

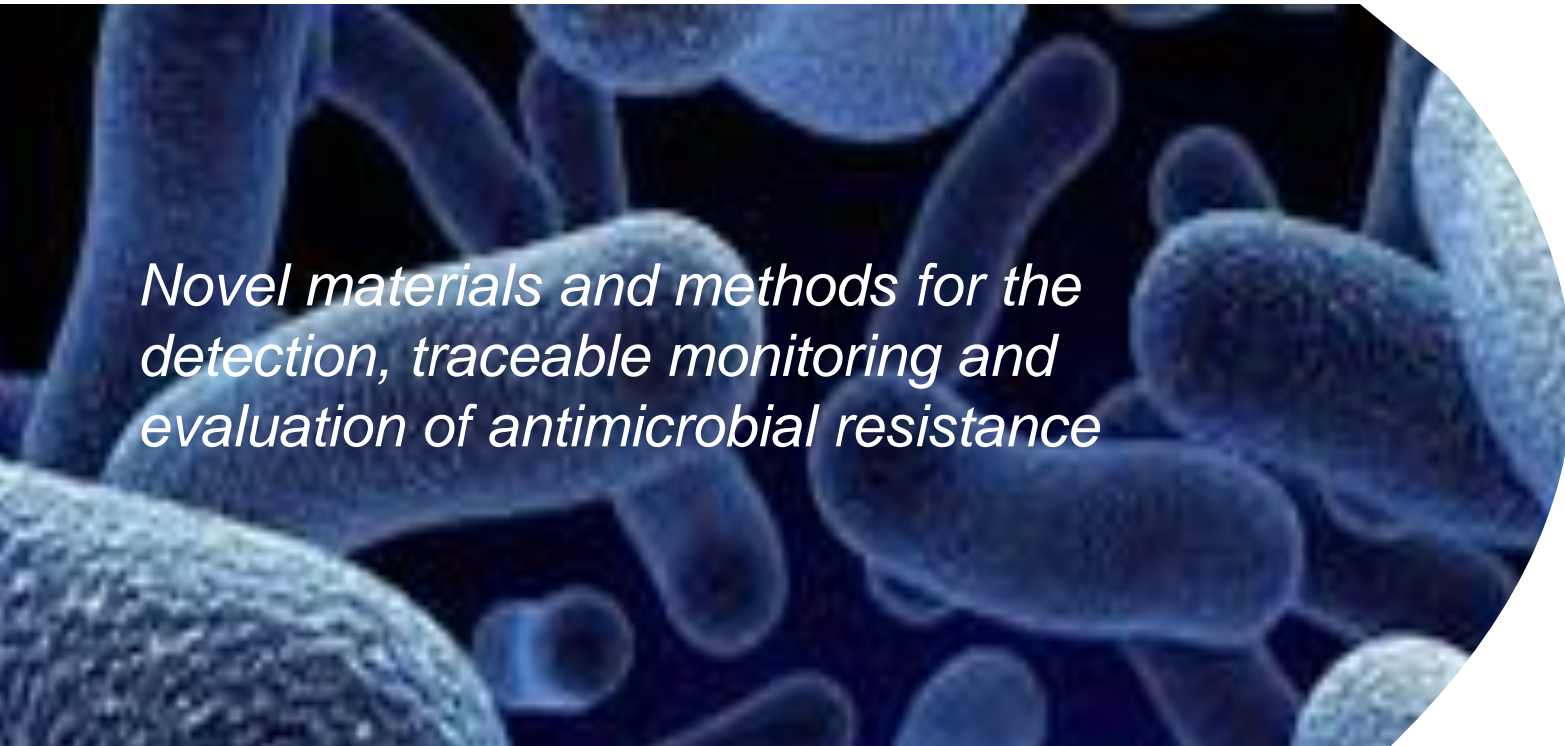
# Implications of quantification in TB molecular diagnosis



Jim Huggett



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



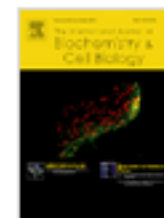
# Quantification of Mtb load in Tuberculosis to guide prognosis and predictive monitoring

- 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



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Medicine in focus

## Tuberculosis: amplification-based clinical diagnostic techniques

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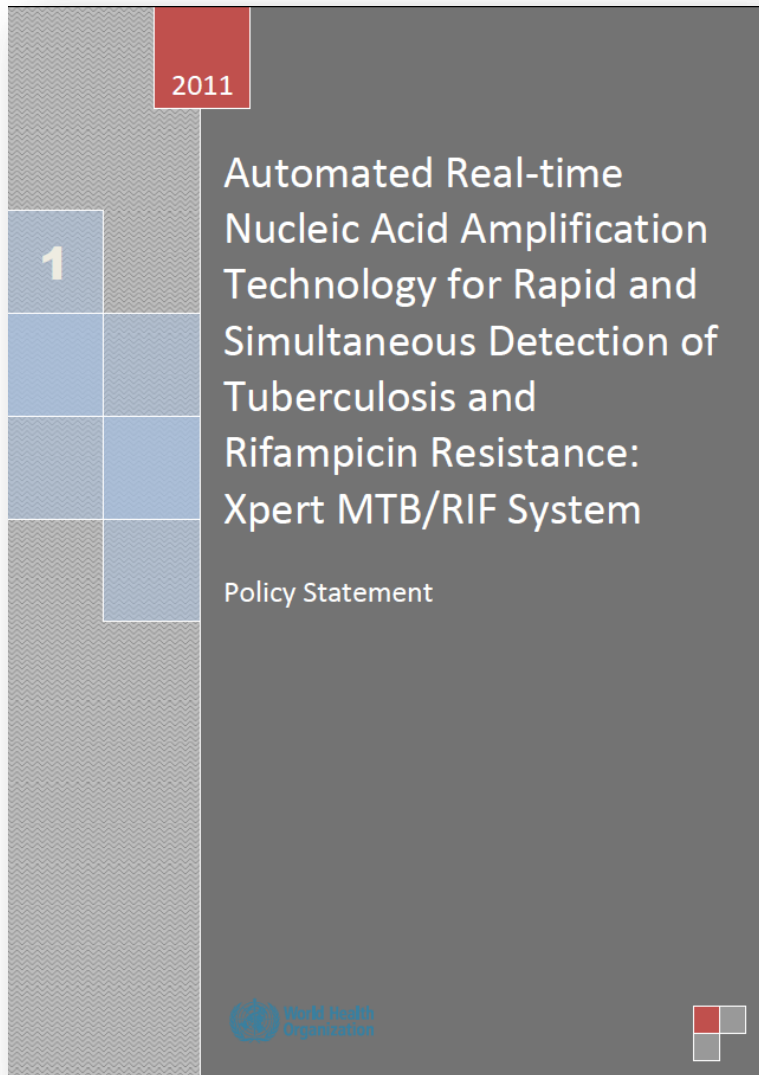
<sup>b</sup> Department of Medical Microbiology, Royal Free and University College Medical School, University College London, Royal Free Campus, London NW3 2PF, UK

Available online 8 April 2003

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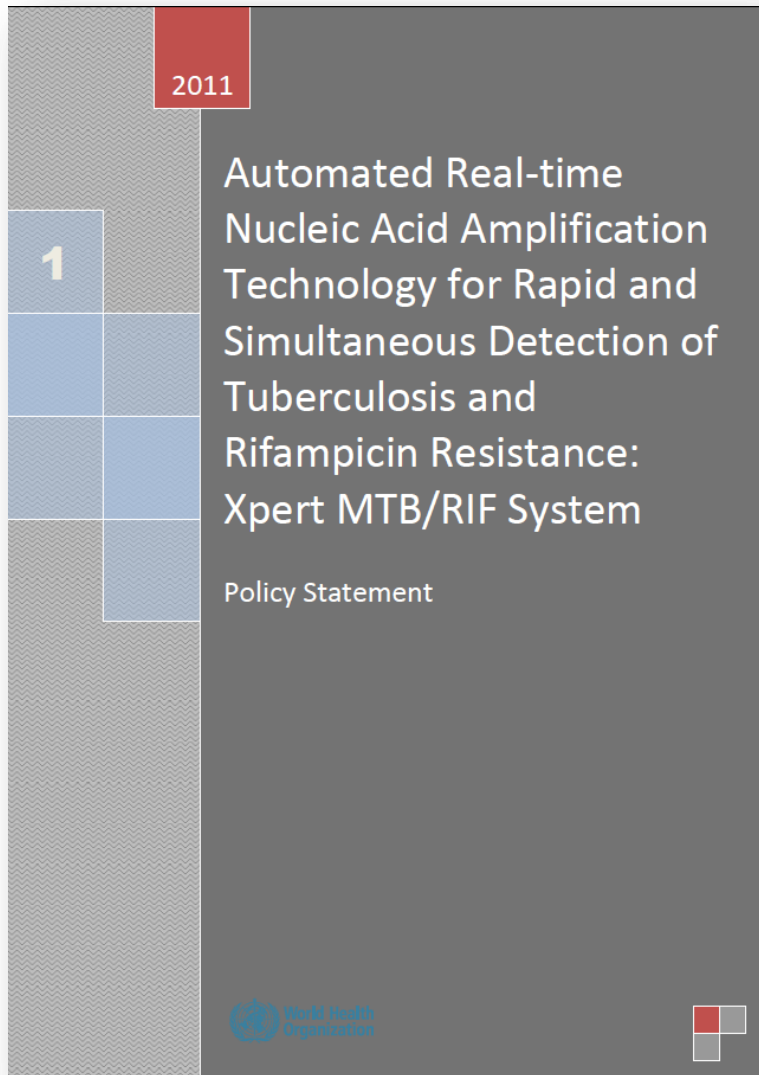


## Xpert RIF/MTB



# Quantification?

Xpert RIF/MTB



# 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

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

Alison S. Devonshire,<sup>†</sup> Isobella Honeyborne,<sup>‡</sup> Alice Gutteridge,<sup>†,||</sup> Alexandra S. Whale,<sup>†</sup> Gavin Nixon,<sup>†</sup> Philip Wilson,<sup>§</sup> Gerwyn Jones,<sup>†</sup> Timothy D. McHugh,<sup>‡</sup> Carole A. Foy,<sup>†</sup> and Jim F. Huggett<sup>\*,†,‡</sup>

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# Measured fold difference between materials

10000

Devonshire et al. *BMC Infectious Diseases* (2016) 16:366

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

1

dPCR

Lab α

Lab β

Lab γ

Lab 1

Lab 2

Lab 3

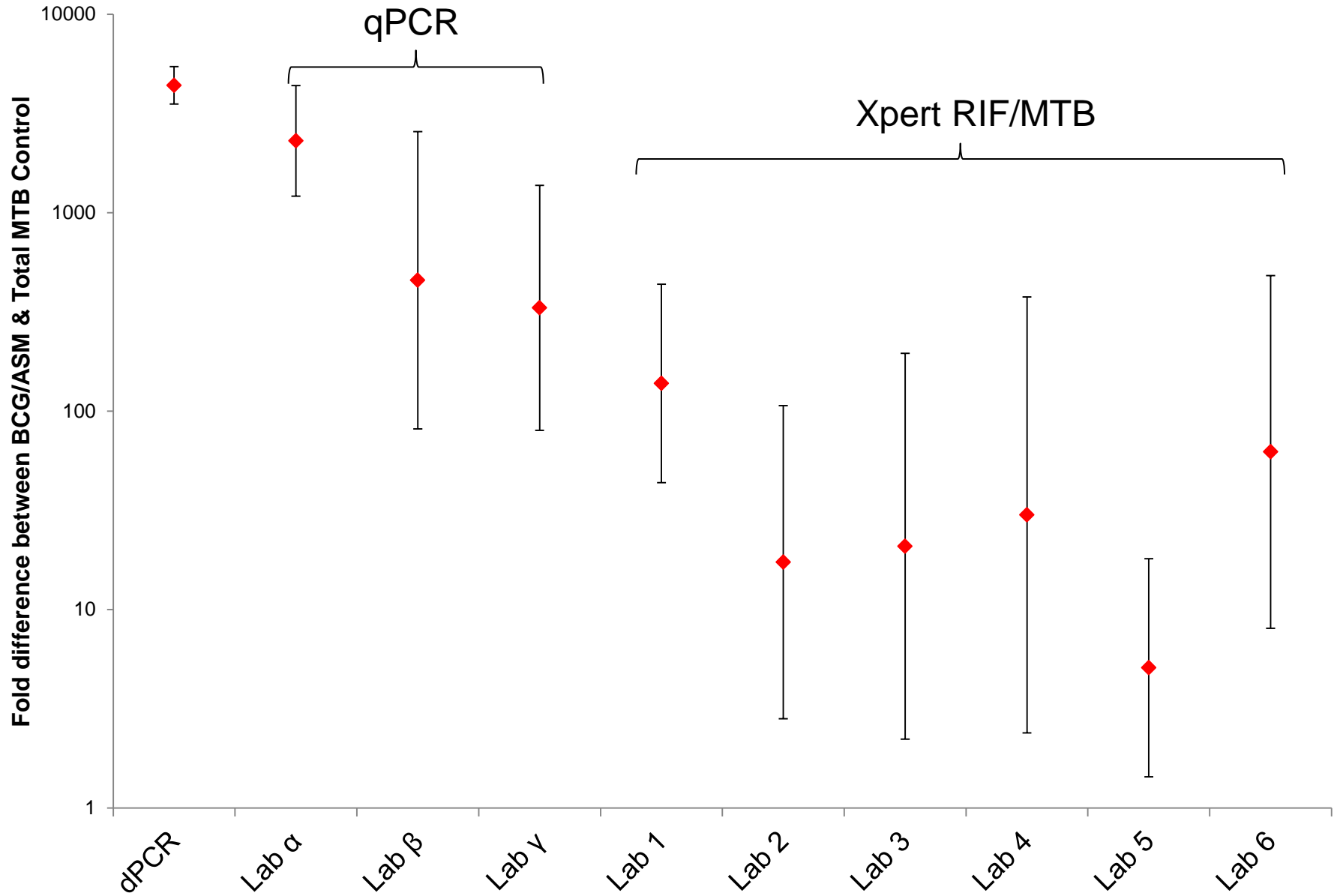
Lab 4

Lab 5

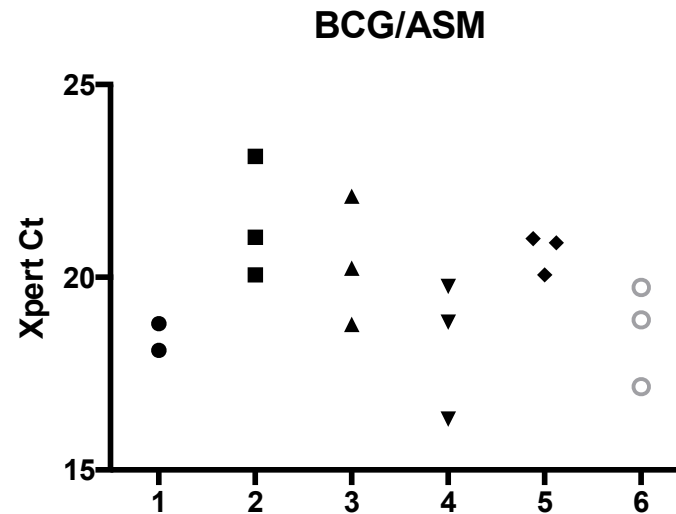
Lab 6



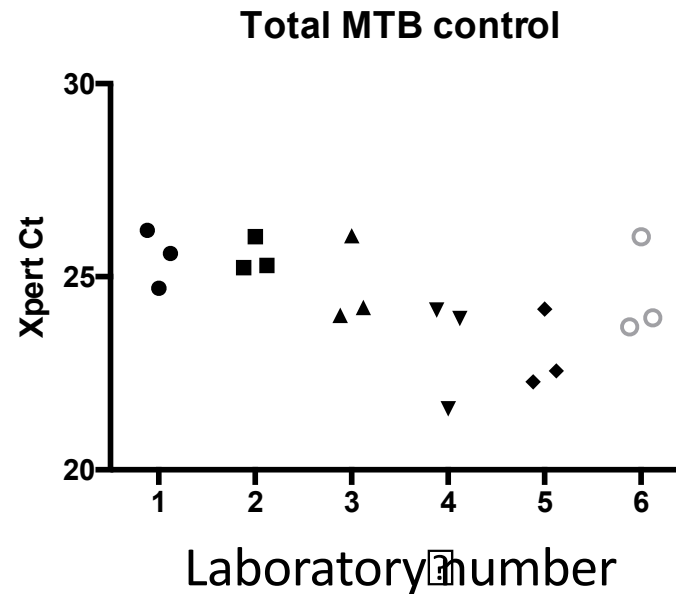
# Measured fold difference between materials



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

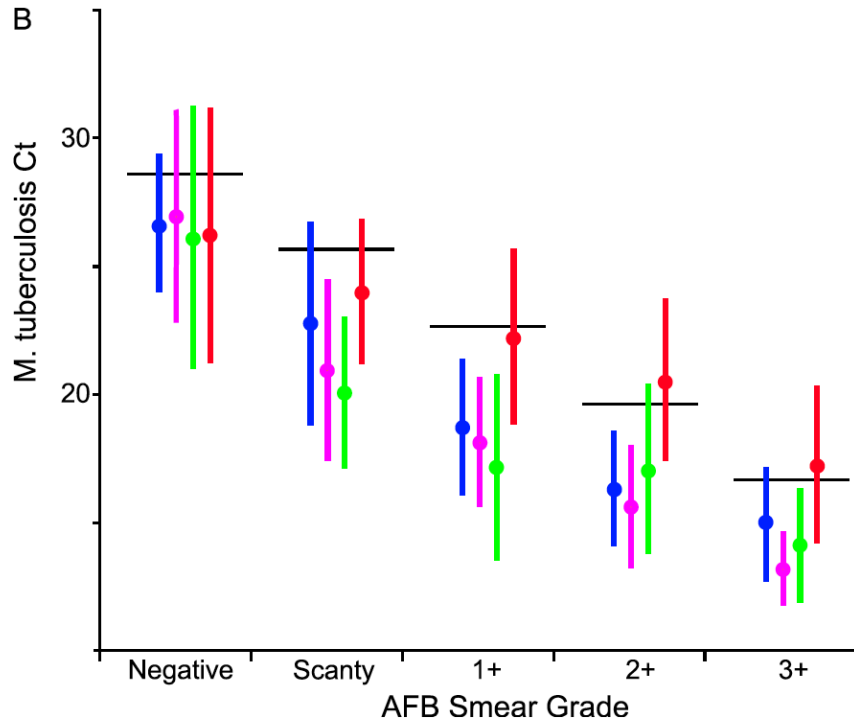
**Conclusions:** Xpert MTB/RIF quantitation offers a new, standardized approach to measuring bacterial burden in the sputum of patients with tuberculosis.

<sup>1</sup>Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, Georgia; <sup>2</sup>Department of Clinical Microbiology, University of California, San Francisco, California; <sup>3</sup>Forsyth Institute, Cambridge, Massachusetts; <sup>4</sup>South African Medical Research Council, Durban, South Africa; <sup>5</sup>Special Treatment Institution for Detainees with Tuberculosis, Baku, Republic of Azerbaijan; <sup>6</sup>Cepheid, Sunnyvale, California; and <sup>10</sup>Foundation for Innovative New Diagnostics, Geneva, Switzerland

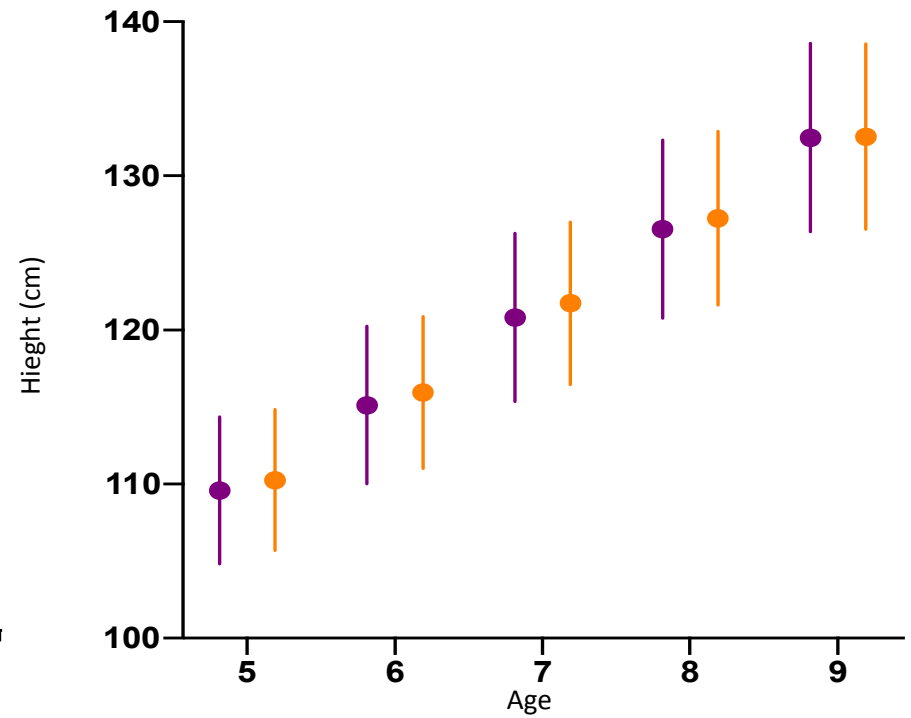
Am J Respir Crit Care Med Vol 184. pp 1076–1084, 2011



Ct against smear



Height against age



WHO statistics

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

Xavier A. Kayigire,<sup>a</sup> Sven O. Friedrich,<sup>b</sup> Amour Venter,<sup>c</sup> Rodney Dawson,<sup>d</sup> Stephen H. Gillespie,<sup>e</sup> Martin J. Boeree,<sup>f</sup> Norbert Heinrich,<sup>g,h</sup> Michael Hoelscher,<sup>g,h</sup> Andreas H. Diacon,<sup>b</sup> 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  $\pm$  standard deviation [SD]), time to culture positivity (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,  $5.72 \pm 1.00$ ; TTP,  $116.0 \pm 47.6$ ;  $C_T$ ,  $19.3 \pm 3.88$ ) to day 7 (log CFU,  $-0.55 \pm 1.24$ ,  $P = 0.0024$ ; TTP,  $11.580 \pm 4.135$ ,  $P = 0.0001$ ;  $C_T$ ,  $12.03 \pm 0.316$ ,  $P = 0.091$ ). Culture-based methods are superior to PCR for the quantification of early antituberculosis treatment effects in sputum.

**$C_T$  was not significantly discriminative**

>300 samples

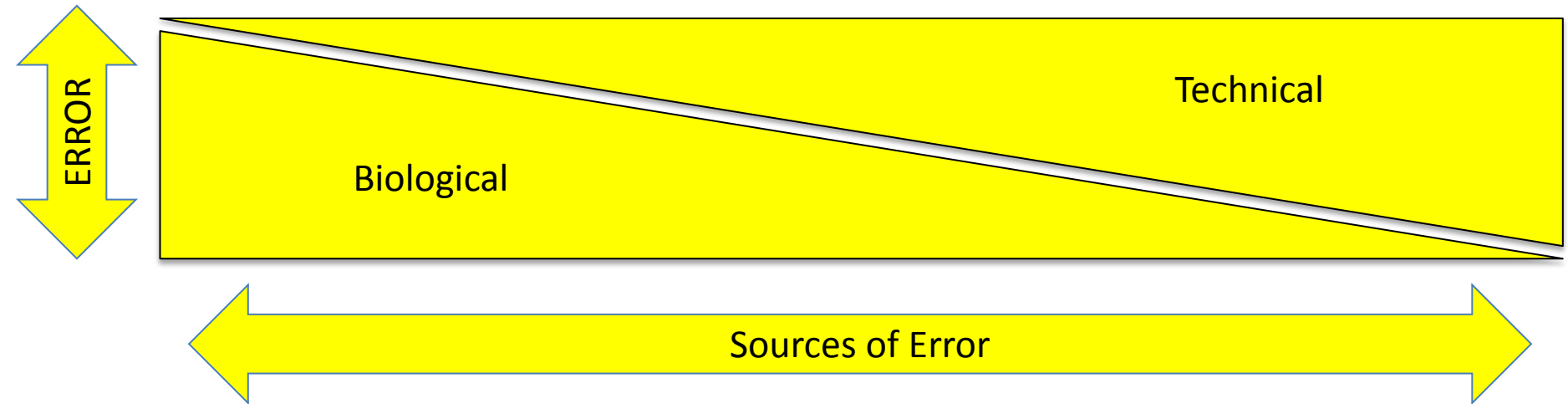
# Summary



- 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



# Acknowledgements

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- Sushma Patel

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- Jernej Pavšič

Participants of inter laboratory study

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Professor Marcus du Sautoy,

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

15HLT07 AntiMicroResist



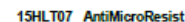
## Overview

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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**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, achievements of modern medicine, and while new therapies to treat resistant pathogens are being developed, the diagnostic tools required to guide their application are equally lacking. This clinically driven research will apply innovative metrological concepts for developing quantitative higher order metrology materials to the improved application of diagnostic testing to the detection and

to antimicrobial treatment threaten effective prevention of an increasing range of nity account for ~25,000 European deaths per annum. A recent review estimates AMR oggering 45 % of global deaths by 2050 (<http://amr-review.org/home>). In recognition of the problem, several European activities to monitor detection and treatment of AMR have the European Centre for Disease Control (ECDC) interactive database for clinical (microbial resistance (EARS-Net).

yes, there is still a vital stakeholder need for methods to be developed and improved in

diagnose patients with infections that do need antimicrobials

ing of innovative antimicrobials

association with The World Bili... Resistance (WAAAR) we do not have the diagnostic evidence AMR<sup>®</sup> a fact that is why the lack of mechanisms to tools that do exist. The most of traceable measurement of infectious diseases such as HIV. While some do exist, there are no that could improve the utility of reference material validation for clinical testing. Resistance is even less of external quality assurance. It is acknowledged by for Traceability in Laboratory that the development of reference measurement systems for infectious disease R is a key requirement to support both comparable clinical measurement and with the IVDR regulation. The objectives of AMR MicroResist aim to address these issues by developing reference methods and materials to underpin the development and application of reference methods and materials to support the measurements required for AMR<sup>®</sup>.

Public

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