TB diagnostic advances

Claudia Denkinger, MD PhD MSc DTMH
Head of TB Programme at FIND

Union meeting, 2018, The Hague
Vision for TB diagnostics in 2022 to achieve EndTB targets

First point of contact/Level 0/1
- Close the gap of 3.6 missing millions

1. Triage test
Or Ideally
2. Highly sensitive stand-alone detection test

Dedicated unit/Level 1/2
- Increase the number of patients receiving DST from currently on 29%

1. TB confirmation with rapid integrated DST for critical drugs to drive regimen decisions
2. (Incipient TB test)

Reference level/Level 3
- Inform individualized therapy

Comprehensive DST to cover the extended portfolio of old and new drugs
<table>
<thead>
<tr>
<th>Early development</th>
<th>Late or completed development</th>
<th>On pathway to WHO evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular – Detection/DST</strong></td>
<td><strong>Molecular – Detection/DST</strong></td>
<td><strong>Abbott – RealTime MTB RIF/INH</strong></td>
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<tr>
<td>Hain – FluoroType MBDXR Ver 1.0</td>
<td>Akorni – TruArrayTruDx2000 MDR/XDR-TB</td>
<td>Becton-Dickinson – BD MAX MDR-TB</td>
</tr>
<tr>
<td>Several acad./comp. – Low cost Easy to Use NGS EMPE Dx – mflDx MDR/XDR-TB</td>
<td>Veredus Laboratories – VeroMTB</td>
<td>Hain – FluoroType MBDXR Ver 1.0</td>
</tr>
<tr>
<td>LifeArc/Univ. St Andrews – Molecular Bacterial Load Assay</td>
<td>CapitoBio – Mycobacteria RT PCR</td>
<td>Roche – cobas MTB-RIF/INH</td>
</tr>
<tr>
<td><strong>Culture-based – Detection/DST</strong></td>
<td><strong>Culture-based – Detection/DST</strong></td>
<td>Bioneer – AccuPower TAMDR RT PCR</td>
</tr>
<tr>
<td>BNP Middlebrook (NanoLogix) MYCOLOR TK BNP (Salubris, USA)</td>
<td>QuantaMatrix – QMAC DST</td>
<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td><strong>Cellular Response/Transcriptomic – Detection/Latent and latent to active progression</strong></td>
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<td><strong>FRIZ</strong></td>
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<td>Akorni – TruArrayTruDx2000 MDR/XDR-TB</td>
<td>Cepheid – Xpert XDR</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>FRZ Biochem – MDR-TB</td>
<td>Cepheid – OMNI</td>
<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td>Bioneer – POC for MDR/XDR-TB</td>
<td>Several groups – Preprocessing molecular stool</td>
<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td>MicuBiomed – Rapid POCT for MDR-TB</td>
<td>Univ. of Washington – Sample collection molecular buccal swab</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>QuantumDx – Q-POC TB/MDR TB</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Genedrive – MTB/RIF</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>InSilixa – HYDRA-1k</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Blink – BLINK ONE</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>SelfDiagnostics Deutschland – TB MultiTest Mobidag – Novodag</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td><strong>Automated Microscopy &amp; Imaging – Detection</strong></td>
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<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td>Advenio Tecnosys – RiView-TB</td>
<td>ID-FISH Technology – ID-FISH assay</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Qiagen – QFT-Predict</td>
<td>Delft Imaging Systems – CAD4TB</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Qiagen – QIA-TB Signature</td>
<td>Cure.ai – Cure Chest X-rays for TB</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Biomérieux/Bioaster – Host signature</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td><strong>Breath Biomarker – Detection</strong></td>
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<td><strong>FRIZ</strong></td>
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<tr>
<td>Menosana – BreathLink</td>
<td>Rapid Biosensor Systems – TB Breathalyser</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Avisa – BreathTest</td>
<td>The eNose Company – Aeonose</td>
<td><strong>FRIZ</strong></td>
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<tr>
<td>Technion – Breath analysis instrument</td>
<td><strong>FRIZ</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Antigen, Antibody and Biomarker detection – Detection</strong></td>
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<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td>E.g. TransDot, Precision Bio – Host markers in blood</td>
<td>Salus Discovery – TB Flow</td>
<td>Fujifilm – Sensitive LAM</td>
</tr>
<tr>
<td>E.g. NanoPin – MTB-antigens in blood</td>
<td>Global Good – High sensitivity TB rapid Ox</td>
<td><strong>FRIZ</strong></td>
</tr>
<tr>
<td>Several acad./comp. – cfDNA in blood/urine</td>
<td>Unisa – TB Ox</td>
<td><strong>FRIZ</strong></td>
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<td>E.g. Omunits, AppGenex – Antibody tests</td>
<td><strong>FRIZ</strong></td>
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**High complexity assays**

**Moderate complexity assays**

**Low complexity assays**

**Low complexity assays**

**Moderate complexity assays**

**High complexity assays**
Non-sputum based tests for diagnosis or triage

Early identification of patients with TB or at high-risk of TB on easy to access samples ideally at level 0/1

Determine TB LAM Ag (urine) for HIV co-infected with low CD4 counts (Alere)

Negative recommendation for Serological assays by the WHO

Next-generation LAM POC assays (urine, blood)
- Fujifilm/FIND
- Salus
- and others

Blood host marker POC tests
- TransDot signature (ScreenTB consortium)
- SomaLogic signature (SomaLogic, FIND)
- and others

Computer-aided detection (X-ray)
- Delft
- Qure.ai

Active TB
- QFT-Predict (Qiagen)
- QIA-TB Signature (Qiagen)
- mRNA Signatures (Stanford, Zak et al.)
- T-cell Immune Profiling (BD)
- RTT TB (Lophius)
- Incipient TB Assay (Abbott)

Latent TB
- Incipient TB tests (blood)
- cfDNA in blood or urine

Breath Tests and Skin Patches
- Technion
- RBS
- eNose
- others

Removing current bottleneck: RTI TB (Lophius)

Early identification of patients with TB or at high-risk of TB on easy to access samples ideally at level 0/1

Pediatric TB Disposable Squeeze Bottle for Stool Processing prior Xpert (FIND, U Bord, KNCV, others)

TB antigen POC assays (blood)
- Arizona State Univ.
- Wyss Inst./Harvard
- and others

2017

2018

2019

2020-2025

Pediatric TB Disposable Squeeze Bottle for Stool Processing prior Xpert (FIND, U Bord, KNCV, others)

Disclaimer: Images & time estimates are to be taken as indicative only.

Source: http://www.delft.care


Source: https://www.whatisepigenetics.com

Source: http://lnbd.technion.ac.il


Source: https://www.whatisepigenetics.com
# Landscape Triage & stand-alone detection at POC

## Host Markers

<table>
<thead>
<tr>
<th>Marker cat.</th>
<th>Perf</th>
<th>Cost</th>
<th>Complex</th>
<th>Maturity</th>
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<tr>
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<td>Dis/Dev</td>
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<td><img src="image" alt="Rating" /></td>
<td>Discov</td>
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<tr>
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<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Dis/Dev</td>
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<tr>
<td>X-ray (CAD)</td>
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<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Valid</td>
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</table>

## Pathogen

<table>
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<tr>
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<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Market</td>
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<tr>
<td>cfDNA</td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Discov</td>
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<tr>
<td>LAM</td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Dev.</td>
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<tr>
<td>MTB-antigens</td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Discov</td>
</tr>
<tr>
<td>Breath/Skin tests</td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td><img src="image" alt="Rating" /></td>
<td>Valid</td>
</tr>
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</table>

- Tech feasibility
- POC suitability
- Performance
- Achievable cost
Enhanced Sensitivity to Detect TB in all HIV+
Lipoarabinomannan (LAM) is one of the most promising non-DNA TB biomarker
Fujifilm Silver Amplification Technology achieves a low pg/ml cut-off
Around 30% increased sensitivity over existing POC LAM assay

High Specificity for Immediate Treatment Initiation

Designed for the POC in LMIC’s where patients seek care
Urine non-invasive and easy to collect
Rapid time-to-result <60 minutes
Simple for healthcare workers with minimal training
Instrument-free and safe

Affordable and Scalable
Proven mass manufacturability

Expected High Patient Impact
Accuracy of FujiLAM is superior to AlereLAM in HIV+

### FujiLAM performance:

- 70.4% sensitivity in HIV+ inpatients across CD4 strata
- 28.1% higher than AlereLAM and superior
- 95.7% specificity against the Composite Reference Standard
- Specificity: no significant difference to AlereLAM

*meta-analysis including three cohorts

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More details in TB/HIV late-breaker on Friday at 2pm

https://ssrn.com/abstract=3254479
Non sputum based triage testing on POC Platform progressing in development

<table>
<thead>
<tr>
<th>Goal</th>
<th>Status</th>
<th>On-going</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Non-sputum based</td>
<td>✓ Non-biased proteomic approach</td>
<td>• Development on lateral flow platform</td>
</tr>
<tr>
<td>• Rule-out TB</td>
<td>✓ Biomarker discovered &amp; tested</td>
<td>• Feasibility study ongoing</td>
</tr>
<tr>
<td>• Independent of HIV status</td>
<td>✓ Suitable industry partner with POC identified &amp; Reagents developed</td>
<td>☐ Prototype: 2019Q3</td>
</tr>
<tr>
<td>☐ Children?</td>
<td></td>
<td>☐ Design locked: 2020Q1</td>
</tr>
</tbody>
</table>

Target Population: Children & adult
Setting/User: L0/L1
Cost: < 2 USD
Time-to-result: < 30 min
Cytokine + panel by African/European consortium promising but more data and proof of POC suitability required

- **Biased discovery/ cytokine panel:**
  - 7-marker signature > 3-4-marker possible?

- **Dx performance evaluation limited**
  - Sens. 94%, Spec. 73%, AUC 0.91 (training set)
  - Ongoing validation

- **In the process of developing LFA assay**
  - Instrument required
  - Multiplexing complex
  - Requires quantitation

- **Cost target achievable??**
  - Unclear at this point but seems unlikely to be <2USD COGS

- **POC possible??**
  - Fast: < 30 min
  - Simple-to-use

- **Challenges:**
  - Investigation thus far only in Africa

Awoniyi J Infect 2016
Chegou Thorax 2016
RNA signatures meet triage performance but NOT operational & cost targets

**Sweeney meta-analysis**
- Identified 3 gene signature
  - GBP5, DUSP3, and KLF2
  - Roles in immune regulation & infection response

**Dx performance meets minimal TPP**
- Sens. 95%
- Spec. 47%, AUC 0.83

**Multiple possible manufacturers:**
- Classical RT-qPCR
- Requires multiplexing

**POC unable to meet**
- 30 min – 2 hours achievable
- Platform necessary
- Some sample prep necessary (lysis, digestion, RNA stabilization)

**Cost target **NOT** achievable**
- At least 5 USD COGS

Conclusions similar for other published/unpublished RNA signatures:
- G. Walzl: 2 or 4-gene signature
- S. Kaufmann: 4-gene signature
- ACS COR: 4 or 16-gene signature

Suliman AJRCCM 2018
Zak Lancet 2016
Maertzdorf Genes Immun 2011
Improving Pediatric TB diagnosis through better access to testing

The problem

- In 2017, ~1 million children with TB
- 234,000 children died of TB (incl. 40,000 children with HIV).

Problem:

- Lack of effective diagnostic tests that can be performed on easily accessible samples
- Lack of availability of quality TB diagnosis in primary care and private sector

The solution?

1. Open bottle. Use spoon to collect sample and place in bottle
2. Pipette buffer & reagent from bottles (if necessary)
3. Shake 20 times & wait 30 minutes. Shake again briefly
4. Replace lid with custom filtration lid
5. Invert and squeeze bottle to dispense filtrate into cartridge
Incipient TB – risk of progression

- Current products (IGRA and TST): 2-3% PPV of existing products to detect latent TB

Optimum TPP
PPV ~16%

16-gene transcriptomic COR
PPV ~7%

Minimum TPP
PPV ~6%

TST/IGRA
PPV ~2% / ~3%

Minimum

Optimum

COP

IGRA

TST

Zak et al. 2016

Development of a Target Product Profile (TPP) and a framework for evaluation for a test for predicting progression from tuberculosis infection to active disease

2017
Several companies are working on products with higher PPV («driven» by high-income country market)

Market Entry ≥2020

**Products in pipeline**
- QFT-Plus and QFT-Predict (Qiagen)
- QIA-TB Signature (Qiagen)
- T-cell Immune Profiling (BD)
- RTT TB (Lophius)
- Incipient TB Assay (Abbott)
- and others

**Biomarkers:**
- RNA signatures
- IFN-γ release after T-cell stimulation with new antigens
- Cell differentiation markers (eg. CD27)
- Cytokine levels in blood (eg. IP-10)
Diversification of sputum-based testing and drug susceptibility testing (DST)

Centralized DST (Abbott, BD, Hain, Roche, Bioneer)

POC DST (QuantuMDx, Bioneer, MicoBiomed, Akonni, ...)

Line probe assays:
- MTBDRplus and id (Hain Lifescience)
- Lipa MTBDR (Nipro)
- TBModule (AID)

Liquid culture:
- MGIT (BD)
- TREK Sensitive (ThermoFisher)
- Mycolor TR BNP (Salubris)

NAAT:
- Xpert MTB/RIF (Cepheid)
- TB-LAMP (Eiken)
- Mycobacteria RT PCR (CapitalBio)
- Anyplex MTB/XDR (Seegene)
- Infiniti MDR TB (Autogenomics)
- VereMTB/Rif/Inh (Vereus Laboratories)
- MeltPro MDR (Zeesan Biotech)
- Genadive TB/Rif (Epistem)
- AccuPower TB/MDR (Bioneer)

Enable TEST & TREAT
Match the regimen pipeline

First Xpert follower

Q1 → Enable TEST & TREAT → Match the regimen pipeline

Hybridisation (Scanogen)

Whole genome Sequencing from sputum

End-to-End targeted sequencing solutions from sputum

DISCLAIMER: Images & time estimates are to be taken as indicative only.
Informing regimen selection at the point-of-care aligned with novel TB regimen development

Studies starts in Q2 2019

Tuberculosis Patient

Level 0
Community/POC

Level 1
Microscopy centre

Level 2
District hospital

Level 3
Reference centre

Established in LMICs >120 countries

Omnicon

GeneXpert

TB detected/FL regimen

INH/FQ/AG

Short/new regimen

Individualized Rx/further testing

**Sensitivity for S+ C+ TB**

+17% (95%CI +10, +25)

-3.2% (95%CI -4.7, -2.1)

**Specificity for TB**

-6 -5 -4 -3 -2 -1 0 +1 +2

-6 -5 -4 -3 -2 -1 0 +1 +2

**Delta Sensitivity**

N=549

**Delta Specificity**

Xie NEJM 2017
Dorman Lancet ID 2018
Diversification of the TB molecular diagnostics market

**MOLBIO**
- Indian product
- First competitor to Cepheid
- Recommended in India
- Now going into WHO trials with FIND

**BLINK**
- POC platform
- Rapid and affordable testing
- Combine molecular and immunoassay
- Semi-open business model
- In feasibility studies

**BD, ROCHE, ABBOTT, BIONEER**
- Centralized
- DST to 1st line drugs
- Comparative analytical study by FIND ongoing followed by WHO review and trials
Novel TB regimen guidelines – are we prepared from the diagnostics side

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEDICINE</th>
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<tbody>
<tr>
<td><strong>Group A:</strong></td>
<td>Include all three medicines (unless they cannot be used)</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin QR</td>
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<tr>
<td></td>
<td>Moxifloxacin</td>
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<tr>
<td></td>
<td>Bedaquiline(^1,4)</td>
</tr>
<tr>
<td></td>
<td>Linezolid(^2)</td>
</tr>
<tr>
<td><strong>Group B:</strong></td>
<td>Add both medicines (unless they cannot be used)</td>
</tr>
<tr>
<td></td>
<td>Clofazimine</td>
</tr>
<tr>
<td></td>
<td>Cycloserine QR</td>
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<tr>
<td></td>
<td>Terizidone</td>
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<tr>
<td><strong>Group C:</strong></td>
<td>Add to complete the regimen and when medicines from Groups A and B cannot be used</td>
</tr>
<tr>
<td></td>
<td>Ethambutol</td>
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<tr>
<td></td>
<td>Delamanid(^3,4)</td>
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<td></td>
<td>Pyrazinamide(^5)</td>
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<tr>
<td></td>
<td>Imipenem-cilastatin QR</td>
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<tr>
<td></td>
<td>Meropenem(^6)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (OR Streptomycin)(^7)</td>
</tr>
<tr>
<td></td>
<td>Ethionamide QR</td>
</tr>
<tr>
<td></td>
<td>Prothionamide</td>
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<tr>
<td></td>
<td>p-aminosalicylic acid</td>
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</tbody>
</table>

**FQ:** Integrated assays in development

**BDQ:** at least 3 large targets: *mmpR*, *atpE*, *pepQ*; up-& downregulating mutations?

**Linezolid:** at least 3 large targets: rplC, rrl, ??

- RIF and INH are critical for first line regimens
- FQ are critical for second line regimens
- No rapid DST is available for Bedaquiline, Linezolid, Clofazimine or Cycloserine
ReSeqTB – a global clinical knowledgebase for TB

- Built by the TB Community to Serve the TB Community
- Rapidly predict phenotypic antimicrobial resistance from Mtb sequences
- Hosted by the WHO by end-2018
- Includes data analysis, management, QC/QA and reporting

**Input**
- Whole Genome & Targeted sequencing
- Integrates key TB databases (e.g. WHO surveillance, Cryptic)

**Analysis**
- Sequence alignment
- Variant calling & validation

**Interpretation**
- Phenotypic-genotypic correlations
- Confidence-graded mutations

**Reporting**
- Drug resistance surveillance
Informing individualized therapy for resistant TB

**GOAL:**

1st step: Replace Phenotypic TB DST with Rapid, Culture-free NGS for Surveillance

2nd step: Clinical Diagnosis of Drug resistant TB
Diagnostics in context - Ideal care envisioned

Level 0
- Active case finding

Level 1
- Triage testing
- Confirmatory testing + regimen selection

Level 2/3
- Individualized care

Optimized use of data to support service delivery, device management, surveillance and quality assurance

Optimized placement of tools through diagnostic network and patient pathway analysis

Patient support services and measures to offset costs
Conclusions

- Significant progress has been made over the last year towards target of reaching the ‘missing millions’ with two parallel developments: Triage and stand-alone detection but high-risk remains

- While integrate systems for DST are needed to inform regimen selection, sequencing is the likely future for comprehensive, rapid and adaptable DST
Thank you

Slides prepared with:
• Tobias Broger
• Anita Suresh
• Romain Wyss
• SJ Loveday