MOLECULAR DIAGNOSTICS:
EXPANDING ACCESS TO DRUG-RESISTANCE TUBERCULOSIS TESTING THROUGH CULTURE-FREE NGS

Andrea Cabibbe, PhD MGH

Research Associate,
Ospedale San Raffaele,
Tuberculosis Supranational Reference Laboratory in Milan

I.R.C.C.S. Ospedale San Raffaele

Novel diagnostics for infectious diseases, 25-26 March 2024, Royal Society, London

The need

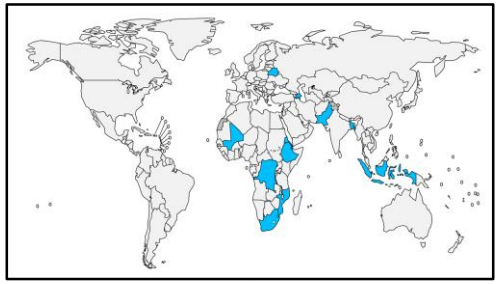
- **Only 2 in 5 people** with MDR/RR-TB were known to have been diagnosed and enrolled on treatment in 2021
- Recently the WHO endorsed the use of a novel all-oral 6-month **BPaLM** regimen in people suffering from MDR/RR-TB, including those with additional resistance to fluoroquinolones (pre-XDR-TB) > better outcomes and quality of life, shorter duration
- However, there is need to expand access to drug-resistance testing, including to the drugs that constitute the best available regimens recommended for drug-susceptible and drug-resistant TB
- The current WHO-recommended rapid diagnostics (WRDs) cannot detect resistance to all the drugs in these two types of regimens in a single test to inform treatment decisions

Increasing access to early and accurate diagnosis using a molecular WHO-recommended rapid diagnostic test

Global TB report 2023

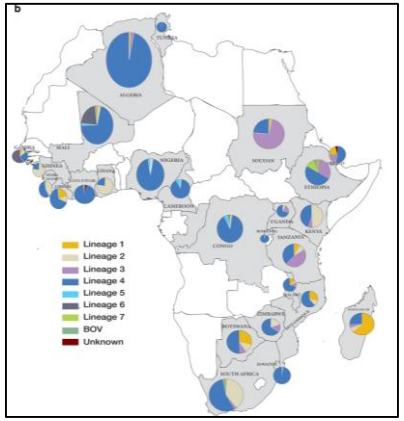
The solution (?)

- **Next-generation sequencing** is introduced as a cost-effective and comprehensive tool for DST, as well as offering additional valuable epidemiological information (WHO 2020)

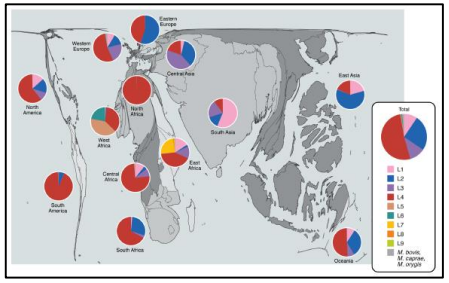


WHO

DR survey and surveillance



Laamarti, Sci Data 2023



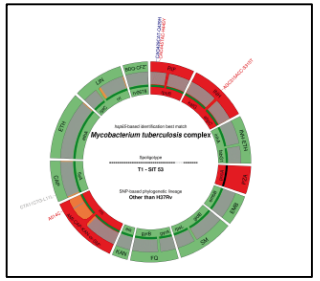
Koleske, J Clin Invest 2023

phylogenetic

evolution

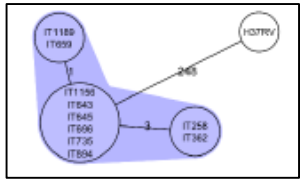
All-in one

DR-TB detection



Deeplex Myc-TB

outbreak investigation

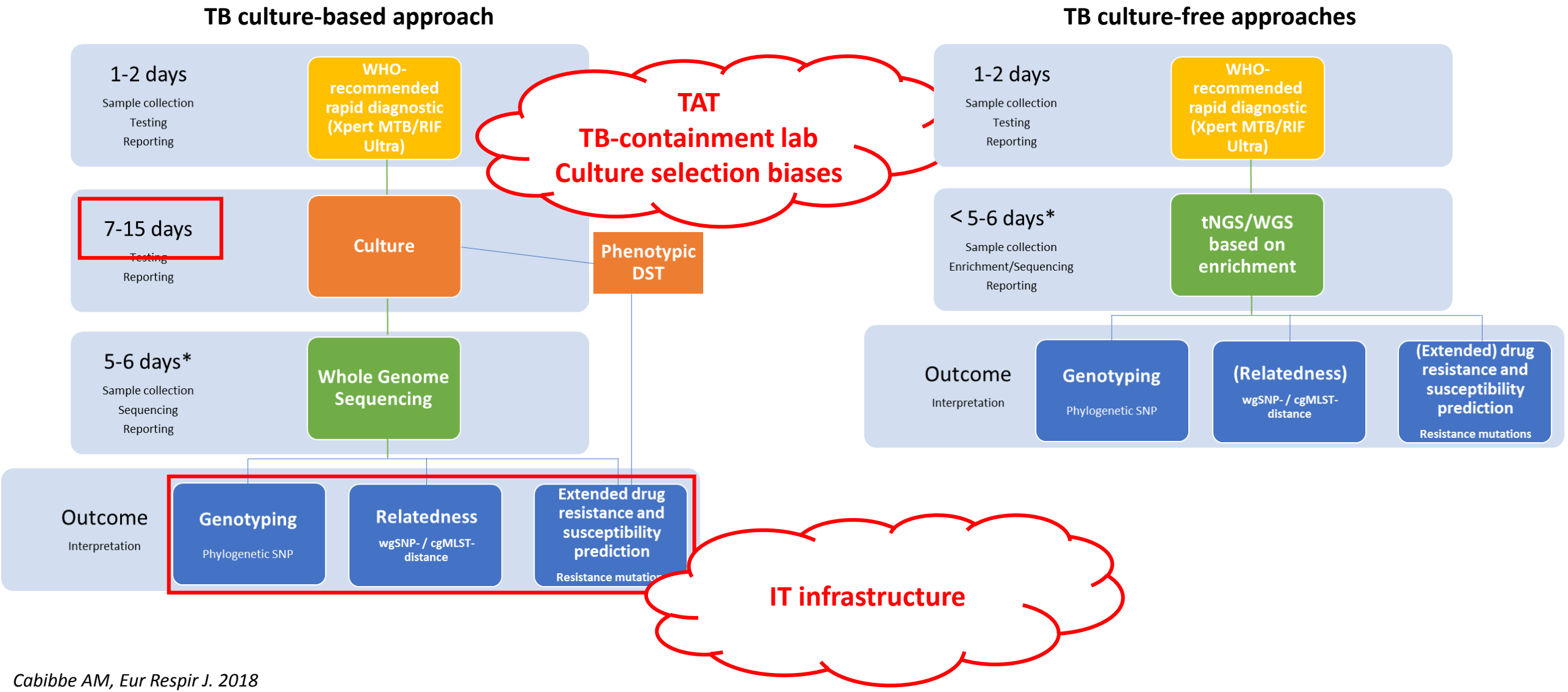


Ridom SeqSphere

Other: **research, validation of novel assays…**

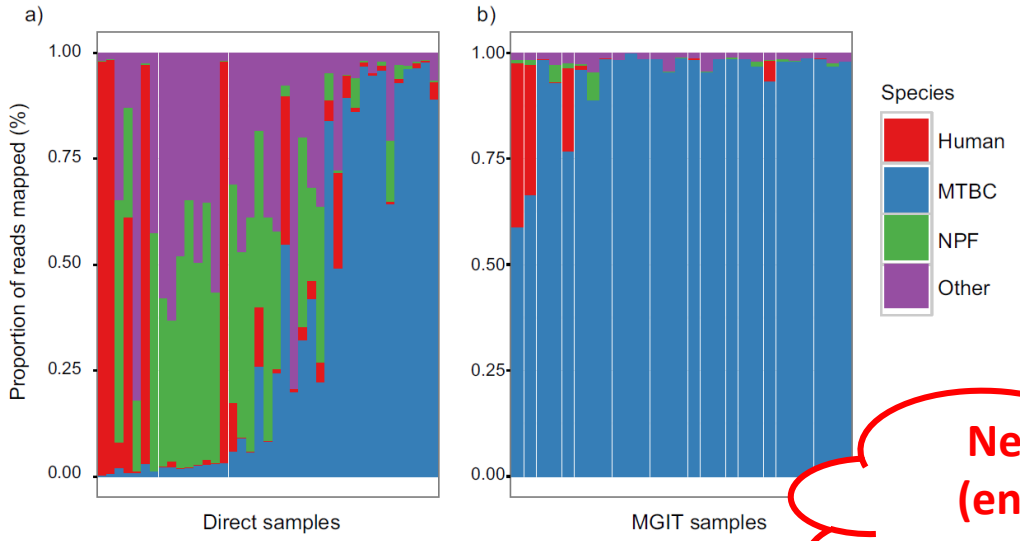
NGS tests offer great potential to provide comprehensive resistance detection matched to modern treatment regimens

The NGS-based workflows



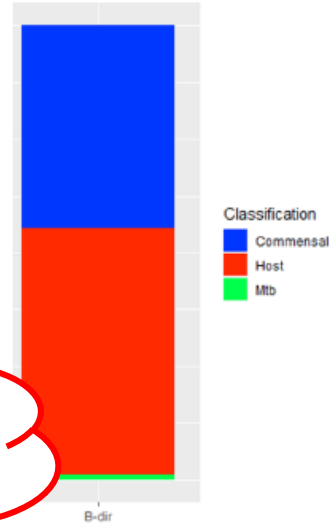
Cabibbe AM, Eur Respir J. 2018

The challenge for TB culture-free NGS approaches



Votintseva, J Clin Microbiol 2017

Need to enrich the (entire) genomes of target pathogens

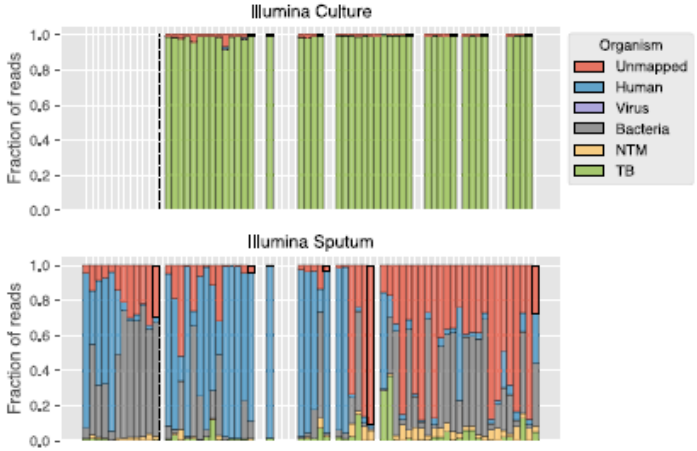


Mann, J Clin Microbiol 2023

Table 1 Sample characteristics and sequencing results.

Sample	ZN grade	DNA concentration in extract (µg/mL)	Total no. reads	% reads aligning to human genome
K1	3+	27.8	989,442	73.71
K2	3+	2.28	2,170,640	78.46
K3	2+	71	1,617,808	99.3
K4	2+	250	1,204,408	97.22
K5	2+	7.7	1,537,676	74.17
K6	2+	48.8	2,411,708	97.47
K7	1+	25	2,818,238	50.59
K8	1+	0.63	1,851,892	20.29

Doughty, PeerJ 2014

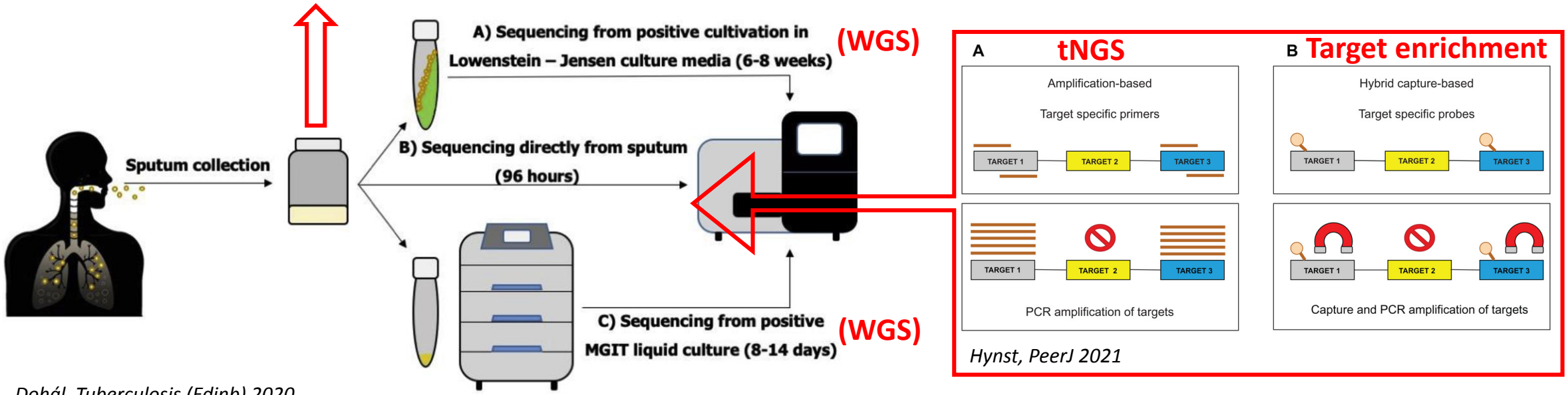


Nilgiriwala, J Clin Microbiol 2023

NGS enrichment approaches

Enrichment of MTB cells, e.g.:

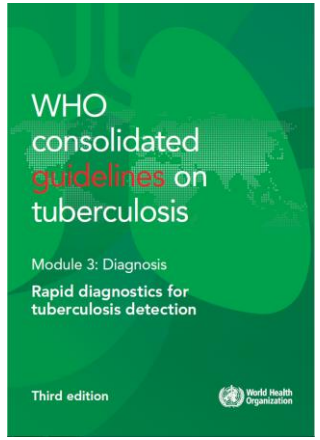
Antibody-based systems; Magnetic carriers with immobilized affinity ligands; capturing onto liquid or solid surface



Dohál, Tuberculosis (Edinb) 2020

tNGS (1)

- ✓ **Targeted next-generation sequencing was found to be accurate**
- ✓ **Targeted next-generation sequencing was found to be cost-effective depending on context**
- ✓ **Targeted next-generation sequencing was found to be acceptable and implementable under routine conditions, despite inherent complexity**

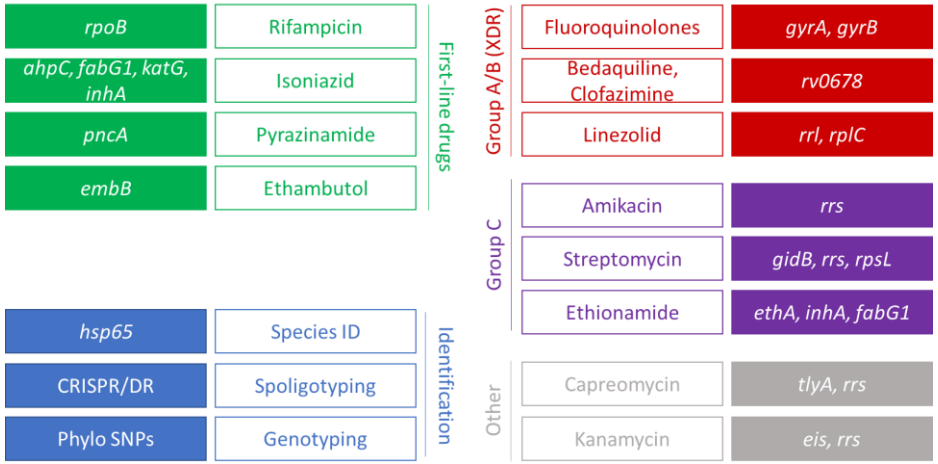


Among people with RR-TB: rifampicin, isoniazid, pyrazinamide, ethambutol, ***fluoroquinolones, bedaquiline, linezolid****, clofazimine, amikacin and streptomycin*

Tool (Developer)	Type	Accuracy*	Time to Result/ Instrument	Price	Status
Deeplex Myc-TB (GenoScreen, France)	tNGS: up to 15 drugs including BDQ & LZD	Sputum: SE: 93.1–98.5% SP: 95.3–98.5%[106] (WGS reference standard)	1-2 days	Test: $50[107]	WHO recommended in 2023[108]
DeepChek Assay 13-Plex KB DST (ABL Diagnostics, France)	tNGS: up to 13 drugs including BDQ	Sputum: Not available	29 hours[109]	Not available	Undergoing evaluation
NanoTB (Oxford Nanopore Technologies, UK)	tNGS: RIF, INH, FQ, AMK, LZD, STM	Sputum: RIF, INH, FQ SE: > 94% SP: > 99%[110]	< 24 hours	Not available	WHO-recommended in 2023
TBSeq (ShengTing Biotech, China)	tNGS: up to 16 drugs	Sputum: SE: 94.8% SP: 97.9%[111]	1-2 days	Not available	WHO-recommended in 2023
CleanPlex (Paragon Genomics, USA)	tNGS: Not available	Sputum: Not available	1-2 days	Not available	Undergoing evaluation
Tuberculini (Clemedi Deutschland GmbH, Germany)	tNGS: up to 12 drugs	Sputum: SE: 84% SP: 95%[112]	< 24 hours	Not available	Undergoing evaluation

WHO recommended

Pipeline Report 2023 (Treatment Action Group)



Deeplex Myc-TB

- ✓ Priority to Smear positive; Xpert medium/high
- ✓ Heteroresistance < 5-10%

tNGS (2)

Strengths	Weaknesses
❖ Multi-purpose, multi-disease	❖ Need of genotypic-phenotypic associations
❖ Suitable from various sample types	❖ Turnaround time depends on sample referral and sequencing capacity/multiplexing
❖ Rapid (faster turnaround times than conventional pDST testing)	❖ Start-up costs
❖ Kit-based and user-friendly analysis tools (improved standardization)	❖ Currently not feasible at peripheral level
❖ Deep level of genetic information enabling "precision"	❖ Procurement and supply chain
	❖ Need of specialized and trained personnel

Opportunities	Threats
❖ Less phenotyping in routine testing	❖ Borderline mutations
❖ High predictive value for drug-resistance	❖ Confidence-grading of mutations requires large and representative datasets
❖ Huge research on innovative NGS technologies	❖ Support to clinicians
❖ Development of lists of confidence-graded mutations reflecting on routine Nucleic Acid Amplification Tests	❖ Not all resistance mechanisms can be explored (e.g., gene expression, structural changes)
❖ Interrogates resistance to additional anti-microbials not routinely tested in national algorithms	❖ Information technology (IT) infrastructure
❖ Research outcomes	❖ Cost-effectiveness to be demonstrated
	❖ Efficient and timely results reporting
	❖ To achieve sustainability

This table provides a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of the use of genomics in TB control and care. The SWOT analysis helps to identify strategies to maximize the advantages and mitigate the disadvantages of having tNGS capacity for TB implemented.

de Araujo, Cabibbe, Mhuulu, Front. Public Health. 2023

Target enrichment (1)

Doughty, PeerJ 2014. Differential osmotyc lysis of human cells and shotgun metagenomics

Brown, J Clin Microbiol. 2015. Biotinylated RNA baits (SureSelectXT)

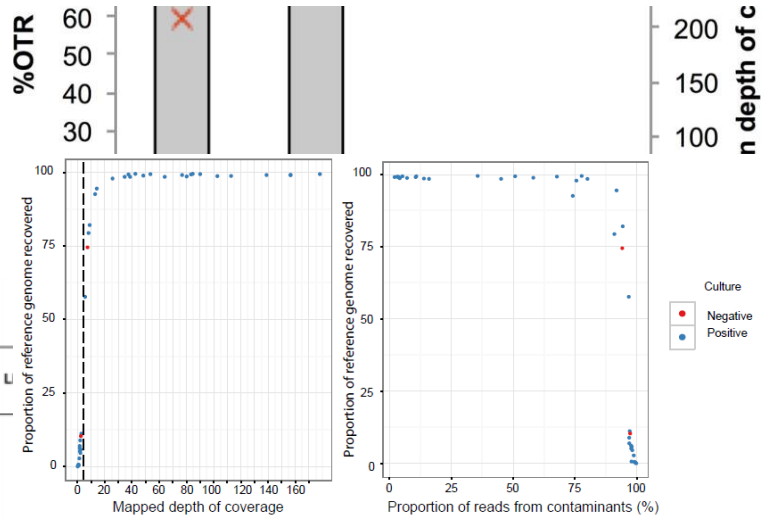
Votintseva, J Clin Microbiol. 2017. Removal of eukaryote DNA and other inhibitors, and direct WGS

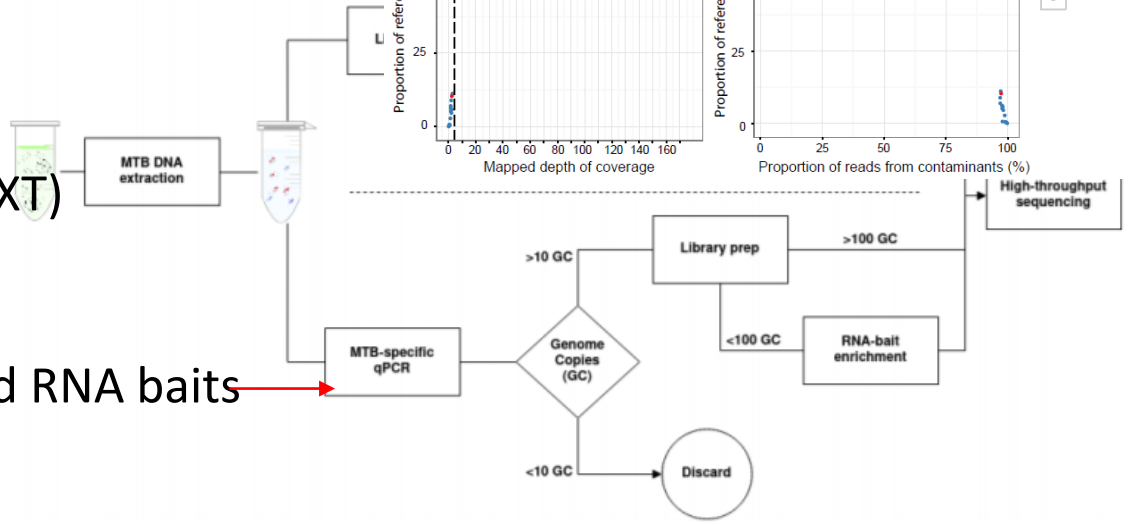
Nimmo, Int J Infect Dis. 2017. Biotinylated RNA baits (SureSelectXT)

Goig, Lancet Micr 2020. Combined differential lysis + biotinylated RNA baits (myBaits)

Soundararajan, Tube 2020. Mtb-specific tiling oligonucleotide probes

Sample	Under relaxed mapping conditions		
	Bases aligning to H37Rv	Coverage of H37Rv	Average read depth
K1	410,228	0.093	2.2
K2	5,685,901	1.289	2.3
K3	99,643	0.023	1.3
K4	40,019	0.009	1.9
K5	732,623	0.166	2.5
K6	94,023	0.021	2.3
K7	1,366,309	0.310	11.4
K8	1,725,816	0.391	7.7

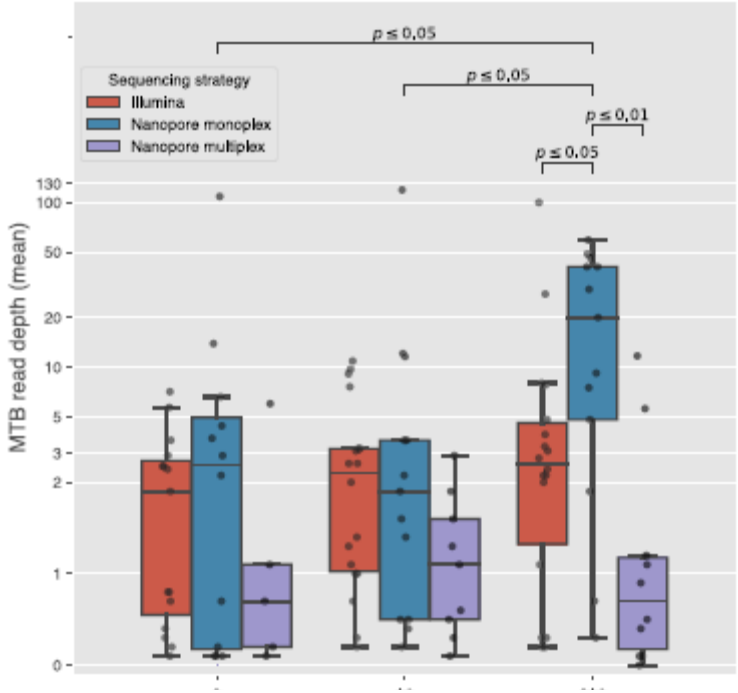




Target enrichment (2)

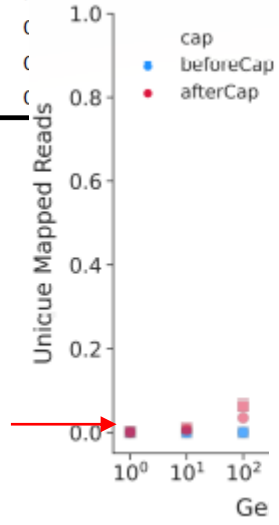
George, J Clin Microbiol. 2020. DNA thermo-protection buffer during heat inactivation and direct WGS.

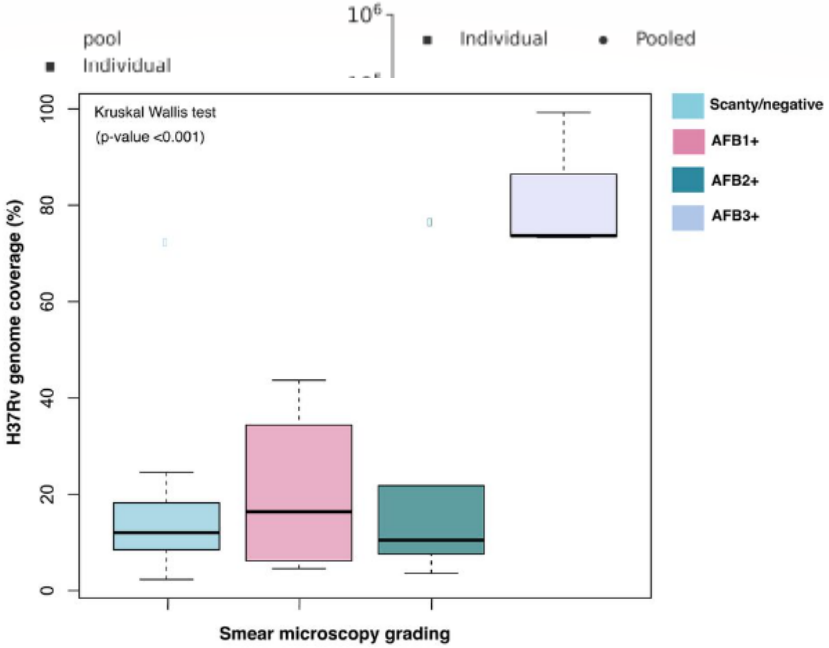
Nilgiriwala, J Clin Microbiol. 2023. DNA thermo-protection buffer and direct WGS on Illumina or ONT.



Mann, J Clin Microbiol. 2023. Benzonase/DNase and Twist target capture probes

Treatment	Mtb copy number/ng	Mtb treated vs control *P* value	Host copy number/ng	Host treated vs control *P* value	Mtb DNA:host DNA ratio
Control	212.3 ± 59.4	NA[b]	6,168 ± 1,638	NA	1:29
Wash	3,151 ± 1,369	(			
DNase	2,862 ± 733.4	(			
Benzonase	11,721 ± 7,096	(			





Sundararaman, mBio 2023: Circular Nucleic acid Enrichment Reagent synthesis (DNA baits)

Vasanthaiah, preprint 2024. TB-Bead kit and direct WGS

Take-home messages

Critical steps for direct MTB WGS:

- Sputum / sediment sample input (ideally the whole volume)
- Selection / enrichment of bacterial cells (ideally MTB) over other population (e.g. human) present in the direct sample
- Selection / enrichment of bacterial DNA (ideally MTB) in the direct sample (recovery rate), quality of extracted MTB DNA (absence of inhibitors for PCR or enzymatic reactions of library prep protocols)
- Handling: number of steps / automation / costs

Current stage:

✓ *tNGS is standardized (WHO approved commercial kits) but doesn't enable extended analyses*

✓ *Enrichment of whole genomes would enable higher resolution: however, lenghty and expensive procedures; genome coverage not yet good enough for clinical deployment*