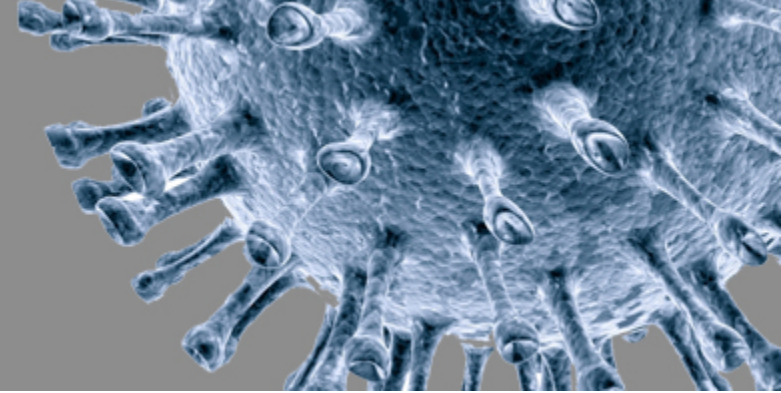




Metrology to support infectious
disease diagnostics
Funded by the European Metrology Research Programme

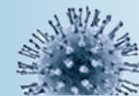
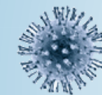
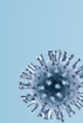


Metrological traceability for Molecular Diagnostics

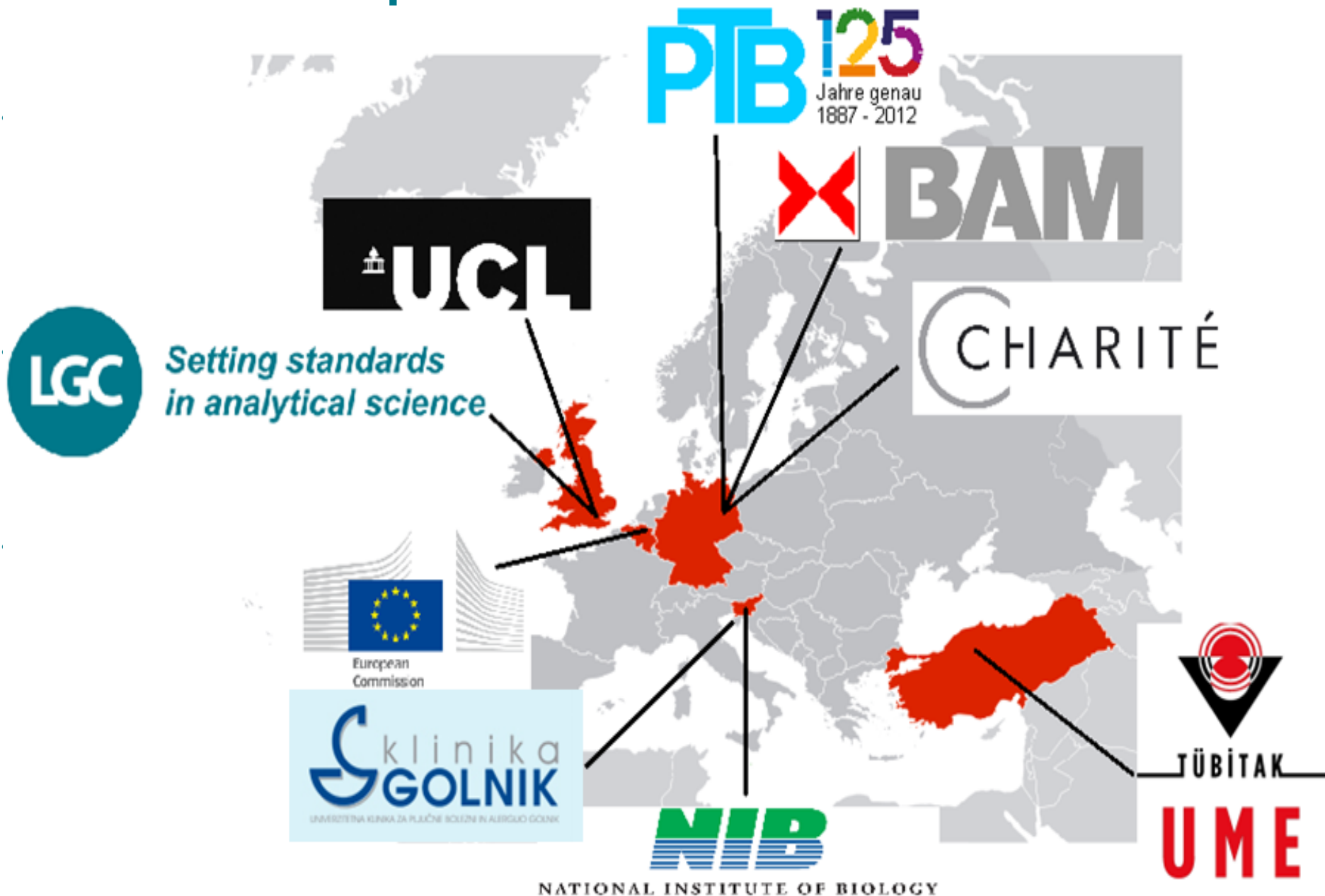
Jim Huggett

Principal Scientist, Nucleic Acid Research
LGC, Queens Road, United Kingdom

INFECTMET: (www.INFECTMET.lgcgroup.com)



INFECTMET partners





**Le Système
international d'unités**
The International
System
of Units

SI

8^e édition
2006

Bureau
international
des poids
et mesures

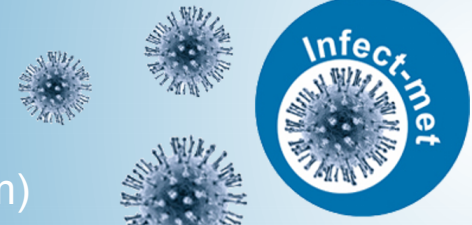
Organisation
intergouvernementale
de la Convention
du Mètre

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.*

WHY NOW?

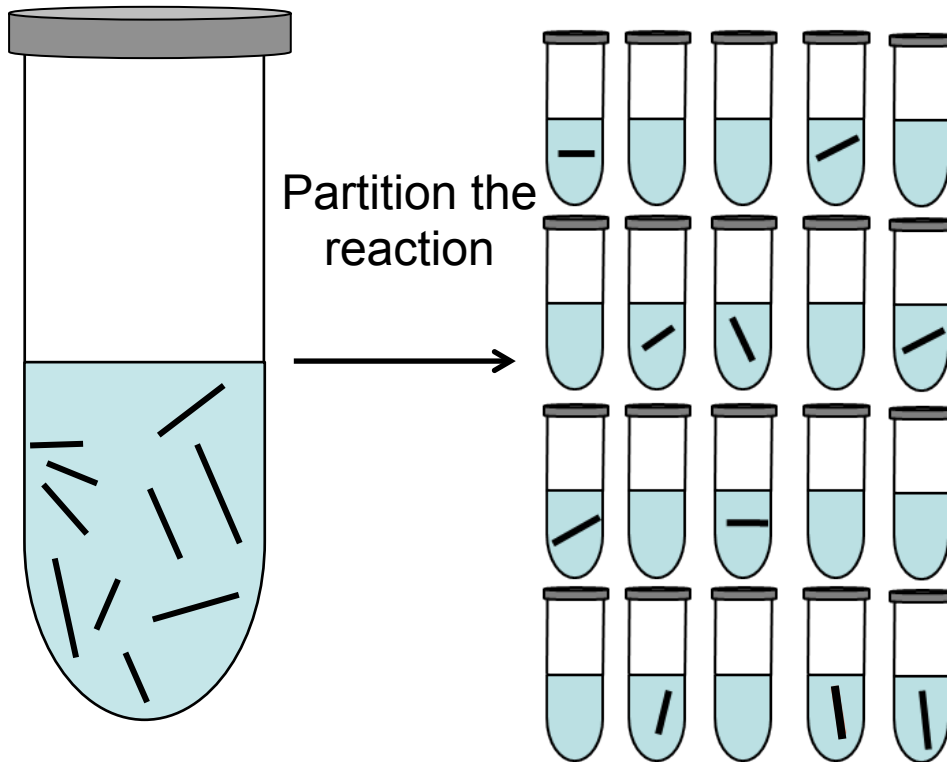
INFECTMET: (www.infectmet.lgcgroup.com)



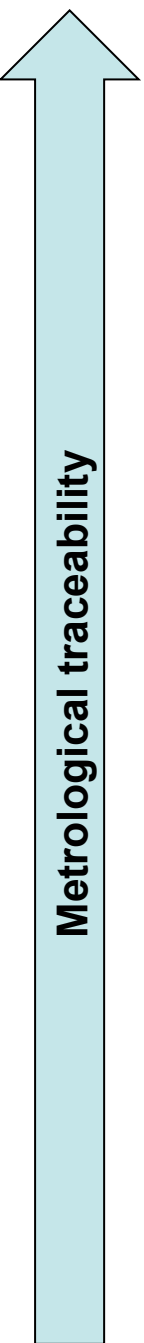
Digital PCR (dPCR)

qPCR 1 × 20 μ l reactions

dPCR 20 × 1 μ l reactions

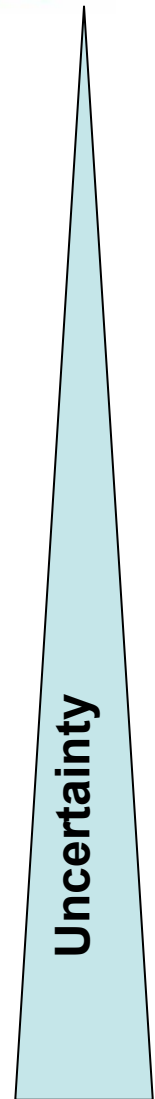
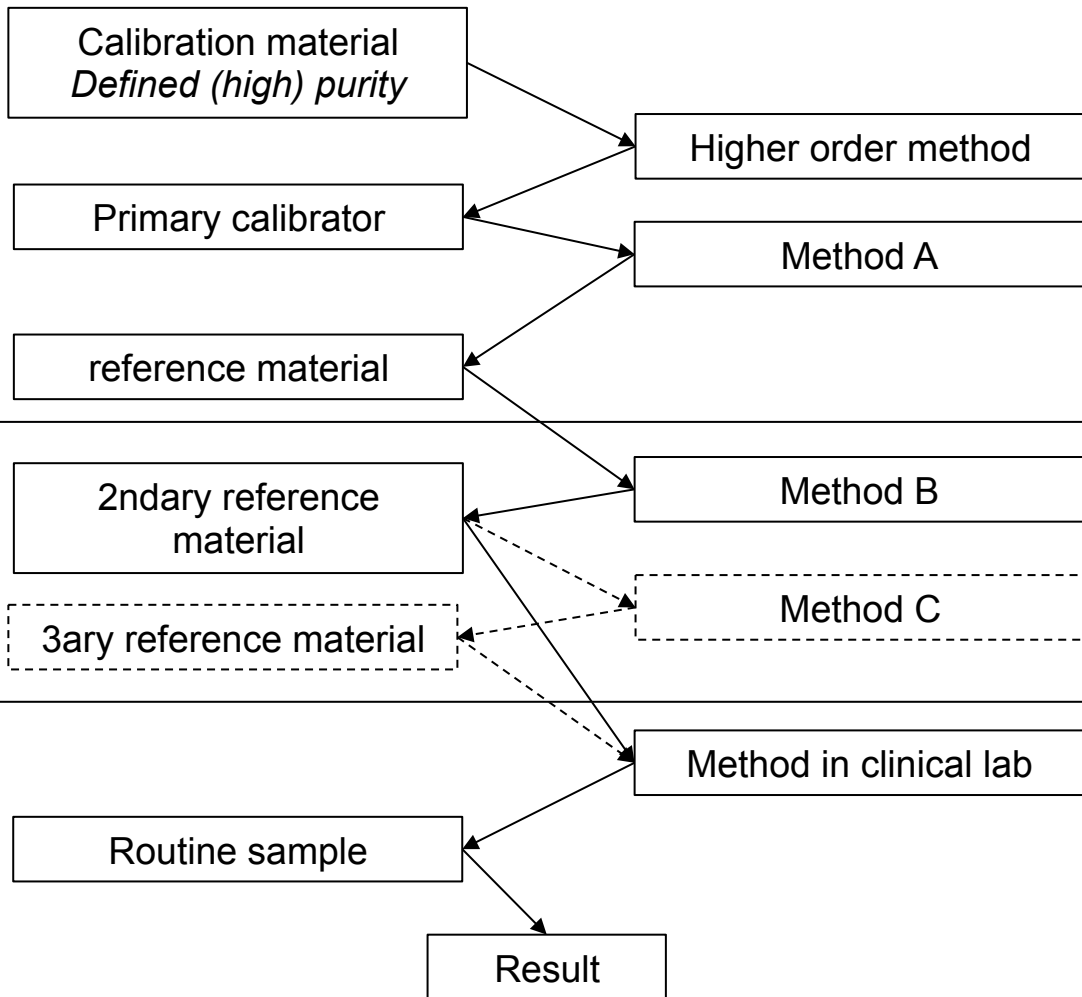


- Limiting dilution
 - Some reaction contain 0 templates
- PCR performed as normal using standard real-time PCR chemistry
- Absolute quantification
 - +ve or –ve reactions
 - Poisson statistics to account for multiple targets per partition (> 1)



Materials

Procedures



Molecular quantification



IU/ml plasma

Materials

Procedures

reference material

2ndary reference material

3ary reference material

qPCR

qPCR

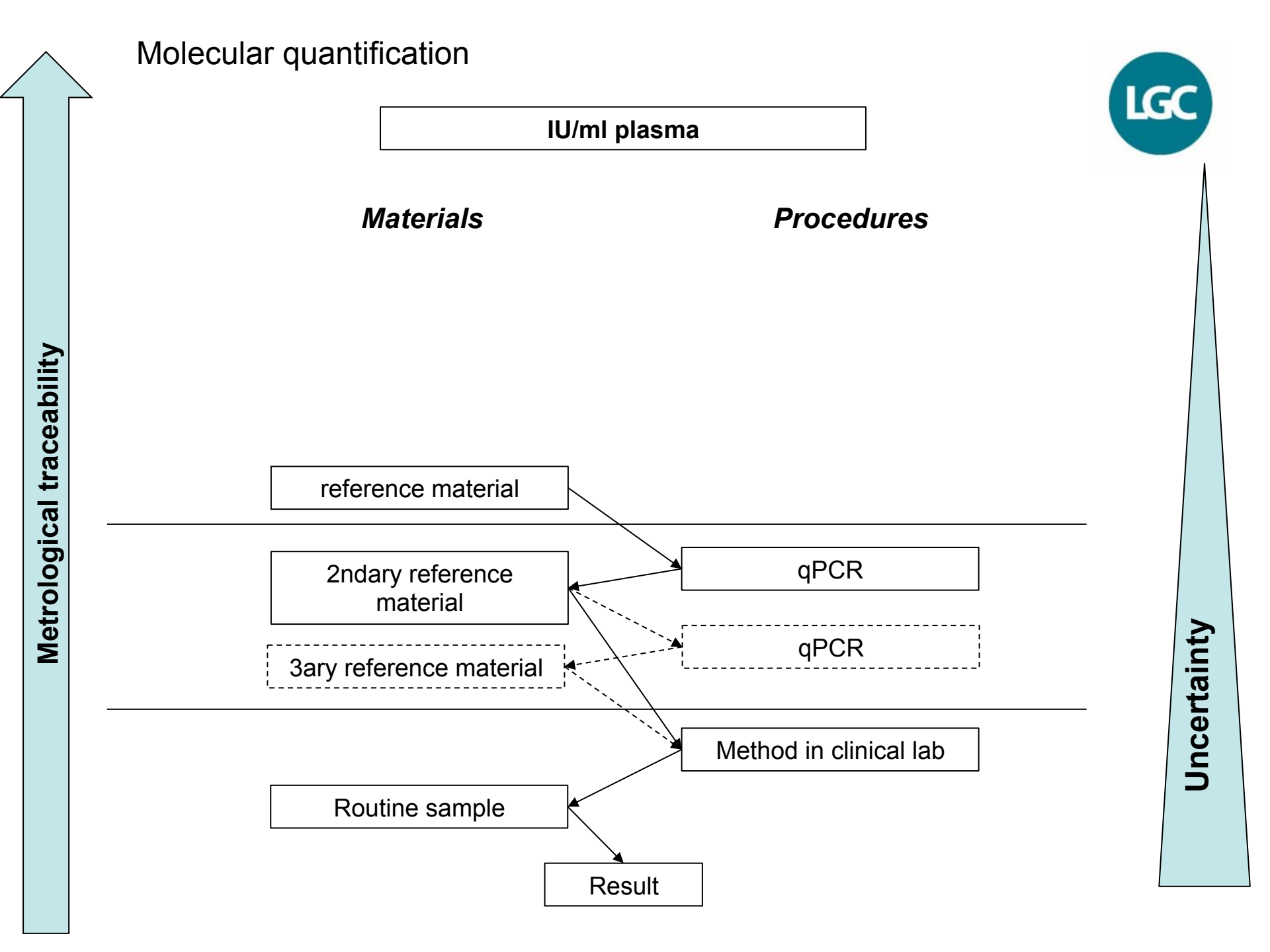
Method in clinical lab

Routine sample

Result

Metrological traceability

Uncertainty



Molecular quantification



Copies

Materials

Procedures

Calibration material
(E.g. dNMPs) *Defined
(high) purity*

Primary calibrator

reference material

2ndary reference material

Weight

Physicochemical method

dPCR

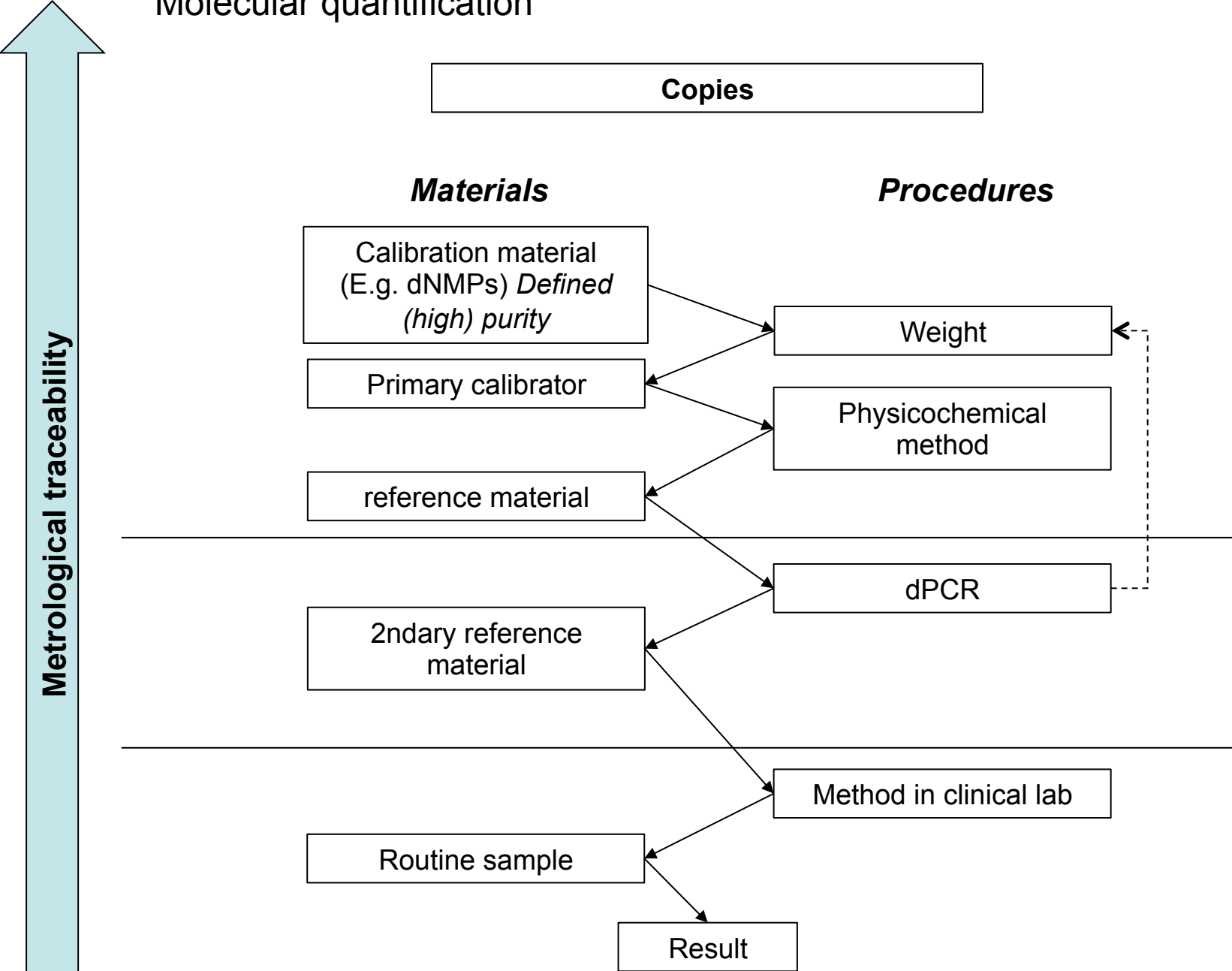
Method in clinical lab

Routine sample

Result

Metrological traceability

Uncertainty





SI traceable via counting

Materials

Procedures

Calibration material
Defined (high) purity

Reference material

2ndary reference material

Routine sample

dPCR

dPCR/qPCR

Method in clinical lab

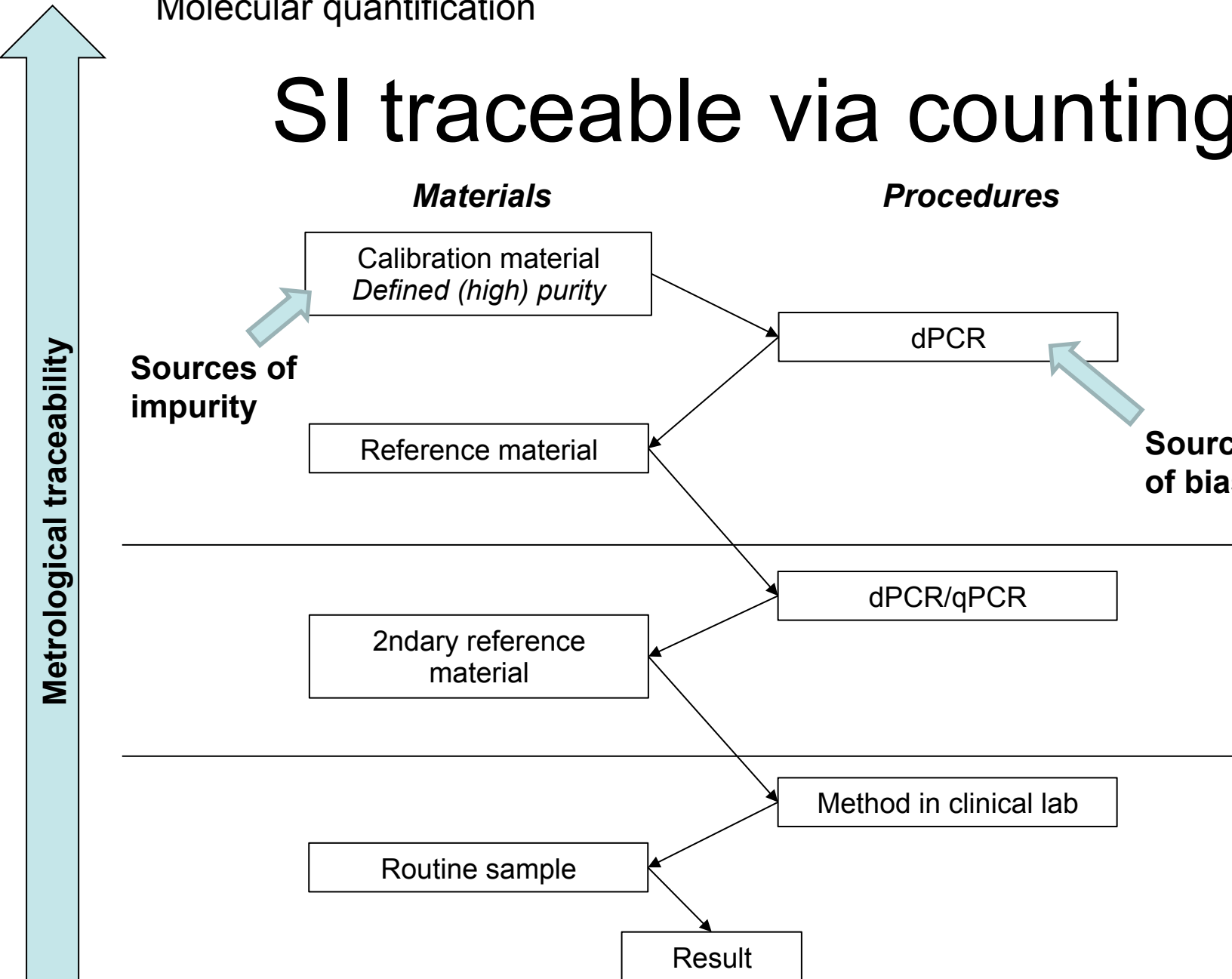
Result

Sources of impurity

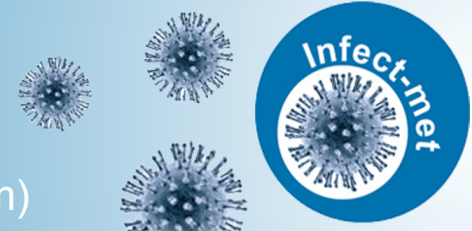
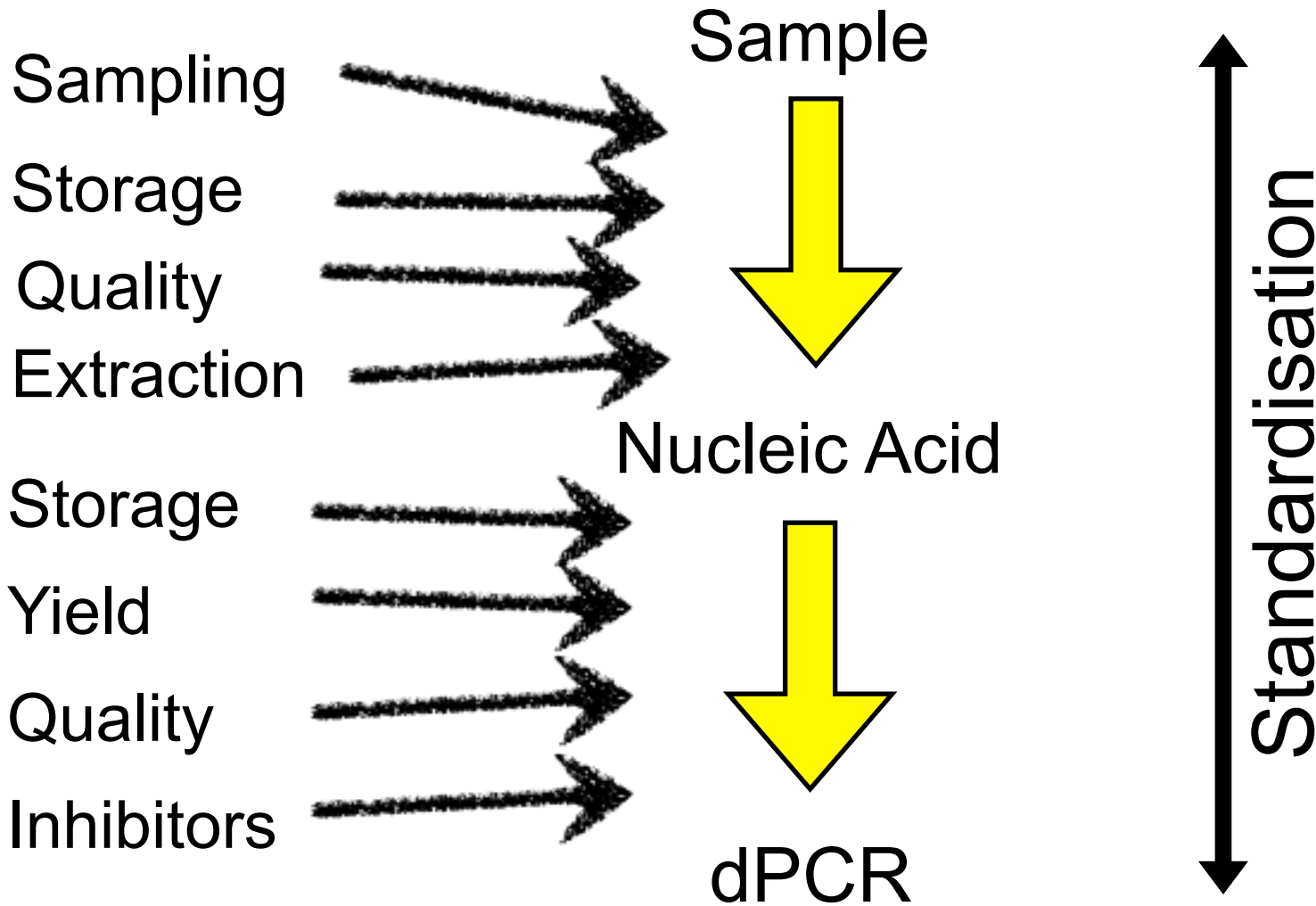
Sources of bias

Metrological traceability

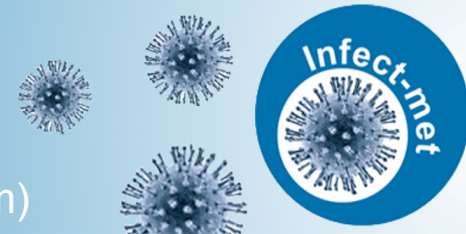
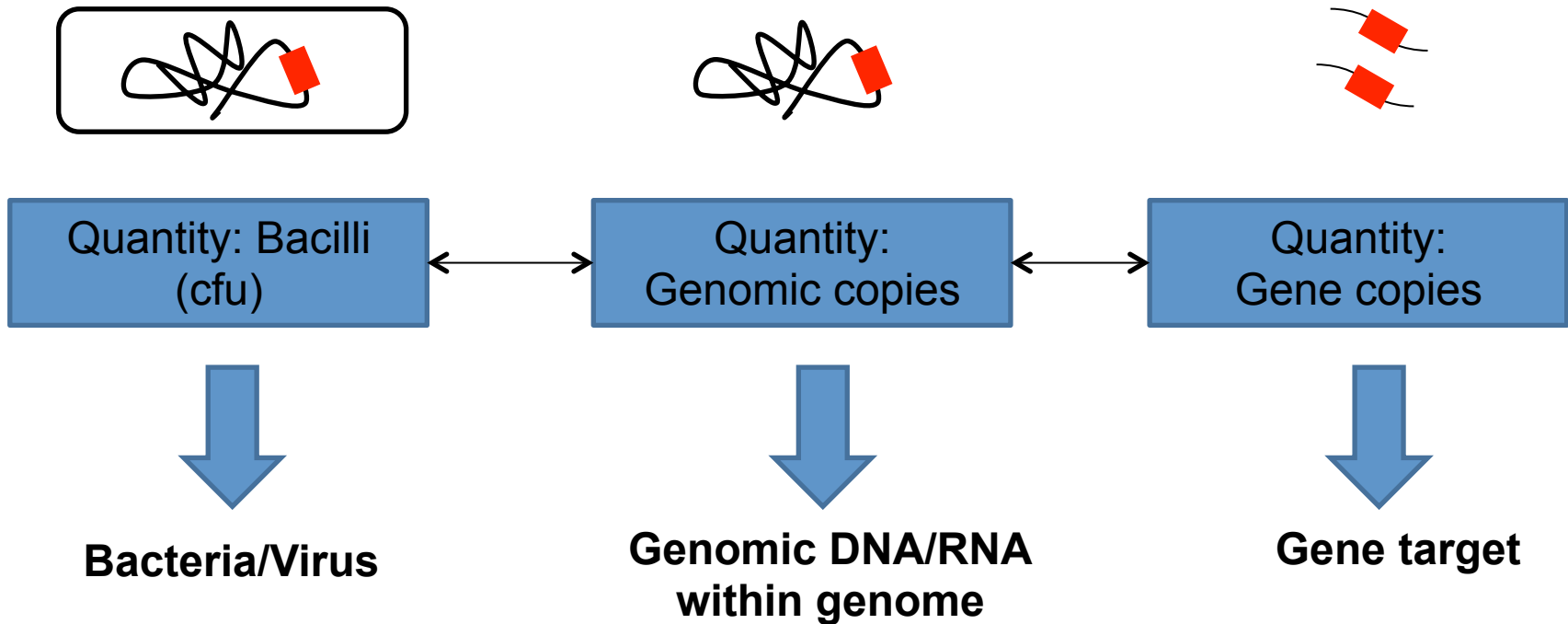
Uncertainty



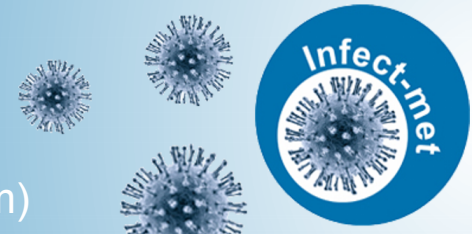
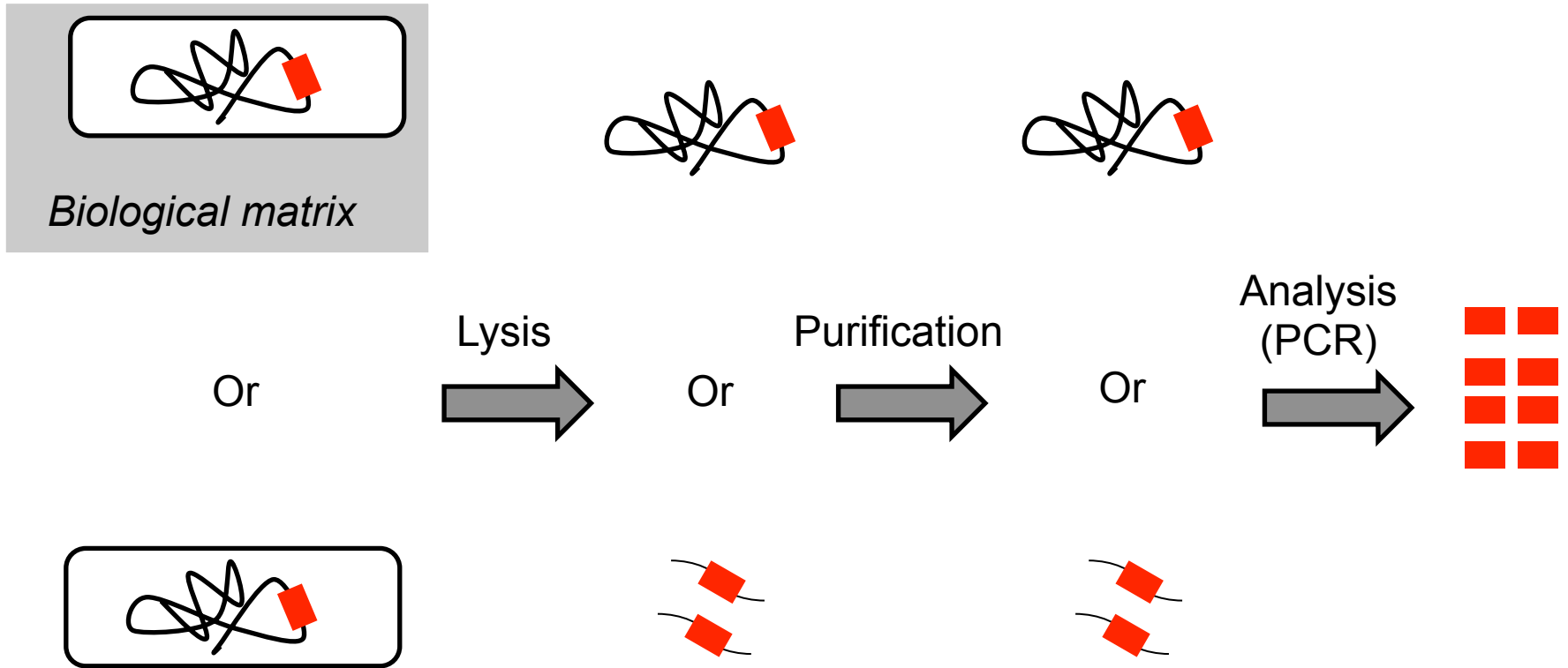
Molecular Analysis



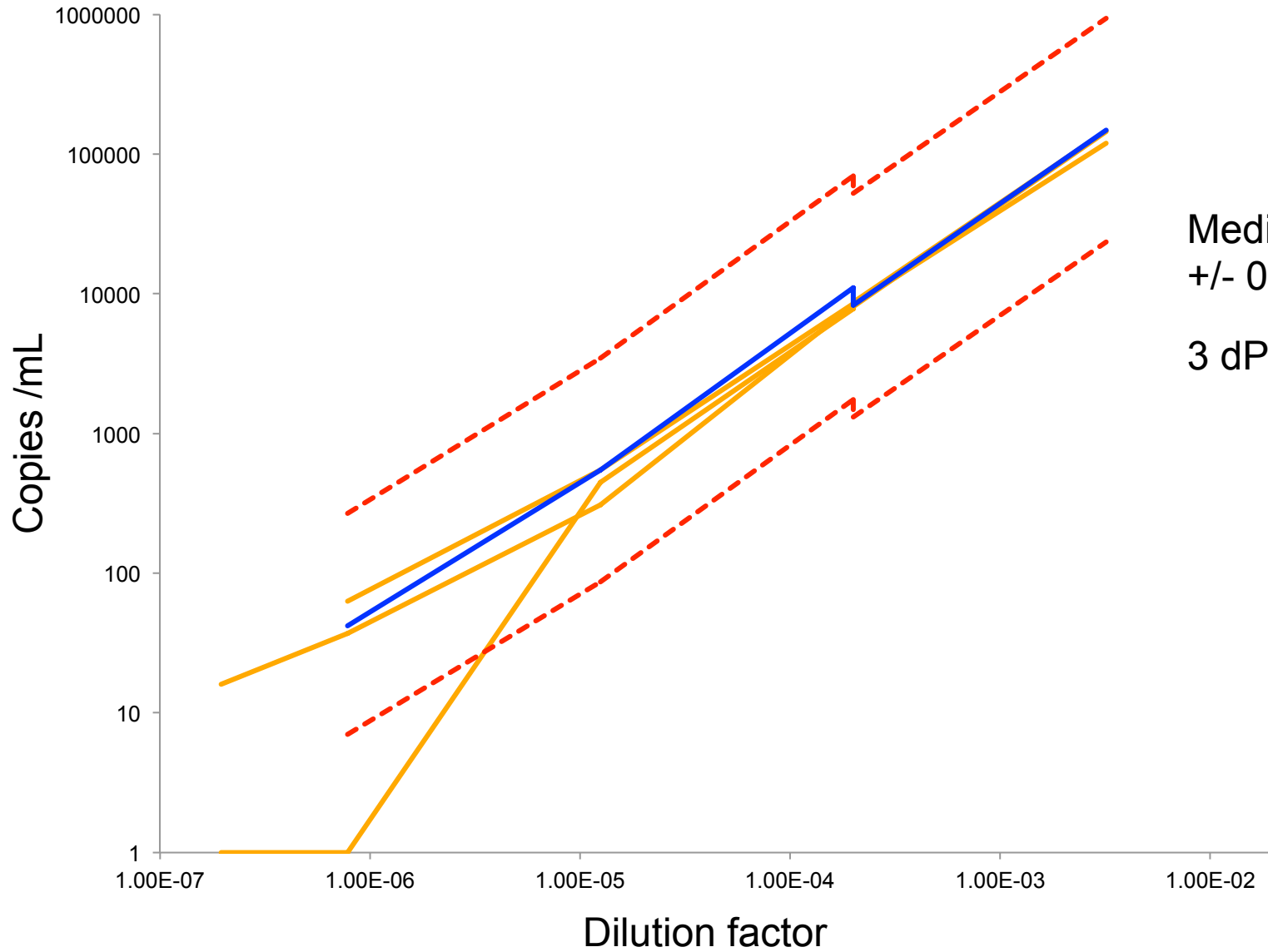
Materials for full analytical workflow



Materials for full analytical workflow

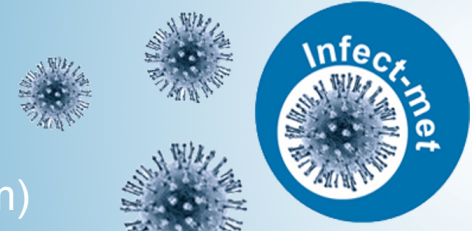


Human Cytomegalovirus



Median values
 $\pm 0.8 \log_{10}$
3 dPCR results

Tuberculosis

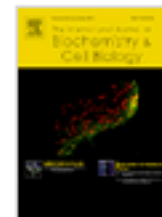




ELSEVIER

The International Journal of Biochemistry & Cell Biology

Volume 35, Issue 10, October 2003, Pages 1407–1412



Medicine in focus

Tuberculosis: amplification-based clinical diagnostic techniques

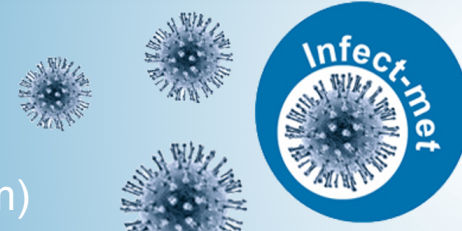
Jim F Huggett^a,  , Timothy D McHugh^{b, 1}, , Alimuddin Zumla^{a, 2}, 

^a Centre for Infectious Diseases, Royal Free and University College Medical School, University College London, Windeyer Building, 46 Cleveland Street, London W1T 4JF, UK

^b Department of Medical Microbiology, Royal Free and University College Medical School, University College London, Royal Free Campus, London NW3 2PF, UK

Available online 8 April 2003

 [Show less](#)



Xpert RIF/MTB

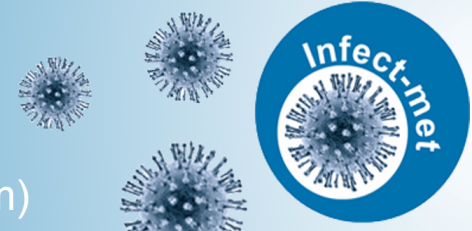




2011

1

Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System

Policy Statement



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

Alison S. Devonshire,[†] Isobella Honeyborne,[‡] Alice Gutteridge,^{†,||} Alexandra S. Whale,[†] Gavin Nixon,[†] Philip Wilson,[§] Gerwyn Jones,[†] Timothy D. McHugh,[‡] Carole A. Foy,[†] and Jim F. Huggett^{*,†,‡}

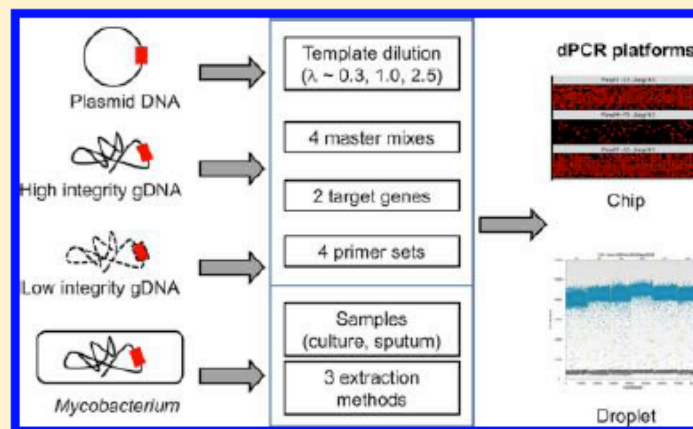
[†]Molecular and Cell Biology Team, LGC, Teddington, Middlesex TW11 0LY, United Kingdom

[‡]Centre for Clinical Microbiology, Department of Infection, Royal Free Campus, University College London, London NW3 2PF, United Kingdom

[§]Statistics Team, LGC, Teddington, Middlesex TW11 0LY, United Kingdom

S Supporting Information

ABSTRACT: Digital PCR (dPCR) offers absolute quantification through the limiting dilution of template nucleic acid molecules and has the potential to offer high reproducibility. However, the robustness of dPCR has yet to be evaluated using complex genomes to compare different dPCR methods and platforms. We used DNA templates from the pathogen *Mycobacterium tuberculosis* to evaluate the impact of template type, master mixes, primer pairs and, crucially, extraction methods on dPCR performance. Performance was compared between the chip (BioMark) and droplet (QX100) formats. In the absence of any external calibration, dPCR measurements were generally consistent within ~2-fold between different master mixes and primers. Template DNA integrity could influence dPCR performance: high molecular



M. bovis BCG in synthetic sputum

CFU data:

Mean: $1.09\text{E}+07 \pm 1.52\text{E}+06$ (14%)

300 units prepared in synthetic sputum.

Molecular homogeneity & Short term stability complete, Long term stability ongoing

Journal of Medical Microbiology (2005), 54, 667–676

DOI 10.1099/jmm.0.45969-0

Correspondence
Ute Römling
Ute.Romling@mtc.ki.se

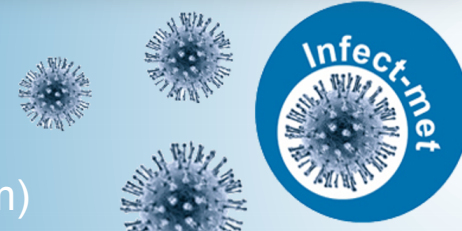
Microcolony formation: a novel biofilm model of *Pseudomonas aeruginosa* for the cystic fibrosis lung

Dinesh D. Sriramulu,^{1,2} Heinrich Lünsdorf,³ Joseph S. Lam⁴
and Ute Römling¹

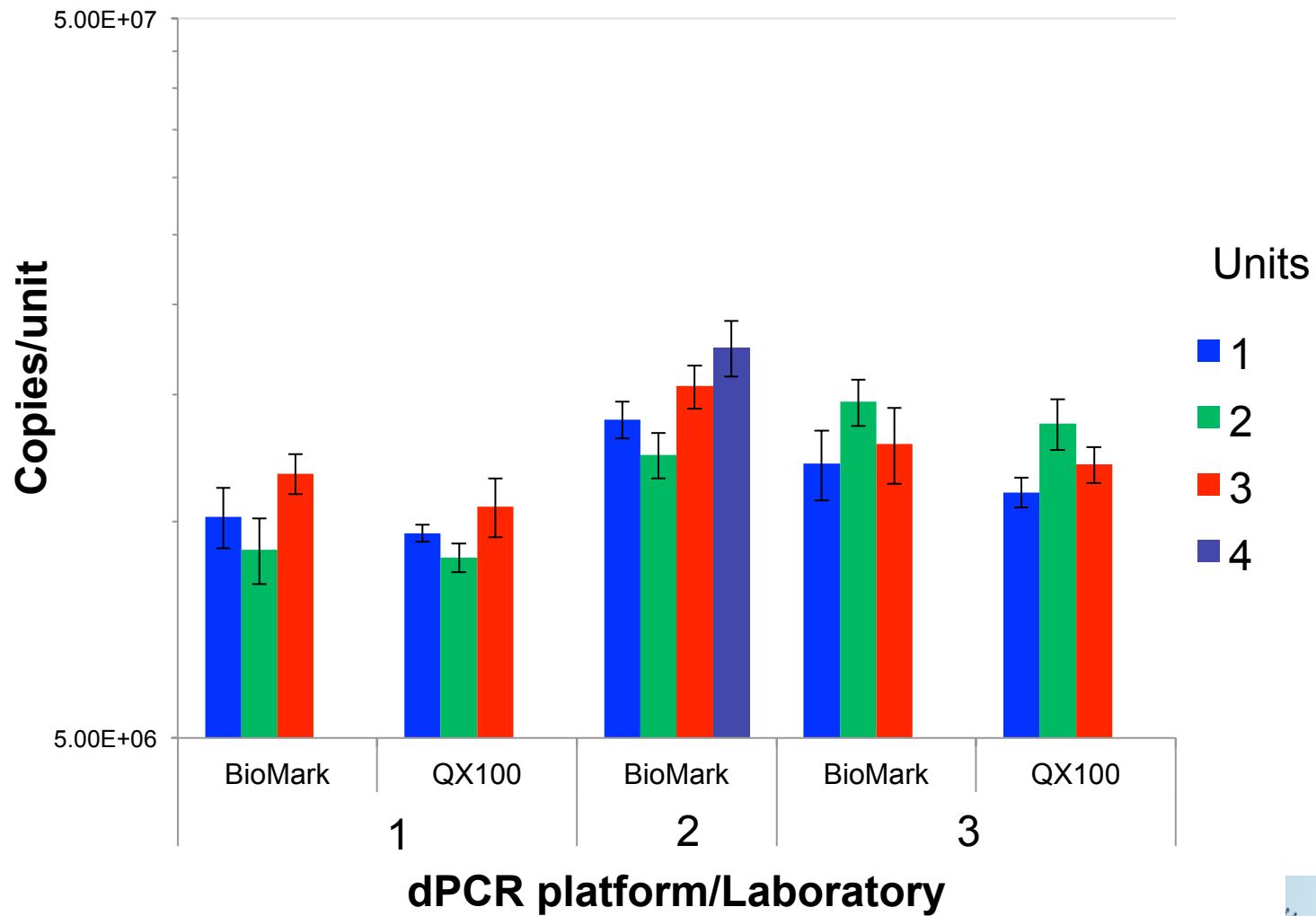
¹Microbiology and Tumor Biology Center (MTC), Karolinska Institutet, 17177 Stockholm, Sweden

^{2,3}Department of Cell Biology and Immunology² and Department of Microbiology³, Gesellschaft für Biotechnologische Forschung, 38124 Braunschweig, Germany

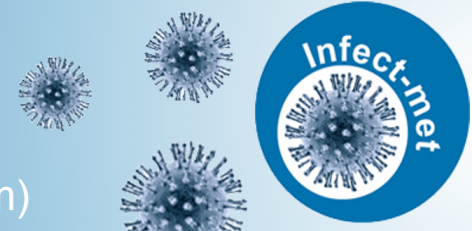
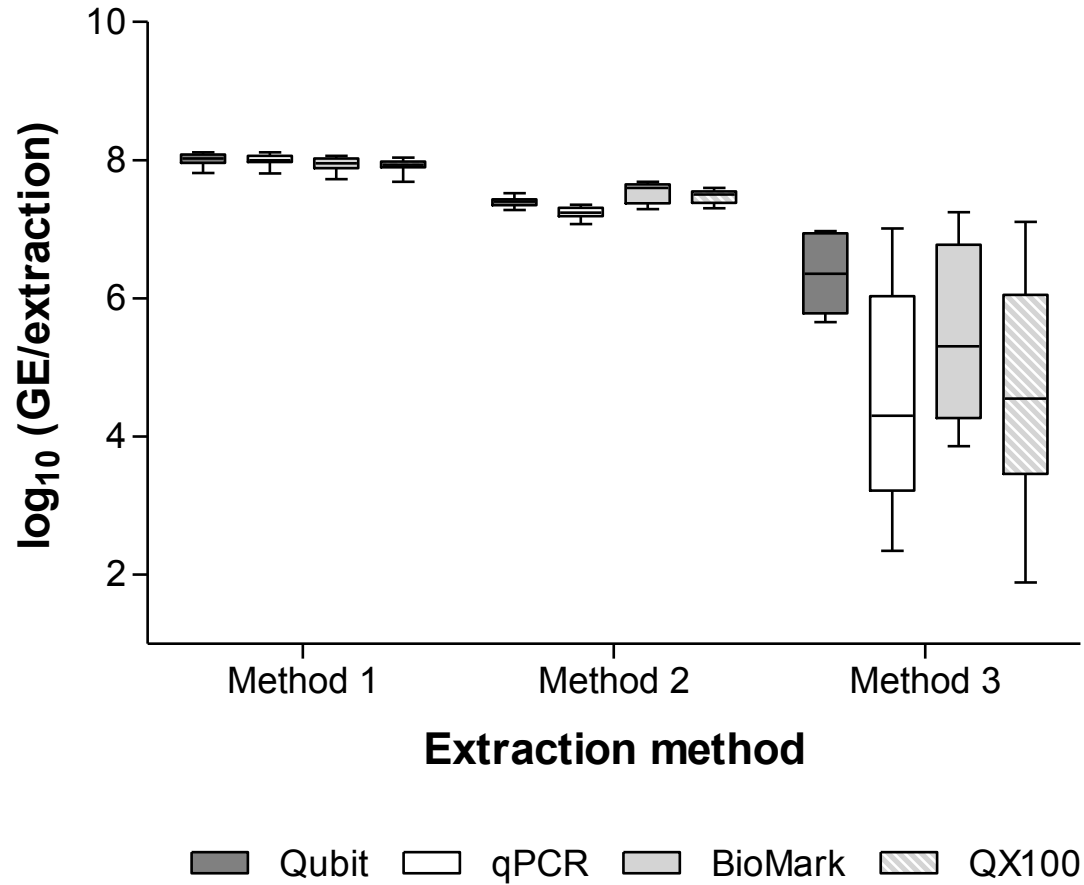
⁴Department of Microbiology, University of Guelph, Canada N1G2W1



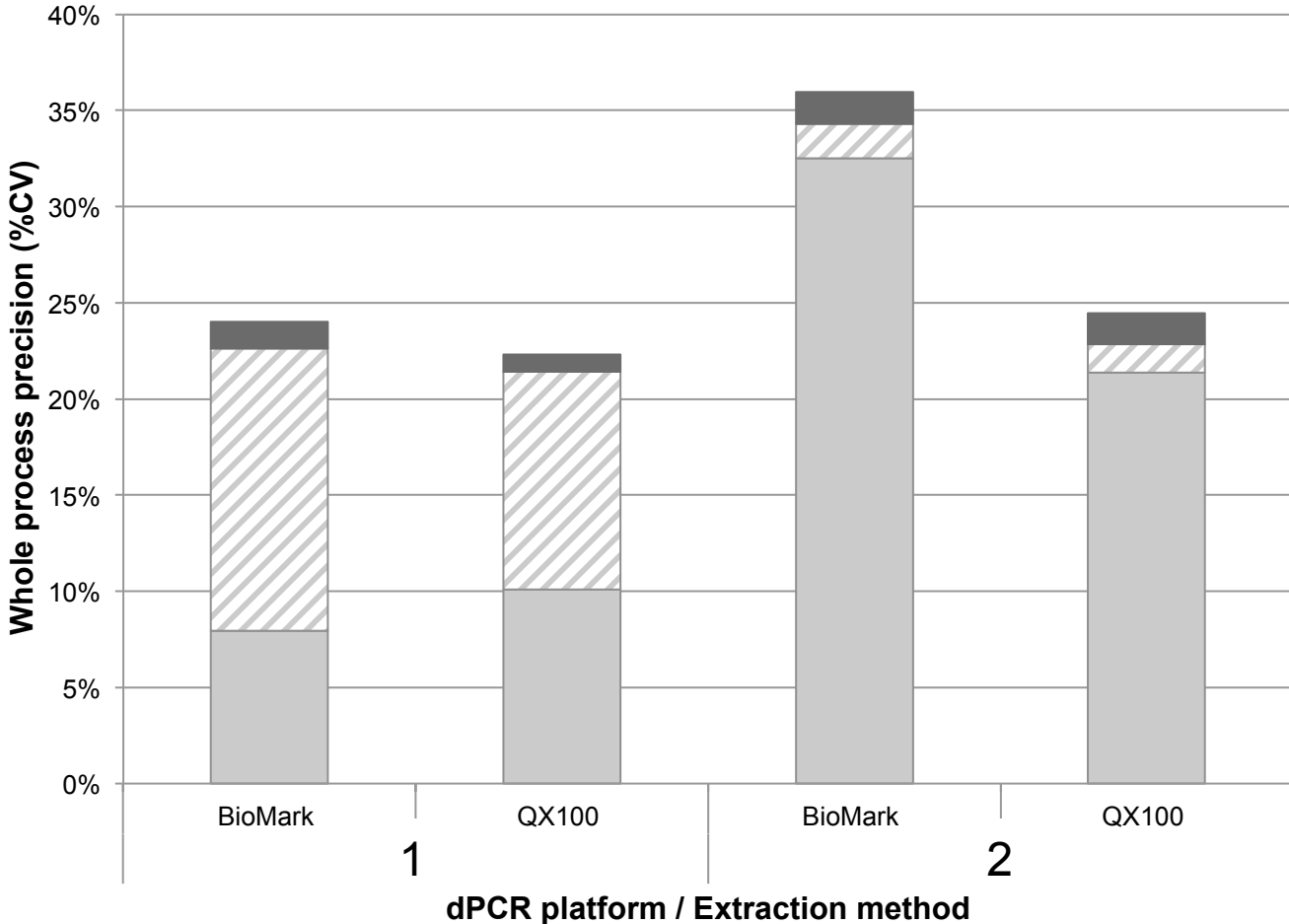
dPCR platforms/laboratories



Extraction methods



Precision



■ Between day ▨ Within day ■ Measurement (dPCR)



Quantification

Xpert RIF/MTB



A Multisite Assessment of the Quantitative Capabilities of the Xpert MTB/RIF Assay

Robert Blakemore¹, Pamela Nabeta¹⁰, Amy L. Davidow², Viral Vadwai⁵, Rasim Tahiri⁸, Vanisha Munsamy⁷, Mark Nicol⁴, Martin Jones⁹, David H. Persing⁹, Doris Hillemann³, Sabine Ruesch-Gerdes³, Felicity Leisegang⁴, Carlos Zamudio⁶, Camilla Rodrigues⁵, Catharina C. Boehme¹⁰, Mark D. Perkins¹⁰, and David Alland¹

Conclusions: Xpert MTB/RIF quantitation offers a new, standardized approach to measuring bacterial burden in the sputum of patients with tuberculosis.

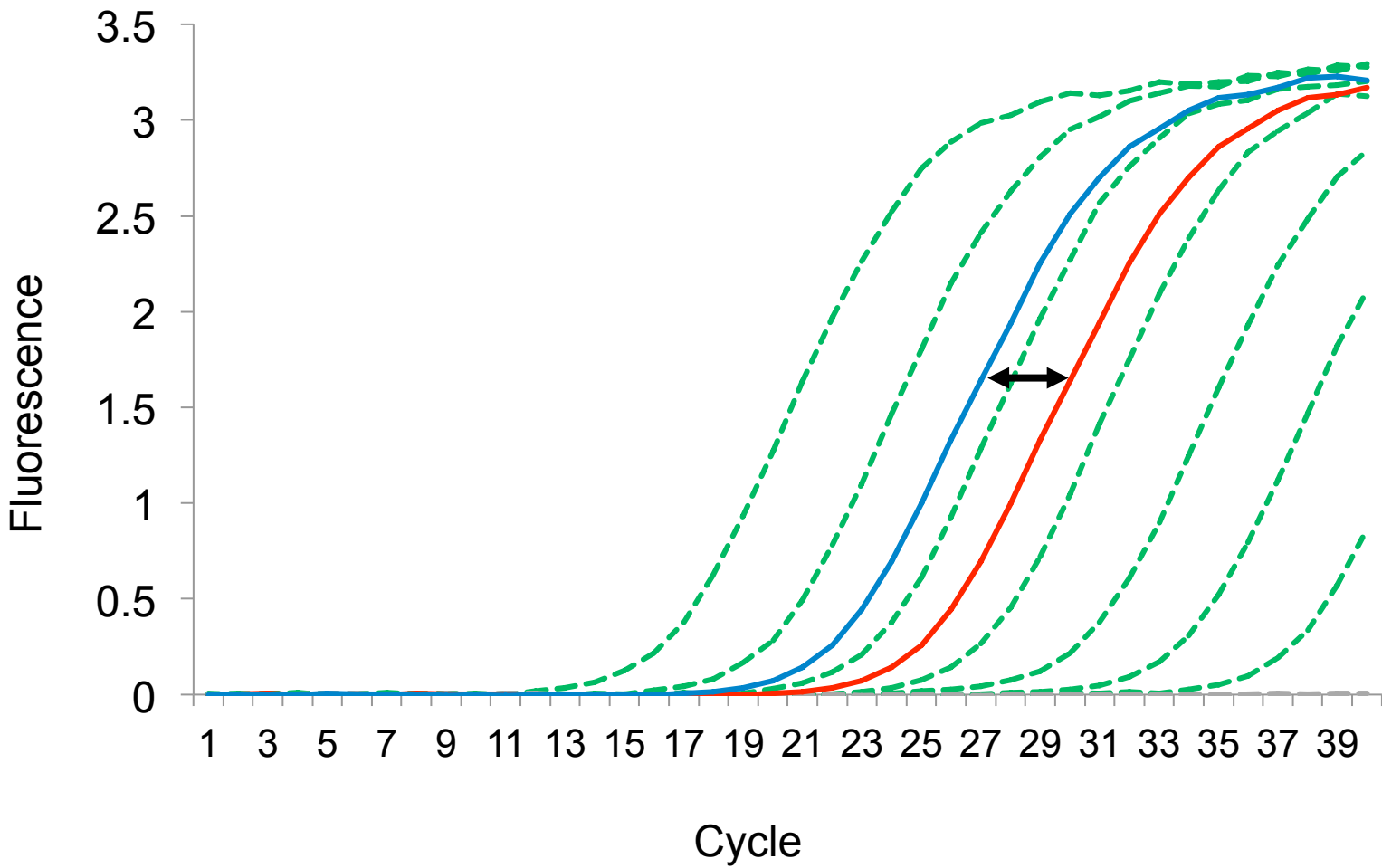
¹Division of Preventive Epidemiology, Forsyth Institute, Laboratory Medicine, South African Medical Research Council, Durban, South Africa; ⁸Special Treatment Institution for Detainees with Tuberculosis, Baku, Republic of Azerbaijan; ⁹Cepheid, Sunnyvale, California; and ¹⁰Foundation for Innovative New Diagnostics, Geneva, Switzerland

Am J Respir Crit Care Med Vol 184. pp 1076–1084, 2011

activity (TTP, in hours [mean \pm SD]) in liquid culture, and Xpert MTB/RIF cycle thresholds (C_T , n [mean \pm SD]). The ability to discriminate treatment effects between groups was analyzed with one-way analysis of variance (ANOVA). All measurements showed a decrease in bacterial load from mean baseline (log CFU, 5.72 ± 1.00 ; TTP, 116.0 ± 47.6 ; C_T , 19.3 ± 3.88) to day 7 (log CFU, -0.55 ± 1.24 , group effects was found with TTP at day 7 and day 14 ($F = 9.012$, $P < 0.0001$, and $F = 11.580$, $P < 0.0001$), followed by log CFU ($F = 4.135$, $P = 0.0024$, and $F = 7.277$, $P < 0.0001$). C_T was not significantly discriminative ($F = 1.995$, $P = 0.091$, and $F = 1.203$, $P = 0.316$, respectively). Culture-based methods are superior to PCR for the quantification of early antituberculosis treatment effects in sputum.

C_T was not significantly discriminative



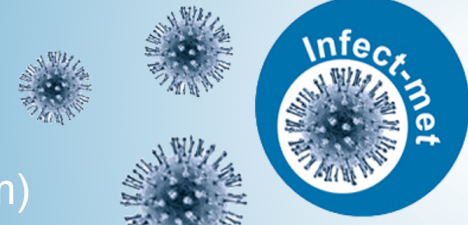


AMPLIRUN[®] TOTAL MTB CONTROL (SPUTUM)

For research use only

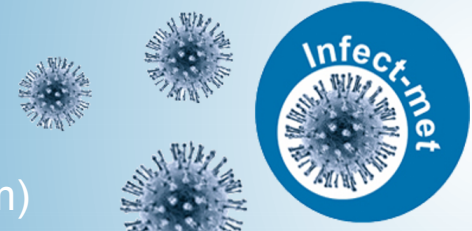
MBTC013: Inactivated *Mycobacterium tuberculosis* (MTB) cells formulated to mimic human sputum specimen and intended to validate and control sample processing, analysis and detection in nucleic acid assays using the product as an external run control.

4,000 copies/vial (2,500-10,000 copies/vial)



Inter-laboratory comparison

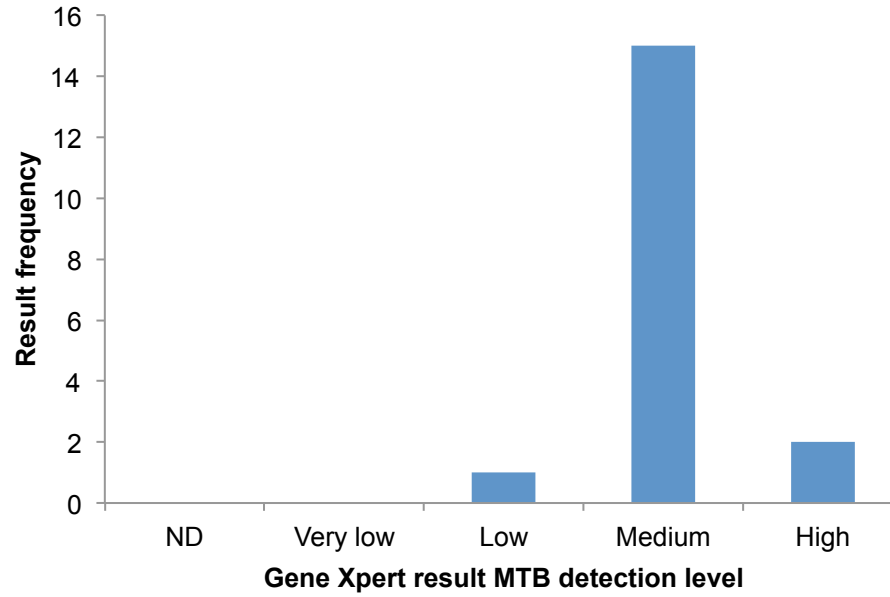
- Materials sent to eight clinical laboratories (three vials of each material per analysis)
 - Three perform qPCR
 - Six perform Xpert RIF/MTB



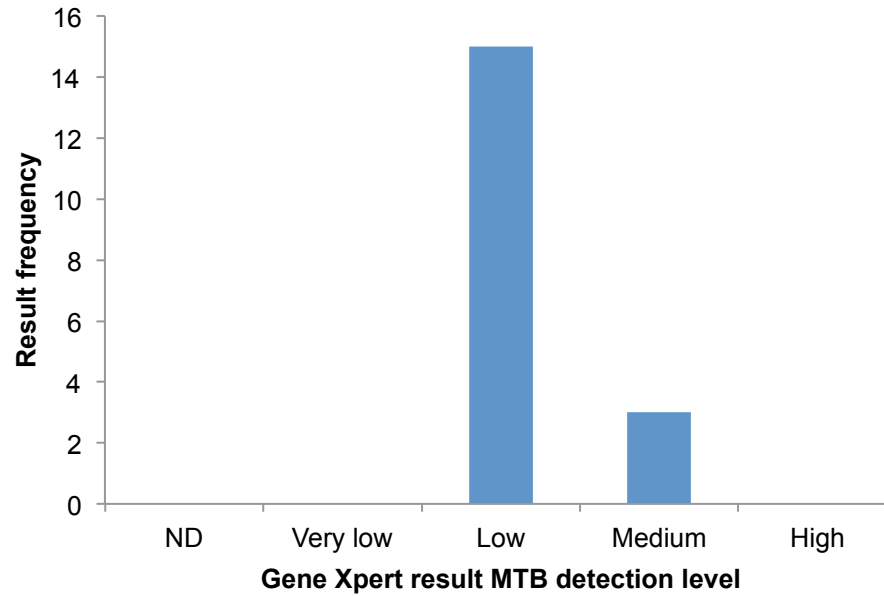
Xpert RIF/MTB



BCG/ASM

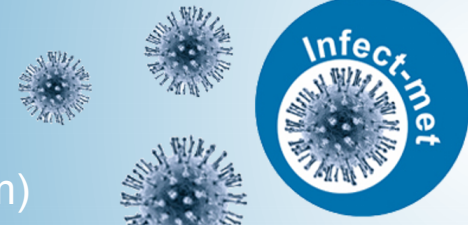


Total MTB Control



Summary

- Concept of developing SI traceable reference measurement system based on nucleic acid enumeration demonstrated
- dPCR with optimised extraction has proven to be reproducible and robust in laboratory comparisons
 - **Potential for dPCR as reference method**
- DNA copy number enumeration by dPCR can form a reliable and informative basis for range of different measurements in molecular diagnostics
 - **Potential for dPCR value assignment of RMs**
- INFECTMET publications available for download:
 - <http://infectmet-lgcgroup.com>



Acknowledgements

LGC

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- Simon Cowen
- Denise O'Sullivan
- Alexandra Whale
- Alice Gutteridge
- Gerwyn Jones
- Carole Foy
- Helen Parkes

University College London

- Tim Mchugh, Isobella Honeybourne
- Jeremy Garson & Kathryn Harris

Charite/GBD

- Heinz Zeichhardt, Hans-Peter Grunert

Vircell

- Pablo Mendoza

Bio-Rad

- Svilen Tzonev/Viresh Patel/DBC

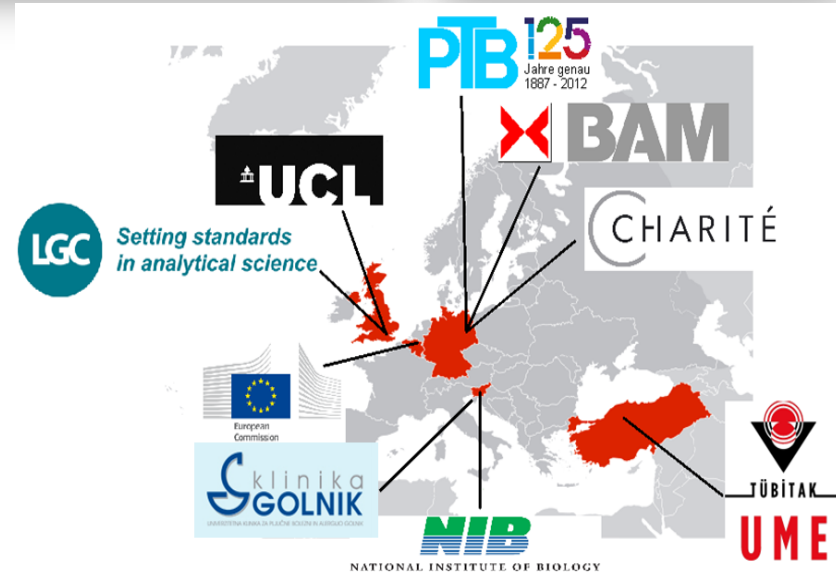
EMRP

European Metrology Research Programme
Programme of EURAMET



The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

National
Measurement
System



JRC

- Heinz Schimmel
- Maria Karczmarczyk

NIB

- Mojca Milavec
- Jernej Pavšič

Acknowledgements

EMRP

European Metrology Research Programme
Programme of EURAMET



The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

National
Measurement
System



Inter Laboratory Comparison

Great Ormond Street Hospital

NUI Galway

Forschungszentrum Borstel

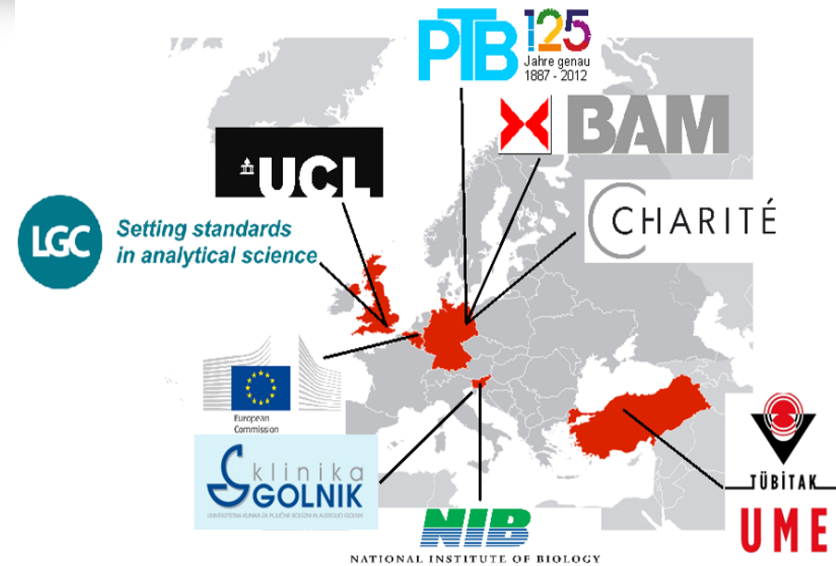
KCMC/KCRI

Lancet Laboratories

San Raffaele Scientific Institute

TASK Applied Science

University College London



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