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
Noutin Michodigni (Noutin)	There are various NGS technologies Including Illumina, Nanopore and PacBio platforms Choice of the sequencing platform is not only limited to wet lab, but also bioinformatics infrastructure, data storage and staff training Deeper coverage is essential for variant calling	Quality assurance programme
Atang Bulane	There are different plat form for NGS	Library prep section

Lusia Mhuulu	NGS workflow (sample preparation, library preparation & sequencing reaction) Application of NGS in TB.	When do you use DNA quality/DNA quantity during the NGS process. Is there any tNGS EQA available in Africa? Which NGS technology is ideal for drug resistance testing and drug resistance surveillance?
Cecilia Mbebe	Advantages of NGS over routine molecular tests and pDST: turnaround time, range of mutations analyzed, and study of transmissions. Workflow: sample collection, DNA extraction, library preparation, etc. The choice of platform depends heavily on the lab's capacity and the objectives set. Several items are required by the WHO for NGS implementation, including equipment, SOPs, and quality indicators, etc	In the NGS flowchart for clinical samples, I saw WGS. How is DNA extracted from clinical samples for WGS? Sequencing quality control Advantages of the NGS platform vs. sequencing products
Shola Able-Thomas	Detailed WHO guidelines for the implementation of next-gen TB sequencing and characterisation of drug-resistance mutations.	How are drug-resistant mutations classified? What are the processes involved that determine whether a mutation confers resistance to a drug? Are there cases where certain mutations only confer resistance under certain conditions?
Chioma Kunle-Ope	1. In choosing NGS platform, the choice depends on Sample volume, Turnaround time and Data requirements 2. In NGS, DNA extraction steps is critical in generating high quality	1. NGS workflow - Library preparation 2. Different illumina platforms - How to select the best platform

	sequencing data 3. NGS provides comprehensive DR-TB profiles/prediction matched to modern treatment regimens, and it overcomes long TAT from first-/second-line phenotypic DST	
N'da Kouame Nazaire KOUADIO	1. NGS is a powerful technology that provides comprehensive information and predictive insights relevant to treatment regimens, potentially leading to more effective therapies through the identification of drug resistance patterns. 2. WGS has also been widely used for drug resistance surveillance.	Which of the two approaches, tNGS and WGS, is more powerful for drug resistance prediction?
Johnny Norman	Implementing NGS requires more than just a sequencing machine, it needs trained staff, SOPs, quality assurance, Bioinformatics capacity, data management, and integration into TB surveillance and clinical systems	I do not yet fully understand how a laboratory or national TB programme decides the best way to implement NGS, especially how to choose the right sequencing platform, whether to use WGS or targeted NGS, and how to plan for the required infrastructure, staffing, costs, data management, and long-term sustainability

		https://www.tbnet.eu/webinars?wix-vod-comp-id=comp-kzf9khep
Kelvin Smith Ofosu-Darko	1. Implementing NGS in low-burden settings is difficult, but possible, with proper planning 2. Incorporation of WGS into TB patient care improves treatment outcomes significantly 3. All sequencing platforms have their advantages and disadvantages. The goals of the research/ application must be considered when selecting a sequencing platform	Bridge amplification in Illumina Sequencing https://youtu.be/EDVKxSNdSic?si=AGyMMXwPdMlHdJjR Sequencing using BGI and MGI platforms https://youtu.be/havS4lupIsc?si=Sx1dpR3e9UA9DoCk
Samuel Chumane	Applications of MTB NGS: Surveillance of resistant strains, outbreak research and phylogenetic ecolocation studies General workflow: sample collection, conservation/culture, DNA extraction, library preparation and sequencing The development and implementation of means for detecting resistance to linezolid, bedaquiline, nitroimidazole and pyrazinamide in countries with a high TB burden should be a priority.	Do you know if is possible to obtain good tNGS results for extrapulmonary samples? Just out of curiosity!

Guy Arnault R MFOUMBI IBINDA	-Role of NGS -Tools to address epidemiological surveillance of MDR-TB -NGS workflow	-NGS quality assessment -Deeplex vs NGS -Deeplex catalogue update
Amy Dziedzorm Dzadey	NGS implementation must consider the disease burden in the region of interest In high burden areas it will be more useful for prospective drug resistance profiling while in low burden areas it will be for surveillance and transmission analysis. Platform choice influences TAT, workload and cost NGS should complement other diagnostic platforms instead of replacing them	Is it likely that tNGS may miss novel mutations in DR-TB cases as compared to WGS? How does WHO collate the cataloging for drug resistance mutations and how often is this revised? How can laboratories obtain political support or government funding aside grant funding for WGS and tNGS especially considering cost and sustainability?
Peggy-Estelle M. Tientcheu	The different platforms that can be used for TB sequencing. The different parameters to consider to make sure the chosen platforms meet your objectives	
Nneka Onyejepu	NGS as a tool for national DR survey and surveillance as it can screen a whole array of drug resistant genes even for the newer TB regimens	How do you drum up political support for the implementation of NGS in-country. Kindly share your thoughts and maybe experience
Justice Ohene Amofa	How NGS (Next-Generation Sequencing) can be used to detect drug-resistant mutations in *Mycobacterium tuberculosis*and support	Could long-read sequencing eventually replace Illumina for routine TB surveillance? How might AI or machine learning improve resistance prediction beyond current mutation catalogues?

	outbreak surveillance.	What unsolved problems in TB genomics are most urgent right now? Could within-host evolution models improve treatment monitoring? How close are we to predicting treatment outcomes directly from genomic data? What are the biggest blind spots in current TB genomic surveillance systems? How do recombination-free assumptions in MTBC shape phylogenetic methodology?
Olga Shavuka	I learned about the full NGS workflow, from sample collection and DNA extraction to sequencing and data analysis. NGS is very useful for detecting drug resistance, studying TB transmission, and supporting outbreak investigations. Compared to routine molecular tests and phenotypic DST, NGS provides faster and more detailed resistance information. The choice of sequencing platform depends on the laboratory capacity, study objectives, turnaround time, cost, and data requirements.	I may not fully understand how laboratories manage and store the large amount of sequencing data generated by NGS.
Michelle Amma Yeboah Amoako-Nimako	·1.Strategic planning is essential for implementing NGS in TB care, especially in low-burden settings. Successful adoption depends on aligning NGS use with regional disease burden supporting	How can political support be gotten for the implementation of NGS for TB

	prospective drug-resistance profiling in high-burden areas and surveillance plus transmission analysis in low-burden areas. 2. Whole-genome sequencing (WGS) improves TB patient management and outcomes when integrated appropriately. However, NGS should complement, not replace, existing diagnostic platforms within the TB care pathway. 3. Sequencing platform selection must be goal-driven. Each platform has strengths and limitations, and the choice directly affects turnaround time, workload, and overall cost	
Samuel Sorie	I have learnt a lot Mycobacterium Tuberculosis NGS provides better clarity and understanding of TB drug resistance patterns as opposed to the known traditional methods - it also has a shorter TAT than phenotypic DSTs. I appreciate the tools developed by WHO for implementing NGS nationally as in the case of Sierra Leone - this tool covers key aspects to consider when thinking about implementing NGS and will be useful for us in Sierra Leone as we look into NGS implementation. Based	WELL technically and per equipment principle, what to consider before deciding on what platform you can to procure or use

	on the considerations highlighted, we can decide to either use WGS or tNGS.	
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