

# Day1 session 1.1- Mycobacterium tuberculosis NGS made easy: data analysis step-by-step -

We would like you to think for a couple of minutes about what you have heard. Also, we would like to assess the quality of our teaching in order to improve the quality of this training. Please name 1 to 3 things that you have learned so far and 1 to 3 that you have not fully understood. Thank you!

## Day 1 Session - Overview of NGS technologies & TB specific NGS solutions - Webinar

(We might use these answers in our Q&A, if you want to leave us your name before your answer that is usually helpful)

Your Name	Name 1 to 3 things that you have learned so far	Name 1 to 3 things that you have not fully understood
Pacome ABDUL ACHIMI	<ul style="list-style-type: none"><li>1- The difference between the long read sequencing and the short reads sequencing</li><li>2- The requirement for the NGS implementation</li><li>3- The NGS technology</li></ul>	<ul style="list-style-type: none"><li>1-the difference between the tNGS and the WGS</li><li>2-How can we select the type of sequencing based on the sample</li></ul>
Catherine Sacopon	<ul style="list-style-type: none"><li>1. The use of NGS in tuberculosis which includes transmission analysis, DR-TB surveys, can be used for future treatment changes, informing the use of modern</li></ul>	

	<p>DR-TB regimens, and comprehensive diagnosis of DR-TB - because it overcomes the limitations of phenotypic testing (long TAT, requirement for complex BSL 3 labs), and the limitations of some WHO-recommended rapid diagnostics which have limited resistance-associated target regions</p> <ol style="list-style-type: none"> <li>2. I also learned about studies from other countries on how they used WGS to optimize therapy, enhance treatment outcomes, and reduce toxicity - for high burden countries.</li> <li>3. I also learned how WGS can be used in low-incidence MDR countries wherein they averted the need for AST of &gt;80-85% of samples</li> </ol>	
Ameenah Salihu	<ol style="list-style-type: none"> <li>1. How to choose your sequencing platform</li> <li>2. The role of NGS in MDR TB surveillance</li> <li>3. How to implement a NGS TB country surveillance including setting up a technical group and monitoring.</li> </ol>	<p>1.tNGs</p> <p>2. How MDRs develop in patients who have never been exposed to those Line of antimicrobials</p> <p>3. How exactly TB drug resistance prediction is done using NGS</p>
Guy Arnault R	<ol style="list-style-type: none"> <li>1. The role of NGS in</li> </ol>	<ol style="list-style-type: none"> <li>1. tNGS vs WGS</li> </ol>

MFOUMBI IBINDA	MDR-TB diagnostic 2. Diagnostic vs surveillance tools to fight drug resistance 3. The key components of Staff to handle NGS	2. Miseq vs Miniseq 3. Relationship between PZA ETH in drug resistance
Afsana Akter Rupa	1. The role of NGS in TB diagnosis and treatment 2. The differences among different platforms of NGS 3. Details NGS workflow	1. Advantage and disadvantage of tNGS and WGS in case of TB 2. Can we use miniseq mid output for WGS?
Micheska EPOLA	1- The role of NGS in MDR surveillance 2- steps to implement sequencing in the diagnostic algorithm and surveillance of MDR-TB cases 3- the different platforms and technologies for whole genome sequencing	Sequencing platforms (Illumina, Oxford Nanopore): what are the differences, when should you use them?
Imen Bouzouita	NGS technologies : illumina, Nanopore, PacBio..	It was clear. Thank you for these presentations
Marco Pardo Freire	The differences between NGS technologies and how their implementation can be applied in different aspects of managing Mtb from diagnosis to surveillance	WGS vs tNGS: When to use one or the other and how it can impact the final information obtained
Mark Gutiérrez Pareja	1. NGS platforms available for the study of DR-TB (Illumina, BGI, Nanopore, PacBio...)	

	<p>2. Applicability of NGS in the diagnosis and surveillance of TB</p> <p>3. Needs (equipments, training, costs...) for the implementation of NGS in the TB laboratories</p>	
Abraham Ali	The importance of NGS for TB diagnosis and surveillance	
	What are the consideration in selecting sequencing platform	
	WHO guide for NGS implementation for TB sequencing	
Annisa Meliana	NGS Platforms Workflows (Long reads vs Short Reads), Considerations of use	<p><b>WHO Mutation of Catalogue</b></p> <p>I've been using tNGS by Deeplex and MGI ATOPlex tech in my country (Indonesia), and i still not fully understand the interpretation of mutation classification in the WHO Catalogue. Furthermore, i'd like to know how Indonesia could contribute to increase the uncertain significance mutation since my country have not contribute to the mutation catalogue data at all. Meanwhile Indonesia is the second tb burden case in the world.</p>
	NGS Role in tuberculosis	
	WHO guidance for NGS Implementation	
Naphatcha Thawong	Overview of the NGS, NGS workflow and detail, NGS types and considerations, the use of NGS data	All good
Dania Saeed	-NGS workflow and platforms,	

	<p>-considerations, benefits of using NGS for DR detection and surveillance.</p>	
Ma. Lyka Padiernos	<ol style="list-style-type: none"> <li>1. There are different NGS platforms that can be used for identification of drug resistance in tuberculosis. Depending on the identified purpose or needs of the facility (i.e research or clinical diagnosis), one can consider a Short-Read Sequencing platform or a Long-Read Sequencing platform. Available resources/ materials/ budget are also critical in choosing what type of workflow a facility should adapt.</li> <li>2. One of the advantages of NGS is the faster TAT over phenotypic DST. Also, certain drug resistance such as PZA resistance can be accurately identified using NGS. I realized that it may be because the PZA drug is more active in an acidic environment but <i>M.tuberculosis</i> grows poorly in low PH, causing a problematic set-up for phenotypic testing.</li> </ol>	<p>None, It was well presented. But I hope specific examples were given when highlighting the advantages of NGS. Data from studies/ researches or actual experiences of the laboratories who have implemented NGS were shared more in detail.</p>

	<p>3. Phylogenetic analysis may be used for monitoring of locally transmitted strains.</p>	
Claudia Gutierrez	<p>1. Different applications of NGS.</p> <p>2. Overview of different NGS platforms, advantages, limitations and the rationale for choosing the technology depending on the objectives and setting.</p> <p>3. Recommendations for the introduction and use of tNGS.</p>	<p>Look deeper into different NGS technologies.</p>
Desmond O. Ntiamoah	<p>1. Using NGS can improve treatment outcomes by providing a comprehensive profile of DR-TB thereby informing future treatment regimens, especially in high-burden regions</p> <p>2. I have also learnt that every strategy used in the implementation of NGS for TB must reflect the local burden of the disease.</p>	
Lilian Nwagbara	<p>1. The current roles and future potential of NGS in the detection and surveillance of DR-TB.</p> <p>2. Equipment and reagents that are required for NGS setup and ToR for</p>	<p>1. How can WGS be successfully implemented in wider DR-TB studies considering the large dataset involved?</p> <p>2. What determines the sequencing technology and bioinformatics workflow I use for my samples?</p>

	<p>key players in end-to-end implementation of NGS workflow eg Senior Scientists, Molecular Biologists, and Bioinformaticians.</p> <p>3. Key quality indicators for NGS workflow particularly the bioinformatics aspect starting with sequencing results.</p>	
Nabila Ismail	<p>1. The number of available resources and where to find them to address any questions I have around sequencing and implementation</p> <p>2. The other available tNGS technologies besides Deeplex</p>	<p>This may be an opinion, but on one of the slides Andrea spoke to, he mentioned that NGS is the fastest way to screen multiple drugs, but phenotypic testing can also screen multiple drugs if multiple DST assays are set up and also NGS technologies, especially WGS require a pure culture anyway, which is the starting point for DST as well. I think both assay types have advantages and disadvantages.</p> <p>In most cases, tNGS may not be as useful as WGS, should we still be motivating for the use of tNGS and why?</p>
Bernice Fumilayor Sawyerr	<p>1. I learnt about NGS, its importance over other sequencing techniques, and the workflow of <i>Mycobacterium tuberculosis</i> and the reasons for each step.</p> <p>2. Relevance of NGS for drug-resistant TB and the various requirements needed for high-level Integration and implementation.</p> <p>3. I learnt the essential steps</p>	

	needed for the effective use of the WHO Implementation Manual for integrating NGS in national TB.	
Adegun Oluwafemi Joseph	<ol style="list-style-type: none"> <li>1. NGS and its implementation in TB</li> <li>2. NGS workflow from DNA extraction to NGS analysis.</li> <li>3. Difference between short and long reads.</li> </ol>	
Olga Shavuka	<ol style="list-style-type: none"> <li>1. In high TB burden countries, WGS can be used to get quick results to help with patient care. In low burden countries, it is especially useful for surveillance</li> <li>2. WGS works by breaking the DNA into small pieces, and then puts the pieces back together to get the full sequence.</li> </ol>	<ol style="list-style-type: none"> <li>1. How gaps and errors are handled in the process of joining the small fragments to make a full sequence.</li> </ol>
Lorraine Boois	<ol style="list-style-type: none"> <li>1 I understand that we can use tNGS/WGS for rapid and more precise detection of resistance</li> </ol>	<ol style="list-style-type: none"> <li>2 What I havent fully grasped yet is the difference between short and long reads</li> </ol>
Veronica Medrano	<ol style="list-style-type: none"> <li>1. The use, utility and benefits of NGS for M. tuberculosis drug resistance analysis, treatment guidance and transmission analysis. The limits and disadvantages of phenotypic testing and WHO recommended rapid</li> </ol>	<p>It was all clear. Thank you</p>



