# The TB diagnostic pipeline

**Claudia Denkinger, MD PhD MSc DTMH**
**Head of TB Programme at FIND**
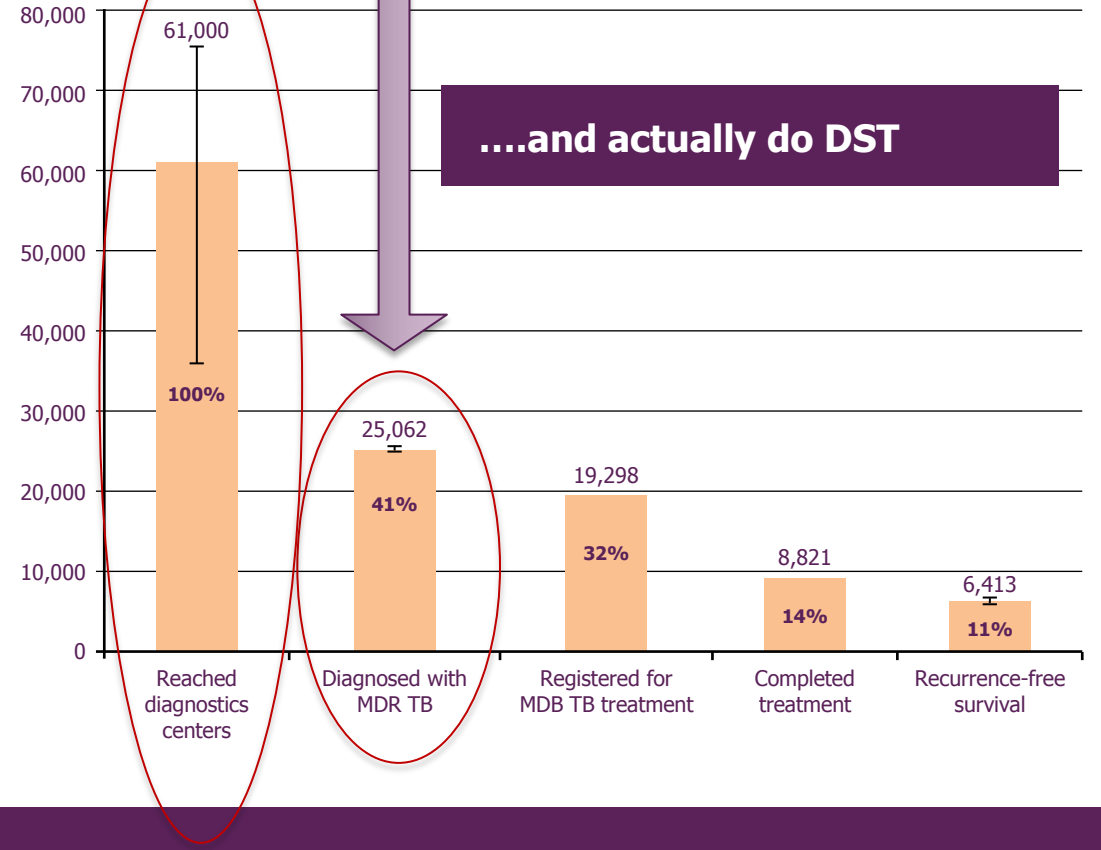
*11th October 2017, Union Meeting, Guadalajara*

# What are the diagnostic gaps in the care cascade?



1. **Detect more TB cases**
2. **Refer presumed MDR for testing.....**

....and actually do DST

**The Tuberculosis Cascade of Care in India's Public Sector: a Systematic Review and Meta-analysis**

Ramnath Subbaraman[1,2]*, Ruvandhi R. Nathavitharana[3,4], Srinath Satyanarayana[5,6], Madhukar Pai[5], Beena E. Thomas[7], Vineet K. Chadha, Kiran Rade[9], Soumya Swaminathan[10], Kenneth H. Mayer[3,11]

**Left chart (0 – 4,000,000)**

| Category | Value | Percent |
|---|---|---|
| Prevalent cases | 2,700,000 | 100% |
| Reached TB Diagnostic centers | 1,938,027 | 72% |
| Diagnosed with TB | 1,629,906 | 60% |
| Registered for treatment | 1,417,838 | 53% |
| Completed treatment | 1,221,764 | 45% |
| Recurrence-free survival | 1,049,237 | 39% |

**Right chart (0 – 80,000)**

| Category | Value | Percent |
|---|---|---|
| Reached diagnostics centers | 61,000 | 100% |
| Diagnosed with MDR TB | 25,062 | 41% |
| Registered for MDB TB treatment | 19,298 | 32% |
| Completed treatment | 8,821 | 14% |
| Recurrence-free survival | 6,413 | 11% |

# The needs in TB diagnostics

Meet patients where they present to care

Identify TB with highly accurate tests

Identify incipient TB before symptoms appear

Enable universal DST that informs regimen selection

Area of Interest

Overall Sensitivity

Specificity

TPP2: Triage test (Rule-out)

TPP 1: Rapid biomarker-based non-sputum-based test for

Optimal ★
Minimal ◆
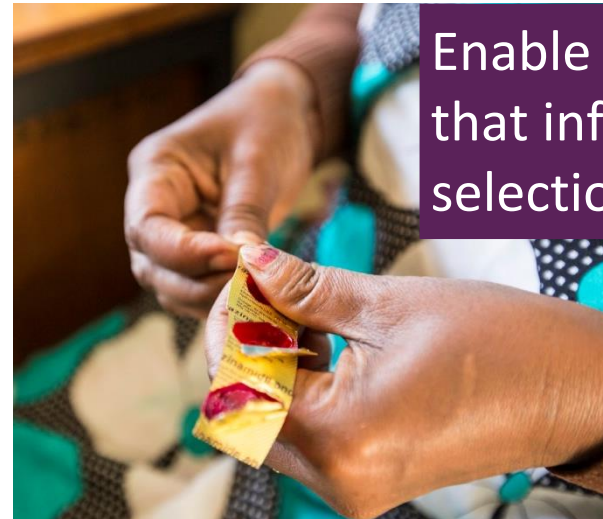
# Without finding the missing TB and MDR-TB cases, we will not bend the curve(s) and achieve End TB targets

Only 60% of TB cases detected and notified: 4.3 of 10.4 million TB cases missed

Only 20% MDR cases diagnosed or treated: 448,000 of 580,000 MDR cases missed



Current global TB incidence trend: -1.5%/year;

Optimal use of current and emerging dx tools + universal health coverage

-10%/year by 2025

Introduce new tools: Level 0 test, vaccine, identification & treatment for active and incipient TB

-5%/year

-17%/year

Rate per 100,000/year

TB incidence

DS-TB

MDR-TB

XDR-TB
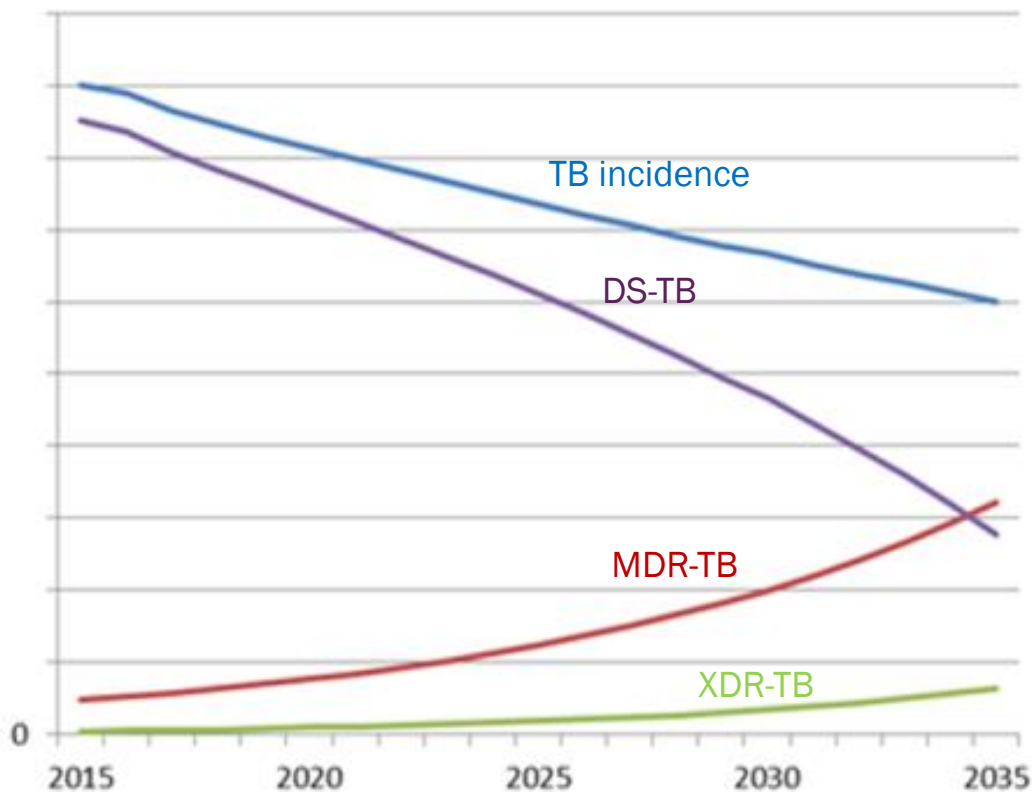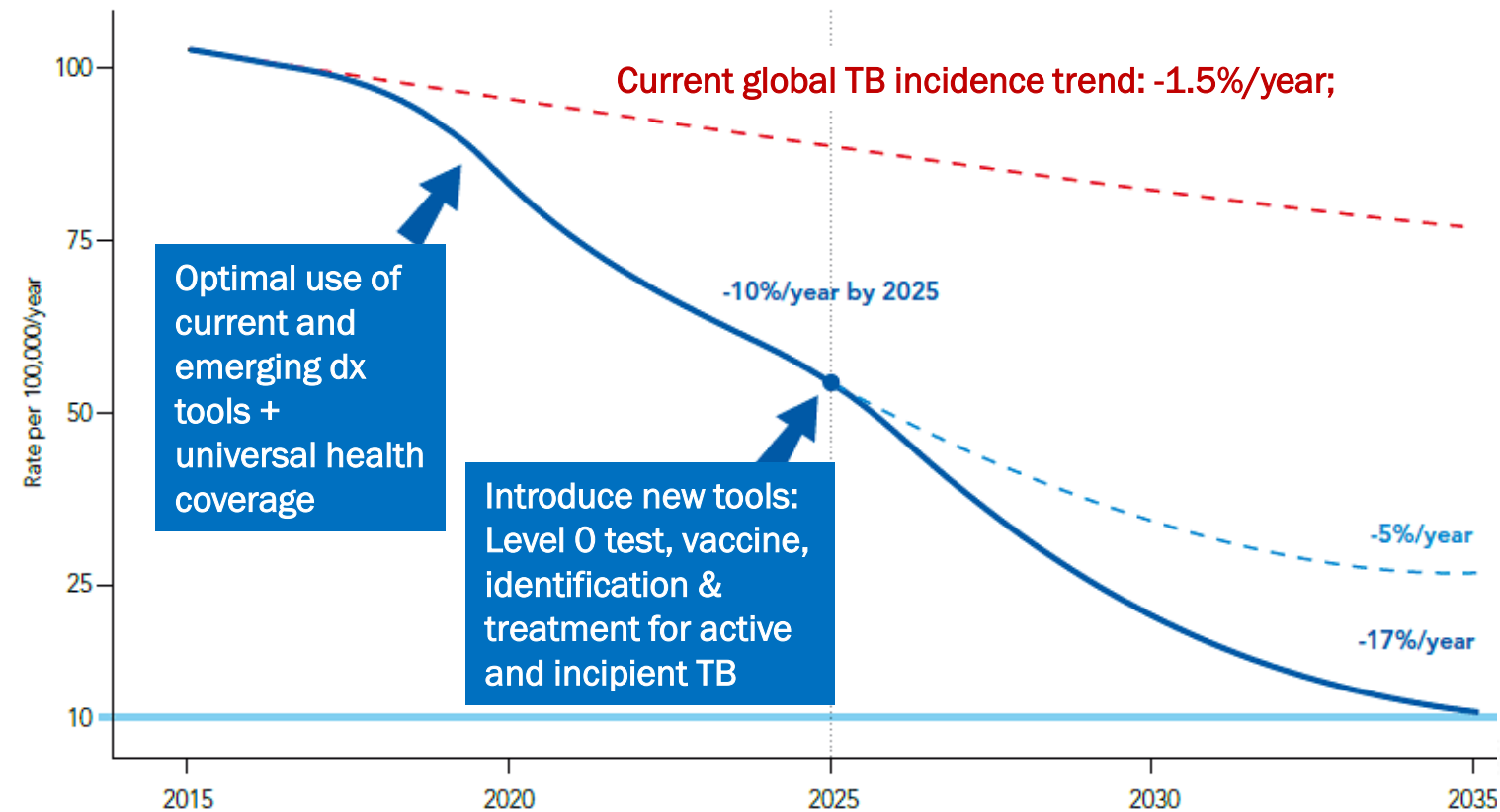
# Vision for TB diagnostic in 2020 – in 3 years!!



**First point of contact**
**Level 0/Level 1**

**Dedicated unit**
**Level 1/Level 2**

**Reference level/**
**Level 3**

1. Triage test at level 0/1
2. Highly sensitive detection at level 1

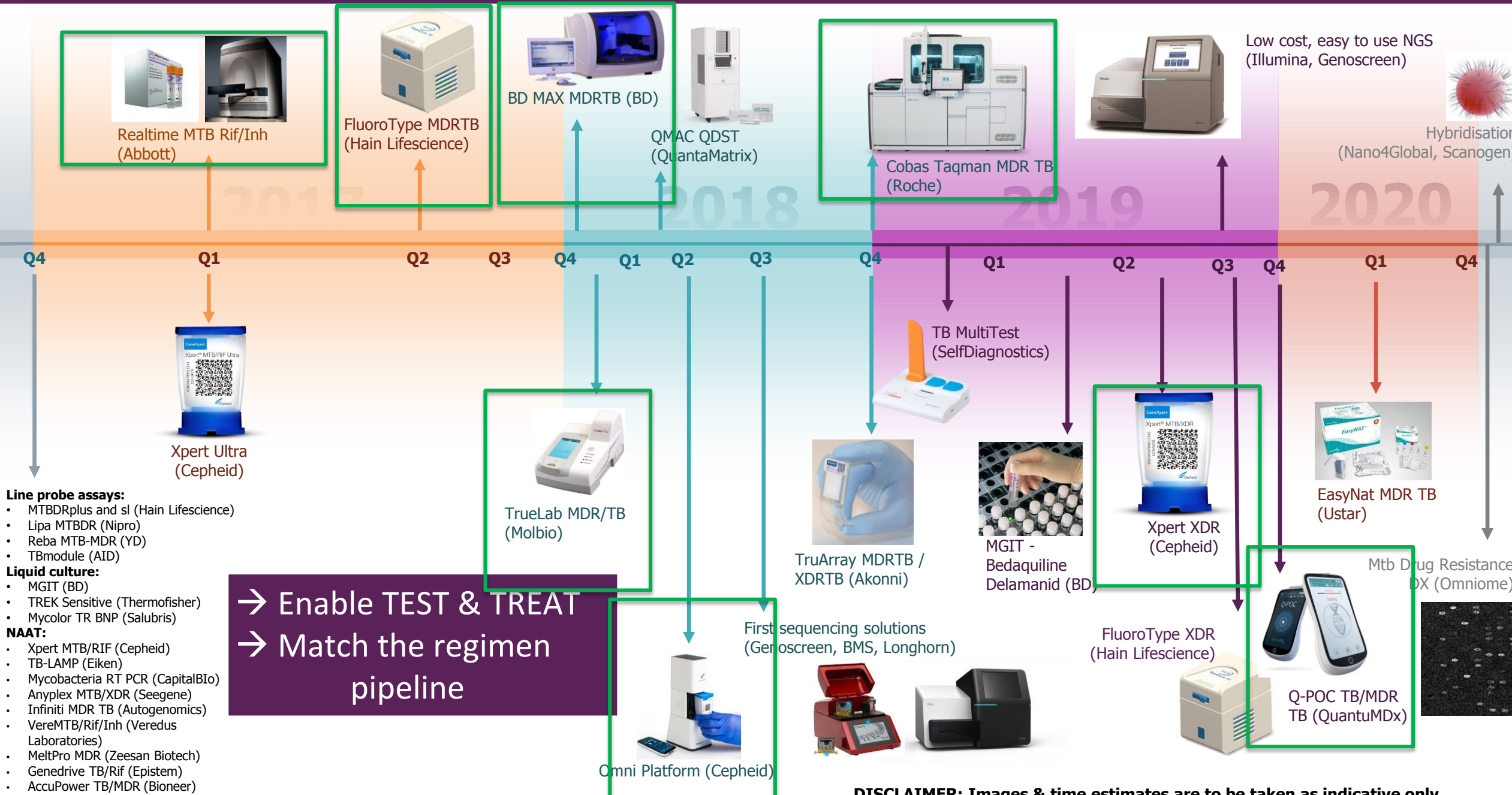TB confirmation with rapid integrated DST for critical drugs to drive regimen decisions

Comprehensive DST to cover the extended portfolio of old and new drugs

Fully integrated, connectivity-enabled solutions linking to treatment & systems strengthening; active case finding

TB

# Diversification of sputum-based testing and drug susceptibility testing (DST)



**Realtime MTB Rif/Inh (Abbott)**

**FluoroType MDRTB (Hain Lifescience)**

**BD MAX MDRTB (BD)**

QMAC QDST (QuantaMatrix)

**Cobas Taqman MDR TB (Roche)**

Low cost, easy to use NGS (Illumina, Genoscreen)

Hybridisation (Nano4Global, Scanogen)

**2017**  **2018**  **2019**  **2020**

Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q4

**Xpert Ultra (Cepheid)**

TB MultiTest (SelfDiagnostics)

**Xpert XDR (Cepheid)**

EasyNat MDR TB (Ustar)

**Line probe assays:**
- MTBDRplus and sl (Hain Lifescience)
- Lipa MTBDR (Nipro)
- Reba MTB-MDR (YD)
- 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 (Veredus Laboratories)
- MeltPro MDR (Zeesan Biotech)
- Genedrive TB/Rif (Epistem)
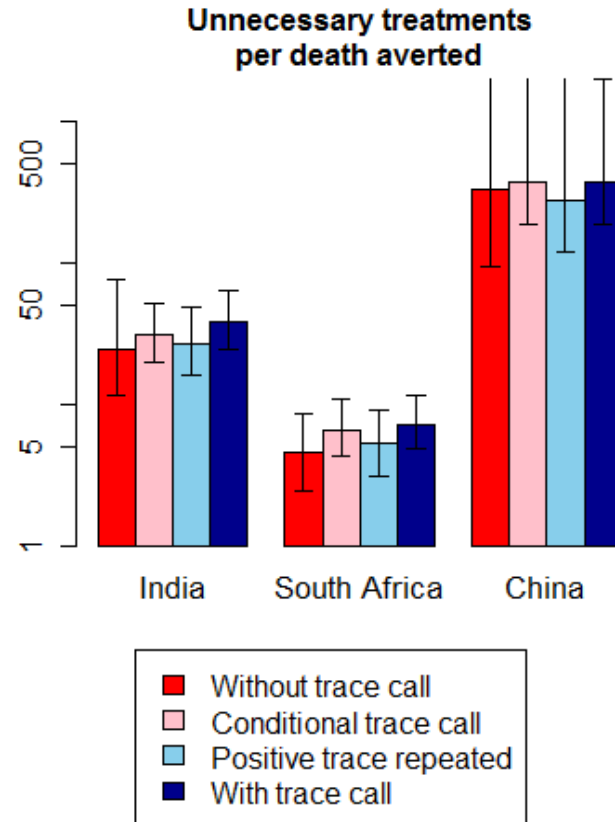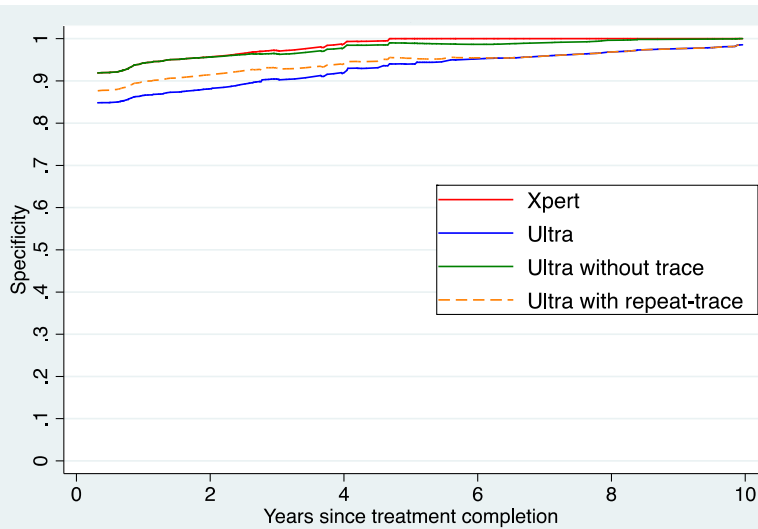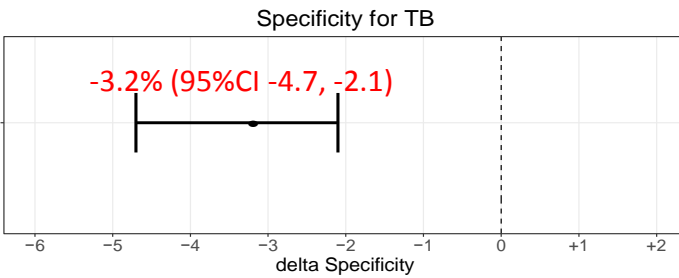- AccuPower TB/MDR (Bioneer)

**TrueLab MDR/TB (Molbio)**

TruArray MDRTB / XDRTB (Akonni)

MGIT - Bedaquiline Delamanid (BD)

FluoroType XDR (Hain Lifescience)

Mtb Drug Resistance DX (Omniome)

→ Enable TEST & TREAT
→ Match the regimen pipeline

First sequencing solutions (Genoscreen, BMS, Longhorn)

Q-POC TB/MDR TB (QuantuMDx)

Omni Platform (Cepheid)

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

# Xpert MTB/RIF Ultra – benefits & challenges



**Sensitivity for S−C+ TB**

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

NI-margin

delta Sensitivity

**Specificity for TB**

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

delta Specificity

- Study: 10 sites; 8 country study
  - Reference standard: 4-6 cultures
  - Direct comparison to Xpert

**Unnecessary treatments per death averted**

India    South Africa    China

- Without trace call
- Conditional trace call
- Positive trace repeated
- With trace call

Xpert Ultra
8

4         3

6

0

Xpert MTB/Rif          MGIT Culture

0              1

TB Negative n=107

Xpert
Ultra
Ultra without trace
Ultra with repeat-trace

Specificity

Years since treatment completion
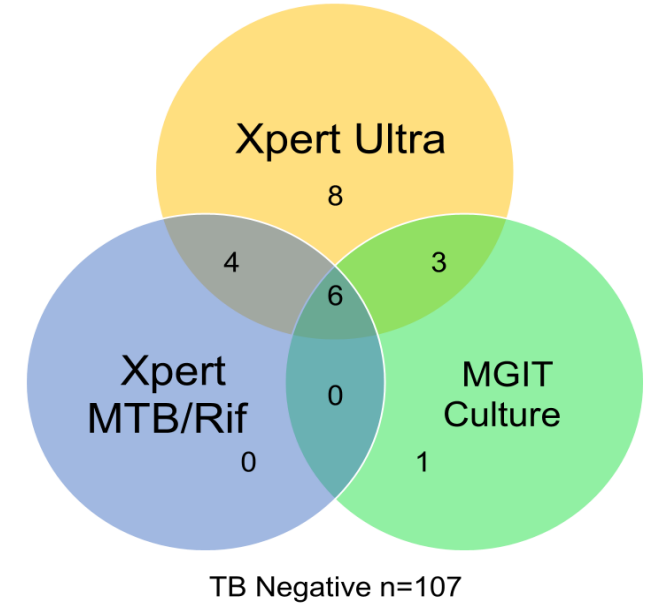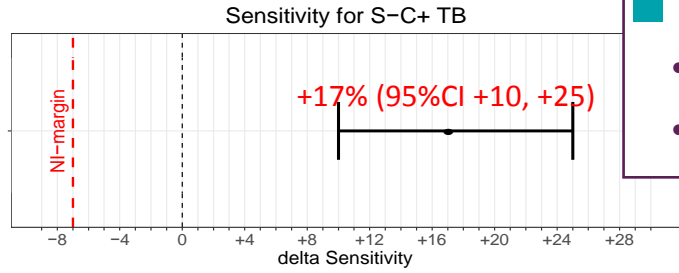
- TB meningitis study in Uganda
  - Ultra 95% sensitive vs Xpert & culture 45%

FIND Lancet ID submitted
FIND WHO report – available on FIND website
Kendall PLOS Med submitted
Bahr N Lancet ID 2017

# Molbio





- ■ TruePREP and TrueLAB utilizing a single-copy and multi-copy target
  - Ongoing studies with CTD assessing sensitivity
  - Ongoing operational study at microscopy center level with FIND
  - Ongoing performance assessment with FIND on frozen samples
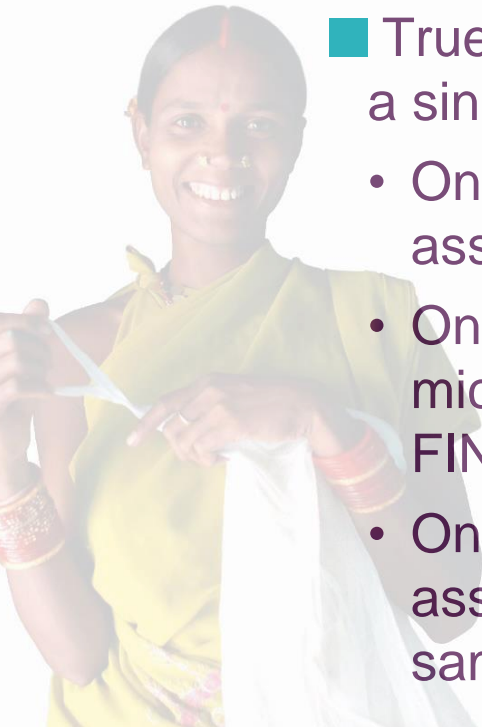
# QuantumDx

- ■ Sample processing and concentration using Dielectrophoresis (Capture-XT)
- ■ Molecular detection using Q-POC targeting 2 and 1 single-copy target
- ■ Time to market Q42019

| FIND Sample # | Q-POC |
|---|---|
| (3+) | + |
| (S-C+) | + |
| (S-C+) | + |
| (3+) | + |
| (3+) | + |
| (S-C+) | + |
| (Neg) | - |
| (2+) | + |
| (1+) | + |
| (3+) | + |
| (2+) | + |

# Centralized DST

- On WHO pathway for 2018

- RIF/INH resistance detection

- HAIN **Fluorotype**® **MTBDR** (CE-marked)

- Abbott RealTi*me* MTB (CE-Marked)

- BD Max MDR-TB

- Roche COBAS TaqMan

# Expanding the DST portfolio to enable new regimens

**■ Cepheid Xpert XDR cartridge, 2019**

- Will detect resistance to INH, FQ & Aminoglycosides
- Time to result approx. 90min
- Will require 10 colour modules (planned availability at launch on GeneXpert and Omni)

Hain FluoroType MTB/XDR**, 2019**

- Separate sample prep (manual or automated)
- On FluoroType XT platform – high throughput, centralized
- Time to result 2.5-4.5hrs + sample prep

Test principle of FluoroType® MTBDR

DNA extraction | Amplification and detection | Results in approx. 3 hours

Xie NEJM 2017

# Cepheid XDR

TB/RIF → (RIFs) → TB detected/FL regimen

TB/RIF → (RIFr) → INH/FQ/AG → (S) → Short regimen

INH/FQ/AG → (R) → Individualized Rx/ further testing

## Xpert XDR sensitivity and specificity compared against DNA sequencing by drug

| Drug | Xpert XDR | DNA Seq: Mutation | DNA Seq: No mutation | Sensitivity % (95% CI) | Specificity % (95% CI) |
|---|---|---|---|---|---|
| isoniazid[1] | Mutation | 151 | 0 | 98.1 | 100.0 |
| | No mutation | 3 | 149 | | |
| fluoro-quinolones[2] | Mutation | 91 | 0 | 95.8 | 100.0 |
| | No mutation | 4 | 208 | | |
| kanamycin[3] | Mutation | 39 | 0 | 92.7 | 100.0 |
| | No mutation | 3 | 256 | | |
| amikacin[3] | Mutation | 30 | 0 | 96.8 | 100.0 |
| | No mutation | 1 | 267 | | |
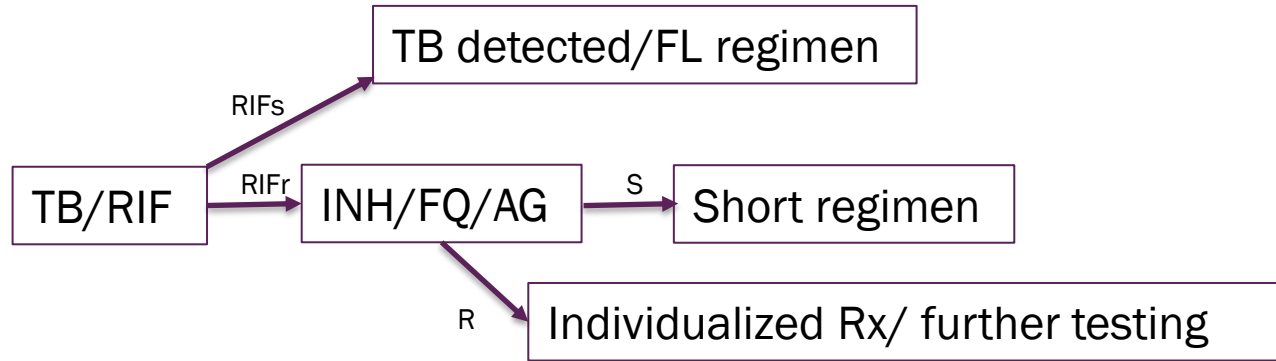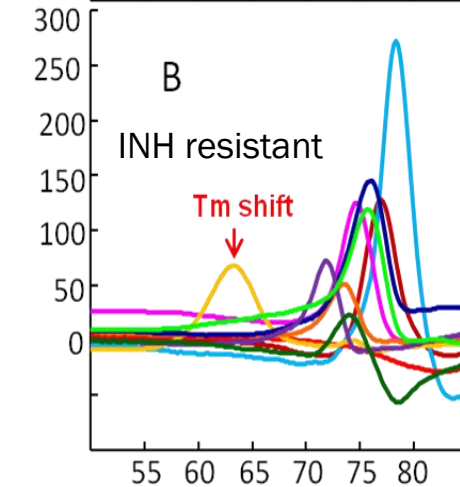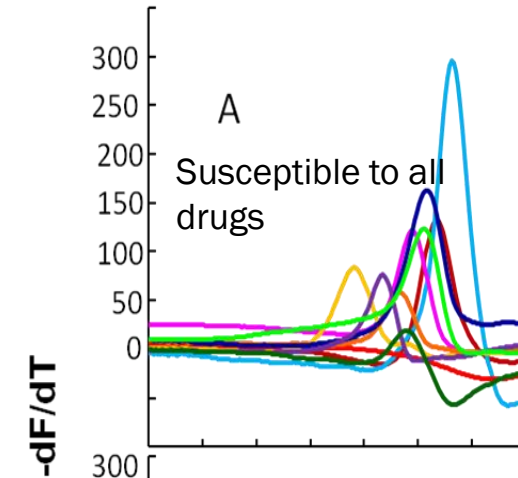
Assay design: 3-Phase, 8-plex, nested PCR, melt-curve analysis and sloppy beacon detection (10 color probe design).

Differentiates 32 mutant sequences in gyr A, gyr B, katG and rrs genes and the promotors of inhA and eis genes.

A — Susceptible to all drugs

B — INH resistant — Tm shift

rrs, inhA, gyrA1, gyrA2, katG, gyrB1, gyrB2, eis1, eis2, IC

# Sequencing – rapid and expanded DST



**Sample Preparation** → **NGS assay** → Targeted NGS

**Sequencing** → **Analysis** → Comprehensive molecular DST

WGS
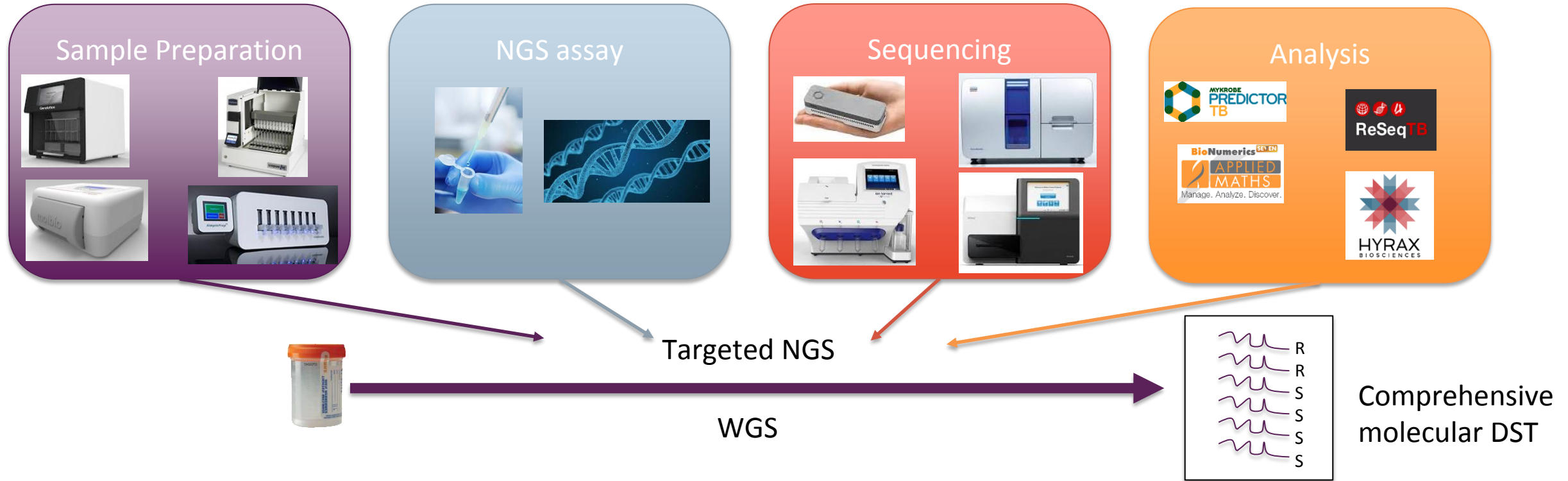
- Target product profiles close to completion
- Sample preparation – DNA extraction direct from sputum: Head to head evaluation of automated instruments
- TB sequencing assay – Head to head evaluation of 4 assays on Illumina, Thermo Fisher & Qiagen Seq tech
- NGS analysis – ReSeqTB: an effort in partnership with WHO, CPTR and BMGF
- Support for simplification of workflow

# Genotypic DST – what we know and don't know

Mutations in 8 Mtb gene regions predict clinically relevant drug resistant phenotypes with high specificity

| TB Drug/Drug Class | Gene/Regions | Sensitivity | Specificity | Best Dx use | |
|---|---|---|---|---|---|
| RIF | *rpoB* | ~93% | 97% | Rule in/out | ✔ |
| INH | *katG, inhA* | ~80-96% | 94% | Rule in | |
| FQ (LEV, OFX) | *gyrA/gyrB* | | | | |
| Injectables (KAN, AMK, CAP) | *eis, rrs* | | | | |
| PZA | *pncA* | | | | |
| Bedaquiline, Clofazamine | *atpE, pepQ* | | | | |
| Delamanid, Pretomanid | *ddn, fgd1, fbiA/B/C* | | | | |

WHO expert review on phenotypic reference standards and MICs integrating PK/PD and patient outcome information to revise critical concentrations
→ Better alignment with genotypic DST

Note: Low sensitivities thought to reflect limitations genetic causes of resistance.

# 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

**Active TB**

**Latent TB**

**2017**

**2018**

**2019**

**2020-2025**

**Pediatric TB Disposable Squeeze Bottle for Stool Processing prior Xpert**
(FIND, Elma Fdn & partners)

**Incipient TB tests (blood)**
- QIA-Predict (Qiagen)
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**Breath Tests and Skin Patches**
- breathtec/Technion
- RBS
- eNose

**Determine TB LAM Ag (urine)** for HIV co-infected with low CD4 counts
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**Next-generation LAM POC assays (urine, blood)**
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- and others

**Blood host marker POC tests**
- TransDot signature (ScreenTB consortium)
- SomaLogic signature (SomaLogic, FIND)
- and others
*Source: http://precision-bio.com/en/images/sub/sub2_1_3.jpg*

**TB antigen POC assays (blood)**
- Arizona State Univ.
- Tufts Univ.
- and others

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

**cfDNA in blood or urine**
- Standford Univ.
- Cornell Univ.
- Karius
- and others

**Negative recommendation for Serological assays by the WHO**

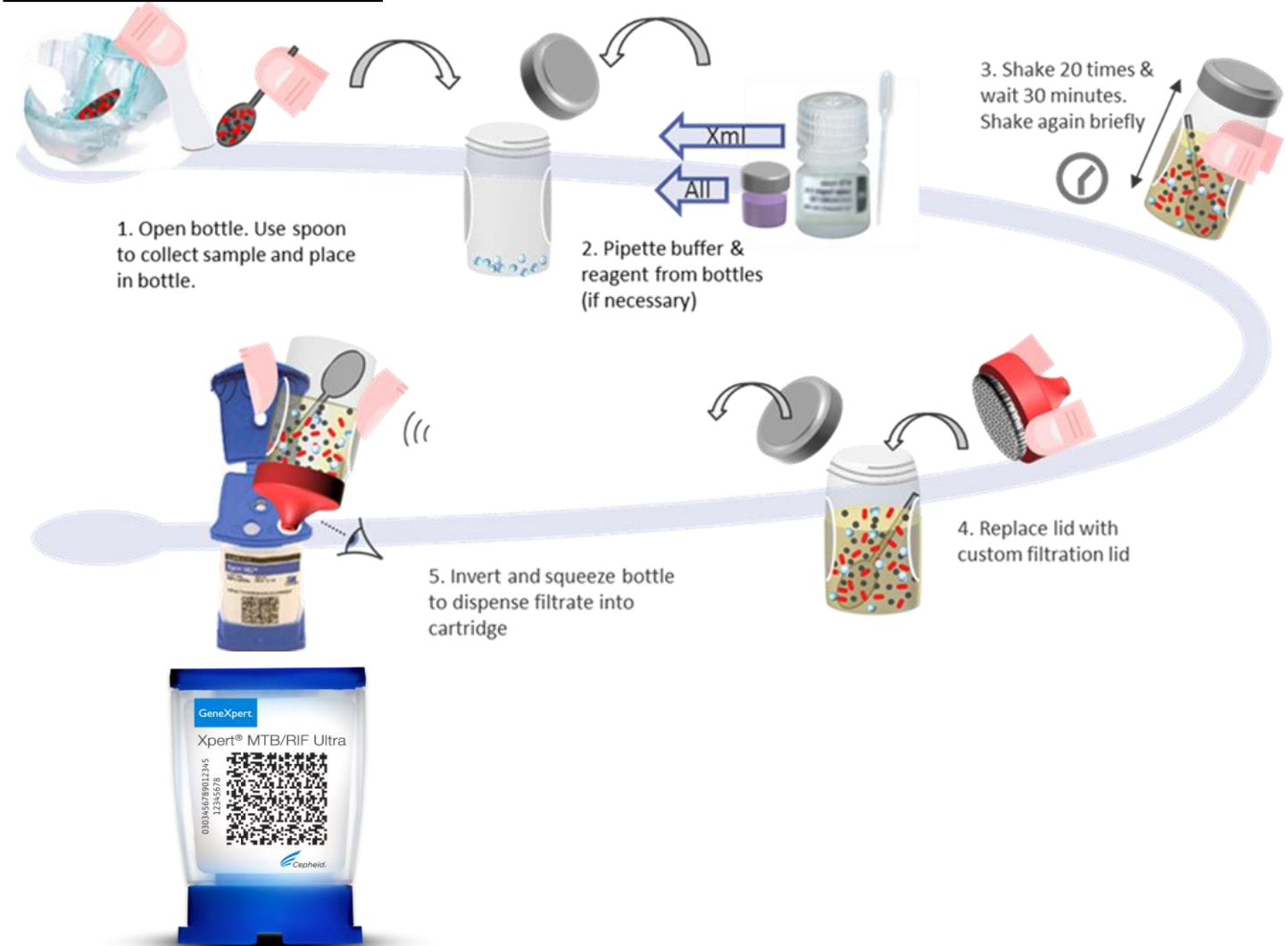**DISCLAIMER: Images & time estimates are to be taken as indicative only.**

# Stool testing with molecular tools

## The problem

- In 2015, ~ 1 million children with TB
- 170'000 children died of TB (excluding children with HIV).
- Lack of **effective** diagnostic tests that can be performed on **easily accessible** samples
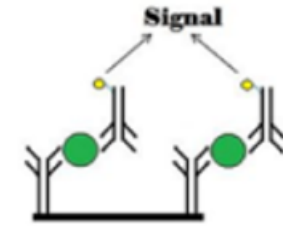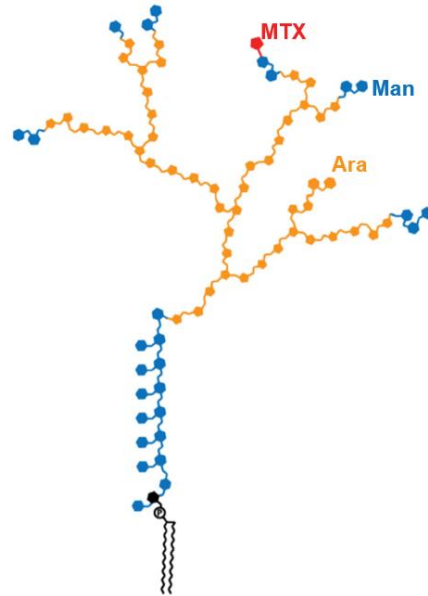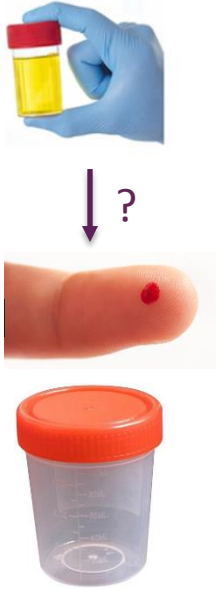
| Xpert vs. Culture | Sensitivity | Specificity |
|---|---|---|
| Respiratory | 71.4% (29.0%, 96.3%) | 98.1% (94.5%, 99.6%) |
| Swab rectal | 42.9% (9.9%, 81.6%) | 100.0% (97.7%, 100.0%) |
| 0.6 g stool | 71.4% (29.0%, 96.3%) | 98.7% (95.5%, 99.8%) |

## 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

GeneXpert
Xpert® MTB/RIF Ultra

# LAM – Progress on the development of a next generation test



### New sample types

- LAM in **sputum** for treatment monitoring and active case detection (CE-marked Otsuka ELISA, Sensitivity S+C+ 100%, S-C+ 70%, Specificity 94%)
- **LAM in serum requires pre-treatment** (Crawford et al. 2017, MSD unpublished)

### LAM structures in clinical samples

- **LAM structures differ** per sample type (unpublished)
- **Epitope mapping** and MS structural analysis lead to a better understanding of the most abundant epitopes per sample type (Univ. of Alberta, Colorado State Univ.)

### New reagents and detection platforms

- Generation of new **high affinity antibodies** (Rutgers Univ., AbCellera, FIND, OHSU, KI)
- Assays & platforms that reach **lower LOD's** (Fujifilm, GG/IVT, Univ. of Utah)
- **Sample prep./LAM concentration** devices (Salus Discovery, CERES Nano)
- Will lower LOD's lead **to higher sensitivity?** (more data expected this year)

# Blood-based markers – complex, high risk development



**Host proteins** · **Host RNA** · **Cellular stimulation** · **Pathogen protein** · **Antibodies**

| Concept | Feasibility | Development | Validation | Trialing |
|---|---|---|---|---|
| Complex signature<br>Signatures<br>FTO | Reagents transfer<br>Sample prep<br>Access | Reagents performance<br>No standard<br>COGS | Diagnostic perf.<br>Reproducibility | Diagnostic perf.<br>Usability |

**RISK**
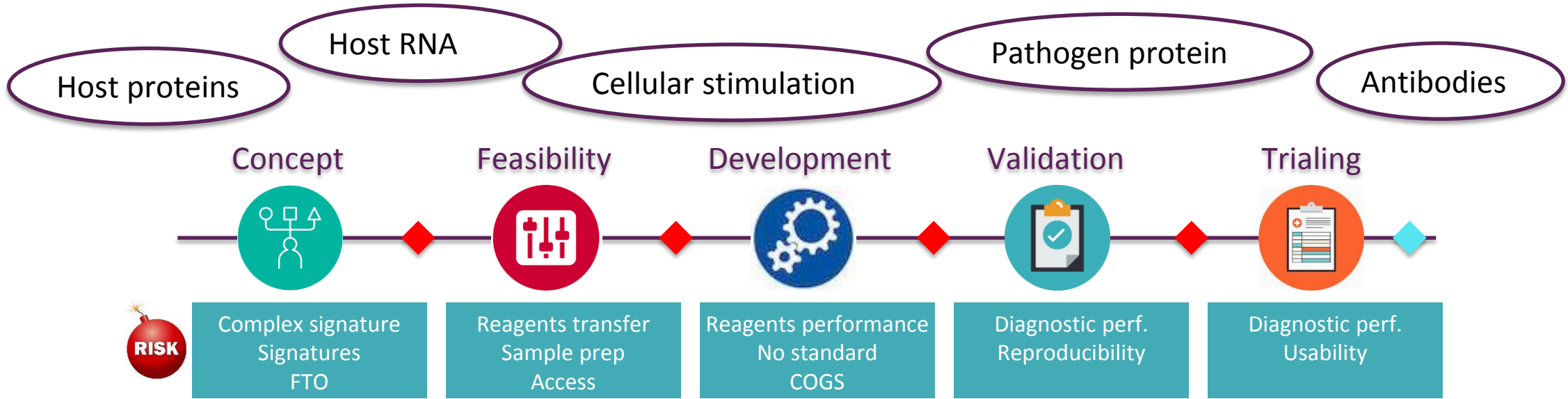
**SomaLogic**
- Non-biased proteomic search
- 1500 sera
- 4000 proteins
- 3-9 markers

?
Performance
Operational characteristics
Cost

# Breath testing

## RBS and eNose solutions selected for feasibility study

Cough/aerosol collection combined with immunoassay based antigen detection [McNerney et al. 2010]

Rapid Biosensor Systems

The eNose company

Metal-oxide-based olfactory sensor [Bruins et al. 2013]
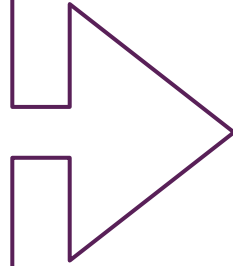
- Prospective, multicentre study to assess the performance, operational characteristics and acceptability of the two selected testing platforms

- 2 countries with medium/high prevalence of TB: Peru and South Africa

- ~240-360 participants enrolled
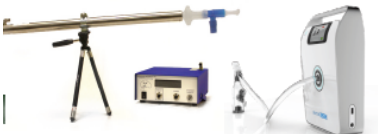
Portable GC coupled to surface acoustic wave (SAW) detection (Philllips, 2013)

$^{13}C$-urea is converted to $^{13}CO_2$ in the patients lung if bacteria are present and detected in a spectrometer (Jassal 2010)

Nanomaterial-based sensors conducting a measurable electrical signal upon TB VOC adsorption (patent WO2014068554A1)

### Timeline

24 weeks | 16 weeks | 5 weeks

Platforms testing ( already completed in South Africa) | Culture results | Data analysis study report

September 2017 | December | March | April 2018
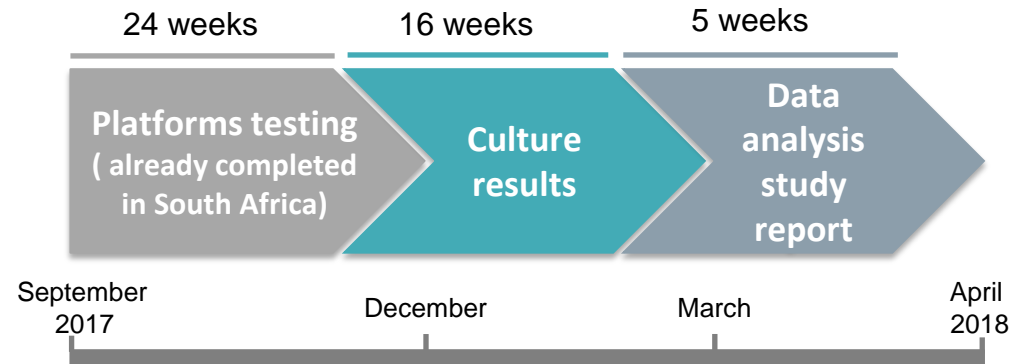
# Computer-assisted reading of CXR

**CHEST RADIOGRAPHY IN TUBERCULOSIS DETECTION**

Summary of current WHO recommendations and guidance on programmatic approaches
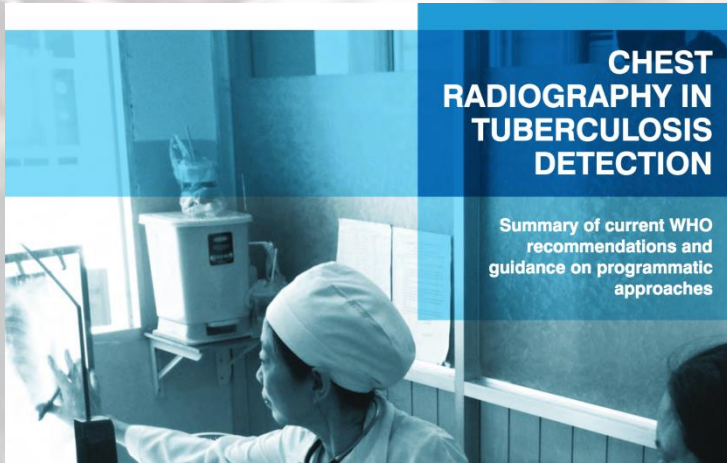
*"CAD can be used for TB detection for research, ideally following a protocol that contributes to the required evidence base for guideline development."*

CAD4TB

CAD4TB Heat Map

Abnormal

Qure.ai

Symptomatic screening

Immigration screening

Prevalence surveys
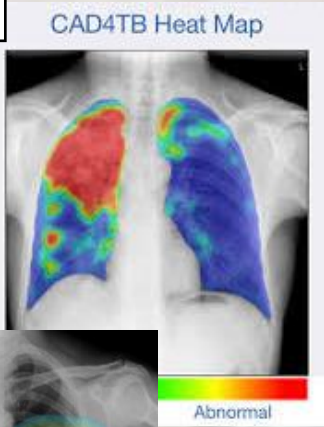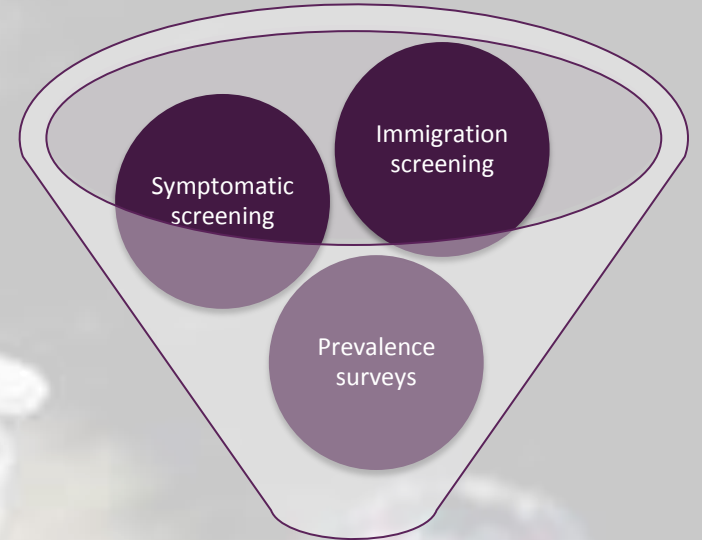
**Standardized panel of DICOM files**
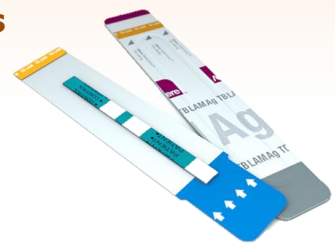
- Rapid comparative assessment of versions and new tools
- Assessment across different use cases: triage versus screening
- Reduction of bias

Khan ERJ 2016

# Incipient TB

**Detecting TB before symptoms appear**

**2017**    **2018**    **2019**    **2020-2025**

**Pediatric TB Disposable Squeeze Bottle for Stool Processing prior Xpert**
(FIND, Elma Fdn & partners)

Active TB

Latent TB

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

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

**Breath Tests and Skin Patches**
- breathtec/Technion
- RBS
- eNose

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

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- and others

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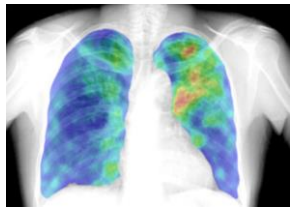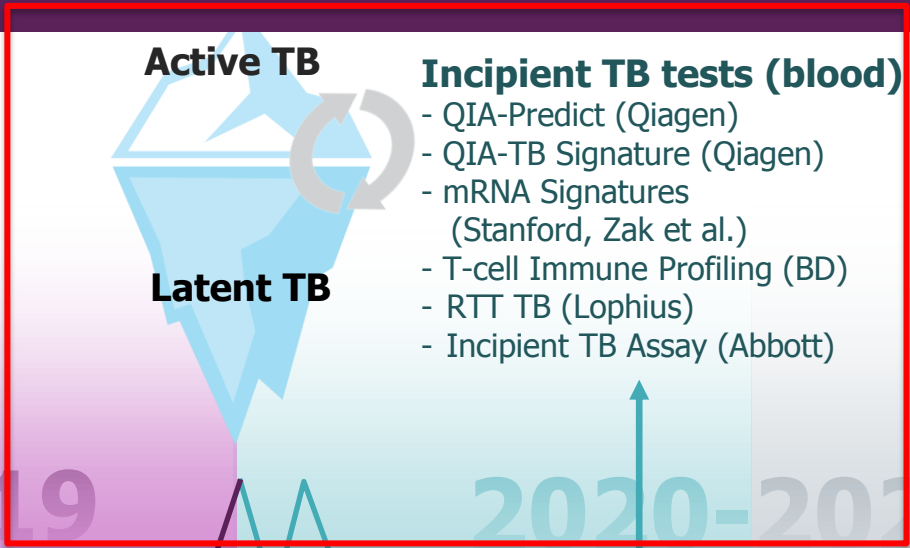
*Source: http://www.delft.care*

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

*Source: http://precision-bio.com/en/images/sub/sub2_1_3.jpg*

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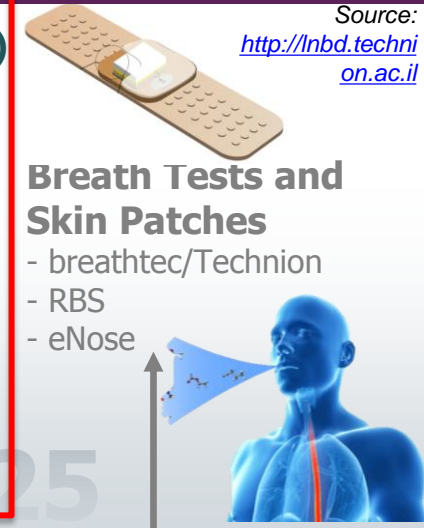- Tufts Univ.
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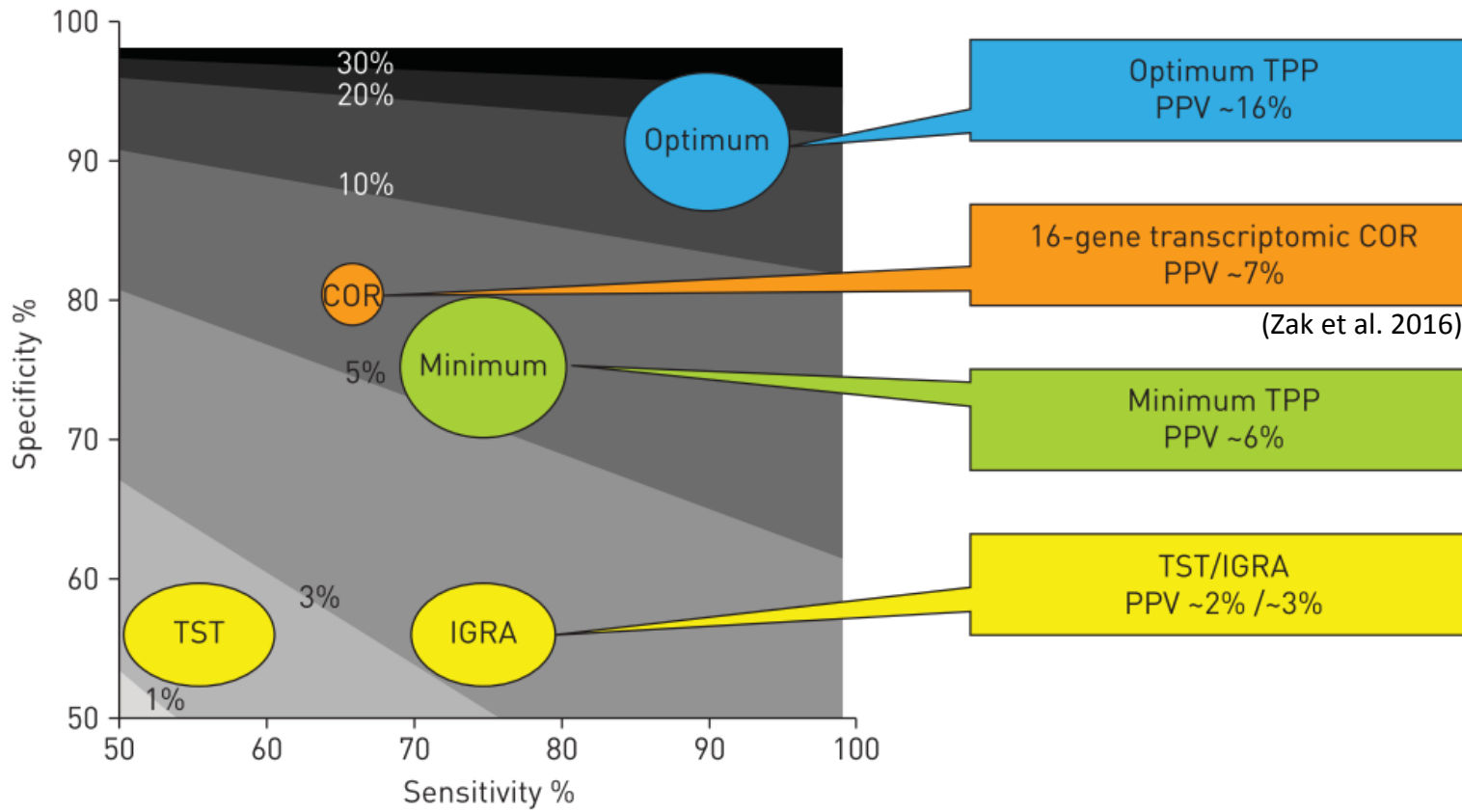- and others
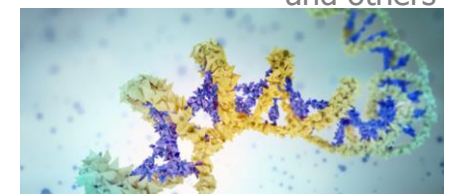
*Source: https://www.whatisepigenetics.com*

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

# Incipient TB –risk of progression

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

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

- Market Entry ≥2020
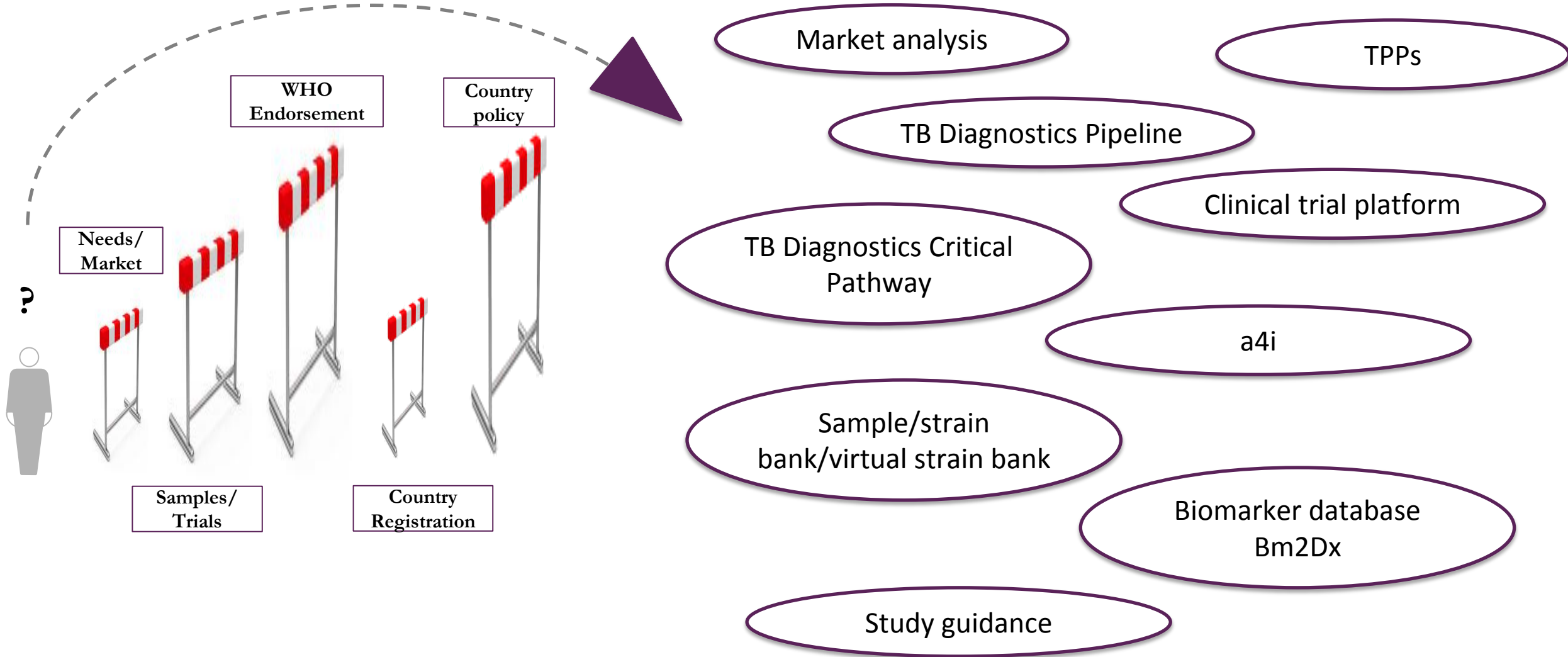


**Products in pipeline**
- QFT-Plus and QFT-Predict (Qiagen)
- QIA-TB Signature (Qiagen)
- T-cell Immune Profiling (BD)
- RTT TB (Lophius)
- Incipient TB Assay (Abbott)
- and others

**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)

# Overcoming key bottlenecks – supportive tools

Needs/Market

WHO Endorsement

Country policy

Samples/Trials

Country Registration

Market analysis

TPPs

TB Diagnostics Pipeline

Clinical trial platform

TB Diagnostics Critical Pathway

a4i

Sample/strain bank/virtual strain bank

Biomarker database Bm2Dx

Study guidance

# TB Diagnostics Critical Pathway

- A virtual knowledge management tool for TB diagnostics
  - Realistic guide for innovators along the pathway to market
  - Identify key stakeholders & resources at each stage
  - Providing key information: TPPs, reports, market data, guidance for clinical trials, and documents used for WHO review
- A communication tool for the TB community at large
- A living document, updated regularly
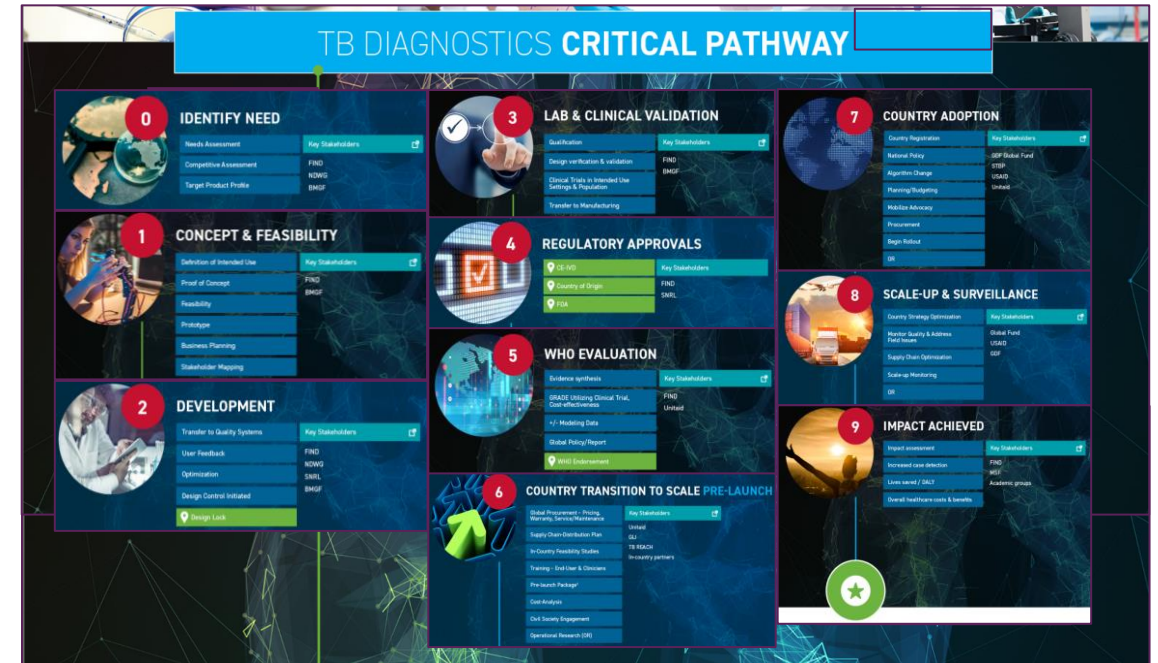


www.tbdxpathway.org

Beta version – your feedback welcome!

# TB diagnostics pipeline

- Is a comprehensive source of information on TB diagnostics activities across the different stages of the diagnostics critical pathway

- A communication tool for the TB community at large

- A living document, updated regularly

https://www.finddx.org/dx-pipeline-status/
https://www.finddx.org/dx-pipeline-timeline/

Beta version – your feedback welcome!

The TB Diagnostic Pipeline Tracker will guide developers and implementers to further innovation, rollout and uptake

# Strain and virtual strain bank



Inactivated strains for assessment of
- LOD
- Drug resistance

**Molecular Developers Toolset**
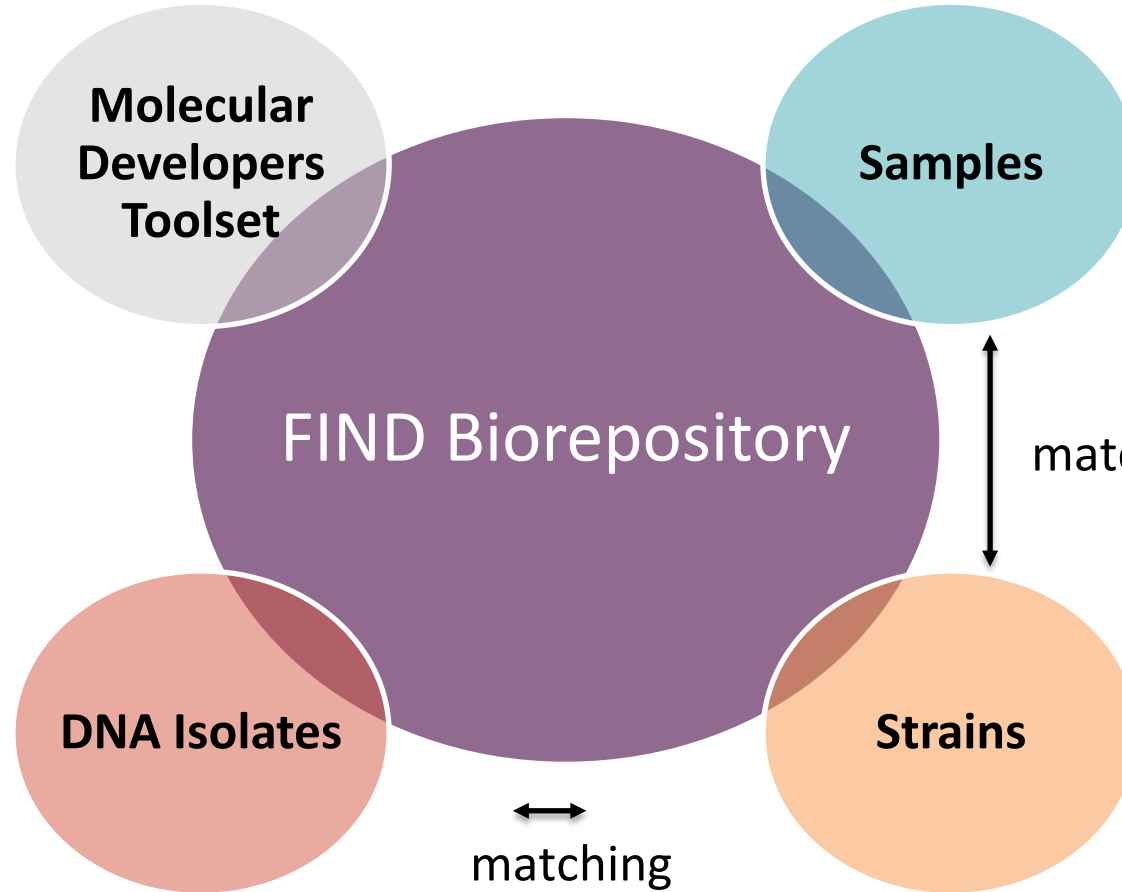
**Samples**
- Sputum
- Urine
- Plasma & Serum
- PAX gene tubes
- PBMC

## FIND Biorepository

matching

- MDR
- Pre-XDR

**DNA Isolates**

matching

**Strains**
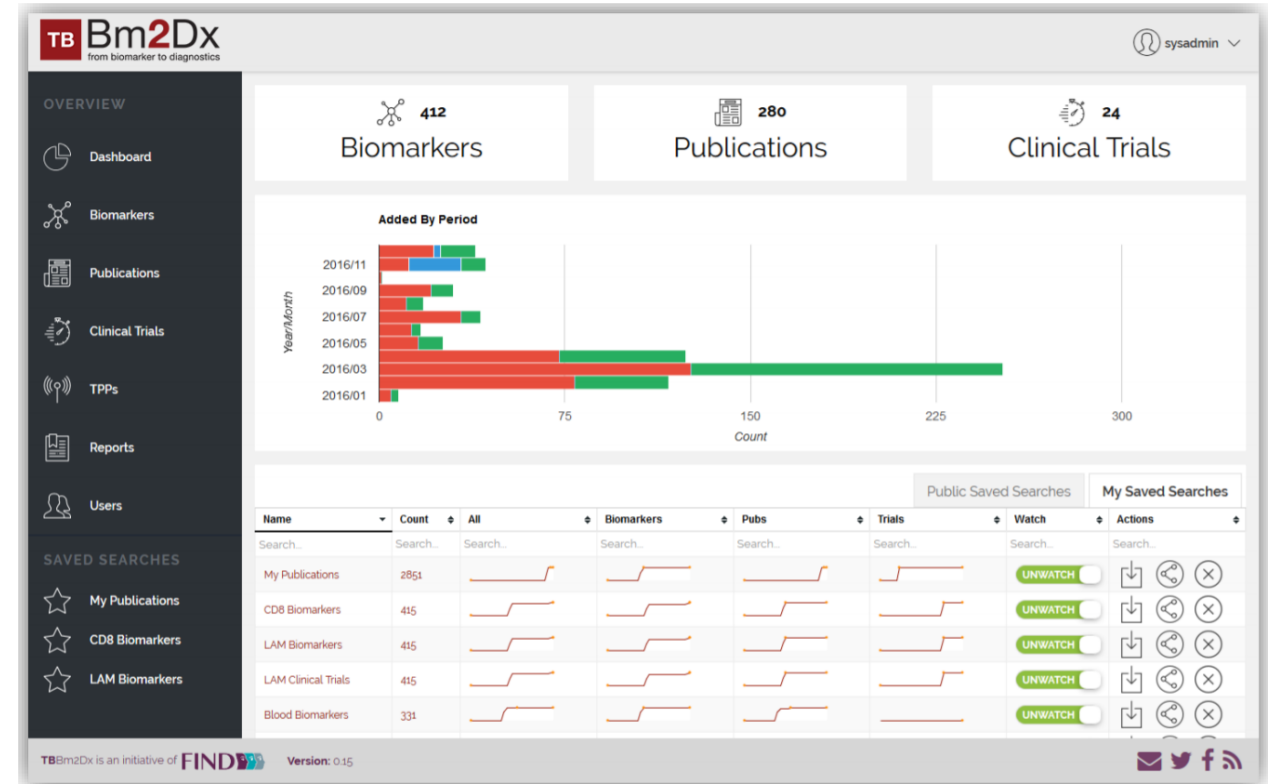- MDR
- Pre-XDR

**Bm2Dx — from biomarker to diagnostics**

**FUNCTIONALITY**
- **Synthesize** biomarker information from published and unpublished sources
- Allow the **analysis** of biomarkers in a user-friendly and **flexible manner**
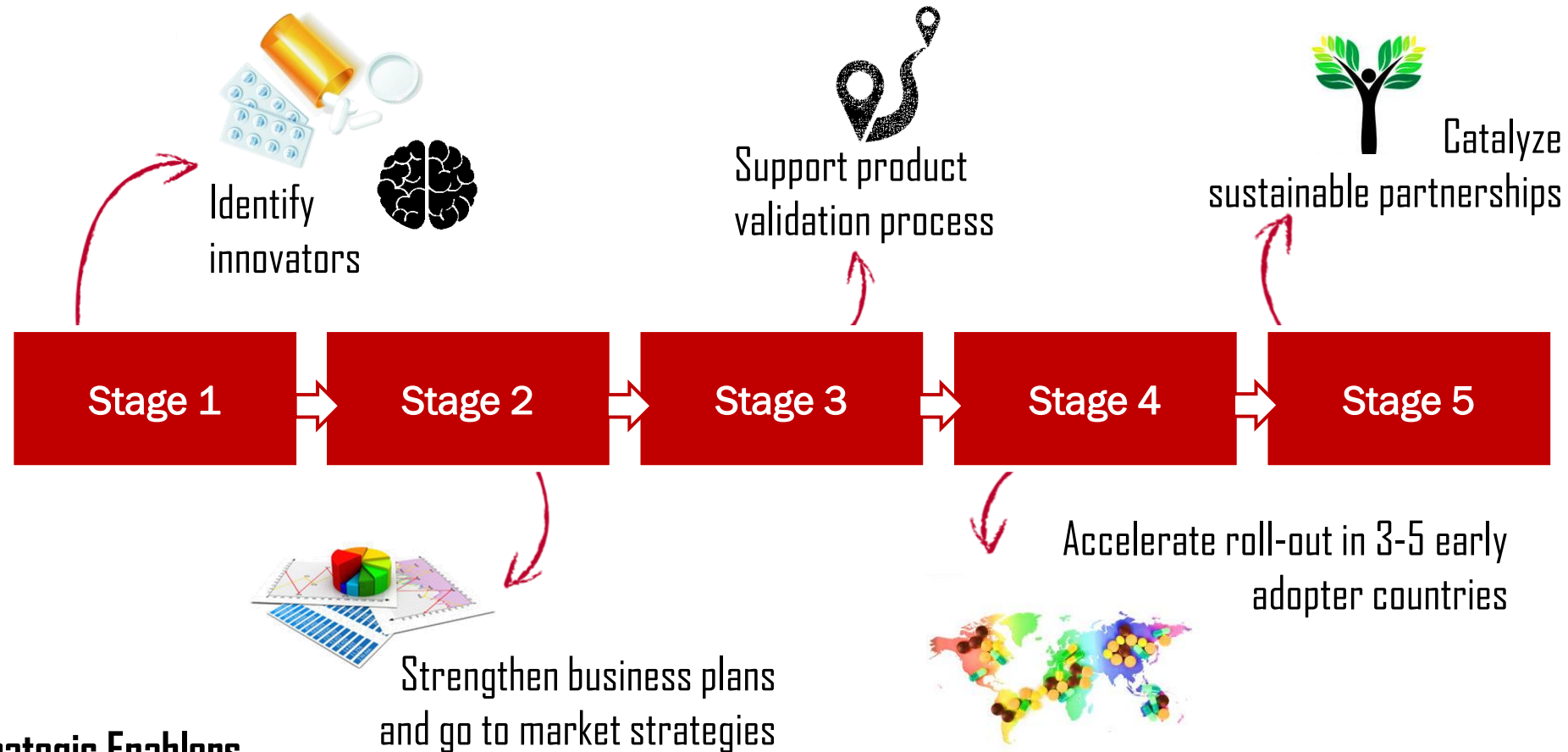- Allow to explore **combinations of biomarkers**

**VALUE**
- **One-stop resource** for relevant biomarker information
- **Accelerate the development of new diagnostics** through linkage to biobanks and clinical trials platform
- Facilitate **better decision making** to **focus resources**

Bm2Dx public version launch: January 2018

**a4i consists of 5 sequential stages**

Identify innovators

Support product validation process

Catalyze sustainable partnerships

| Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |

Strengthen business plans and go to market strategies

Accelerate roll-out in 3-5 early adopter countries

**Strategic Enablers**

- Diagnosis remains a key challenge for the End TB Strategy
- We need to reach patients with diagnostic tests where they first present
- Novel tools need to match treatment guidelines and regimen pipeline.

# QUESTIONS?

**THANK YOU**