

LabCoP EXTENDED ECHO SESSION

Closing the gap in drug-resistant TB diagnosis: the critical role of sequencing

Presenter: Anita Suresh, Head of Genomics & Sequencing Unit, FIND

Date: 22 May 2025

tNGS overview

1. With this modern method of diagnosing TB patients, is there still a need to use microscopy?

A: The method discussed, tNGS is primarily for diagnosis of drug resistance following TB diagnosis. Smear microscopy is still being used as an initial diagnostic test to detect acid-fast bacilli including TB in high-burden, low-resource settings due to its low cost and high access, but it has low sensitivity (especially when bacterial load is less than 10,000 organisms/ml sputum sample), and is being replaced or at least confirmed by molecular TB testing wherever feasible and affordable.

2. May I ask what is the sensitivity and specificity of genomic sequencing to specimens from HIV patients coinfecting with TB?

A: The Seq&Treat study did not have sensitivity/specificity results stratified by HIV status. From a sequencing failure rate standpoint, amongst participants with HIV, 80-85% of samples had successful sequencing results for drug resistance. While sample sequencing *failures* appear dependent on bacterial load (lower the load, more likely failure to sequence), the *accuracy* of drug resistance prediction, when sequence results are obtained, is likely not associated with bacterial load in the same way that failure is - if sequence results are obtained, they are highly accurate across diverse bacterial loads.

3. Are there any reservations with tNGS testing for children? I am looking forward to hearing about the recommendations in the TB HIV co-infected group, Thank You

A: [https://www.thelancet.com/journals/laninf/article/PIIS1473-3099\(24\)00586-3/abstract](https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(24)00586-3/abstract). The results of the Seq&Treat clinical evaluation published in the Lancet (you need to register for free access) show the performance of tNGS stratified by HIV status. Regarding testing in children - the starting material for tNGS is a decontaminated sputum sample. It is often difficult to obtain sputum samples from children, but if it is available then there should not be any issues with tNGS testing. I think there are some ongoing studies testing the suitability on tNGS from other samples, including from stool - which if used for testing children.

4. Having an MDR patient complete drugs then after six months they test MTB positive. Shall we start as a new patient for treatment or not?

A: That's a great question. Further investigation would be needed before treating the patient as a new case. It will be important to understand whether it is a case of relapse (same MDR-TB strain) or re-infection (new TB strain). Depending on how MDR-TB was diagnosed the first time (phenotypic DST, rapid molecular tests, NGS) it would be helpful to compare those data with the results from the same tests after 6 months when patient tested positive again. If it's the same strain, then it would be treated as a recurrent MDR-TB and if it's a new strain the treatment would depend on the resistance profile obtained using appropriate DST methods.

5. Would you consider WGS from cultures a more suitable approach than tNGS from respiratory samples in settings with low (drug-resistant) TB prevalence—such as in parts of Europe—where tracking transmission is critical for TB elimination efforts and sequencing capacity is well established? what are pros and cons?

A: Yes, many low-prevalence settings with established sequencing capacity, such as in Australia, Netherlands, UK, Italy, Germany, New York State – are already implementing WGS to get a full resistance profile and to inform surveillance and tracking. Starting from culture allows for sequencing from extrapulmonary TB samples. There are also ongoing efforts to speed up the process through sample enrichment or the use of early positive cultures for the WGS workflows. For pros and cons, here's some suggested reading:

- <https://www.sciencedirect.com/science/article/pii/S1201971221002514>
- <https://www.nature.com/articles/s41598-021-94297-z>
- <https://www.sciencedirect.com/science/article/pii/S1201971221002009>

6. What about extrapulmonary TB lesions, is it handled like pulmonary one in genotyping, resistance and mutations?

A: There are not enough studies on NGS from extrapulmonary samples yet but given the correlation of sequencing success with higher bacterial load, it is likely that the low bacterial load inherent in such samples would require some form of enrichment of the DNA or culture to get successful sequencing done.

7. Is DNA extraction performed on a raw sample, or we need to culture the same first, not sure if recovery will be sufficient especially for cases with low bacteraemia.

A: For tNGS, one can perform extraction from raw or sedimented sample, without the need for culture. See in conjunction with answers above on correlation of sequencing success with bacterial load.

8. How do non-coding regions of the genome contribute to MTB gene regulation and is overall genomic functionality identified with tNGS?

A: The catalogue of mutations published by WHO includes some mutations in the non-coding regions of the genome - typically in promoter regions - that are associated with resistance to certain drugs and can act as molecular markers to predict resistance.

9. Do we have a list of mutations conferring drug resistance TB in bioinformatic tools? If yes, how many are out there?

A: Depends on the bioinformatics tool. TB Profiler for example mentions their mutations list on the website. <https://tbdr.lshtm.ac.uk/>. Which mutations are being used to predict resistance? Resistance is predicted using the curated tbdb database. This database has been tested using over 17,000 samples with genotypic and phenotypic data. You can read more about the markers present in the library at the time of testing here or you can look at the up-to-date CSV file here. For a list of tools, you can refer to this review: <https://pubmed.ncbi.nlm.nih.gov/40258039/>

10. Apart from the Genoscreen assay is there another panel that is being recommended for tNGS for TB

A: Yes, the WHO consolidated guidelines published in March 2024 include AmPORE-TB from ONT and TBseq for specific drugs.
<https://www.who.int/publications/i/item/9789240089488>

Cost-effectiveness

11. I'm particularly interested in understanding the cost-effectiveness of the sequencing technologies used in the project, and how these approaches can be adapted for implementation in resource-limited settings that are highly affected by tuberculosis.

A: Here's some literature addressing cost-effectiveness, definitely an area where more work is ongoing and more real-world data is needed from settings of intended use!

- a. <https://pubmed.ncbi.nlm.nih.gov/39810935/>
- b. <https://pmc.ncbi.nlm.nih.gov/articles/PMC10478709/>
- c. <https://www.medrxiv.org/content/10.1101/2025.03.13.25323893v1.full.pdf>
- d. <https://pmc.ncbi.nlm.nih.gov/articles/PMC9973455>

12. The shorter turn-around times compared with the conventional methods we have available is impressive. Could you please speak about the cost per test and what technological/infrastructural upgrades we might need for our existing or new labs?

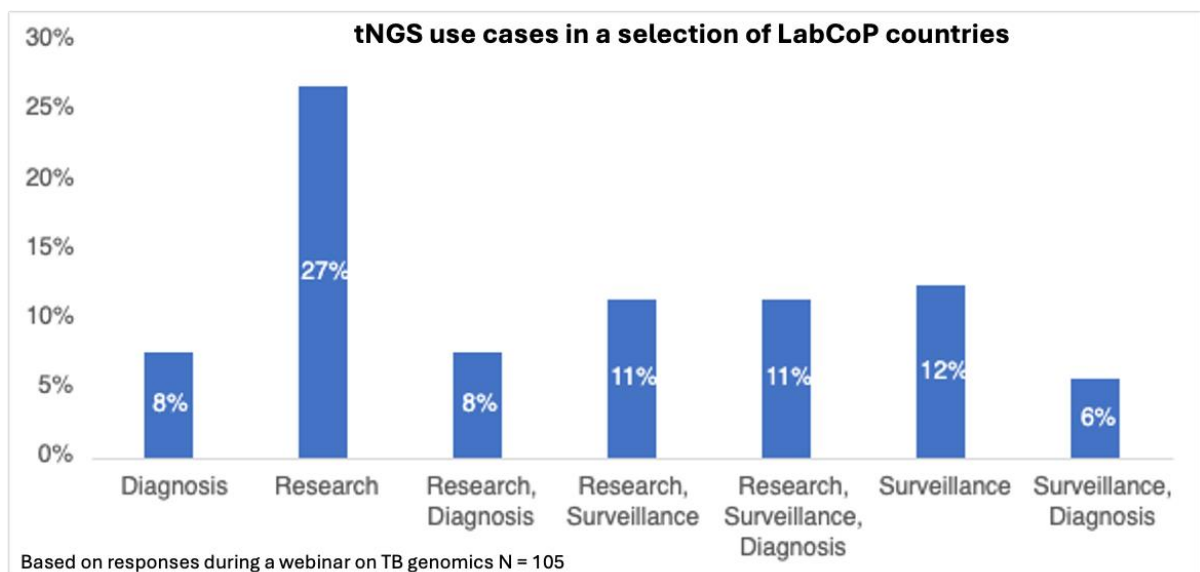
A: The cost of test is estimated in the range of \$60-\$120 at scale. More information can be found in the operational manual for NGS from WHO:

- a) <https://www.who.int/publications/i/item/9789240089501>
- b) <https://pubmed.ncbi.nlm.nih.gov/39810935/>
- c) [https://www.thelancet.com/journals/laninf/article/PIIS1473-3099\(24\)00263-9/fulltext](https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(24)00263-9/fulltext)

13. Thank you for the informative session. What support or resources can you direct us to for getting reagents to initiate TB targeted sequencing in our country by leveraging existing oxford nanopore platforms

A:

https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://www.stoptb.org/sites/default/files/imported/document/gli_sequencing_faqs_0.pdf&ved=2ahUKewjUxeOUUnreNAXR9QIHhc6WG0cQFnoECBkQAQ&usg=AOvVaw1pwg8d7wp-shQL0h1stYvu. The GLI has put together a useful list of resources including factsheets for some of the tNGS solutions.



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Webinar 2: TB Genomics Series: Anita Suresh: FIND