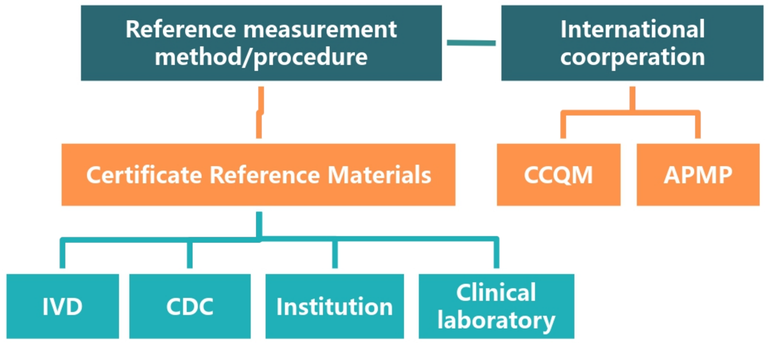
Best Practice of Biometrology to Support Nucleic Acids Detection of Infection Disease

Lianhua Dong, Chunyan Niu, Xia Wang, Yongzhuo Zhang, Yunhua Gao, Xinhua Dai*
Center for Advanced Measurement Science, National Institute of Metrology, Beijing 100029, China
Contact Email: donglh@nim.ac.cn, Tel: +86-10-64524959

Introduction

The worldwide spread of new pandemic diseases raise new chanlenge to biometrology. In response to the demand for metrology in the context of the SARS-CoV-2 epidemic, National Institute of Metrology (NIM), China has undertaken a series of initiatives in the field of infectious disease testing, ranging from cutting-edge research to practical application.



Series of initiatives in the field of infectious disease testing taken by NIM

Reference Measurement Procedures (RMPs)

Three reference measurement procedures (RMP) were developed and published at JCTLM specify the operating specifications for SARS-CoV-2 (open reading frame 1ab, *ORF1ab* and nucleocapsid protein, *N* and Envelope, *E* gene) RNA copy number concentration by reverse transcription digital PCR (RT-dPCR).

List of reference measurement methods / procedures listed in the JCTLM database

Analyte	Reference measurement method / procedure	Measurement principle / technique
SARS-CoV-2 RNA N gene	NIM Reference measurement procedure for SARS-CoV-2 "N" gene measurement JCTLM DB identifier C18RMP12	Digital PCR
SARS-CoV-2 RNA E gene	NIM Reference measurement procedure for SARS-CoV-2 "E" gene measurement JCTLM DB identifier C20RMP1	Digital PCR
SARS-CoV-2 RNA ORF1ab gene	NIM Reference measurement procedure for SARS-CoV-2 "ORF1ab" measurement JCTLM DB identifier C20RMP2	Digital PCR

Certified Reference Materials (CRMs)

Certified Reference Materials including *in-vitro* transcribed RNA, genomic RNA and Pseudoviral RNA of SARS-CoV-2 and different variants (GBW09298~GBW09300, GBW09310~GBW09318) have been developed and widely applied for practical application. GBW09298 and GBW09299 (also known as GBW(E) 091089 and GBW(E) 091090) have been listed as available higher-order certified reference materials in the JCTLM database.

List of Certified Reference Materials (CRMs) for SARS-CoV-2 RNA

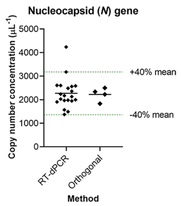
Code	Name
GBW09298~ GBW09299	Reference Material for In-Vitro Transcribed RNA of SARS-CoV-2
GBW09300	Reference Material for Pseudoviral RNA of SARS-CoV-2
GBW09310~ GBW09314	Reference Material for SARS-CoV-2 Subgenomic Nucleocapsid and Envelope RNA
GBW09315	Reference Material for SARS-CoV-2 Beta B.1.351 Variant Genomic RNA
GBW09316	Reference Material for SARS-CoV-2 Delta B.1.617 Variant Genomic RNA
GBW09317	Reference Material for SARS-CoV-2 Gamma P.1 Variant Genomic RNA
GBW09318	Reference Material for SARS-CoV-2 Omicron BA.1.1 Variant Genomic RNA
GBW(E)091098~ GBW(E)091099	Reference Material for for SARS-CoV-2 Genomic RNA

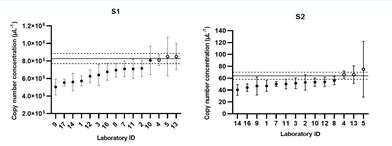
International Coorperations

CCQM Activities

To investigate the international comparability in nucleic acid measurement and supporting CMC claim, NIM successfully coordinated two rounds' comparisons for SARS-CoV-2 (CCQM-P199B and CCQM-K181) with LGC, NIBSC and NIST.

CCQM-P199B:
Results from part of the study demonstrating that laboratories performing molecular RT-dPCR methods are able to perform with good agreement with each other and the SI-traceable orthogonal methods (isotope-dilution mass spectrometry and single molecule flow cytometry), the latter providing the basis for the RT-dPCR method's claimed accuracy. The RT-dPCR results were compared with orthogonal methods using different concentrations of the same RNA material. The different concentrations were prepared using gravimetric dilution. Diamonds show individual laboratories' results with solid black line (mean) and dotted green lines +/-40% of mean value. (https://www.bipm.org/)

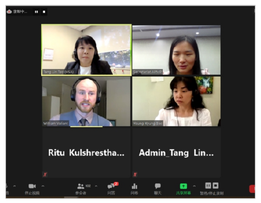




CCQM-K181: Study Material 1 and 2 consensus values, reported results and uncertainties.
Solid line shows the recommended consensus value and dotted line represents its expanded uncertainty. Participants results are displayed as values (circles) and expanded uncertainty (k=2). Open circles represent RT-dPCR corrected for the suspected bias observed between RT-dPCR and IDMS results.

APMP Activities

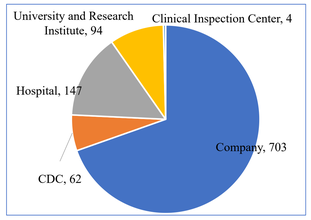
To support other NMIs in APMP region to establish the capability in measurement of SARS-CoV-2, an international training course of "Accurate Measurement of SARS-CoV-2 and Development of Nucleic Acids Reference Material" was organized by NIM incorporated with HSA, KRISS and NIST.

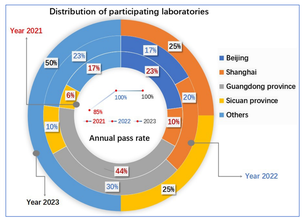


Applications of CRMs in China

Application in supporting tracebility and Proficiency Testing

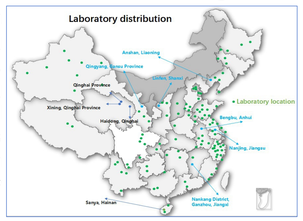
These CRMs have been used by more than **1,000** institutes in China. Three rounds of proficiency testing for SARS-CoV-2 RNA and its different variants were conducted to enhance the capacity and guarantee the consistency of testing facilities in China from 2021 to 2023. The correct rate has raised from 85% to 100%.





Supports for monitoring of SARS-CoV-2 in urban sewage

In 2023-2024, NIM support China CDC in the ongoing monitoring SARS-CoV-2 in urban sewage. This support includes providing simulated samples of pseudoviral virus to **162** paticipants, as well as enriching the virus in urban sewage and employing highly accurate measurement methods.



Conclusion

Reference measurement methods/procedures have been established in the field of infectious disease detection, whose reliability can be insured by international comparisons. Certified Reference Materials (CRMs) have been developed to support the traceability demands of stakeholders. The whole framework can be used as the best practice of biometrology to support nucleic acids detection of infection disease and get prepared for future epidemic.

References

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