# WHO recommended tNGS, its application at the diagnostic algorithm and implementation consideration

.

Alexei Korobitsyn
Diagnostic Team, GTB

World Health Organization

# Pipeline of TB diagnostics/WHO guidelines

2010

2015

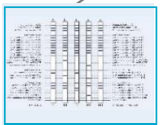
TB LAM RDT (Alere)

MTB/RIF(Gene Xpert; Cepheid)

2016

MTB/RIF Ultra (GeneXpert; Cepheid) *

1st line drugs LPA – TB (2nd gen) (Hain Lifescience; NIPRO Corporation)



2nd line drugs LPA – TB (Hain Lifescience)

2017

TrueNat TB & RIF (Molbio) *

TB LAMP (EIKEN)

2018

2019

Stool processing solutions

Centralized assays TB, INH & RIF (Roche, BD, Hain, Abbott)

2020

New IGRAs (Qiagen, Wantai)

MTB/XDR 10 color system (GeneXpert; Cepheid)

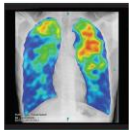
2021

Genoscholar PZA-TB (Nipro)

2022

TB antigen based skin tests (Cy-TB, C-TST, Diaskintest) *

CAD solutions fpr CXR interpretation



2023

Targeted Sequencing

Low complexity NAATs (Xpert, TrueNAt)

2024

Other samples: Oral swabs

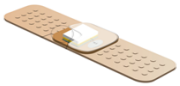
New cartridge-based technologies

2025+

New sampling approaches

Absorbent strips in face masks

Breath tests & skin patches



Ultra sensitive LAM

# Comparison of different sequencing approaches

rpoB RRDR hotspot 81bp

rpoB gene >3500bp

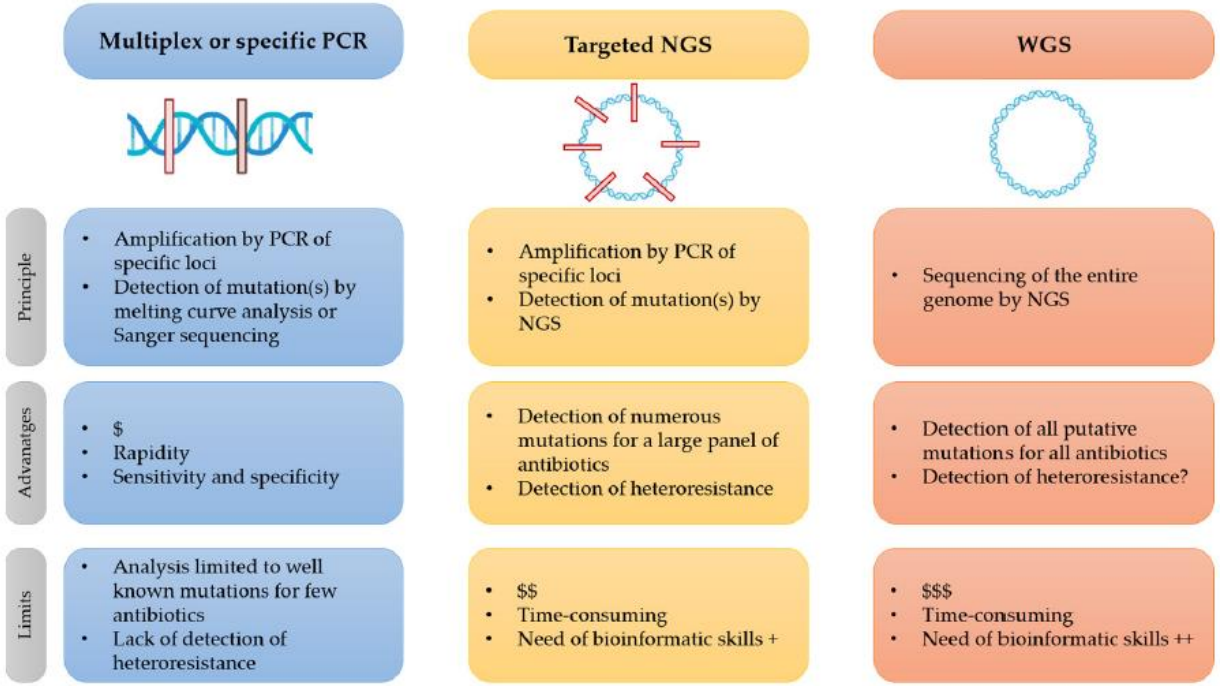



Figure 1. Advantages and limits of molecular techniques for detection of resistance mutations in MTB.

Mtb genome > 4 million bp

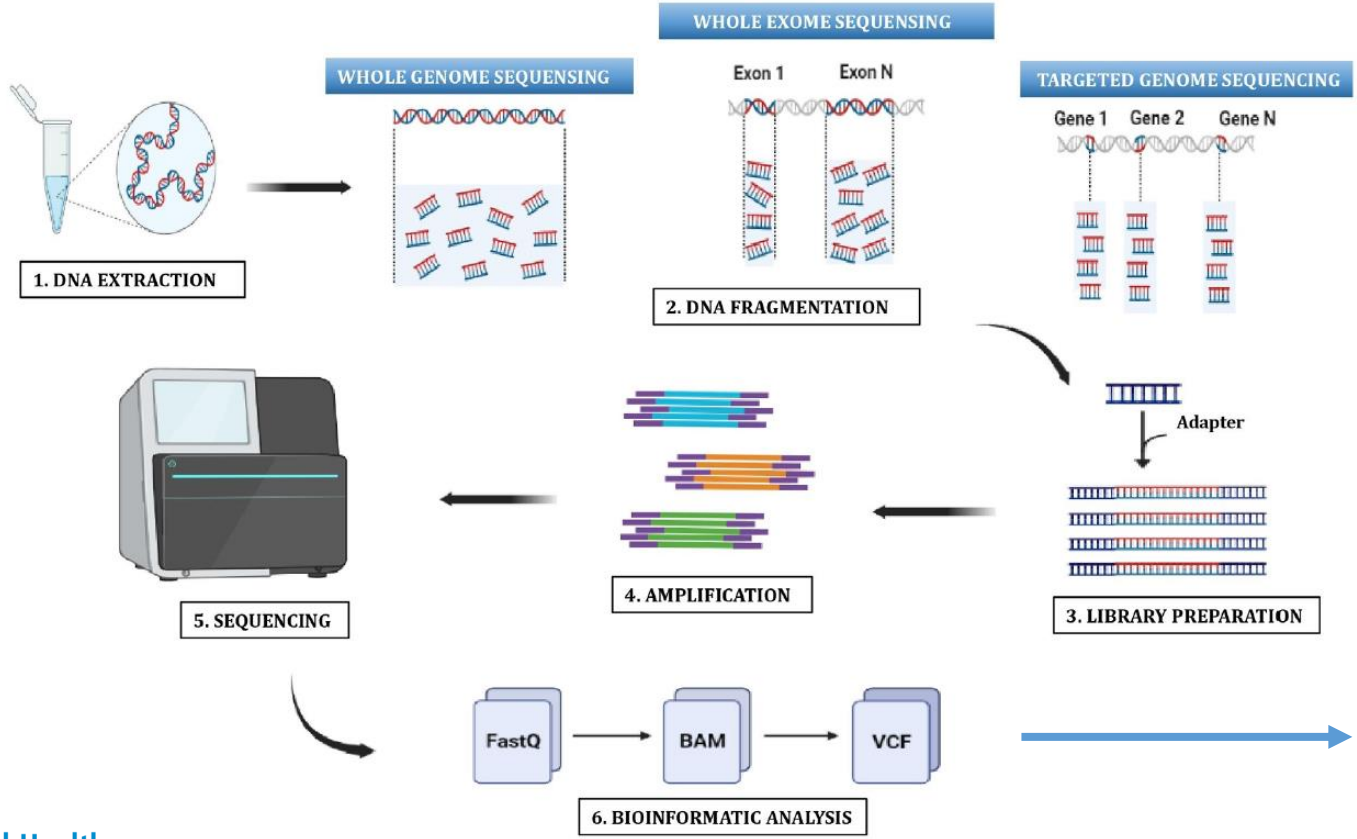
World Health Organization

Beviere, et al. The Role of Next-Generation Sequencing (NGS) in the Management of Tuberculosis: Practical Review for Implementation -in Routine. Pathogens **2023**, 12, 978. https://doi.org/10.3390/pathogens12080978

# Sequencing process

- Depth of coverage
- Width of coverage

NB input DNA quantity critical





World Health Organization

Satam, et al. Next-Generation Sequencing Technology: Current Trends and Advancements. Biology **2023**, 12, 997. https://doi.org/10.3390/biology12070997

# Class of targeted NGS products

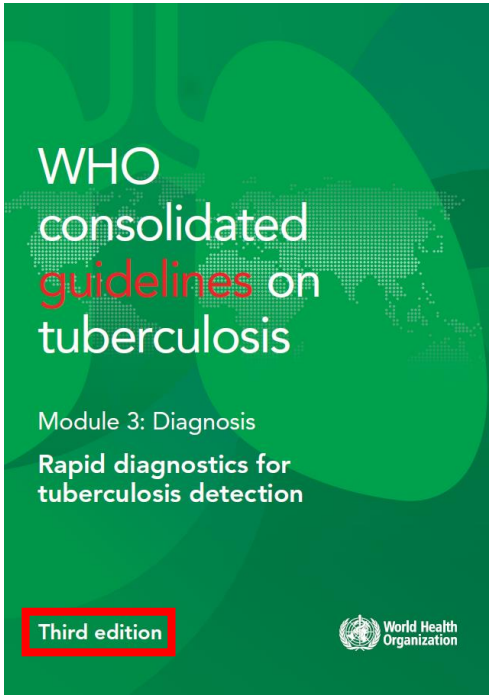
- Uses massively parallel sequencing to detect resistance to TB drugs
- Starting from a processed clinical sample
- Ending with an end-user report that relates detected *M.tb* mutations to the presence (or absence) of drug resistance, based on the interpretation of a standard catalogue of mutations.

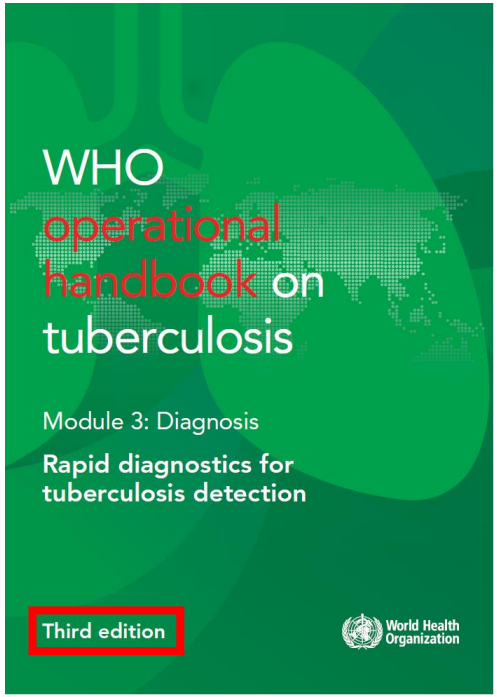
# WHO GDG process

- In 2022, WHO has commissioned a series of systematic reviews of published and unpublished data on the class of targeted NGS products that are commercially developed for TB drug resistance detection.
- The systematic reviews included data on diagnostic accuracy, economic information, and qualitative evidence on feasibility, acceptability, equity and end-user values and preferences.
- WHO convened a GDG on 2–5 May 2023 to discuss the findings of the systematic reviews and make recommendations on this technology.
- 25th July WHO has issued rapid communication including early results of the process
- Issue of full recommendations: March 2024

World Health Organization

# WHO guidelines and handbook on diagnostics for TB




World Health Organization

https://www.who.int/publications/i/item/9789240089488

# Products included in the evaluation

- **Deeplex® Myc-TB** (GenoScreen): for rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin.
- **AmPORE-TB®** (Oxford Nanopore Technologies): for rifampicin, isoniazid, fluoroquinolones, linezolid, amikacin and streptomycin.
- **TBseq®** (ShengTing Biotech): for ethambutol.

# The test performance among people with RR- TB

- Accurate for isoniazid, levofloxacin, moxifloxacin, pyrazinamide and ethambutol (pooled sensitivity ≥ 95%)
- Acceptable for bedaquiline (68%), linezolid (69%), clofazimine (70%), amikacin (87%) and pyrazinamide (90%)
- The specificity was ≥ 95% for all drugs except streptomycin (75%)

# Recommendations (bacteriologically confirmed TB)

In people with bacteriologically confirmed PTB, targeted NGS technologies may be used on respiratory samples to diagnose resistance to RIF, INH, Fq, Z and E rather than culture-based pDST. *(Conditional recommendation, certainty of evidence moderate [INH and Z], low [RIF, Fq and E])*

**Remarks**

- Priority should be assigned to those at higher risk of resistance to first-line treatment medications, including individuals who:
  - continue to be smear or culture positive after 2 or more months of treatment, or experience treatment failure;
  - have previously had TB treatment,
  - are in contact with a person known to have resistance to TB drugs; or
  - reside in settings or belong to subgroups where there is a high probability of resistance to either RIF, INH or FQ (used in new shorter regimens), or where there is a high prevalence of *M. tb* strains harbouring mutations not detected by other rapid molecular tests.

# Recommendations (rifampicin-resistant TB)

In people with bacteriologically confirmed RR PTB, targeted NGS technologies may be used on respiratory samples to diagnose resistance to INH, Fq, Bdq, Lzl, Cfz, Z, E, Amk and S rather than culture-based pDST. *(Conditional recommendation, certainty of evidence high [INH, Fq and Z], moderate [E], low [Bdq, Lzl, Cfz and S], very low [Amk])*

**Remarks**

- Priority should be given to those at a higher risk of resistance to medications used for the treatment of RR-TB, including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment or have experienced treatment failure;
  - have previously had TB treatment, including with the new and repurposed drugs;
  - are in contact with a person known to have resistance to TB drugs, including the new and repurposed drugs; or
  - have pre-XDR-TB with resistance to fluoroquinolones.

# Implementation considerations (general)

- **Regulatory approval** from national regulatory authorities or other relevant bodies is required before implementation of these diagnostic tests.
- In its current format, targeted NGS is a high-complexity test most suitable for centralized laboratories equipped with **specialised skills and infrastructure**.
- Targeted NGS tests **do not replace existing rapid tests that are more accessible and easier to perform** for detecting resistance to **RIF, INH and FQ**.
  - However, if targeted NGS can be performed rapidly, it can be considered as an alternative initial option for prioritized populations.
- Priority should be given to samples with a **high bacillary load** as determined by initial bacteriological tests (e.g. semi-quantitative high/medium or smear-positive grading).
  - In situations where the bacillary load is low (e.g. semi-quantitative low/very low/trace or smear-negative grading), the recommendations still hold, although rates of indeterminate results are likely to be higher;
  - therefore, pDST is likely still required for samples with a low bacillary load.

# Implementation considerations (BDQ, LZD, CFZ)

Since sensitivity for BDQ, LZD and CFZ resistance is **suboptimal**, consideration of the **pretest probability** is important in interpreting the targeted NGS results for these drugs. Further testing of samples with a susceptible result (using culture-based pDST) would be warranted, particularly when the risk of resistance is high. Since specificity is high, a result that indicates resistance may be used to guide the therapy, particularly among those at risk for resistance.

**Low pre-test prob**

- **Negative predictive value good**

**High pre-test prob**

- **Positive predictive value is good**

**Table 65**: Should TNGS be used to diagnose drug resistance to bedaquiline (BDQ) (pDST) in patients with bacteriologically confirmed rifampin-resistant pulmonary TB disease?

| | |
|---|---|
| Sensitivity | 0.68 (95% CI: 0.43 to 0.93) |
| Specificity | 0.97 (95% CI: 0.94 to 1.00) |

| Prevalences | 1% | 3% | 5% |
|---|---|---|---|

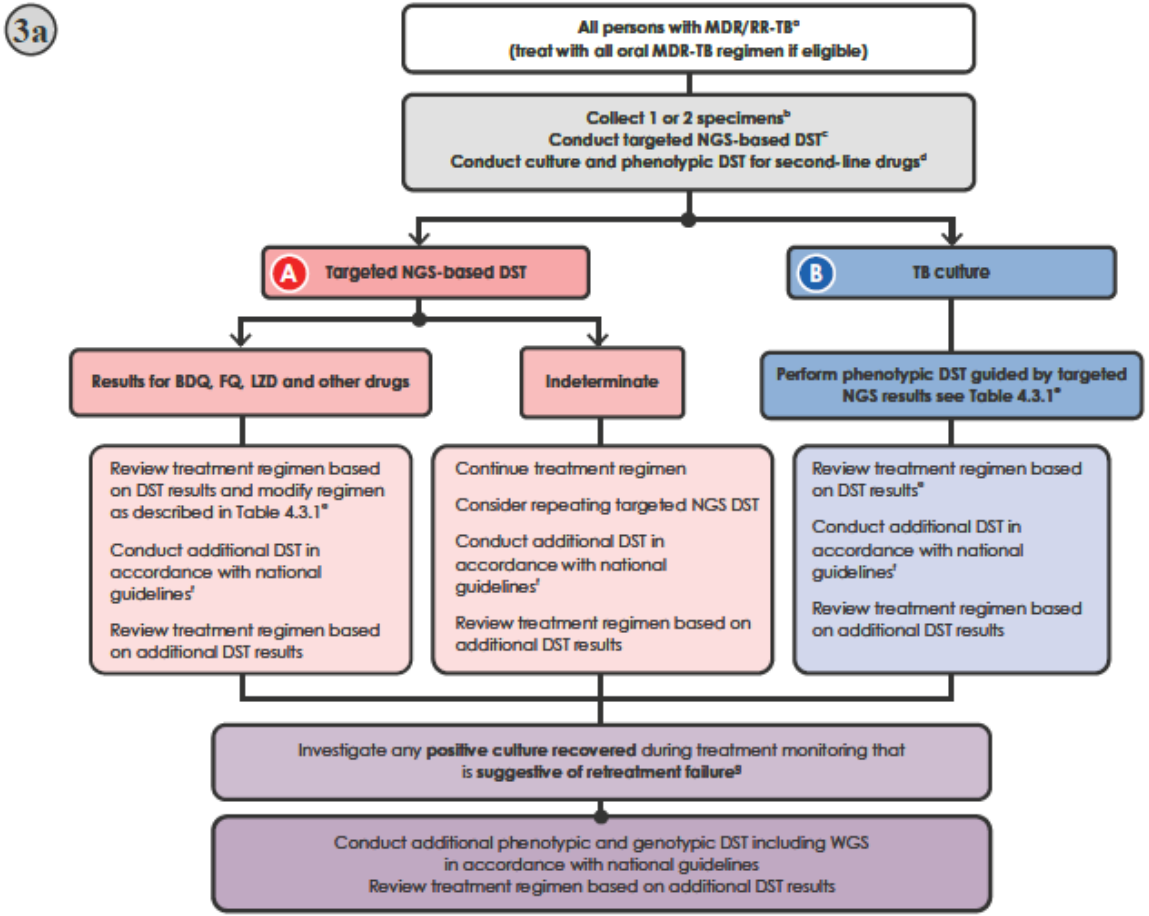
| Outcome | № of studies (№ of patients) | Study design | Factors that may decrease certainty of evidence | | | | | Effect per 1,000 patients tested | | | Te accur Co |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Risk of bias | Indirectness | Inconsistency | Imprecision | Publication bias | pre-test probability of1% | pre-test probability of3% | pre-test probability of5% | |
| **True positives** (patients with drug resistance to bedaquiline (BDQ) (pDST)) | 3 studies 31 patients[a] | cross-sectional (cohort type accuracy study) | not serious[b] | not serious | not serious[c] | very serious[d] | none | 7 (4 to 9) | 20 (13 to 28) | 34 (22 to 47) | ⊕⊕◯ Lo |
| **False negatives** (patients incorrectly classified as not having drug resistance to bedaquiline (BDQ) (pDST)) | | | | | | | | 3 (1 to 6) | 10 (2 to 17) | 16 (3 to 28) | |
| **True negatives** (patients without drug resistance to bedaquiline (BDQ) (pDST)) | 4 studies 519 patients[e] | cross-sectional (cohort type accuracy study) | not serious[b] | not serious | not serious | not serious | none | 960 (931 to 990) | 941 (912 to 970) | 922 (893 to 950) | ⊕⊕ Hig |
| **False positives** (patients incorrectly classified as having drug resistance to bedaquiline (BDQ) (pDST)) | | | | | | | | 30 (0 to 59) | 29 (0 to 58) | 28 (0 to 57) | |

**b. Explanations**

a. This model is not controlled for CT value as that variable was collinear in the original model
b. Prevalence of resistance to bedaquiline across data used in the model was 6% (CI 4% to 8%)
c. One study had very low sensitivity but it only had 3 resistant samples. It identified 0/3.
d. Very wide 95% confidence intervals for sensitivity
e. This model is not controlled for rifampicin resistance as this variable was collinear in the original model. Instead, the data have been restricted to isolated that are resistant to rifampicin by Xpert, and then controlled for CT value.

World Health Organization

# Updated diagnostic algorithm using tNGS



# Conclusion

- The new recommendations on targeted NGS marks a major shift in the diagnosis of drug-resistant TB
  - Addresses important gaps in the diagnosis of new and repurposed drugs despite the less optimal performance
- The cost and feasibility are potential barriers but offer important opportunities post-Covid
  - Many countries have sequencers available and have experience
- The best implementation approach is yet to be defined but will likely be context-dependent
- More evidence is needed on the new drugs and the impact of targeted NGS on patient important outcomes

# Acknowledgements


- WHO Global TB Programme
  - Nazir Ismail,
  - Alexei Korobitsyn,
  - Carl-Michael Nathanson
- Other WHO staff at HQ, Regions and Country offices
- Guideline Development Group & other experts
- Staff of national TB programmes in Member States
- USAID, Unitaid, The Global Fund

# Thank you


World Health Organization