Regional Workshop on Accelerated Implementation of WHO Guidelines on TB Prevention, Diagnosis, and Drug-Resistant TB (DR-TB) Treatment

Implementation of tNGS for DR-TB screening in the region of Karalkapakstan, Uzbekistan

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World Health Organization

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KNCV TB PLUS

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Content

Background

- Intro TB diagnosis
- MSF Program in Karakalpakstan
- Diagnosis of DRTB in Karakalpakstan
- Testing in in Karakalpakstan

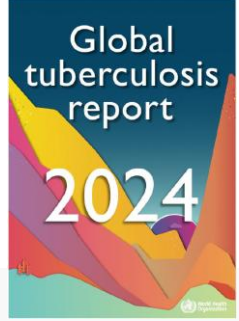
tNGS in Nukus TB1 Hospital lab

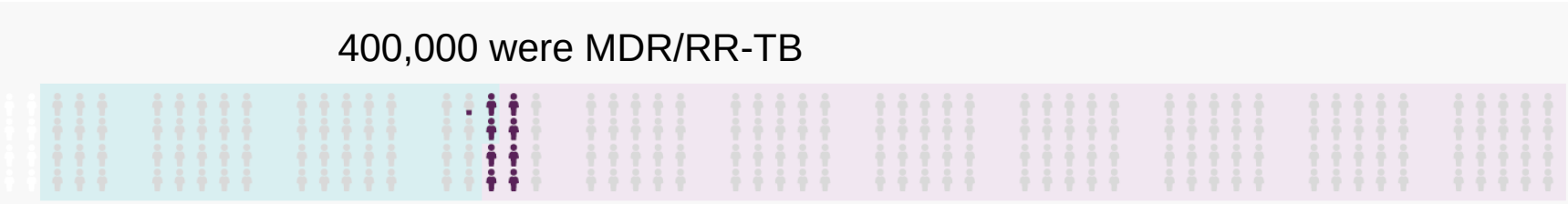
- Preparation
- Implementation
- Routine testing (planning)

Implementation Challenges

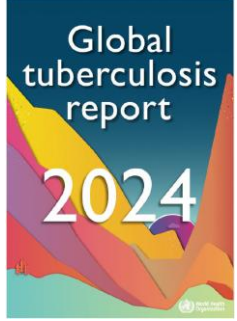
Current Benefits and Future Planning

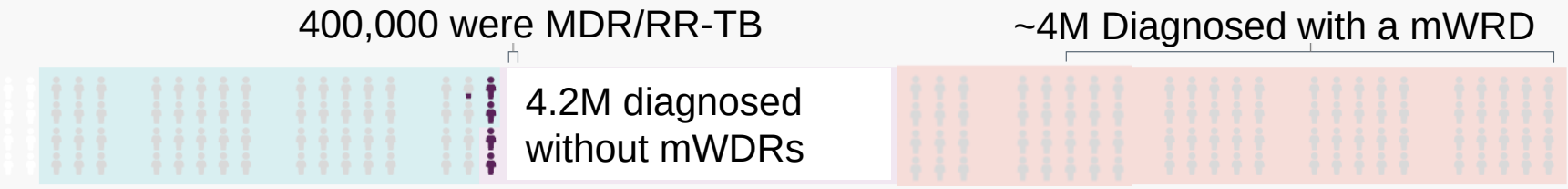
Global State of TB Diagnosis



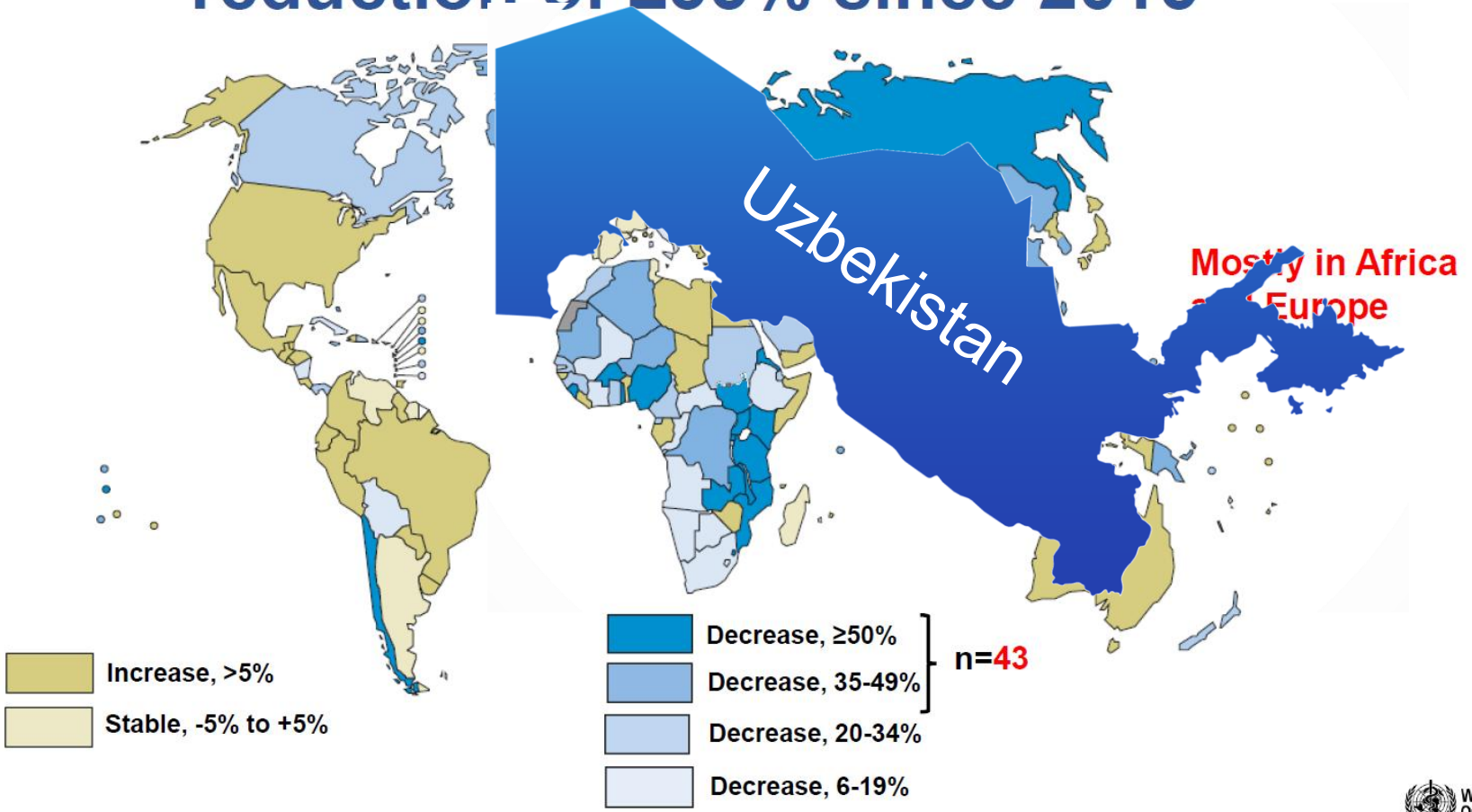


Global State of TB Diagnosis





43 countries estimated to have achieved reduction of ≥35% since 2015



World Health Organization

MSF Program in Karakalpakstan

Implementing a DOTS to support TB care

Comprehensive TB care for all
- Management DRTB cases

END of TB PRACTECAL trial

Integration of BDQ/LZD/MFX DST in routine

Implementation of tNGS for routine diagnosis

1998-2010 **2016** **2023**

1998 **2010** **2022** **2024**

MDR-TB burden and care

Capacity building

Community engagement

Advocacy and research

TB Laboratory renovated

TB PRACTECAL trial start

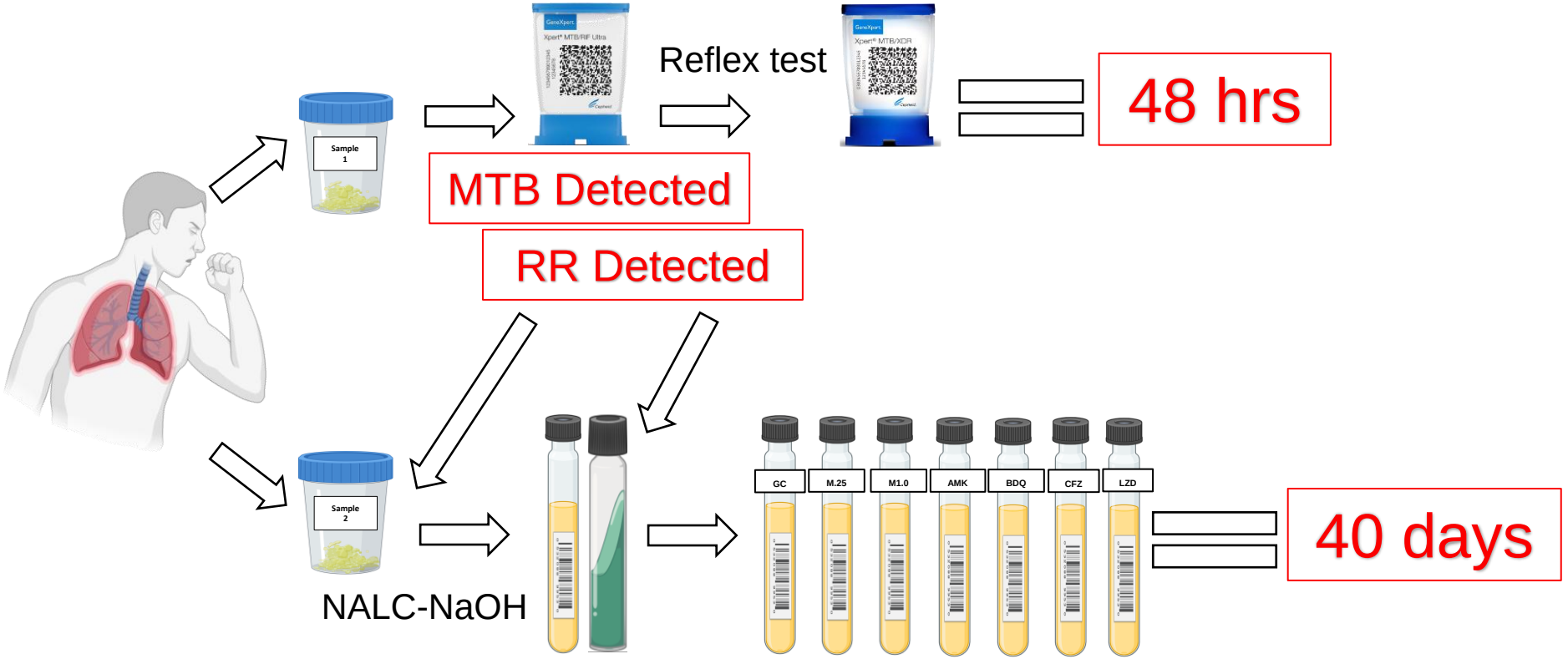
TB Lab enrolled EQA program

Full implementation of BPaL(M) treatment

Management of XDR-TB

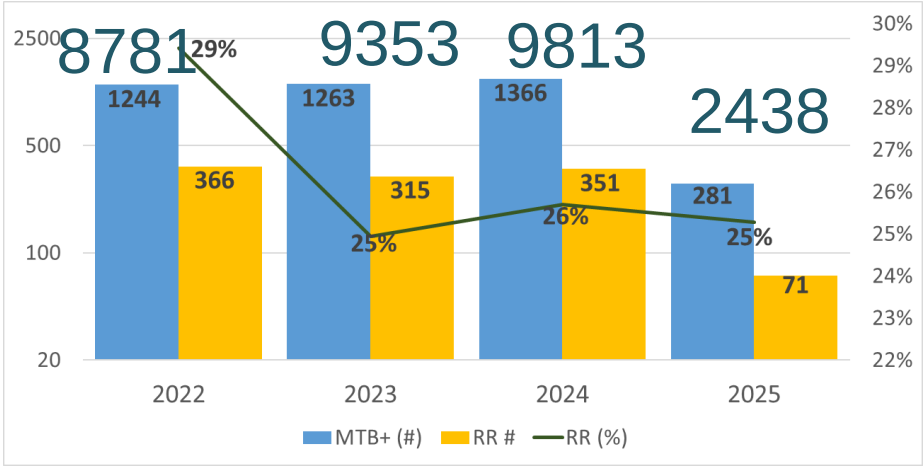
Validation of tNGS for DRTB detection

Diagnosis of DRTB in Karakalpakstan

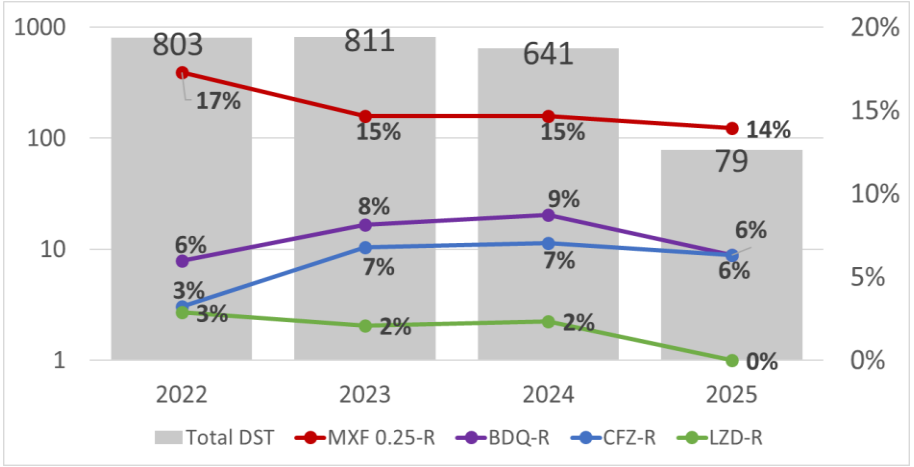


Testing in Karakalpakstan

Xpert Rif-R Detected



DST for New/Repurpose Drugs



Is the Next Generation Sequencing the solution for DR-TB detection in KKP?

- ✓ Fast diagnosis for SL and new drugs TB resistance profile (Short TAT)
- ✓ Comprehensive (includes rare mutations, TB strain types,)
- ✓ Lineages-epidemiology and surveillance

Targeted NGS in Karakalpakstan Approach

Preparation

Implementa-tion

Routine testing

Sustainability

Supplies
Quality Assurance
Technical support and troubleshooting

NGS Roadmap and Challenges

2019

Initiation of NGS project

NGS working group created

2020

Recruitment of NGS implementer

Site infrastructure rehabilitation

Ordering equipment and reagents

PAUSE

Major delay in reagent procurement. Due to COVID and Easter EU conflict

Delay recruiting NGS implementer

2023

Recruitment of NGS Lab Implementer

Preparation of SOPs

Illumina iSeq100 installation

Recruitment of NGS lab tech

Training started

2024

Pilot 1: retrospective testing of RR isolates (from 2022 – 2023)

Pilot 2: Pilot using routine samples (test algorithm and flow)

Nov 2024

Implementation of tNGS for routine diagnosis

Preparation

Human Resources

- International mobile staff was identified for initial assessment and implementation
- Recruitment of Lab Technician

Readiness assessment

- Carried by IMS:
  - Check infrastruture needs
  - Supplies required
  - Identification of suppliers and setting up contracts

Budgeting

Rehabilitation space and updating data systems

Placing initial order

Reagents and Systems Selected

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DNA Extraction and Purification

Multiplex PCR kit

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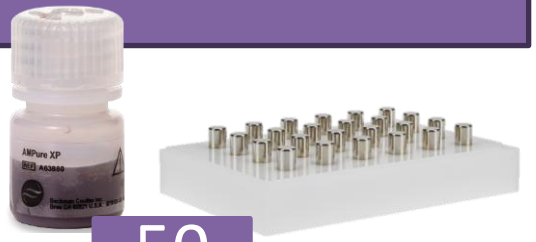

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Purification post-PCR/library

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Library Prep Kit

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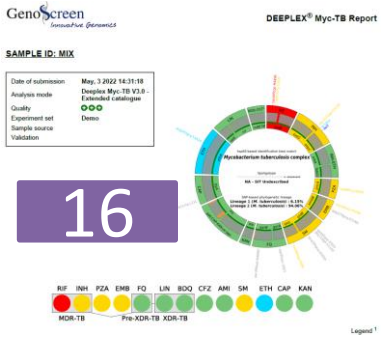
Sequencer and Cartridge



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Results Reporting

Implementation

Initial staff training

- Implementer training in Deeplex workflow at ITM
- Training of the lab staff
  - Implementer
  - Genoscreen trainer

Finalization SOPs and Job Aids including translations to Karakalpak

Pilot 1: retrospective testing of RR isolates

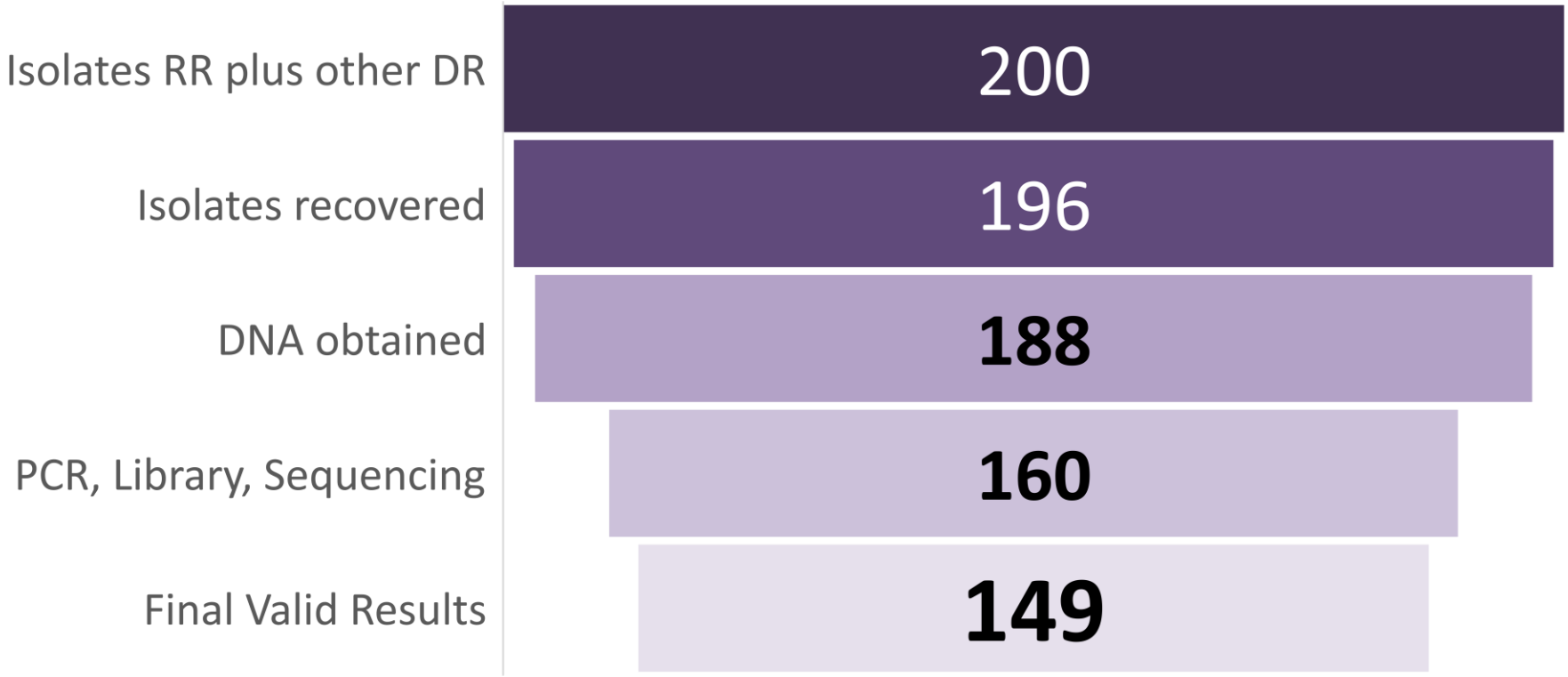
- Verification
- Feasibility

Pilot 2: Pilot using routine samples

- Fine-tune the methods using sample sediment
- Test algorithm and workflow




Setup Pilot 1: Test Verification using Isolates



Results Pilot 1: Test Verification using Isolates

Drugs	Sensitivity (%) CI95	Specificity (%) CI95	PPV (%)	NPV (%)	Accuracy (%)	LR+	LR-
LFX	83 (53-100)	98 (93-100)	83	98	96	33.3	0.17
MFX 0.25	83 (53-100)	98 (93-100)	83	98	96	33.3	0.17
MFX 1.0	80 (45-100)	95 (86-100)	67	98	93	16.4	0.21
BDQ	88 (77-99)	100	100	97	97	>100	0.17
CFZ	100	95 (91-98)	71	100	95	17.3	0.06
LZD	67 (13-100)	100	100	99	99	>100	0.33
AMK	80 (45-100)	95 (88-100)	67	97	93	16	0.21

Pilot 1: Messages

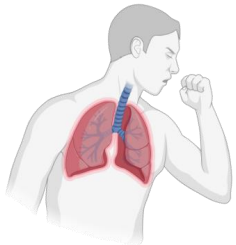
Lessons learned

- Extraction Protocol was modified (digestion lysozyme overnight)
- Takes a few runs to get used to the flow from DNA extraction to sequencing
- Contamination was observed, some troubleshooting was needed
- Issues with coverage and depth *rrs* genes (AMK)

Conclusions from Pilot 1

- Great performance for all drugs (except LZD – due to low number of LZN-R isolates)
- tNGS Deeplex can be used to “Rule In” resistance to all drugs tested.
- tNGS Deeplex can be used to “Rule Out” resistance to all drugs tested except BDQ and LZD
  - Limited information on mutations associated with resistance
  - Limited number of strains with DST-R

Setup Pilot 2: Test Algorithm and Workflow



Person Suspecting of TB

2 Samples

NALC-NaOH

Xpert Ultra SM1

MTB Detected

RR Detected

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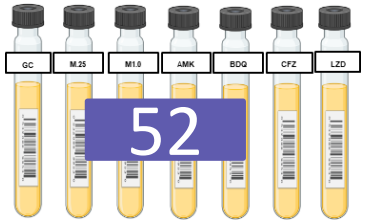
DST



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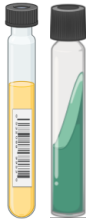
Culture



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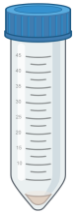
SM1: Xpert Ultra Ct <20

OR

SM2: Smear +



"Sample 2" sediment

27

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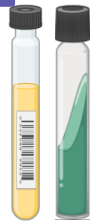
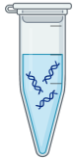
SM1: Xpert Ultra Ct >20

AND

SM2: Smear -



Culture+



DNA Extraction

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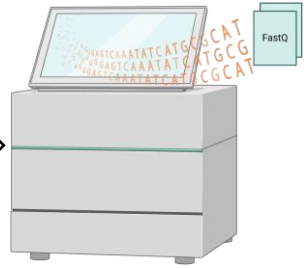

Sequencing

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Results Pilot 2: Test Workflow and Algorithm

Drugs	Sensitivity (%) CI95	Specificity (%) CI95	PPV (%)	NPV (%)	Accuracy (%)	LR+	LR-
LFX	97 (91-100)	94 (90-98)	83	99	95	16.4	0.03
MFX 0.25	93 (86-100)	100	100	97	98	>100	0.07
MFX 1.0	100	82 (75-88)	39	100	84	5.4	<0.0001
BDQ*	100	100	100	100	100	>100	<0.0001
CFZ*	100	100	100	100	100	>100	<0.0001
LZD 0 R	Unsufficient Data						
AMK	100	99 (97-100)	96	100	99	70.4	0.02

Pilot 2: Messages

Lessons learned

- Extraction Protocol was modified for a second time
- Issues with coverage and depth *rrs* genes (AMK) were higher when dealing with sediments
- Must add other criteria to keep a decent TAT during routine use. Priority other DR cases by rWRDs
- Despite adding additional samples. TAT of results are almost 2 weeks
- Some uncharacterized mutations needed double check using 2023 WHO catalogue

Conclusions from Pilot 2

- Workflow and algorithm was successfully tested with minor adjustments
- Very few resistant strains on this cohort
- tNGS to screen DR on new drugs is promising but closed monitoring is recommended since Pilot 1 indicated good to moderate sensitivity due to test coverage

Routine Testing

Lessons learnt and optimization from pilots

- Modifications were done to SOPs to improve results
- Modified indications for testing

Diagnostic algorithm tNGS

- Same as recommended by WHO Handbook 3

Considerations for testing (weekly testing)

- ALL Xpert Ultra Rif-R results (priority 1)
- Suspects of treatment failure (priority 2)
- INH-R samples or isolates (priority 3)
- DR-TB with missing drug test (PZA)

Validation results and their delivery to clinicians

- Simple report was developed to delivery of results

Enroll tNGS in the current EQA program with Gauting

- First batch submitted and successfully passed in December 2024

DNA Extraction

Multiplex PCR
(Deeplex Myc-TB)

Post-PCR Purification

Library Preparation

Sequencing

Sustainability

Assess impact of current global funding climate in the decentralization and access to tNGS

To incorporate tNGS as part of the NTP and NTRL planning

Future utilization of tNGS to include other applications (routine, surveillance, research, etc.)

Offer tNGS to other regions besides KKP

Long term planning:

- Scaling up
- Identification of additional funding
- Work in coordination with NRL

Implementation Challenges and Mitigation Measures

Infrastructure

- Lab infrastructure were insufficient
  - Additional rehabilitation ⇨ unidirectionality
  - Reorganization of space
- Internet connectivity was modified

Supplies

- Supply and procurement of short shelf life reagents
- Supply team working hard to ensure ALL required items are available.
- Constant communication

Trainings

- Baseline molecular biology knowledge
- Language barrier (software and interface are in English

Technical (already discussed in the pilots)

- DNA Extraction
- Issues with rrs gene
- Uncharacterized mutations
- Contamination observe

Data Management and Reporting

- Instrument storage is limited.
- Incorporating NGS in routine reporting required updating the LIS

Current Benefits and Future Plans

Comprehensive Test

- MTB Identification + Lineage + 13 Drugs

Turn Around Time (TAT)

- Sample sediments between 7-14 days
- Isolates between 18-21 days

Mutations and Surveillance

- Undertanding the mutations, frecuency and distribution would be important to intervention strategies

Potential Research Tool

- Epidemiological and Research studies.
  - Surveillance analysis of data obtained from the pilot 1
- Contribute into the growing mutation catalogue

Patient Management

- Monitor the performance of routine testing of tNGS by using key indicators
- Shorten the TAT of diagnosis will increase the likelihood of treatment success

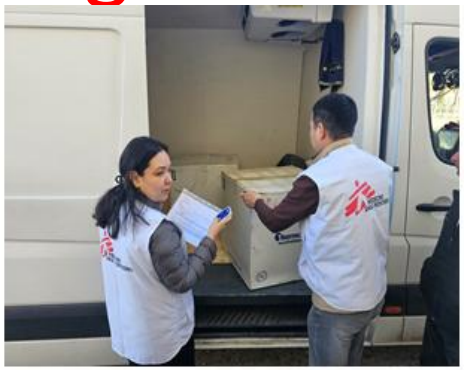
Advocacy

- Influence market on cost reduction and access

Acknowledgments

Nukus Lab Team

Data & Lab Team, MSF



Supply Team, MSF

Biomed Team, MSF

Mercy Oluya
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(Former Consultant for NGS)

Gulnara Tolepova
(NGS Lab Technologist)

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