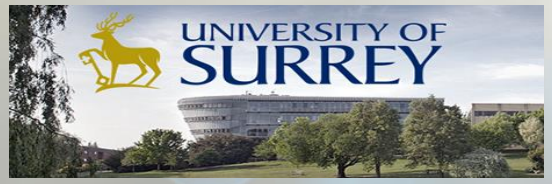


dPCR as a reference measurement procedure for viral detection and quantification

Jim Huggett

Principal Scientist (NML) & Senior Lecture (University of Surrey)

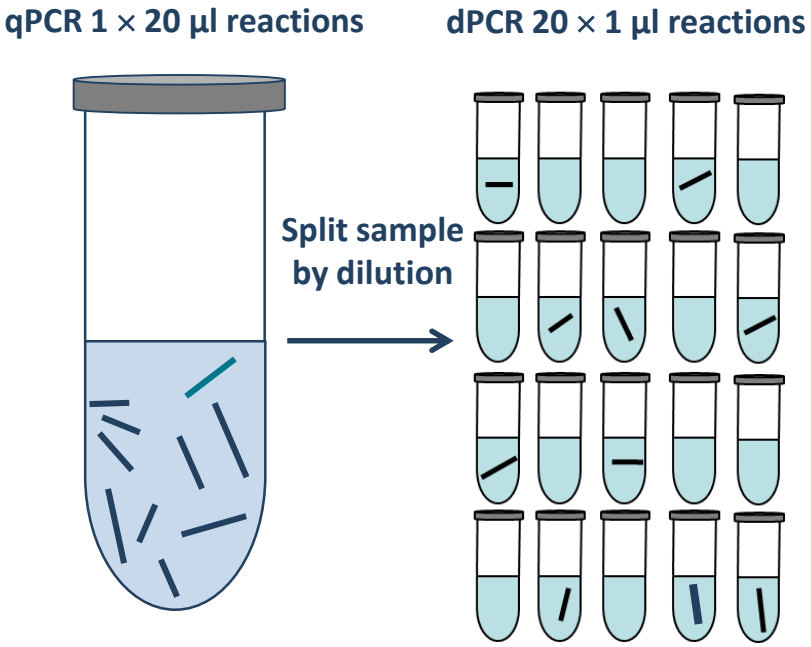
National Measurement Laboratory (NML)

Measurement matters

UNIVERSITY OF SURREY

LGC

What is Digital PCR?



- 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)

Advantages

- Does not require standard curve
- Reduced susceptible to inhibitors
- High sensitivity when measuring rare variants
- Very confident low level measurement

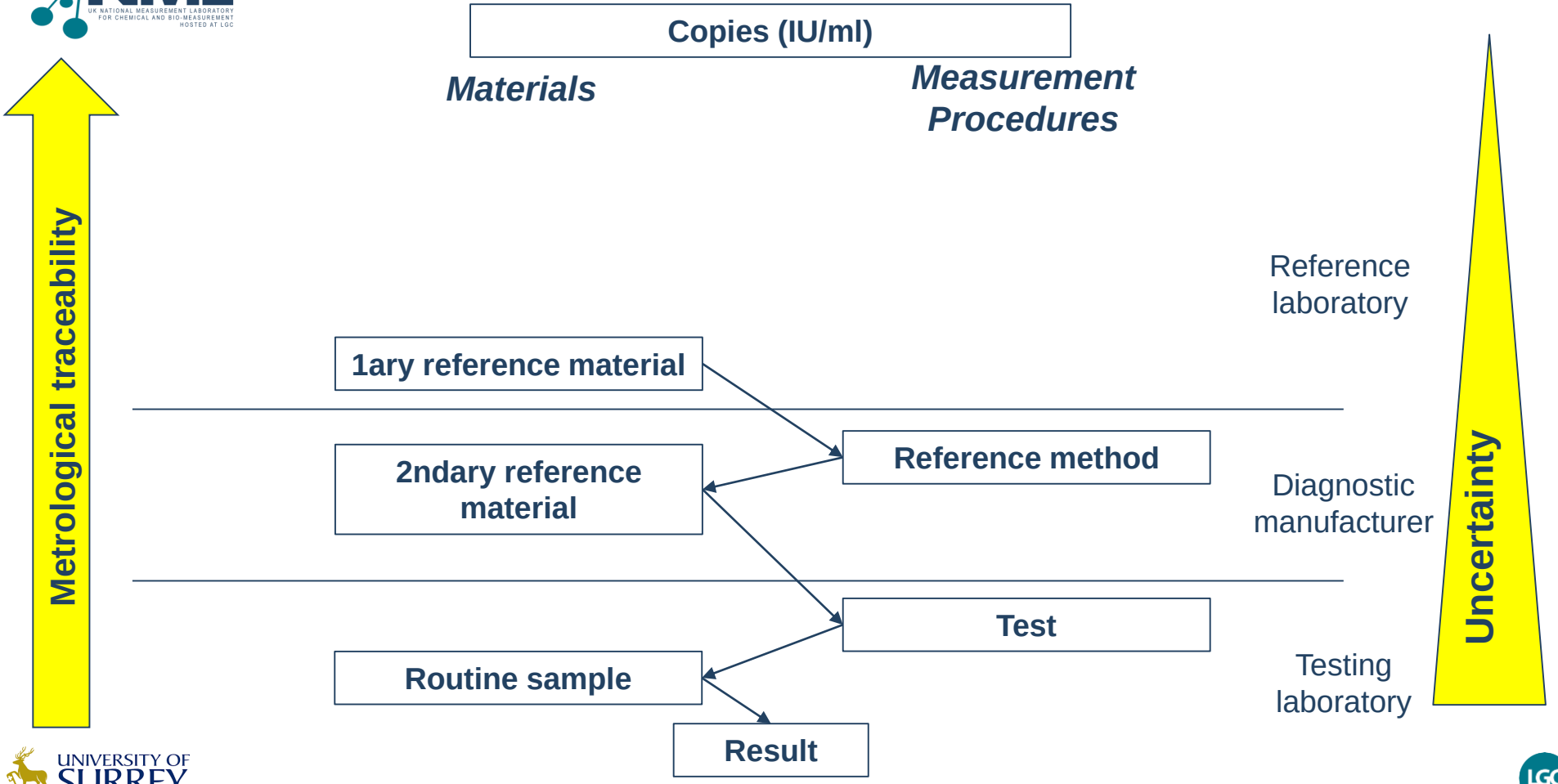
Disadvantages

- Complex/not currently automatable
- It’s PCR. So you need to know what to look for
- Limited reaction volume
- Setting thresholds can be challenging (Viruses)

Reference measurement procedure?

Molecular measurement

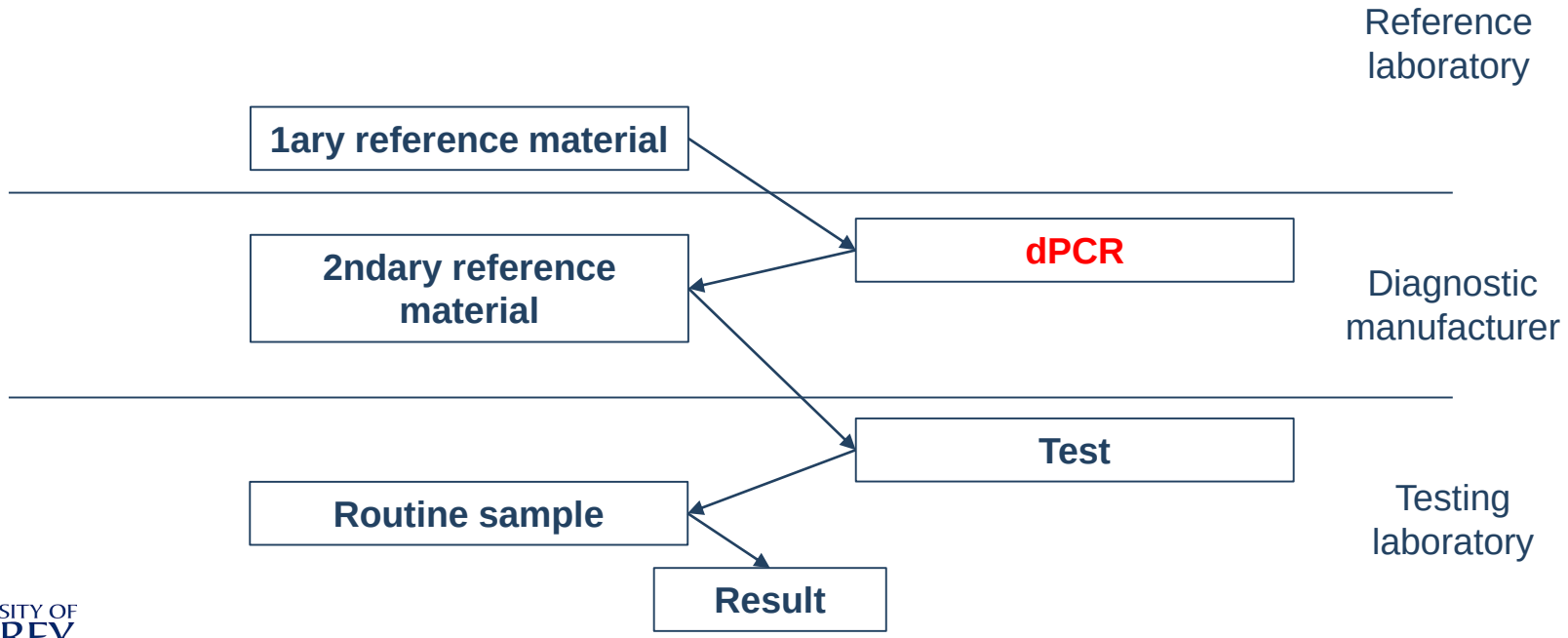
Adapted from ISO17511: 2003



Molecular measurement

Copies (IU/ml)

Materials ***Measurement Procedures***



Anal Bioanal Chem (2017) 409:2601–2614
DOI 10.1007/s00216-017-0206-0

CrossMark

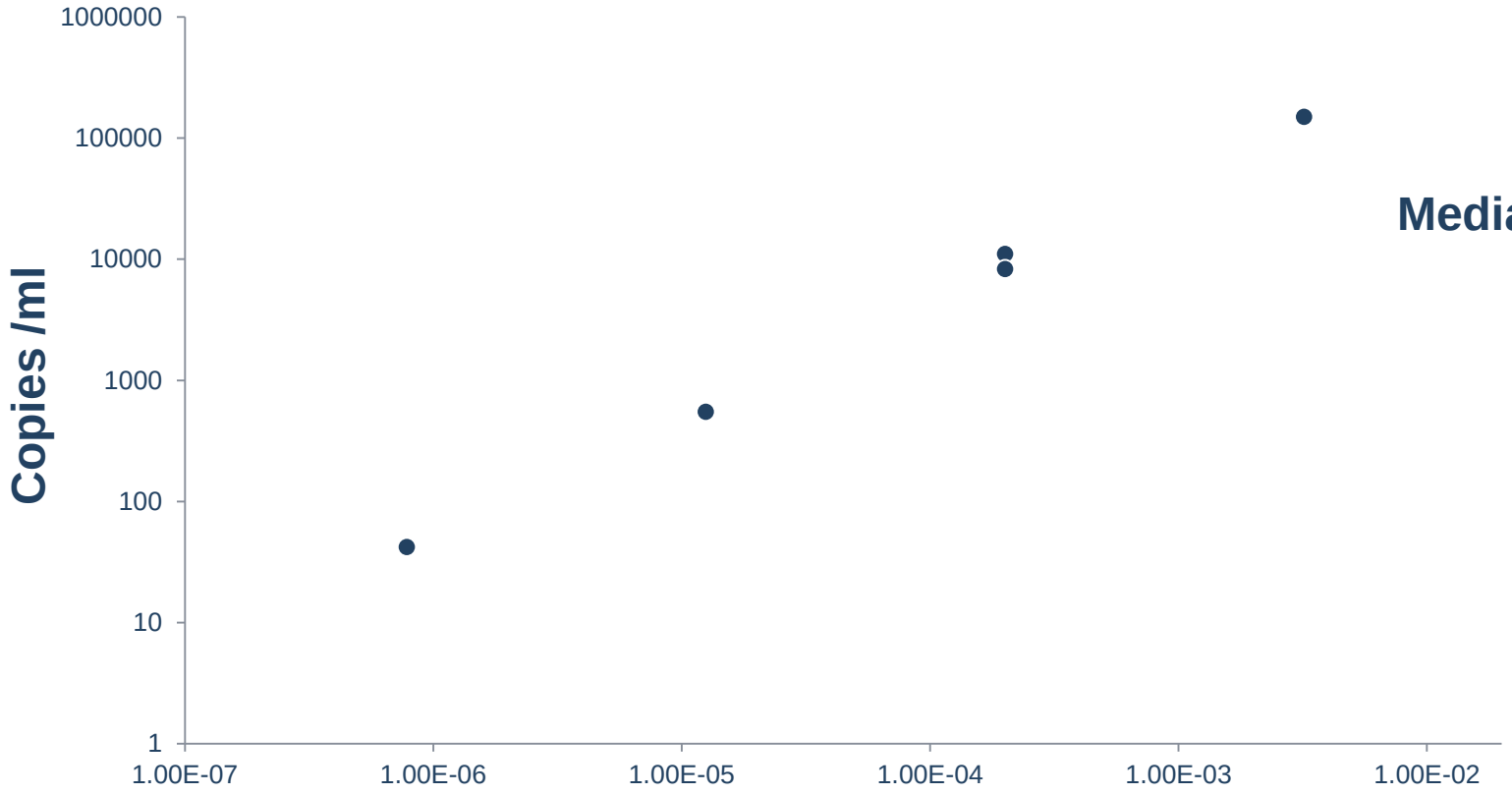
RESEARCH PAPER

Inter-laboratory assessment of different digital PCR platforms for quantification of human cytomegalovirus DNA

Jernej Pavšič[1,2] · Alison Devonshire[3] · Andrej Blejec[1] · Carole A. Foy[3] · Fran Van Heuverswyn[4] · Gerwyn M. Jones[3] · Heinz Schimmel[4] · Jana Žel[1] · Jim F. Huggett[3,5] · Nicholas Redshaw[3] · Maria Karczmarczyk[4] · Erkan Mozioğlu[6] · Sema Akyürek[6] · Müslüm Akgöz[6] · Mojca Milavec[1]

Training Program (368)
with low concentrated samples

Main EQA scheme (365)
(subject to RiliBÄK-B3)



Median values

Training Program (368)
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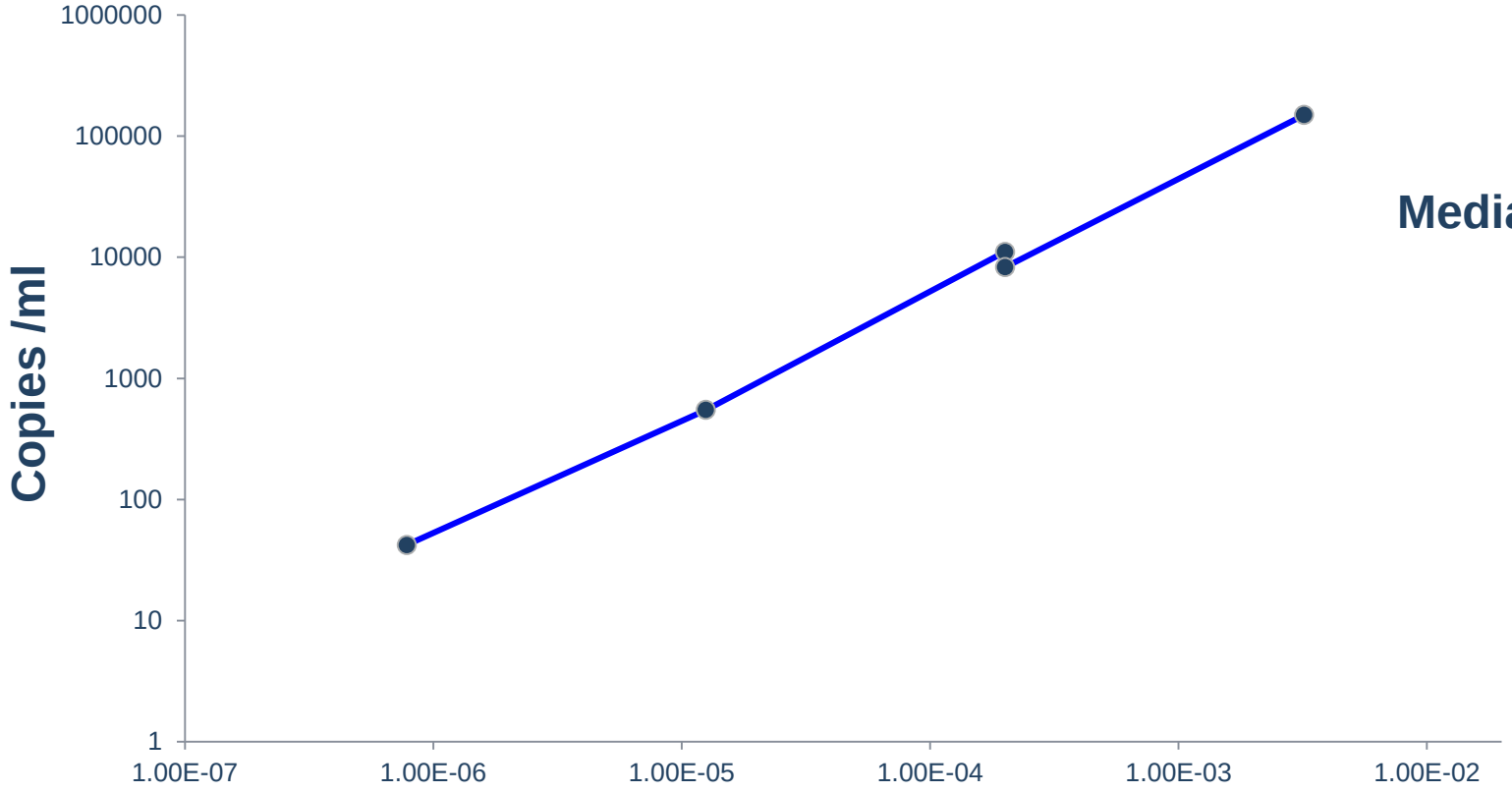
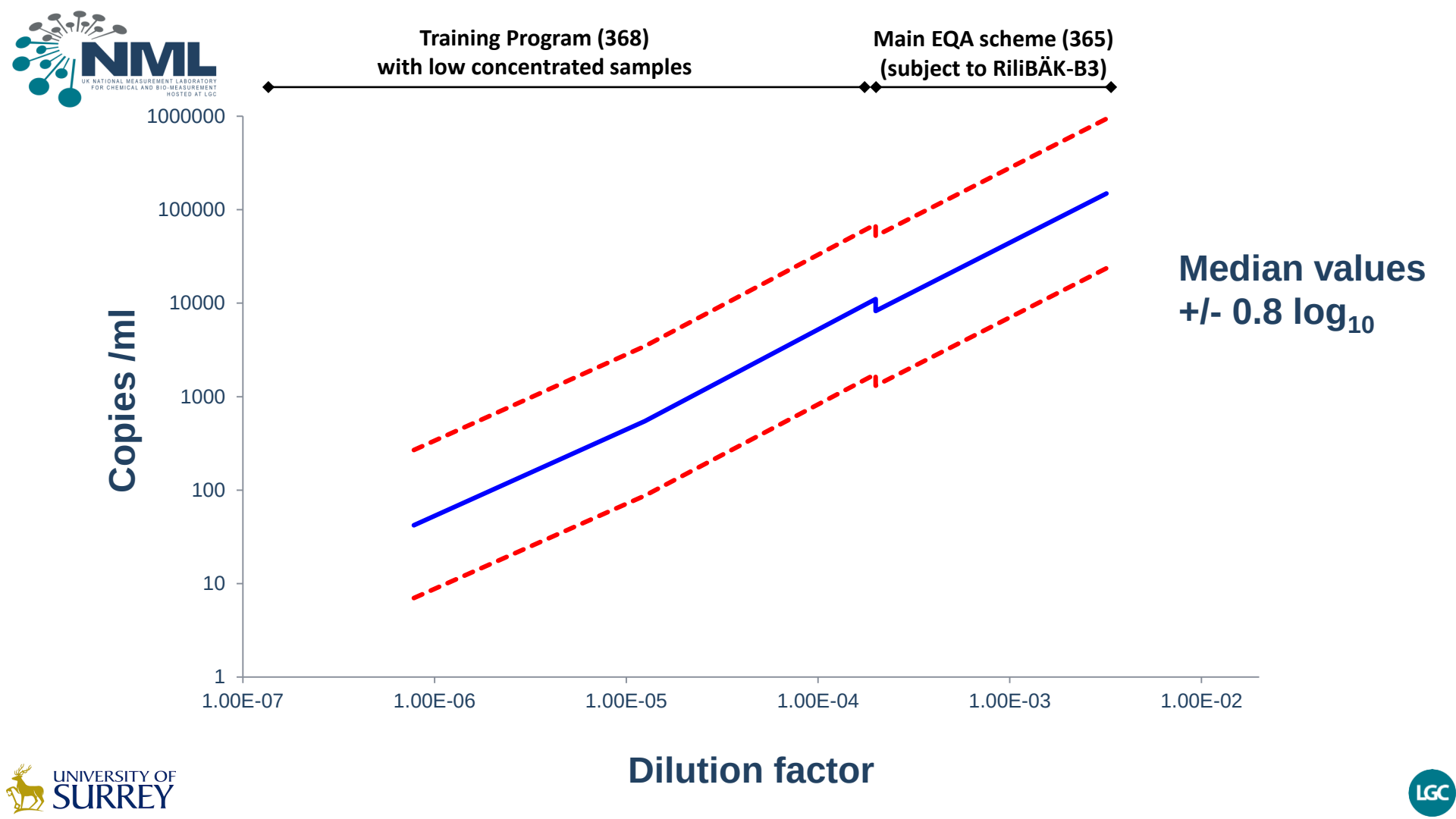
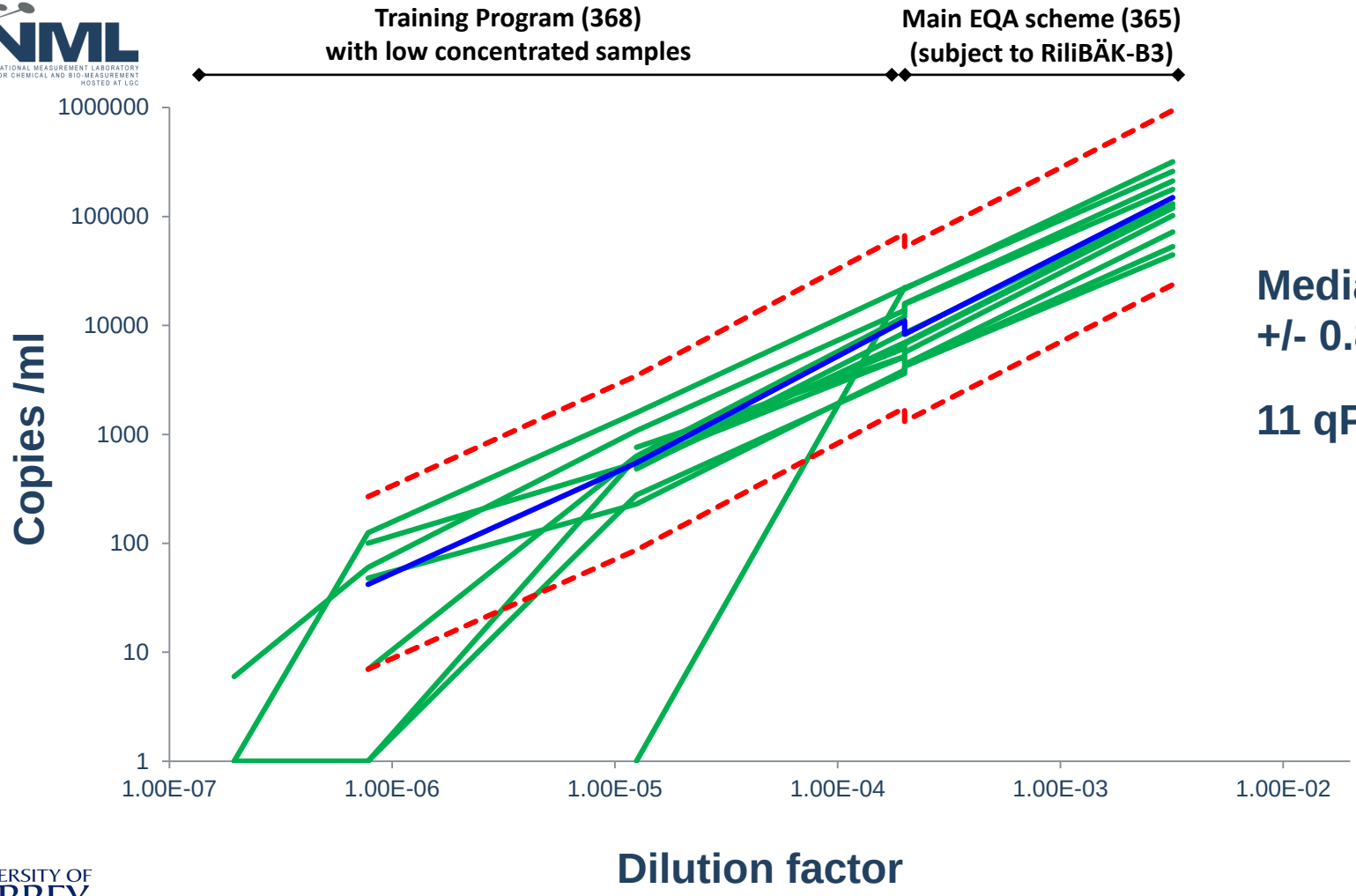


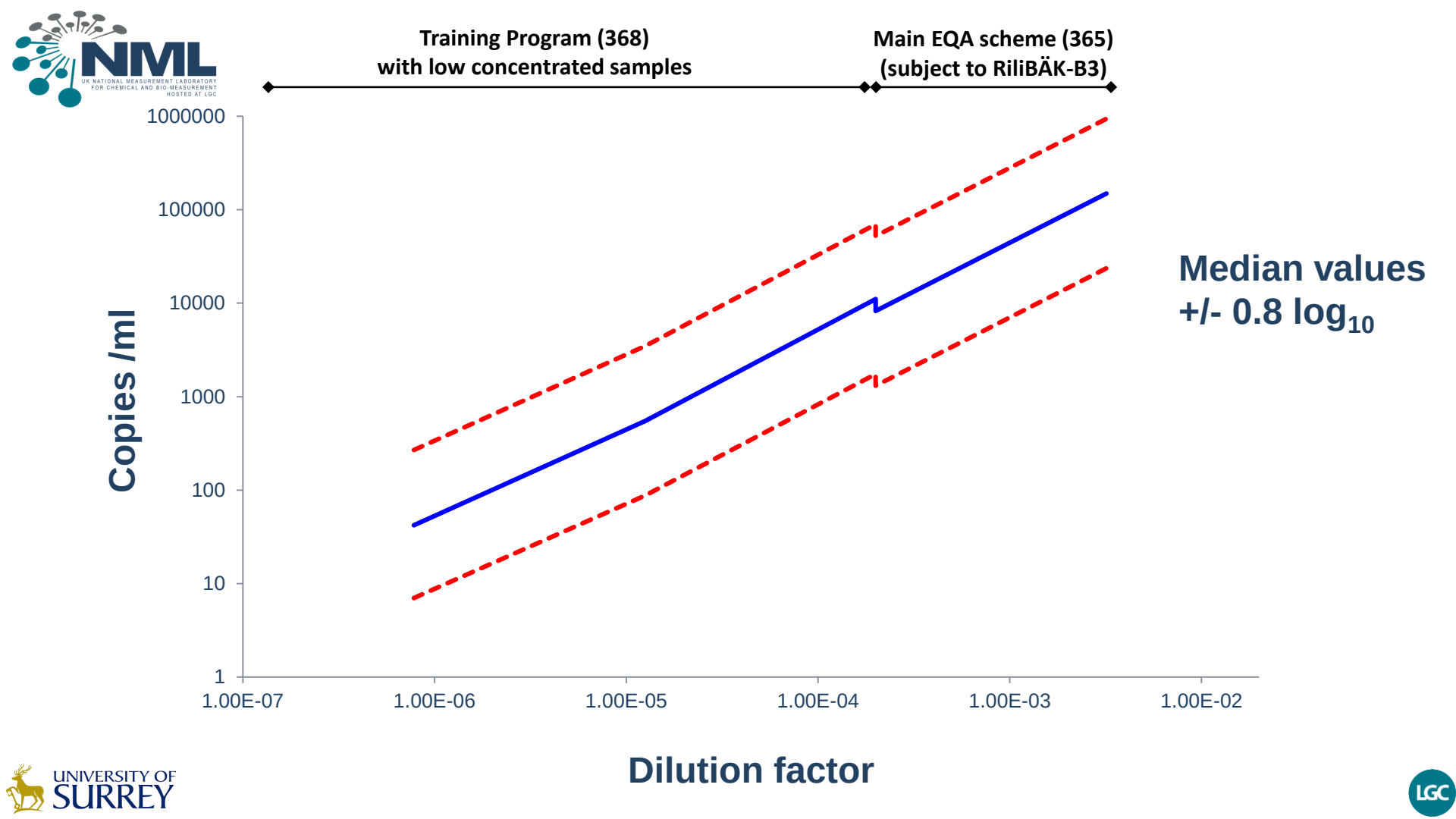
Median values


Training Program (368)
with low concentrated samples
Main EQA scheme (365)
(subject to RiliBÄK-B3)
1000000
100000
10000
1000
100
10
1
Copies /ml
1.00E-07
1.00E-06
1.00E-05
1.00E-04
1.00E-03
1.00E-02
Dilution factor
Median values
+/- 0.8 log10



Median values
+/- 0.8 $\log_{10}$

11 qPCR results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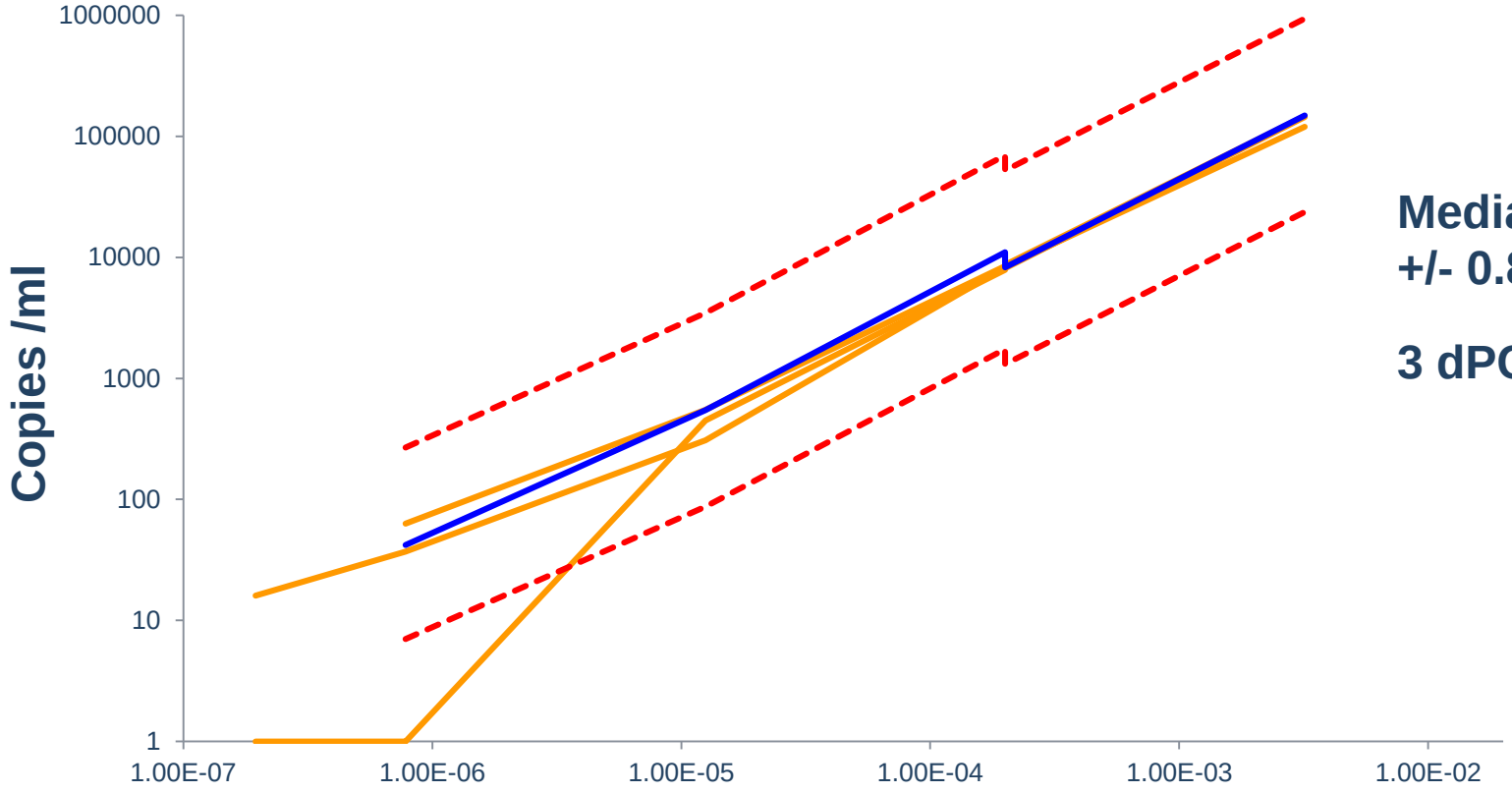


Median values
+/- 0.8 $\log_{10}$

3 dPCR results

Participation in INSTAND hCMV EQA schemes since 2014

Number	Scheme Date	Date Performed	INSTAND EQA Schemes	Project	dPCR Participants
1	Sep-14	Sep-14	365, 368	InfectMet	LGC, NIB, JRC
2	Nov-14	Dec-14	365, 994	InfectMet	LGC, NIB, JRC
3	N/A	Jun-16	N/A	NA	LGC
4	Nov-16	Oct-17	349	AMR	NIB
5	Mar-17	Jun-17	365, 368	AMR	LGC, NIB
6	Jun-17	Sep-17	365 and 4 other samples	AMR	LGC, NIB
7	Sep-17	Dec-17	365 and 1 other sample	AMR	NIB
8	Nov-17	Dec-17	365	AMR	NIB
9	Mar-18	May-18	365, 368 and 2 other samples	AMR	LGC, NIB, PTB

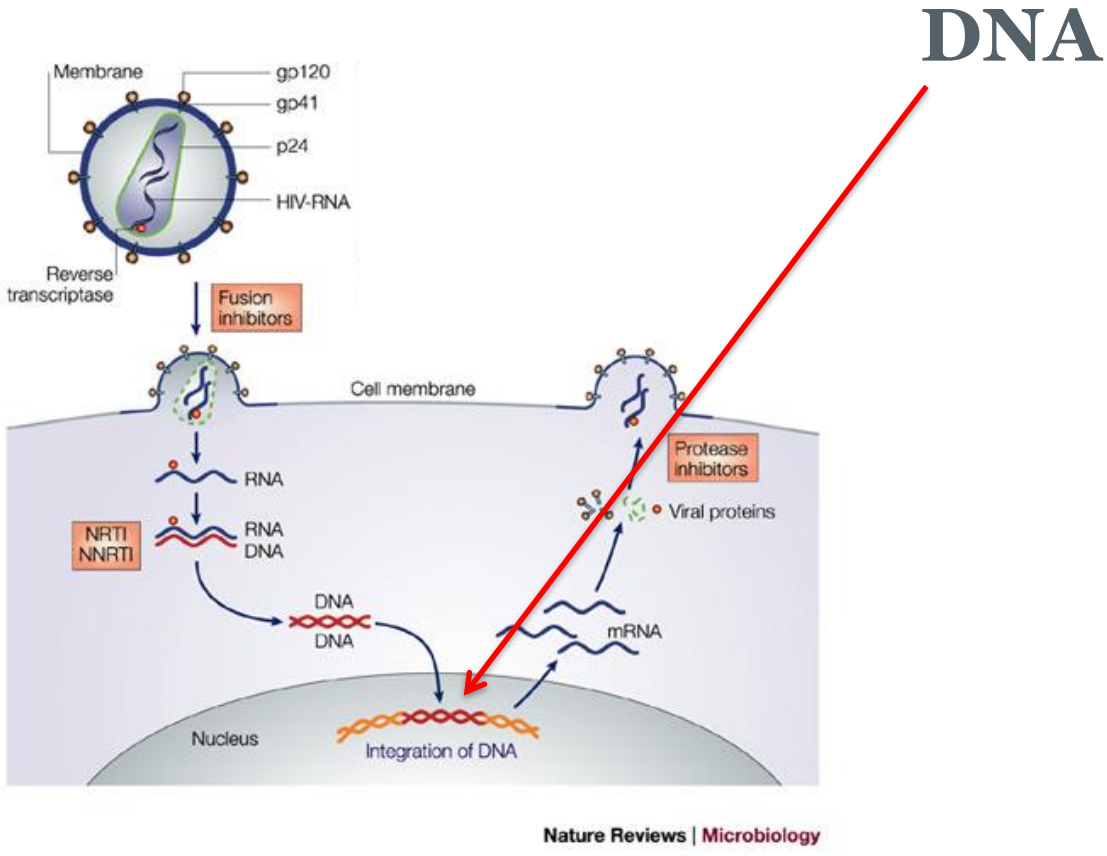
JRC - Belgium (EU)

LGC - UK

NIB - Slovenia

PTB - Germany

The HIV Life Cycle



www.nature.com/scientificreports

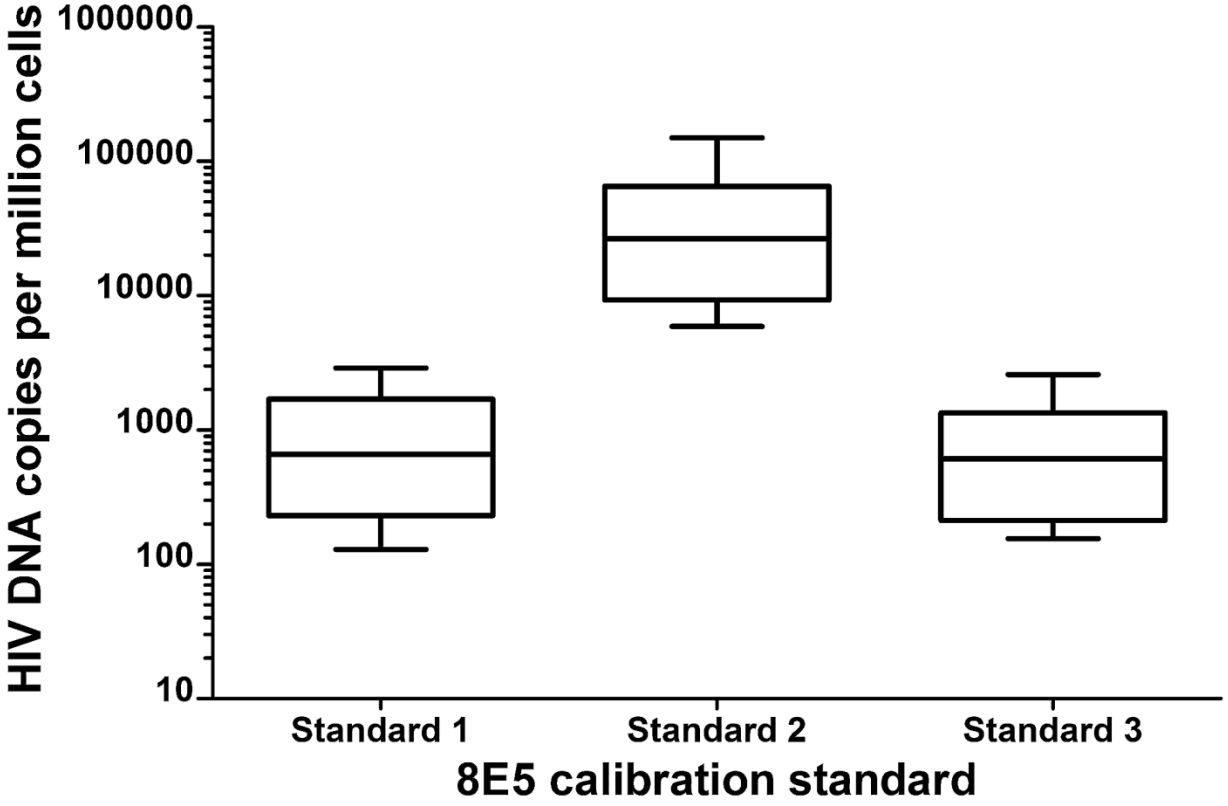
OPEN

Instability of 8E5 calibration standard revealed by digital PCR risks inaccurate quantification of HIV DNA in clinical samples by qPCR

Received: 14 December 2016
Accepted: 22 March 2017
Published online: 26 April 2017

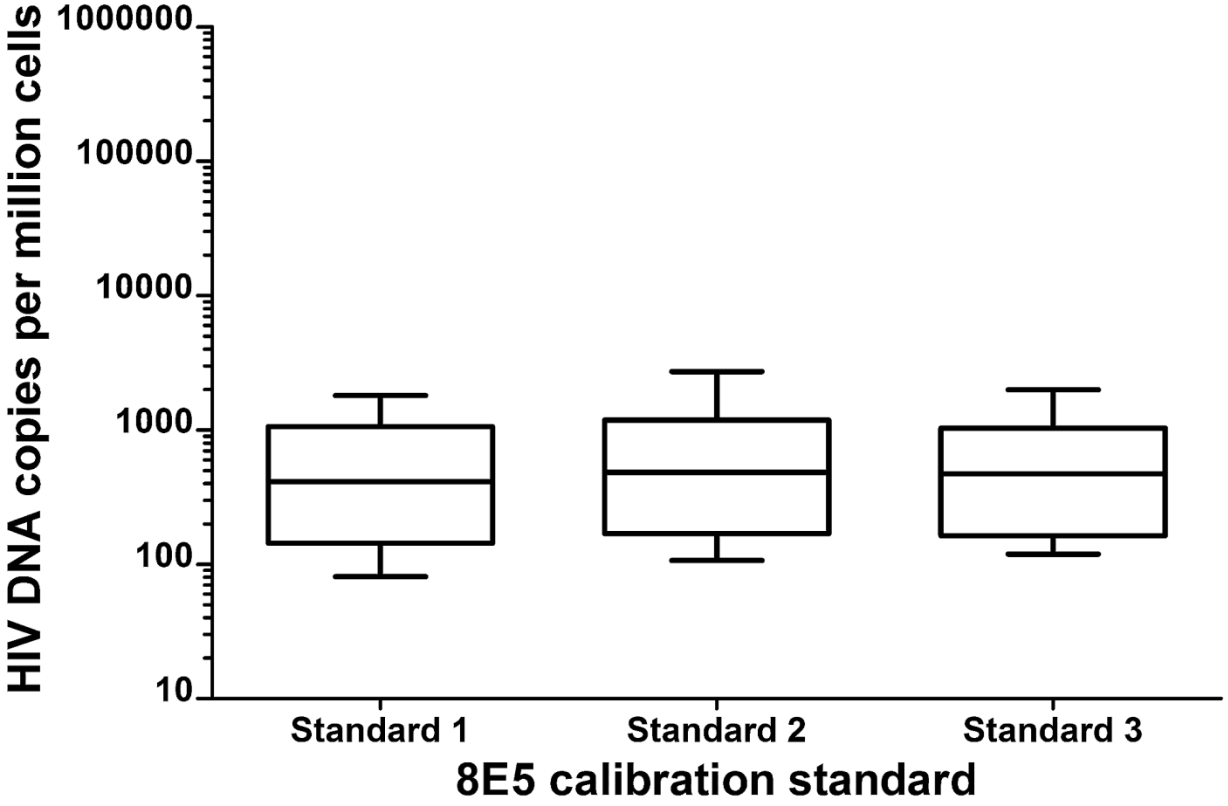
Eloise Busby[1], Alexandra S. Whale[1], R. Bridget Ferns[2], Paul R. Grant[3], Gary Morley[1], Jonathan Campbell[1], Carole A. Foy[1], Eleni Nastouli[3,4], Jim F. Huggett[1,5] & Jeremy A. Garson[2,6]

HIV DNA in HIV +ve/viral load –ve patients



Busby *et al., Nature Scientific Reports 2017*

HIV DNA in HIV +ve/viral load –ve patients

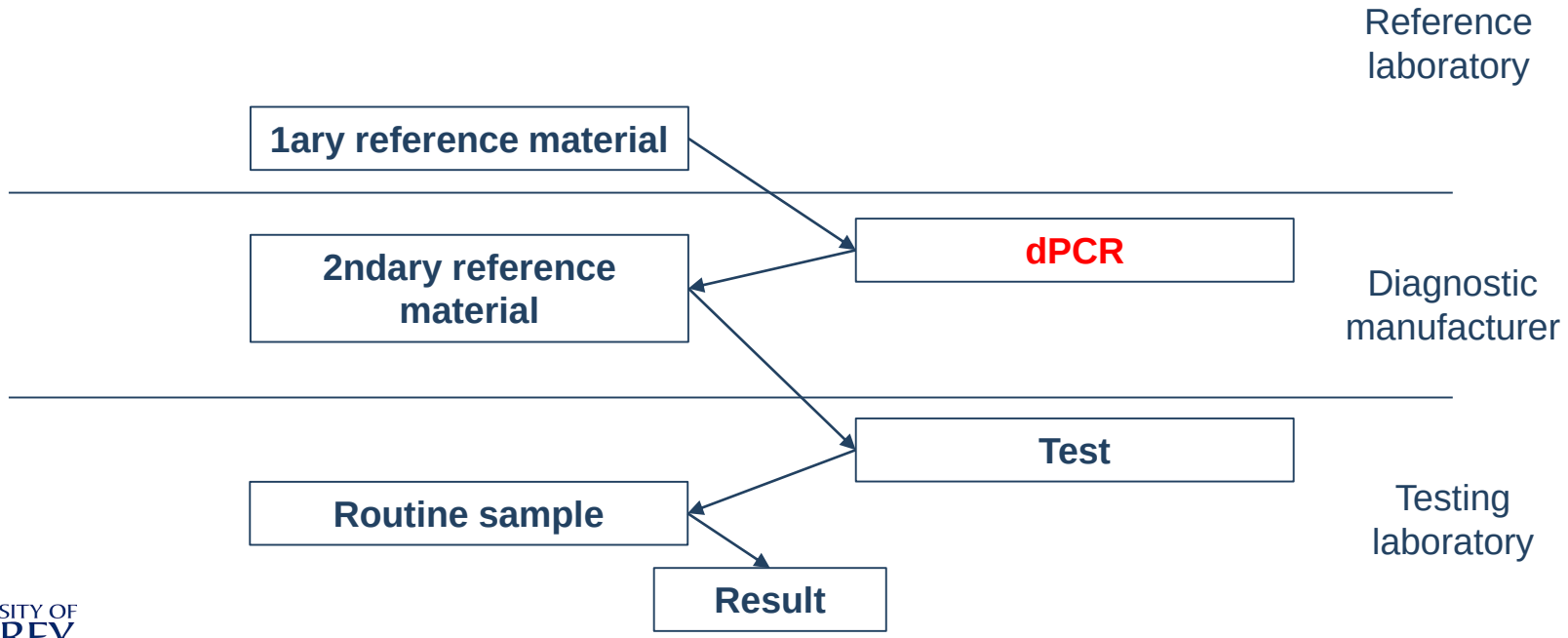


Busby *et al., Nature Scientific Reports 2017*

Molecular measurement

Copies (IU/ml)

Materials ***Measurement Procedures***

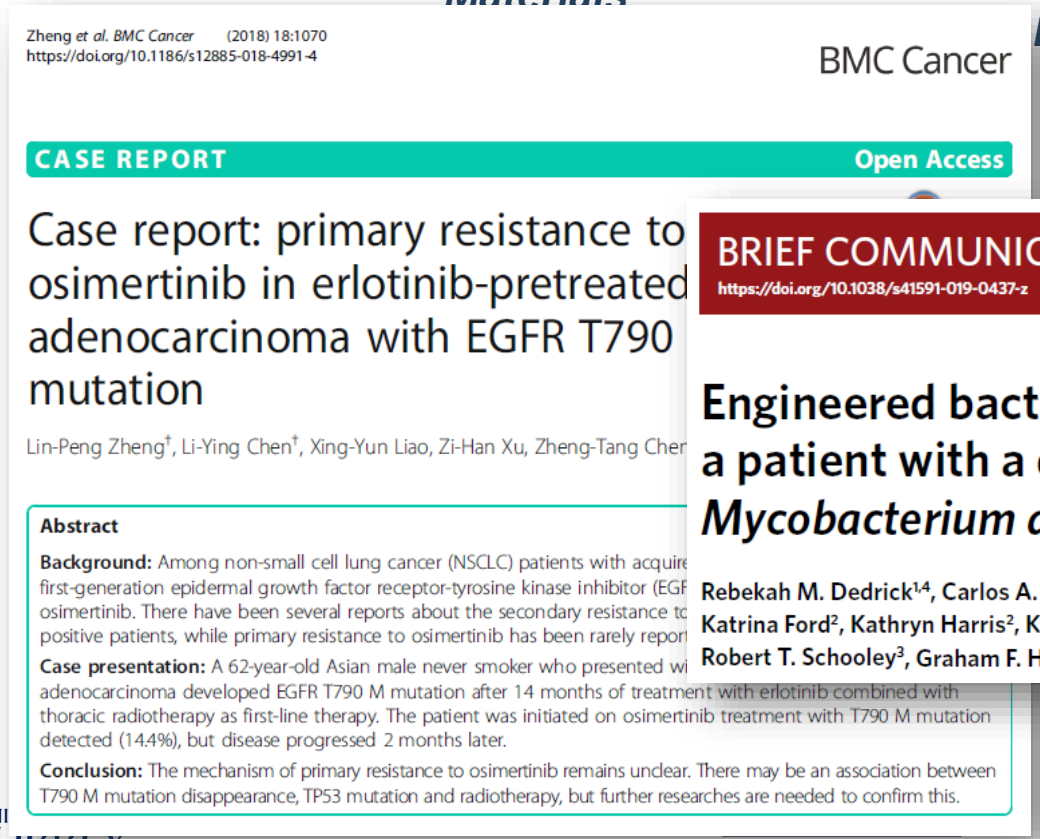


Molecular measurement

Copies (IU/ml)

Materials

Measurement Procedures



Zheng *et al. BMC Cancer* (2018) 18:1070
https://doi.org/10.1186/s12885-018-4991-4

BMC Cancer

CASE REPORT Open Access

Case report: primary resistance to osimertinib in erlotinib-pretreated adenocarcinoma with EGFR T790 mutation

Lin-Peng Zheng†, Li-Ying Chen†, Xing-Yun Liao, Zi-Han Xu, Zheng-Tang Chen

Abstract

Background: Among non-small cell lung cancer (NSCLC) patients with acquire first-generation epidermal growth factor receptor-tyrosine kinase inhibitor (EGF osimertinib. There have been several reports about the secondary resistance to positive patients, while primary resistance to osimertinib has been rarely report

Case presentation: A 62-year-old Asian male never smoker who presented wi adenocarcinoma developed EGFR T790 M mutation after 14 months of treatment with erlotinib combined with thoracic radiotherapy as first-line therapy. The patient was initiated on osimertinib treatment with T790 M mutation detected (14.4%), but disease progressed 2 months later.

Conclusion: The mechanism of primary resistance to osimertinib remains unclear. There may be an association between T790 M mutation disappearance, TP53 mutation and radiotherapy, but further researches are needed to confirm this.

BRIEF COMMUNICATION
https://doi.org/10.1038/s41591-019-0437-z

nature medicine

Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*

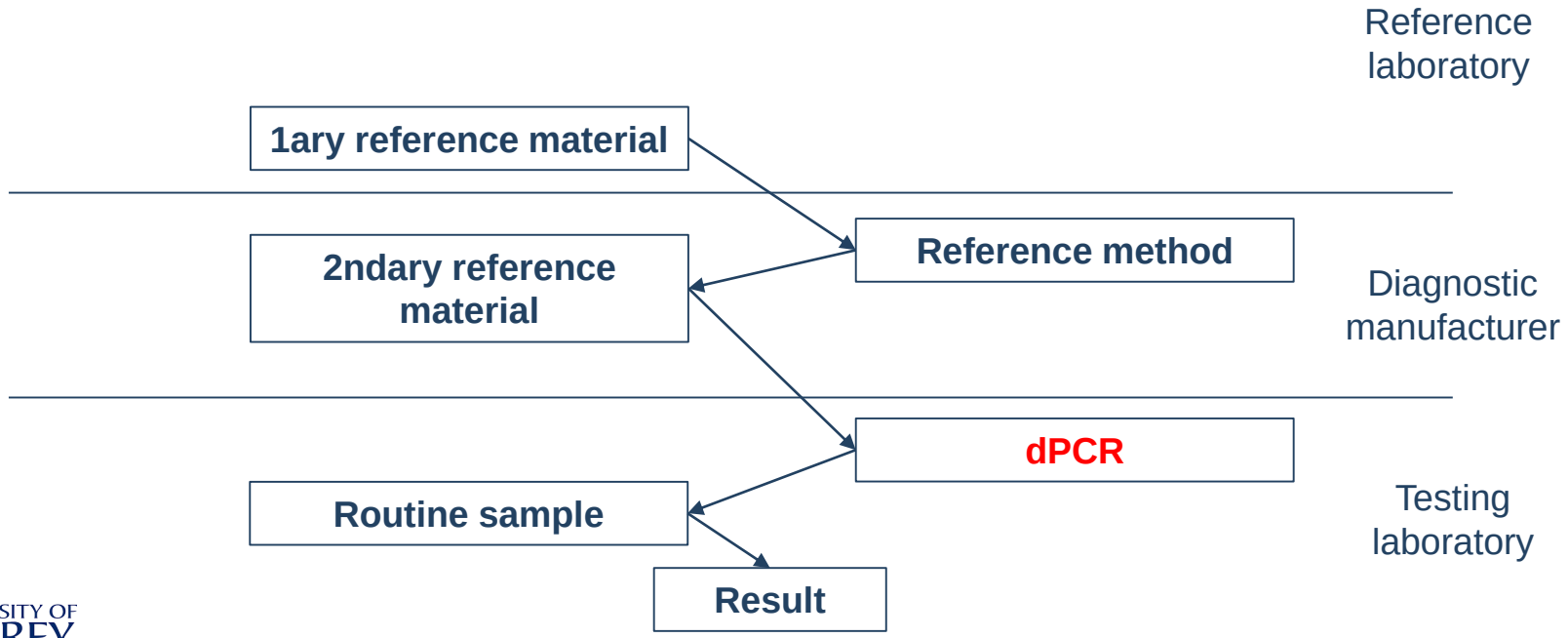
Rebekah M. Dedrick[1,4], Carlos A. Guerrero-Bustamante[1,4], Rebecca A. Garlena[1], Daniel A. Russell[1], Katrina Ford[2], Kathryn Harris[2], Kimberly C. Gilmour[2], James Soothill[2], Deborah Jacobs-Sera[1], Robert T. Schooley[3], Graham F. Hatfull[1]* and Helen Spencer[2]*

Testing laboratory

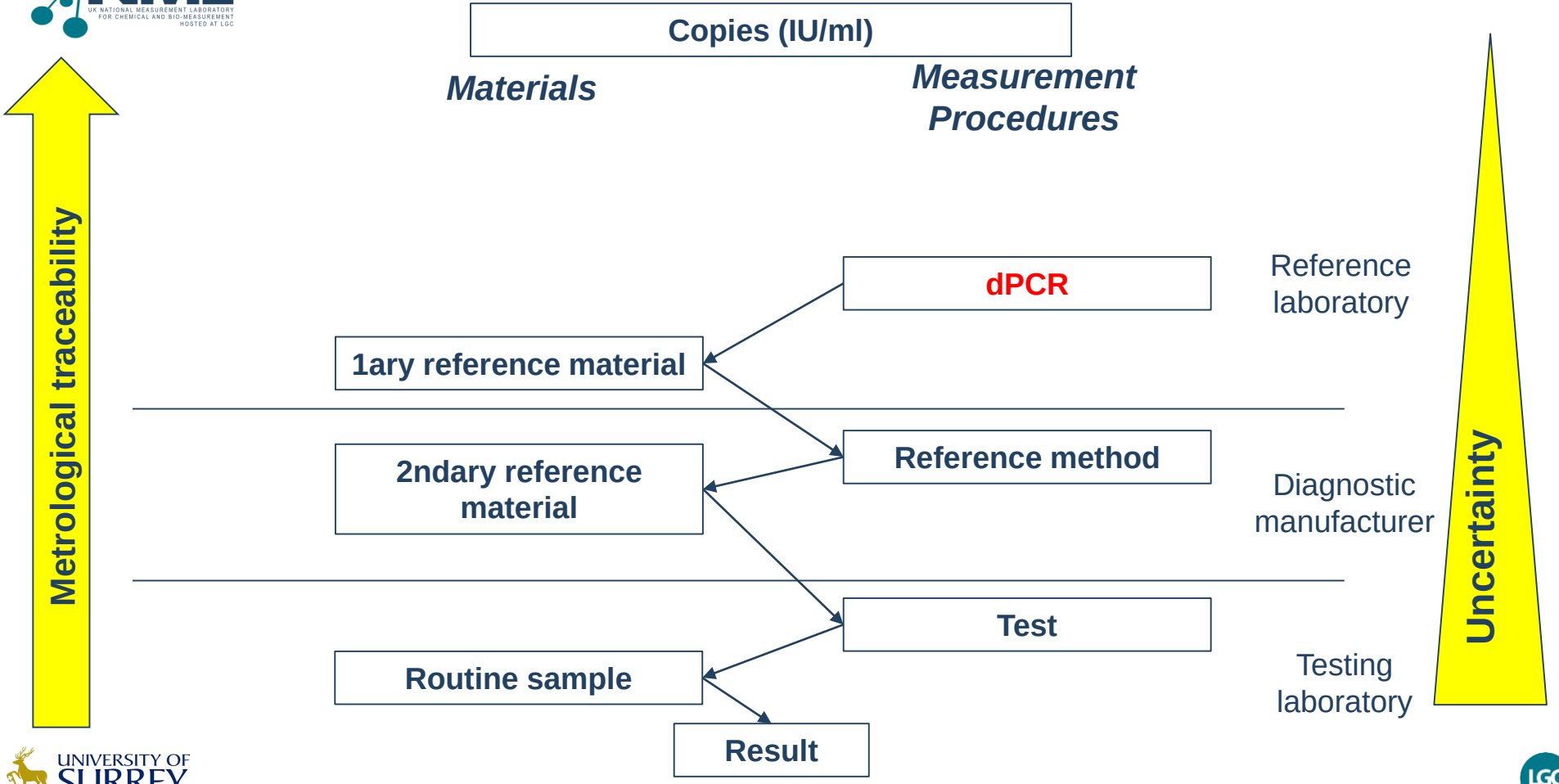
Molecular measurement

Copies (IU/ml)

Materials ***Measurement Procedures***



Molecular measurement



traceability

analytical chemistry

Article

pubs.acs.org/ac

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,[*,†,‡] Lianhu
Mojca Milavec,[∥] Muslum Akgoz,[⊥] Erkan Mozioğl
Janaina J. de V. Cavalcante,[▽] Roberto Becht Flat
Jacob McLaughlin,[○] Kerry Emslie,[*,○] Alexandr
Margaret C. Kline,[¶] Jo Lynne Harenza,[¶] and Pet

tary reference material

analytical chemistry

Article

pubs.acs.org/ac

Highly Reproducible Absolute Quantification of *Mycobacterium tuberculosis* Complex by Digital PCR

shire,[†] Isobella Honeyborne,[‡] Alice Gutteridge,[†,∥] Alexandra S. Whale,[†] Gavin Nixon,[†]
Gerwyn Jones,[†] Timothy D. McHugh,[‡] Carole A. Foy,[†] and Jim F. Huggett[*,†,‡]

Biology Team, LGC, Teddington, Middlesex TW11 0LY, United Kingdom
Microbiology, Department of Infection, Royal Free Campus, University College London, London NW3 2PF,
C, Teddington, Middlesex TW11 0LY, United Kingdom

Clinical Chemistry 64:9
1296–1307 (2018)

Special Report

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] Müslüm Akgöz,[4] Carla Divieto,[5] Maria Paola Sassi,[5] Hua-Jun He,[6] Kenneth D. Cole,[6]
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*†] and Alison S. Devonshire[1*†]

Test

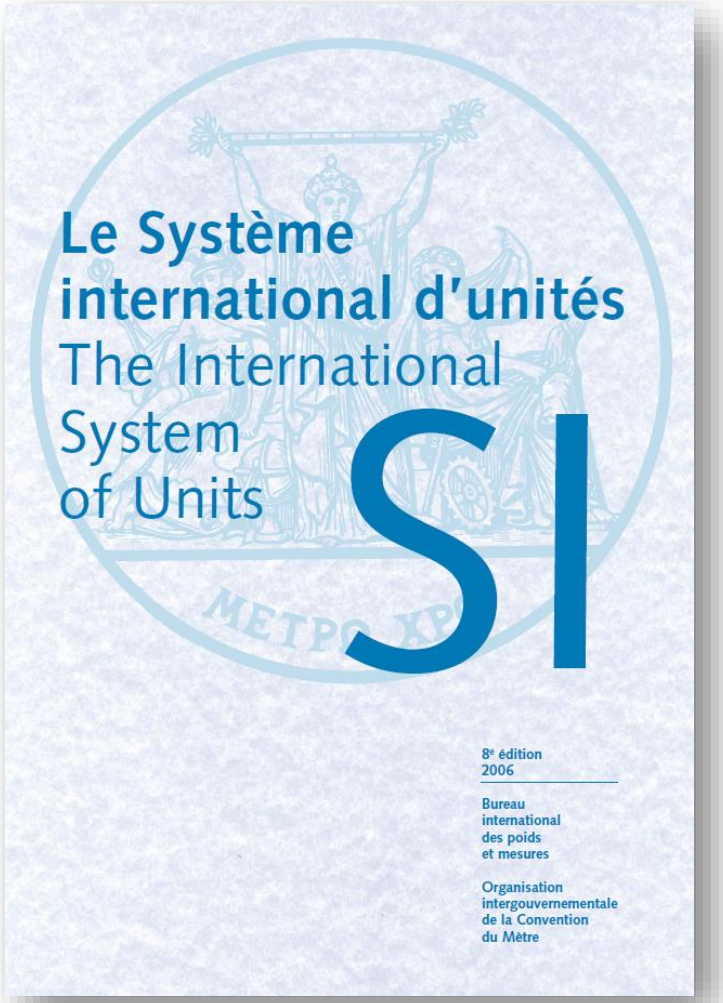
Testing laboratory

Uncer

SURREY

LGC

Le Système International d'Unités (SI)


Le Système international d'unités
The International System of Units
SI
8e édition
2006
Bureau international des poids et mesures
Organisation intergouvernementale de la Convention du Mètre

2.2.3 Units for dimensionless quantities, also called quantities of dimension one

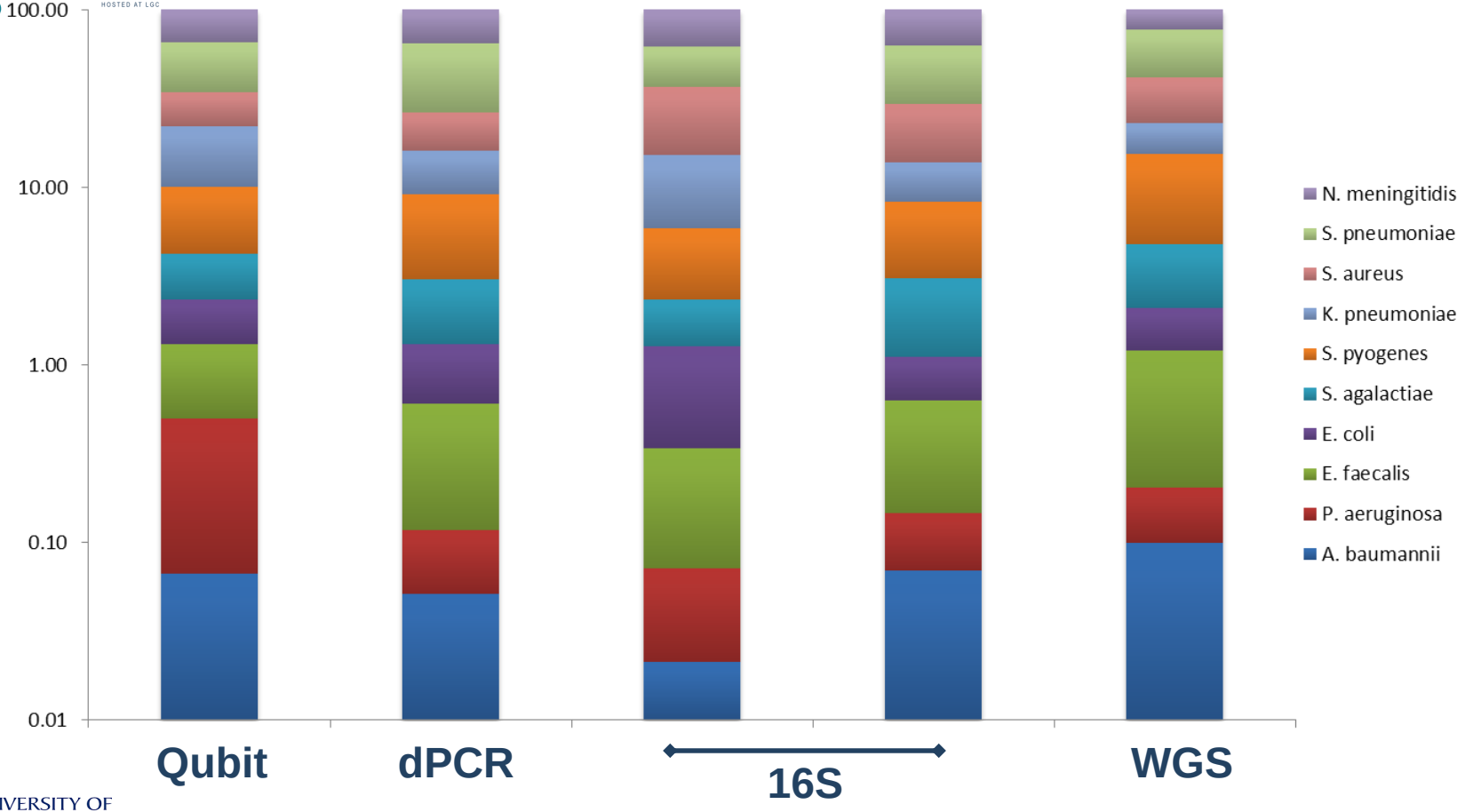
"Another class of dimensionless quantities are numbers that represent a count, such as a number of molecules, degeneracy (number of energy levels), and partition function in statistical thermodynamics (number of thermally accessible states). All of these counting quantities are also described as being dimensionless, or of dimension one, and are taken to have the SI unit one, although the unit of counting quantities cannot be described as a derived unit expressed in terms of the base units of the SI. For such quantities, the unit one may instead be regarded as a further base unit".

SI brochure 8th edn.

dPCR as a reference measurement procedure for viral detection and quantification

- dPCR offers high accuracy (SI traceable) quantitative measurement with unprecedented reproducibility
- With further development dPCR may offer an important alternative to current molecular methods
- dPCR could be used as a reference measurement procedure to support the use of other molecular methods

Wider microbial molecular measurement: Metagenomics



Int. J. Mol. Sci. 2014, 15, 21476-21491; doi:10.3390/ijms151121476

Acknowledgements

Department for Business, Energy & Industrial Strategy

FUNDED BY BEIS

CCQM NAWG

Bureau International des Poids et Mesures

EMPIR EURAMET

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

NML

- Alison Devonshire
- Alexandra Whale
- Denise O'Sullivan
- Simon Cowen
- Helen Parkes
- Carole Foy
- Alison Woolford

NIST

- Peter Vallone
- Kenneth Cole
- Megan Cleveland

GBD

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

NIBSC

- Neil Almond
- Clare Morris

UCL (Hospitals)

- Eleni Nastouli
- Tim Mchugh
- Jeremy Garson

PTB

- Andreas Kummrow
- Annabell Plauth

NIB

- Tania Dreo
- Mojca Milavec
- Jernej Pavšič

Anti Micro Resist

Bio SITrace

Infect-met

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Thank you

Starts in September 2019

jim.huggett@lgcgroup.com