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Current and future technical options for the diagnosis of drug resistant tuberculosis.

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UN GENERAL ASSEMBLY HIGH-LEVEL MEETING ON ENDING TB 26 Sept 2018, New York

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The WHO End TB Strategy

The End TB Strategy at a glance

VISION	**A WORLD FREE OF TB** — zero deaths, disease and suffering due to TB			
GOAL	END THE GLOBAL TB EPIDEMIC			
INDICATORS	MILESTONES		TARGETS	
	2020	2025	SDG 2030[a]	END TB 2035
Percentage reduction in the absolute number of TB deaths (compared with 2015 baseline)	35%	75%	90%	95%
Percentage reduction in the TB incidence rate (compared with 2015 baseline)	20%	50%	80%	90%
Percentage of TB-affected households experiencing catastrophic costs due to TB (level in 2015 unknown)	0%	0%	0%	0%

PRINCIPLES

1. Government stewardship and accountability, with monitoring and evaluation
2. Strong coalition with civil society organizations and communities
3. Protection and promotion of human rights, ethics and equity
4. Adaptation of the strategy and targets at country level, with global collaboration

PILLARS AND COMPONENTS

1. **INTEGRATED, PATIENT-CENTRED CARE AND PREVENTION**
   - A. Early diagnosis of TB including universal drug-susceptibility testing, and systematic screening of contacts and high-risk groups
   - B. Treatment of all people with TB including drug-resistant TB, and patient support
   - C. Collaborative TB/HIV activities, and management of comorbidities
   - D. Preventive treatment of persons at high risk, and vaccination against TB
2. **BOLD POLICIES AND SUPPORTIVE SYSTEMS**
   - A. Political commitment with adequate resources for TB care and prevention
   - B. Engagement of communities, civil society organizations, and public and private care providers
   - C. Universal health coverage policy, and regulatory frameworks for case notification, vital registration, quality and rational use of medicines, and infection control
   - D. Social protection, poverty alleviation and actions on other determinants of TB
3. **INTENSIFIED RESEARCH AND INNOVATION**
   - A. Discovery, development and rapid uptake of new tools, interventions and strategies
   - B. Research to optimize implementation and impact, and promote innovations

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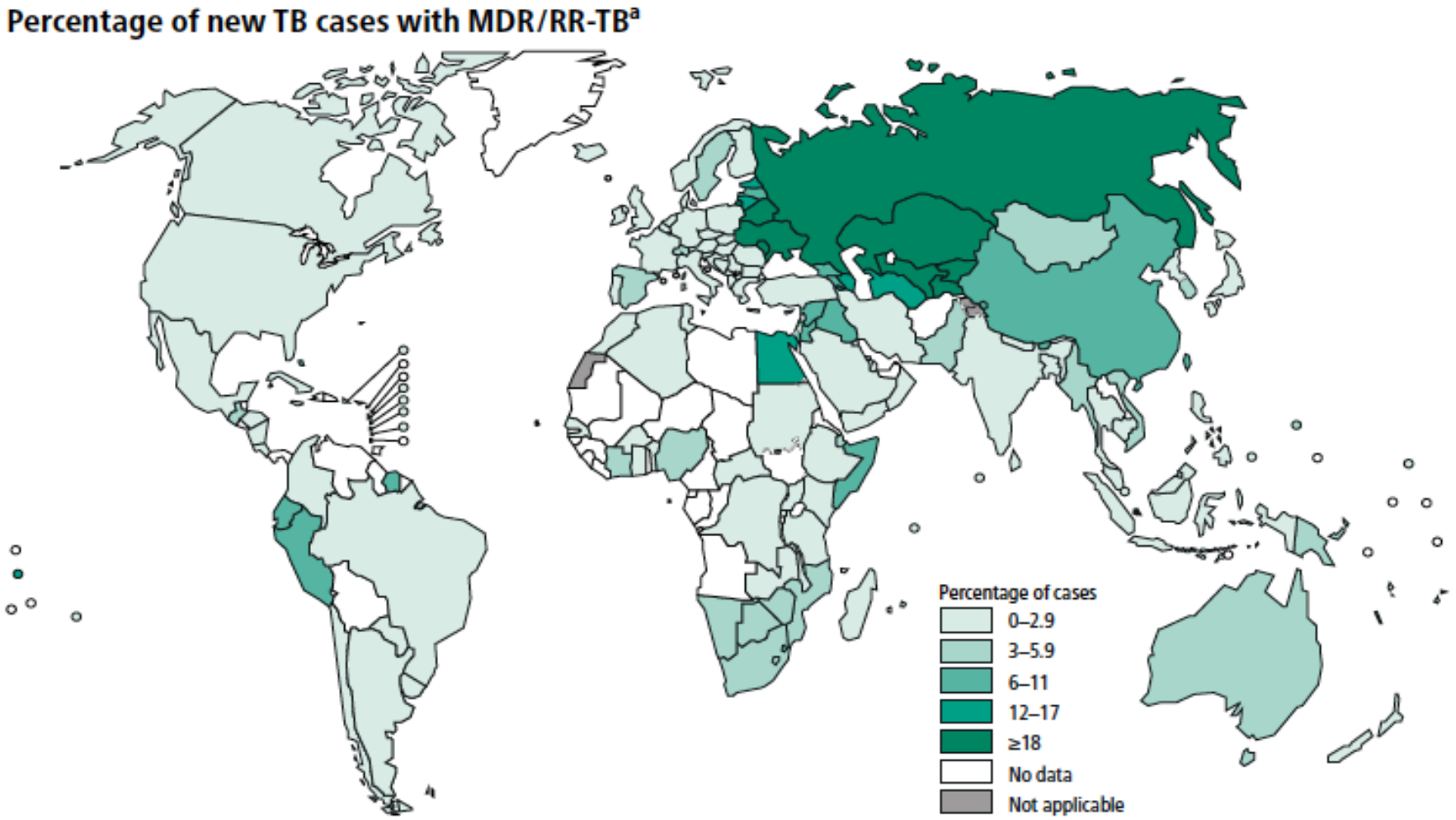
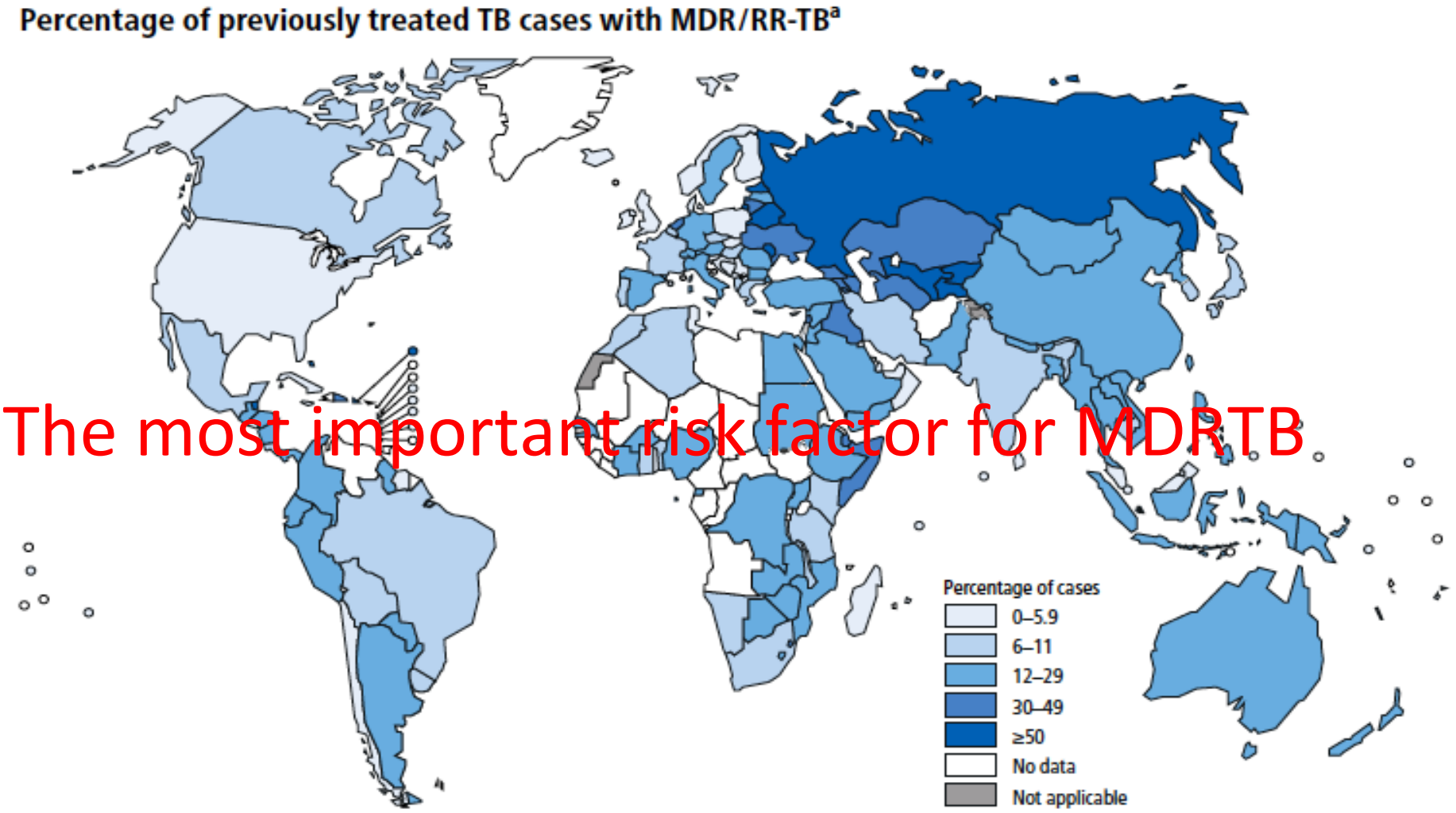
% of new TB cases which are MDR/RR



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before 2002 are not shown.

% of previously treated TB cases which are MDR/RR



The most important risk factor for MDRTB

[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before 2002 are not shown. The high percentages of previously treated TB cases with MDR-TB in Bahamas, Belize, French Polynesia, Puerto Rico and Sao Tomé and Principe refer to only a small number of notified cases

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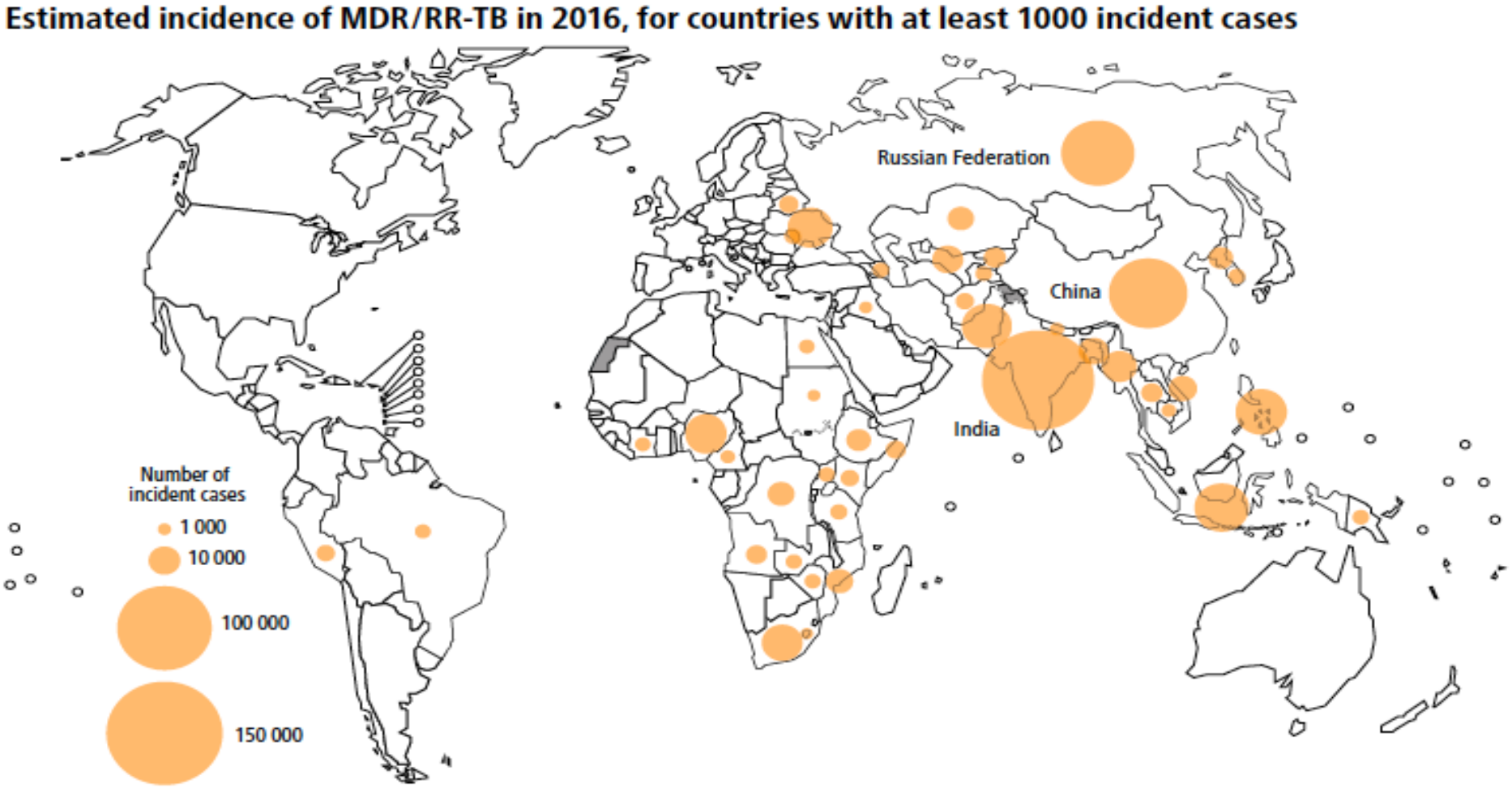
MDR cases: importance of cases as well as rates



Different numbers as important as rates eg Estonia vs Russia

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Know MDRTB infection rate

In 2016, global coverage for rifampicin resistance testing= 33% for new TB patients and 60% for previously treated TB patients,

So 41% overall (up from 31% in 2015).

Why? Surveillance

Public Health

Clinical

Argument 10 years ago eg Paul Farmer

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WHO High MDRTB Burden countries (n=30)

The top 20 by estimated absolute number:

Bangladesh
China
DPR Korea
DR Congo
Ethiopia
India
Indonesia
Kazakhstan
Kenya
Mozambique
Myanmar
Nigeria
Pakistan
Philippines
Russian Federation
South Africa
Thailand
Ukraine
Uzbekistan
Viet Nam

Additional 10 by estimated rate per 100 000 population and minimum number of 1000 cases per year (in alphabetical order):

Angola
Azerbaijan
Belarus
Kyrgyzstan
Papua New Guinea
Peru
Republic of Moldova
Somalia
Tajikistan
Zimbabwe
(

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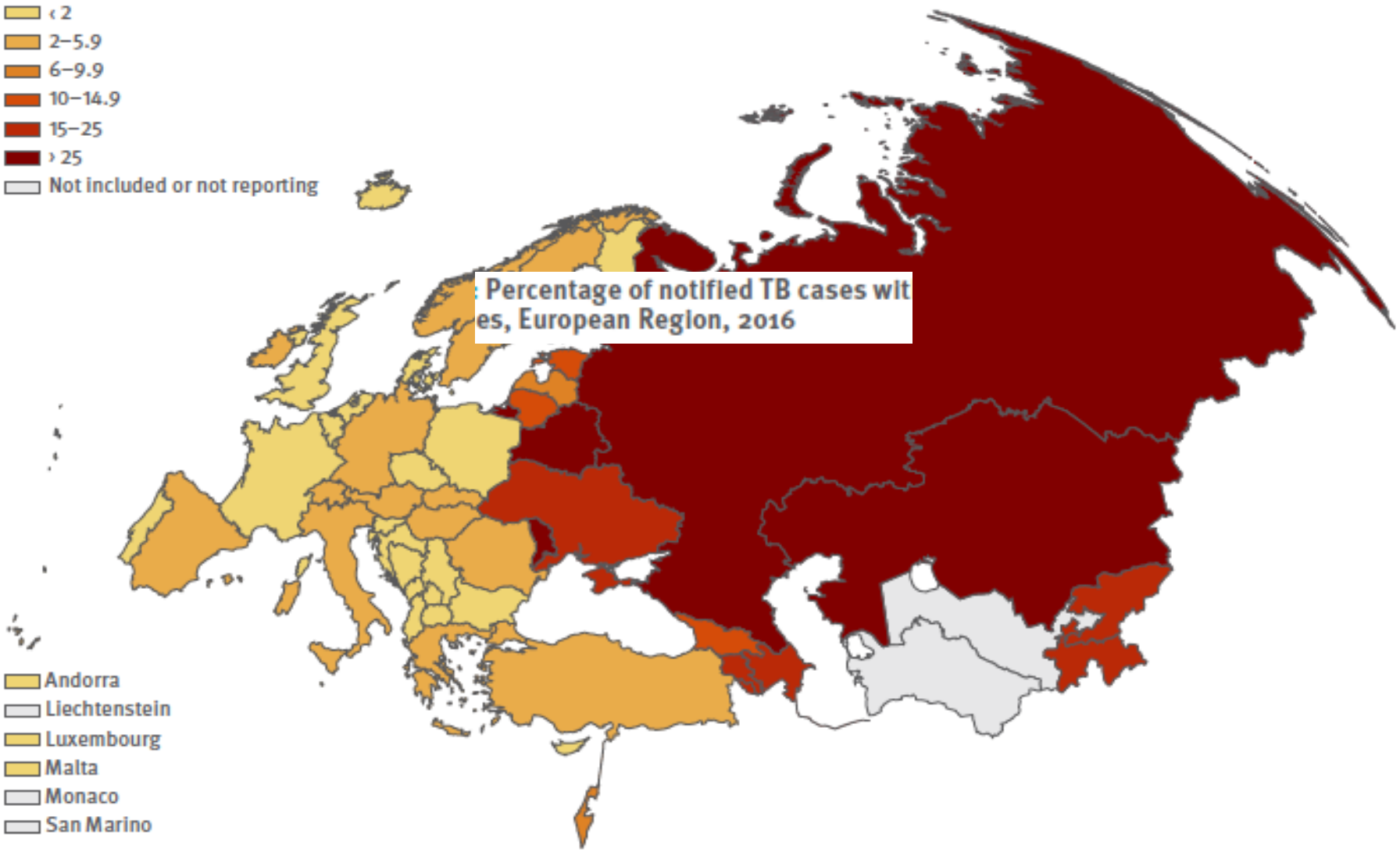
Euro: % Notified Pulmonary TB cases proven to have MDRTB (lab testing)



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MDRTB in E Europe

- 9 of 30 of world's highest MDRTB burden countries are in Eastern Europe/Central Asia:
- In 2015, an estimated 16% of people newly diagnosed TB and 48% of people previously treated for TB had multi-drug resistant TB (MDR-TB), accounting for an estimated 74,000 cases.

WHO Target Product Profiles and molecular testing

WHO target product profiles for new molecular assays for M. tuberculosis require more than 90% sensitivity and 95% specificity.

(High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva: World Health Organization, 2014 (http://www .who .int/ tb/ publications/ tpp_report/ en/))

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New approach: international organisations, NGOs, Industry

- Global need: about active TB, not latent TB
- Molecular tests endorsed by WHO:
- Have tools that rapidly (within 1 day):

- Line probe assays (TB, RIF, INH from sputum eg
- Genotype MTBDRplus
- GenXpert (TB, RIF from sputum)
- LAMP, WGS
- DIAGNOSTIC TARGET PRODUCT PROFILES

- …and other samples
- …UK and most of the EU/EEA >90% MTB is drug susceptible…

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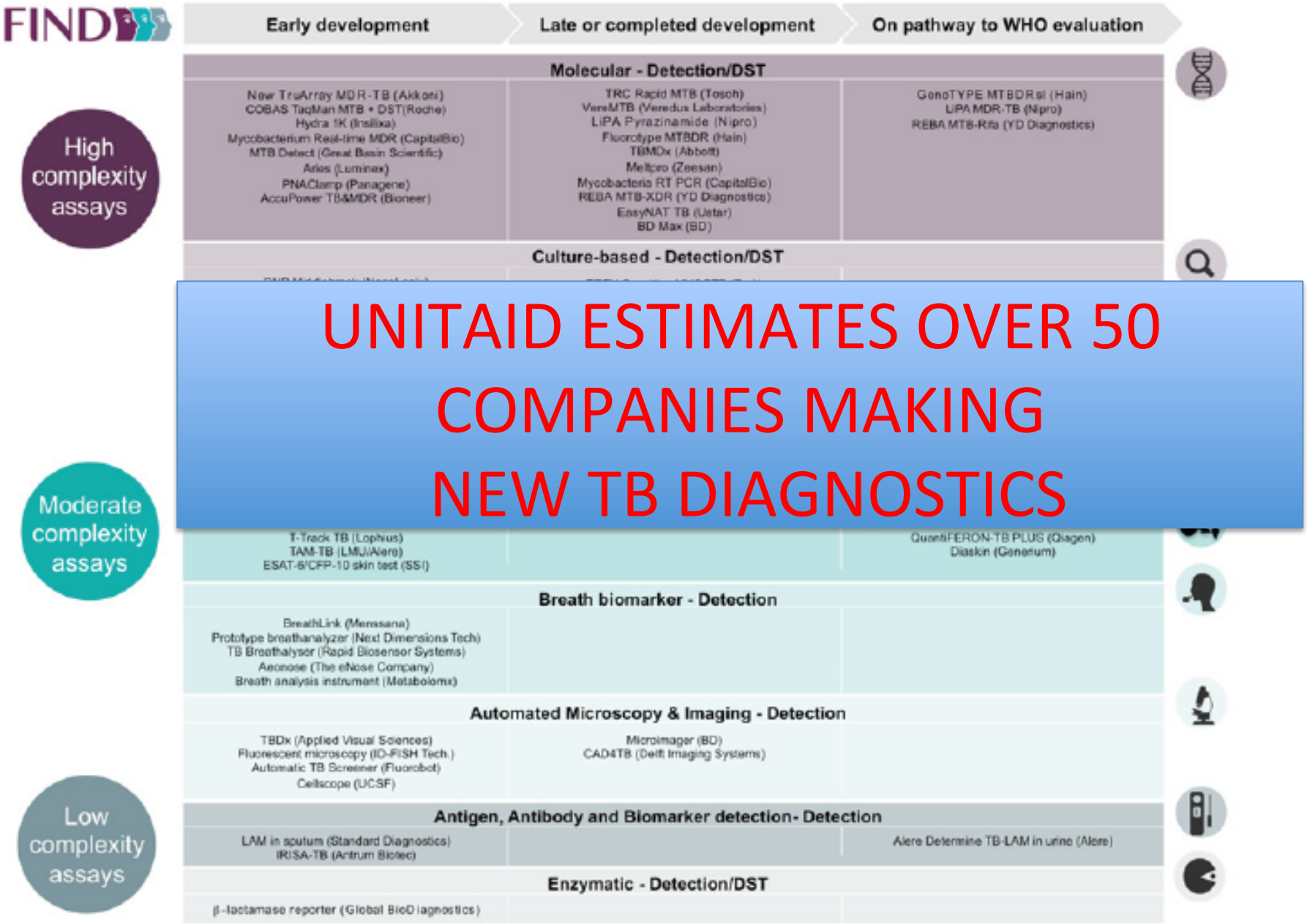
Figure 4. Current FIND TB diagnostics pipeline listing the development phases and the types of technologies in development or evaluation



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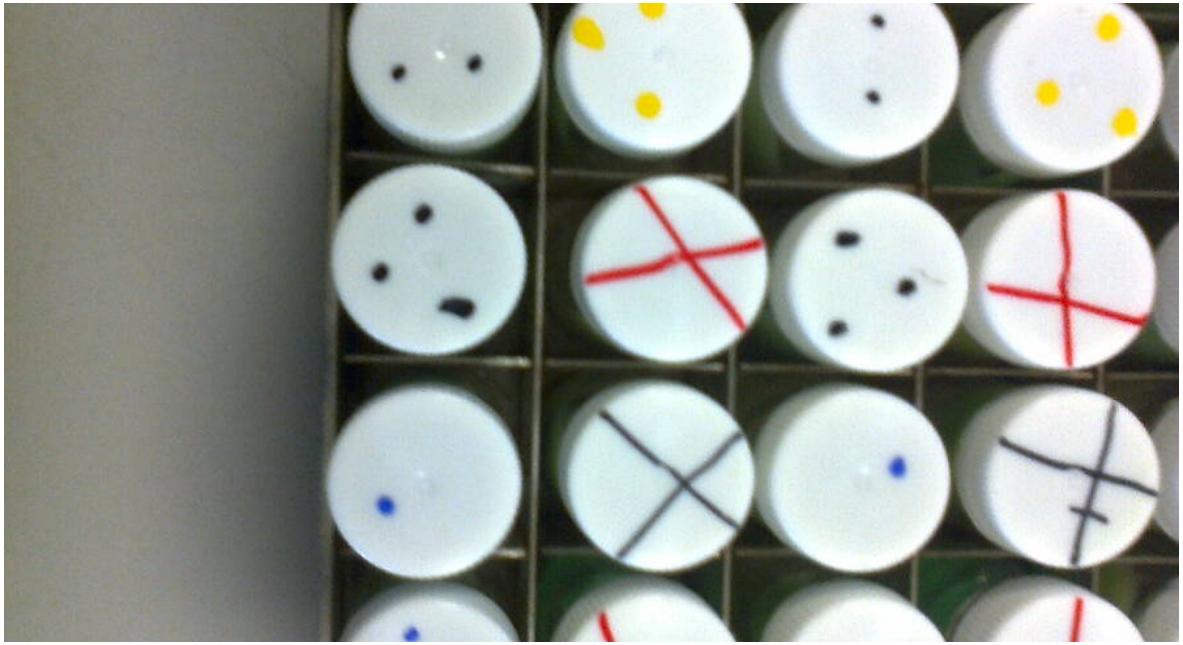
MTB susceptibility testing on LJ

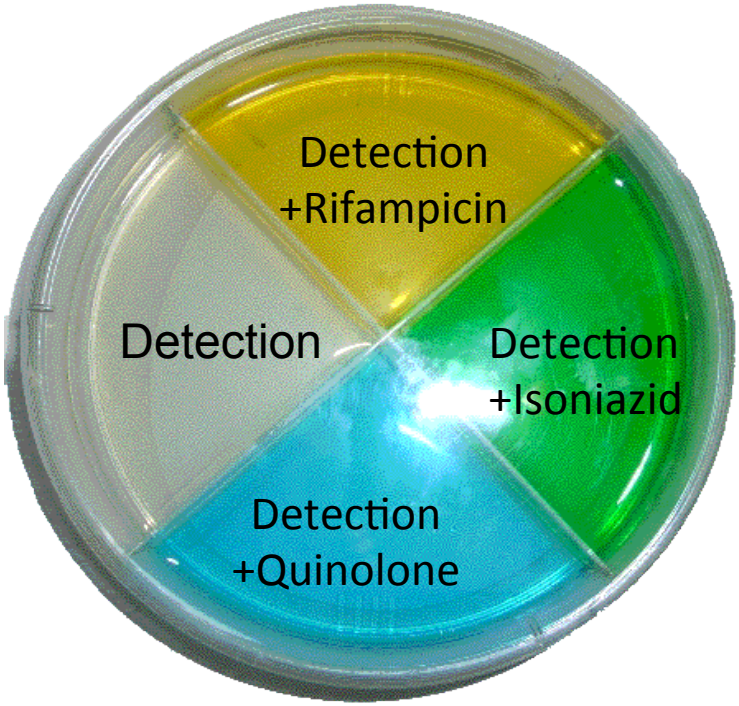


1: Add

Add disinfectant to sputum pot & wait ~20-60 minutes

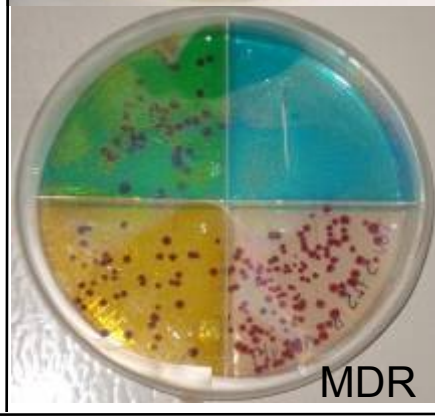
2: Transfer

Use disposable pipette to apply directly to selective thin layer agar plate. Permanently seal & incubate in air



3: Inspect

Glance at plate 2-3x/week for 3-4 weeks then discard



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 811–818 Vol. 44, No. 3
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.3.811–818.2006

Evaluation of MGIT 960-Based Antimicrobial Testing and Determination of Critical Concentrations of First- and Second-Line Antimicrobial Drugs with Drug-Resistant Clinical Strains of *Mycobacterium tuberculosis*

Annika Krüüner,[1,2] Malcolm D. Yates,[1] and Francis A. Drobniewski[1]*

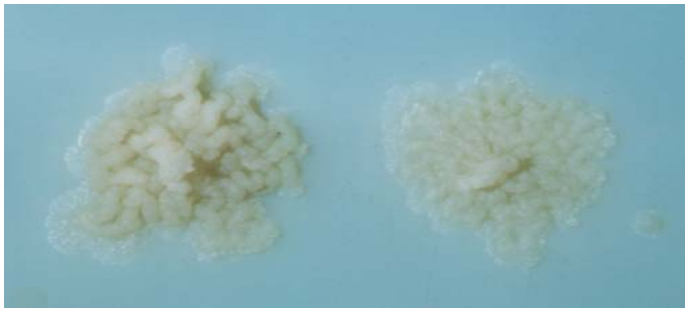
Health Protection Agency, Mycobacterium Reference Unit, Clinical Research Centre, Barts and the London School of Medicine, Queen Mary College, University of London, 2 Newark Street, London, United Kingdom E1 2AT,[1] and Tartu University Clinics, United Laboratory, Department of Mycobacteriology, Tartu, Estonia[2]

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 688–692 Vol. 44, No. 3
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.3.688–692.2006

Multicenter Laboratory Validation of the BACTEC MGIT 960 Technique for Testing Susceptibilities of *Mycobacterium tuberculosis* to Classica Second-Line Drugs and Newer Antimicrobials

Sabine Rüsch-Gerdes,[1]* Gaby E. Pfyffer,[2] Manuel Casal,[3] Maureen Chadwick,[4] and Salman Siddic

National Reference Center for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany[1]; Department of Medical Microb Luzern General Hospital, Lucerne, Switzerland[2]; Mycobacteria Reference Center, Faculty of Medicine, University of Cordoba, Cordoba, Spain[3]; Royal Brompton Hospital, London, United Kingdom[4]; and Becton Dickinson Diagnostic Systems, Sparks, Maryland[5]



Health Technology Assessment 2007; Vol. 11: No. 3

A systematic review of rapid diagnostic tests for the detection of tuberculosis infection

J Dinnes, J Deeks, H Kunst, A Gibson, E Cummins, N Waugh, F Drobniewski and A Lalvani

January 2007

Health Technology Assessment
NHS R&D HTA Programme
www.hta.ac.uk

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Different molecular methods

- Cobas Amplicor TB Roche- PCR 16sRNA
- MTD Gen-Probe TMA of rRNA
- BD ProbeTec SDA IS6110, 16sRNA
- Eiken LAMP Isothermal amplification +uv fluorescence
- Artus Realart Real time PCR
- +DST
- Innolipa RifTB
- Hain Lifesciences MTBDRPlus
- Cepheid GeneXpert

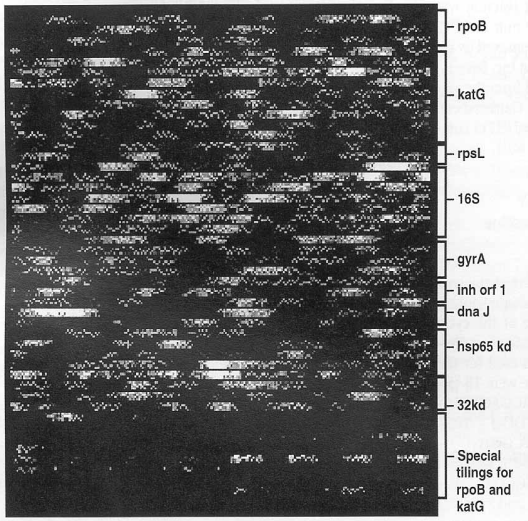
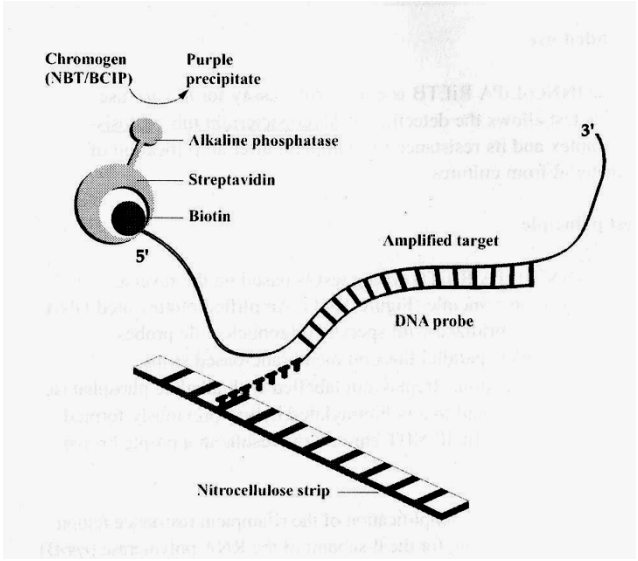
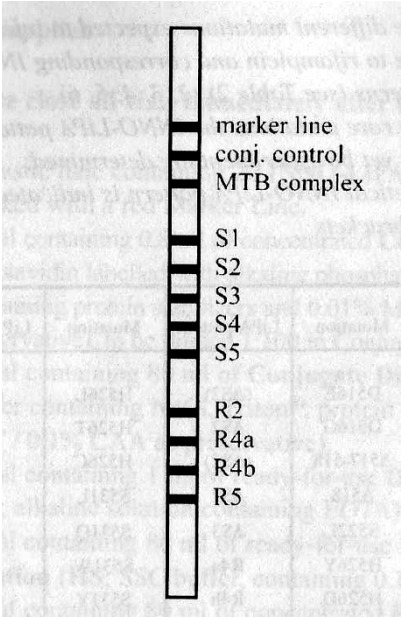


Figure 6 A high-density oligonucleotide array used to genotype 731 bp of *rpoB*, 2286 bp of *katG*, 356 bp of *rpsL*, 1683 bp of 16S, 731 bp of *gyrA*, 281 bp of *inh* orf, 341 bp of *hsp* 65 kd, 1097 bp of *dnaJ*, and 1279 bp of *32 Kd* genes. Additionally, specific insertion, deletions, and missense mutations in *rpoB* and *katG* are interrogated by the alternative allele-specific oligonucleotide probes at the bottom of the chip.

Rapid diagnosis of resistance to RIF and INH: molecular line-probe assays

- DNA extraction from cultures and clinical specimens (sputum);
- PCR amplification of fragments of genes associated with drug resistance;
- Hybridization with the DNA probes on membranes;
- Development, reading and interpretation of results





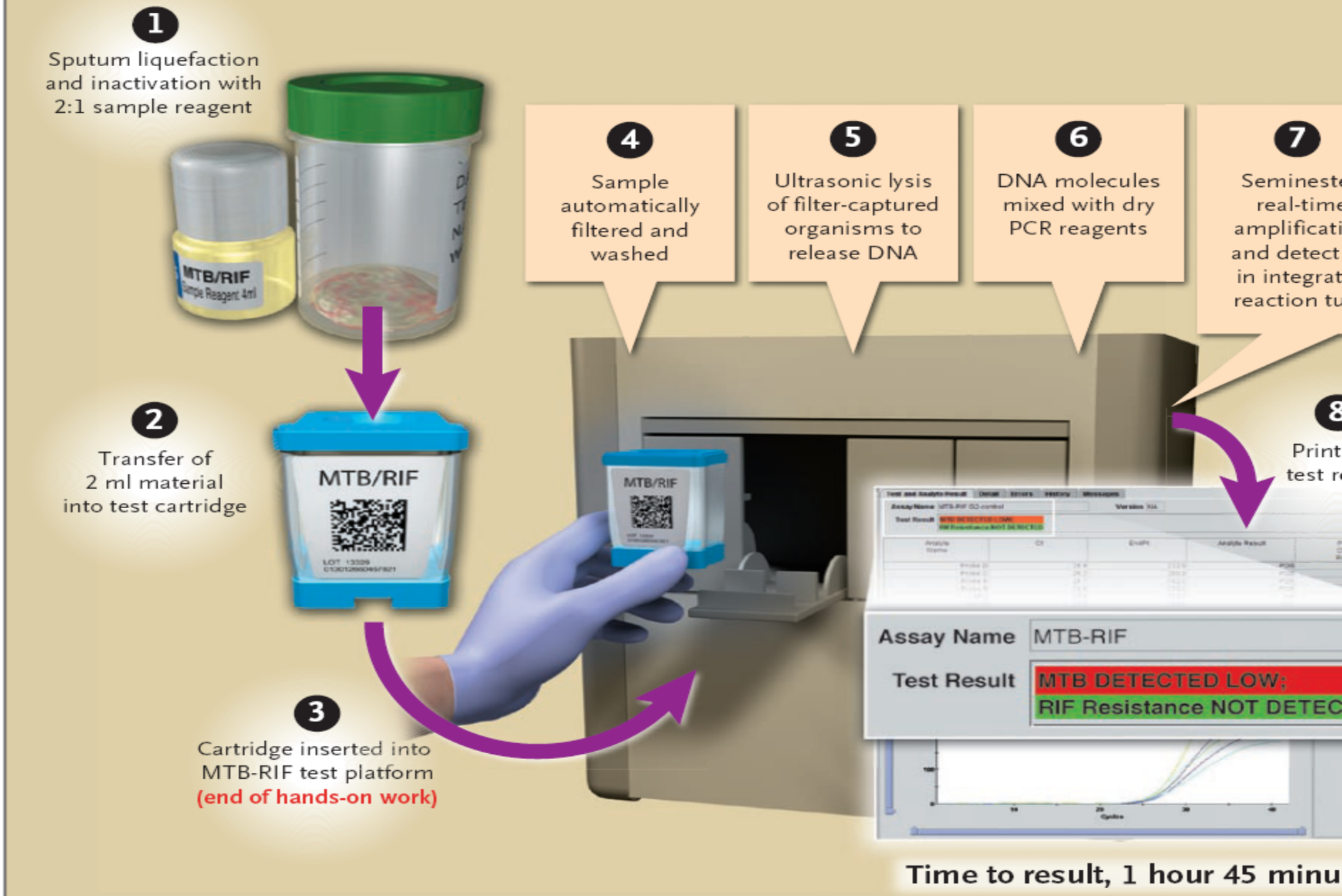


Figure 2. Assay Procedure for the MTB/RIF Test.

Cepheid GeneExpert NEJM

2/3 samples randomly processed with *NALC and NaOH before microscopy, solid and liquid culture,* and the MTB/RIF test, and one specimen used for direct testing with microscopy and the MTB/RIF test.

Among culture-positive patients, single, direct MTB/RIF test identified 551/561 patients with smear-positive TB (98.2%) and 124/171 with smear-negative TB (72.5%).

Boehme C et al New Eng J Medicine 1 Sept 2010

Multicenter validation studies:

JCM Journals.ASM.org

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XDR

Detection of Resistance to Second-Line Antituberculosis Drugs by Use of the Genotype MTBDR*sl* Assay: a Multicenter Evaluation and Feasibility Study

Olga Ignatyeva,[a] Irina Kontsevaya,[a] Alexander Kovalyov,[a] Yanina Balabanova,[a,b] Vladislav Nikolayevskyy,[b] Kadri Toit,[c] Anda Dragan,[d] Daniela Maxim,[e] Svetlana Mironova,[a] Tiina Kummik,[c] Ionela Muntean,[d] Ekaterina Koshkarova,[a] and Francis Drobniewski[b]

Samara Oblast Tuberculosis Dispensary, Samara, Russia[a]; Queen Mary College, Barts and the London School of Medicine, University of London, London, United Kingdom[b]; Tartu University Hospital, Tartu, Estonia[c]; Pneumophtisiology

The rate of multidrug-resistant (MDR) and ext
countries of the former USSR. The availability
drugs is vital for adequate patient management
that of phenotypic drug susceptibility testing (
rium tuberculosis isolates at four sites in Easter
The sensitivity for the detection of resistance t
77.3% and 92.3%; however, it was much lower
The test specificity was over 82% for all drugs.
individual second-line drugs and can be recom
for the detection of kanamycin resistance need

Multidrug-resistant (MDR) and extensivel
(XDR) tuberculosis (TB) has become a
global TB control due to difficulties in diagnosi
the high rates

JCM Journals.ASM.org

Diagnostic Accuracy of the GenoType MTBDR*sl* Assay for Rapid Diagnosis of Extensively Drug-Resistant Tuberculosis in HIV-Coinfected Patients

Irina Kontsevaya,[a] Olga Ignatyeva,[a] Vladyslav Nikolayevskyy,[b] Yanina Balabanova,[b] Alexander Kovalyov,[a] Andrey Kritsky,[a] Olesya Matskevich,[a] Francis Drobniewski[b,c]

Samara Oblast Tuberculosis Dispensary, Samara, Russia[a]; Queen Mary College, Barts and the London School of Medicine, University of London, London, United Kingdom[b]; University College, London, United Kingdom[c]

The Russian Federation is a high-tuberculosis (TB)-burden country with high rates of *Mycobacterium tuberculosis* multidrug
le diagnosis for
mance of the
obtained from 90
ll drugs, while
o the presence of
(not commonly
specimens from

of laboratory infra-
as biosafety consid-
s, limit its potential
mited settings (11)

Have LPA for diagnosing XDRTB (aminoglycosides and FQs from cultures and heavily smear positive specimens). WHO recommends for primary speceimens and cultures now

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Metanalysis Hain Sl (Feng et al Plos One 2013)

- 14 independent studies from 11 articles Among these 14 studies, 3 tested clinical specimens, rest used clinical isolates.
- Summarized sensitivity was 0.87, 0.83, 0.82, 0.44, and 0.68 for FQs, amikacin, capreomycin, kanamycin, and ethambutol, respectively.
- Specificity was 0.97, 1.00, 0.97, 0.99, and 0.80, respectively.
- Concluded that MTBDRsl showed good accuracy for detecting drug resistance to fluoroquinolones, amikacin and capreomycin, but it may not be an appropriate choice for kanamycin and ethambutol.

Single Point-of-care Multidrug Resistant Tuberculosis Test

Introduction and Purpose

Technology

Fluorescent probes that bind to specific gene sequences in a temperature dependent order. The shape of this fluorescence response is characteristic of the particular gene sequence.

Sample preparation

Advanced sample preparation chemistries based on Microsens's proprietary magnetic bead extraction technology integrated into single use cartridge.

Instrument

The Enigma® MiniLab is designed for direct processing of clinical samples operated by non-laboratory trained personnel in a range of resource poor clinical settings including field hospitals, local clinics and outreach/screening centres.

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UNITAID-Biomarkers

- Lot interest for POC tests but still challenges to success
- Alere LAM-very limited use in severe HIV pos, low CD4 count,
- Volatile component tests progressing but limited evaluations of performance

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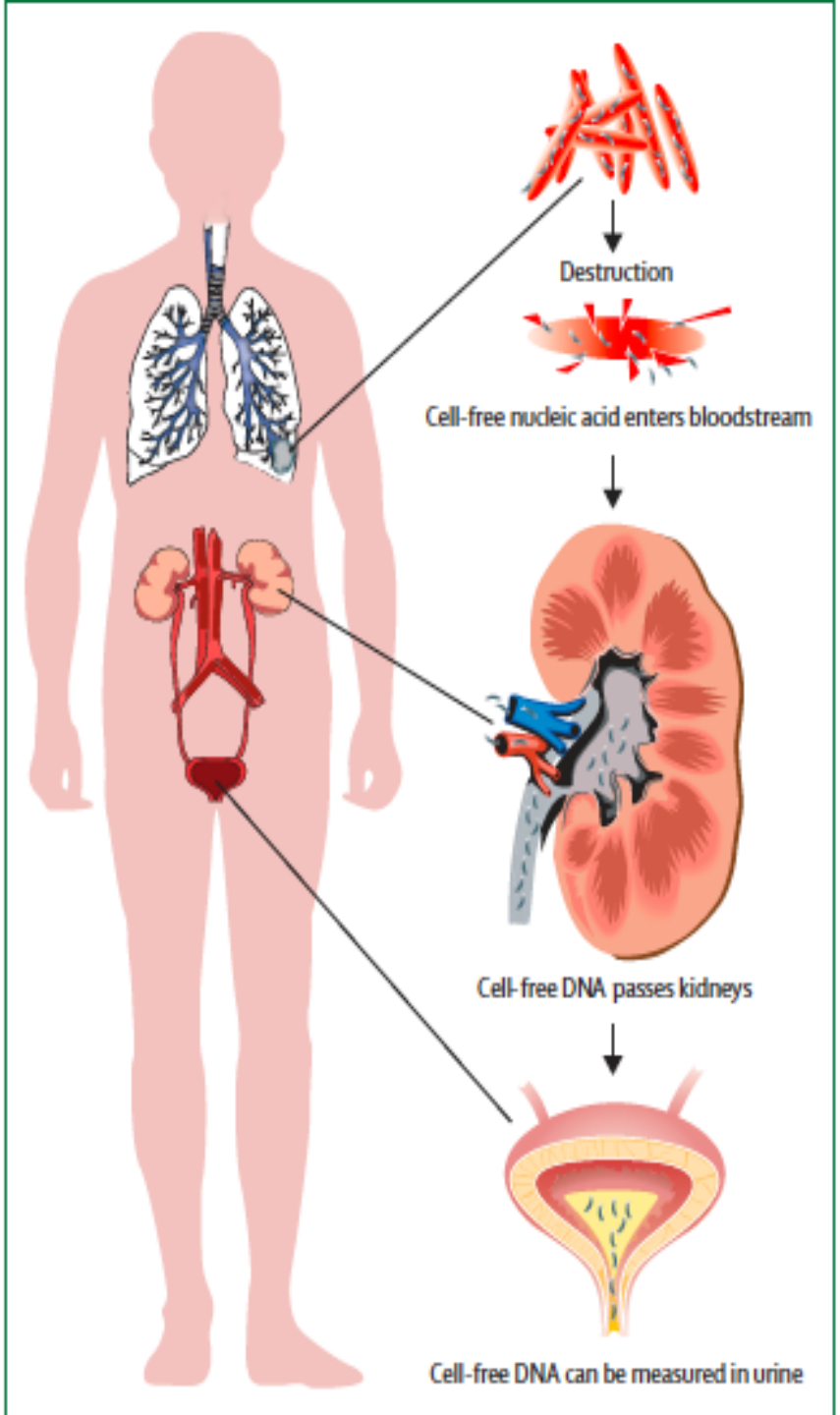
Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods

Clare Green, Jim F Huggett, Elizabeth Talbot, Peter Mwaba, Klaus Reither, Alimuddin I Zumla

Figure 1: Transrenal DNA production in a patient with pulmonary tuberculosis
M tuberculosis bacilli from infective foci in the lungs are destroyed by the immune response releasing cell-free nucleic acids in plasma. The smaller sized cell-free nucleic acids pass through the kidney during filtration to produce transrenal DNA, which can be measured in urine by nucleic acid amplification techniques.



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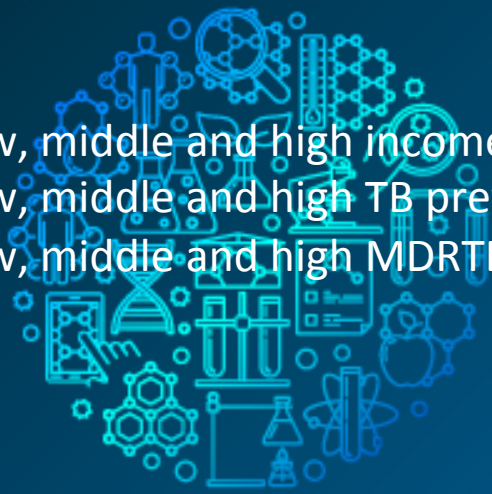
World Health Organization
REGIONAL OFFICE FOR Europe

Всемирная организация здравоохранения
Европейское региональное бюро

Algorithm for laboratory diagnosis and treatment-monitoring of pulmonary tuberculosis and drug-resistant tuberculosis using state-of-the-art rapid molecular diagnostic technologies



For low, middle and high income countries
For low, middle and high TB prevalence countries
For low, middle and high MDRTB prevalence countries

Expert opinion of the European Tuberculosis Laboratory Initiative core group members for the WHO European Region

Алгоритм лабораторной диагностики и мониторинга лечения туберкулеза легких и туберкулеза с лекарственной устойчивостью на основе применения современных быстрых молекулярных методов

Экспертное заключение членов основной группы Европейской лабораторной инициативы, подготовленное для Европейского региона ВОЗ

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Key message

The emphasis is on the rational use of rapid tests from patient specimens, as close to the patient as technically possible,

Rapid molecular diagnosis as an initial method for all cases with clinical suspicion of TB, to be applied in all countries of the Region. One specimen, second for culture.

With high MDR-TB rates being present in Eastern Europe, every presumptive TB case could also be an MDR-TB case

most appropriate therapeutic and infection control strategies can be instituted

GenXpert, LPA, (LAMP) ..

Less exclusion re labs doing PCR and insistence on NRL and regional tests

Role for culture and phenotypic DST (and limited microscopy)

Pharmacokinetics/Pharmacodyamics (Pk/Pd)

- Underpinning some of the WHO treatment changes
- PK describes the behaviour of a drug in a body. Drug is absorbed (A) into systemic circulation, distributed (D) throughout body including tissues and site of infection; metabolised (m) usually in the liver and excreted (E) by the kidneys into the urine. Parameters form the PK model
- PD describes the pharmacological effect ie efficacy of drug on pathogen and on patient as toxicity. Desire the max effect (Emax)
- Correlation of drug concentration and efficacy: (1) area under curve related to MIC (AUC/MIC) (2) max concentration during dosing interval in relation to MIC (Cmax/MIC) (3) time concentration exceeds MIC during the dosing interval (%T.MIC)
- Need to know Critical Concentrations for any antibiotic
- Hollow fiber and other models eg bioelectrospray

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3-D bioelectrospray cell culture model

- *ex vivo* model incorporating extracellular matrix
- More "physiological"
- Multiple uses
- Microfluidics: pharmacokinetics, drugs with specific kinetics

mbio.asm.org/content/8/1/e02073-16.full.pdf

eered Three-Dimensional Cell Culture Platform Integrated with Microfluidics To... 1 / 14

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RESEARCH

A Bioengineered Three-Dimensional Cell Culture Platform Integrated with Microfluidics To Address Antimicrobial Resistance in Tuberculosis

Magdalena K. Bielecka,[a] Liku B. Tezera,[a] Robert Zmijan,[b] Francis Drobniewski,[c] Xunli Zhang,[b,e] Suwan Jayasinghe,[d] Paul Elkington[a,e]

NIHR Respiratory Biomedical Research Unit, Clinical and Experimental Sciences Academic Unit, Faculty of Medicine, University of Southampton, Southampton, United Kingdom[a]; Faculty of Engineering, University of Southampton, Southampton, United Kingdom[b]; Department of Infectious Disease, Imperial College London, London, United Kingdom[c]; BioPhysics Group, UCL Institute of Biomedical Engineering, UCL Centre for Stem Cells and Regenerative Medicine and UCL Department of Mechanical Engineering, University College London, London, United Kingdom[d]; Institute for Life Sciences, University of Southampton, Southampton, United Kingdom[e]

ABSTRACT Antimicrobial resistance presents one of the most significant threats to

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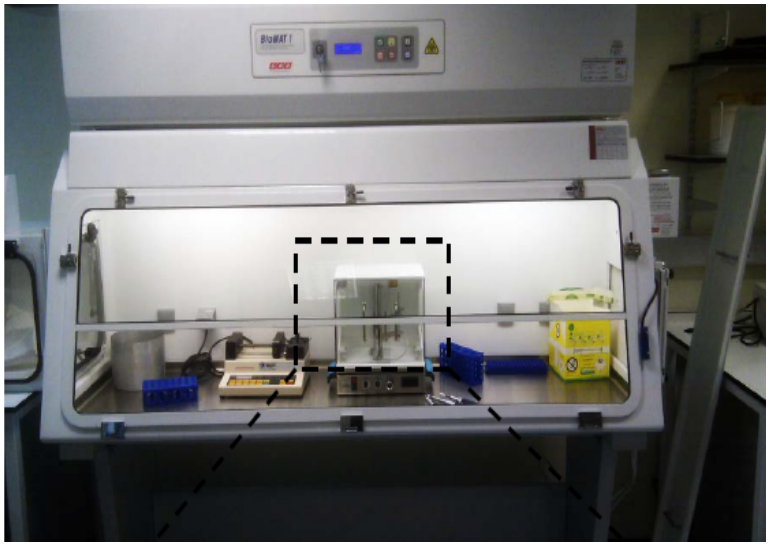
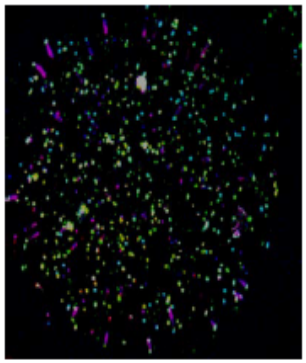
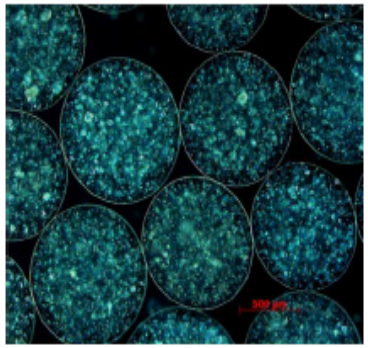
3-D bioelectrospray cell culture model

- Isolation of PMBCs
- Infecting with MTB Lux+
- Mixing with alginate-collagen matrix
- Bioelectrostatic system
- Bioluminescence measurement







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Modified WHO MDRTB Regimens

GROUP	MEDICINE	Abbreviation
Group A: Include all three medicines (unless they cannot be used)	Levofloxacin OR Moxifloxacin	Lfx Mfx
	Bedaquiline[1,4]	Bdq
	Linezolid[2]	Lzd
Group B: Add both medicines (unless they cannot be used)	Clofazimine	Cfz
	Cycloserine OR Terizidone	Cs Trd
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol	E
	Delamanid[3,4]	Dlm
	Pyrazinamide[5]	Z
	Imipenem-cilastatin OR Meropenem[6]	Ipm-Cln Mpm
	Amikacin (OR Streptomycin)[7]	Am (S)
	Ethionamide OR Prothionamide	Eto Pto
	p-aminosalicylic acid	PAS

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Modified WHO MDRTB Regimens

GROUP	MEDICINE	Abbreviation
Group A: Include all three medicines (unless they cannot be used)	Levofloxacin OR Moxifloxacin	Lfx Mfx
	Bedaquiline[1,4]	Bdq
	Linezolid[2]	Lzd
	Amikacin (OR Streptomycin)[7]	Am (S)
	Ethionamide OR Prothionamide	Eto Pto
	p-aminosalicylic acid	PAS

Emphasis on oral regimens. Kanamycin out. Streptomycin in, Greater role for clofazamine, cycloserine, delamanid, bedaquiline, levofloxacin equal to moxifloxacin

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Starting point

- WGS is vital for research
- WGS excellent for understanding phylogeny and evolution—not considered further
- WGS is useful for providing data to set policy
- WGS is useful for ID and DST

Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage

Matthias Merker[1], Camille Blin[2,3], Stefano Mona[2,3], Nicolas Duforet-Frebourg[4], Sophie Lecher[5–8], Eve Willery[5–8], Michael G B Blum[4], Sabine Rüsch-Gerdes[9], Igor Mokrousov[10], Eman Aleksic[11], Caroline Allix-Béguec[12], Annick Antierens[13], Ewa Augustynowicz-Kopeć[14], Marie Ballif[15], Francesca Barletta[16], Hans Peter Beck[17], Clifton E Barry III[18], Maryline Bonnet[19], Emanuele Borroni[20], Isolina Campos-Herrero[21], Daniela Cirillo[20], Helen Cox[22], Suzanne Crowe[11,23,24], Valeriu Crudu[25], Roland Diel[26], Francis Drobniewski[27,28], Maryse Fauville-Dufaux[29], Sébastien Gagneux[17], Solomon Ghebremichael[30], Madeleine Hanekom[31], Sven Hoffner[32], Wei-wei Jiao[33], Stobdan Kalon[34], Thomas A Kohl[1], Irina Kontsevaya[35], Troels Lillebæk[36], Shinji Maeda[37], Vladyslav Nikolayevskyy[27,28], Michael Rasmussen[36], Nalin Rastogi[38], Sofia Samper[39], Elisabeth Sanchez-Padilla[19], Branislava Savic[40], Isdore Chola Shamputa[18], Adong Shen[33], Li-Hwei Sng[41], Petras Stakenas[42], Kadri Toit[43], Francis Varaine[44], Dragana Vukovic[40], Céline Wahl[12], Robin Warren[31], Philip Supply[5–8,12,46], Stefan Niemann[1,45,46] & Thierry Wirth[2,3,46]

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NGS for WGS

- No cloning of template DNA into vectors.
- De novo assembling initially-more complex and expensive than re-sequencing
- Relatively short reads (approx 400bps) sequenced and stitched together by complex bioinformatics
- Genome sequencing of more than 100 pathogen genomes within 2 days (but from the culture=time)
- Most applicable/straightforward where reference genome completed and re-sequencing and comparing against this template

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Strategies

- Early diagnosis
- New drugs
- New drug *regimens ie novel combinations*
- Systems creating MDRTB, XDRTB need fixing or new drugs will be lost
- MDRTB is endemic in some areas eg 25-50% of cases—strategy must be different

nature genetics

Imperial College London

Genomic analysis identifies targets of convergent positive selection in drug-resistant *Mycobacterium tuberculosis*

Maha R Farhat[1,28], B Jesse Shapiro[2–5,28], Karen J Kieser[6], Razvan Sultana[7], Karen R Jacobson[8,9], Thomas C Victor[9], Robin M Warren[9], Elizabeth M Streicher[9], Alistair Calver[10], Alex Sloutsky[11], Devinder Kaur[11], Jamie E Posey[12], Bonnie Plikaytis[12], Marco R Oggioni[13], Jennifer L Gardy[14], James C Johnston[15], Mabel Rodrigues[16], Patrick K C Tang[16], Midori Kato-Maeda[17], Mark L Borowsky[18,19], Bhavana Muddukrishna[18,19], Barry N Kreiswirth[20], Natalia Kurepina[20], James Galagan[2,21–23], Sebastien Gagneux[24,25], Bruce Birren[2], Eric J Rubin[6], Eric S Lander[2], Pardis C Sabeti[2–4,6] & Megan Murray[26,27]

"We also found evidence of positive selection in an additional 39 genomic regions in resistant isolates. "

Genome sequencing of 161 *Mycobacterium tuberculosis* isolates from China identifies genes and intergenic regions associated with drug resistance

Hongtai Zhang[1,2,14], Dongfang Li[3,4,14], Lili Zhao[5,6,14], Joy Fleming[1,14], Nan Lin[7], Ting Wang[1], Zhangyi Liu[3], Chuanyou Li[8], Nicholas Galwey[1], Jiaoyu Deng[9], Ying Zhou[1], Yuanfang Zhu[3], Yunrong Gao[1], Tong Wang[5], Shihua Wang[7], Yufen Huang[3], Ming Wang[1], Qiu Zhong[10], Lin Zhou[10], Tao Chen[10], Jie Zhou[11], Ruifu Yang[3], Guofeng Zhu[12], Haiying Hang[1], Jia Zhang[1], Fabin Li[13], Kanglin Wan[5,6], Jun Wang[3], Xian-En Zhang[2,9] & Lijun Bi[1]

"72 new genes, 28 intergenic regions (IGRs), 11 nonsynonymous SNPs and 10 IGR SNPs with strong, consistent associations with drug resistance."

Published in final edited form as:
Nat Genet. 2014 March ; 46(3): 279–286. doi:10.1038/ng.2878.

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Evolution and transmission of drug resistant tuberculosis in a Russian population

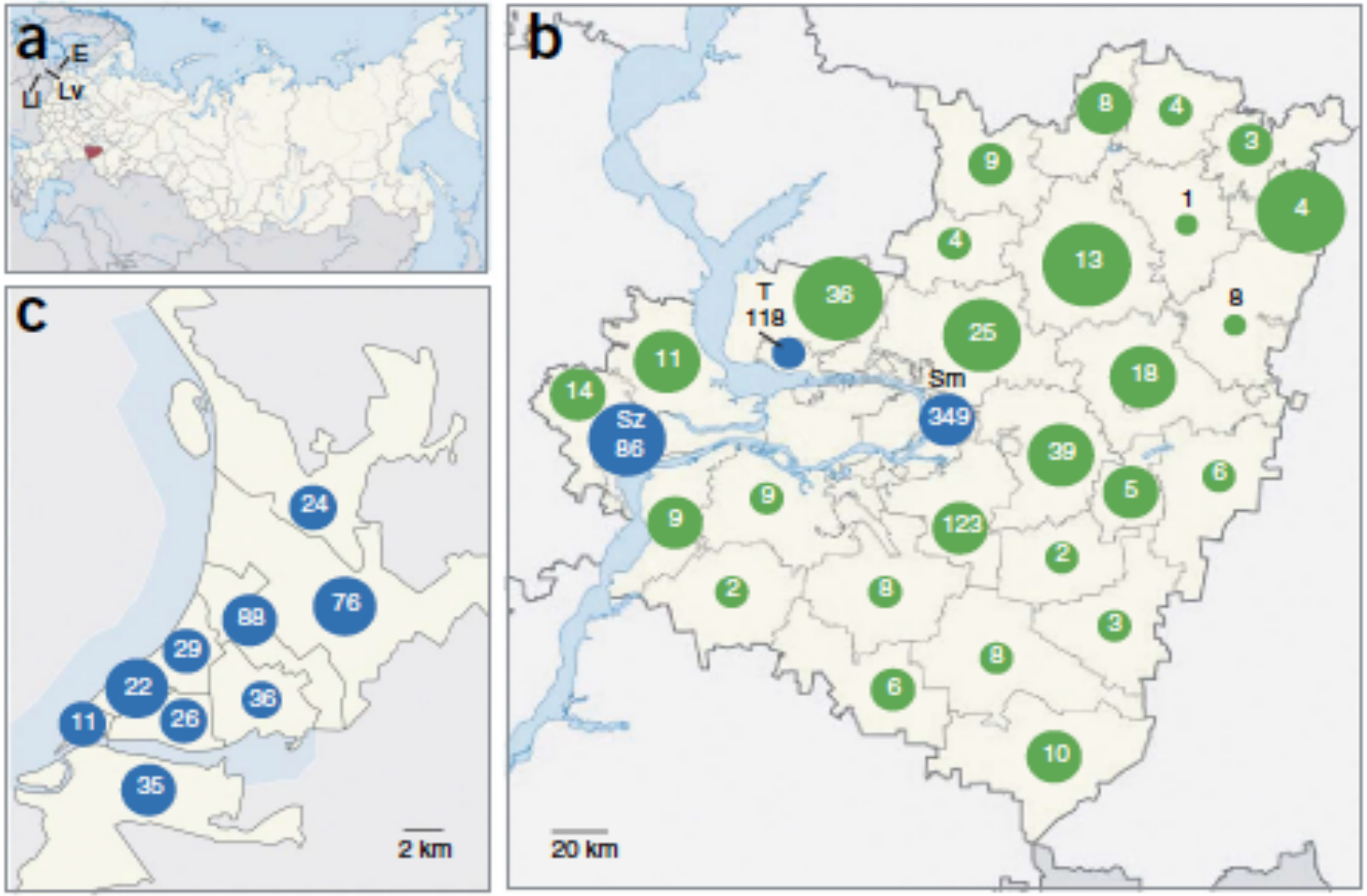
Nicola Casali[1], **Vladyslav Nikolayevskyy**[1], **Yanina Balabanova**[1], **Simon R Harris**[2], **Olga Ignatyeva**[3], **Irina Kontsevaya**[3], **Jukka Corander**[4], **Josephine Bryant**[2], **Julian Parkhill**[2], **Sergey Nejentsev**[5], **Rolf D Horstmann**[6], **Timothy Brown**[1], and **Francis Drobniewski**[1,7,*]

Nature Genetics 2014

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study

Timothy M Walker, Thomas A Kohl*, Shaheed V Omar*, Jessica Hedge*, Carlos D O Elias, Phelim Bradley, Zamin Iqbal, Silke Feuerriegel, Katherine E Niehaus, Daniel J Wilson, David A Clifton, Georgia Kapatai, Camilla Ip, Rory Bowden, Francis A Drobniewski, Caroline Allix-Béguec, Cyril Gaudin, Julian Parkhill, Roland Diel, Philip Supply, Derrick W Crook, E Grace Smith, A Sarah Walker, Nazir Ismail, Stefan Niemann†, Tim E A Peto†, and the Modernizing Medical Microbiology (MMM) Informatics Group‡*

Lancet Infectious Diseases 2015



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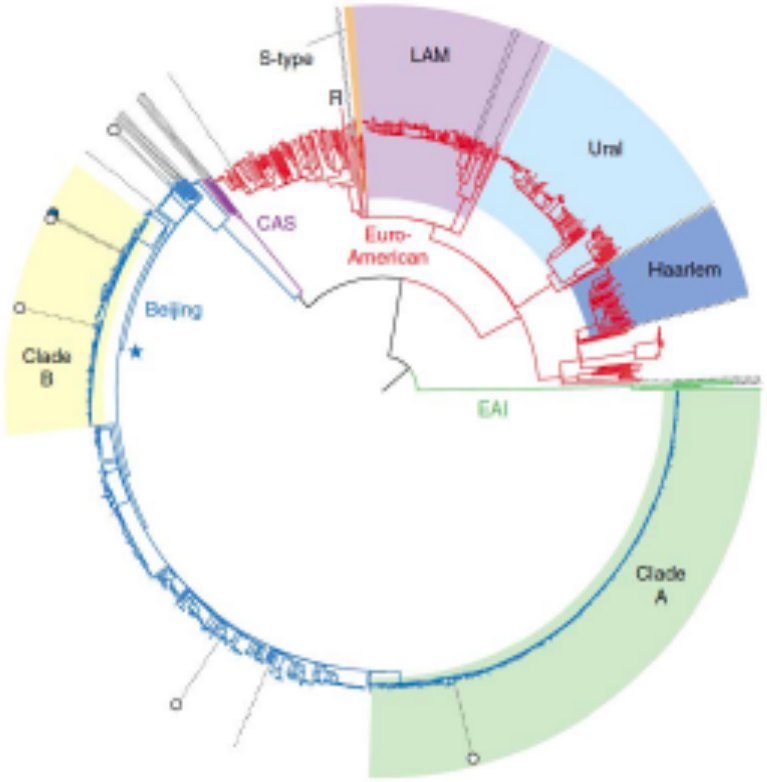


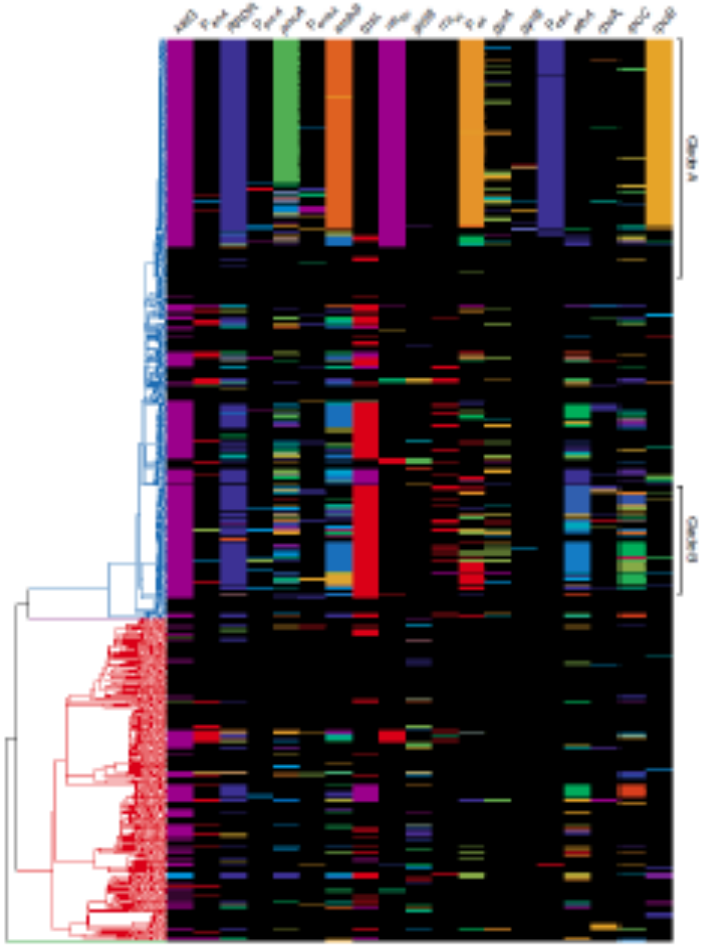


Figure 2 Maximum-likelihood phylogeny of 1,035 *M. tuberculosis* isolates based on 32,445 variable sites.

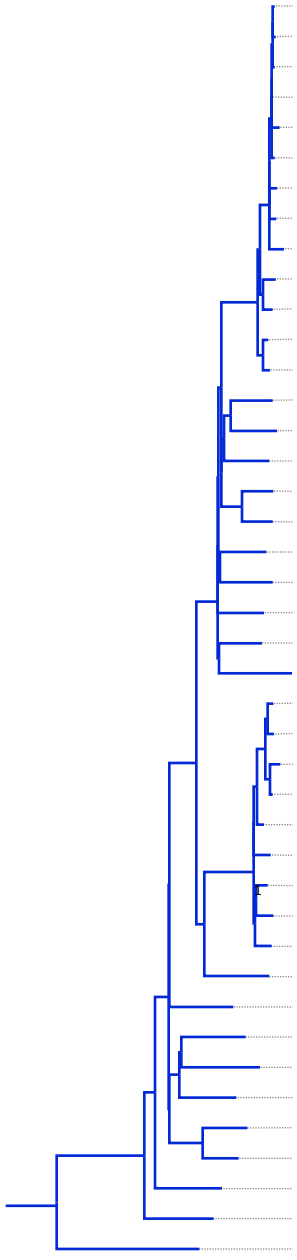
Evolution and transmission of drug-resistant tuberculosis in a Russian population

Nicola Casali[1], Vladyslav Nikolayevskyy[1], Yanina Balabanova[1], Simon R Harris[2], Olga Ignatyeva[3], Irina Kontsevaya[3], Jukka Corander[4], Josephine Bryant[2], Julian Parkhill[2], Sergey Nejentsev[5], Rolf D Horstmann[6], Timothy Brown[1] & Francis Drobniewski[1,7]

nature genetics

published online 26 January 2014; doi:10.1038/ng.2878

Drug resistance phenotypes and genotypes



ID London	RIF	rpoB	INH	katG	mabA	PZA	pncA	EMB	embA	embB	STR	rpsL	rrs	CAP	KAN	rrs	eis	MOX	OFL	gyrA
08-14471	R	S450L	R	S315T	-	S	I6L	S	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14559	R	S450L	R	S315T	-	ND	I6L	R	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14558	R	S450L	R	S315T	-	S	I6L	R	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14543	R	S450L	R	S315T	-	ND	I6L	R	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14304	R	S450L	R	S315T	-	ND	I6L	R	-	D354A	R	-	517	S	R	-	-37	R	R	-
08-14370	R	S450L	R	S315T	-	S	I6L	R	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14487	R	S450L	R	S315T	-	R	A3E	R	-	D354A	R	-	517	S	R	-	-37	R	R	D94A
08-14391	R	S450L	R	S315T	-	R	W119R	R	-	D354A	R	-	517	S	R	-	-37	S	S	-
08-14300	R	S450L	R	S315T	-	ND	-	R	-12	D354A	R	-	517	S	R	-	-37	R	R	D94N
08-14546	R	S450L	R	S315T	-17	S	V44G	R	-	M306V	R	-	517	S	S	-	-12	R	R	D94G
08-14514	R	S450L	R	S315T	-	S	F58L	R	-	M306V	R	K43R	517	S	S	-	-14	S	S	-
08-14477	S	-	S	-	-	S	-	S	-	-	R	K43R	-	ND	ND	-	-	ND	ND	-
08-14357	S	-	S	-	-	ND	-	S	-	-	S	-	-	ND	ND	-	-	ND	ND	-
08-14569	S	-	R	S315T	-	ND	-	S	-	E504D	R	K88M	-	ND	ND	-	-	ND	ND	-
08-14443	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-	ND	ND	-
08-14567	S	-	R	S315T	-	S	-	R	-12	M306V	R	K43R	-	S	S	-	-	S	S	-
08-14310	R	L430P/H445N	R	S315T	-15	ND	V139A	R	-	M306V	R	K88R	-	R	R	1401	-	R	R	D94G
08-14493	R	S450L	R	S315T	-	R	-11	R	-	M306V	R	K43R	-	R	R	-	-37	R	R	-
05-08961	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-	ND	ND	-
08-14358	S	-	S	-	-	ND	-	S	-	-	S	-	-	ND	ND	-	-	ND	ND	-
08-14329	R	S450L	R	S315T	-	ND	S59P	R	-	H1002R	R	K43R	-	S	R	1401	-	S	S	-
08-14354	R	S450L	R	S315T	-	S	-	S	-	-	R	K88R	-	R	S	-	-	ND	S	-
08-14556	R	L430P/S431G/H445Q	R	S315T	-	R	-	R	-	M306V	S	K43R	-	S	R	-	-37	R	R	A90V
08-14377	R	S450L	R	S315T	-	S	-	R	-	M306V	R	K43R	-	S	R	-	-10	R	R	-
08-14521	R	S450L	R	S315T	-	R	V128G	R	-	M306V	R	K43R	-	S	R	-	-	S	S	-
08-14350	R	S450L	R	S315T	-	ND	-11	R	-	M306V	R	K43R	-	R	R	-	-10	R	R	D94A
08-14337	R	S450L	R	S315T	-	ND	-	R	-	M306V	R	K43R	-	S	S	-	-10	S	S	-
08-14303	R	S450L	R	S315T	-	ND	-11	R	-	M306I	R	K43R	-	R	R	1401	-	R	R	-
08-14306	R	S450L	R	S315T	-	ND	D136Y	R	-	M306I/Q497R	R	K43R	-	R	R	-	-12	R	R	D94G
08-00483	R	S450L	R	S315T	-	R	-	R	-	M306V	R	K43R	-	S	R	-	-10	S	S	-
08-14361	R	S450L	R	S315T	-	S	T47A	S	-	N296H	R	K43R	-	S	R	-	-10	R	R	-
08-14486	R	S450L	R	S315T	-	S	A102V	R	-16	-	R	K43R	-	S	R	1401	-	R	R	-
08-14365	S	-	R	S315T	-	S	-	S	-16	-	R	K43R	-	S	S	-	-	S	S	-
05-03798	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-	ND	ND	-
05-10261	S	-	S	-	-	S	-	S	-	-	R	K43R	-	ND	ND	-	-	ND	ND	-
05-06899	R	D435V	R	S315T	-	R	-11	R	-	P404S	R	K43R	-	S	S	-	-	S	S	-
05-03633	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-			
05-12611	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-			
05-09310	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-			
05-03802	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-			
06-01971	S	-	S	-	-	S	-	S	-	-	S	-	-	ND	ND	-	-			
08-14389	R	S450L	R	S315T	-	R	T135P	R	-	Q497R	R	K43R	-	R	R	-	-			

Clade A

Clade B

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Compensation

Whole-genome sequencing of rifampicin-resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations in RNA polymerase genes

Iñaki Comas[1,8], Sonia Borrell[2,3], Andreas Roetzer[4], Graham Rose[1], Bijaya Malla[2,3], Midori Kato-Maeda[5], James Galagan[6,7], Stefan Niemann[4] & Sebastien Gagneux[2,3]

N=10strains+invitro

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Nat Genet. 2014 March ; 46(3): 279–286. doi:10.1038/ng.2878.

Evolution and transmission of drug resistant tuberculosis in a Russian population

Nicola Casali[1], Vladyslav Nikolayevskyy[1], Yanina Balabanova[1], Simon R Harris[2], Olga Ignatyeva[3], Irina Kontsevaya[3], Jukka Corander[4], Josephine Bryant[2], Julian Parkhill[2], Sergey Nejentsev[5], Rolf D Horstmann[6], Timothy Brown[1], and Francis Drobniewski[1,7,*]

N=1000strains

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study

Timothy M Walker, Thomas A Kohl*, Shaheed V Omar*, Jessica Hedge*, Carlos D O Elias, Phelim Bradley, Zamin Iqbal, Silke Feuerriegel, Katherine E Niehaus, Daniel J Wilson, David A Clifton, Georgia Kapatai, Camilla Ip, Rory Bowden, Francis A Drobniewski, Caroline Allix-Béguec, Cyril Gaudin, Julian Parkhill, Roland Diel, Philip Supply, Derrick W Crook, E Grace Smith, A Sarah Walker, Nazir Ismail, Stefan Niemann†, Tim E A Peto†, and the Modernizing Medical Microbiology (MMM) Informatics Group‡*

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- 2010- 2013, WGS of 2099 MTB strains, examined 23 gene mutations associated with drug-resistance
- predict phenotypic DST result for a validation set of 1552 MTB genomes.
- predicted 89·2% of the validation-set phenotypes with a mean 92·3% Sensitivity and 98·4% specificity.
- 10·8% of validation-set phenotypes could not be predicted as uncharacterised mutations present.
- As in-silico comparison, resistance mutations had higher sensitivity than 3 line-probe assays (85·1% vs 81·6%).

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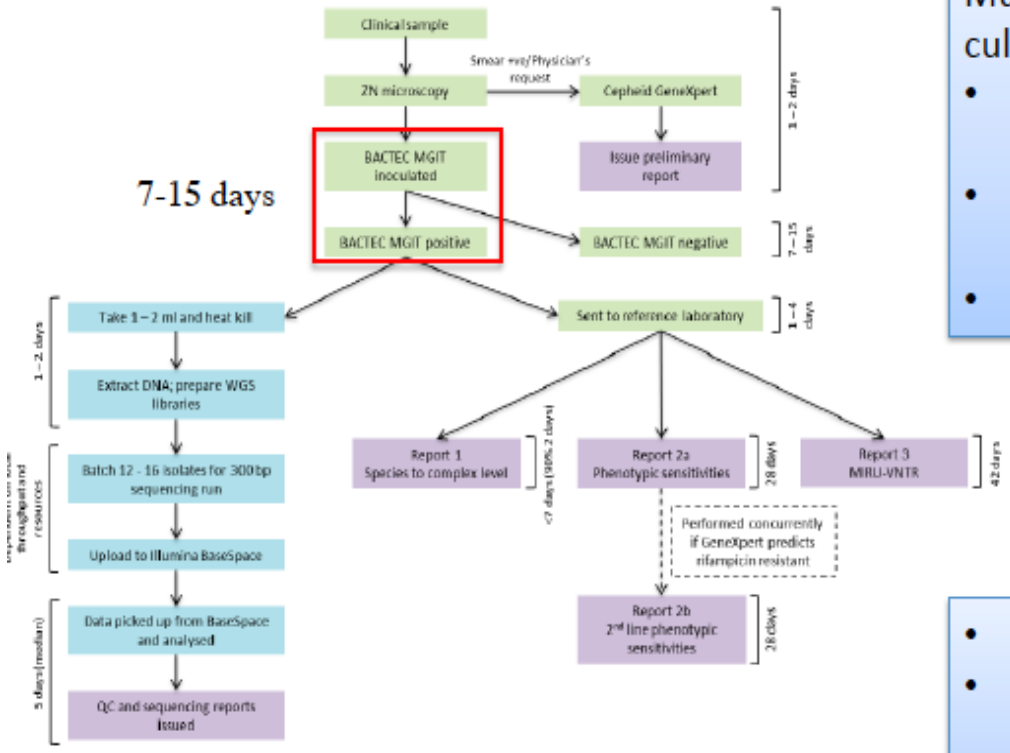
Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study

Louise J Pankhurst*, Carlos del Ojo Elias*, Antonina A Votintseva*, Timothy M Walker*, Kevin Cole, Jim Davies, Jilles M Fermont, Deborah M Gascoyne-Binzi, Thomas A Kohl, Clare Kong, Nadine Lemaitre, Stefan Niemann, John Paul, Thomas R Rogers, Emma Roycroft, E Grace Smith, Philip Supply, Patrick Tang, Mark H Wilcox, Sarah Wordsworth, David Wyllie, Li Xu, Derrick W Crook, for the COMPASS-TB Study Group†



Multicentric study on WGS from newly positive culture:

- Prediction of species and drug susceptibility with 93% accuracy
- Full WGS Dx, incl. genetic relatedness, median 21 days faster than classical Dx
- 7% less costly annually than current workflow

- Primary culture still needed : delayed Dx
- WGS on clinical samples: low multiplexing/ coverage depth or capture system (Brown, JCM, 2015) not cost-effective (yet)

Cryptic 10 000 Genomes project

(NEJM 2018)

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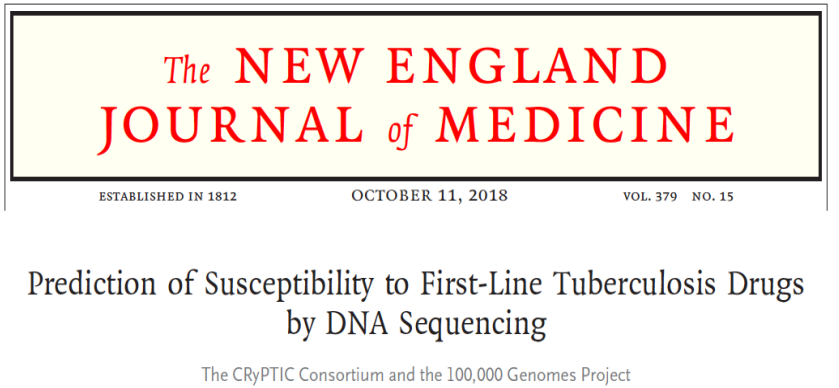

- 10,209 isolates analysed by WGS.
- 23 collections 16 countries
- Resistance to isoniazid, rifampicin, ethambutol, and pyrazinamide correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity, respectively,
- Susceptibility to same drugs correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity.
- Of 7516 isolates with complete phenotypic DST profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted.
- Among 4037 phenotypic profiles that were predicted to be pansusceptible, 3952 (97.9%) were correctly predicted.

WHO Target Product Profiles and 10000 genomes

- WHO target product profiles for new molecular assays for M. tuberculosis require more than 90% sensitivity and 95% specificity.

(High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva: World Health Organization, 2014 (http://www .who .int/ tb/ publications/ tpp_report/ en/))

- Overall, both these targets were met for all drugs with the exception of specificity for ethambutol (93.6%)--phenotyping is an imperfect standard, particularly for isolates with embB mutations.

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A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*

Miotto et al ERJ 2017

TABLE 3 List of confidence-graded mutations associated with phenotypic drug resistance as determined by best confidence values

Drug (phenotypic testing)	Gene	High-confidence mutations	Moderate-confidence mutations	Minimal-confidence mutations	No association with resistance
First-line					
Rifampicin (R)	*rpoB*	F505V+D516Y, S512T, Q513H+L533P, Q513-F514ins, **Q513K**, **Q513L**, **Q513P**, **F514dupL**, M515I+D516Y, **D516A**, **D516F**, **D516G**, D516G+L533P, D516ins, D516N, **D516V**, Del N518, **S522Q**, **H526C**, **H526D**, H526F, **H526G**, **H526L**, **H526R**, **H526Y S531F**, **S531L**, S531Q, **S531W**, S531Y, D626E	**D516Y**, **S522L**, H526P, **L533P**	**L511P**, **H526N**, **I572F**	
Isoniazid (H)	*inhA-mabA*	g-102a[#,¶]	**c-15t**		**g-102a**[#,¶], t-80g, *g-47c*, **T4I**
	katG	S315I, **S315N**, **S315T**, pooled frameshifts and premature stop codons			A110V, **R463L**, L499M
	furA				**L68F**
	mshA		A187V[#,¶]		**N111S**
Second-line (group A)					
Moxifloxacin (MFX)	*gyrA*	G88C, **A90V**, **S91P**, **D94A**, **D94G**, **D94N**, **D94Y**			E21Q, **S95T**, G247S, G668D, V712L
Ofloxacin (OFX)/ levofloxacin (LFX)	*gyrA*	**G88A**, **G88C**, **S91P**, **A90V**, **D94A**, **D94G**, **D94H**, **D94N**, **D94Y**	D89N		E21Q, **T80A**, **S95T**, G247S, G668D, V712L
	gyrB	E459K, **A504V**			
Second-line (group B)					
Amikacin (AM)	*rrs*	**a1401g**, **g1484t**			
Kanamycin (KM)	*eis*	**c-14t**, **g-10a**		g-37t, c-12t	**a1338c**
	rrs	a514c[#], **a1401g**, c1402t, g1484t			
	rrs+eis	*rrs* c517t[#] + *eis* g-37t			
Capreomycin (CM)	*rrs*	**a1401g**, **c1402t**, **g1484t**			**c517t**
	tlyA	**N236K**, **pooled frameshifts and premature stop codons**			D149H
Streptomycin (S)	*rpsL*	**K43R**, K43T, **K88Q**, **K88R**, T40I			
	rrs	a1401g[#], **a514c**, a514t, c462t, c513t, **c517t**			
	gidB		**E92D**[#,¶]		**L16R**, **V110G**, pooled frameshifts and premature stop codons
Second-line (group C)					
Ethionamide and prothionamide (ETO/PTO)	*inhA*	c-15t+I194T, c-15t+S49A	**c-15t**		
	ethA				Q347Stop
Second-line (group D)					
Pyrazinamide (Z)	*pncA*	t-12c, **a-11g**, t-7c, **A3E**, L4S, **I6T**, V7G, D8E, **D8G**, D8N, **Q10P**, **D12A**, **D12N**, **C14R**, G17D, L19P, G24D, **Y34D**, **A46V**, **K48T**, **D49G**, D49N, **H51Q**, H51R, P54S, **H57D**[¶], H57P, **H57R**, **H57Y**, S59P, P62L, P62Q, **D63G**, S66P, **S67P**, W68C, **W68R**, **H71D**, H71Q, **H71Y**, **C72R**, **T76P**, **H82R**, **L85P**, **L85R**, **F94L**, **F94S**, K96N, K96R, G97C, **G97D**, **G97S**, **Y103H**, **S104R**, **G108R**, L116P, **L116R**, **L120P**, R123P, V125F, **V125G**, **V128G**, G132A, G132D, G132S, A134V, **T135N**, **T135P**, H137P, C138Y, **V139G**, **V139L**, **Q141P**, **T142A**, **T142K**, **T142M**, **indel - R148ins (inframe)**, L151S, **V155G**, L159P, T160P, G162D, T168P, **L172P**, M175T, **M175V**, V180F, V180G, **Pooled frameshifts and premature stop codons**	**V7G**, Q10R, **P54L**, **W68G**, K96E, K96T, A171E, M175I	**D12G**, F58L, H71R, I133T, **V139A**	**indel - c-125del**, **I31T**, **L35R**, **T47A**, **I6L**, K48T, T114M

The table includes all the mutations graded according to the proposed standardised approach for providing confidence levels to their association with phenotypic drug resistance.

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REVIEW

Whole genome sequencing of *Mycobacterium tuberculosis* for detection of recent transmission and tracing outbreaks: A systematic review

Vlad Nikolayevskyy [a, b, *, 1], Katharina Kranzer [a, c, d, 1], Stefan Niemann [d, e], Francis Drobniewski [a, b]

Systematic review was conducted …studies published between 01/01/2005 and 30/11/2014. A total of 12 publications were included.

WGS
detects
of <6 S
perform
using u

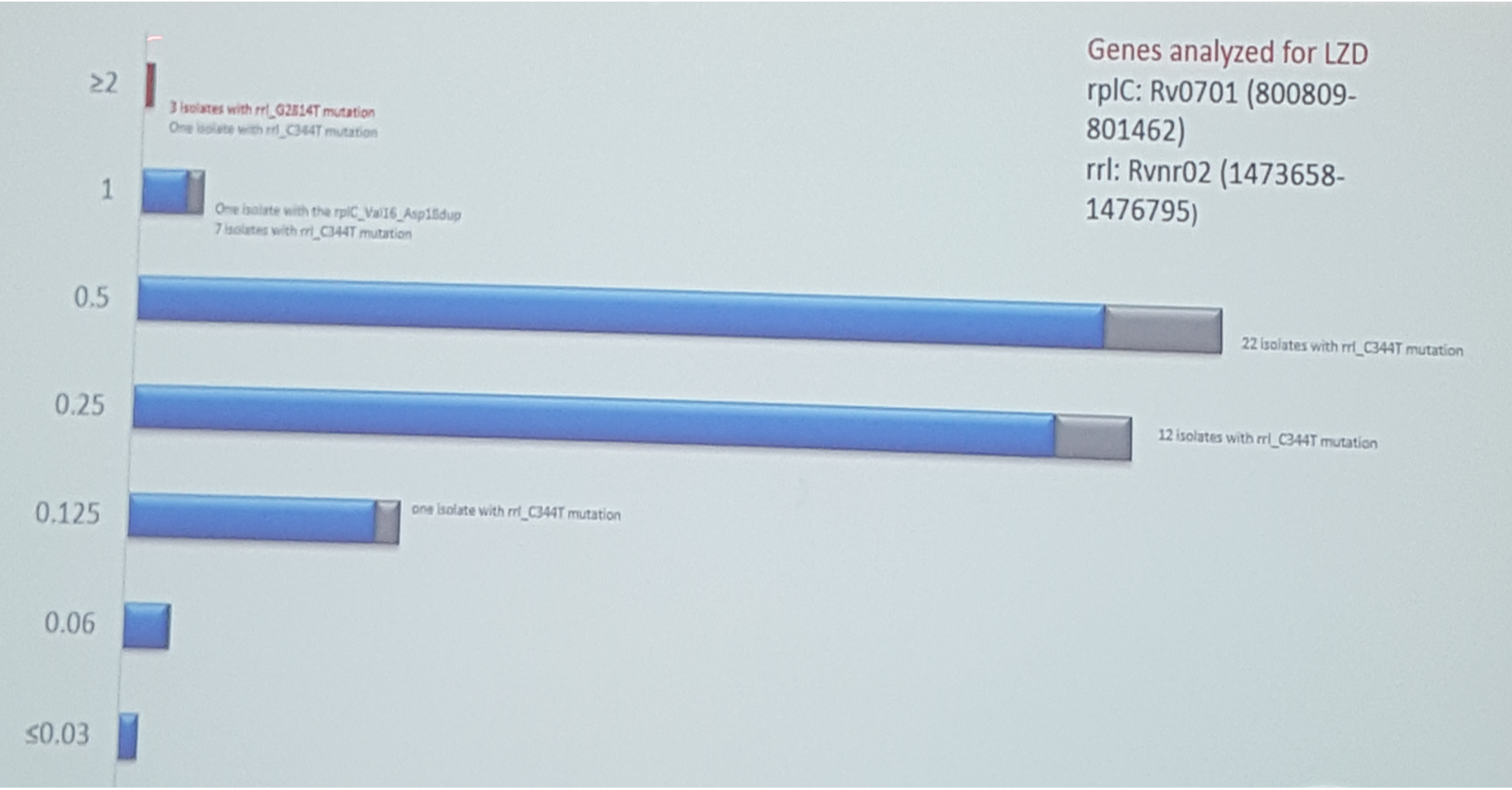
Several systematic reviews have looked at drug susceptibility testing by WGS and molecular epidemiology/transmission by WGS.

[6,15

which may provide answers to specific questions such as transmission chains and cross-border transmission and may evolve into a practical instrument used to cover the majority of laboratory diagnoses, including identification, provide indication of drug resistance testing and epidemiological typing. However, its impact in routine day-to-day public health and clinical investigations remains to be demonstrated.

Using WGS to define resistance genes

(Italy-LZD-Courtesy D Cirillo)



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How good are any tests: Mislabelling (10000 genomes study NEJM 2018)

Mislabeling of laboratory samples contributed to discrepant results.

Possibility was assessed for each collection on the basis of the proportion of isolates that were excluded because of katG S315T or rpoB S450L mutations being classified as SENSITIVE rather than RESISTANT, the discrepancy rate within the collection, and the prevalence of antimicrobial resistance.

Overall, approximately 43% of discrepancies for isoniazid and 12% of discrepancies for rifampicin were thereby judged to be attributable to mislabeling.

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WGS from sputum (Doughty et al 2015)

- Proof-of-concept study in 2015
- 2015, Doughty et al. extracted M. tuberculosis
- DNA directly from clinical samples
- Sequenced it with Illumina MiSeq
- However, while TB could be diagnosed, the DNA obtained was insufficient for DST because of contamination with human DNA

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WGS from sputum directly

(Brown et al JCM 2015)

- WGS data on resistance mutations and strain typing for transmission, but previously only from cultured M. tb.
- Utilising biotinylated RNA baits, designed for MTB DNA to capture full genomes directly from sputum samples, allowing WGS.
- 24 smear-positive sputum samples, from UK and Lithuania with matched culture sample
- TB sequencing data obtained directly from all 24 sputa: 20 were high quality (>20x depth and >98% genome covered).
- Turnaround time about 50 hours

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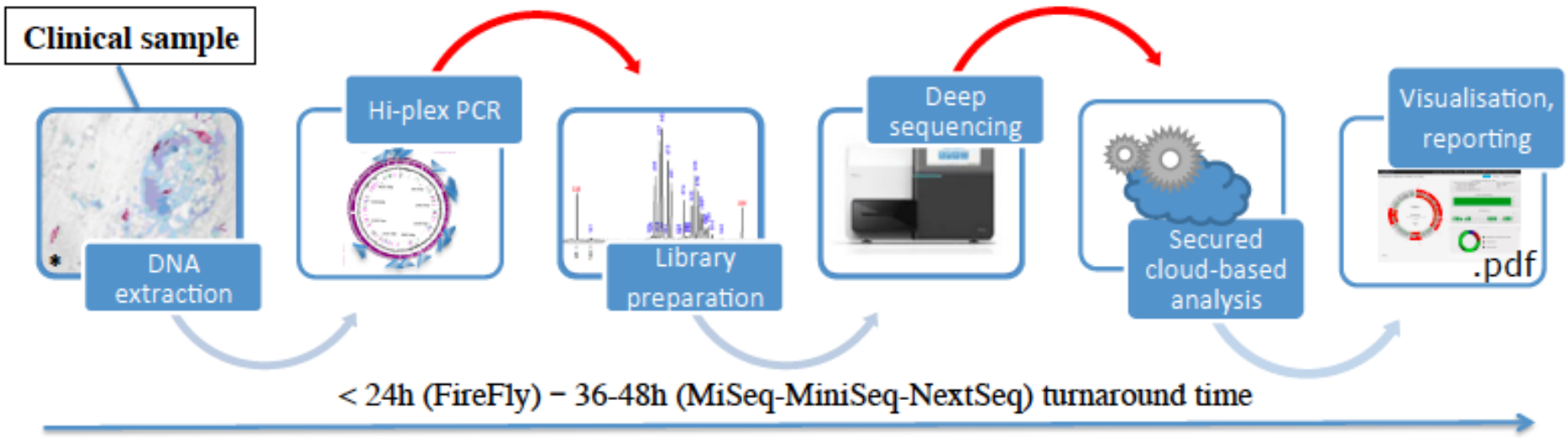
WGS from sputum (Votintseva et al 2017)

- Extract DNA from sputum without enrichment.
- Sequencing using MiSeq sequencing
- Turnaround time 44 hours
- Cheaper than Brown et al –(£100 vs 203)
- 95% were identified as M. tuberculosis
- overall quality metrics were lower than in Brown et al 2015
- depth of coverage of 12 x with 90% genome coverage for 21/37 (57%) of the smear-positive, culture-positive samples.

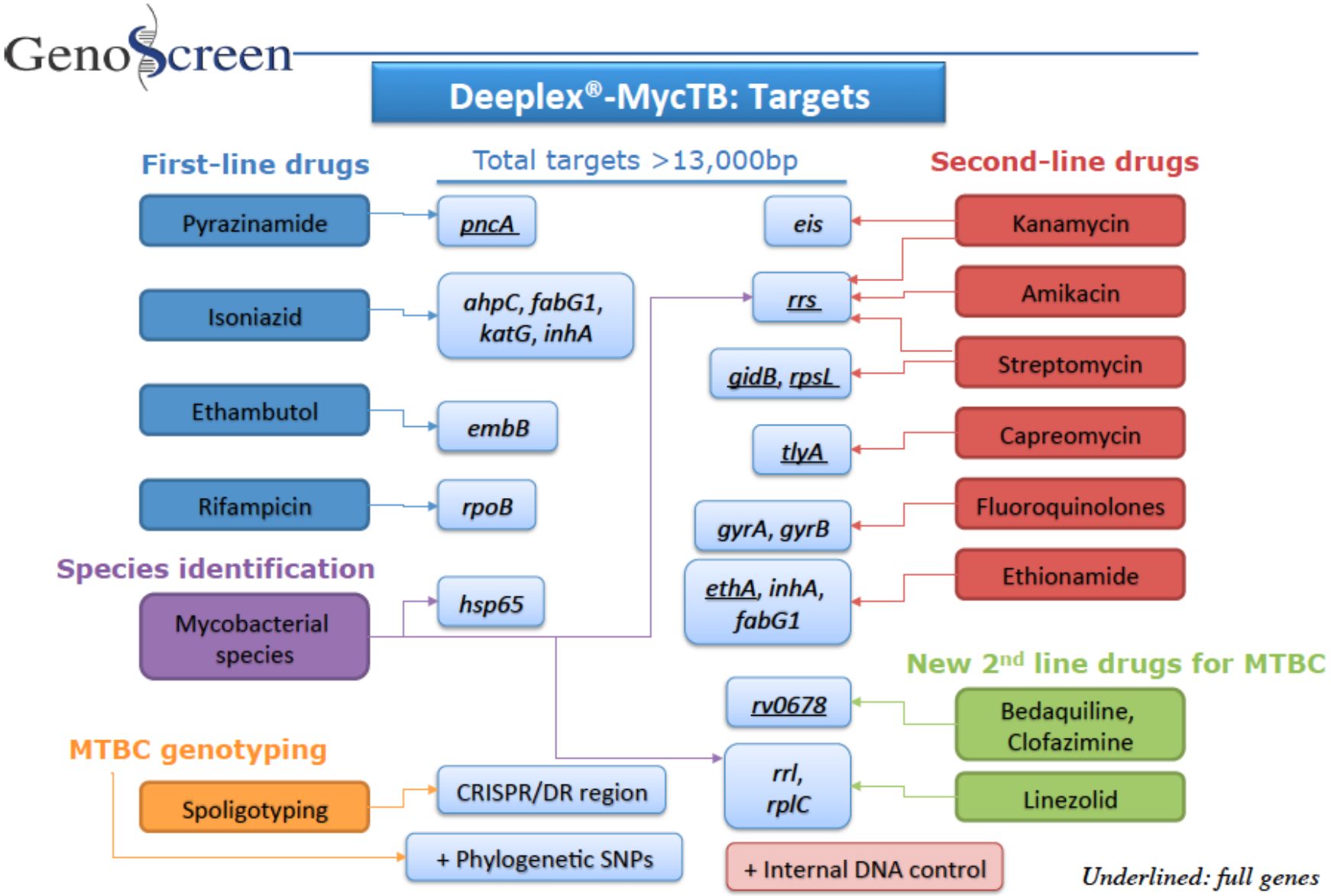
Deeplex®-MycTB, an all-in-one NGS-based diagnostic test for *M. tuberculosis*



- Targeted NGS of single 24-plex amplification of main drug resistance targets, plus species identification and MTBC genotyping targets
- Deep sequencing for sensitive detection of heteroresistance
- Scalable: from 1 to 8 (FireFly), to 50/90 (MiSeq/MiniSeq) and 384 samples (NextSeq)/run
- Fast, easy-to-use NGS data analysis and reporting on highly secured, high performance cloud

*Photo credit: WHO, The Natural History of Pulmonary Tuberculosis, Facilitator Guide', 2001

Courtesy P Supply



Courtesy of P Supply

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Conclusion-WGS and Diagnostics 3

- No EQA for NGS; lot operator settings for analysis ie “home-brew” system
- Replace bioinfomatics ie completely automated process
- Need improvements for low DNA conc in clinical specimens—starting to happen
- Databases/storage handling solutions
- 20-40% of treated TB cases have no culture or any laboratory result

UK

Sputum

No rifampicin or isoniazid resistance

Treat 6 month standard regime

Culture

WGS or targetted

rifampicin or isoniazid resistance

Culture

Phenotypic DST

Results to clinician

MDRTB Treat

GLOBAL

Sputum

- Xpert, Line probe assays

No rifampicin or isoniazid resistance

Treat 6 month standard regime

Rifampicin resistance → **Culture** → **WGS or targetted**

Culture → **Phenotypic DST** → **Results to clinician**

MDRTB Treat

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But…UNITAID-2015

- So most high burdens are still using microscopy for *diagnosis: 22 HBC=77.6 m sputum smears= $137m* ***cost=performed 43,000 centers***
- *POC or "big lab"*
- *Need for "big lab" reduced as cat 3 reduced*
- *NGS requires "big lab" due to infrastructure*

Audience: "Health economics"

- Test with 100% sensitivity, 100% specificity
- Excellent patient trial, 10, 000 TB suspects, patient sputum; "perfect test"
- TB is a significant problem in your country

- What are you going to recommend about introducing it to your Minister/WHO?
- What else would you like to know?

Audience: "Health economics"

- Test with 100% sensitivity, 100% specificity
- Excellent patient trial, 10, 000 TB suspects, patient sputum;"perfect test"
- TB is a significant problem in your country

- Costs $100,000 per test

Audience: "Health economics"

- Test with 100% sensitivity, 100% specificity
- Excellent patient trial, 10, 000 TB suspects, patient sputum;"perfect test"
- TB is a significant problem in your country

- Costs $10 per test

Audience: "Health economics"

- Test with 100% sensitivity, 100% specificity
- Excellent patient trial, 10, 000 TB suspects, patient sputum; "perfect test"
- TB is a significant problem in your country

- Costs $50 per test

Cost-effectiveness of Xpert (Choi et al 2013)

- What is the the cost-effectiveness of implementing Xpert in low TB prevalence countries eg USA?
- Evaluated the cost-effectiveness of incorporating Xpert into TB diagnostic algorithms in the USA compared to existing diagnostics.
- A decision-analysis model compared current TB diagnostic algorithms in the United States to algorithms incorporating Xpert.

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COSTS OF DIAGNOSTIC TESTS PER SAMPLE

Diagnostic test	Cost of consumables US$ (% total)	Cost of equipment US$ (% total)	Labor cost US$ (% total)	Overhead cost US$ (% total)*	Total cost per sample [range]†
Decontamination/ concentration	4.93 (66)	0.17 (2)	1.70 (23)	0.68 (9)	7.48 [2.58–12.88]
Smear microscopy	0.92 (23)	0.09 (2)	2.69 (66)	0.37 (9)	4.07 [2.35–5.95]
MGIT	15.02 (42)	2.87 (8)	14.16 (40)	3.51 (10)	35.56 [17.29–52.60]
DST	57.00 (56)	23.43 (23)	11.99 (12)	9.26 (9)	101.68 [19.60–166.37]
MTD®	70.37 (77)	1.50 (2)	11.30 (12)	8.32 (9)	91.49 [26.08–320.42]
Xpert® MTB/RIF	74.60 (76)	13.94 (14)	4.78 (5)	4.78 (5)	98.10 [20.24–838.46]‡

Choi et al 2013

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Cost-effectiveness of Xpert (Choi et al 2013)

- Despite existing mycobacterial culture as the reference in USA diagnostic algorithms, adding Xpert leads to a gain in QALYs
- QALYS in patients a result of more rapid diagnosis and treatment of active TB, and less unnecessary treatment in cases of false-positive smear microscopy
- TB diagnostic algorithms incorporating Xpert in the United States are highly cost-effective and based on real not discounted cost
- Laboratory costs increase by over 60% per patient compared to no molecular testing.
- But Xpert into diagnostic algorithms in the USA would be cost-saving from a health systems perspective

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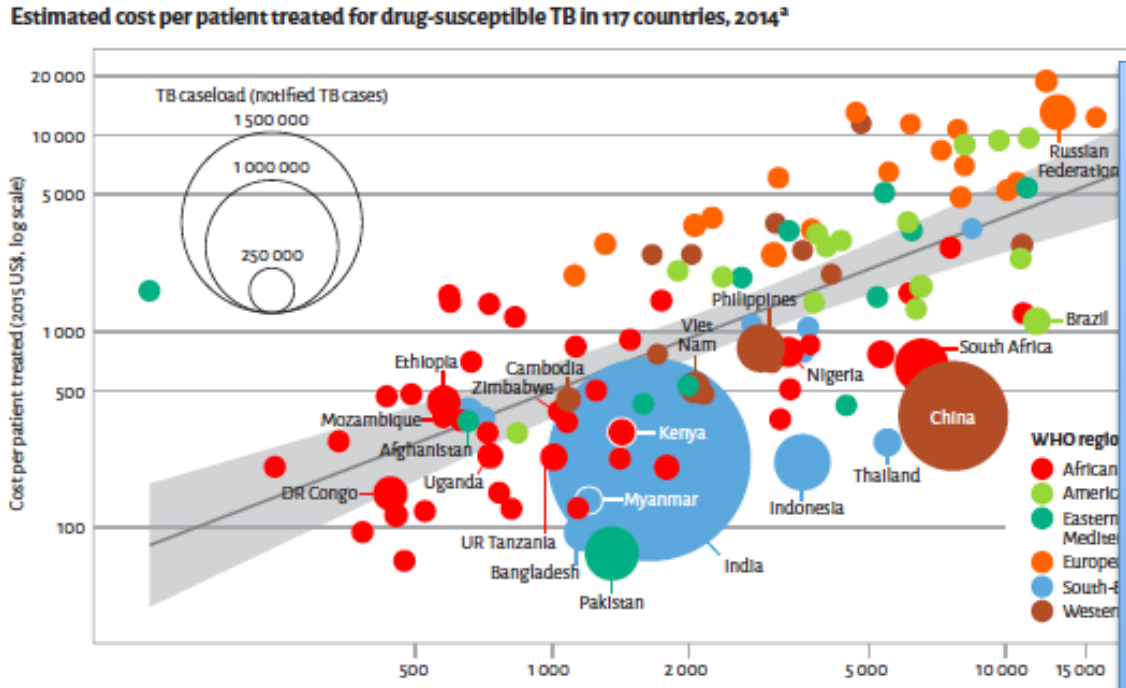

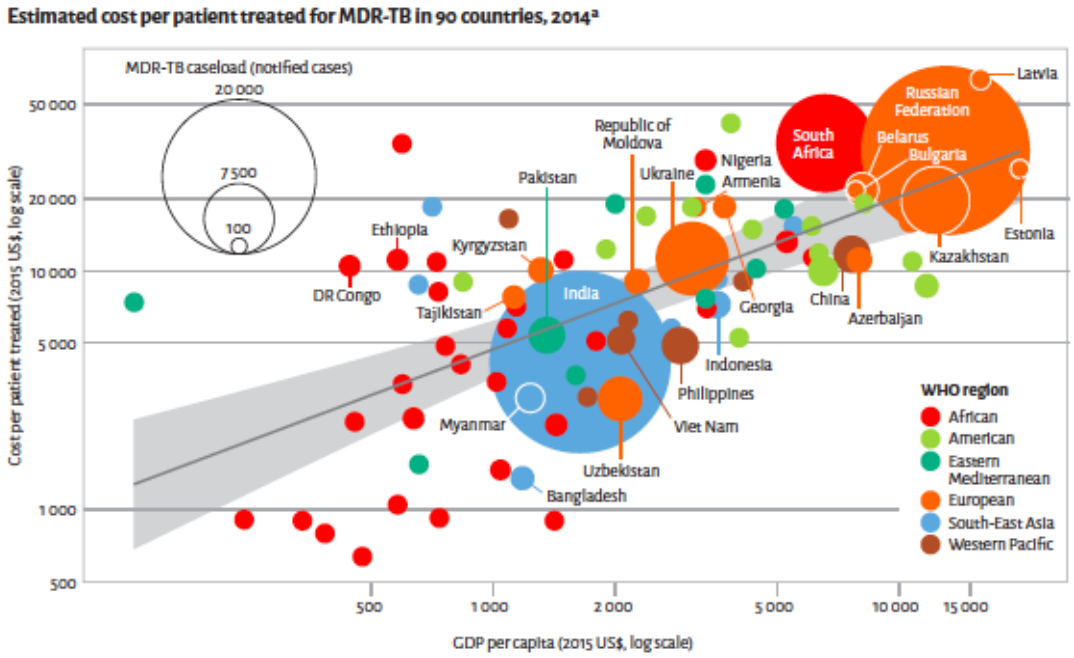

[a] Limited to countries with at least 20 patients on second-line treatment in 2014.

Cost per patient for drug-susceptible TB in2014 ranged from US$ 100–500 in most countries with high burden of TB. Cost per patient for MDRTB was typically US$ 5000 –10000

(WHO Global TB Report 2015)

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