**Report from the CCQM Nucleic Analysis Working Group for the period (April 2019 – 2020)**

During this period, the 9st CCQM-NAWG meeting was held at INRIM on 3rd and 4th of October 2019, attended by 22 delegates from 16 institutes and the 10th meeting took place virtually on 6th, 12th, 14th May 2020. Jim Huggett and Maxim Vonsky started their terms as Chair and Vice Chair respectively. On the 6th of May 2020 it was agreed that Megan Cleveland would be the Rapporteur until the October 2023. Under the new leadership, there is a drive to expand metrological considerations from where, in nucleic acid measurement, they are more established (such as food testing) into areas that would benefit from such approaches (e.g. molecular diagnostics and bio-manufacturing/synthetic biology). The NAWG will also aim to further establish capacity and confidence amongst NMIs who are newer to molecular methods thereby establishing the CCQMs global expertise and leadership in molecular measurement. The NAWG will also seek to increase impact via interaction with stakeholders by highlighting the potential role of molecular reference measurement systems for molecular measurement.

# Ongoing Key comparisons and pilot studies

# CCQM NAWG P184(NAWG) Copy number concentration and fractional abundance of a mutation (SNV or INDEL) mixed with wild-type DNA

The aim of CCQM P184 is to support traceable measurement of the copy number concentration and copy number concentration ratio (sometimes termed fractional abundance or variant allele frequency (VAF)) of a biologically-relevant gene mutations (SNV and INDEL) when present in a background of wild-type DNA. This will evaluate variability between laboratories in determining copy number concentration and fractional abundance and provide evidence for Calibration and Measurement Claims by participating laboratories. This study has been completed and the report was written over this period with some additional experiments performed to better understand some of the findings. The draft will be distributed to NAWG members in 2020.

1. **CCQM P199 (NAWG) HIV-1 RNA copy number quantification**

The aim of CCQM P199 is to support higher order measurement of RNA copy number concentration, in particular viral RNA quantification. Human Immunodeficiency Virus-1 (HIV-1) was chosen as the model for the study as it is major pathogen which is quantified clinically to guide treatment and monitor resistance, and is reported as an ‘absolute’ concentration (such as copies/mL plasma). The measurand for the study was the RNA copy number concentration of the *gag* gene (expressed in copies per µL (c/µL)), corresponding to a specific region of 1467 bases within the HIV-1 genome.

Applying the design of earlier study CCQM P154 (Absolute Quantification of DNA), P199 aimed to compare approaches based on molecular counting (digital PCR and single molecule flow cytometry) with more established chemical analysis methods traceable to the mol. The National Measurement Laboratory hosted at LGC (NML) coordinated the study with the support of NIBSC. To facilitate comparison of orthogonal methods, the coordinating laboratory prepared two materials by serial of a single stock of *in vitro* synthesised RNA, which were suitable for reverse transcription digital PCR (RT-dPCR) (Study Material 1 containing ~103 copies/ul) and chemical and flow cytometric methods (Study Material 2 containing ~109 copies/ul). A third Study Material containing purified HIV-1 viral genomic RNA was generated from viral stocks used to prepare the WHO 3rd and 4th International Standard (IS) for HIV-1 (NIBSC). Thirteen laboratories participated in the analysis of Study Material 1, the results of which were compared with the results of four laboratories performing orthogonal methods (HPLC-UV, ID-MS and single molecule flow cytometry) by extrapolating the results based on the gravimetric dilution used to prepare Study Material 1 (**Figure**). Inter-laboratory reproducibility (CV) of RT-dPCR results was 21%, which is impressive considering all laboratories developed assays independently and without calibration; and offers a significant improvement compared to diagnostic tests which offer vary quantitatively by an order of magnitude. The extrapolated values assigned to Material 2 using orthogonal techniques were higher on average compared to the RT-dPCR results, suggesting potential sources of negative bias affecting RT-dPCR and/or positive bias affecting orthogonal approaches (such as the sensitivity of ID-MS to nucleotide impurities). Follow-up work is ongoing to investigate these.

**Figure**: Comparison of CCQM P199 participant results for RT-dPCR (Study Material 1) and orthogonal techniques (Study Material 2). Key: participant results for Study Material 1 (◆); extrapolated results for Study Material 2 taking into account 106-fold dilution (◼). Grey dash line shows median of nominated results for Study Material 1. Orange dashed line shows median of extrapolated results for Study Material 2. Error bars reflect expanded uncertainty.

# CCQM NAWG P199.b: SARS-CoV-2 copy number quantification

CCQM NAWG P199b will expand on capabilities demonstrated in the P199 study (HIV-1 RNA copy number quantification) for **targeted RNA copy number concentration** and **viral gene quantification**. This pilot was fast tracked in response to the COVID-19 pandemic will build NMI knowledge and capabilities for also establishing candidate reference measurement procedures for detecting the *viral genome of the causative agent of* *the Covid-19 pandemic (2019-nCoV, termed as SARS-CoV-2).* This willexpedite the ability of NMIs/DIs to demonstrate capability for reference measurement of SARS-CoV-2 Reference Materials (RMs), Quality Control (QC) materials and sources of whole viral genome materials enabling them to support diagnostic manufacturers, clinical laboratory-developed tests and international test standardization efforts.

1. **Development of the NAWG for the 2020s**

Drs Cleveland (NIST), Milavec (NIB) and Bae (KRISS) have been drafting an update to the NAWG Process Guidance Document. Most sections of the document are complete, but the following sections needed further work:

1. Strategic approach for selecting NAWG comparisons
2. Preparing proposals for NAWG comparisons

Drs Huggett and Vonsky prepared a ‘NAWG member questionnaire 2020: Information gathering to support NAWG comparison study prioritization and design’ survey to priorities future activities. This was due to be distributed prior to and presented at the April 2020 meeting. This has been delayed and due to COVID-19 and will be presented in October 2020.

**Jim Huggett and Maxim Vonsky -05-2020**