Advancing tuberculosis drug resistance diagnosis with targeted next-generation sequencing

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Global TB burden

10,8 M
incident TB cases

400 000
incident DR-TB cases

1,25 M
deaths

180 000
DR-TB cases detected

Out of 10 people with drug-resistant TB

4 diagnosed and enrolled on treatment

Global TB report, WHO, 2024

Current TB diagnostic assays

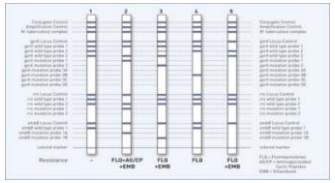
Microscopy

- Low sensitivity and specificity
- No information on resistance

Culture

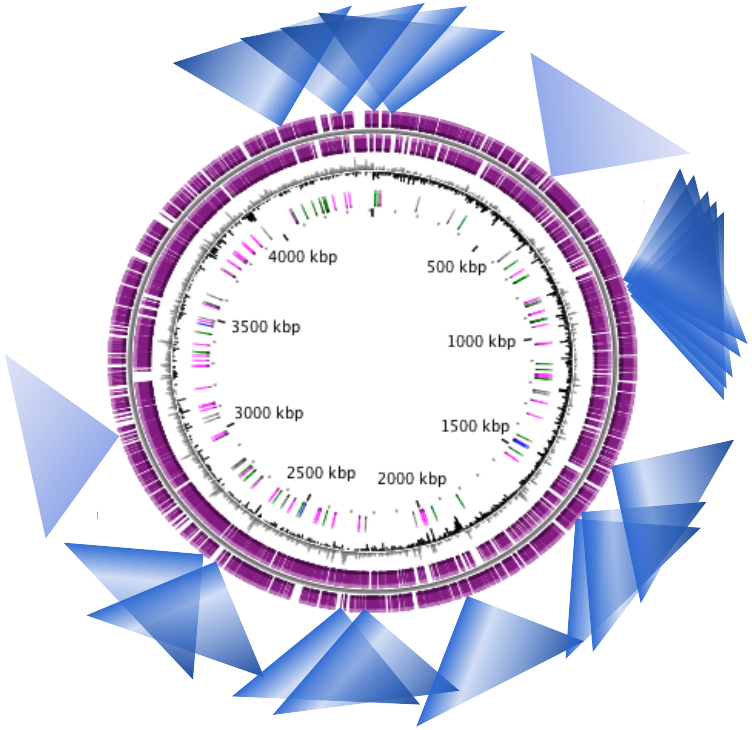
- Slow (~7 weeks)
- Multiple tests needed for ID and DST

Classical molecular tests

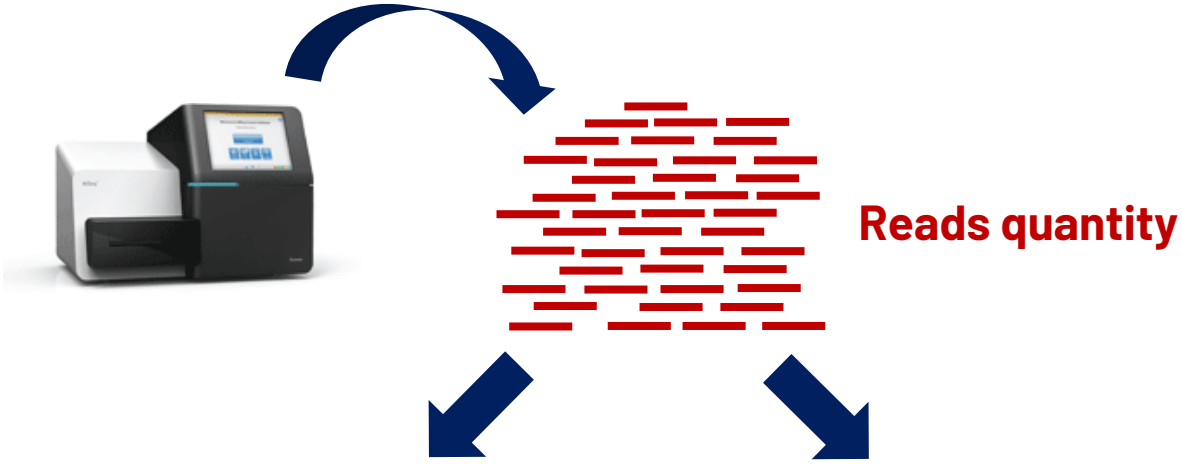


- Only few common variants in few genes

Targeted sequencing to detect antimicrobial resistance

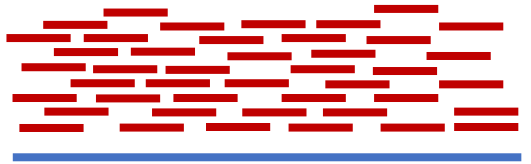


➔ **Custom panels of genes/targets**

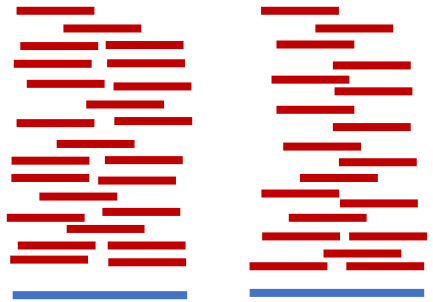


WGS
Lower coverage depth

Targeted sequencing
Higher coverage depth



Whole genome



Targets

Flexible & streamlined workflow

24 - 48h

For resistance surveillance

THE LANCET
Infectious Diseases

ARTICLES | VOLUME 18, ISSUE 12, P1350-1359, DECEMBER 01, 2018

Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study

Ndivhuho A Makhado, MSc • Edith Matabane, MSc • Mauro Faccin, PhD • Claire Pinçon, PhD • Agathe Jouet, PhD • Fairouz Boutachkourt, BSc • et al. Show all authors • Show footnotes

Retrospective screening of 249 isolates reported as INH-mono R from 4 South African provinces :

- **37 (15%) with *RpoB* I491F RIF R mutation missed by conventional tests, reclassifying them as MDR**
- Additional genotypic resistance to EMB and PZA
- Most with same genotype, also detected in e-Swatini isolates
- Multiple mutations in BDQ resistance associated target *Rv0678*
- Clonal outbreak confirmed by WGS, indicating positive selection on BDQ target

For routine diagnostics

Regional lab → NRL

13/10/21 — 20/10/21 — 17-19/11/21 — 26/11/21 — 02/12/21 — 10/01/22

HIV neg 48 y man
Born in Morocco

Cavitary pulmonary TB
100 AFB/field

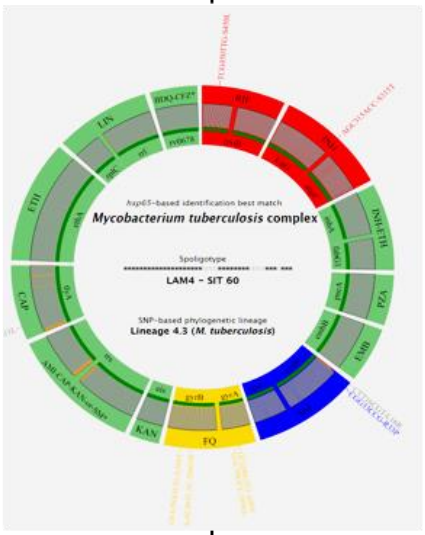
First line pDST

R: RIF, INH

Start of probabilistic treatment:
MXF-LNZ-BDQ-CYC-CFZ

Strain received

LPA test
MTBDRplus/MTBDRslv2:
R: RIF, INH
No *gyrA* or *gyrB* mutations detected



Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Confidence	PMID
gyrA	7570	GCG90GTG	10.1	87.50	A90V	FQ	High // High	ReSeqTb
gyrA	7582	GAC94GCC	14.9	160.75	D94A	FQ	High // High	ReSeqTb
gyrB	6620	GAC461CAC	9.7	566.75	D461H	FQ	High	
gyrB	6749	GCG504ACG	3.7	152.25	A504T	FQ	n/a	
katG	2155168	AGC315ACC	99.9	341.75	S315T	INH	High	ReSeqTb
rpoB	761155	TCG450TTG	99.9	4832.75	S450L	RIF	High	ReSeqTb

R: RIF, INH + FQ (hetero)
S: BDQ, CFZ, LNZ

Weekly National Consilium:
MXF replaced by DLM

pDST
Proportion method/MGIT:

FQ hetero R confirmed
(1%, 2µg/ml MXF)
& R: RIF, INH & SM R
All other drugs S

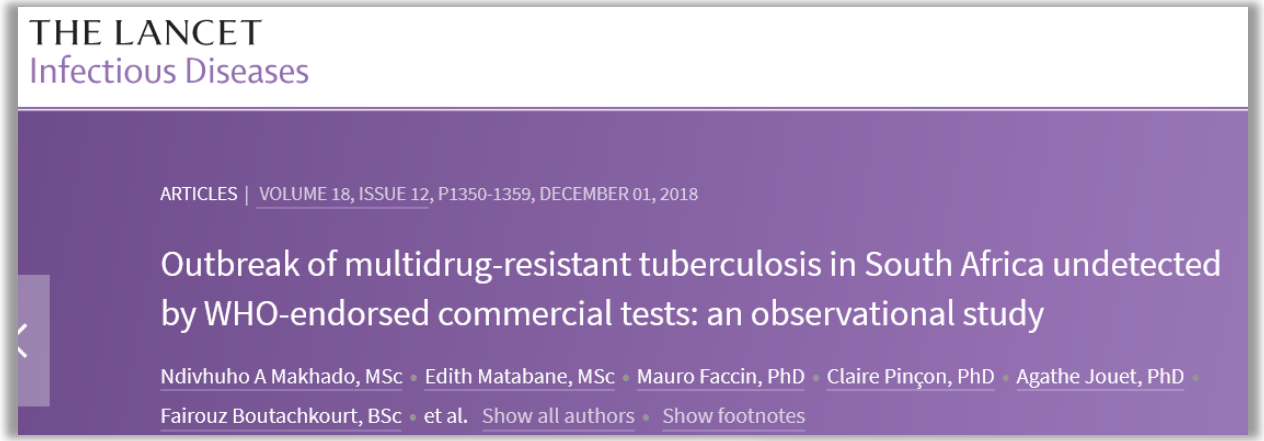
Adapted MDR-TB treatment informed 6 weeks before pDST results

PITIÉ SALPÉTRIÈRE NRL FRANCE
F. MOREL, A. AUBRY & TEAM

Track record

20+

PUBLICATIONS



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Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study

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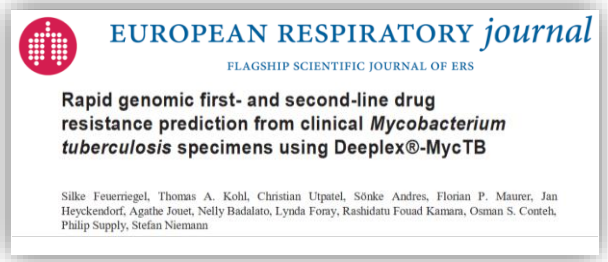
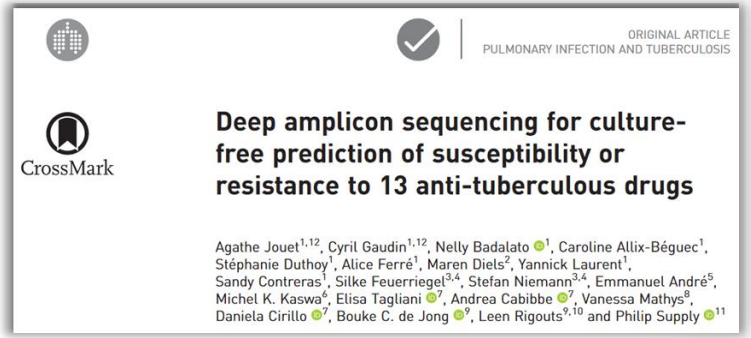


EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Rapid genomic first- and second-line drug resistance prediction from clinical *Mycobacterium tuberculosis* specimens using Deeplex®-MycTB

Silke Feuerriegel, Thomas A. Kohl, Christian Utpatel, Sönke Andres, Florian P. Maurer, Jan Heyckendorf, Agathe Jouet, Nelly Badalato, Lynda Foray, Rashidatu Fouad Kamara, Osman S. Conteh, Philip Supply, Stefan Niemann

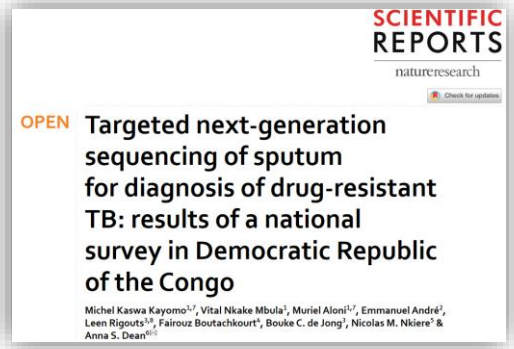


ORIGINAL ARTICLE
PULMONARY INFECTION AND TUBERCULOSIS

CrossMark

Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs

Agathe Jouet[1,12], Cyril Gaudin[1,12], Nelly Badalato[1], Caroline Allix-Béguec[1], Stéphanie Duthoy[1], Alice Ferré[1], Maren Diels[2], Yannick Laurent[1], Sandy Contreras[1], Silke Feuerriegel[3,4], Stefan Niemann[3,4], Emmanuel André[5], Michel K. Kaswa[6], Elisa Tagliani[7], Andrea Cabibbe[7], Vanessa Mathys[8], Daniela Cirillo[7], Bouke C. de Jong[9], Leen Rigouts[9,10] and Philip Supply[11]



SCIENTIFIC REPORTS
nature research

OPEN

Targeted next-generation sequencing of sputum for diagnosis of drug-resistant TB: results of a national survey in Democratic Republic of the Congo

Michel Kaswa Kayomo[1,7], Vital Nkake Mbula[1], Muriel Aloni[1,7], Emmanuel André[2], Leen Rigouts[3,8], Fairouz Boutachkourt[4], Bouke C. de Jong[2], Nicolas M. Nkiere[5] & Anna S. Dean[6]

Genome Medicine

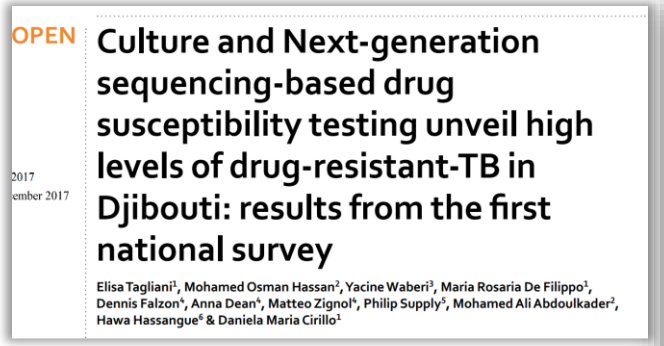
Rapid molecular diagnostics of tuberculosis resistance by targeted stool sequencing

Doctor B. Sibandze[1,2,3,4†], Alexander Kay[2,3*†], Viola Dreyer[5,6†], Welile Sikhondze[4,7†], Qiniso Dlamini[2,3], Andrew DiNardo[3], Godwin Mtetwa[2,3], Bhekumusa Lukhele[2,3], Debrah Vambe[7], Christoph Lange[3,6,8,9], Muyalo Glenn Dlamini[1], Tara Ness[3], Rojelio Mejia[10], Barbara Kalsdorf[6,8,9,11], Jan Heyckendorf[6,9,12], Martin Kuhns[13], Florian P. Maurer[6,13,14], Sindisiwe Dlamini[1], Gugu Maphalala[1], Stefan Niemann[5,6†] and Anna Mandalakas[3,6,8*†]

Int J Tuberc Lung Dis. 2021 Jan 1;25(1):43-51. doi: 10.5588/ijtld.20.0558.

First molecular-based anti-TB drug resistance survey in Eritrea

A B Mesfin [1], Z Z Araia [1], H N Beyene [1], A H Mebrahtu [1], N N Suud [2], Y M Berhane [2], D T Hailu [2], A Z Kassahun [3], O T Auguet [4], A S Dean [4], A M Cabibbe [5], D M Cirillo [5]

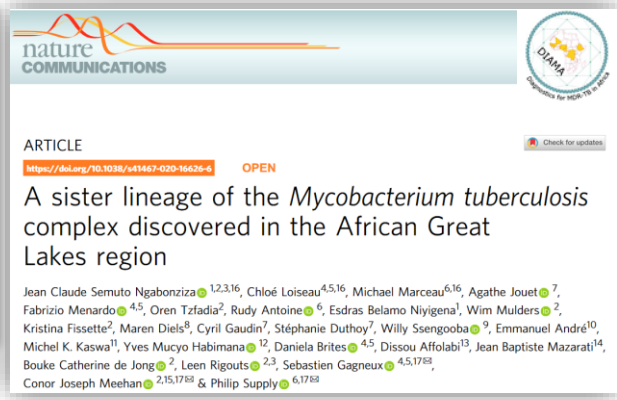


OPEN

2017
ember 2017

Culture and Next-generation sequencing-based drug susceptibility testing unveil high levels of drug-resistant-TB in Djibouti: results from the first national survey

Elisa Tagliani[1], Mohamed Osman Hassan[2], Yacine Waberi[3], Maria Rosaria De Filippo[1], Dennis Falzon[4], Anna Dean[4], Matteo Zignol[4], Philip Supply[5], Mohamed Ali Abdoulkader[2], Hawa Hassangue[6] & Daniela Maria Cirillo[1]



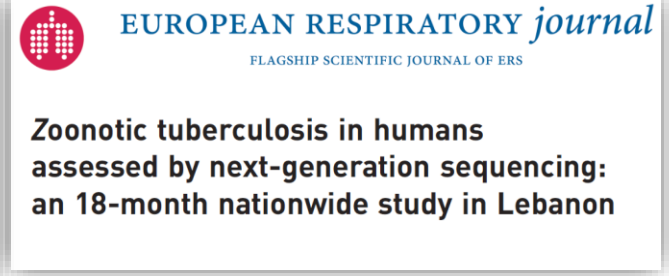
nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-16626-6 OPEN

A sister lineage of the *Mycobacterium tuberculosis* complex discovered in the African Great Lakes region

Jean Claude Semuto Ngabonziza[1,2,3,16], Chloé Loiseau[4,5,16], Michael Marceau[6,16], Agathe Jouet[7], Fabrizio Menardo[4,5], Oren Tzfadia[2], Rudy Antoine[6], Esdras Belamo Niyigena[1], Wim Mulders[2], Kristina Fissette[2], Maren Diels[8], Cyril Gaudin[7], Stéphanie Duthoy[7], Willy Ssengooba[9], Emmanuel André[10], Michel K. Kaswa[11], Yves Mucyo Habimana[12], Daniela Brites[4,5], Dissou Affolabi[13], Jean Baptiste Mazarati[14], Bouke Catherine de Jong[2], Leen Rigouts[2,3], Sebastien Gagneux[4,5,17✉], Conor Joseph Meehan[2,15,17✉] & Philip Supply[6,17✉]



EUROPEAN RESPIRATORY *journal*
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Zoonotic tuberculosis in humans assessed by next-generation sequencing: an 18-month nationwide study in Lebanon

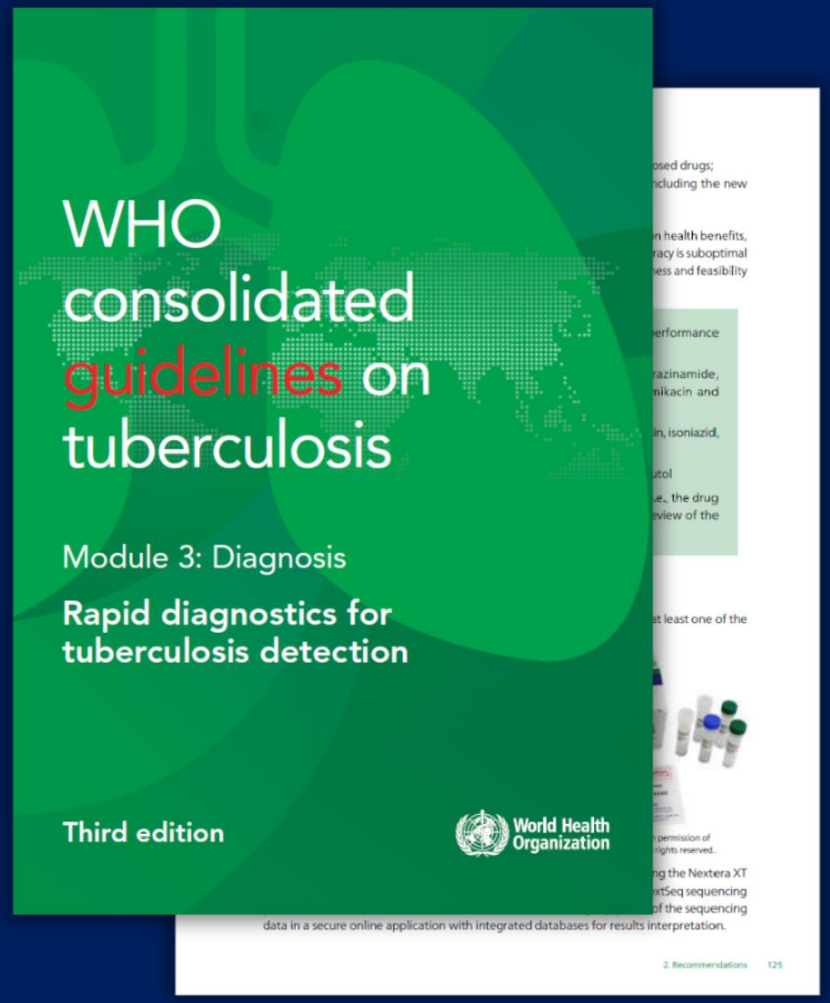
Independent evaluation

WHO endorsement

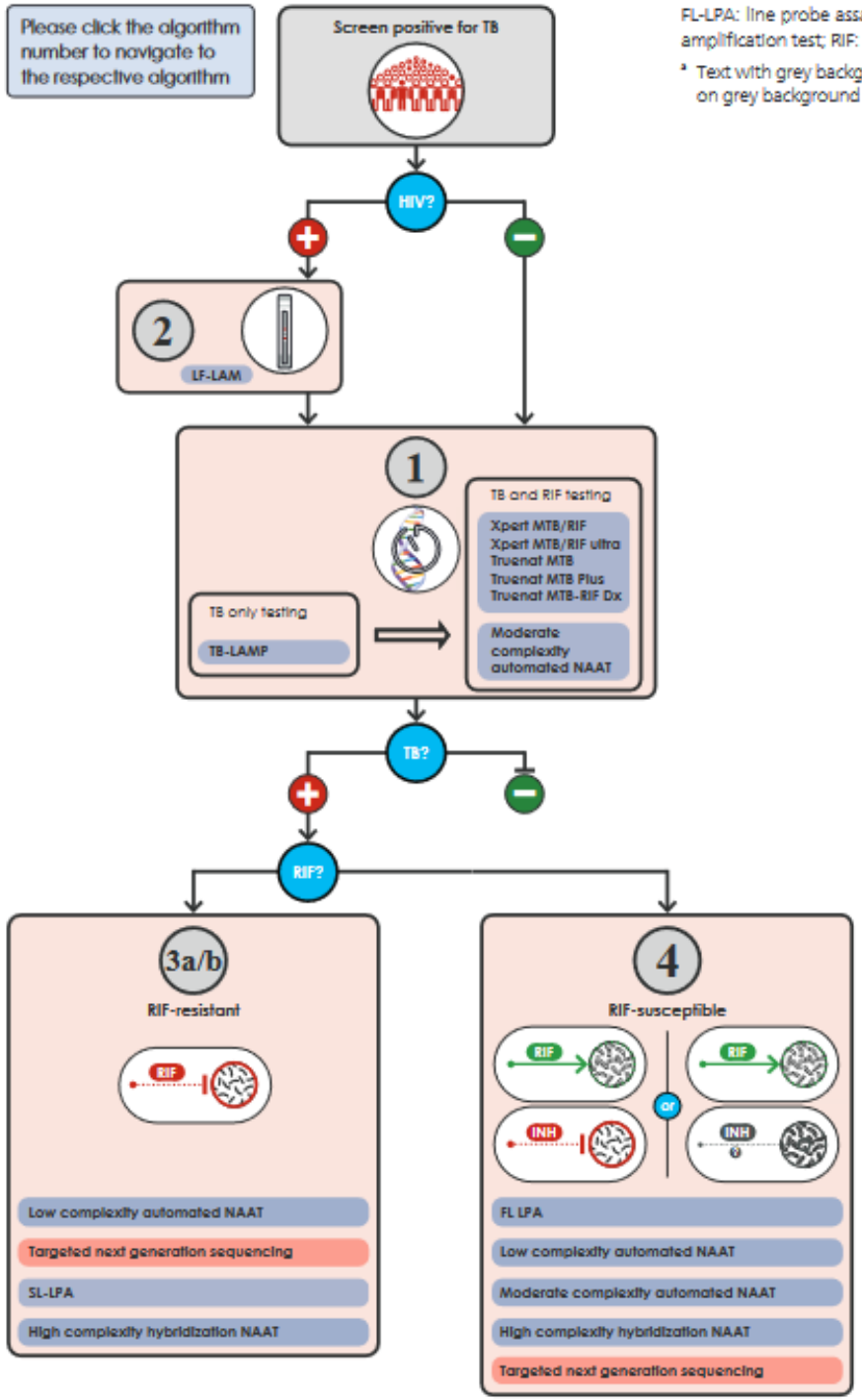
tNGS found to be:
- Accurate
- Cost-effective
- Acceptable and implementable under routine conditions

"Innovative diagnostic options for people with drug-resistant TB, such as targeted NGS, are increasing thanks to manufacturer engagement and research generating new evidence," said Dr Tereza Kasaeva, Director of WHO's Global TB Programme.

"Ensuring that everyone in need can obtain a rapid and accurate diagnosis of drug resistant TB will save lives and reduce suffering"

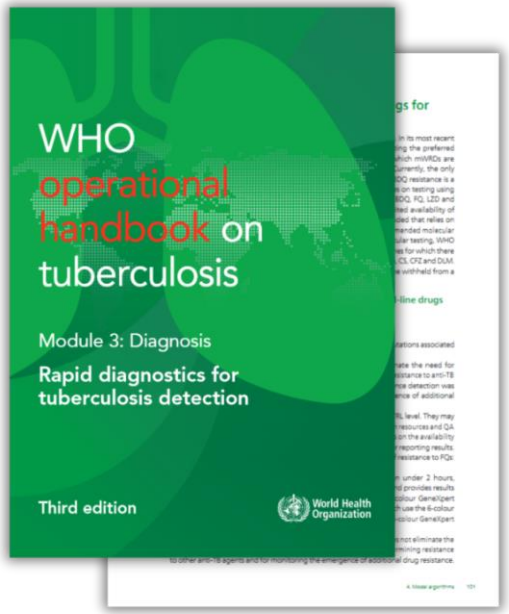


Model algorithms



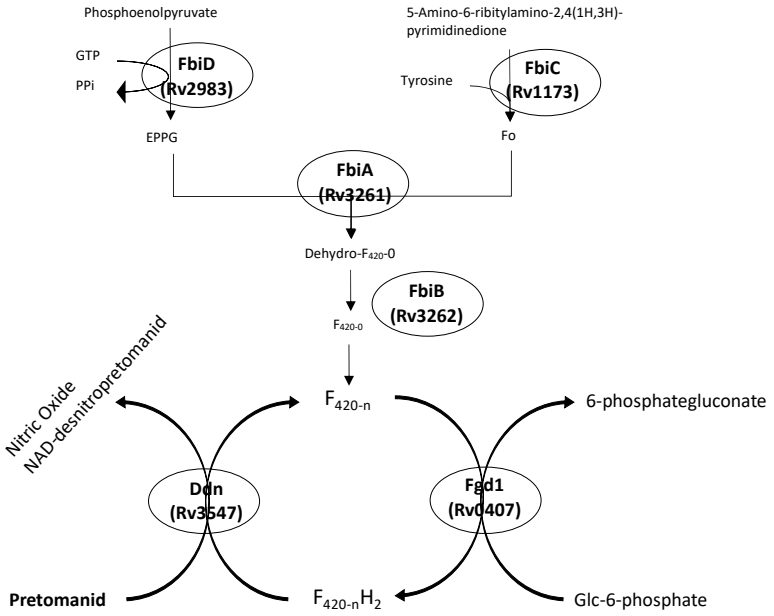
FL-LPA: line probe assay for first-line drugs; INH: isoniazid; LF-LAM: lateral flow lipoarabinomannan assay; NAAT: nucleic acid amplification test; RIF: rifampicin; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis.

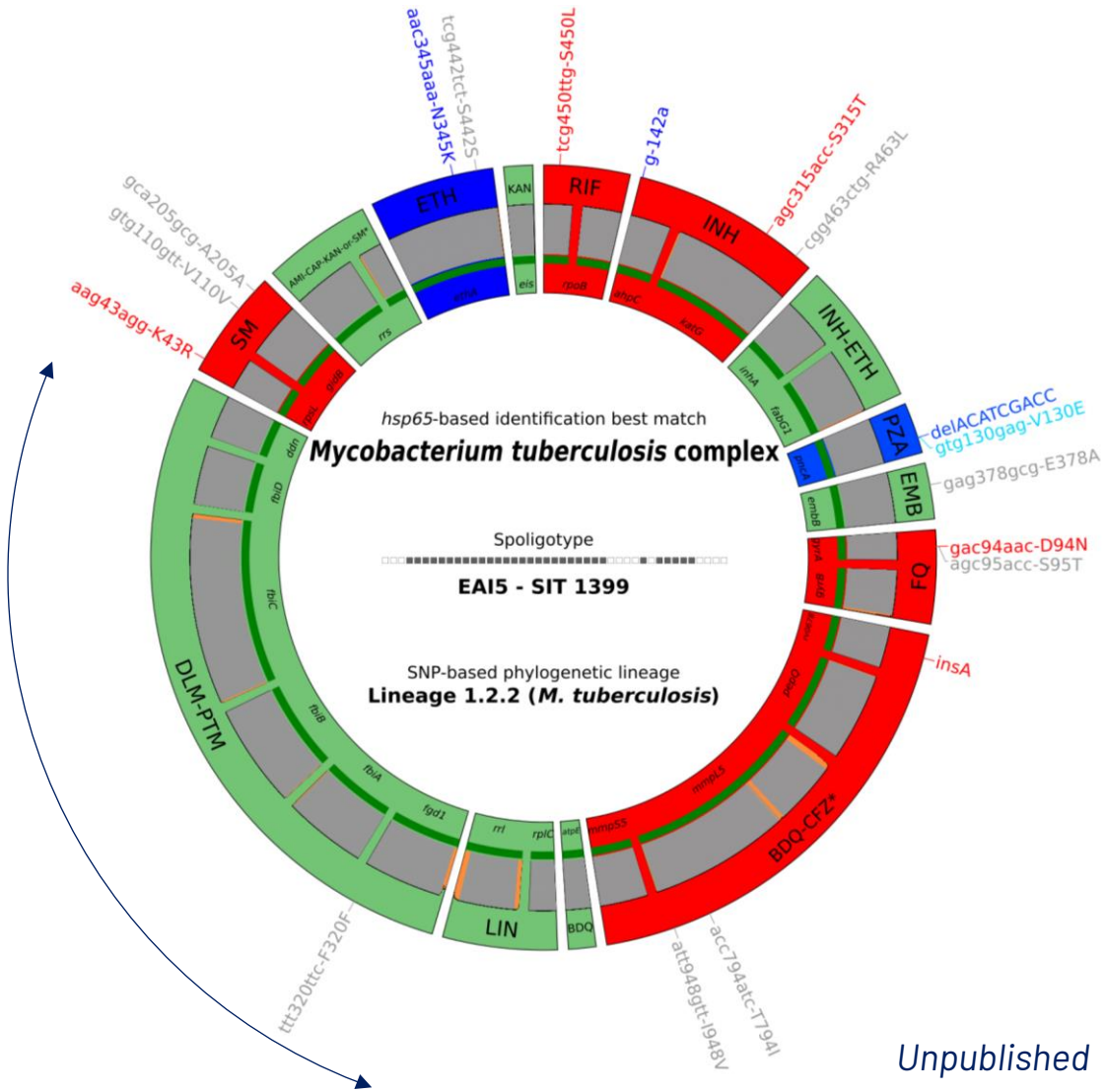
[a] Text with grey background: currently recommended tests, text with orange background: newly recommended tests. Numbers on grey background refer to the model algorithms.



tNGS as an adaptable technology

- Entire coding sequences of *ddn*, *fbiA-D* and *fgd1* plus (part of) promoter regions
- Mutation catalogue integrating knowledge on mode of action





Unpublished

Rapid and comprehensive knowledge of drug resistance / susceptibility is key to optimize treatment

Reflex test after TB/RR-MDR diagnosis

- Fast / reliable / comprehensive **drug resistance and susceptibility prediction**
- For **optimized treatment decision** guidance
- For **drug resistance surveillance**

Technology applicable to other **challenging pathogens** and **adaptable**

- *Mycobacterium leprae* and *Helicobacter pylori*
- New design for TB to include additional targets (new drugs)
- Evolving mutation catalogues