2nd Course on Principles of Molecular Microbiological Diagnostics, ESCMID Postgraduate Education Course

Quantification

Jim Huggett

Quantification

In mathematics and empirical science, **quantification** (or quantitation) is the act of counting and measuring that maps human sense observations and experiences into members of some set of numbers. **Quantification** in this sense is fundamental to the scientific method.

Metrology

- Metrology is the science of measurement
  - **Scientific or fundamental metrology**

    The establishment of quantity systems, unit systems, units of measurement, the development of new measurement methods, realisation of measurement standards and the traceability from these standards to society.
  - **Applied, technical or industrial metrology**

    The application of measurement science to manufacturing, clinical and other applications and their use in society, ensuring instrument suitability, their calibration and quality control.
  - **Legal metrology**

    Underpins results for statutory measurement to support the needs for (e.g.) protection of health, public safety, the environment, enabling taxation, protection of consumers and fair trade.

Measurement information needed

- Identity
  - Organism X is present
- Identity and Quantity
  - Organism X is present at abundance Y
- Identity, Quantity and Confidence (Uncertainty)
  - Organism X is present at abundance Y measured with confidence Z

When may quantification be necessary

- Diagnosis
- Stratification
- Prognostic monitoring

Quantitative methods used in Molecular Microbiological Diagnostics

- Nucleic acid amplification tests (NAAT)
  - qPCR
  - Quantitative isothermal amplification methods
- Sequencing
  - Next Generation(s) Sequencing
- Others
  - Arrays
  - Branched DNA

Relative vs absolute quantification

- Relative
  - qPCR, NGS, Arrays, Fluorescence
- Absolute
  - CFU counting, Absorbance, digital PCR
- Convert 'relative' to 'absolute' using calibrators
- Calibrators represent a simple idea that is complicated to implement

Achilles heal of quantification (measurement)

Standardisation

Standardisation

- Enables
  - Comparison of measurement
  - Robust findings
  - Better conclusions
- Standardisation is essential to measurement
- Standardisation is often taken for granted

Molecular diagnosis research

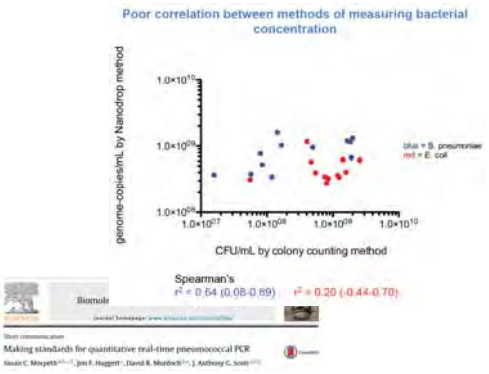
- Developing novel methods
- Point of care
- Improve current approaches
- Challenges of standardisation

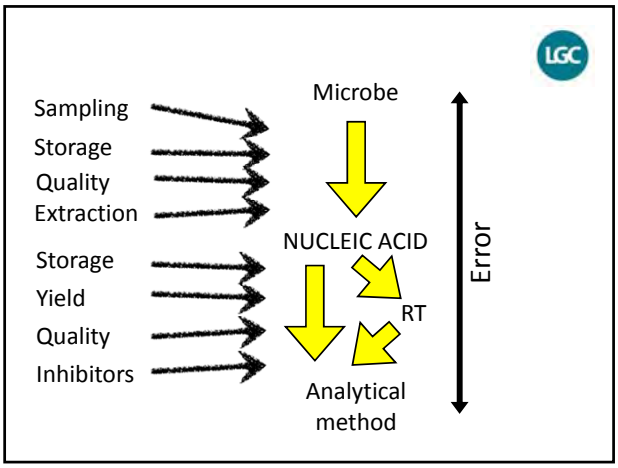
Excitement

What unit?

- Colony (Plaque) Forming Units
- Genomes/Genome equivalents
- International Units (IU)
- Instrument specific units (e.g. Cq, or Ct, for qPCR)

What if different units disagree?





What is the solution

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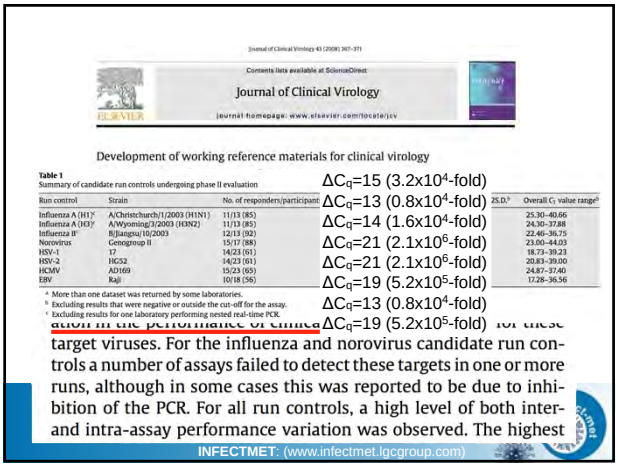
Journal of Clinical Virology

Development of working reference materials for clinical virology

Table 1
Summary of candidate run controls undergoing phase II evaluation

Run control	Strain	No. of responders/participants	SD.[b]	Overall C_T value range[b]
Influenza A (H1)[c]	A/Christchurch/1/2003 (H1N1)	11/13 (85)		25.30–40.66
Influenza A (H3)[c]	A/Wyoming/3/2003 (H3N2)	11/13 (85)		24.30–37.88
Influenza B[c]	B/Jiangsu/10/2003	12/13 (92)		22.46–36.75
Norovirus	Genogroup II	15/17 (88)		23.00–44.03
HSV-1	17	14/23 (61)		18.73–39.23
HSV-2	HG52	14/23 (61)		20.83–39.00
HCMV	AD169	15/23 (65)		24.87–37.40
EBV	Raji	10/18 (56)		17.28–36.56

[a] More than one dataset was returned by some laboratories.
[b] Excluding results that were negative or outside the cut-off for the assay.
[c] Excluding results for one laboratory performing nested real-time PCR.

ation in the performance of clinica target viruses. For the influenza and norovirus candidate run controls a number of assays failed to detect these targets in one or more runs, although in some cases this was reported to be due to inhibition of the PCR. For all run controls, a high level of both inter- and intra-assay performance variation was observed. The highest

ΔC_q=15 (3.2x10^4-fold)
ΔC_q=13 (0.8x10^4-fold)
ΔC_q=14 (1.6x10^4-fold)
ΔC_q=21 (2.1x10^6-fold)
ΔC_q=21 (2.1x10^6-fold)
ΔC_q=19 (5.2x10^5-fold)
ΔC_q=13 (0.8x10^4-fold)
ΔC_q=19 (5.2x10^5-fold)

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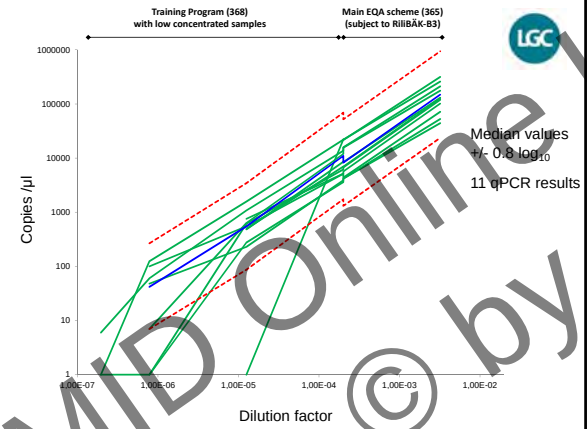
Virus Genome Detection of CMV

Main-EQA Scheme (365) and Training Program (368) – September 2014

Main EQA scheme (365)		
Sample No.	**Sample source**	**Dilution Dilution factor: 4 x Y**
365069*	plasma pool of healthy blood donors spiked with a lysate of CMV infected cells (isolate of a patient)	1 : 313*
365070 = 365072	negative plasma pool of healthy blood donors	----
**365071*,$ **	plasma pool of healthy blood donors spiked with a lysate of CMV infected cells (isolate of a patient)	1 : 5 000*,$
365072 = 365070	negative plasma pool of healthy blood donors	----

Training Program (368)		
Sample No.	**Sample source**	**Dilution Dilution factor: 4 x Y**
368005*,$	plasma pool of healthy blood donors spiked with a lysate of CMV infected cells (isolate of a patient)	1 : 5 000*,$
368006*		1 : 5 120 000*
368007*		1 : 1 280 000*
368008*		1 : 80 000*

CHARITÉ INSTAND ICBS



What about bacteria?

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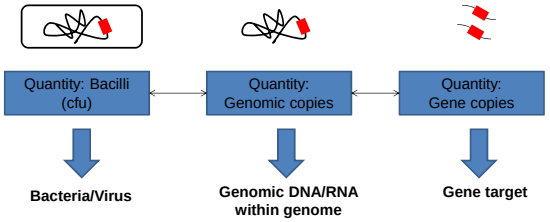
Materials for full analytical workflow



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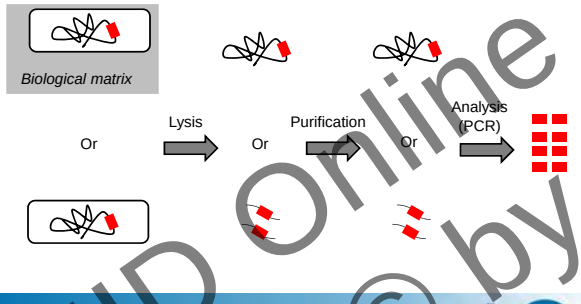
Materials for full analytical workflow



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Tuberculosis

INFECTMET: (www.infectmet.lgcgroup.com)

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

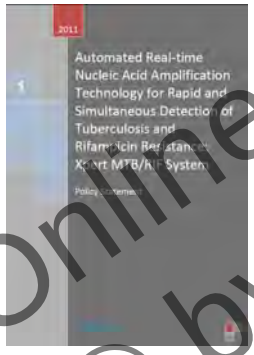
Jim F Huggett[a], Timothy D McHugh[b, 1], Alimuddin Zumla[a, 2]

[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



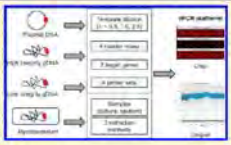
analytical chemistry

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

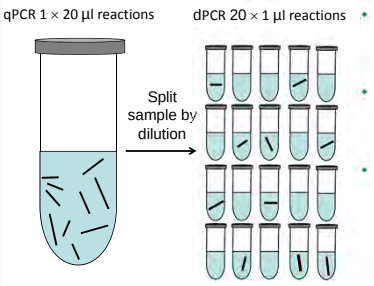
Alison S. Devonshire,[†] Isobella Honeyborne,[‡] Alice Gutteridge,[†,§] Alexandra S. Whale,[†] Gavin Nixon,[†] Philip Wilson,[§] Gerwyn Jones,[†] Timothy D. McHugh,[‡] Carole A. Foy,[†] and Jim F. Huggett*[,†,⊥]

[†]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

Supporting Information

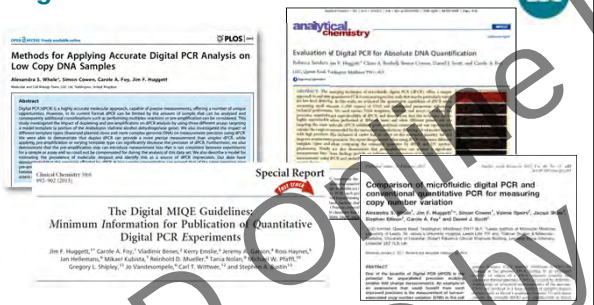


What is Digital PCR?



- 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)

Digital PCR



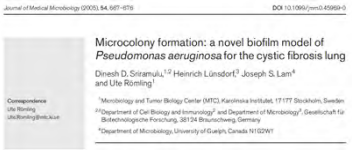
"The ability to measure extremely low concentrations of specific DNA sequences, independent of a standard curve, with high precision, in a complex background, is unique to dPCR"

M. bovis BCG in synthetic sputum

CFU data:
Mean: 1.09E+07 ± 1.52E+06 (14%)

300 units prepared in synthetic sputum.

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



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vircell MICROBIOLOGISTS

LGC

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.

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JCM

Direct Comparison of Xpert MTB/RIF Assay with Liquid and Solid

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

Robert Blakemore[1], Pamela Nabeta[10], Amy L. Davidow[2], Viral Vadwai[3], Rasim Tahirli[8], Vanisha Munsamy[7], Mark Nicol[4], Martin Jones[9], David H. Persing[9], Doris Hillemann[3], Sabine Ruesch-Gerdes[3], Felicity Leisegang[4], Carlos Zamudio[6], Camilla Rodrigues[5], Catharina C. Boehme[10], Mark D. Perkins[10], and David Alland[1]

[1]Di
Pre
[2]Fc
Lat
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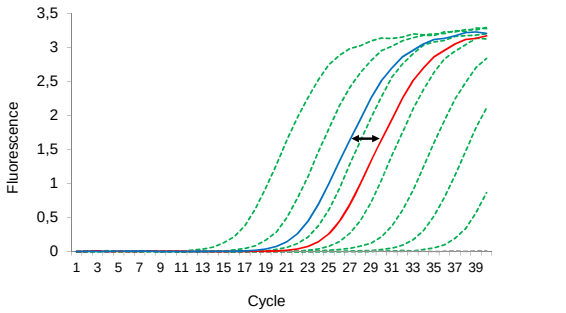
Sou

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

Azerbaijan; [7]Cepheid, Sunnyvale, California; and [10]Foundation for Innovative New Diagnostics, Geneva, Switzerland

INFECTMET: (www.infectmet.lgcgroup.com)



Interlaboratory comparison

- Materials sent to eight clinical laboratories
  - Three perform qPCR
  - Six perform Xpert MTB/RIF

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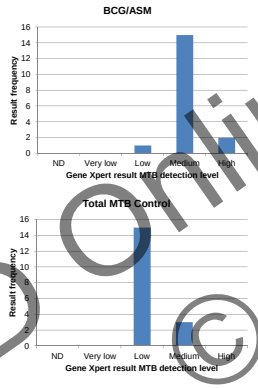
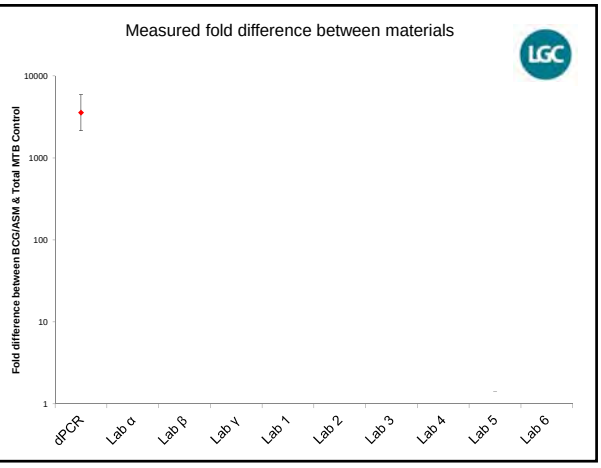
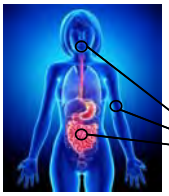




Metagenomics for microbial analysis



The microbiome as a human organ

-Oral (10^{10}) bacteria
-Skin (10^{12}) bacteria
-Intestine (10^{14}) bacteria

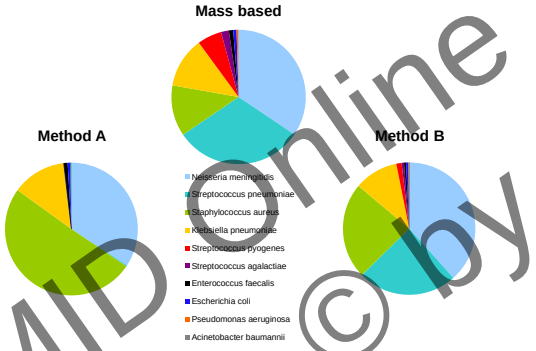
- Microbiomes play a crucial role in health
- Implicated in many diseases
- Microbiome analysis will define:
  - How they affect health and disease
  - Role in Diagnostics/Prognostic monitoring
  - Therapeutic manipulation

Human microbiome 8,000,000+ genes

Human genome 23,000 genes

>360 times

Method comparison



Accuracy?

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Article

Assessing the Accuracy of Quantitative Molecular Microbial Profiling

Denise M. O'Sullivan [1,*], Thomas Laver [2,3], Sasithon Temisak [1,3], Nicholas Redshaw [1], Kathryn A. Harris [3], Carole A. Foy [1], David J. Studholme [2] and Jim F. Huggett [1]

[1] Molecular Biology, LGC Ltd., Queens Road, Teddington TW11 0LY, UK;
E-Mails: sasithon.temisak@lgcgroup.com (S.T.); nicholas.redshaw@lgcgroup.com (N.R.); carole.foy@lgcgroup.com (C.A.F.); jim.huggett@lgcgroup.com (J.F.H.)
[2] Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK;
E-Mails: twl207@exeter.ac.uk (T.L.); d.j.studholme@exeter.ac.uk (D.J.S.)
[3] Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital for Children NHS Trust, Great Ormond Street, London WC1N 3JH, UK;
E-Mail: Kathryn.harris@gosh.nhs.uk

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Summary. Quantification

- Quantitative considerations influence all measurement.
- To develop useful quantitative molecular methods we need to understand technical sources of bias and variance.
- This will improve technical accuracy and allow us to determine what is biologically possible.
- For pre clinical methods to be successfully translated, we must consider technical reproducibility and mechanisms for standardisation.

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University College London