The role of reference measurement procedures in supporting EQA

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National Measurement Laboratory (NML)

Measurement matters

National Metrology Institutes

- National Metrology Institutes (NMIs) and Designated Institutes (DIs)

Metrology

/mɪˈtrɒlədʒi/

noun

the scientific study of measurement.

The BIPM: An International Organization for Metrology

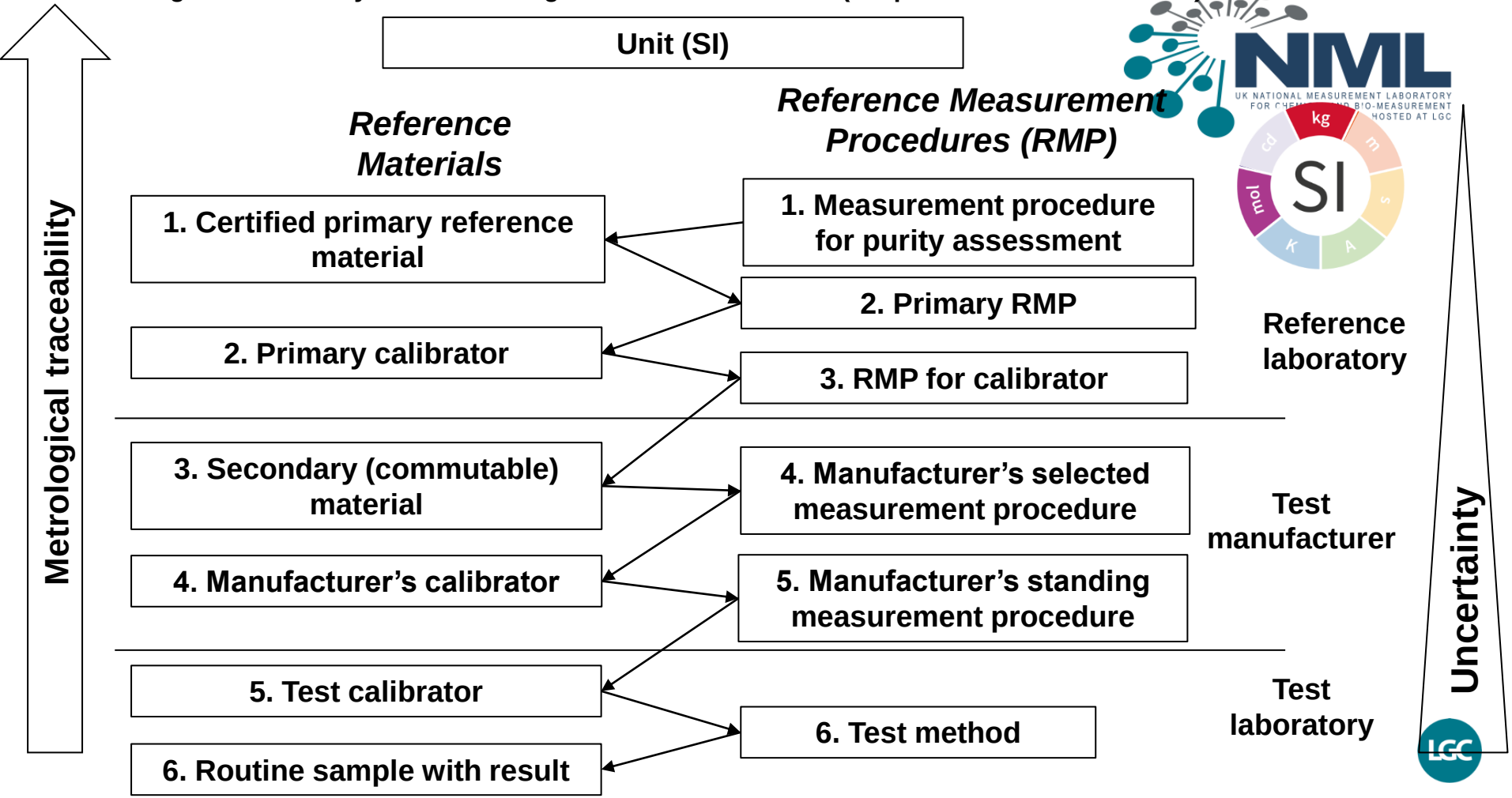
"The BIPM is an intergovernmental organization established by the Metre Convention, through which Member States act together on matters related to measurement science and measurement standards".

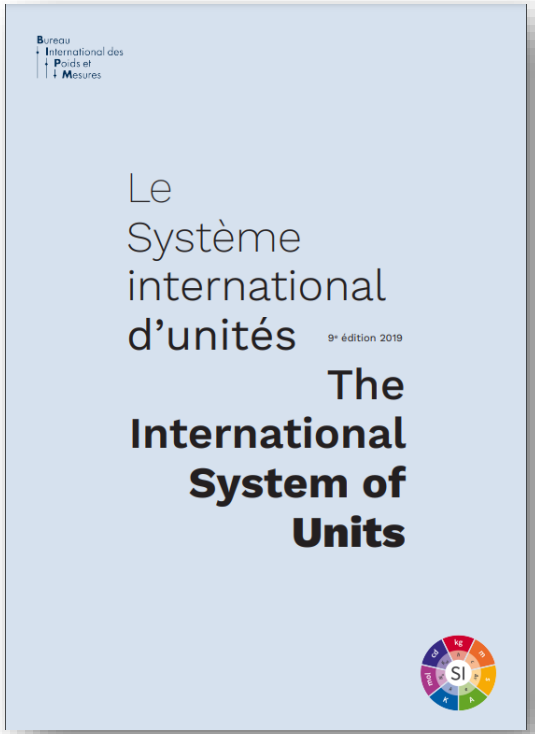
- **Founded in Paris in 1875 by 17 Member States and based at the *Pavillon de Breteuil* in Parc St Cloud, Sèvres, France**
- **Now involving over 100 states and economies as Members or Associates.**





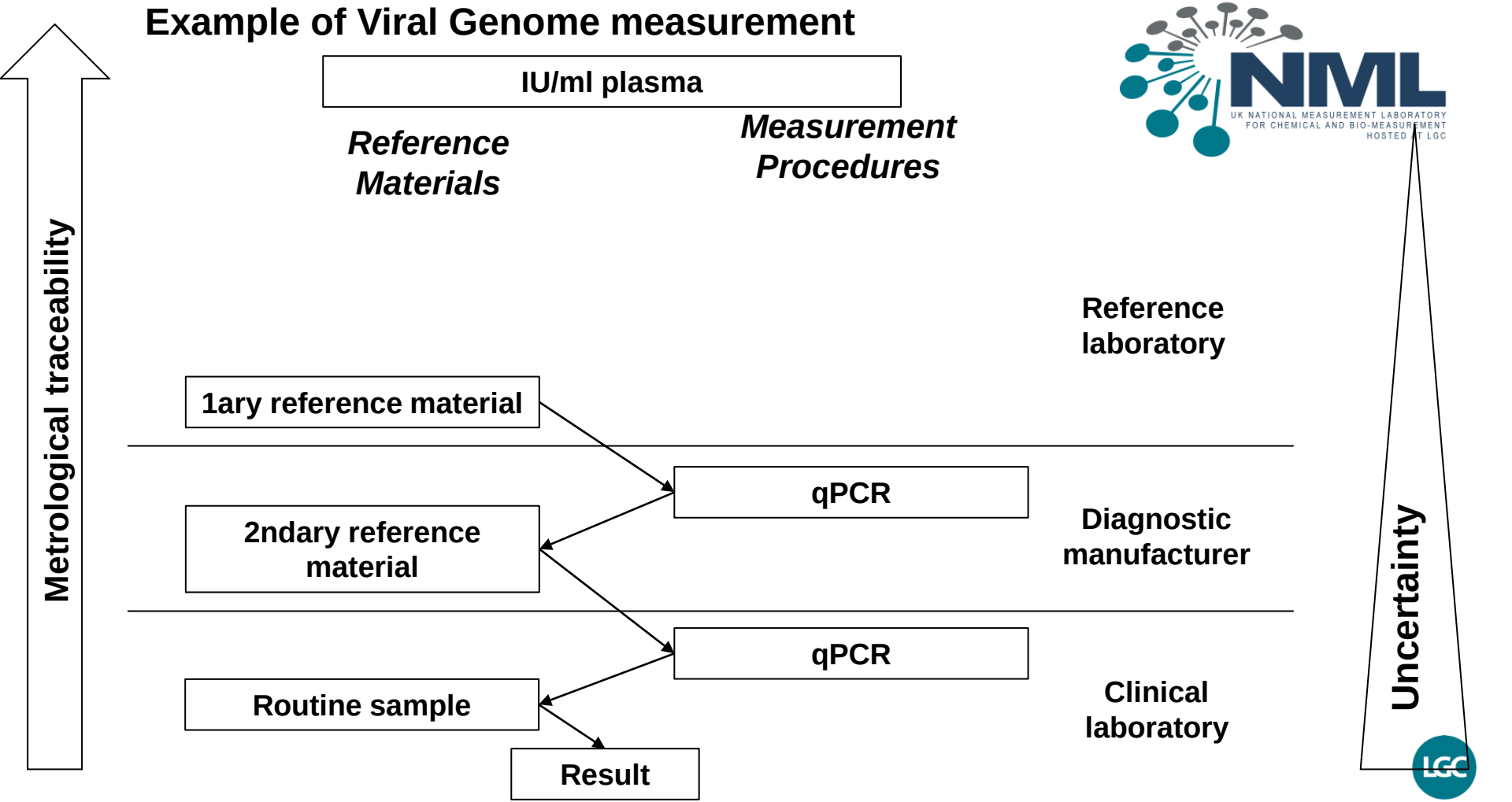
Metrological traceability for in vitro diagnostic medical devices (adapted from ISO17511:2021)



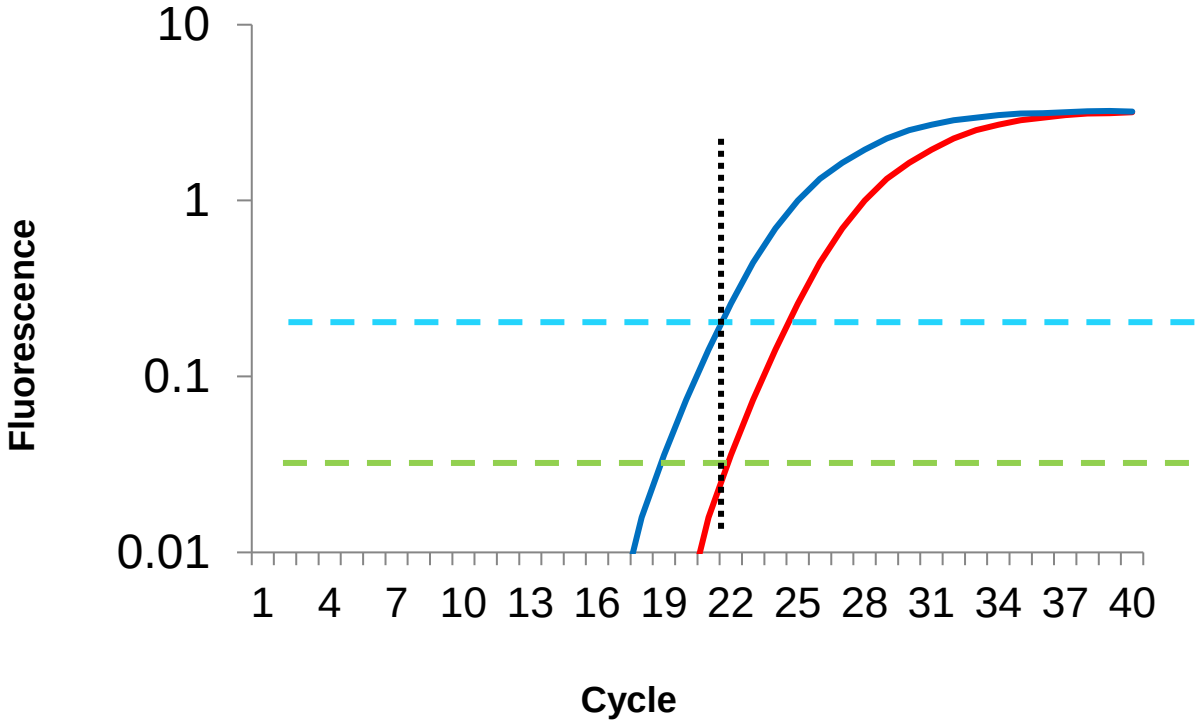


There are also some quantities that cannot be described in terms of the seven base quantities of the SI, but have the nature of a count. Examples are a number of molecules, a number of cellular or biomolecular entities (for example copies of a particular nucleic acid sequence), or degeneracy in quantum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

Example of Viral Genome measurement

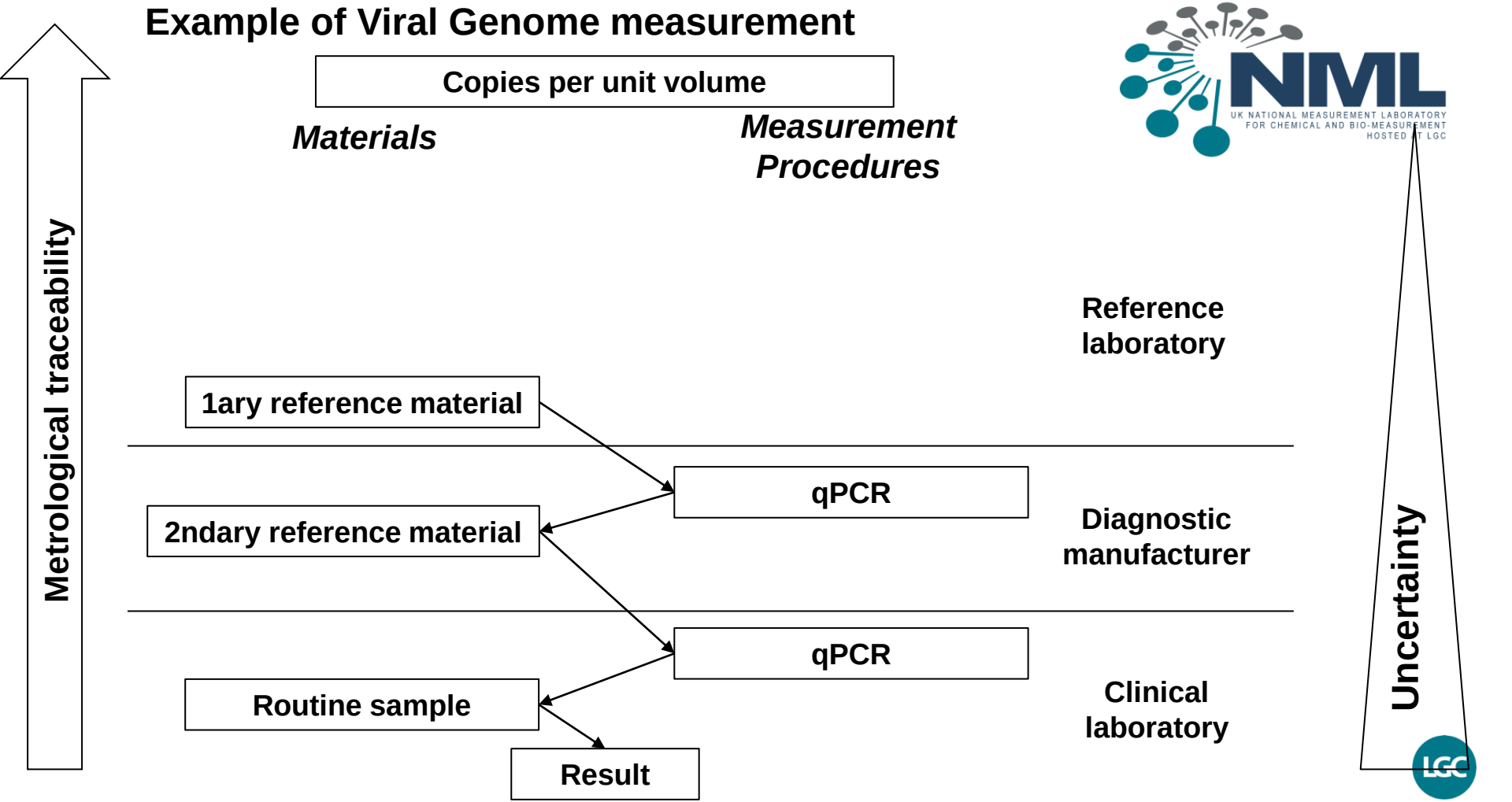


Reverse transcription real time quantitative PCR (RT-qPCR)

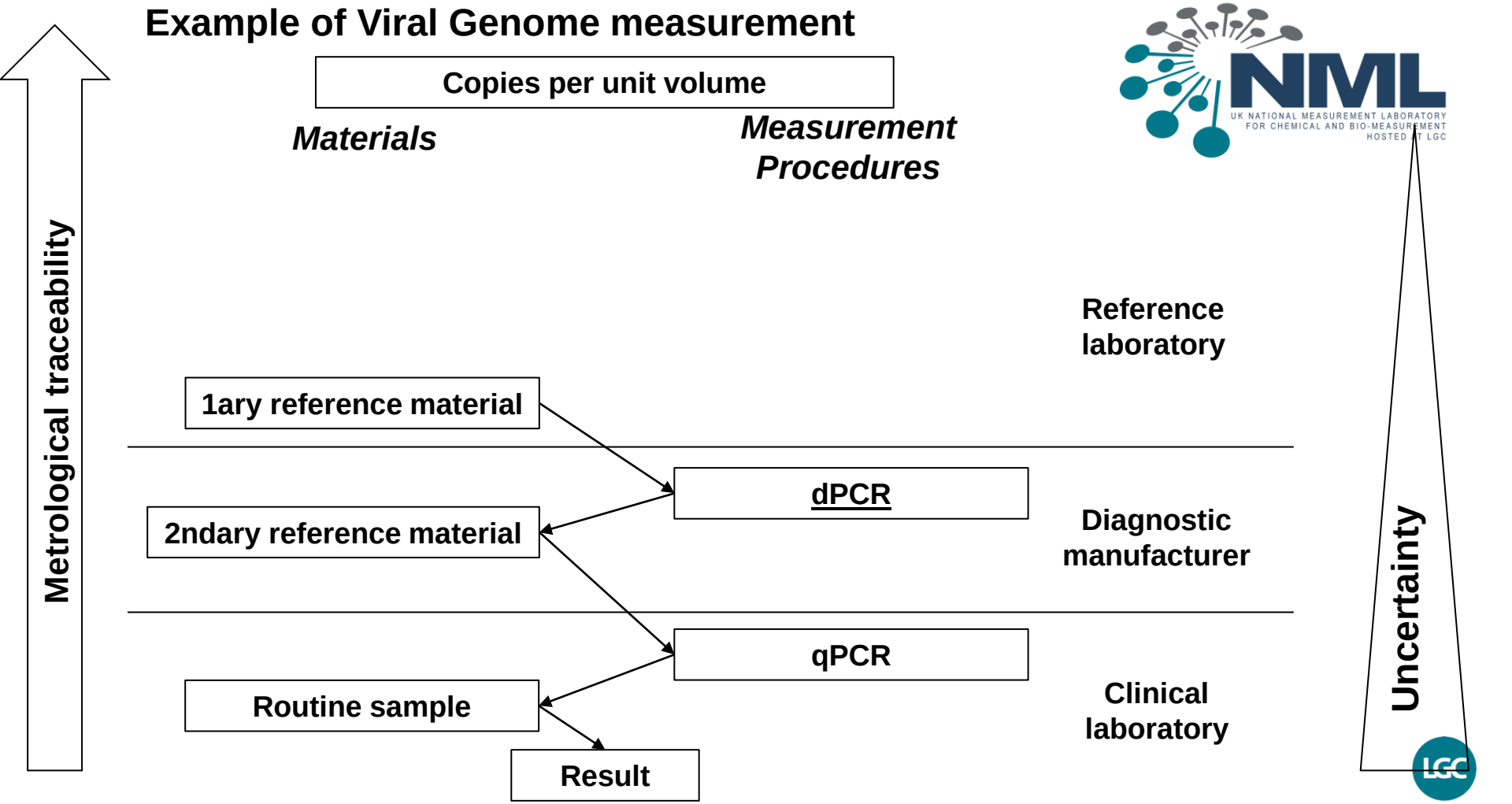


Both have a C_q (C_t) of 22

Example of Viral Genome measurement

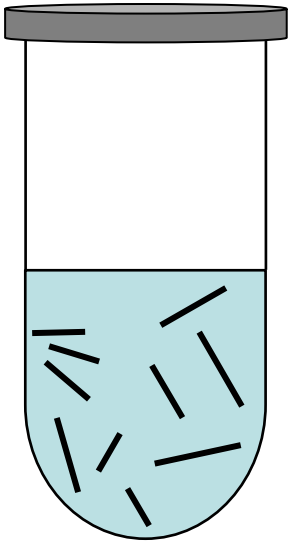


Example of Viral Genome measurement



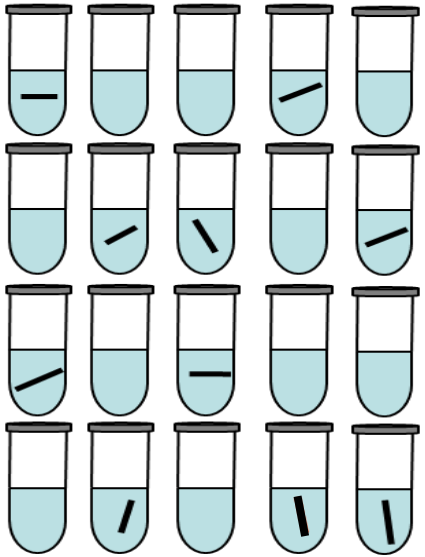
Digital PCR

Real-time PCR
1 × 20 µL reaction



Split sample by dilution

Digital PCR
20 × 1 µL reactions



- **Absolute counting method**
  - Counts nucleic acid molecules based on sequence
  - Binary output
- **Absolute quantification**
  - It is calibration free for quantification (unlike most methods that are relative)
  - High sensitivity
  - Predictable precision
- **Value assignment for calibrators or reference materials**

dPCR as a reference method

Clin Chem (2018) 64(9):1296-1307

Assessment of Digital PCR as a P
Measurement Procedure to Sup
Precision Medicir

Alexandra S. Whale,[1†] Gerwyn M. Jones,[1†] Jernej Pavšič,[2,3] Ta
Sema Akyürek,[4] Müslüm Akgöz,[4] Carla Divieto,[5] Maria Paola Sass
Young-Kyung Bae,[7] Sang-Ryoul Park,[7] Liesbet Deprez,[8] Philippe Cor
Raquel Larios,[10] Simon Cowen,[11] Denise M. O'Sullivan,[1] Claire A.
Carole A. Foy,[1] Alison J. Woolford,[1] Helen Parkes,[1] Jim F. Hugget

H White[1,2]

Methods (2022) 201:65-73

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

The performance of human cytomegalovirus digital PCR reference measurement procedure in seven external quality assessment schemes over four years

Mojca Milavec[a,*], Jernej Pavšič[a], Alexandra Bogožalec Košir[a], Gerwyn M. Jones[b], Denise M. O'Sullivan[b], Alison S. Devonshire[b], Fran Van Heuverswyn[d,1], Maria Karczmarczyk[d,2], Jannika Neeb[e,3], Annabell Plauth[e], Philippe Corbisier[d], Heinz Schimmel[d], Andreas Kummrow[e], Jörg Neukammer[e,4], Carole A. Foy[b], Martin Kammel[f,g], Hans-Peter Grunert[h], Heinz Zeichhardt[f,g,h], Jim F. Huggett[b,c]

Methods (2022) 201:34-40

An Assessment of the Reproducibility of Reverse Transcription Digital PCR Quantification of HIV-1

Samreen Falak*[1], Rainer Macdonald[1], Eloise J Busby[2], Denise M O'Sullivan[2], Mojca Milavec[3], Annabell Plauth[1], Martin Kammel[4,5], Heinz Zeichhardt[4,5], Hans-Peter Grunert[6], Andreas Kummrow *[1], Jim F. Huggett*[2,7]

Open Access

improve the
ve molecular
tuberculosis

loneyborne[2], Gerwyn Jones[1], Maria Karczmarczyk[3],
Mendoza[5], Heinz Schimmel[3], Fran Van Heuverswyn[3],
ni[6], Kathryn Harris[7], Marinus Barnard[8,9],
allis[11], Keshree Pillay[11], Thomas Barry[12], Kate Reddington[12],
anettin Yalçınkaya[14], Muslum Akgoz[14], Jana Žel[4],
[1,2,15]*

Bureau International des Poids et Mesures

Database of higher-order reference materials, measurement methods/procedures and services

JCTLM
Accurate results for patient care

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UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

Results of the search

Your search criteria produced 1 result.
For more information on a reference measurement method/procedure for a given Analyte/Matrix (or Material)/Measurement principle (or technique) combination, select one or more of the options below.

Select all items from the list

Sort by : Analyte | Measurement principle/technique | Matrix/Material

Select	Analyte	Measurement principle/technique	Matrix/Material
☐	KRAS DNA wild type sequence and gene mutation	Digital PCR	calibration solution

Deselect all items from the list

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Modify your search criteria

gnostics

e materials, measurement

esterol

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Select an analyte category

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Results of the search

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Select all items from the list

Sort by : Analyte | Measurement principle/technique | Matrix/Material

Select	Analyte	Measurement principle/technique	Matrix/Material
☐	Human cytomegalovirus (HCMV) DNA sequence	Digital PCR	other

Deselect all items from the list

View

Modify your search criteria

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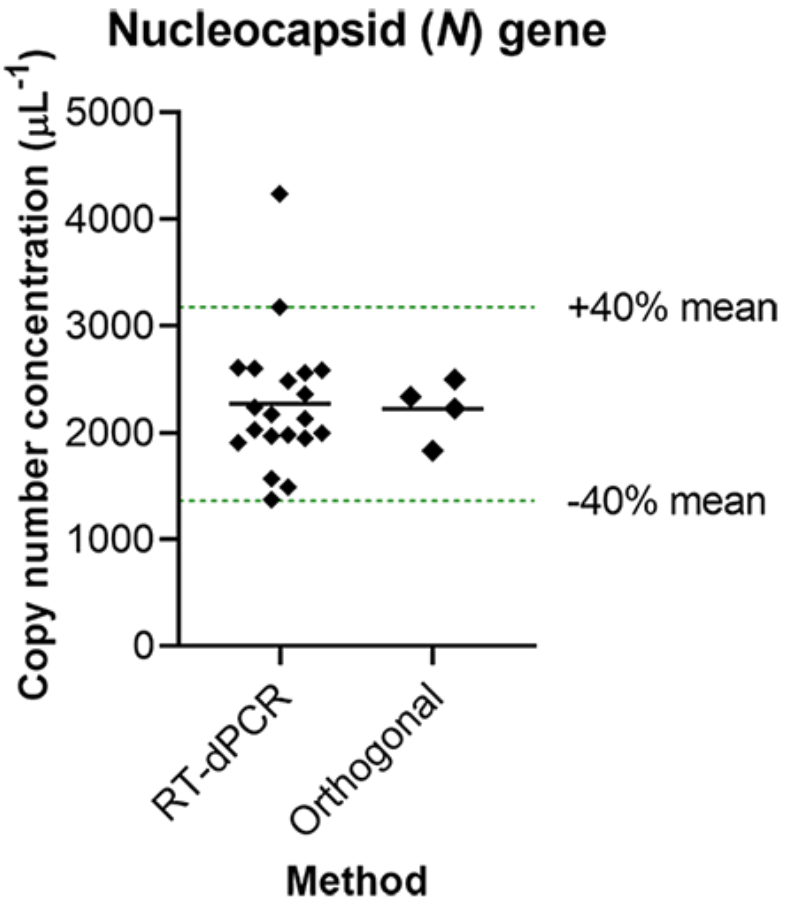
CCQM-P199b: SARS-CoV-2 copy number quantification

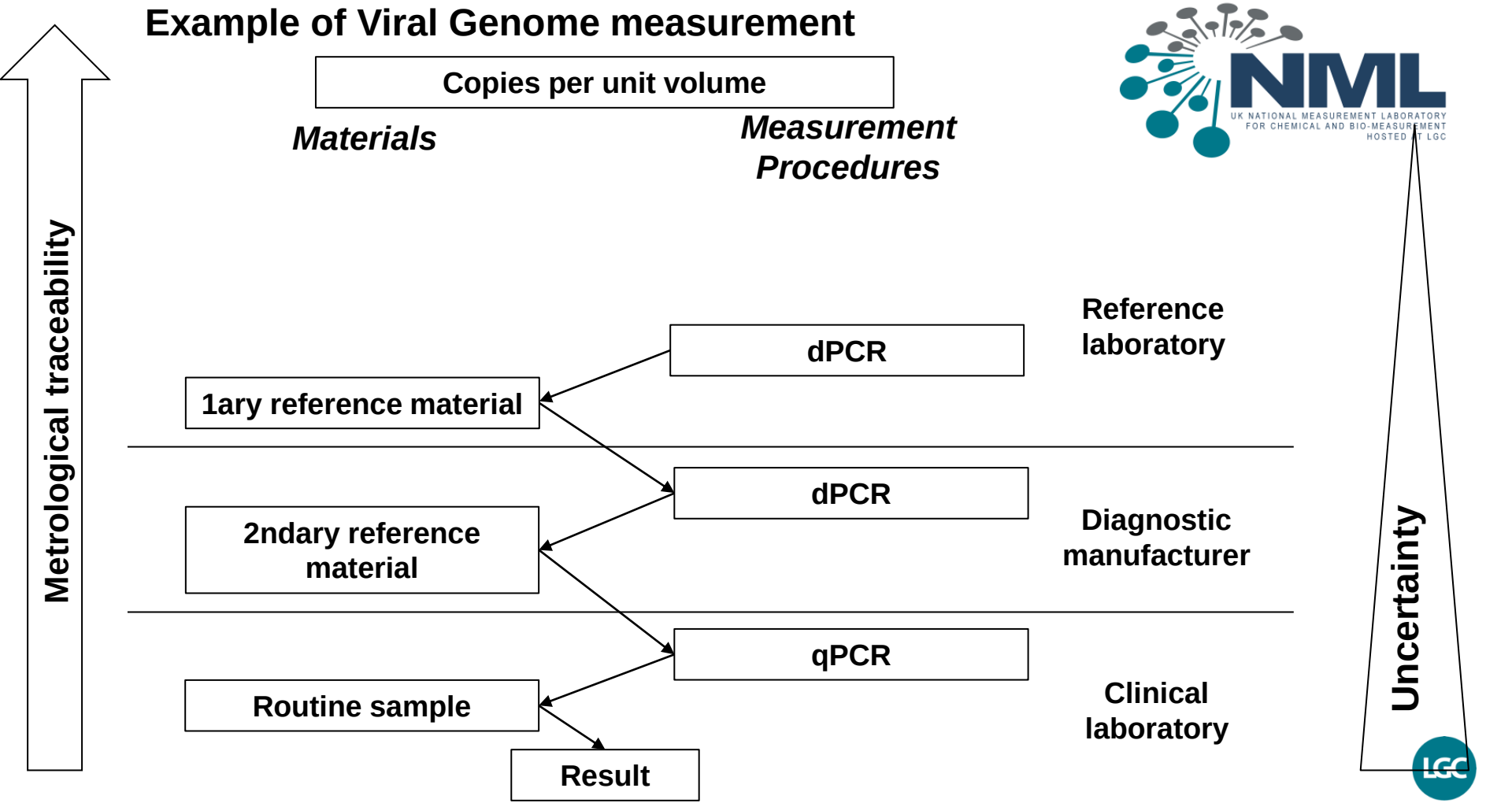


CCQM
NUCLEIC ACID ANALYSIS WORKING GROUP

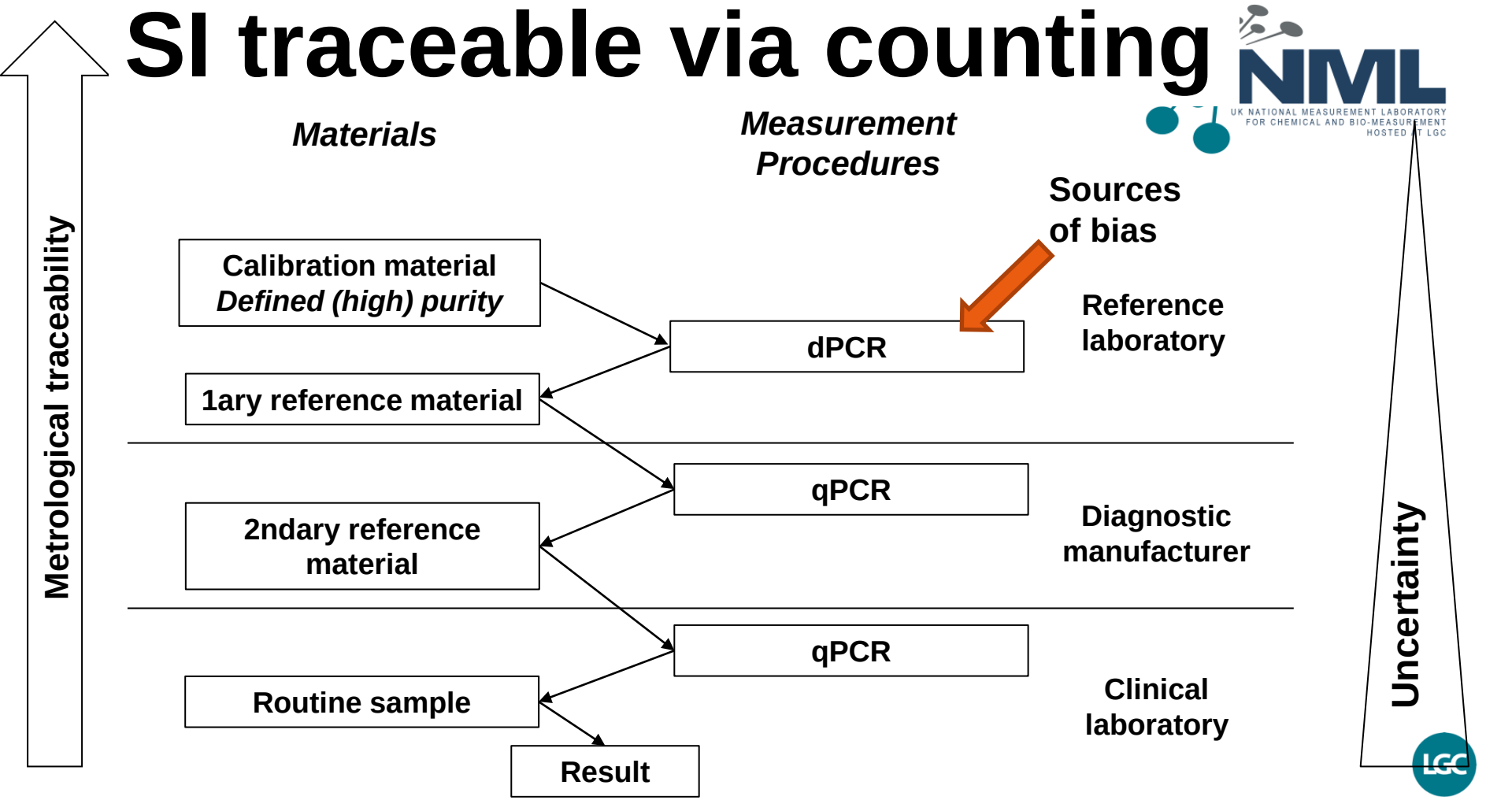
- **21 Laboratories were told the sequence of the two genes to measure**
- **RNA in buffered solution provided/**
  i. **No recommended assays**
  ii. **No calibrators provided**

Bureau International des Poids et Mesures

Example of Viral Genome measurement



SI traceable via counting



What am I counting (Virus genome)?

Whole molecule (functional genome)

CTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGAAGCTC
GATGCGACATGATCATGCGGATCGCATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGC
TCGATGCGACATGATCATGCGGATCGATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAT
GATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGA
GCTCGATGCGACATGATCATGCGGATCGAG

Whole molecule (defective genome)

CTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGAAGCTC
GATGCGACATGATCATGCGGATCGCATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGC
TCGATGCGACATGATCATGCGGATCGATGATCATGCGGATCGAGCTCGATGCGACATGATCATAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGC
GACATGATCATGCGGATCGATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATG
CGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAG

Fragment

CTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGAAGCTC
GATGCGACATGATCATGCGGATCGCATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGCTCGATGCGACATGATCATGCGGATCGAGC
TCGATGCGACATGATCATGCGGATCGATG

SARS-CoV-2 testing

Specimen → Sampling/ transport/ storage → Viral genome purification → Detection of viral genome

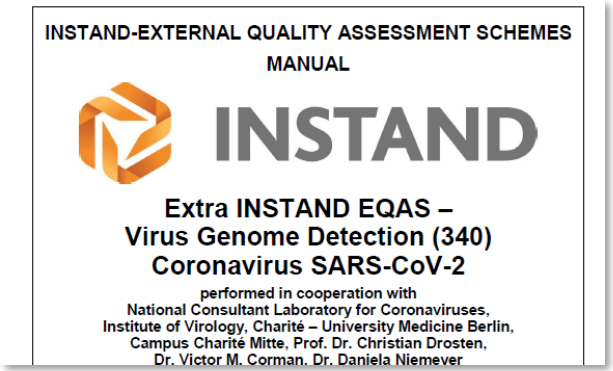
SARS-CoV-2 testing

1. Analysis only
2. Full analytical process
3. Full diagnostic process

? → Specimen → Sampling/ transport/ storage → Viral genome purification → Detection of viral genome

Error

Digital PCR supporting characterisation of EQA materials


INSTAND-EXTERNAL QUALITY ASSESSMENT SCHEMES MANUAL

INSTAND

Extra INSTAND EQAS –
Virus Genome Detection (340)
Coronavirus SARS-CoV-2

performed in cooperation with
National Consultant Laboratory for Coronaviruses,
Institute of Virology, Charité – University Medicine Berlin,
Campus Charité Mitte, Prof. Dr. Christian Drosten,
Dr. Victor M. Corman, Dr. Daniela Niemeyer

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IQVD

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

PTB
Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

Table 3: Qualitative results –
Summary of sample properties, target values, results, success rates, medians of Ct/Cp/Cq/CN values as well as the reported minimum Ct/Cp/Cq/CN value and reported maximum Ct/Cp/Cq/CN value – differentiated according to the targeted gene region

Sample no.	Sample properties	Expected qualitative result for SARS-CoV-2	Gene region	Correct results per reported results differentiated by gene region	Reported Ct/Cp/Cq/CN-results differentiated by gene region median (min – max)
Sample 340059[$,*]	SARS-CoV-2 1 : 1 000 diluted sample not evaluated[$]	positive	E	373/373 (100%)	22.6 (16.8-34.0)
			N	165/167 (98.8%)	23.6 (17.9-34.9)
			ORF1a	45/46 (97.8%)	22.2 (20.8-28.7)
			ORF1ab	48/48 (100%)	21.8 (10.9-29.1)
			RdRP	185/185 (100%)	23.8 (10.0-34.5)
			S	100/100 (100%)	21.8 (17.5-27.8)
			n.s.[§]	64/64 (100%)	22.6 (9.4-33.0)
		total		980/983 (99.7%)[$]	22.8

Table 5 (continued): Quantitative r

Sample No.		340059*,$
Sample properties		SARS-CoV-2 positive
Dilution		1 : 1 000*
Method / gene region	Participant No.	copies/ml
qPCR / N gene	1467	23 217 000
	4973	3 811 423
	4973	78 599 453
	40839	644 900
	58803	450 276
	67794	57 428 571

Different gene targets
10^2-10^7 fold difference

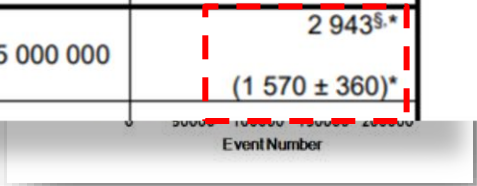
Same gene target
Differences of > 2 log

LGC

Strategy for EQA scheme June/July 2020 dPCR assigned SARS-CoV-2 concentration of samples from sensitivity panel

Table 1: EQA Scheme Virus Genome Detection - Coronaviruses incl. SARS-CoV-2 June/July 2020 - Summary of sample properties and target values

Program	Group	RiliBÄK	Sample	Sample properties			
				qualitative	virus	dilution	Target value of all methods copies/ml § robust mean calculated from EQAS data (* via dPCR assigned quantitative value)
			340066*,1	positive* not evaluated	SARS-CoV-2[1] (inactivated)	(a) 1 : 5 000 000	2 943§,* (1 570 ± 360)*



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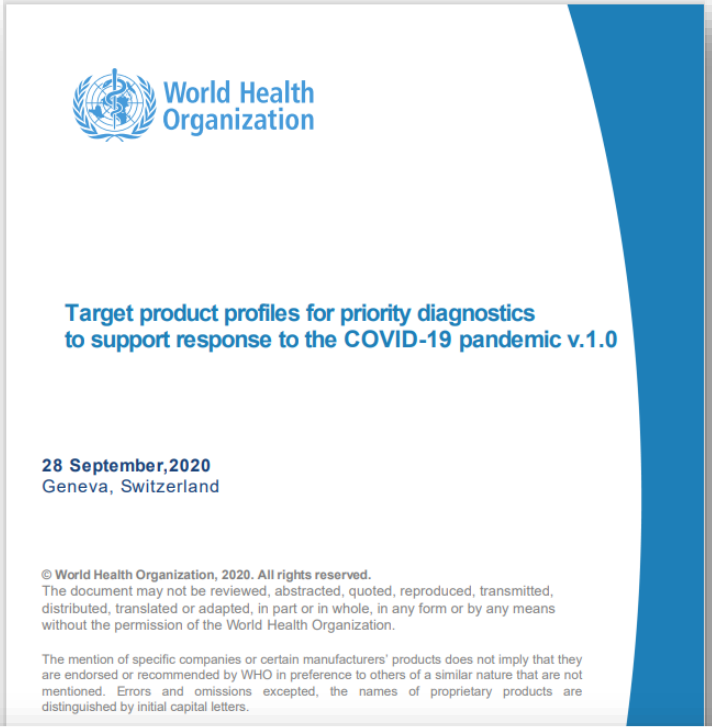
TPP


World Health Organization

Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0

28 September,2020
Geneva, Switzerland

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A target product profile (TPP) outlines the desired 'profile' or characteristics of a target product that is aimed at a particular disease or diseases. TPPs state intended use, target populations and other desired attributes of products, including safety and efficacy-related characteristics. Such profiles can guide product research and development (R&D)

https://www.who.int/observatories/global-observatory-on-health-research-and-development/analyses-and-syntheses/target-product-profile/links-to-who-tpps-and-ppcs

Key Feature	Acceptable	Desirable
Analytical sensitivity/Limit of detection	equivalent to 10^6 genomic copies/mL or Ct ≈ 25-30	equivalent to 10^4 genomic copies/mL or Ct≈>30

Value assignment for Quality Assurance

PLOS ONE

RESEARCH ART

RNA ref
RNA loa
towards

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Simon Cowen
Andreas Kumm
Martin Kamme

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Infektiologiezentr
Pathogens, Berlir
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Medical Science,
13 Materials Mea
Standards and Te
America, 14 Phys
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Applied Sciences

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Citation: Vierbaum L, Wojtalewicz N, Grunert H-P, Lindig V, Duehring U, Drosten C, et al. (2022) RNA reference materials with defined viral RNA loads of SARS-CoV-2—A useful tool towards a better PCR assay harmonization. PLoS ONE 17(1): e0262656. https://doi.org/10.1371/journal.pone.0262656

Editor: Paulo Lee Ho, Instituto Butantan, BRAZIL

Received: August 24, 2021

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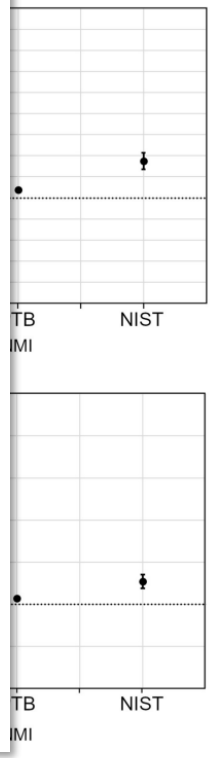
Results of German external quality assessment schemes for SARS-CoV-2 antigen detection

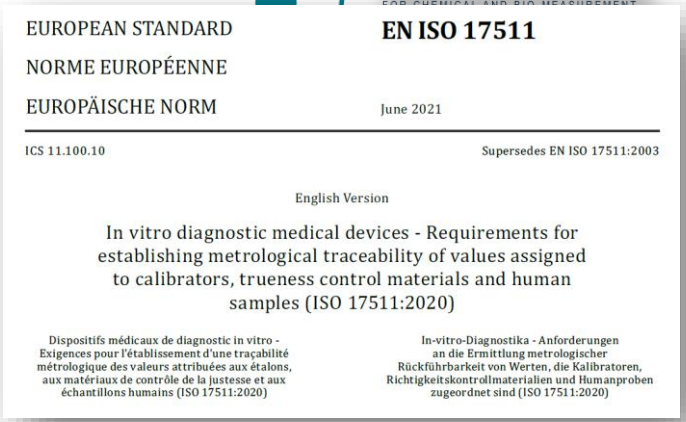
Laura Vierbaum[1,13], Nathalie Wojtalewicz[1,13], Hans-Peter Grunert[3], Anika Zimmermann[2], Annemarie Scholz[2], Sabine Goseberg[1], Patricia Kaiser[1], Ulf Duehring[3], Christian Drosten[4], Victor Corman[4], Daniela Niemeyer[4], Holger F. Rabenau[4], Martin Obermeier[5], Andreas Nitsche[6], Janine Michel[7], Andreas Puyskens[7], Jim F. Huggett[8,9], Denise M. O'Sullivan[8], Eloise Busby[8], Simon Cowen[8], Peter M. Vallone[10], Megan H. Cleveland[10], Samreen Falak[11], Andreas Kummrow[11], Ingo Schellenberg[1,12], Heinz Zeichhardt[1,2,3] & Martin Kammel[1,2,13]

The COVID-19 pandemic illustrated the important role of diagnostic tests, including lateral flow tests (LFTs), in identifying patients and their contacts to slow the spread of infections. INSTAND performed external quality assessments (EQA) for SARS-CoV-2 antigen detection with lyophilized and chemically inactivated cell culture supernatant of SARS-CoV-2 infected Vero cells. A pre-study demonstrated the suitability of the material. Participants reported qualitative and/or quantitative antigen results using either LFTs or automated immunoassays for five EQA samples per survey. 711 data sets were reported for LFT detection in three surveys in 2021. This evaluation focused on the analytical sensitivity of different LFTs and automated immunoassays. The inter-laboratory results showed at least 94% correct results for non-variant of concern (VOC) SARS-CoV-2 antigen detection for viral loads of ≥ 4.75×10^6 copies/mL and SARS-CoV-2 negative samples. Up to 85% had success for a non-VOC viral load of ~ 1.60×10^6 copies/mL. A viral load of ~ 1.42×10^7 copies/mL of the Delta VOC was reported positive in > 96% of results. A high specificity was found with almost 100% negative SARS-CoV-2 antigen results for HCoV 229E and HCoV NL63 positive samples. Quantitative results correlated with increasing SARS-CoV-2 viral load but showed a broad scatter. This study shows promising SARS-CoV-2 antigen test performance of the participating laboratories, but further investigations with the now predominant Omicron VOC are needed.



Conclusion

- **Reference measurement procedures (RMP) can complement material standards to aid quantification and identification,**



EUROPEAN STANDARD **EN ISO 17511**

NORME EUROPÉENNE

EUROPÄISCHE NORM June 2021

ICS 11.100.10 Supersedes EN ISO 17511:2003

English Version

In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples (ISO 17511:2020)

Dispositifs médicaux de diagnostic in vitro - Exigences pour l'établissement d'une traçabilité métrologique des valeurs attribuées aux étalons, aux matériaux de contrôle de la justesse et aux échantillons humains (ISO 17511:2020)

In-vitro-Diagnostika - Anforderungen an die Ermittlung metrologischer Rückführbarkeit von Werten, die Kalibratoren, Richtigkeitskontrollmaterialien und Humanproben zugeordnet sind (ISO 17511:2020)

Acknowledgements

NML
- Eloise Busby
- Gerwyn Jones
- Daniel Evans
- Alison Devonshire
- Alexandra Whale
- Ana Fernandez-Gonzalez
- Carole Foy
- Alison Woolford
- Simon Cowen
- Jim Huggett

GBD/IQVD
- Heinz Zeichhardt,
- Martin Kammel
- Hans-Peter Grunert
- Ulf Dühring

NIB
- Mojca Milavec
- Alexandra Bogozalec

NIBSC/MHRA
- Clare Morris
- Emma Bentley
- Neil Almond

NIST
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- Peter Vallone

UK-HSA
- Jade Cogdale
- Maria Zambon

PTB
- Andreas Kummrow
- Samreen Falak

Department for Science, Innovation & Technology

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EMPIR

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The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Thank you

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