How can measurement science assist in improving AMR diagnostics research and development?

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INFECTMET: (www.INFECTMET.lgcgroup.com)

Measurement Science?

- 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**

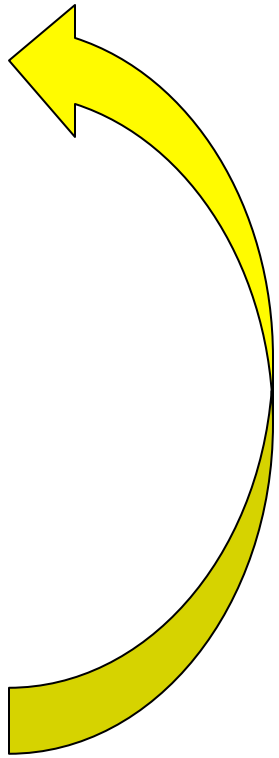
  The application of measurement science to manufacturing, clinical and other applications and their use in society, ensuring instrument suitability, their calibration and quality control.

  – **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

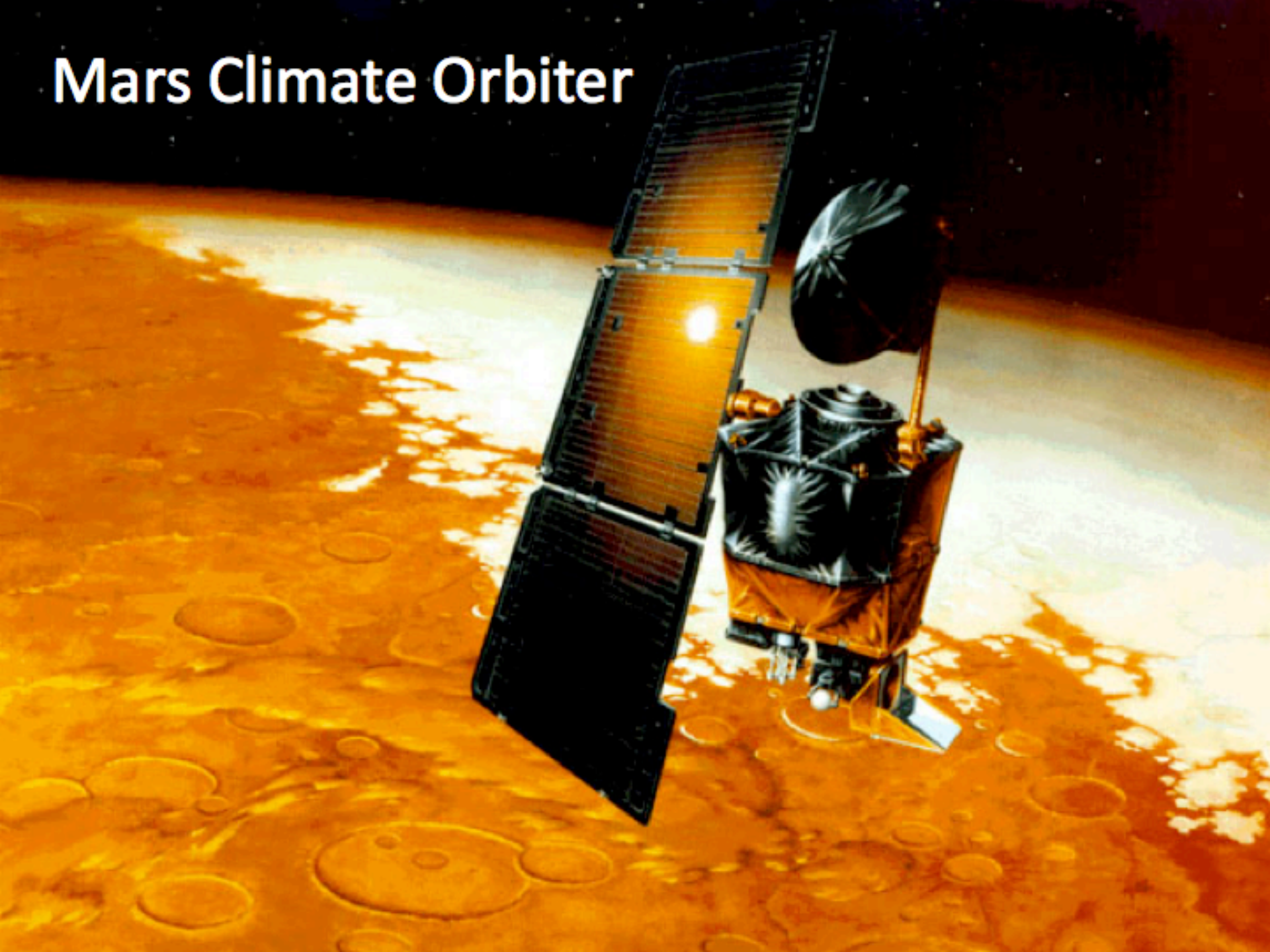
- Identity
  - Organism X is present
- Identity and Quantity
  - Organism X is present at abundance Y
- Identity, Quantity and Confidence (Uncertainty)
  - Organism X is present at abundance Y measured with confidence Z



What unit?

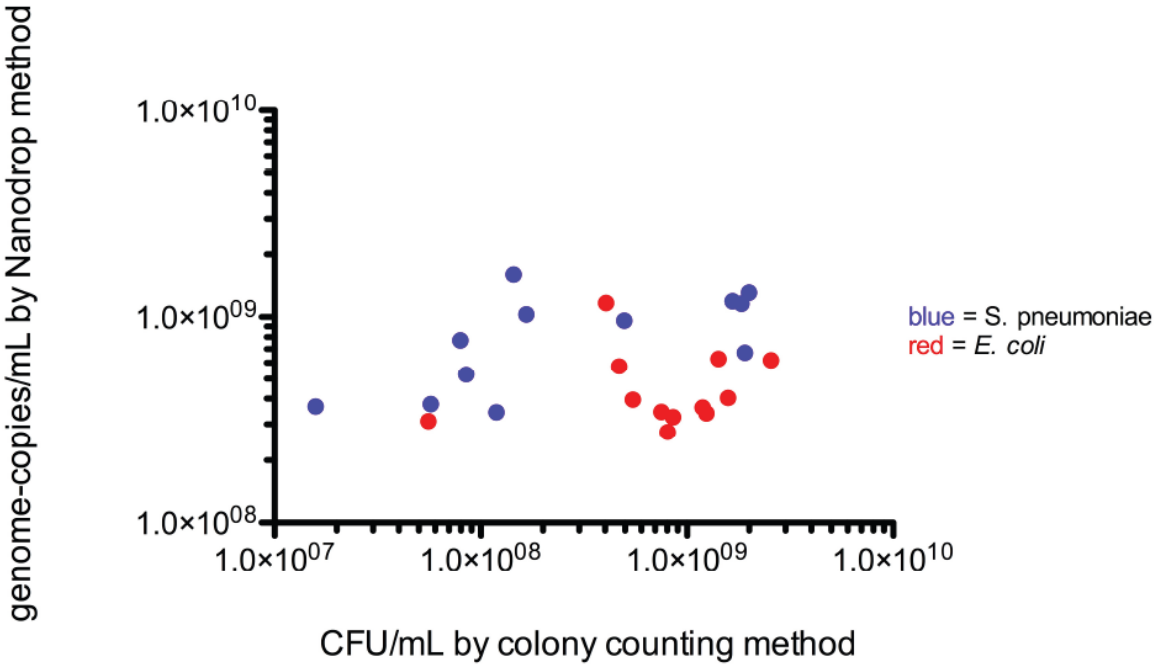
- Colony (Plaque) Forming Units
- International Units (IU)
- Optical density, fluorescent units
- Genomes/Genome equivalents
- Instrument specific units (e.g. Cq, or Ct, for qPCR)

Mars Climate Orbiter



What if different units disagree?

Poor correlation between methods of measuring bacterial concentration



Spearman's

r^2 = 0.64 (0.08-0.89) r^2 = 0.20 (-0.44-0.70)

ELSEVIER
Biomole
journal homepage: www.elsevier.com/locate/bdq

Short communication

Making standards for quantitative real-time pneumococcal PCR

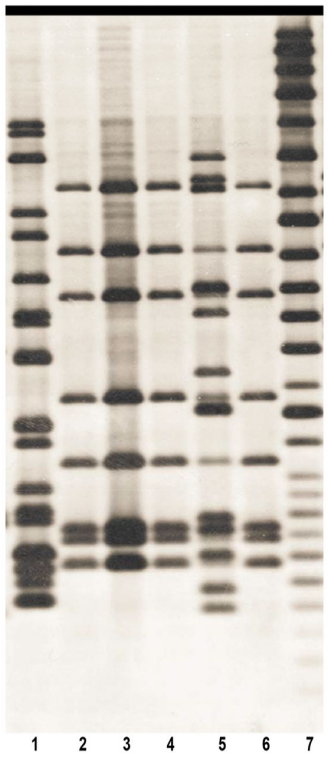
Susan C. Morpeth[a,b,*,1], Jim F. Huggett[c], David R. Murdoch[d,e], J. Anthony G. Scott[a,b,f]

Examples of Methods for measuring AMR

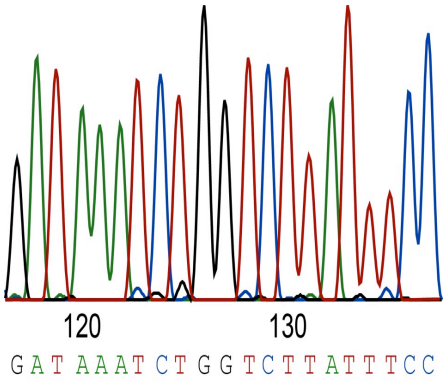
- Dilution method (broth and agar dilution method)
- Disk-diffusion method
- E-test
- Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test
- Molecular methods

Specific DNA detection methods

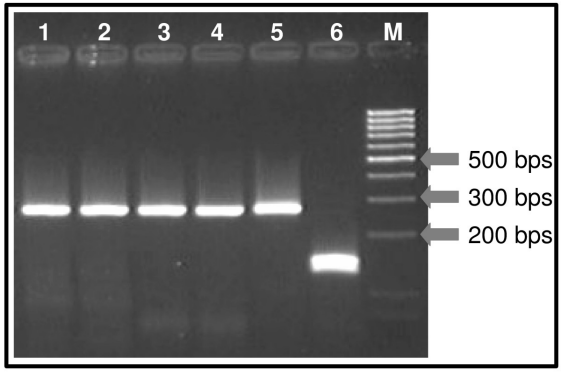
Hybridisation



Sequencing



NAAT/PCR



Quantification of Mtb load in Tuberculosis to guide prognosis and predictive monitoring

- Strong requirement for biomarker(s) to assist treatment
  - Informing treatment of individual
- Useful in evaluating new therapies/regimens
  - Speeding up analysis of outcome (culture –ve after 2 months)
- Quantitative assessment of microbial load investigated
  - Smear positivity grading
  - Colony forming units
  - Time to positivity
  - Molecular quantification
    - gDNA
    - RNA

Xpert RIF/MTB

Cepheid®

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

World Health Organization

Quantification?

Xpert RIF/MTB

2011

Automated Real-time

JCM
Journals.ASM.org

Direct Comparison of Xpert MTB/RIF Assay with Liquid and Solid Mycobacterial Culture for Quantification of Early Bactericidal Activity

Xavier A. Kayigire,[a] Sven O. Friedrich,[b] Amour Venter,[c] Rodney Dawson,[d] Stephen H. Gillespie,[e] Martin J. Boeree,[f] Norbert Heinrich,[g,h] Michael Hoelscher,[g,h] Andreas H. Diacon,[b] on behalf of the Pan African Consortium for the Evaluation of Anti-tuberculosis Antibiotics

Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa[a]; Division of Medical Physiology, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa[b]; MRC Centre for Molecular and Cellular Biology, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa[c]; Division of Pulmonology, Department of Medicine, University of Cape Town Lung Institute, Groote Schuur Hospital, Cape Town, South Africa[d]; School of Medicine, University of St. Andrews School of Medicine North Haugh, St. Andrews, United Kingdom[e]; Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands[f]; Division of Infectious Diseases and Tropical Medicine, Medical Centre of the University of Munich (LMU), Munich, Germany[g]; DZIF German Centre for Infection Research, Munich, Germany[h]

The early bactericidal activity of antituberculosis agents is usually determined by measuring the reduction of the sputum mycobacterial load over time on solid agar medium or in liquid culture. This study investigated the value of a quantitative PCR assay for early bactericidal activity determination. Groups of 15 patients were treated with 6 different antituberculosis agents or regimens. Patients collected sputum for 16 h overnight at baseline and at days 7 and 14 after treatment initiation. We determined the sputum bacterial load by CFU counting (log CFU/ml sputum, reported as mean ± standard deviation [SD]), time to culture positivity (TTP, in hours [mean ± SD]) in liquid culture, and Xpert MTB/RIF cycle thresholds (C_T, n [mean ± 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, P = 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

Inter-laboratory comparison

analytical chemistry

Article

pubs.acs.org/ac

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*[,†,‡]

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Measured fold difference between materials

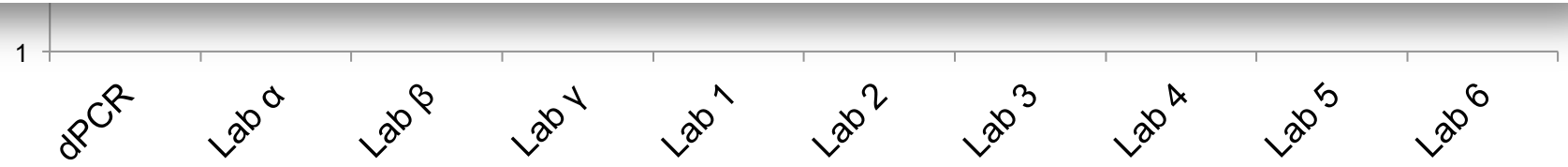
Devonshire *et al. BMC Infectious Diseases* (2016) 16:366
DOI 10.1186/s12879-016-1696-7

BMC Infectious Diseases

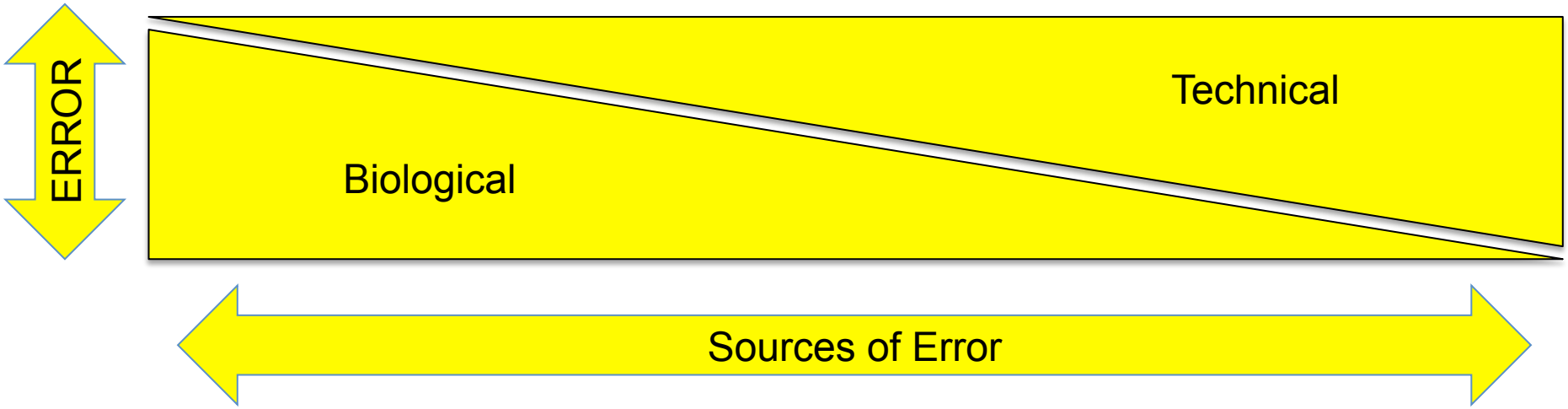
RESEARCH ARTICLE Open Access

The use of digital PCR to improve the application of quantitative molecular diagnostic methods for tuberculosis

Alison S. Devonshire[1†], Denise M. O'Sullivan[1†], Isobella Honeyborne[2], Gerwyn Jones[1], Maria Karczmarczyk[3], Jernej Pavšič[4], Alice Gutteridge[1], Mojca Milavec[4], Pablo Mendoza[5], Heinz Schimmel[3], Fran Van Heuverswyn[3], Rebecca Gorton[2], Daniela Maria Cirillo[6], Emanuele Borroni[6], Kathryn Harris[7], Marinus Barnard[8,9], Anthenette Heydenrych[8,9], Norah Ndusilo[10], Carole L. Wallis[11], Keshree Pillay[11], Thomas Barry[12], Kate Reddington[12], Elvira Richter[13], Erkan Mozioğlu[14], Sema Akyürek[14], Burhanettin Yalçınkaya[14], Muslum Akgoz[14], Jana Žel[4], Carole A. Foy[1], Timothy D. McHugh[2] and Jim F. Huggett[1,2,15*]



Technical vs Biological error



Diagnosis of drug resistance

- Culture
- PCR based molecular methods
- ?Sequencing?

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Press release

England world leaders in the use of whole genome sequencing to diagnose TB

From: Public Health England
Published: 28 March 2017

Whole genome sequencing (WGS) is now being used to identify different strains of tuberculosis (TB), announced Public Health England today.

This is the first time that WGS has been used as a diagnostic solution for managing a disease on this scale anywhere in the world. This builds on WGS based services for public health investigation of infectious diseases, which offer the opportunities for faster, cheaper and more accurate diagnostics than other testing methods.

The technique, developed in conjunction with the University of Oxford, means patients can be treated with precisely the right medication more quickly. Where previously it could take up to a month to confirm a diagnosis of TB, confirm the treatment choices and to detect spread between cases, this can now be done in just over a week by PHE's Birmingham laboratory. This slows the spread of the disease and boosts the fight against anti-microbial resistance (AMR).

Experience from clinical trials: relapse vs re-infection

REMoxTB

- 1931 patients
  - 17 treatment failures
  - 122 relapses
  - 58 re-infections
- Defined by MIRU-VNTR
- Performed sub-analysis of 47 patients
  - Prior to outcome data

Gillespie et al NEJM 2014
Bryant et al LRM 2013

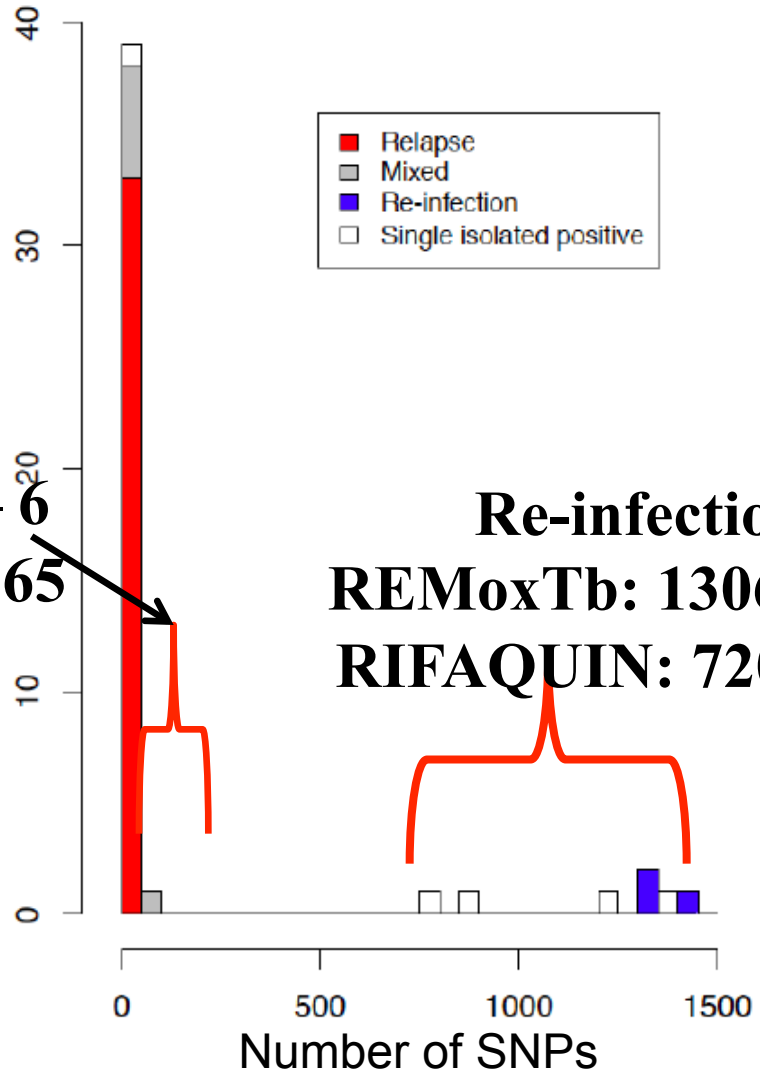
RIFAQUIN

- 827 patients randomised
  - 33 relapse
  - 9 re-infection
  - 4 culture confirmed treatment failure
- Defined by MIRU-VNTR

Jindani et al NEJM 2014

Thank you Professor Tim McHugh

Defining the groups: trail data



Relapse:
REMoxTb: 0 – 6
RIFAQUIN: 0- 65

Re-infection:
REMoxTb: 1306 – 1419
RIFAQUIN: 720 - 1400

Phelan *et al. Genome Medicine* (2016) 8:132
DOI 10.1186/s13073-016-0385-x

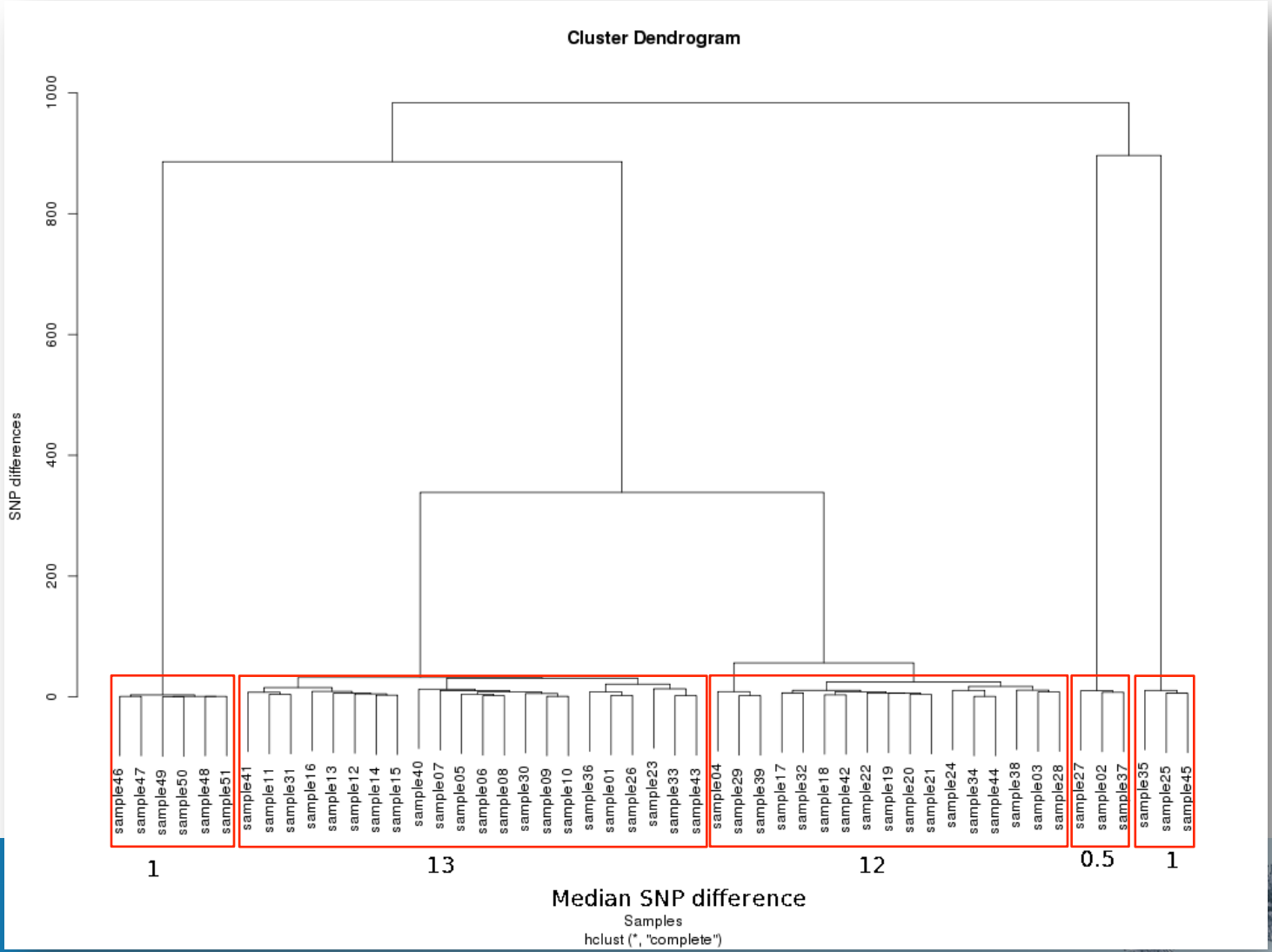
Genome Medicine

RESEARCH Open Access

The variability and reproducibility of whole genome sequencing technology for detecting resistance to anti-tuberculous drugs

Jody Phelan[1†], Denise M. O'Sullivan[2†], Diana Machado[3†], Jorge Ramos[3], Alexandra S. Whale[2], Justin O'Grady[4], Keertan Dheda[5], Susana Campino[1], Ruth McNerney[5†], Miguel Viveiros[3†], Jim F. Huggett[2,6†] and Taane G. Clark[1,7*†]

Cultures of XDR TB



Same data, different pipeline

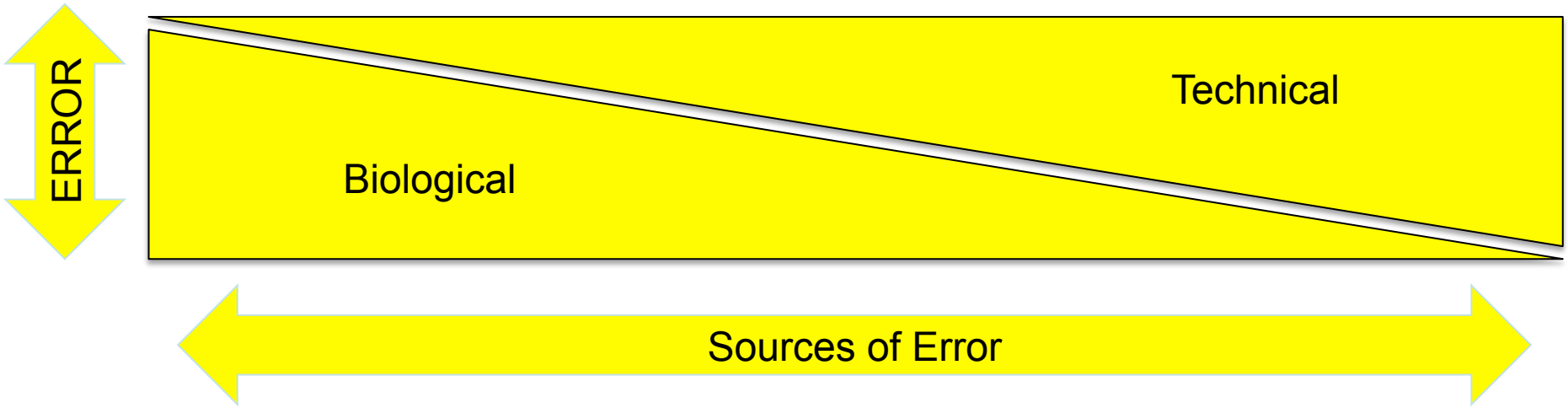
Phenotypic resistance vs predicted using different informatics tools for assigning resistance from sequence data

Sample	Year[a]	Lineage	Spoligo. family	Drug susceptibility test phenotype														
				INH	RIF	STR	ETB	PZA	RFB	ETH	AMK	CAP	OFX	MOX	PAS	LZ	KAN[b]	Resistance phenotype
POR1	2007	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	R	S	R	XDR-TB
POR2	2007	4.1.1.1	X2	R	R	S	S	S	R	R	S	S	S	S	S	S	-	MDR-TB
POR3	2007	4.3.4.2	LAM1	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR4	2007	4.3.4.2	LAM1	R	R	R	R	R	R	R	R	S	R	R	S	S	R	XDR-TB
POR5	2007	4.3.4.2	LAM4	R	R	R	R	R	R	R	S	S	S	S	S	S	-	MDR-TB
POR6	2008	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR7	2009	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR8	2012	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR9	2011	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	R	S	R	XDR-TB
POR10	2013	4.2.1	Ural H3/4	R	R	R	R	R	R	R	S	S	S	S	S	S	R	MDR-TB
H37Rv	-	4.9	H37RV	S	S	S	S	S	S	S	S	S	S	S	S	S	-	Pan-susceptible

Informatics tool

- Mykrobe Predictor
- TBProfiler

Technical vs Biological error

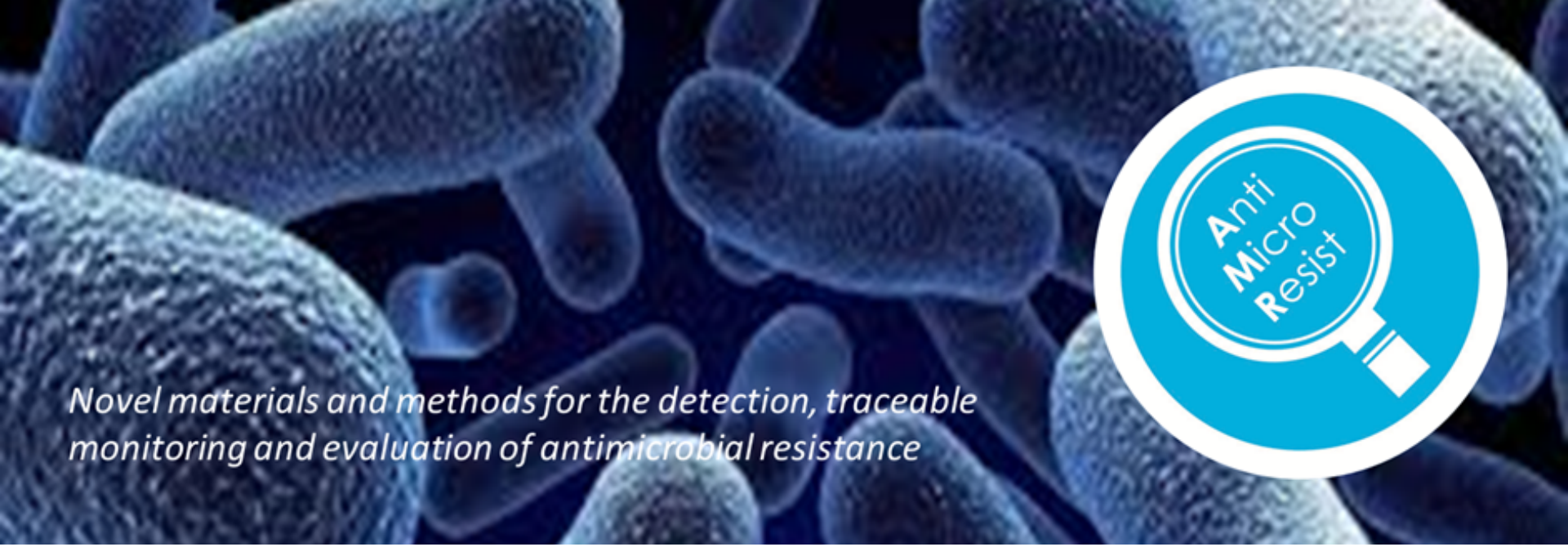


Summary: accuracy and NGS

- Molecular methods (sequencing) can play a major role in improving our understanding & management of TB
- Like any method sequencing is prone to error
- These errors need to be understood to maximise the impact of such methods

How can measurement science assist in improving AMR diagnostics research and development?

- Molecular methods offers the potential for monitoring of bacteria to guide treatment with advances in quantification and sequencing providing many new opportunities
- However, our findings suggest more work is required during research and development to define the technical and biological/clinical error
- A systematic approach that characterises sources of error would provide a more robust understanding of the potential of a given measurement
- If this is not done these opportunities could be missed



Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

http://www.lgcgroup.com/EMPIR-antimicroresist/

INFECTMET: (www.INFECTMET.lgcgroup.com)

Infect-met

AntiMicroResist

- Sequencing
  - MDR & XDR TB
  - ESBLs & CRE
  - Microbiomes
  - HIV

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