

CCQM P199b SARSCoV2 RNA Copy Number Quantification: a Pilot Under Pressure

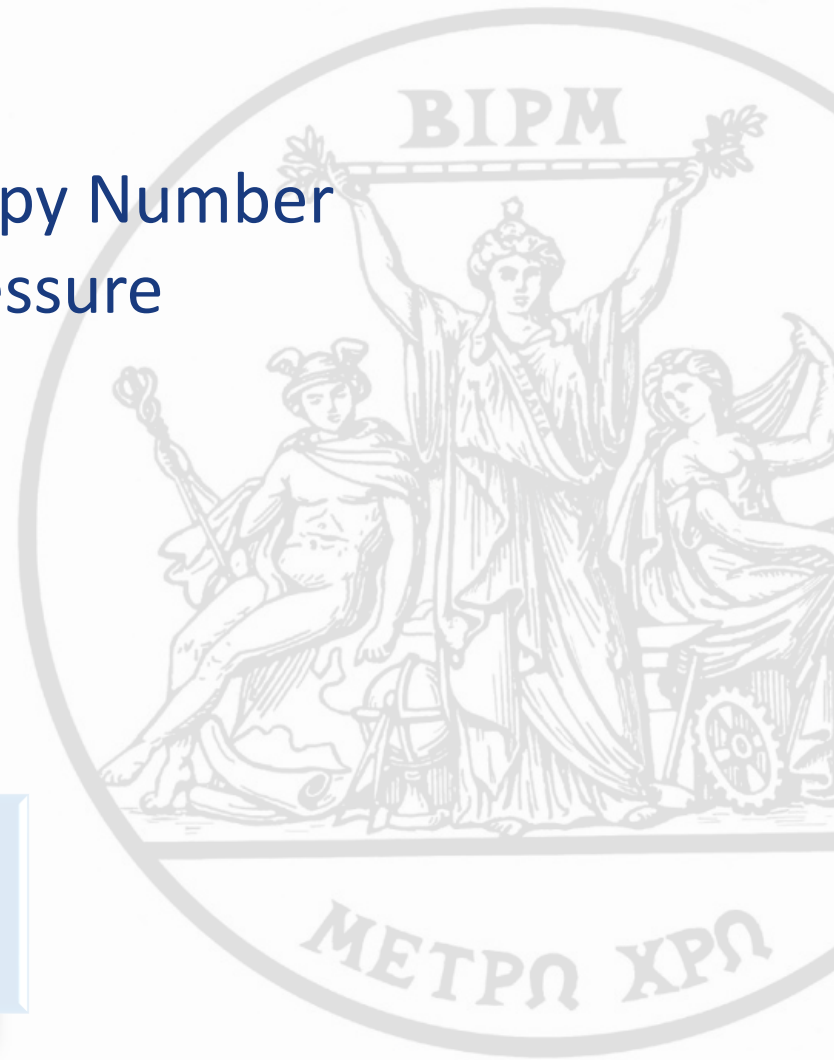
4th November 2021

Jim Huggett

Chair: CCQM Nucleic Acid
Analysis Working group

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International des
Poids et
Mesures

 **CCQM**
NUCLEIC ACID
WORKING GROUP



New CCQM - WG Leadership 12th April 2019-2023

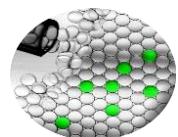
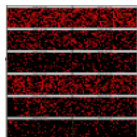
President: S-R. Park, CIPM

Executive Secretary: R. Wielgosz, BIPM

Working Groups :

	WG Chair		Deputy Chair	
– Organic Analysis (OAWG)	L. Mackay	NMIA	K. Lippa	NIST
– Gas Analysis (GAWG)	P. Brewer	NPL	S. Lee	KRISS
– Inorganic Analysis (IAWG)	M. Winchester	NIST	P. Fisicaro	LNE
– Electrochemical Analysis (EAWG)	S. Seitz	PTB	T. Asakai	NMIJ
– Cellular Analysis (CAWG)	J. Campbell	LGC	Boqiang Fu	NIM
– Protein Analysis (PAWG)	J. Melanson	NRC	C. Swart	PTB
– Nucleic Acids Analysis (NAWG)	J. Huggett	LGC	M. Vonsky	VNIIM
– Surface Analysis (SAWG)	T. Fujimoto	NMIJ	A. Shard	NPL
– Isotope Ratio Metrology (IRWG)	Z. Mester	NRC	J. Vogl	BAM
– <i>Key Comparison and CMC Quality (KCWG)</i>	<i>W.M.(Della) Sin</i>	<i>GLHK</i>	<i>A. Botha</i>	<i>NMISA</i>
– <i>Strategic Planning (SPWG)</i>	<i>S-R. Park</i>	<i>CIPM</i>		
– <i>ad hoc working group on the mole</i>	<i>B. Guettler,</i>	<i>PTB</i>		

Reference Measurement Procedures for MDx



- Demonstration of nucleic acid copy enumeration by digital PCR as a reference measurement procedure through CCQM NAWG comparisons
- Established feasibility metrological traceability (to unit 1) for nucleic acid copy enumeration
- Clarification included in 9th SI brochure
- Significant stakeholder engagement & influence on technology manufacturers

An International Comparison of Enumeration-based Quantification of DNA Copy-concentration Using Flow Cytometric Counting and Digital Polymerase Chain Reaction

Heebong Yoo, Sang-Ryoul Park, Lianhua Dong, Jing Wang, Zhiwei Sui, Jernej Pavšič, Mojca Milavec, Muslim Akgoz, Erkan Moziolu, Philippe Corbisier, Janka Matrai, Bruno Cosme, Janaina Japlassu da Vasconcelos Cavalcante, Roberto Becht Flatschart, Daniel Garrard Burke, Michael Forbes-Smith, Jacob L. H. McLaughlin, Kerry R. Emslie, Alexandra S. Whale, Jim Francis Huggett, Helen Parkes, Margaret Kline, Jo Lynne Harenza, and Peter M Vallone

Anal. Chem., Just Accepted Manuscript • DOI: 10.1021/acs.analchem.6b03076 • Publication Date (Web): 10 Nov 2016
Downloaded from <http://pubs.acs.org> on November 18, 2016

Draft of the ninth SI Brochure, 5 February 2018 • 19

There are also some quantities that cannot be described in terms of the seven base quantities of the SI, but have the nature of a count. Examples are a number of molecules, a number of cellular or biomolecular entities (for example copies of a particular nucleic acid sequence), or degeneracy in quantum mechanics. Counting quantities are also quantities with the associated unit one.

The unit one is the neutral element of any system of units – necessarily and present automatically. There is no requirement to introduce it formally by decision. Therefore, a formal traceability to the SI can be established through appropriate, validated measurement procedures.



Assessment of Digital PCR as a Primary Reference Measurement Procedure to Support Advances in Precision Medicine

Alexandra S. Whale,^{1*} Gerwyn M. Jones,^{1*} Jernej Pavšič,^{2,3*} Tanja Dreo,² Nicholas Redshaw,¹ Sema Akyürek,⁴ Mislav Akgöz,⁵ Carla Oliveira,⁶ Maria Paola Sassi,⁷ Hua-Jun He,⁸ Kenneth D. Cole,⁹ Young-Kyung Bae,⁷ Sang-Ryoul Park,⁷ Liesbet Deprez,⁸ Philippe Corbisier,⁸ Sonia Garrigou,⁹ Valérie Taly,⁹ Raquel Larios,¹⁰ Simon Cowen,¹¹ Denise M. O'Sullivan,¹ Claire A. Bushell,¹ Heidi Goenaga-Infante,¹⁰ Carole A. Foy,¹ Alison J. Woolford,¹ Helen Parkes,¹ Jim F. Huggett,^{1,12,13*} and Alison S. Devonshire^{1,14*}

BACKGROUND: Genetic testing of tumor tissue and circulating cell-free DNA for somatic variants guides patient treatment of many cancers. Such measurements will be fundamental in the future support of precision medicine. However, there are currently no primary reference measurement procedures available for nucleic acid quantification that would support translation of tests for circulating tumor DNA into routine use.

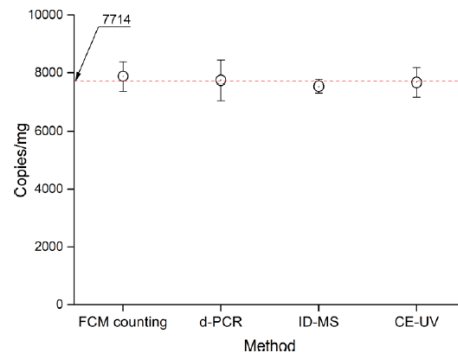
METHODS: We assessed the accuracy of digital PCR (dPCR) for copy number quantification of a reference sequence.

CONCLUSIONS: This work validates dPCR as an SI-traceable reference measurement procedure based on enumeration and demonstrates how it can be applied for assignment of copy number concentration and fractional abundance values to DNA reference materials in an aqueous solution. High-accuracy measurements using dPCR will support the implementation and traceable standardization of molecular diagnostic procedures needed for advancements in precision medicine.

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International Comparison of Enumeration-Based Quantification of DNA Copy-Concentration Using Flow Cytometric Counting and Digital Polymerase Chain Reaction

Hee-Bong Yoo,^{†,‡} Sang-Ryoul Park,^{*,†,‡} Lianhua Dong,[§] Jing Wang,[§] Zhiwei Sui,[§] Jernej Pavšič,^{||} Mojca Milavec,^{||} Muslum Akgoz,[†] Erkan Mozioglu,[†] Philippe Corbisier,[#] Mátrai Janka,[#] Bruno Cosme,[▽] Janaina J. de V. Cavalcante,[▽] Roberto Becht Flatshart,[▽] Daniel Burke,[○] Michael Forbes-Smith,[○] Jacob McLaughlin,[○] Kerry Emslie,^{*,○} Alexandra S. Whale,[◆] Jim F. Huggett,[◆] Helen Parkes,[◆] Margaret C. Kline,[¶] Jo Lynne Harenza,[¶] and Peter M. Vallone[¶]

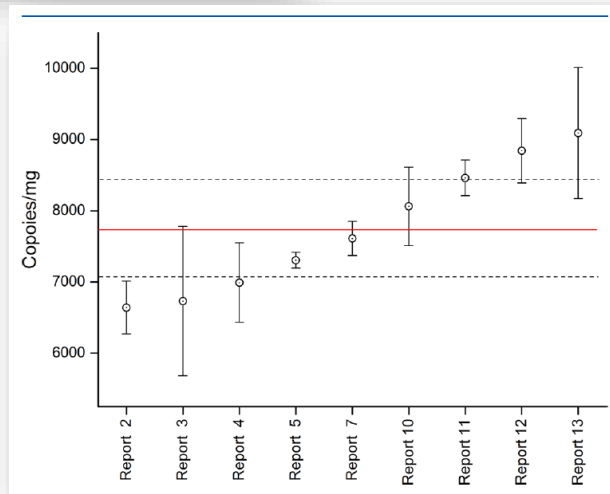
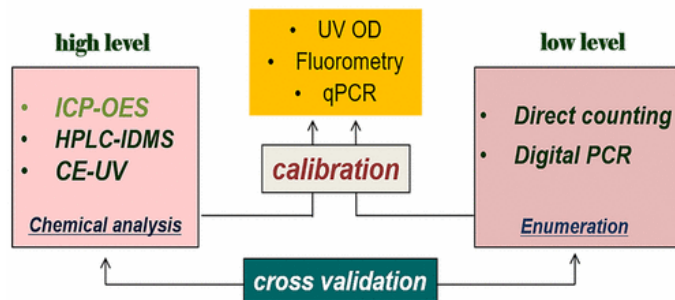


CCQM Nucleic Analysis WG

CCQM-P154: Absolute Quantification of DNA

Standards for DNA Quantification

Bureau
International des
Poids et
Mesures



KRAS G12D DNA SNP

Clinical Chemistry 64:9
1296–1307 (2018)

Special Report



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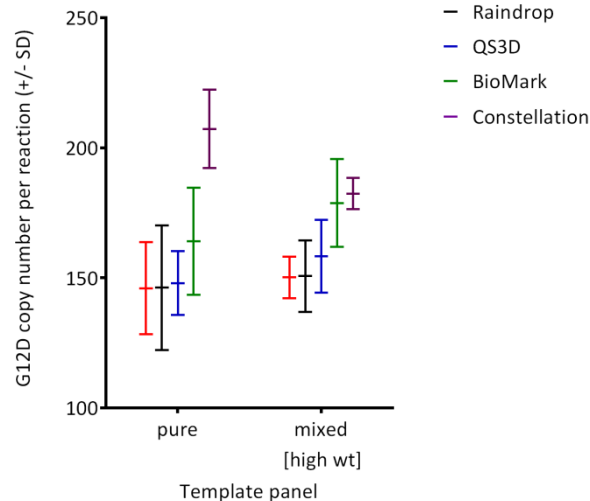
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G12D [low]



JCTLM database: Laboratory medicine and *in vitro* diagnostics

OPEN CALL FOR NOMINATIONS

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- Leaflet

Analyte keyword search for reference materials, measurement methods/procedures and services

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OPEN CALL FOR
NOMINATIONS

Analyte keyword search for reference materials, measurement
methods/procedures and services

Results of the search

Your search criteria produced 1 result.

For more information on a reference measurement method/procedure (or Material)/Measurement principle (or technique) combination, select options below.

Select all items from the list

Sort by : ☒ Analyte ☐ Measurement principle/technique ☐ Matrix/Material

Select	Analyte	Measurement principle/technique	Matrix/Material
<input type="checkbox"/>	KRAS DNA wild type sequence and gene mutation	Digital PCR	calibration solution

Deselect all items from the list

View

Modify your search criteria



Results of the search

Your search criteria produced 1 result.

For more information on a reference measurement method/procedure (or Material)/Measurement principle (or technique) combination, select options below.

Select all items from the list

Sort by : ☒ Analyte ☐ Measurement principle/technique ☐ Matrix/Material

Select	Analyte	Measurement principle/technique	Matrix/Material
<input type="checkbox"/>	Human cytomegalovirus (HCMV) DNA sequence	Digital PCR	other

Deselect all items from the list

View

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Select an analyte category

Nucleic acids Download

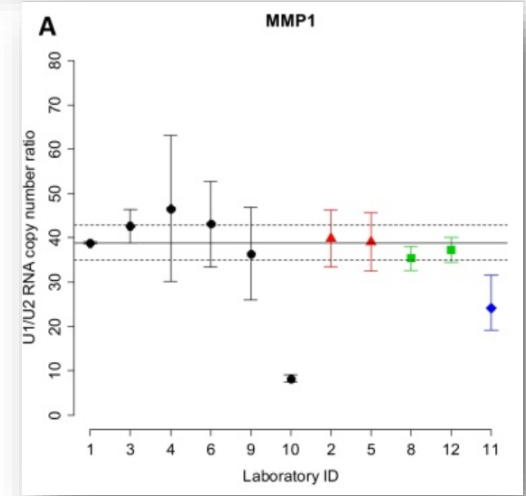
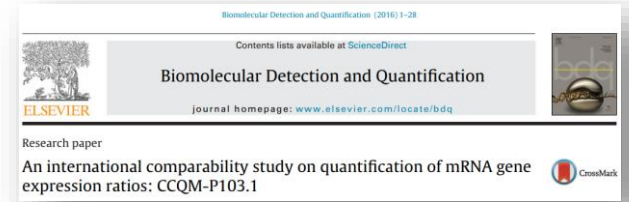
Select a matrix category

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Reference measurement systems. RNA measurement

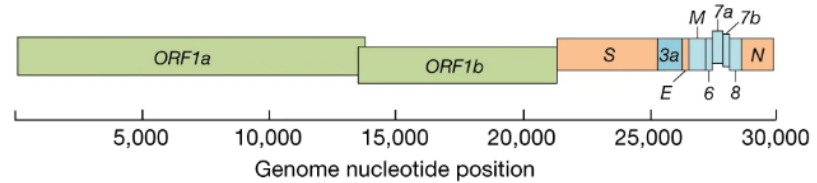
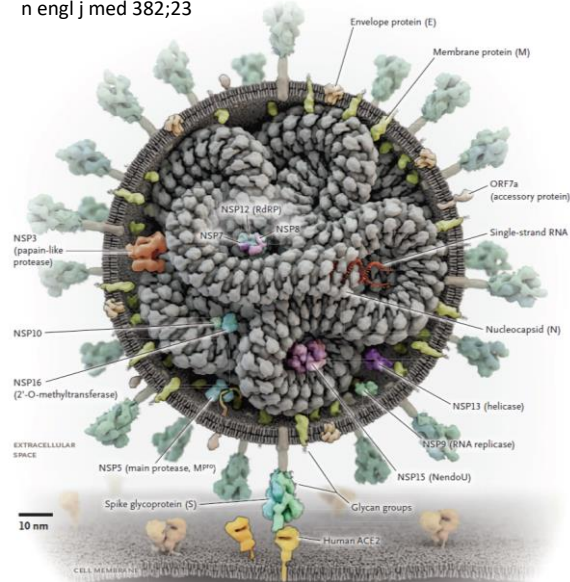
CCQM-P103 & CCQM-P103.1. Measurement of multiplexed biomarker panel of RNA transcripts

CCQM-P155. Multiple cancer cell biomarker measurement



Why should this matter for SARS-CoV-2 diagnosis?

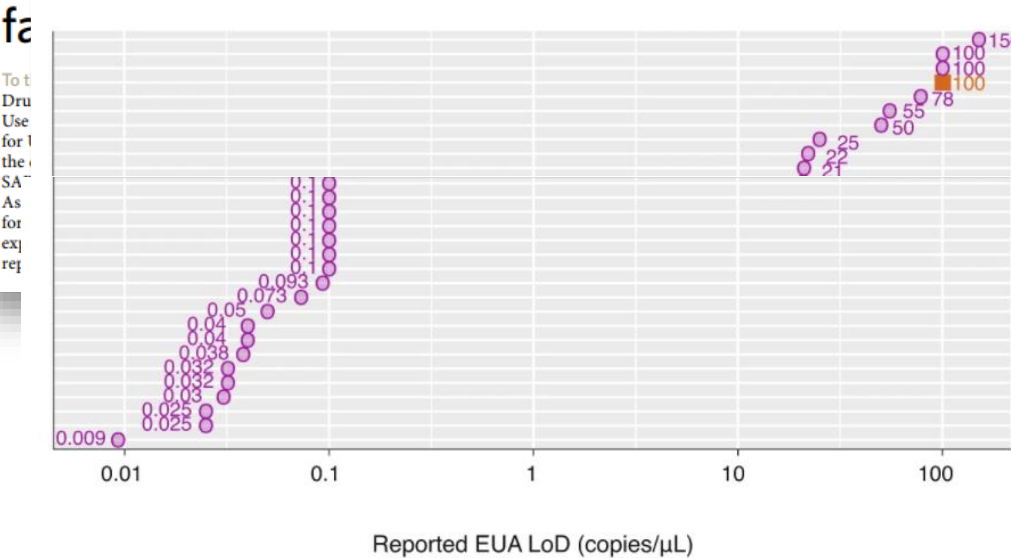
n engl j med 382;23



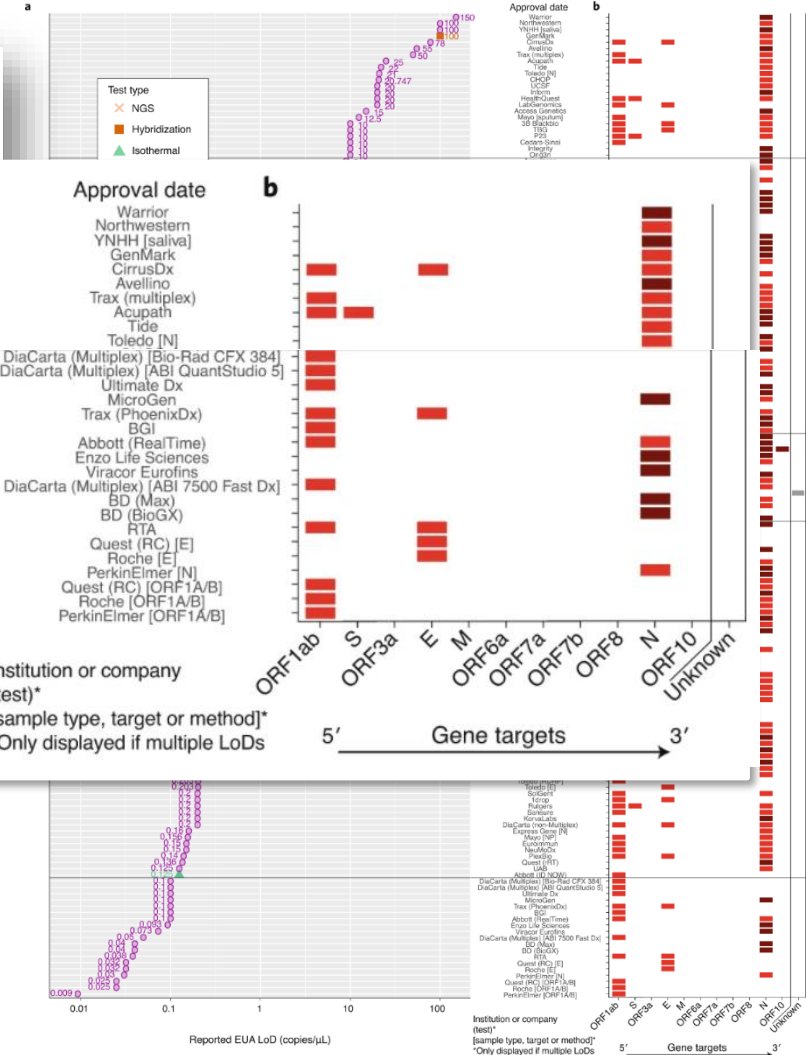
Arguments that have been used to suggest diagnosis targeting SARS-CoV-2 RNA is a simple process

1. “The viral load is high (1,000,000 copies/ml)”
2. “The methods are highly sensitive”
3. “Genome presence (not quantity) is measured”

The COVID-19 XPRIZE and the need for scalable,



Variation in limit of detection = $> 4 \log_{10}$



Differences in measured viral RNA concentration

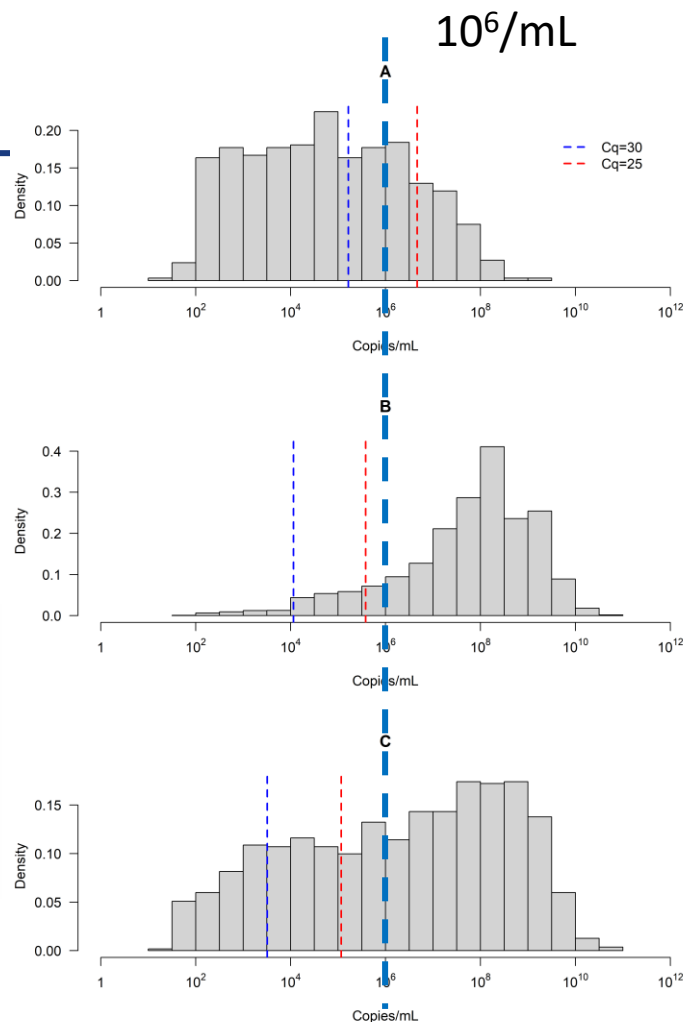
ACCEPTED MANUSCRIPT

The dangers of using Cq to quantify nucleic acid in biological samples; a lesson from COVID-19

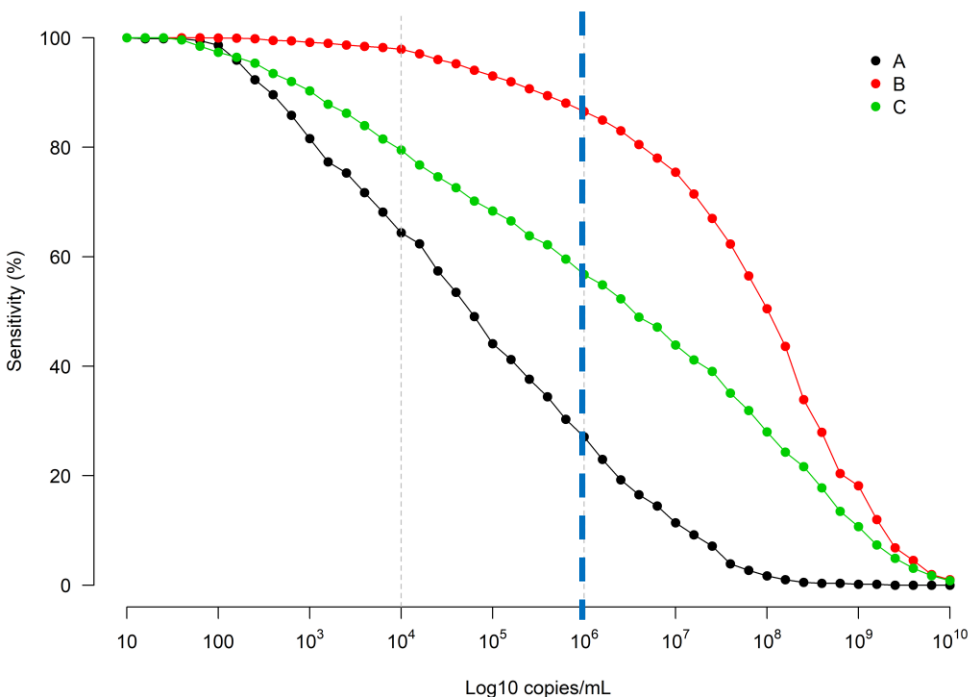
Daniel Evans, Simon Cowen, Martin Kammel, Denise M O'Sullivan, Graham Stewart, Hans-Peter Grunert, Jacob Moran-Gilad, Jasper Verwilt, Jiwon In, Jo Vandesompele ...
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Clinical Chemistry, hvab219, <https://doi.org/10.1093/clinchem/hvab219>

Published: 11 October 2021 [Article history](#)



Impact on Sensitivity



Copies/mL	Lab	Sensitivity %
10 ⁴	A	64.40
	B	97.92
	C	79.51
10 ⁶	A	27.09
	B	86.53
	C	56.75

ACCEPTED MANUSCRIPT

The dangers of using Cq to quantify nucleic acid in biological samples; a lesson from COVID-19

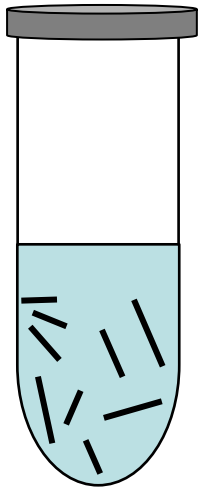
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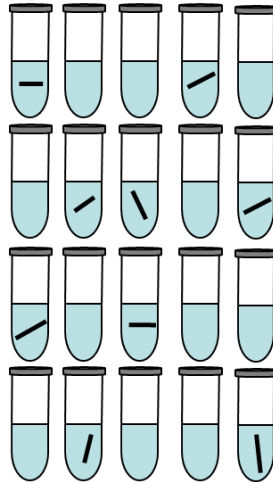
1. Good example of why quantitative measurements (and their standardisation) are needed for evaluation of presence absence
2. Material standards are used in virology for harmonisation, reference measurement procedures also provide a dynamic complementary route for standardisation
3. Historically potential reference measurement procedures for nucleic acid analysis have been limited
4. Digital PCR offers a potential route to provide (SI) traceable value assignment of RNA

qPCR $1 \times 20 \mu\text{l}$ reactions



Split
sample by
dilution →

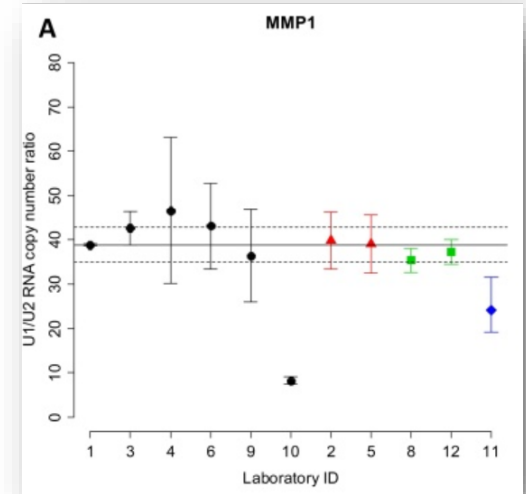
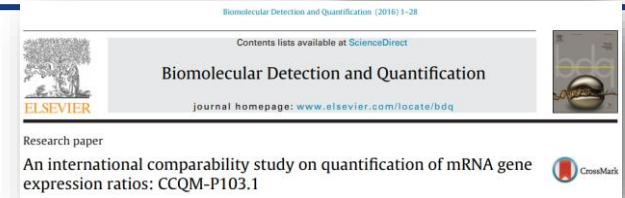
dPCR $20 \times 1 \mu\text{l}$ reactions



- Limiting dilution
 - Some reaction contain 0 templates
- PCR performed as normal using standard real-time PCR chemistry
- Absolute quantification
 - +ve or –ve reactions
 - Poisson statistics to account for multiple targets per partition (> 1)

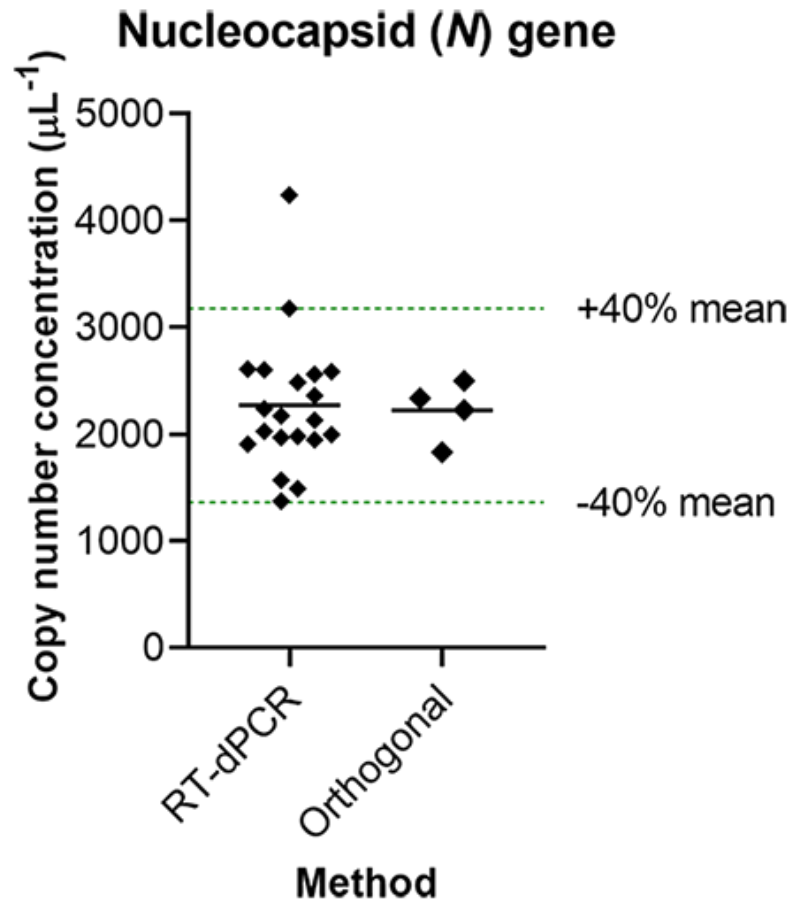
Reference measurement systems. RNA measurement

1. CCQM-P103 & CCQM-P103.1. Measurement of multiplexed biomarker panel of RNA transcripts
2. CCQM-P155. Multiple cancer cell biomarker measurement
3. CCQM-P199: Copy number concentration of HIV-1 RNA genomic sequences
4. CCQM-P199b: SARS-CoV-2 copy number quantification



Coordinated by NML (LGC), NIM China, NIBSC and NIST

1. SARS-CoV-2 RNA synthetic molecules shared between 21 laboratories (16 countries)
 - 1 (pure) high concentration material
 - 3 lower concentration materials (including covering full genome) for molecular analysis
2. Laboratories were told the sequence and the two genes to measure
 - i. No recommended assays
 - ii. No calibrators provided
3. CCQM-P199b took six months from initiation to data submission (draft A complete with feedback being taken)



CRMs/RMs/quality control materials/research grade test materials

- ◆ RNA fragments
 - JRC (Geel), NIST, NIM China, TUBITAK UME, NMIA, KRISS,
- ◆ Genomic RNA
 - NIM China
- ◆ SARS DNA fragment
 - CENAM
- ◆ Whole virus/viral like particle
 - KRISS, NIBSC

Summary

- ♦ The NAWG has worked together to demonstrate reference measurement procedures
 - capable of reproducible and increasingly accurate measurement
- ♦ The RT PCR is able to measure viral RNA in complex mixtures of RNA (such as matrix materials or human extracts).
- ♦ This offers the potential for a reference measurement procedure for pandemic causing viruses

To do

1. Explain why there is a 40% +/- . Possibly due to reverse transcriptase, assay design, experimental setup, other explanations
2. Combine with extraction to conduct reproducible measurements of EQA, RMs.
3. How can this be applied during *next pandemic* (QC materials, real samples)? Case study(ies), make the case.
4. Wider than COVID-19

Acknowledgements



NML

- Alison Devonshire
- Denise O'Sullivan
- Eloise Busby
- Ana Fernandez-Gonzalez
- Gerwyn Jones
- Alexandra Whale
- Simon Cowen

NIST

- Megan Cleveland
- Peter Vallone

NIBSC

- Clare Morris
- Neil Almond

NIMC

- Liana Dong

BIPM

- Robert Wielgosz

GBD

- Heinz Zeichhardt,
- Martin Kammel
- Hans-Peter Grunert

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- Andreas Kummrow
- Samreen Falak

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- Kathryn Harris
- Julianne Brown



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