Diagnosing TB in clinical practice (including DR-TB)? Controversies, Innovation, and Challenges.

IFCC World Lab, Durban, 2017

Keertan Dheda, MB.BCh (Wits), FCP(SA), PhD (Lond), FRCP (Lond)

Professor and Head: Division of Pulmonology,

Department of Medicine, University of Cape Town

Conflict of interest statement: I have received speaker fees, grant funding, and/or free kits/ material from Hain Life Sciences, Alere Diagnostics, Clondiag, Orgenics, Qiagen (Cellestis), Protein Logic, Oxford Immunotec, Antrum Biotech, FIND Diagnostics, Boston Scientific, Nycomed Takeda, Novartis, Cipla, GSK, Astra Zeneca and SSI - however noneof these entities have played a role in study design or publication of data.

Overview

- TB or not TB? If TB, what is the resistance profile?
- Is the patient highly infectious? (contact tracing and infection control)
- Why does this patient have TB? (HIV, HbA1C, Cr, NCD)

- Diagnosis of LTBI (TST, IGRA and C-tb)

- NAAT (Gene Xpert MTB/ RIF; LPA; LAMP etc)
  - Retreatment cases; PPV
  - Contact tracing and infection control
  - Ultra cartridge

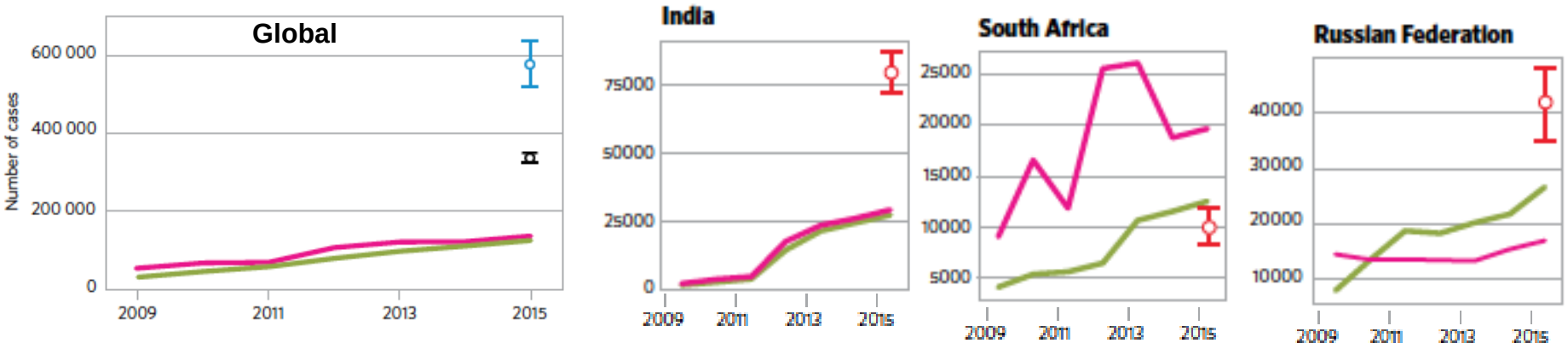
- WGS: implications for diagnostics & precision medicine

- Decentralising diagnostics to practice/ clinic or community: active case finding & triage tests

TB & drug-resistant TB: size of the problem

- TB top ID killer- 3 people die every minute! Over 1 billion people killed over the last 2 centuries!

- ~600 000 (540 000 to 664 000) MDR-TB (RR) cases globally in 2016 (~20% of TB deaths)



Global TB Report, WHO, 2016

- 2015: 51% of MDR-TB globally had resistance to either a FQ, a second-line injectable agent, or both
  (2017= 39% of those treated= FQ or SLID resistance)

- ~20% strains globally resistant to 1 major TB drug

Global TB Report, WHO, 2016

THE LANCET

Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study

Elize Pietersen, Elisa Ignatius*, Elizabeth M Streicher, Barbara Mastrapa, Xavier Padanilam, Anil Pooran, Motasim Badri, Maia Lesosky, Paul van Helden, Frederick A Sirgel, Robin Warren, Keertan Dheda*

Pietersen and Dheda, Lancet, 2014

THE LANCET 2012

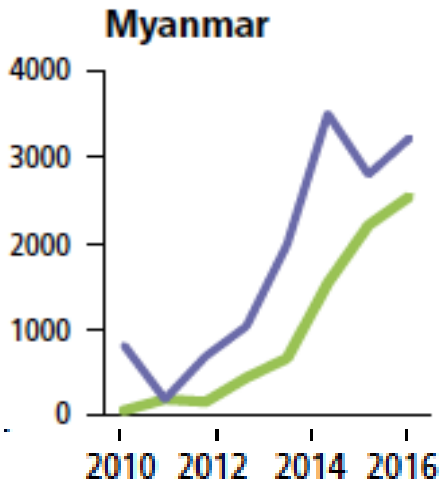
The global rise of extensively drug-resistant tuberculosis: is the time to bring back sanatoria now overdue?

Keertan Dheda, Giovanni B Migliori

Sondalo (1938) - 3500 beds

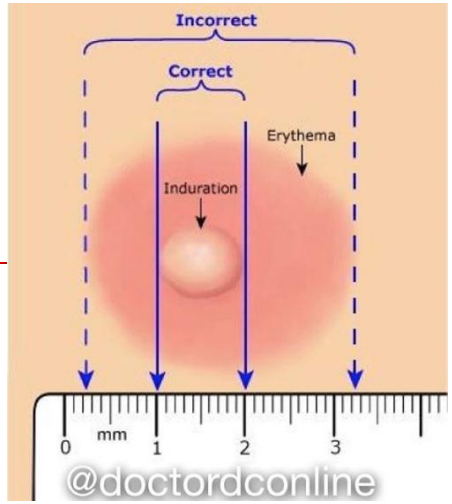
Myanmar (53 million: 45% in informal housing)

- Estimated burden in 2017: 191 000 cases [141-249]
  Incidence: 361/ 100 000 cases (9% HIV-infected)
  (estimated MDR-TB burden= 13 000 (5.1% new and 27% retreatment)
- 139 625 notified
  tested for R resistance= ~15% new and 63% of previous TB)
- Detected: 3213 MDR and 0 XDR
- Treated: 2537 and 5 XDR-TB
  (19.5 % of the total burden!!)



Global TB Report, WHO, 2017 (blue= detected; green= treated)

Diagnosis of LTBI



- TST
- T-SPOT-TB
  (post overnight ELISPOT assay)
- Quantiferon-TB Gold Plus
  (post overnight ELISA readout)

C-TB (ESAT-6 and CFP-10-specific skin test)

EUROPEAN RESPIRATORY *journal*

OFFICIAL SCIENTIFIC JOURNAL OF THE ERS

Sensitivity of C-Tb: a novel RD-1-specific skin test for the diagnosis of tuberculosis infection

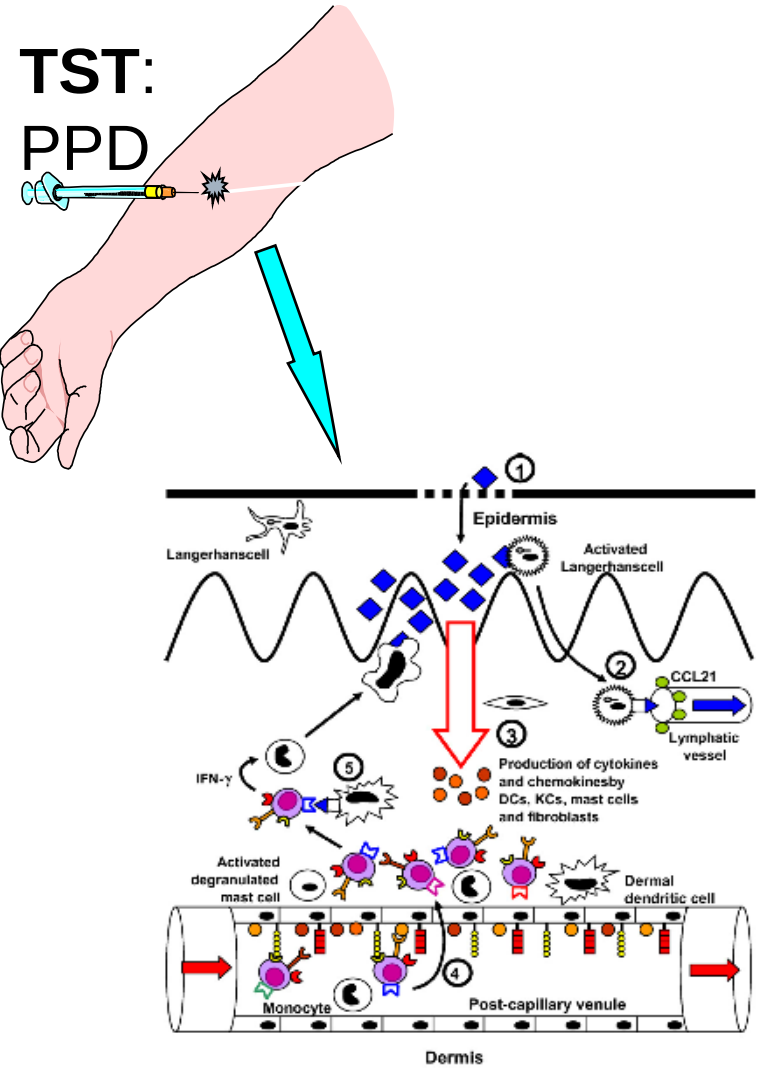
Soren T. Hoff[2], Jonathan G. Peter[1], Grant Theron[1], Mellissa Pascoe[1], Pernille N. Tingskov[3], Henrik Aggerbeck[3], Daniel Kolbus[3], Morten Ruhwald[2], Peter Andersen[2,4] and Keertan Dheda[1,4]

LTBI diagnosis: detection of a memory T cell response

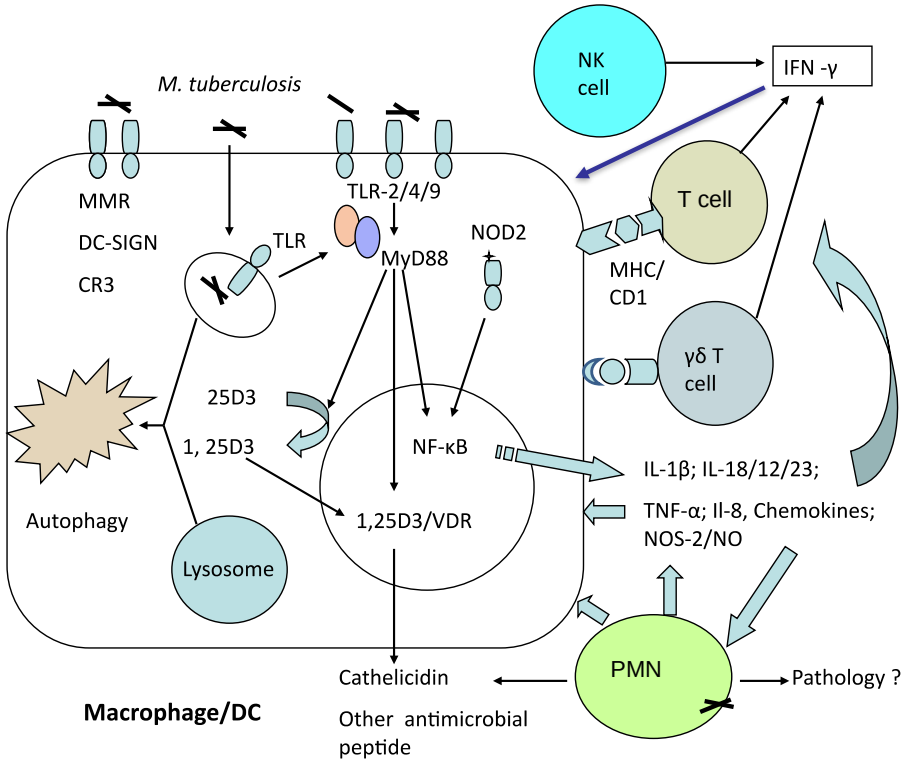
RD1 proteins encoded by gene segments deleted from BCG

Relatively *M.tb* specific *(M. kansasii, M szulgai, M marinum.)*

TST:
PPD



Vukmanovic-Stejic M, Imm Letters, 2006



Dheda K, Respirology, 2010

Indications in low burden settings: screening for LTBI where the risk benefit ratio is likely in favour of testing + treatment

- Contacts of infectious TB cases at risk
- Immuno-suppressive conditions: IMID (TNF), silicosis, HIV, post transplant, dialysis (TST + IGRA)
- Health care workers, prisoners, homeless, drug users
- Immigrants from TB endemic countries (risk stratify when older than 35 years; high incidence= > 150/ 100 000 cases)

Dheda K, Lancet, 2016

WHO guideline on LTBI, 2015

UK NICE guideline, 2016

Public Health England, 2016 (+ Migrant screening 2016)

ATS/ CDC 2000

Indications in high burden settings: screening for LTBI where the risk benefit ratio is likely in favour of testing + treatment

- HIV-infected persons and children under 5 years
- Contacts of infectious TB cases at risk: inform and advise (? CXR)
- Immuno-suppressive conditions: IMID (TNF), silicosis, post transplant, dialysis (TST + IGRA): cover with INH

Dheda K, Lancet, 2016

WHO guideline on LTBI, 2015

UK NICE guideline, 2016

Public Health England, 2016 (+ Migrant screening 2016)

ATS/ CDC 2000

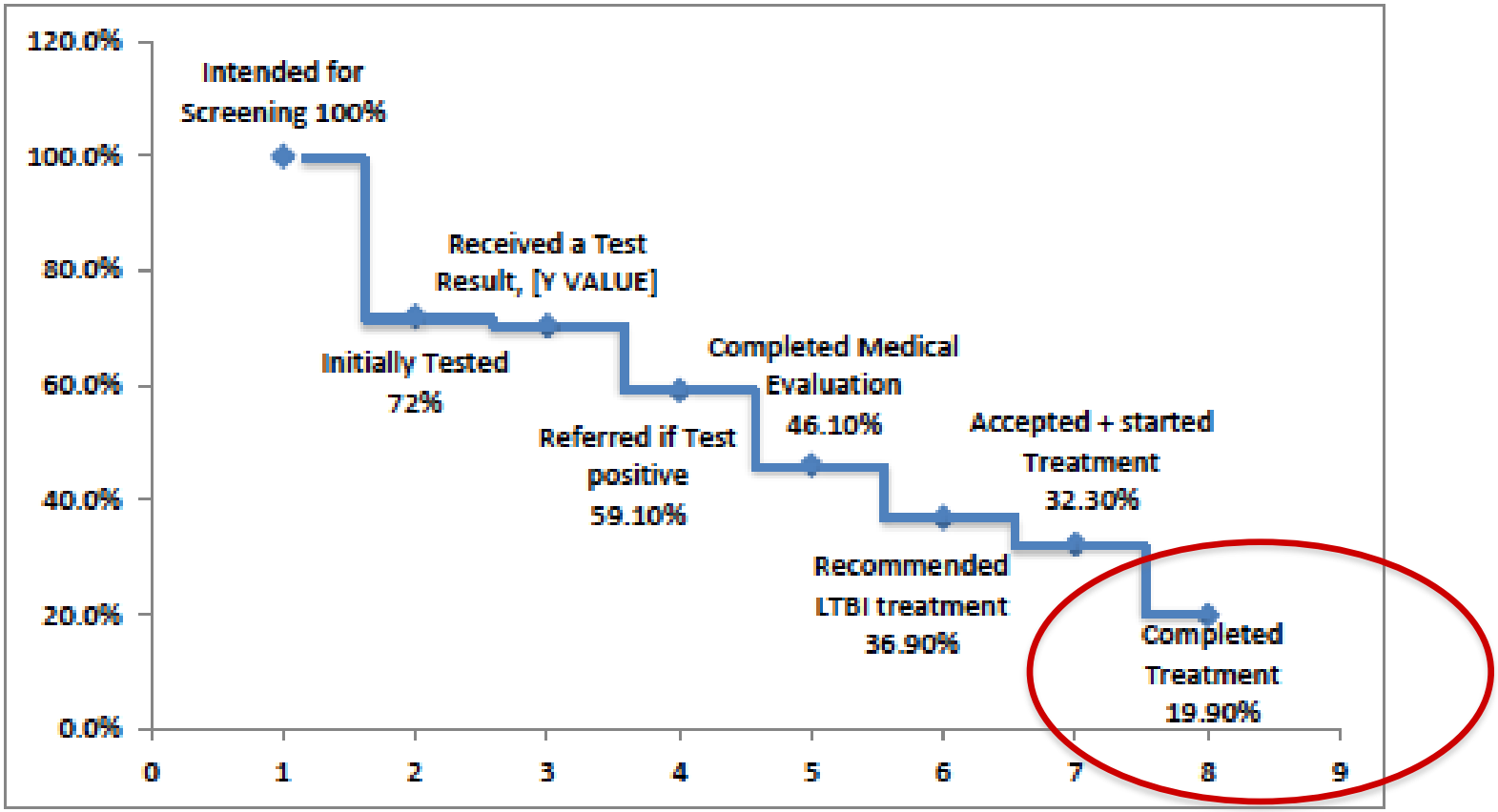
Predictive value of test +ve TST versus +ve IGRAs in longitudinal studies (2 SRs)

PPV IGRA (recommended cut-point) = 2.10 to 2.7%
PPV TST (10mm cut-point) = 1.60 to 1.5%

BOTH IMPERFECT TESTS

Rangaka M, Lancet Infect Dis, 2011
Diel R, Eur Resp J, 2012

LTBI cascade

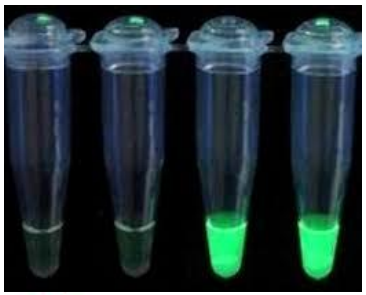


Alsdurf H, Lancet Infect Dis. 2016

Europe: **Smear microscopy ± culture routine**

- Molecular tests (NAAT): alternatives when rapid diagnosis and/ or DST required for clinical or public health reasons

(e.g. high suspicion of MDR/ XDR, or if phenotypic DST likely unavailable within 8 wks)



EIKEN CHEMICAL CO., LTD.

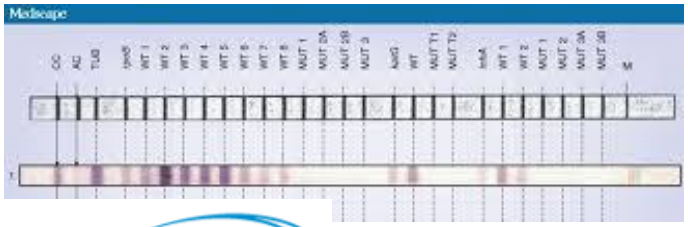
Cepheid®

- Major advance
- rules-in 2/3

smear negative TB and rapid Dx of DR-TB

Abbott

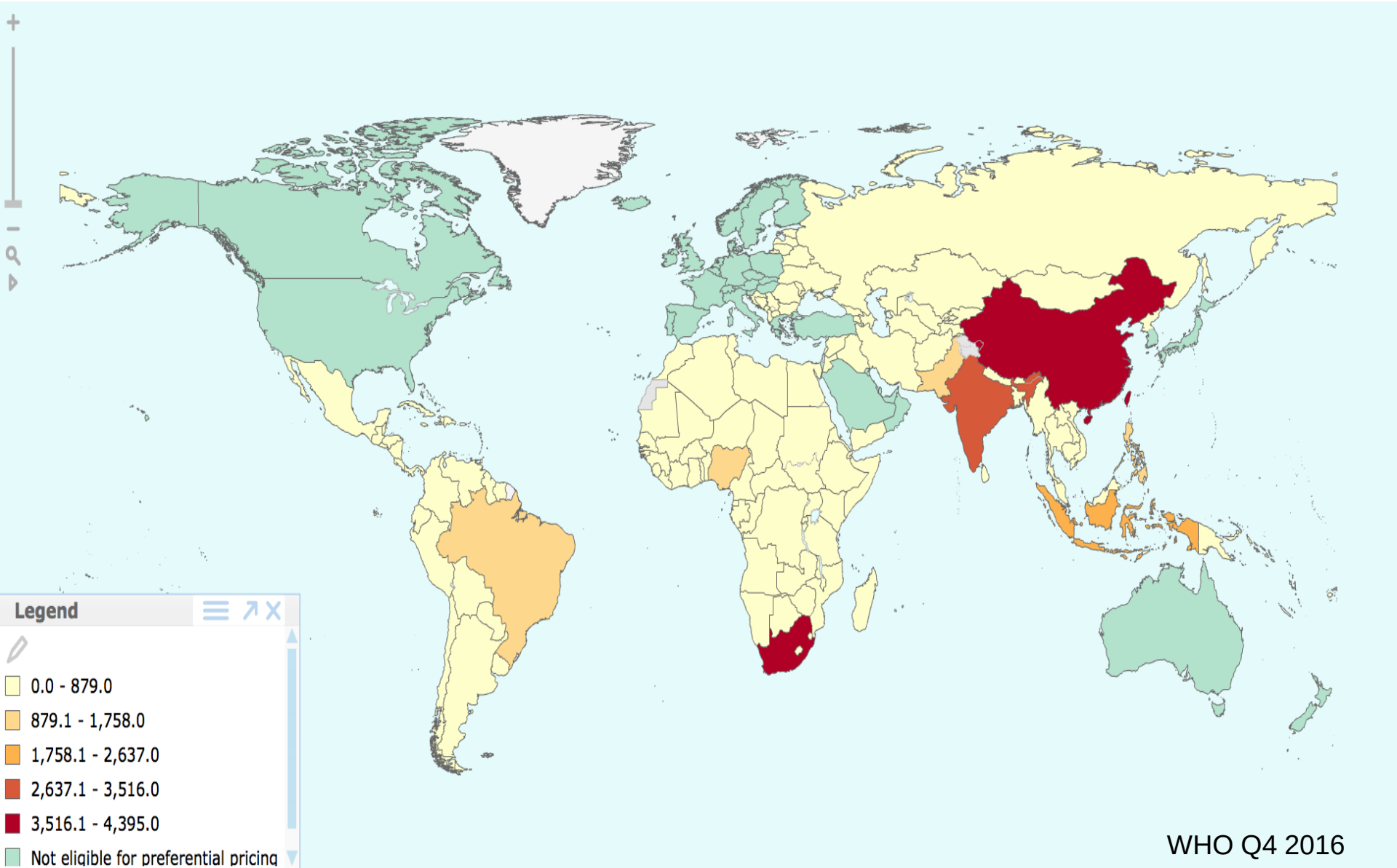
Roche



HAIN LIFESCIENCE

INNOGENETICS
BIOTECHNOLOGY FOR HEALTHCARE

Number of Xpert modules procured



WHO Q4 2016

Key practice points

- PPV of Rif R limited when TB incidence is low

(MDR TB prevalence of 2%= PPV of 50%; 3% MDR prevalence= 60%)

WHO Xpert Implementation Guidance, 2011

- Those with previous TB may yield false positive Xpert results

MAJOR ARTICLE

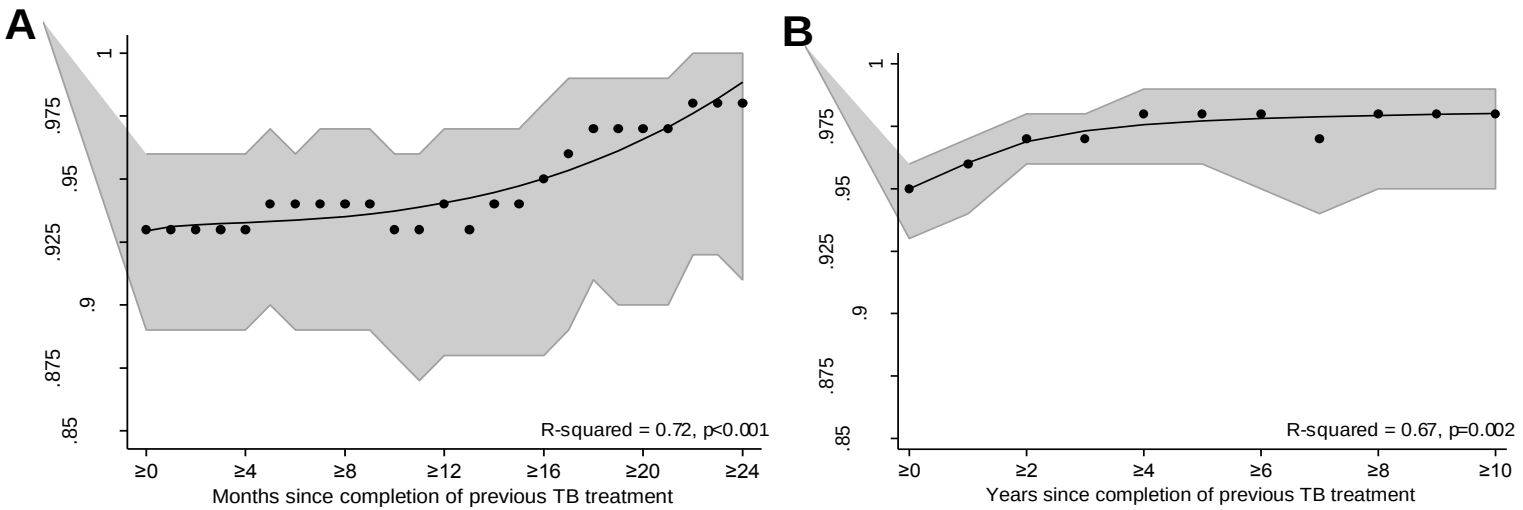
IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

Xpert MTB/RIF Results in Patients With Previous Tuberculosis: Can We Distinguish True From False Positive Results?

Grant Theron,[1,2,a] Rouxjeane Venter,[2] Greg Calligaro,[1] Liezel Smith,[1] Jason Limberis,[1] Richard Meldau,[1] Duncan Chanda,[1,3] Aliasgar Esmail,[1] Jonny Peter,[1] and Keertan Dheda[1,4]

[1]Lung Infection and Immunity Unit, Division of Pulmonology and University of Cape Town Lung Institute, Department of Medicine, University of Cape Town, and [2]DST/NRF of Excellence for Biomedical Tuberculosis Research, and MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa; [3]Institute for Medical Research and Training, Lusaka, Zambia; and [4]Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa

False positive Xpert MTB/RIF results in re-tested patients with previously confirmed tuberculosis



- Re-tested 238 Xpert +ve previously treated patients
- False-positivity rate (Xpert +ve, culture-negative) = 7% (16/238)

- Duration since the initial TB episode of ≤2 years rules in only 50% of false +ves
- Reclassifying “very low positive” to a “negative” result improved specificity [+3%(2-5%)] but reduced test sensitivity [-10%(4-15%)].

Infection control and contact tracing

- Negative PCR (e.g. Xpert) using a good sputum sample suggests no need for isolation (NPV of 99.7% for smear positivity)

Luetkemeyer AF, Clin Infect Dis, 2016

- Xpert bacterial load Readouts (Ct values correlate poorly with smear positivity)

The Use of An Automated Quantitative Polymerase Chain Reaction (Xpert *Mycobacterium tuberculosis*/RIF) to Predict the Sputum Smear Status of Tuberculosis Patients

Grant Theron,[1] Lancelot Pinto,[2] Jonny Peter,[1] Hemant Kumar Mishra,[3] Hridesh Kumar Mishra,[3] Richard van Zyl-Smit,[1] Surendra Kumar Sharma,[3] and Keertan Dheda[1,4,5]

Int J Tuberc Lung Dis. 2017 May 1;21(5):493-502. doi: 10.5588/ijtld.16.0702.

Diagnostic accuracy of the Xpert® MTB/RIF cycle threshold level to predict smear positivity: a meta-analysis.

Lange B[1], Khan P[2], Kalmambetova G[3], Al-Darraji HA[4], Alland D[5], Antonenka U[6], Brown T[7], Balcells ME[8], Blakemore R[9], Denkinger CM[10], Dheda K[7], Hoffmann H[6], Kadyrov A[3], Lemaitre N[11], Miller MB[12], Nikolayevskyy V[13], Ntinginya EN[14], Ozkutuk N[15], Palacios JJ[16], Popowitch EB[12], Porcel JM[17], Teo J[18], Theron G[18], Kranzer K[19].

REPORT FOR WHO

A multicentre non-inferiority diagnostic accuracy study of the Ultra assay compared to the Xpert MTB/RIF assay

Version 1.8 / February 2017

	Sensitivity (95%CI)				Specificity (95%CI)
	Pooled	Smear-negative	HIV-	HIV+	
Xpert	**82.9%** (78.8, 86.4)	**44.5%** (35.4, 53.9)	**89.3%** (83.1, 93.7)	**75.5%** (65.8, 83.6)	**98%** (96.8, 98.8)
Ultra	**87.8%** (84.2, 90.9)	**61.3%** (52, 70.1)	**90.6%** (84.7, 94.8)	**87.8%** (79.6, 93.5)	**94.8%** (93, 96.2)

17% improvement

Sensitivity of Xpert ULTRA compared to Xpert MTB/RIF in smear negative samples that are *M.tb* culture positive (n= 87)

Test performance with CIs and numbers	Xpert MTB/RIF	Xpert ULTRA	P-value
All Patients	63.4% (51.8, 73.7) 45/71	78.4% (68.4, 85.9) 65/83	P = 0.0496
HIV Un-infected	72.5% (54.3, 85.4) 21/29	85.4% (71.6, 93.2) 35/41	P = 0.2305
HIV Infected	57.2% (42.3, 70.9) 24/42	70.8% (55.6, 82.4) 29/41	P = 0.2548

+15%

+13%

+ 13%

- Xpert ULTRA cartridge
  - 2 amplification targets (IS6110 &1081)
  - Larger DNA reaction chamber
  - Addition of 'trace' detection readout
  - Improved fluidics and amplification
  - Melt curve analysis for RIF resistance
  - Ultra LOD is 15.6 CFU (vs 114 CFU for Xpert)

Esmail & Dheda, in preparation

Effect of empiric treatment

- **All RIF^R cases initiated on empiric MDR-TB Rx**
  - Suboptimal Rx of 30% [Pre-XDR (20%(FLQ^R or SLID^R) and XDR (10%)]
  - Suboptimal Rx of 70% MDR-TB

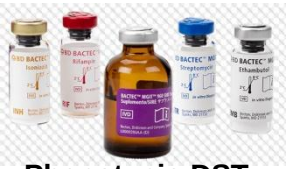
Limitations of existing technologies to predict resistance profiles: DR-TB

GXP gives RIF^R only
- No info on SL drugs

Xpert MTB/RIF

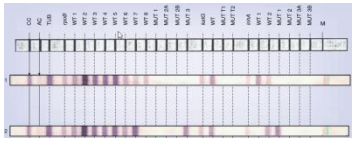
No reliable readouts for PZA & ETH
- In MDR: 60% PZA^R and 50% E^R

Long DST waiting times
- 2 to 3 mths SLIDs and FLQs



Phenotypic DST
MGIT liquid culture

Indeterminate results
- Up to 30% in SM -ve TB
- Readouts of 4 drugs
- contamination



Genotypic DST
Hain LPA - MTBDR*plus*
MTBDRs*l*

Inappropriate Rx initiated

POOR OUTCOMES + AMPLIFICATION OF RESISTANCE

We therefore need a full drug sensitivity profile at diagnosis

SCIENTIFIC REPORTS

OPEN

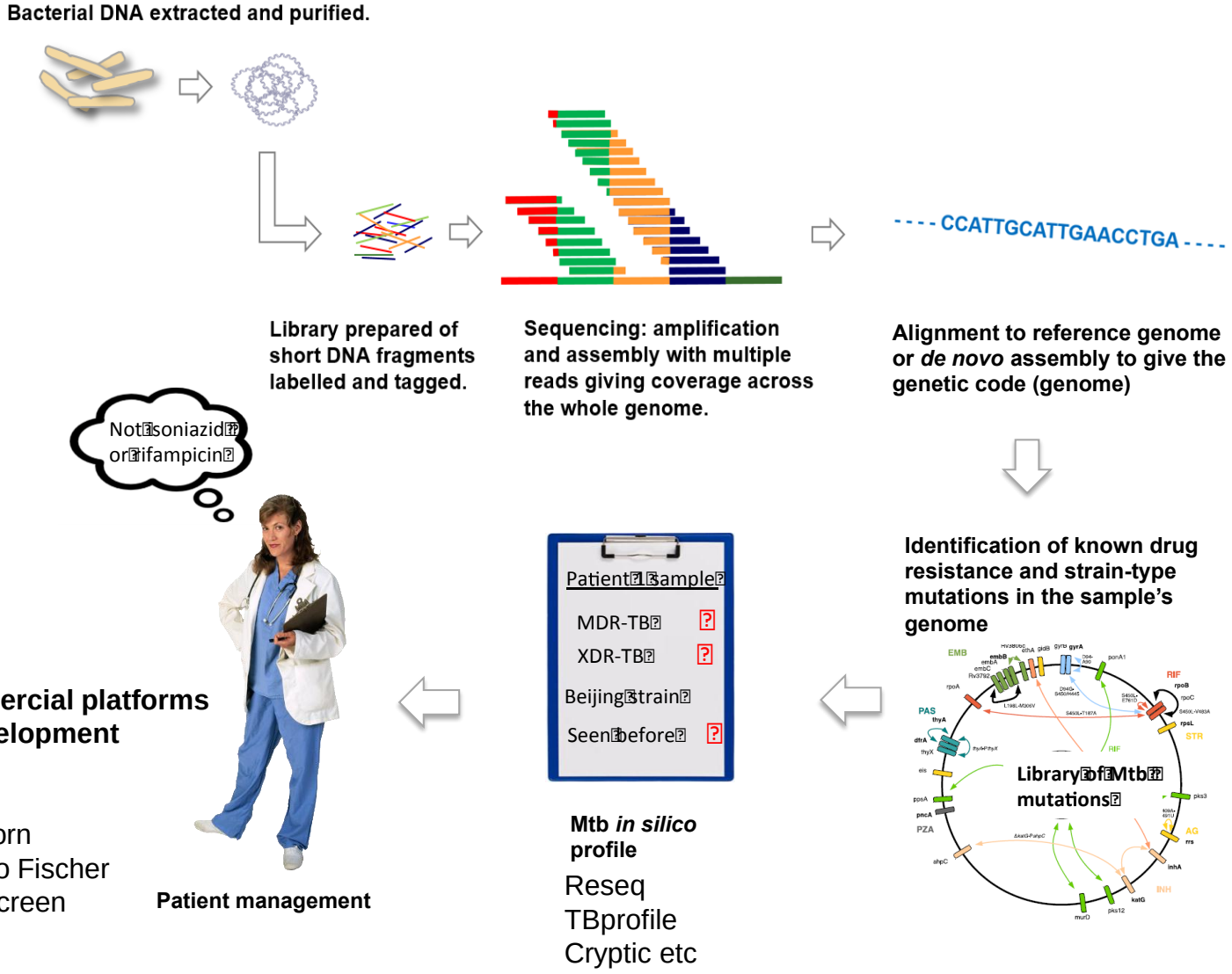
The diagnostic accuracy of the MTBDR*plus* and MTBDR*sl* assays for drug-resistant TB detection when performed on sputum and culture isolates

Received: 11 May 2015
Accepted: 04 November 2015
Published: 10 February 2016

Michele Tomasicchio[1,*], Grant Theron[2,1,*], Elize Pietersen[1], Elizabeth Streicher[2], Danielle Stanley-Josephs[2], Paul van Helden[2], Rob Warren[2] & Keertan Dheda[1,3]

Rule in XDR-TB in 78% of cases

Next generation WGS: precision medicine the next diagnostic frontier

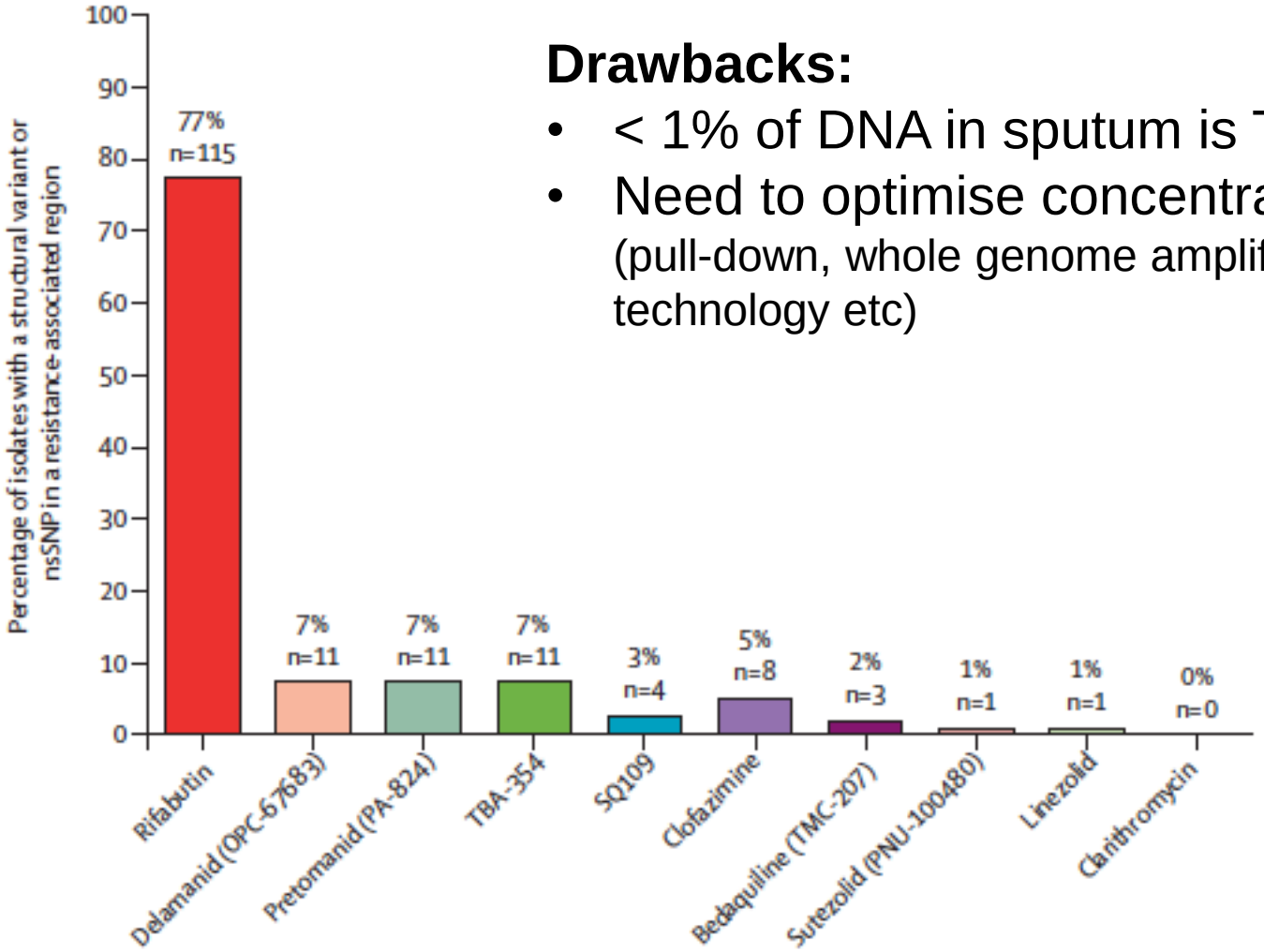


Dheda K, Lancet Resp Med, 2017

THE LANCET
Respiratory Medicine
2017

Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study

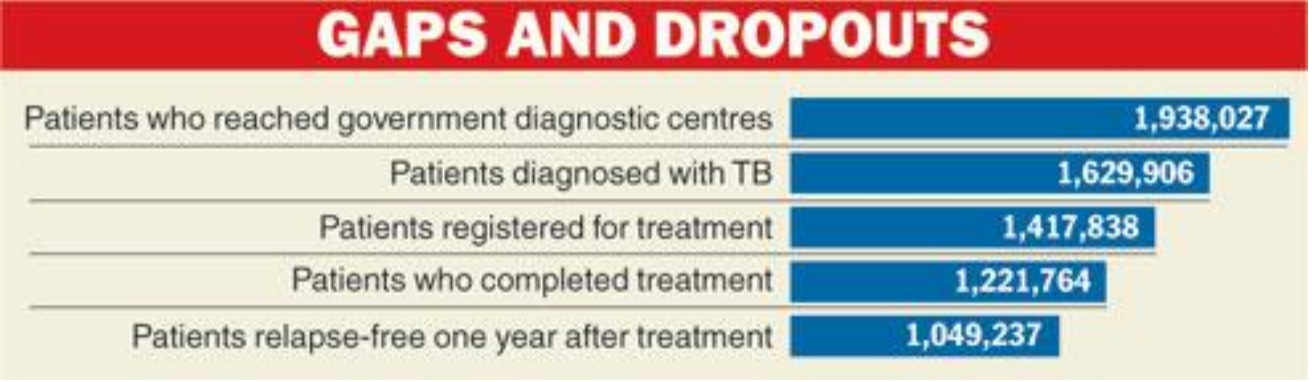
Keertan Dheda, Jason D Limberis*, Elize Pietersen, Jody Phelan, Aliasgar Esmail, Maia Lesosky, Kevin P Fennelly, Julian te Riele, Barbara Mastrapa, Elizabeth M Streicher, Tania Dolby, Abdallah M Abdallah, Fathia Ben-Rached, John Simpson, Liezel Smith, Tawanda Gumbo, Paul van Helden, Frederick A Sirgel, Ruth McNerney, Grant Theron, Arnab Pain, Taane G Clark†, Robin M Warren†*



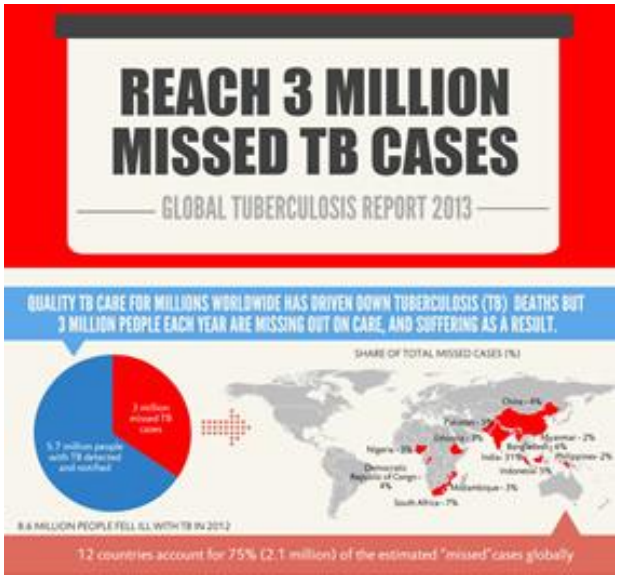
Drawbacks:

- < 1% of DNA in sputum is TB-specific
- Need to optimise concentration of DNA (pull-down, whole genome amplification technology etc)

Dheda K, Lancet Resp Med, 2017



Subbaraman R, PLoS Med, 2017



4.26 million (41%) of ~10.4 million new cases in 2015 went undiagnosed or unreported (worse for DR-TB= drivers of transmission)

RCT of Xpert versus smear microscopy (n=1500 in 4 countries in Africa)

Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial

*Grant Theron, Lynn Zijenah, Duncan Chanda, Petra Clowes, Andrea Rachow, Maia Lesosky, Wilbert Bara, Stanley Mungofa, Madhukar Pai, Michael Hoelscher, David Dowdy, Alex Pym, Peter Mwaba, Peter Mason, Jonny Peter, Keertan Dheda, for the TB-NEAT team**

- Feasible at POC in a clinic and significantly reduces patient drop out

Theron & Dheda, Lancet, 2014

THE LANCET
Infectious Diseases

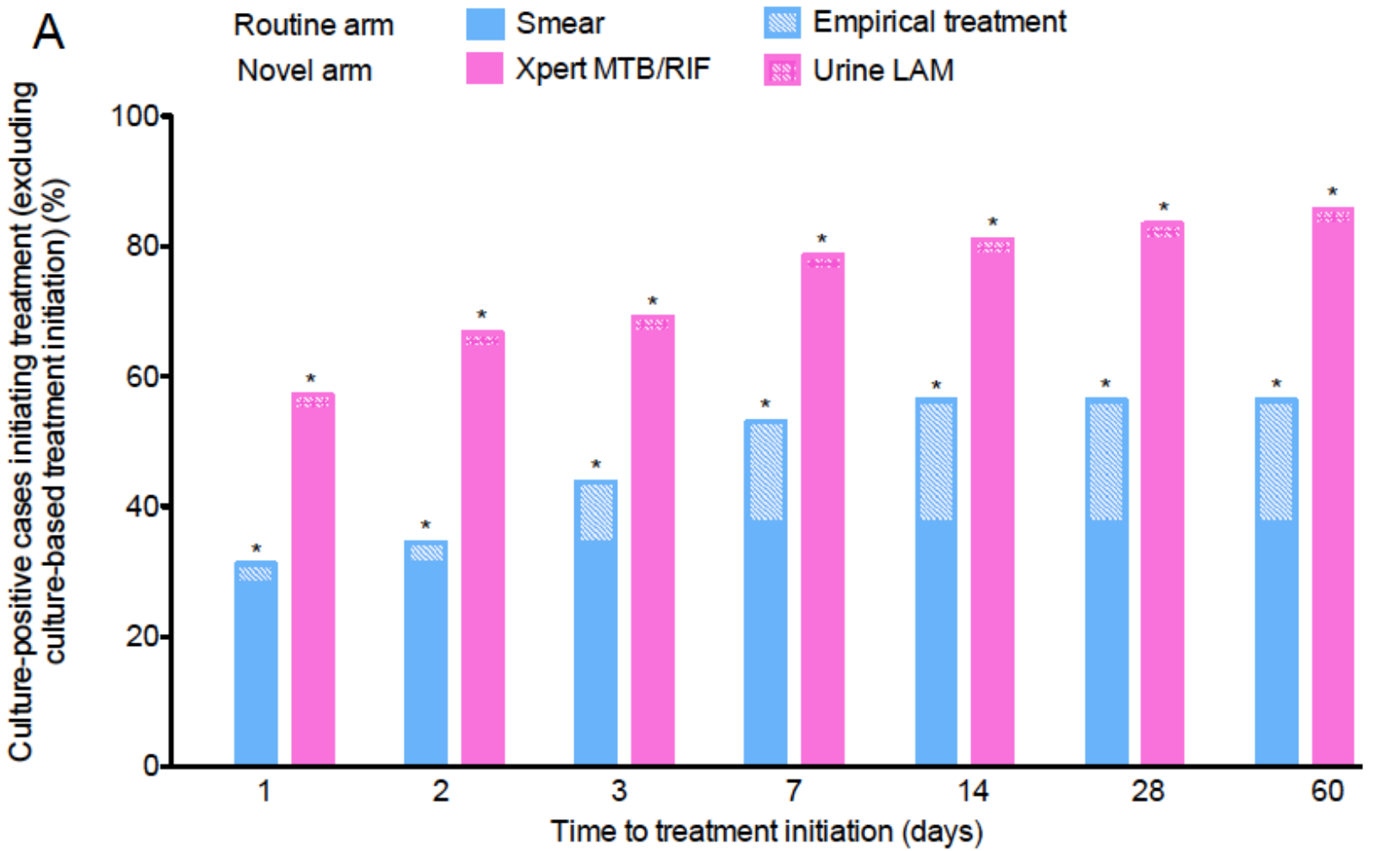
Effect of new tuberculosis diagnostic technologies on community-based intensified case finding: a multicentre randomised controlled trial

2017

Gregory L Calligaro, Lynn S Zijenah*, Jonathan G Peter, Grant Theron, Virginia Buser, Ruth McNerney, Wilbert Bara, Tsitsi Bandason, Ureshnie Govender, Michele Tomasicchio, Liezel Smith, Bongani M Mayosi, Keertan Dheda*


Mobile Clinic for Kids' Heart-health
www.lifefounda


LANGA
LANGA
12A



Routine arm	10/32 (31%)	11/32 (34%)	14/32 (44%)	17/32 (53%)	18/32 (56%)	18/32 (56%)	18/32 (56%)
Novel arm	24/42 (57%)	28/42 (67%)	29/42 (69%)	33/42 (79%)	34/42 (81%)	35/42 (83%)	36/42 (86%)
p-value	**0.0268**	**0.0058**	**0.0289**	**0.0205**	**0.0213**	**0.0105**	**0.0047**

PIMA CD4

Rapid HIV

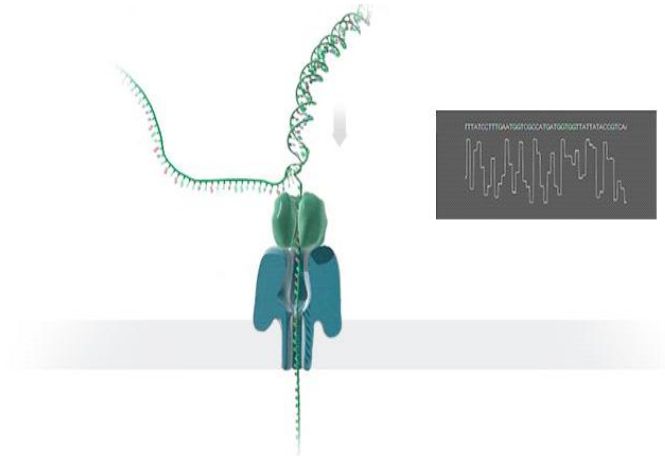
Sputum induction

Xpert OMNI

Xpert OMNI

- A POC version of Gene Xpert
- Small and portable single cartridge system – ideal for a mobile mini clinic
- Battery operated (up to 12 hours) - no need for external power supply

Pore Sequencing



- NanoPore MinIon
  - Small, USB powered device that can be run with a laptop
  - Rapid workflows for some sample types
    - <10 minutes to prepare samples for sequencing
    - Sequencing results can be analysed “in real time”
  - Higher error rate than Illumina and PacBio platforms
- SmidgION

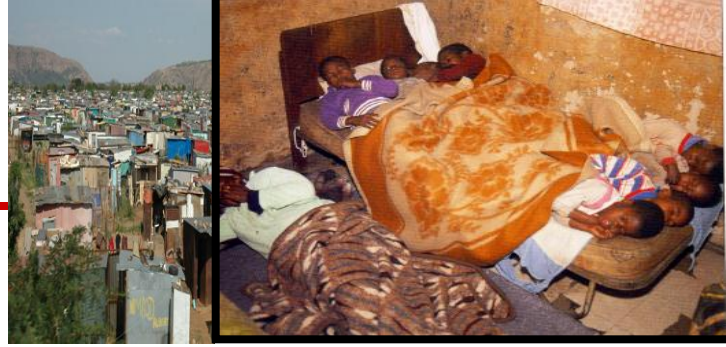
P1.32 **HAND-HELD RAPID WHOLE GENOME NANOPORE SEQUENCING TO PREDICT *NEISSERIA GONORRHOEAE* ANTIBIOTIC SUSCEPTIBILITY: STEPS TOWARDS CLINIC BASED TAILORED ANTIMICROBIAL THERAPY**

[1]Laura T Phillips, [1]Adam Witney, [2]Fernando Izquierdo-Carrasco, [2]Simon Mayes, [2]Amber Wright, [1]Ken Laing, [1]Kate Gould, [1]Marcus Pond, [1]Catherine L Hall, [3]Emma M Harding-Esch, [1]Phillip Butcher, [1]Liqing Zhou, [1]Syed T Sadiq. *[1]St George's University of London, London, UK; [2]Oxford Nanopore Technologies, Oxford, UK; [3]Public Health England, London, UK*

Same-Day Diagnostic and Surveillance Data for Tuberculosis via Whole-Genome Sequencing of Direct Respiratory Samples

Antonina A. Votintseva,[a] Phelim Bradley,[b] Louise Pankhurst,[a] Carlos del Ojo Elias,[b] Matthew Loose,[c] Kayzad Nilgiriwala,[d] Anirvan Chatterjee,[d] E. Grace Smith,[a,c] Nicolas Sanderson,[a] Timothy M. Walker,[a] Marcus R. Morgan,[a] David H. Wyllie,[a,f,h] A. Sarah Walker,[a,i] Tim E. A. Peto,[a,i] Derrick W. Crook,[a,f,h] Zamin Iqbal,[b]

Summary



- Xpert – feasible if placed in a clinic but does not impact TB burden. Rule-in test. PPV. Re-treatment cases.
- **LTBI: PLHIV and children under 5 years (no TST needed)**
- **WGS (precision medicine)**: need to enable sequencing from sputum; more data needed about impact
- For real impact on burden need **ACF** and triage testing:
  ……major research challenge remains the development of a **low cost non-sputum-based screening test**

LIIU 2015

Funding Agencies:

EUFP7

Discovery

NIH Fogerty

South African National Research Foundation

EDCTP

South African MRC