Implementation of next generation sequencing for detection of DR TB in the Kyrgyz Republic.
Early country experiences using tNGS

27th VMC Webinar on TB Diagnostics hosted by WHO EURO

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- 26/04/2024

TB Country profile

Kyrgyzstan's population is 7,3 million

KG remains among the top 30 countries with a high burden of MDR/RR-TB in the world and within the 18 priority countries in the WHO European Region.

Number of MDR TB – 805 out of 4 674 TB cases (2023)

The proportion of MDR-TB among new cases is 26% and among previously treated TB cases is 55%

TB diagnostic network:

- 30 GeneXpert devices and 2 TrueNats
- 6 culture labs, 1 DST lab including phenotypic, LPA and NGS (WGS, tNGS and ONT (validation))

Sample referral sytem covers all country, funded by state budget

I. Implementation of whole genome and targeted sequencing of *M. tuberculosis*

A little bit a story for you…

Stop TB transmission in hospitals (StopTTH) project, funded by USAID:

- Implemented 8 working packages
- Duration of the project: 24 months (June 2017-July 2019)

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ПРИ МИНИСТЕРСТВЕ ЗДРАВООХРАНЕНИЯ
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WHO / GLI - Supranational Reference Laboratory of Tuberculosis

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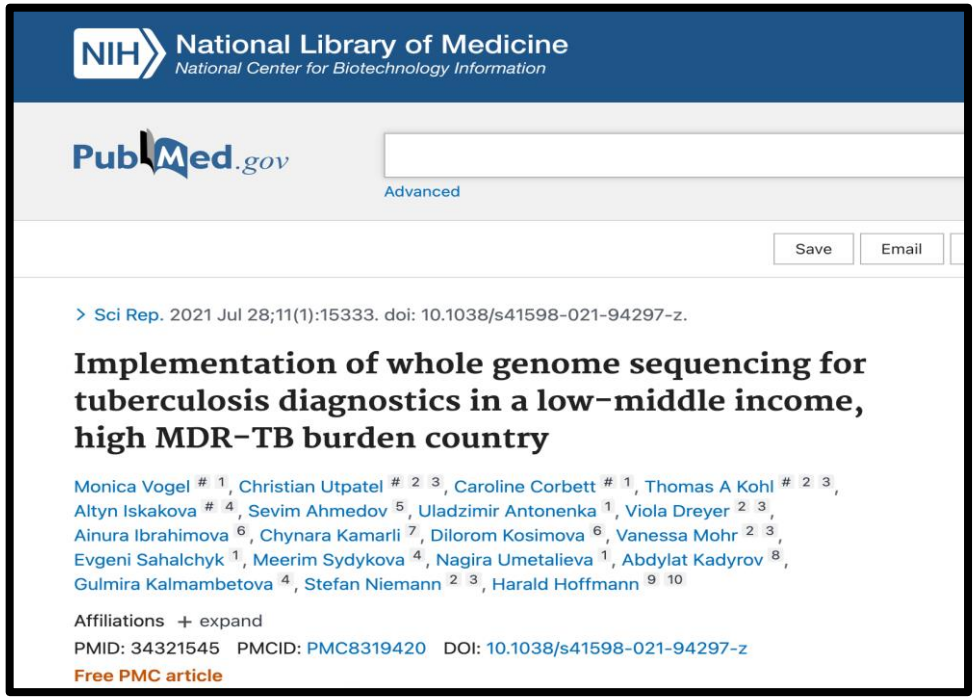
KNCV TUBERCULOSIS FOUNDATION
To eliminate TB

Partner institutions of the STOP TTH project:

- **USAID, Washington DC, USA and CO**
- **Defeat-TB project,** Kyrgyzstan
- **IMLred**: Institute of Microbiology and Laboratory Medicine , Department of Science, Education and Development (IML red GmbH), SNRL, Munich, Germany
- **RCB:** Research Center, Borstel, Germany
- **KNCV,** Challenge TB project
- **National TB Center, Kyrgyzstan**
- **NRL (National Reference Laboratory)**



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> Sci Rep. 2021 Jul 28;11(1):15333. doi: 10.1038/s41598-021-94297-z.

Implementation of whole genome sequencing for tuberculosis diagnostics in a low-middle income, high MDR-TB burden country

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Affiliations + expand

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Free PMC article

Large equipment and kits with associated manufacturer information.

Category	Item (model/brand) additional cost	Manufacturer	Distributor	Catalogue Number	Units	Net price per unit (USD)	Net sub-Total (USD)
Large equipment	Next generation sequencer (MiSeq system V2)	Illumina	Alliance Global	SY-410-1003	1	118,800.00	118,800.00
	– Ultimate 2-year Warranty	Illumina	Alliance Global	IL-SV-420-1008	1	26,039.65	26,039.65
	– Installation and initial instruction	Illumina			1	10,1900.00	11,900.00
	Fluorometer/Spectrophotometer (DS-11FX + Microvolume)	Denovix	BioLabtech,	9027304040	1	12,165.80	12,165.80
	Fragment analyzing system[a] (Advanced Analytical 5200 FA System)	Agilent	Vizamed	FSv2-CE2F	1	47,880.00	47,880.00
	– Installation and initial instruction	Agilent	Vizamed		1	2933.22	2933.22
	Computer[b]	Locally built-to-order	Locally built-to-order		1	1646.96	1646.96
	Uninterrupted Power Supply APC/BR1500GI UPS Por/AVR/1 500 VA/865 W	APC	Ermex		1	700.00	700.00
Sub-total					**8**	**222,065.63**	**222,065.63**
Kit and supplement-ary supplies	dsDNA High Sensitivity Kit—1000 assay	Denovix	BioLabtech	BioLabtech	2	395.00	790.00
	dsDNA Broad Range Kit—1000 assay	Denovix	BioLabtech	BioLabtech	2	455.48	910.96
	Denovix vials 0.5 mL thin wall (500 pc. Box)	Denovix	BioLabtech	TUBE-PCR-0.5-500	6	63.00	378.00
	Fragment Analyzer qualification kit	Agilent	Vizamed	DNF-FSEW-OQ	1	900.60	900.60
	Capillary conditioner	Agilent		DNF-475-0100	1	74.10	74.10
	12 capillary array cartridge 50 µm	Agilent	Vizamed	A2300-1250-3355	1	853.00	853.00
	HS NGS Fragment Analysis Kit 1–6000 bp	Agilent	Vizamed	DNF-474-0500	1	2117.00	2117.00
	MiSeq Reagent Kit v2 (500 cycle)	Illumina	Alliance Global	MS-102-200	17	1452.00	24,684.00
	MiSeq Reagent Kit v3 (600 cycle)	Illumina	Alliance Global	MS-102-3003	6	1896.00	11,376.00
	Nextera XT DNA Sample Preparation Kit (96 Samples)	Illumina	Alliance Global	FC-131-1096	4	3924.00	15,696.00
	Nextera XT Index Kit v2 Set A (96 Indices, 384 Samples)	Illumina	Alliance Global	FC-131-2001	2	1224.00	2448.00
	PhiX Control Kit	Illumina	Alliance Global	FC-110-3001	4	192.00	768.00
Sub-total					**47**		**60,995.66**
Total					**55**		**283,061.29**

Sequencing and reagent costs per sequence

Work-step	Measured costs per sequence[a]	Calculated costs per sequence	
		V2-kits[b]	V3-kits[c]
DNA extraction	$ 4.05	$ 4.05	$ 4.05
Denovix DNA Quant + QC	$ 2.88	$ 1.31	$ 1.31
Library preparation	$ 91.25	$ 59.61	$ 59.61
Fragment analysis/library QC	$ 14.7	$ 3.83	$ 3.83
Sequencing	$ 156.32	$ 90.41	$ 64.53
Shipping	$ 8.13	$ 8.13	$ 8.13
Total	$ 277.34	$ 167.33	$ 141.46

[a]Measured for first 174 sequences.
[b]Theoretical costs based on 16 samples/run.
[c]Theoretical costs based on 30 samples/run.

II. Seq_MDRTB_Net

Use of targeted next-generation sequencing to accelerate antibiotic resistance testing for TB patients in Kyrgyzstan

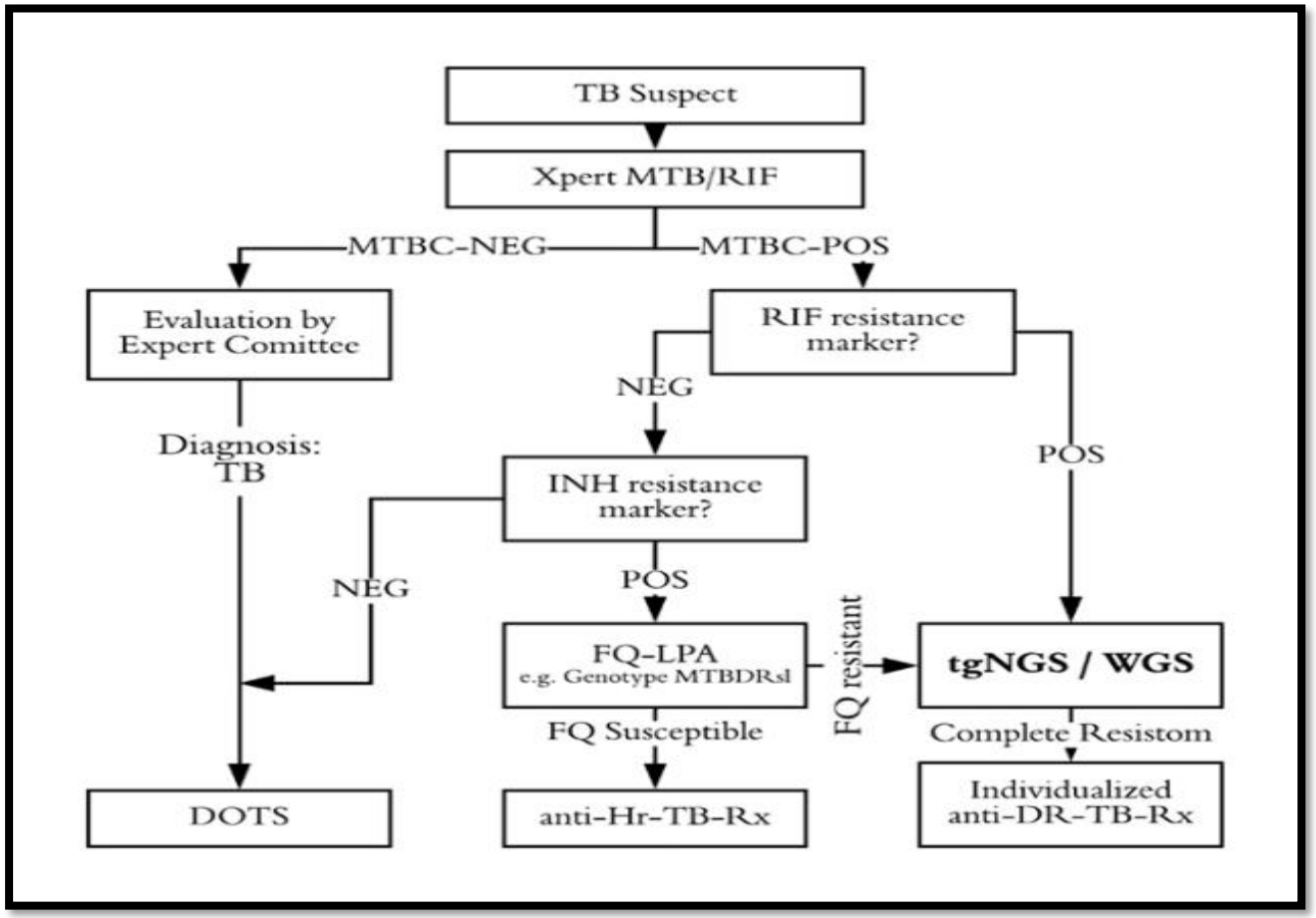
Seq_MTB_Net, funded by the German government

- Duration of the project (2019-2022)

The goals of the study

- Implementation of Targeted Sequencing in the KG NRL
- Collaboration with clinicians on targeted sequencing to improve treatment outcomes

Seq_MDRTB_Net project algorithm



Seq_MDRTB_Net project performance

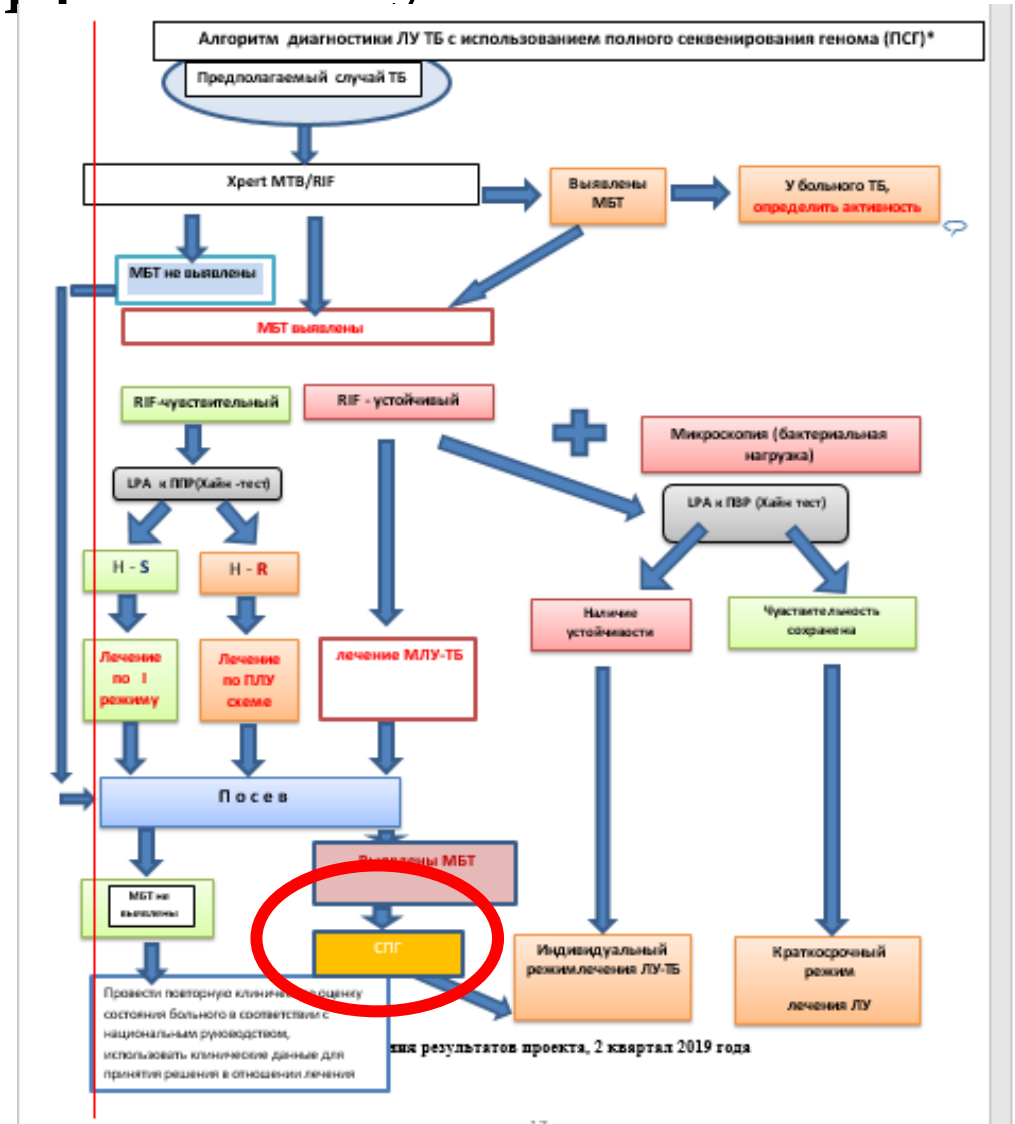
- Over the course of three years (2019-2022) project enrolled 389 patients
- Out of 389 enrolled patients, 237 (60,9%)are male and 152 female (39,1%) aged from 18-89 years old.
- Of the 389 patients that were enrolled
  - 330 samples provided yielded positive culture results.
    - 252 had sufficient library prep quality for sequencing
    - DNA from 235 of these samples were sent to Borstel and analyzed for WGS to determine the association between phenotypic and genetic antibiotic resistance results

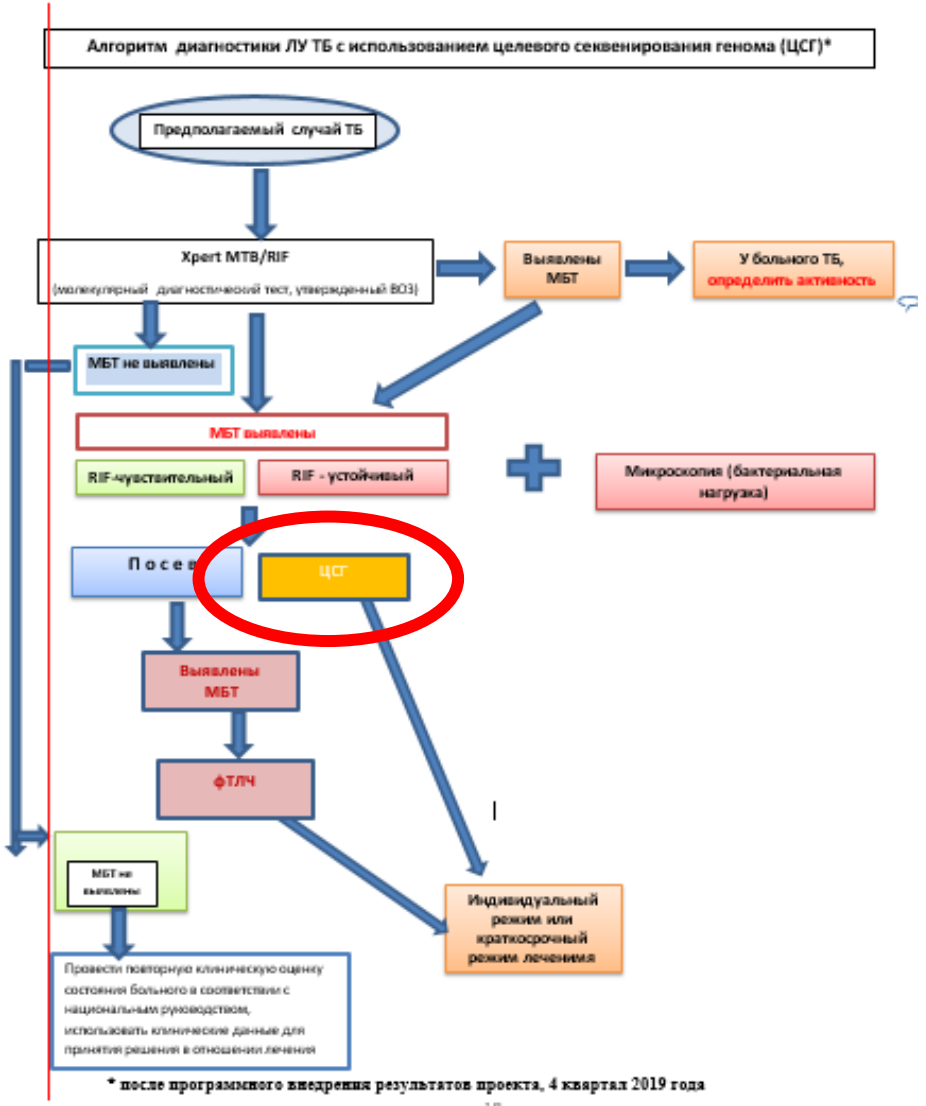
Challenges of Seq_MDRTB_Net project

- the 9-months stock-out of reagents on site caused significant delays for the project from December 2020 – August 2021
  - there are numerous challenges that cause interruptions in the supply of reagents:
    - lengthy shipping times and short shelf-life of reagents
    - sub-optimal communication and follow-up from the regional distributor
- malfunction of the Illumina sequencer in January 2022 due to a damage to an optical spare part
- political situation in Eastern Europe made it problematic to arrange a visit by an official representative of Illumina, Albiogen, based in Russia
- regular technical maintenance of the equipment needs to be addressed

Place of tNGS in National Clinical Guideline

(Approved by MoH in Dec 2023)





III. TA on scaling up tNGS on the programmatic level (funded by USAID)

TA is being provided to support NRL in setting up processes for using NGS in routine TB clinical management and for TB surveillance purposes.

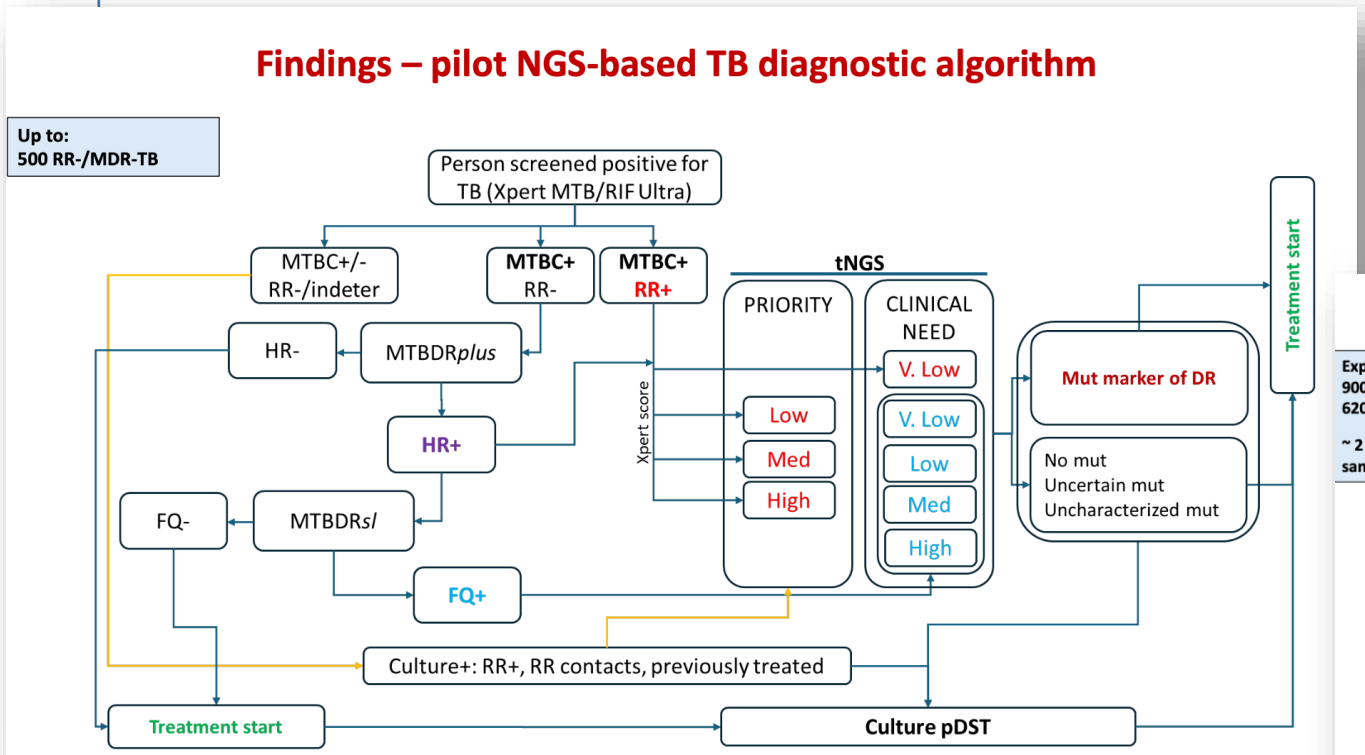
Objectives of the onsite visit:

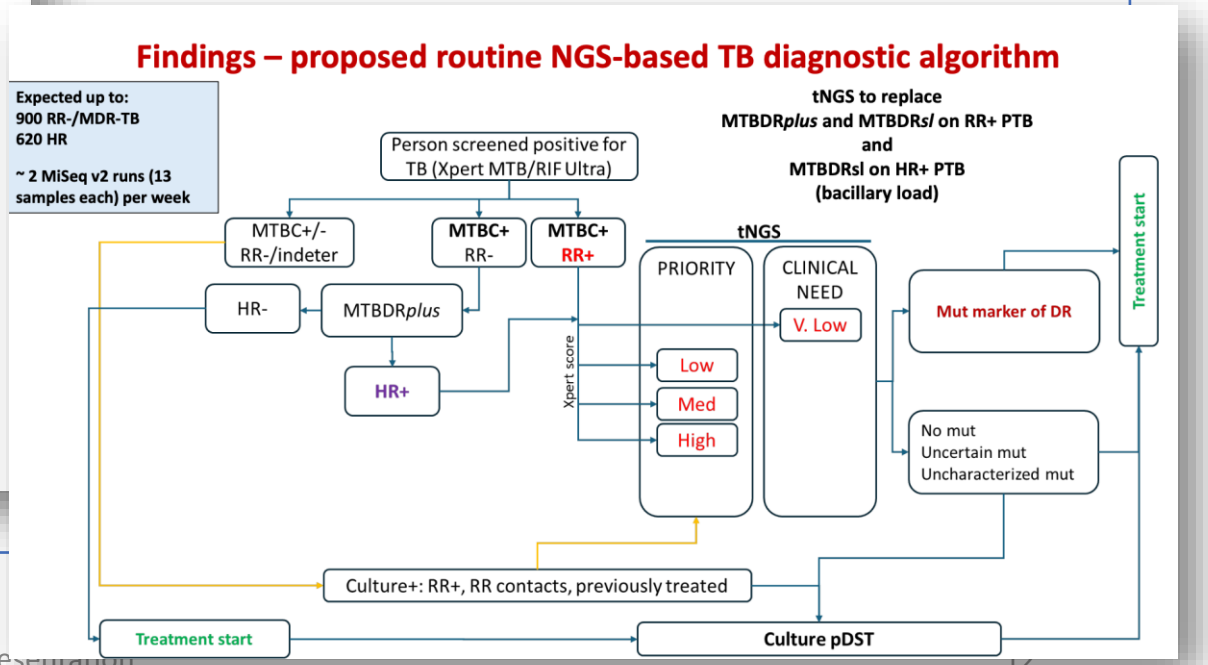
- **Overview of the NGS workflows, targeted NGS (tNGS) solution, WHO mutation catalogue and implementation process**
- **Review of NGS laboratory protocols, practical demonstration on tNGS use, development of NGS clinical report**
- **Assessment of current NGS results vs phenotypic DST results**
- **Assessment of NTP/NRL preparedness to utilize NGS for DR-TB diagnosis and surveillance**
- **Definition of targets and areas of intervention for developing a costed action plan for the use of NGS in the routine DR-TB clinical management and surveillance**

Andrea Cabibbe, Paolo Miotto, TB Supranational Reference Laboratory in Milan, WHO Collaborating Centre in TB Laboratory Strengthening

tNGS based DR TB diagnostic algorithm

(debreaf of A. Cabibbe and Paolo Miotto, 26.02.2024)





At the moment…

- **Action plan is being developed**
- **Systematic discussion on genotypic – phenotypic DST correlation is conducted**

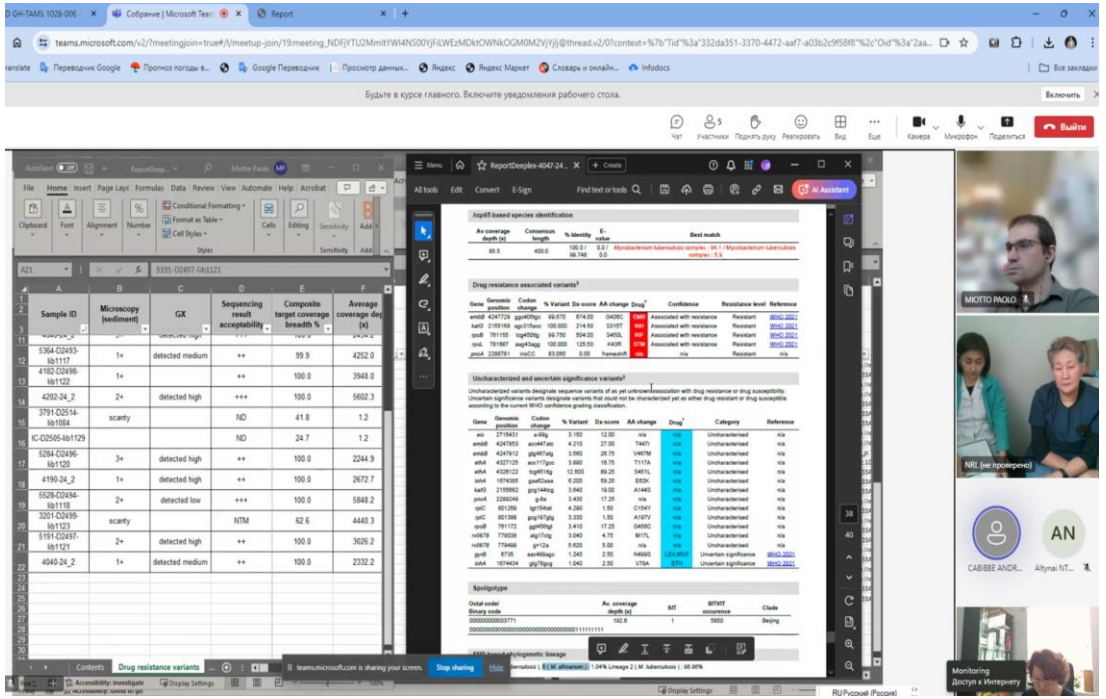
Cross-check reporting of results

Cross-check NGS data (e.g. presence of uncharacterized/uncertain mutations that might imply resistance)

Cross-check pDST quality

Interpretation is drug-dependent (e.g. suboptimal sensitivity of NGS targets; suboptimal reliability of pDST results)

Troubleshooting: consider re-testing of new samples, retesting phenotypically (e.g. with lower CCs)



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Thank you for your attention!

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