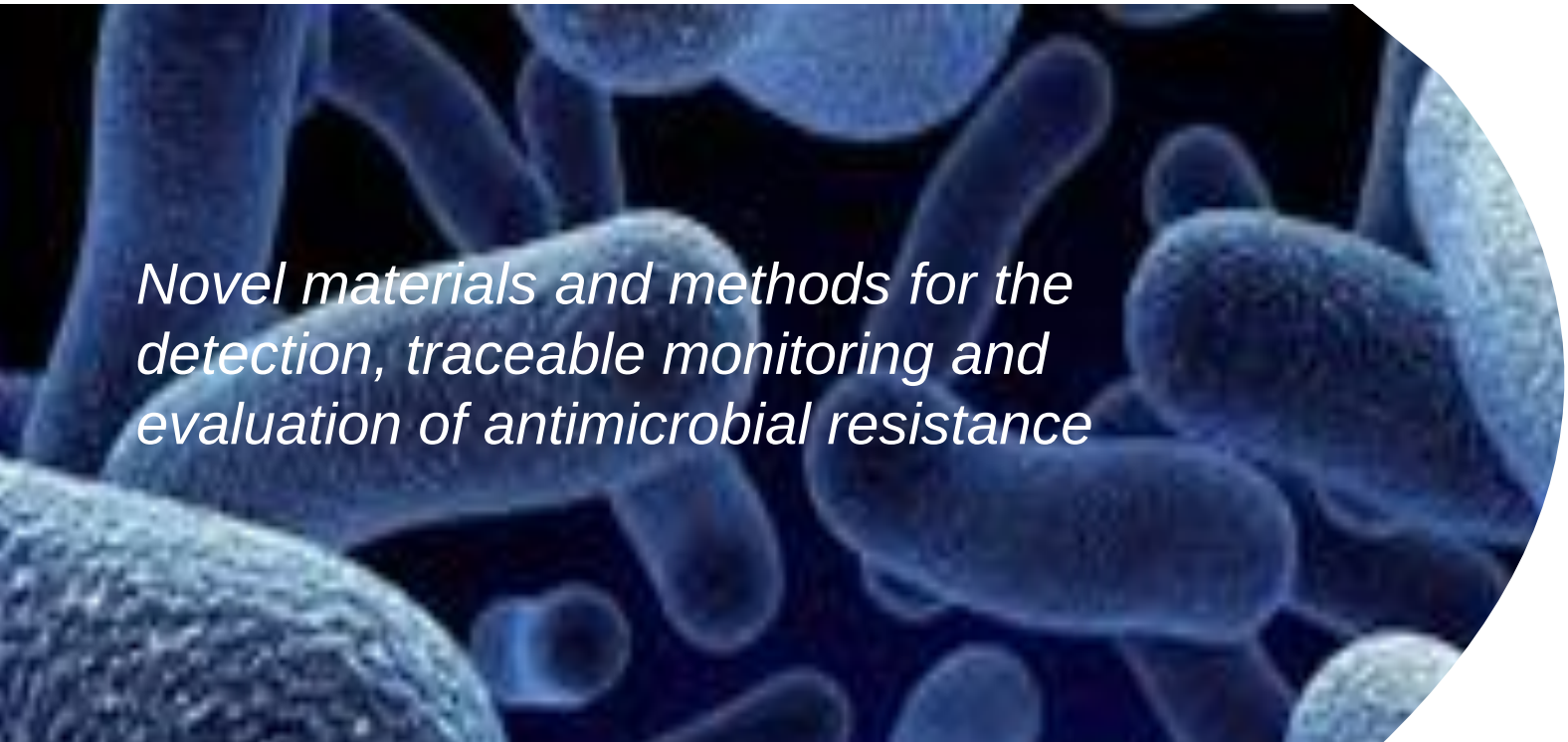
Implications of quantification in TB molecular diagnosis

Jim Huggett



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 (smear –ve after 2 months)
- Quantitative assessment of microbial load investigated
  - Smear positivity grading
  - Colony forming units
  - Time to positivity
  - Molecular quantification
    - gDNA
    - RNA

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

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Available online 8 April 2003

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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

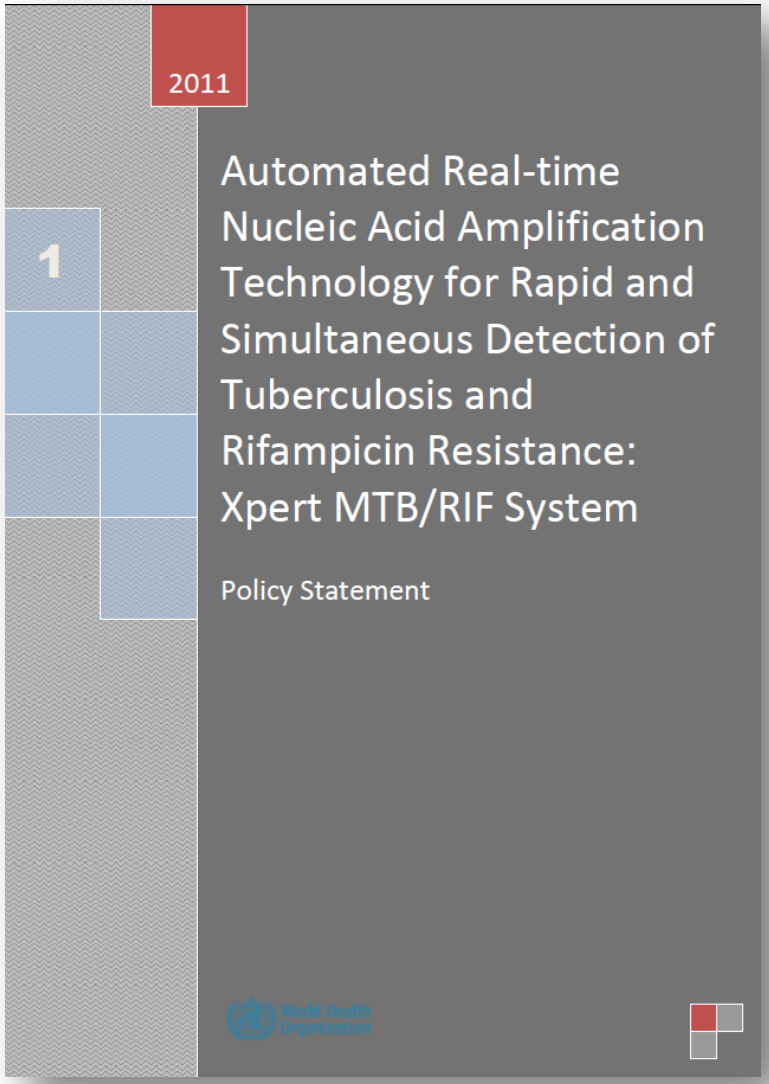
Policy Statement

World Health Organization

Quantification?

Xpert RIF/MTB

Cepheid®



Inter laboratory study to investigate

- The technical error of molecular quantification of Mtb (independent of the patient)
- Quantitative reproducibility
  - Methods (qPCR, Xpert MTB/RIF)
  - Laboratories
- Potential role of EQA materials for quantification of Mtb using molecular methods

Inter-laboratory comparison

- Materials sent to eight clinical laboratories (3 vials of each)
  - Three perform qPCR
  - Six perform Xpert RIF/MTB

- 8 Laboratories

analytical chemistry

Article

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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

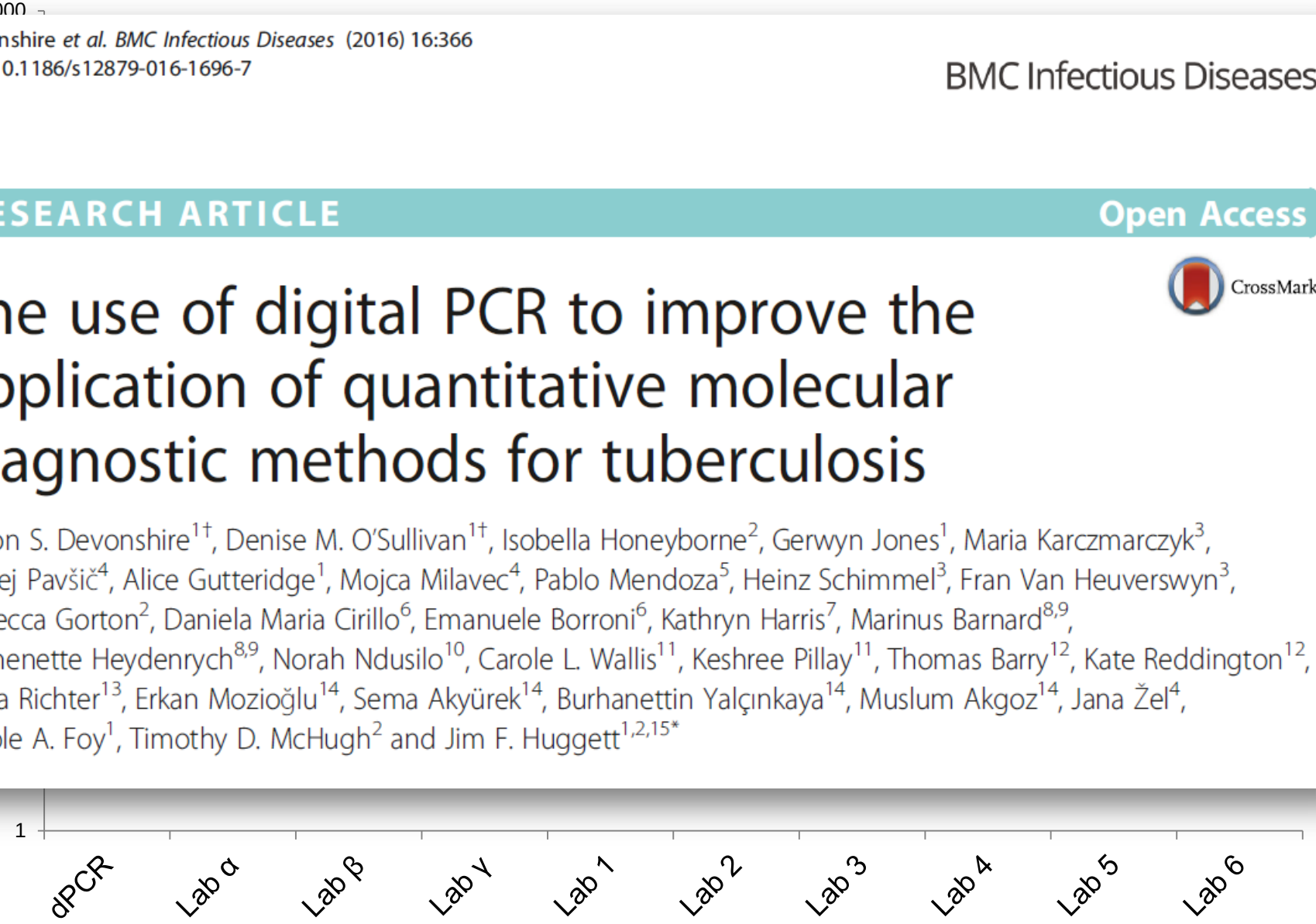
Devonshire *et al. BMC Infectious Diseases* (2016) 16:366
DOI 10.1186/s12879-016-1696-7

BMC Infectious Diseases

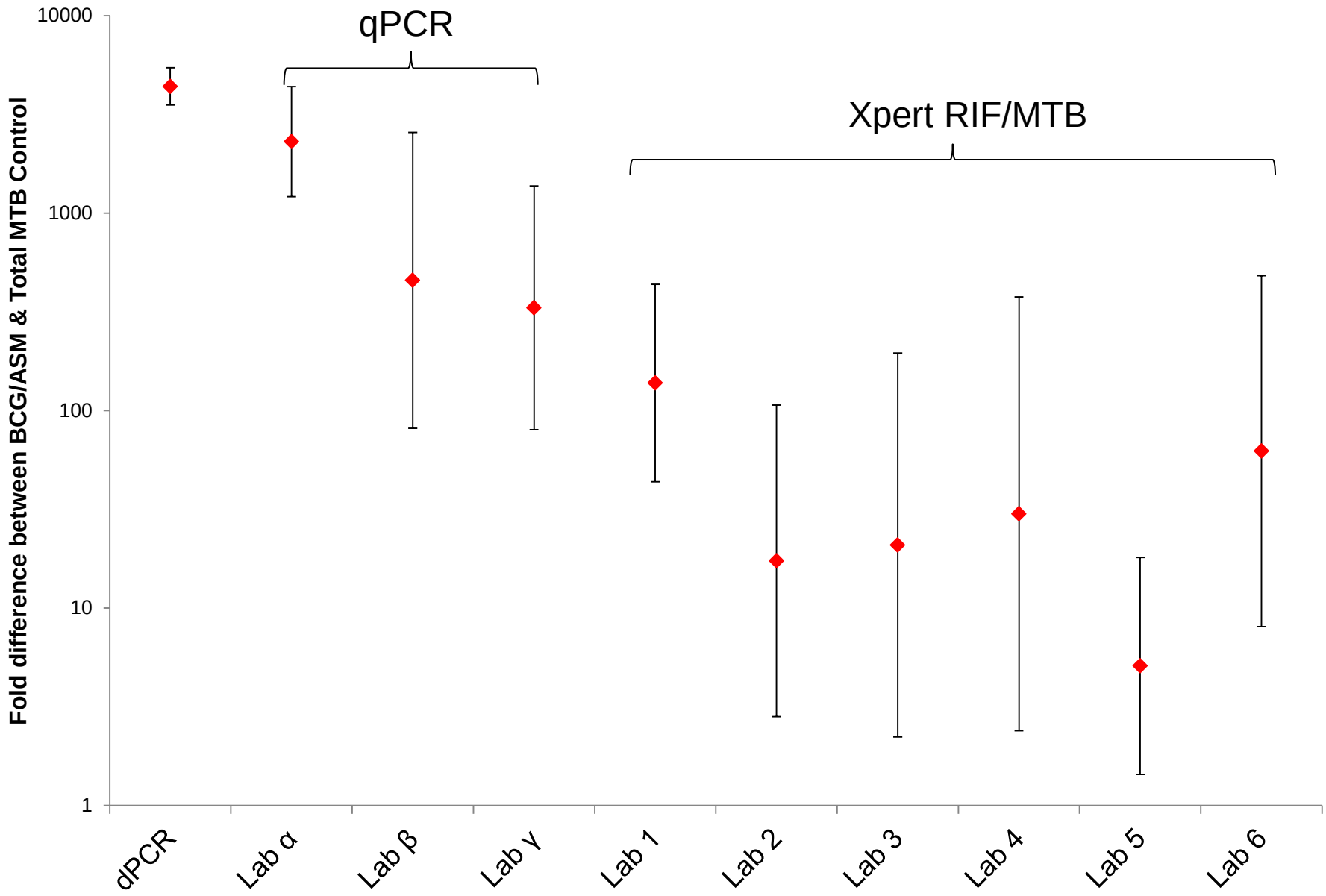
RESEARCH ARTICLE Open Access

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

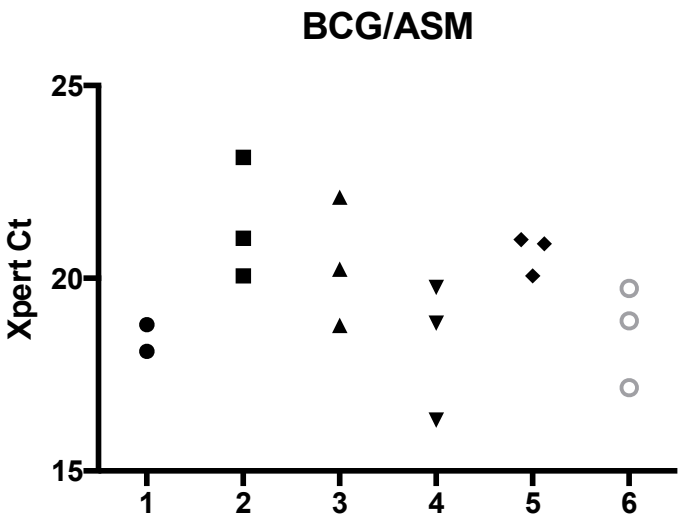
Alison S. Devonshire[1†], Denise M. O'Sullivan[1†], Isobella Honeyborne[2], Gerwyn Jones[1], Maria Karczmarczyk[3], Jernej Pavšič[4], Alice Gutteridge[1], Mojca Milavec[4], Pablo Mendoza[5], Heinz Schimmel[3], Fran Van Heuverswyn[3], Rebecca Gorton[2], Daniela Maria Cirillo[6], Emanuele Borroni[6], Kathryn 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*]



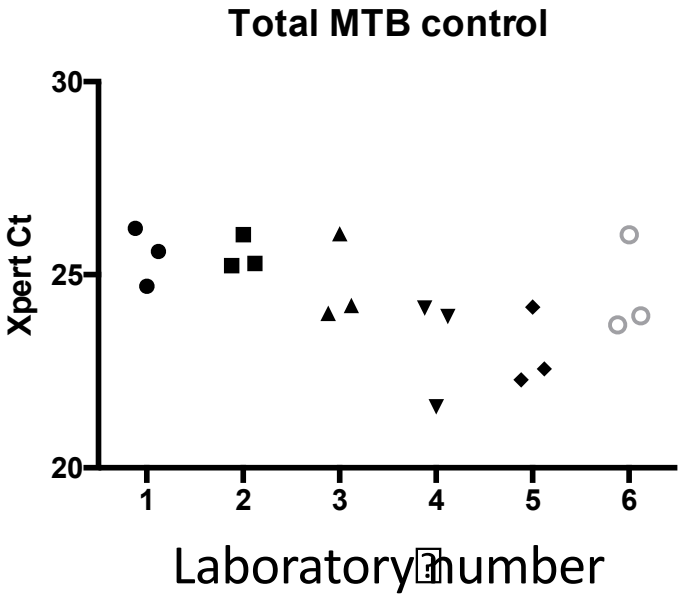
Measured fold difference between materials



One way anova to estimate within and between laboratory SD. Rough estimate of precision



SD = ~1.7 Ct



SD = ~1.4 Ct

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[5], 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]

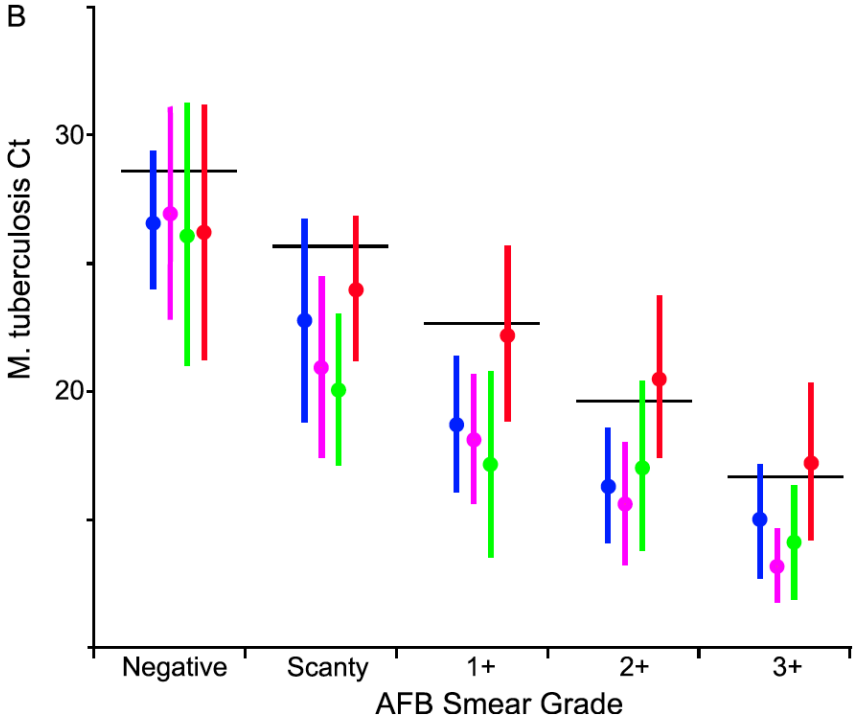
[1]Divisi… rtment of Prever… Jersey; [3]Forsc… lth Labora… stituto de Medic… Research, South African Medical Research Council, Durban, South Africa; [8]Special Treatment Institution for Detainees with Tuberculosis, Baku, Republic of Azerbaijan; [9]Cepheid, Sunnyvale, California; and [10]Foundation for Innovative New Diagnostics, Geneva, Switzerland

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

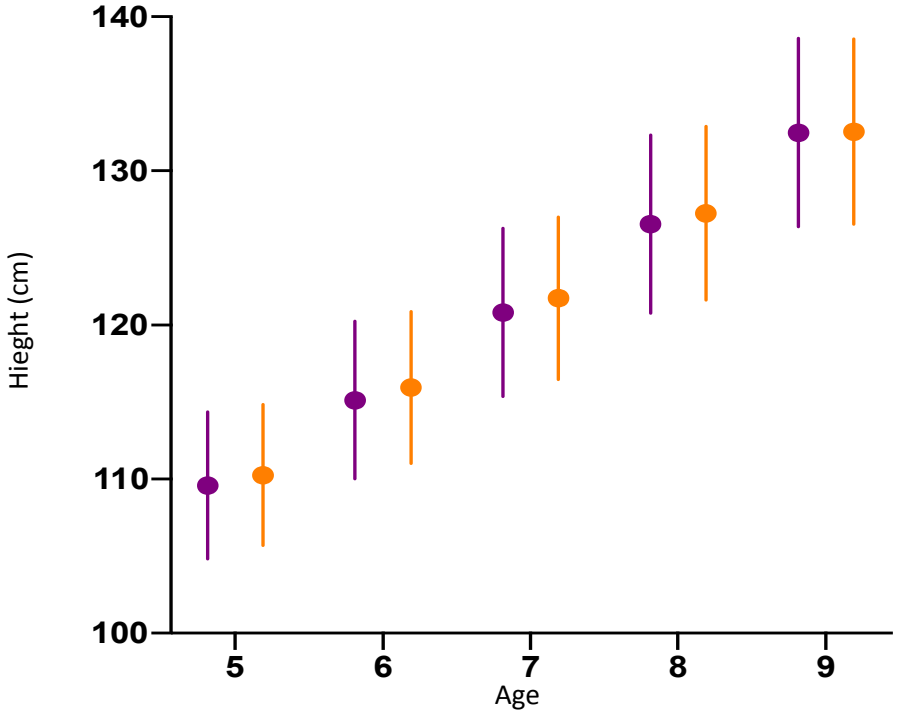
Am J Respir Crit Care Med Vol 184. pp 1076–1084, 2011

>2,000 samples

Ct against smear



Height against age



WHO statistics

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

Culture-based methods are superior to PCR for the quantification of early antituberculosis treatment effects in sputum.

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 C_T was not significantly discriminative og 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.

>300 samples

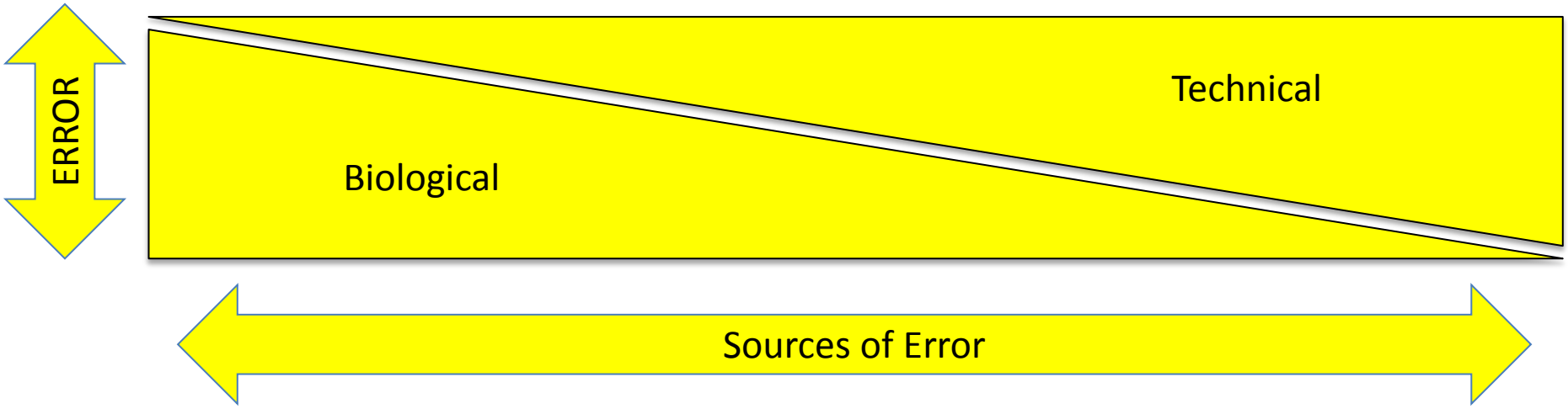
Summary

- Molecular quantification offers the potential for rapid monitoring of bacterial load/viability that could increase the pace of clinical trials

- However, our findings suggest more work is required to understand the sources of technical and biological/clinical noise

- Applying concepts of metrology (the science of measurement) offers a route to better understand the sources of error, improving the identification and translation of such biomarkers

Technical vs Biological error



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Vircell

- Pablo Mendoza

Cepheid

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- Maria Karczmarczyk

NIB

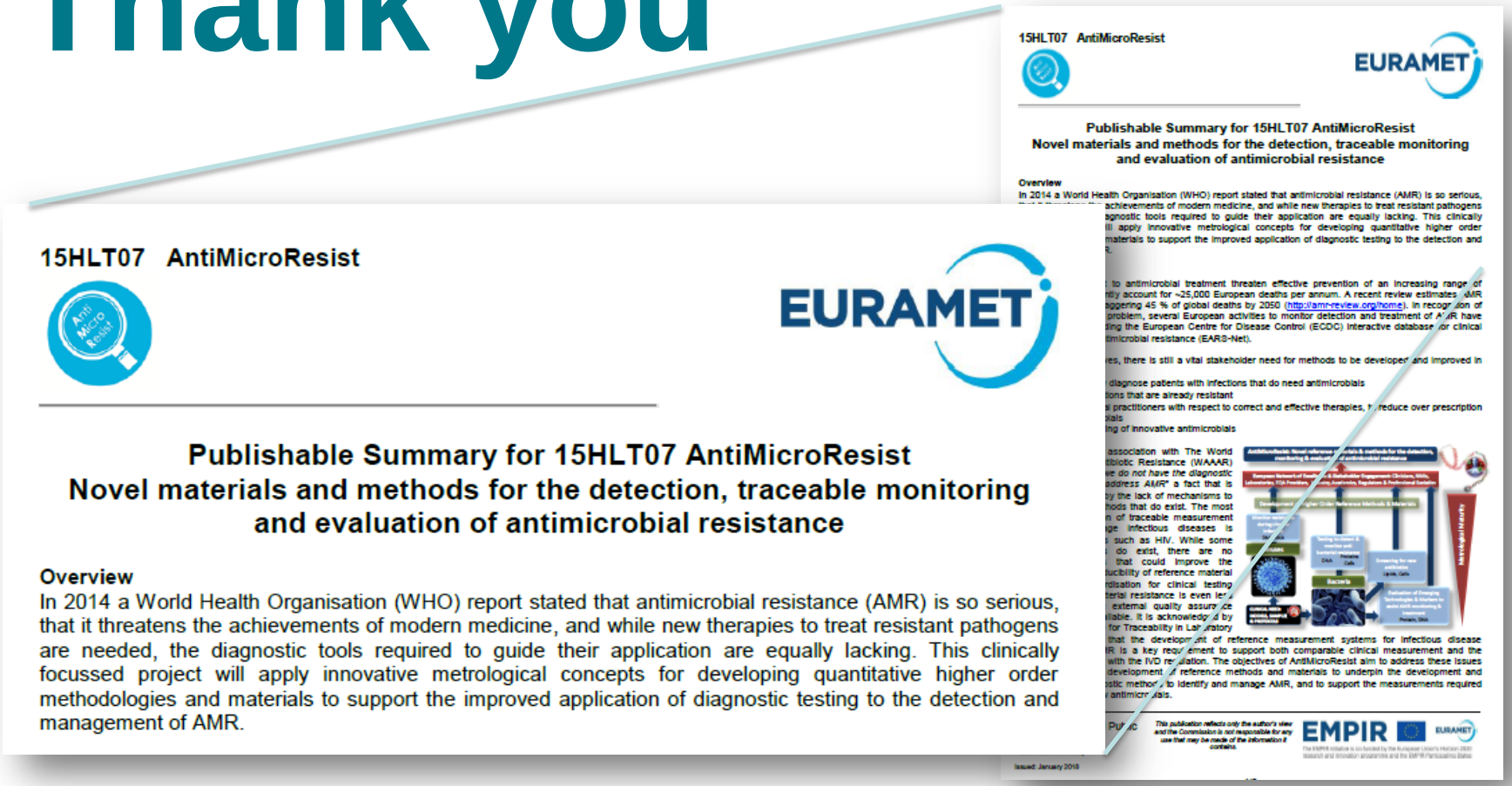
- Mojca Milavec
- Jernej Pavšič

Participants of inter laboratory study

Professor Marcus du Sautoy,

“up to the 1960s we could measure a distance to within an accuracy of one millionth of a meter.

Thank you



15HLT07 AntiMicroResist

EURAMET

Publishable Summary for 15HLT07 AntiMicroResist
Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

Overview

In 2014 a World Health Organisation (WHO) report stated that antimicrobial resistance (AMR) is so serious, that it threatens the achievements of modern medicine, and while new therapies to treat resistant pathogens are needed, the diagnostic tools required to guide their application are equally lacking. This clinically focussed project will apply innovative metrological concepts for developing quantitative higher order methodologies and materials to support the improved application of diagnostic testing to the detection and management of AMR.

New molecular diagnostic interlab study on MDR planned Autumn 2018

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