-based DNA quantification methods. The study was co-led by KRISS, NMIA, IRMM, and NML at LGC. There were 11 participating NMIs and DIs. Unlike previous studies which used relative quantification using quantitative polymerase chain reaction (qPCR), participating laboratories utilized enumeration-based quantification in order to achieve direct absolute quantification of DNA. This enables reliable value assignments of DNA reference materials, and International System of Units (SI) traceability to copy number unit 1 through counting.

In this study, two enumeration-based methods, direct flow cytometric (FC) counting and digital polymerase chain reaction (dPCR), were used to quantify the pBR322 plasmid copy number in a solution at a concentration of several thousand copies per microliter. Unlike DNA measurement methods based on chemical analysis such as mass spectrometry or capillary electrophoresis, direct flow cytometric (FC) counting offers simplicity once the system is set up and optimized. The counting of DNA molecules in a flow stream is independent of extra calibration or added internal standards. Double stranded DNA molecules are stained with a fluorescent intercalating dye and then counted by laser-induced fluorescence detection in capillary flow in a self-instrumented system (Lim et al. 2009). Key achievements in this technology include electrohydrodynamic focusing of DNA molecules at the center of the flow cross-section, optical adjustment for improving signal to noise ratio, self-controlled optical alignment, and exhaustive counting. This strategy applied with precise determination of sample volume allows calibration-free quantification of DNA copy number concentration. Using this optimized enumeration instrument, copy number concentration of target DNA molecules can be readily determined without any biochemical amplification steps.

Laboratories participating in P154 used a variety of methods (Yoo et al. 2016) including FC-based direct counting, digital PCR, and two chemical-analysis methods, which are based on nucleotide quantification: isotope-dilution mass spectrometry (IDMS) and capillary electrophoresis (CE). Nine dPCR results from eight laboratories showed some dispersion where relative standard deviation [RSD] was 11.8%. However the means from digital PCR results were comparable with those of the FC counting method and the chemical analysis methods, corrected for gravimetric dilution factors. When the average value of dPCR

results was compared to the other methods, the RSD of all four methods was 1.8%. This strongly suggested these enumeration methods are valid in absolute quantification of DNA.

II. Towards SI traceable RNA measurement

Ribonucleic acid (RNA) is the other analyte that NAWG is tasked with measuring. RNA provides the genomic sequence for many viruses, is the intermediary molecule used to translate the genetic code into protein sequences, and also provides a range of other structural (ribosomal RNA), regulatory (micro RNAs) and even enzymatic functions (Ribozymes). As DNA, RNA molecules are comprised of a variation of the order of four monomers, allowing similar methods to be applied to different types. Challenges specifically associated with RNA include the fact that it is usually single stranded and can form secondary structures through complementary binding or where molecules like micro RNAs are very small making them difficult to measure using conventional molecular methods. RNA is also difficult to measure due to the fact that most of the methods used in molecular biology measure DNA. Consequently reverse transcription is required to convert RNA into complementary DNA (cDNA).

Over the last decade, the NAWG (and BAWG beforehand) has dedicated considerable effort to ensure the development of RNA measurement capability was also at the forefront of the strategic focus. CCQM P103 (Quantification of an RNA transcript) was the first pilot study to explore the measurement of RNA; it investigated the measurement of an RNA molecule (from the External RNA Controls Consortium [ERCC]). Two unknown concentrations of this RNA molecule were provided to laboratories, along with a calibrator to allow laboratories to perform reverse transcription quantitative PCR. The calibrator was value assigned using UV spectrophotometry with factors such as potential non-target fragments considered in the uncertainty. 11 NMIs/DIs participated in the pilot with majority using RT-qPCR and one applying RT-dPCR (corrected using the calibrator value). The pilot study concluded that most laboratories were able to accurately measure both concentrations of the RNA molecule in good agreement with the assigned value.

CCQM P103.1 (Measurement of multiple RNA transcripts) study was built upon the knowledge generated in P103 by including six ERCCs and three cellular RNA measurements using human RNA derived from cell lines (Devonshire et al. 2016). The use of multiple ERCCs demonstrated that absolute RNA measurement could be widely applied and the inclusion of different concentrations of ERCCs and cellular RNA allowed for ratio-based measurements in the range typically measured by stakeholders. This study saw an increased number of participant laboratories to 14, the majority of which conducted RT-qPCR, although two laboratories submitted RT-dPCR results and one laboratory conducted next generation sequencing (NGS). P103.1 demonstrated that participating laboratories were able to measure target RNA quantities and fractional abundance (ratios) in a complex (total RNA) background with good concordance. Additionally, the study highlighted the lack of a standardized approach for uncertainty calculations, especially when conducting fractional abundance quantification.

CCQM P155 (Multiple cancer cell biomarker measurement) expanded the earlier RNA pilot studies by incorporating whole cell materials for analysis. This necessitated a pre-processing step to purify the nucleic acid prior to molecular analysis and enabled participant laboratories to develop and compare their extraction capabilities. This also allowed the evaluation of the potential contribution of these initial steps to be considered when characterizing uncertainty both in terms of copy number concentration measurements as well as when measuring. CCQM-P155 demonstrated that participant laboratories were able to conduct measurements with good agreement when conducting both fractional abundance and absolute measurements, extending the findings of P103.1 to consider whole cell extracts.

The three preceding RNA studies dealt with both fractional abundance and absolute measurements and saw a serial increase with time in the adoption and application of digital PCR technologies for RNA quantification. The absolute measurement was supported with value assignment using spectrophotometry for the CCQM-P103 series and by RT dPCR for CCQM-P155. Neither approach, at the time, had been demonstrated to be suitable as an SI traceable method. Consequently an unmet need was identified to explore the potential for using higher accuracy SI traceable methods for RNA quantification. Following the success of CCQM P154, for the assessment of SI traceable measurement for DNA, the CCQM-P199 (HIV-1 RNA copy number quantification) was proposed followed by CCQM-P199b (SARS-CoV-2 copy number quantification) which was fast tracked in response to the COVID-19 pandemic. Both P199/P199.b studies demonstrated that NAWG members were capable of high accuracy measurement of RNA molecules in buffered solutions. The P199.b demonstrated SARS-CoV-2 quantification was possible with most laboratories submitting values with +/- 40% of the mean (BIPM 2020). The five RNA studies open the possibility of future key comparisons to demonstrate a wide range of CMCs that allow NMIs to support stakeholders in a variety of fields with future pilot studies needed to further understand challenges associated with matrices and other RNA types, like micro RNAs.

III. Case study III: GMO in Food

The planting of genetically modified (GM) crops has increased significantly since the 1990s. The commercial planting of GM crops contributes to global feed, fiber, food, and fuel production; however, the safety of GM crops and their products are important issues. National legislation regulating GM crop cultivation and its products varies significantly in different jurisdictions. A global reference measurement system, including reference materials and reference methods, must be established to facilitate international trade and to provide information to consumers on GM ingredients content. Nucleic acid analysis methods are commonly used for quantification of GM content in processed food and feed.

The NAWG works to support members in nucleic acid RM development and calibration services for relative GM crop quantity. This process includes DNA extraction from a complex biological matrix followed by measurement of two specific genomic DNA fragments - the GM specific sequence and the endogenous gene specific sequence. Measurements are performed by quantitative real-time PCR (qPCR) with an independent reference material as a calibrant or by digital PCR (dPCR), which is considered a direct SI-traceable method for nucleic acid measurements.

The CCQM K86.c key comparison “Relative quantification of genomic DNA fragments extracted from a biological tissue” was jointly coordinated by the National Research Council of Canada (NRC) and the EU Joint Research Centre, Geel (JRC) and had 12 NMI/DI participants: JRC Geel, NIMT, NMIA, TUBITAK, NMIJ, VNIIM, NML at LGC, INM, NIB, CENAM, GLHK and NIM China (Mester et al. 2020). This study was performed to demonstrate and document the capacity of NMIs/DIs to determine the relative quantity of two specific genomic DNA fragments present in canola powder. This study was a follow-up of previous comparisons - CCQM K86 and CCQM K86.b (Milavec et al. 2020).

Study participants had to perform extraction of genomic DNA followed by accurate detection and quantification of the relative amount of two defined DNA sequences in the extracted genomic DNA. The matrix in the previous CCQM K86 (13 NMI/DI participants) was a maize seed powder, whereas in CCQM

K86.b (9 NMI/DI participants), the matrix was a rice seed powder. Both these matrices are rich (between 75 and 80 g/100g) in polymeric carbohydrate (amylose and amylopectin) and poor in fat (<5 g/100g) requiring adapted DNA extraction methods to remove substances that can hinder the polymerase activity. In the K86.c study, the NAWG decided to challenge laboratories with a plant material with elevated oil/fat content, such as rapeseed. Despite the availability of validated DNA extraction and purification methods, in the frame of K86.c participants had to isolate DNA from the most difficult plant material matrix. CCQM K86.c and a concurrently run pilot study, CCQM P113.4, were conducted with materials provided by JRC Geel (T1) and NRC (T2).

In K86.c, DNA sequence quantification was performed using dPCR. This method requires extraction and purification of genomic DNA before the detection and quantification of the two defined DNA sequences. CCQM K86.c study participants used a wide range of DNA extraction approaches, including phenol-chloroform extraction, CTAB extraction and adsorption by silica and isolation with magnetic beads.

Participants in CCQM-K86.c were required to report the absolute copy number of sequence targets per µL for the T1 and T2 materials and the ratio of both sequence targets expressed in percent, along with the corresponding uncertainty. Almost all participants quantified the respective targets by simplex dPCR, though one participant used duplex dPCR. Considering the complexity of the analytical challenge, the degree of equivalence plots demonstrated a good level of agreement between the laboratories, especially for T1.

CCQM-K86.c supports measurement claims for the quantification of the ratio of the number of copies of specified intact sequence fragments of a length up to 150 nucleotides following extraction from an unprocessed, high fat/oil ground seed matrix, with a copy number ratio from 0.001 to 1.

The complexity of analyzing animal tissues, cultured cells or other similar samples represent a lower level of complexity than the plant based samples studied in K86.c, so this study can also be used as evidence of a general capability for DNA quantitation in those areas.

Another follow-up study, CCQM-K86.d/P113.5, is planned as the measurement space of the food matrix lacks measurements in high protein content matrix. This study utilizes pork matrix in beef matrix as a protein rich matrix model for genomic DNA quantification. In previous studies, raw materials have been studied; however, this study will focus on samples with degraded genomic DNA. K86.d will be used to investigate the DNA extraction process for comparability of measurements for processed food samples. In addition, in this comparison, both DNA copy number ratios and mass fractions will be measured, setting the basis for delivering measurement services on mass fraction (obligatory in some jurisdictions) by the use of a conversion factor.

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5. DOCUMENT REVISION SCHEDULE

Document name; type of revisions; date