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

Francis Drobniewski MBBS PhD
Professor of Global Health and Tuberculosis
f.drobniewski@imperial.ac.uk
UN GENERAL ASSEMBLY HIGH-LEVEL MEETING ON ENDING TB 26 Sept 2018, New York
The WHO End TB Strategy

The End TB Strategy at a glance

<table>
<thead>
<tr>
<th>VISION</th>
<th>A WORLD FREE OF TB — zero deaths, disease and suffering due to TB</th>
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<tbody>
<tr>
<td>GOAL</td>
<td>END THE GLOBAL TB EPIDEMIC</td>
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<tr>
<th>INDICATORS</th>
<th>MILESTONES</th>
<th>TARGETS</th>
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<tbody>
<tr>
<td>Percentage reduction in the absolute number of TB deaths (compared with 2015 baseline)</td>
<td>35%</td>
<td>75%</td>
</tr>
<tr>
<td>Percentage reduction in the TB incidence rate (compared with 2015 baseline)</td>
<td>20%</td>
<td>50%</td>
</tr>
<tr>
<td>Percentage of TB-affected households experiencing catastrophic costs due to TB (level in 2015 unknown)</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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
% of new TB cases which are MDR/RR

Percentage of new TB cases with MDR/RR-TB

- 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
MDR cases: importance of cases as well as rates

Estimated incidence of MDR/RR-TB in 2016, for countries with at least 1000 incident cases

Different numbers as important as rates eg Estonia vs Russia
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
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

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

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...
UNITAID estimates over 50 companies making new TB diagnostics.
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

Detection
Detection
Detection
Detection

+Rifampicin
+Isoniazid
+Quinolone

SUSCEPTIBLE

MDR
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,‡ Malcolm D. Yates,† and Francis A. Drobniewski†

*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,† and Tartu University Clinics, United Laboratory, Department of Mycobacteriology, Tartu, Estonia‡*

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

Sabine Rüsch-Gerdes,† Gaby E. Pfyffer,‡ Manuel Casal,§ Maureen Chadwick,¶ and Salman Siddiq

*National Reference Center for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany†; Department of Medical Microbiology, Lucerne General Hospital, Lucerne, Switzerland‡; Mycobacteria Reference Center, Faculty of Medicine, University of Cordoba, Cordoba, Spain§; Royal Brompton Hospital, London, United Kingdom¶; and Becton Dickinson Diagnostic Systems, Sparks, Maryland*
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
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.

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Print test result

Time to result, 1 hour 45 minutes
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
Detection of Resistance to Second-Line Antituberculosis Drugs by Use of the Genotype MTBDRsL Assay: a Multicenter Evaluation and Feasibility Study

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

Samara Oblast Tuberculosis Dispensary, Samara, Russia; Queen Mary College, Barts and the London School of Medicine, University of London, London, United Kingdom; Tartu University Hospital, Tartu, Estonia; Pneumoptriology...

The rate of multidrug-resistant (MDR) and ext

countries of the former USSR. The availability of dr

gs is vital for adequate patient management. T

among drug susceptibility testing (lino

tuberculosis) isolates at four sites in Ea

t sensitivity for the detection of resistance to 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

Phenotypic resistance testing of drug sensitivities in 504 sputum samples was performed at four sites in Eastern Europe. The diagnostic accuracy was assessed using the Xpert MTB/RIF and I


diagnostic accuracy of the GenoType MTBDRsL Assay for Rapid Diagnosis of Extensively Drug-Resistant Tuberculosis in HIV-Co

Irina Kontsevaya, Olga Ignatyeva, Vladislav Nikolayevskyy, Yanina Balabanova, Alexander Kovalyov, Andrey Kritsky, Olesya Matskevich, Francis Drobniewski

Samara Oblast Tuberculosis Dispensary, Samara, Russia; Queen Mary College, Barts and the London School of Medicine, University of London, London, United Kingdom; University College Hospital, London, United Kingdom

Multidrug-resistant (MDR) and extensivel

tuberculosis (TB) has become a global TB control due to difficulties in diagnos

The Russian Federation is a high-tuberculosis (TB)-burden country with high rates of Mycobacterium tuberculosis multidrug resistance. XDR-TB has become a major global TB control due to difficulties in diagnosing the high rates of drug-resistant cases. XDR-TB is defined as resistance to isoniazid, rifampicin, and at least one of the following four classes of drugs: fluoroquinolones, second-line injectable drugs, and aminoglycosides. The importance of rapid diagnostic tests for XDR-TB has grown due to the increasing drug resistance and the need for early treatment initiation. XDR-TB has become a major global TB control due to difficulties in diagnosing the high rates of drug-resistant cases. XDR-TB is defined as resistance to isoniazid, rifampicin, and at least one of the following four classes of drugs: fluoroquinolones, second-line injectable drugs, and aminoglycosides. The importance of rapid diagnostic tests for XDR-TB has grown due to the increasing drug resistance and the need for early treatment initiation.

Have LPA for diagnosing XDRTB (aminoglycosides and FQs from cultures and heavily smear positive specimens). WHO recommends for primary specimens and cultures now
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.
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.
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
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 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.
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
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/Pharmacodynamics (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
3-D bioelectrospray cell culture model

- *ex vivo* model incorporating extracellular matrix
- More “physiological”
- Multiple uses
- Microfluidics: pharmacokinetics, drugs with specific kinetics
3-D bioelectrospray cell culture model

- Isolation of PMBCs
- Infecting with MTB Lux+
- Mixing with alginate-collagen matrix
- Bioelectrostatic system
- Bioluminescence measurement
## Modified WHO MDRTB Regimens

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<tr>
<td><strong>Group A:</strong> Include all three medicines (unless they cannot be used)</td>
<td>Levofloxacin <strong>OR</strong> Moxifloxacin</td>
<td>Lfx</td>
</tr>
<tr>
<td></td>
<td>Bedaquiline&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>Bdq</td>
</tr>
<tr>
<td></td>
<td>Linezolid&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Lzd</td>
</tr>
<tr>
<td></td>
<td>Clofazimine</td>
<td>Cfz</td>
</tr>
<tr>
<td></td>
<td>Cycloserine <strong>OR</strong> Terizidone</td>
<td>Cs</td>
</tr>
<tr>
<td></td>
<td>Ethambutol</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>Delamanid&lt;sup&gt;3,4&lt;/sup&gt;</td>
<td>Dlm</td>
</tr>
<tr>
<td></td>
<td>Pyrazinamide&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Z</td>
</tr>
<tr>
<td></td>
<td>Imipenem-cilastatin <strong>OR</strong></td>
<td>Ipm-Cln</td>
</tr>
<tr>
<td></td>
<td>Meropenem&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Mpm</td>
</tr>
<tr>
<td></td>
<td>Amikacin <strong>OR</strong> Streptomycin&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Am (S)</td>
</tr>
<tr>
<td></td>
<td>Ethionamide <strong>OR</strong> Prothionamide</td>
<td>Eto Pto</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-aminosalicylic acid</td>
<td>PAS</td>
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<sup>1</sup> Bedaquiline is co-administered with rifampicin and pyrazinamide. 
<sup>2</sup> Linezolid is co-administered with pyrazinamide. 
<sup>3</sup> Delamanid is co-administered with rifampicin and pyrazinamide. 
<sup>4</sup> Moxifloxacin is used instead of levofloxacin when necessary. 
<sup>5</sup> Pyrazinamide is used instead of ethambutol when necessary. 
<sup>6</sup> Meropenem is used instead of imipenem-cilastatin when necessary. 
<sup>7</sup> Streptomycin is used instead of amikacin when necessary.
Modified WHO MDRTB Regimens

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<td>Linezolid(^2)</td>
<td>Lzd</td>
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Emphasis on oral regimens. Kanamycin out. Streptomycin in, Greater role for clofazamine, cycloserine, delamanid, bedaquiline, levofloxacin equal to moxifloxacin.
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 Merker¹, Camille Blin², Stefano Mona², Nicolas Duforet-Frebourg⁴, Sophie Lecher⁵-⁸, Eve Willery⁵-⁸, Michael G B Blum⁴, Sabine Rüscher-Gerdes⁹, Igor Mokrousov¹⁰, Eman Aleksic¹¹, Caroline Allix-Bégué¹², Annick Antierens¹³, Ewa Augustynowicz-Kopeć¹⁴, Marie Ballif¹⁵, Francesca Barletta¹⁶, Hans Peter Beck¹⁷, Clifton E Barry III¹⁸, Maryline Bonnet¹⁹, Emanuele Borroni²⁰, Isolina Campos-Herrero²¹, Daniela Cirillo²⁰, Helen Cox²², Suzanne Crowe¹¹,²³,²⁴, Valeriu Crudu²⁵, Roland Diel²⁶, Francis Drobniewski²⁷,²⁸, Maryse Fauville-Dufaux²⁹, Sébastien Gagneux¹⁷, Solomon Ghebremichael³⁰, Madeleine Hanekom³¹, Sven Hoffner³², Wei-wei Jiao³³, Stobdan Kalon³⁴, Thomas A Kohl¹, Irina Kontsevaya³⁵, Troels Lillebæk³⁶, Shinji Maeda³⁷, Vladyslav Nikolayevsky²⁷,²⁸, Michael Rasmussen³⁶, Nalin Rastogi³⁸, Sofia Samper³⁹, Elisabeth Sanchez-Padilla¹⁹, Branislava Savic⁴⁰, Isdore Chola champuta¹⁸, Adong Shen³³, Li-Hwei Sng⁴¹, Petras Stakenas⁴², Kadri Toit⁴³, Francis Varaine⁴⁴, Dragana Vukovic⁴⁰, Céline Wahl¹², Robin Warren³¹, Philip Supply⁵,¹²,¹⁴,⁶, Stefan Niemann¹,⁴⁵,⁴⁶ & Thierry Wirth²,³,⁴⁶
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
Strategies

- Early diagnosis
- New drugs
- New drug regimens ie novel combinations
- Systems creating MDRTB, XDR TB need fixing or new drugs will be lost
- MDRTB is endemic in some areas eg 25-50% of cases—strategy must be different
Genomic analysis identifies targets of convergent positive selection in drug-resistant *Mycobacterium tuberculosis*


“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 Zhang1,2,14, Dongfang Li3,4,14, Lili Zhao5,6,14, Joy Fleming1,14, Nan Lin7, Ting Wang1, Zhangyi Liu5, Chunyaou Li8, Nicholas Galwey1, Jiaoyu Deng8, Ying Zhou1, Yuanfang Zhu8, Yunriong Gao1, Tong Wang7, Shihua Wang7, Yufen Huang3, Ming Wang1, Qiu Zhong10, Lin Zhou10, Tao Chen10, Jie Zhou11, Ruifu Yang3, Guofeng Zhu12, Haiying Hang1, Jia Zhang1, Fabin Li1, Kanglin Wan5,6, Jun Wang3, Xian-En Zhang2,29 & Lijun Bi1

“72 new genes, 28 intergenic regions (IGRs), 11 nonsynonymous SNPs and 10 IGR SNPs with strong, consistent associations with drug resistance.”
Evolution and transmission of drug resistant tuberculosis in a Russian population

Nicola Casali¹, Vladyslav Nikolayevskyy¹, Yanina Balabanova¹, Simon R Harris², Olga Ignatyeva³, Irina Kontsevaya³, Jukka Corander⁴, Josephine Bryant², Julian Parkhill², Sergey Nejentsev⁵, Rolf D Horstmann⁶, Timothy Brown¹, and Francis Drobniewski¹,⁷,*

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


Lancet Infectious Diseases 2015

Nature Genetics 2014
Evolution and transmission of drug-resistant tuberculosis in a Russian population

Nicola Casali1, Vladyslav Nikolayevskyy1, Yanina Balabanova1, Simon R Harris3, Olga Ignatyeva3,
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Timothy Brown1 & Francis Drobniewski1,6

published online 26 January 2014; doi:10.1038/ng.2878
| ID London | RIF   | rpoB  | INH   | katG  | mabA  | pza   | pncA  | EMB   | embA  | embB  | STR   | rpsL  | rrs   | CAP   | KAN   | rrs   | ets   | MOX   | OFL   | gyrA   |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 08-1447  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    | ND    |
| 08-1455  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1457  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1430  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1451  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1447  | S     | S     | S     | S     |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1456  | S     | S     | S     | S     |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1443  | S     | S     | S     | S     |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1456  | S     | R     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1430  | R     | S     | R     | S31T  | -15   | ND    | V139A|       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1443  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1432  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1434  | R     | S     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1456  | R     | R     | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1452  | R     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1435  | R     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1436  | S     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-0689  | R     | D435V | R     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-0363  | S     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1261  | S     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-0380  | S     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
| 08-1438  | R     | S     | S     | S31T  |       |       |       |       |       |       |       |       |       |       |       |       |       |       | ND    | ND    | ND    | ND    |
Compensation

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

Iñaki Comas¹,⁸, Sonia Borrell²,³, Andreas Roetzer⁴, Graham Rose¹, Bijaya Malla²,³, Midori Kato-Maeda⁵, James Galagan⁶,⁷, Stefan Niemann⁴ & Sebastien Gagneux²,³

Published in final edited form as:

Evolution and transmission of drug resistant tuberculosis in a Russian population

Nicola Casali¹, Vladyslav Nikolayevskyy¹, Yanina Balabanova¹, Simon R Harris², Olga Ignatyeva³, Irina Kontsevaya³, Jukka Corander⁴, Josephine Bryant², Julian Parkhill², Sergey Nejentsev⁵, Rolf D Horstmann⁶, Timothy Brown¹, and Francis Drobniewski¹,⁷,*

N=10strains+invitro

N=1000strains
• 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%).
Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study

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

• 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


• 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.
### Table 3: List of confidence-graded mutations associated with phenotypic drug resistance as determined by best confidence values

<table>
<thead>
<tr>
<th>Drug (phenotypic testing)</th>
<th>Gene</th>
<th>High-confidence mutations</th>
<th>Moderate-confidence mutations</th>
<th>Minimal-confidence mutations</th>
<th>No association with resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-line</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid (IH)</td>
<td>inhA-mabA</td>
<td>g-102a&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>katG</td>
<td>S315I, S315N, S315T, pooled frameshifts and premature stop codons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>msbA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Second-line (group B)</strong></td>
<td>grrB</td>
<td>E459K, A504Y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin (AM)</td>
<td>rrs</td>
<td>a1401g, g1484t</td>
<td>c-14, g-14a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin (KM)</td>
<td>eis</td>
<td>e514c&lt;sup&gt;a&lt;/sup&gt;, a1401g, c1402t, g1484t</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capreomycin (CM)</td>
<td>rrs</td>
<td>a1401g, c1402t, g1484t</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>rpsL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>gidB</td>
<td>a1601g&lt;sup&gt;a&lt;/sup&gt;, a514c, a514g, c1537t, c517t</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Second-line (group C)</strong></td>
<td>inhA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethionamide and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prothionamide (ETO/PTO)</td>
<td>ehaA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table includes all the mutations graded according to the proposed standardised approach for providing confidence levels to their association with phenotypic drug resistance.
Several systematic reviews have looked at drug susceptibility testing by WGS and molecular epidemiology/transmission by WGS.
Using WGS to define resistance genes

(Italy-LZD-Courtesy D Cirillo)
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.
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
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
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 12x 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


Courtesy P Supply
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
Sputum

No rifampicin or isoniazid resistance

Treat 6 month standard regime

Culture

WGS or targeted

rifampicin or isoniazid resistance

Results to clinician

Phenotypic DST

MDRTB Treat

UK
Sputum
- Xpert, Line probe assays

- No rifampicin or isoniazid resistance
  - Treat 6 month standard regime

- Rifampicin resistance
- Culture
- WGS or targetted

- MDRTB
  - Treat

- Culture
- Phenotypic DST
- Results to clinician
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
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 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.
## COSTS OF DIAGNOSTIC TESTS PER SAMPLE

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

Choi et al 2013
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
Cost per patient for drug-susceptible TB in 2014 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)
References and reviews

- WHO Euro (2017) Algorithm for Laboratory Diagnosis of Pulmonary Tuberculosis using State of the Art Rapid Diagnostic Technologies-Expert opinion of the European TB Laboratory Initiative Core Group members for the WHO European Region