Next-generation testing for pulmonary

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Disclosures

- No relevant industry financial relationships

Learning objectives

- Explain current recommendations and available tools for rapid diagnosis of pulmonary TB in the United States and globally

- Improve knowledge of the progress and advances in development of novel diagnostics for pulmonary TB
  - Molecular testing
  - Urine LAM tests

Millions of patients today do not have access to TB diagnosis

Gap: 4.1 million

5.9 million diagnosed and treated

71% detected

WHO Global TB Report 2021
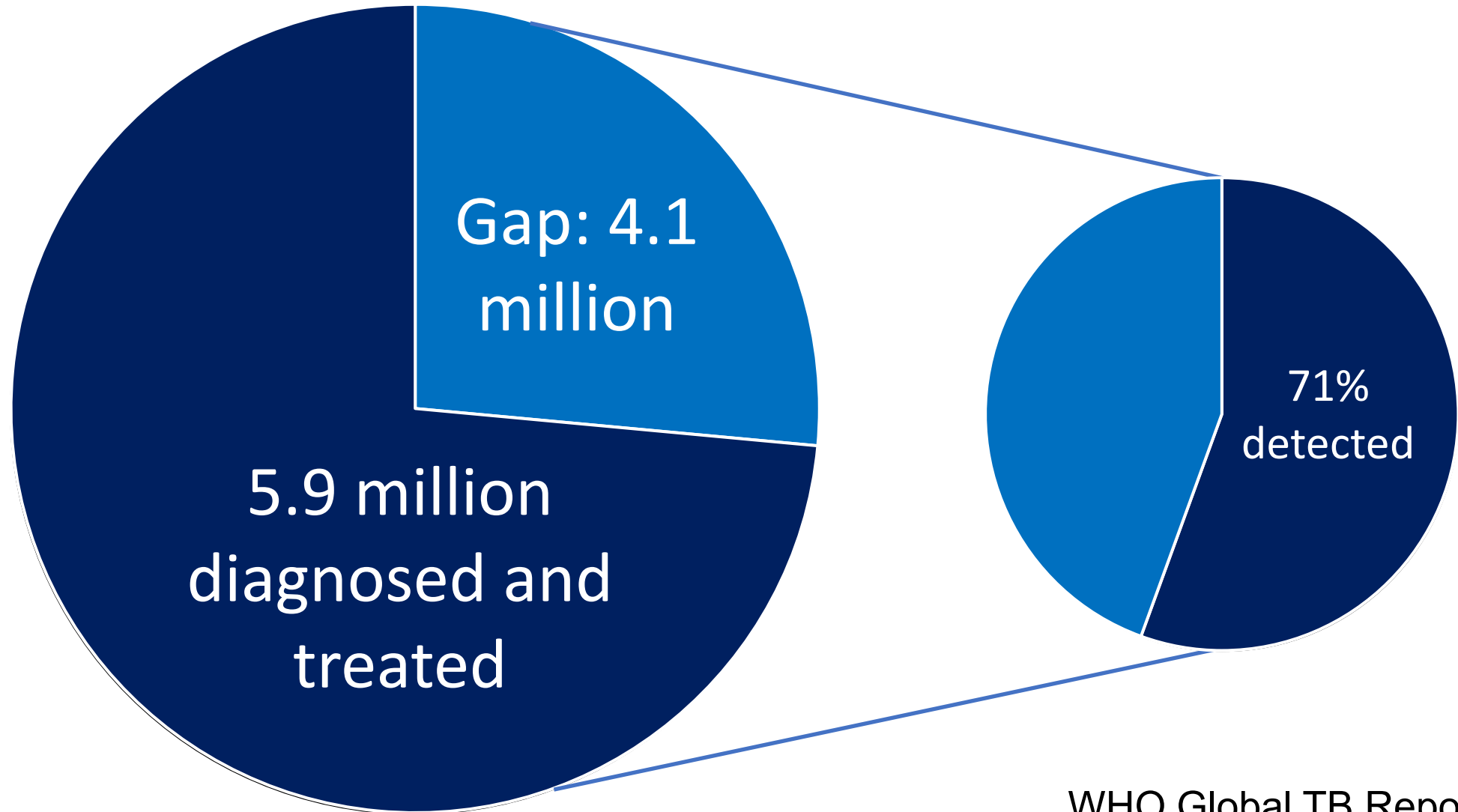
Tests for pulmonary TB: Current guidelines

ATS/IDSA/CDC 2017

- Sputum smear microscopy (strong recommendation)

- Liquid AND solid culture (strong recommendation)

- Molecular test for diagnosis (conditional recommendation)

- Molecular test for RIF +/- INHr (strong recommendation)

WHO 2021

- Rapid molecular test as first-line (strong recommendation)

- Universal testing for RIF +/- INHr (strong recommendation)

- Urine LAM for HIV+ inpatients (strong recommendation)

- Urine LAM for HIV+ outpatients (conditional recommendation)

Rapid tests for pulmonary TB: Current options

United States (FDA-approved)

- Molecular tests
  - Xpert MTB/RIF
  - 2 moderate complexity assays

Global (WHO-endorsed)

- Molecular tests
  - Xpert MTB/RIF
  - TB LAMP*
  - Xpert MTB/RIF Ultra
  - **Truenat MTB/MTB Plus and MTB-RIF***
  - **4 moderate complexity assays***
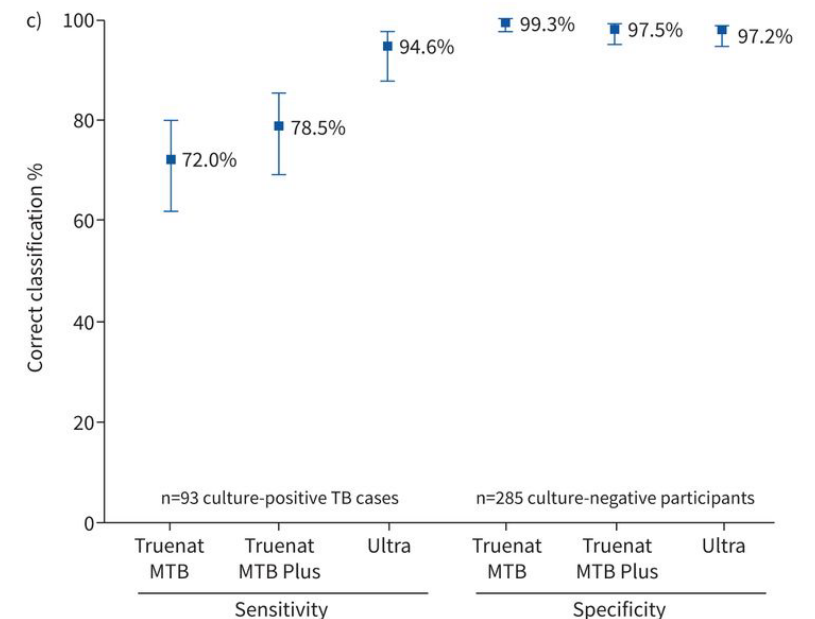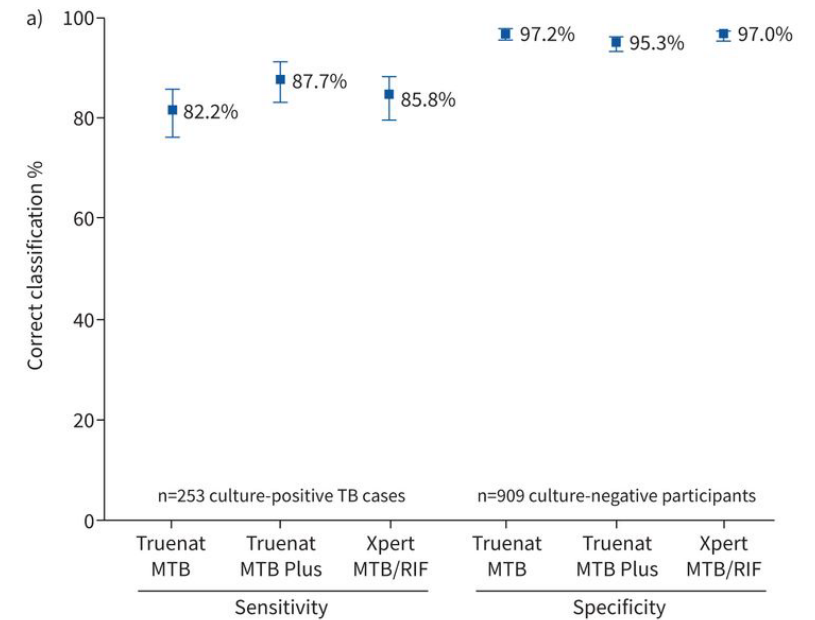- Determine TB-LAM for PLHIV (inpatients, outpatients*)

*Conditional recommendation

Truenat (Molbio, India)

- Automated, battery-operated devices for DNA extraction and PCR

- Disposable PCR chip (MTB, MTB Plus, MTB RIF)

- Results in less than one hour

- Similar performance to Xpert in a trial including 1800 participants at 19 sites in 4 countries

1. Extract — Trueprep Auto
2. Load — Truenat
3. Read — Truelab UnoDx

First serious competitor to Xpert MTB/RIF

a)

n=253 culture-positive TB cases — Sensitivity: Truenat MTB 82.2%, Truenat MTB Plus 87.7%, Xpert MTB/RIF 85.8%

n=909 culture-negative participants — Specificity: Truenat MTB 97.2%, Truenat MTB Plus 95.3%, Xpert MTB/RIF 97.0%

c)

n=93 culture-positive TB cases — Sensitivity: Truenat MTB 72.0%, Truenat MTB Plus 78.5%, Ultra 94.6%

n=285 culture-negative participants — Specificity: Truenat MTB 99.3%, Truenat MTB Plus 97.5%, Ultra 97.2%

Penn-Nicholson A et al, ERJ 2021

Moderate complexity automated PCR tests

Table 2.3: Head-to-head comparisons between index and WHO-endorsed assays for the detection of *M. tuberculosis*

Index test	Smear status	# Datasets (# specimens)	Sensitivity (95% CI)	Specificity (95% CI)
Head to Head comparisons (Abbott MTB and Xpert MTB/RIF)				
Abbott RealTime MTB	All	3 (685)	Range: 79%-100%	Range: 84%-97%
Xpert MTB/RIF	All	3 (685)	Range: 82%-97%	Range: 90%-100%
Head to head comparisons (FluoroType MTB and Xpert MTB/RIF)				
Hain FluoroType MTB	All	1 (296)	89 % (79-95)	60% (53-66)
Xpert MTB/RIF	All	1 (296)	79% (67-87)	94% (90-97)
BD Max MDR-TB	All	1 (575)	95% (92-98)	72% (66-77)
Xpert MTB/RIF	All	1 (575)	95% (92-97)	73% (68-78)
Head to head comparisons (cobas 6800/8800 MTB and Xpert MTB/RIF)				
Roche cobas MTB	All	1 (294)	95%(88-98)	97% (94-99)
Xpert MTB/RIF	All	1 (294)	92% (84-96)	97% (94-99)

Definitions: CI: Confidence interval; # = number of

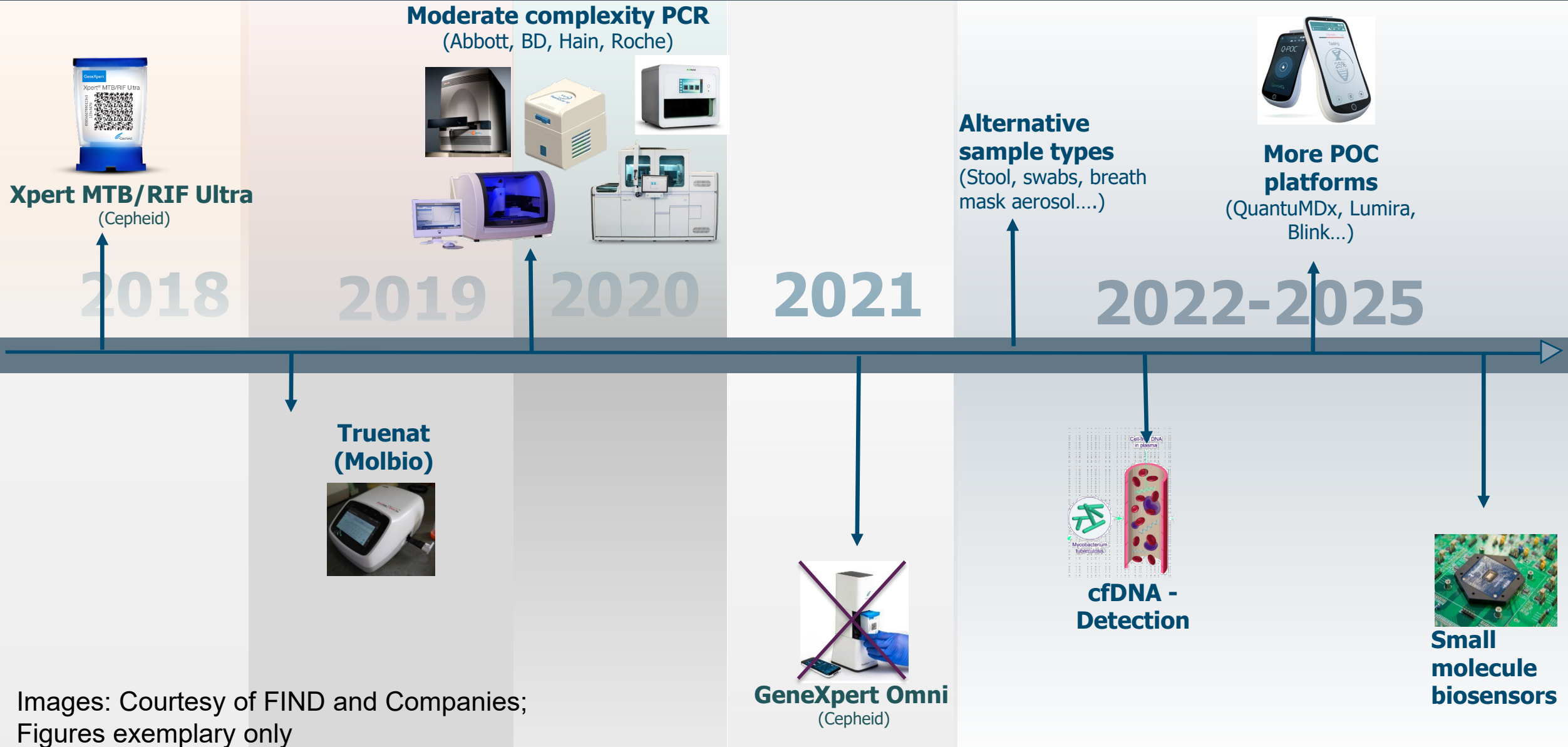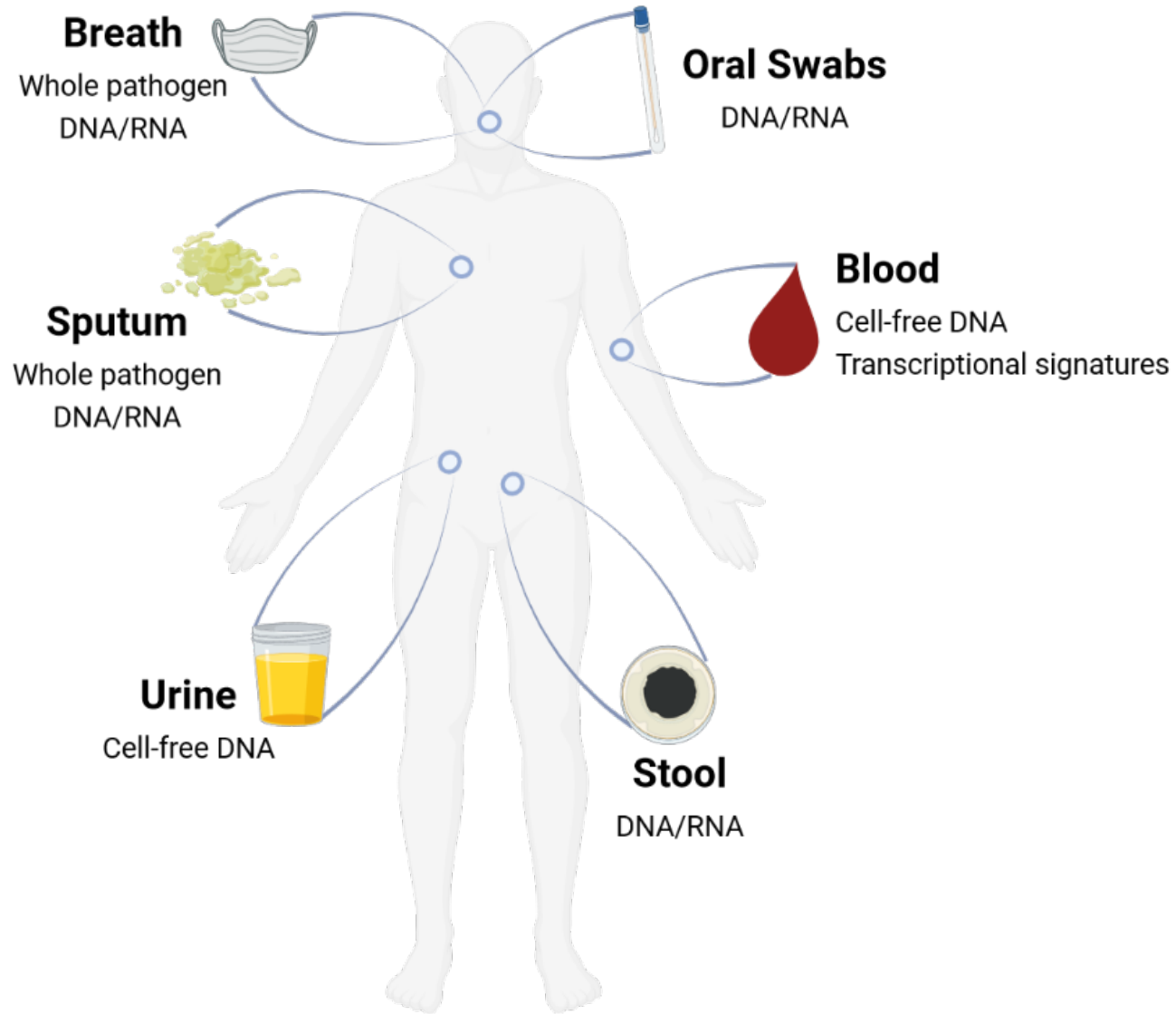Centralized Assays – can we leverage economies of scale?

Kohli M et al, ERJ 2020
WHO Guidelines 2021
de Vos M, J Clin Microbiol 2021

Fast forward: Diversification of molecular testing

Moderate complexity PCR
(Abbott, BD, Hain, Roche)

Alternative sample types
(Stool, swabs, breath mask aerosol….)

More POC platforms
(QuantuMDx, Lumira, Blink…)

Xpert MTB/RIF Ultra
(Cepheid)

2018   **2019**   **2020**   **2021**   **2022-2025**

**Truenat
(Molbio)**

GeneXpert Omni
(Cepheid)

cfDNA - Detection

Small molecule biosensors

Images: Courtesy of FIND and Companies;
Figures exemplary only

Novel Samples Types for Molecular TB Detection

Breath — Whole pathogen DNA/RNA

Oral Swabs — DNA/RNA

Sputum — Whole pathogen DNA/RNA

Blood — Cell-free DNA, Transcriptional signatures

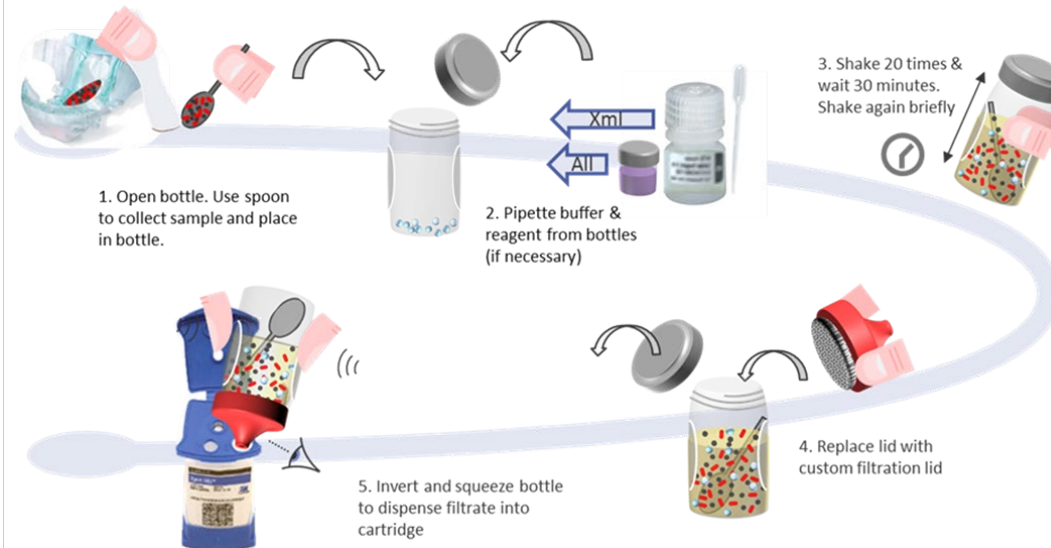Urine — Cell-free DNA

Stool — DNA/RNA

Stool PCR testing – a novel sample type in children

The problem

- |~ **1.1 million** children with TB

- **~220,000** children died of TB (excluding children with HIV)

- No <u>effective</u> tests that can be performed on <u>easily accessible</u> samples

Walters J et al, Clin Micro 2018;
Banada PP et al, PLOSone 2016;
Trial publication, in prep

The solution – stool preprocessing

Process Overview:

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

Diagnostic accuracy against MRS (6 sites in 4 countries)

	N	Sensitivity (95% CI)	Specificity (95% CI)
Ultra/SOS	332	52.1 (38.3-65.5)	97.5 (94.9-98.9)
Ultra/SPK	368	48.3 (35.9-60.8)	97.1 (94.5-98.5)
Ultra/OSF	319	46.8 (33.4-60.8)	97.8 (95.1-99.1)

ORAL SWABS – GOOD ENOUGH?

- Non-invasive, rapid sampling
- Simpler processing relative to sputum

Diagnostic accuracy (N=183 adults with presumed TB)

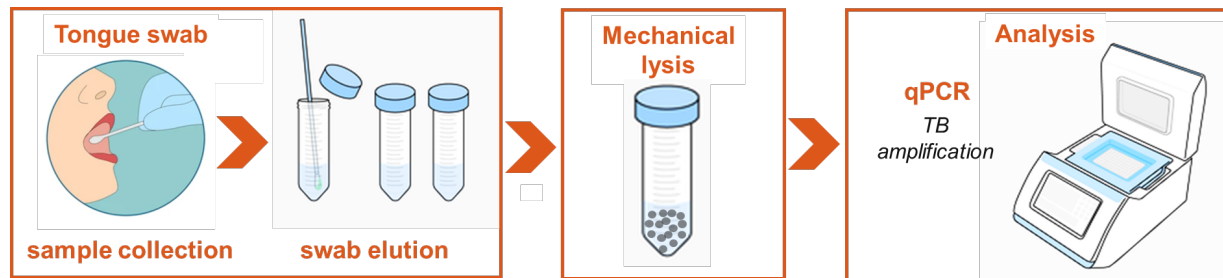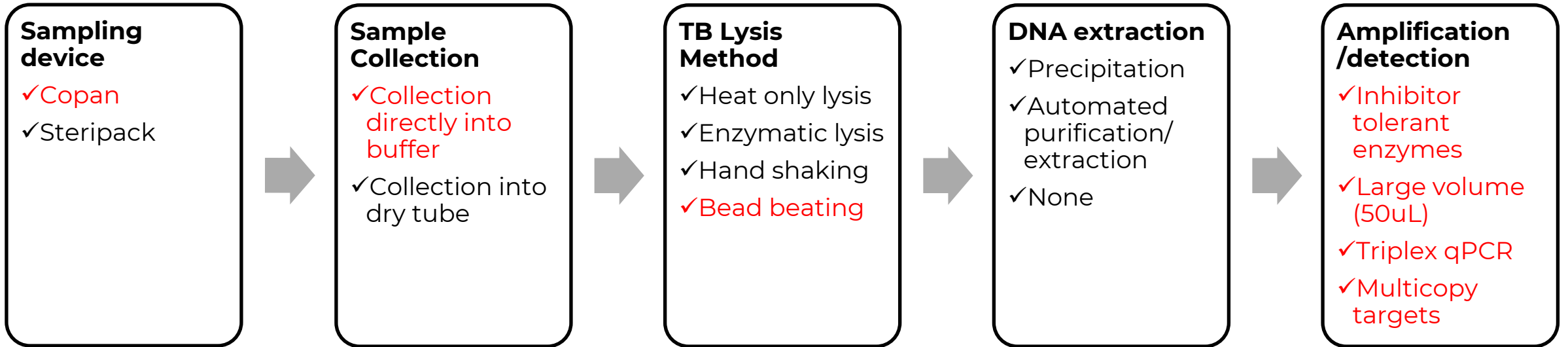|	Sputum Xpert Ultra reference standard	Microbiologic reference standard
Sensitivity	77.8 (64.4-88.0)	72.4 (59.1-83.3)
Specificity	100 (97.2-100)	100 (96.9-100)

Tongue swab collection and processing

Semi-quantitative results: Swab vs sputum Xpert Ultra

		Tongue swab Xpert Ultra (double swab SR method)					Total
		Negative	Trace	Very low	Low	Medium	
Sputum Xpert Ultra[a]	Negative	127	0	0	0	0	127
	Trace	2	0	0	0	0	2
	Very low	6	0	0	0	0	6
	Low	6	3	0	3	0	12
	Medium	0	3	5	7	0	15
	High	0	1	4	14	2	21
	Total	141	7	9	24	2	183

Andama A et al, J Clin Microbiol 2022

IS IT POSSIBLE TO DO BETTER? OPTIMIZED REFERENCE ASSAY

Sampling device
- ✓Copan
- ✓Steripack

➡

Sample Collection
- ✓Collection directly into buffer
- ✓Collection into dry tube

➡

TB Lysis Method
- ✓Heat only lysis
- ✓Enzymatic lysis
- ✓Hand shaking
- ✓Bead beating

➡

DNA extraction
- ✓Precipitation
- ✓Automated purification/ extraction
- ✓None

➡

Amplification /detection
- ✓Inhibitor tolerant enzymes
- ✓Large volume (50uL)
- ✓Triplex qPCR
- ✓Multicopy targets

Tongue swab
sample collection — swab elution

Mechanical lysis

Analysis
qPCR
TB amplification

Clinical Performance vs. Sputum Xpert Ultra		
Xpert Ultra high	100%	(13/13)
Xpert Ultra medium	100%	(13/13)
Xpert Ultra low	100%	(6/6)
Xpert Ultra very low/trace	100%.	(1/1)
Xpert Ultra negative	1.5%	(1/65)
Overall Sens./Spec.	**100% / 98.5%**	

GH+Labs   walimu

Breath aerosol sampling – promising early results

Infected person

Oral / Laryngeal / Bronchial / Alveolar

Breath sampling captures aerosols that carry pathogens that are then detected with molecular tools

Aerosols typically 3 μm size
- Carry pathogens from entire lung
- Travel beyond 1 meter
- Can float in air for hours
- Can be inhaled

Droplets >100 μm size
Travel <1 meter
Fall to the ground in <5 sec
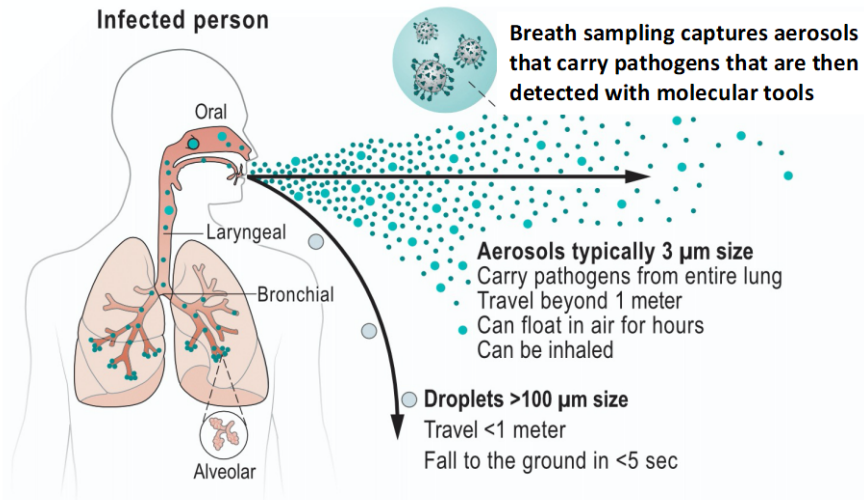
Breath sampling captures human aerosols that carry pathogens. After collection the pathogens' DNA or RNA is detected. (Adapted from Wang.Science.2021;373(6558):eabd9149)
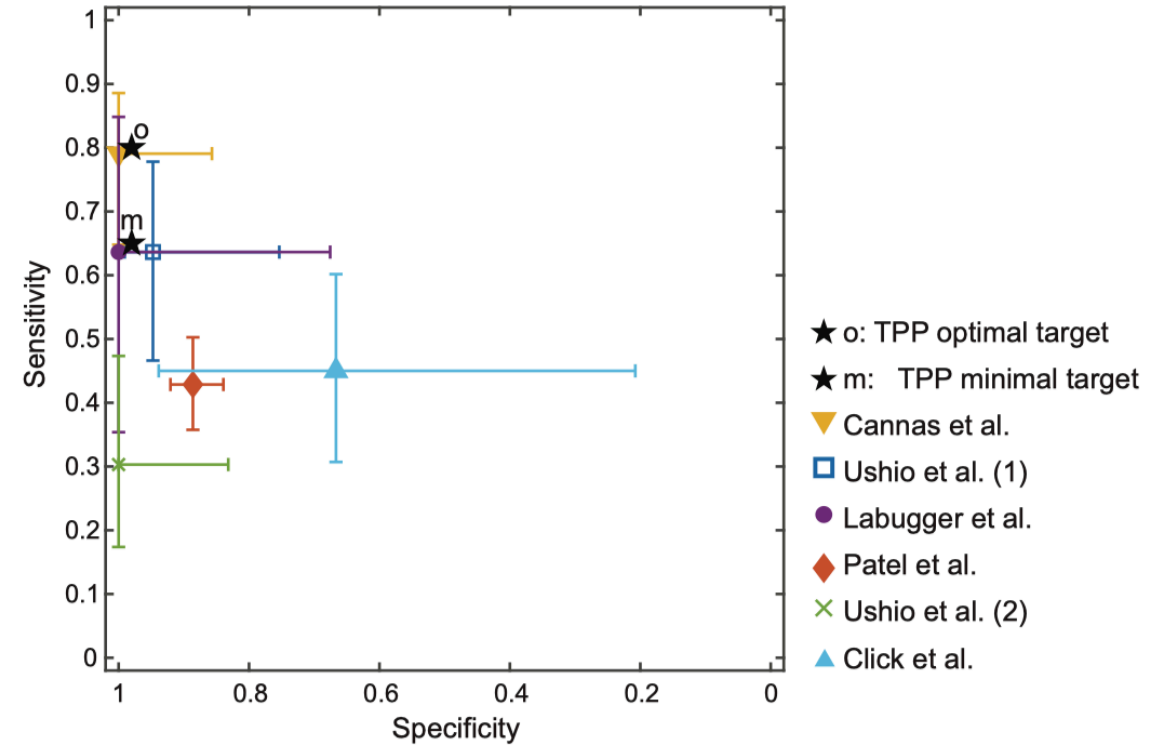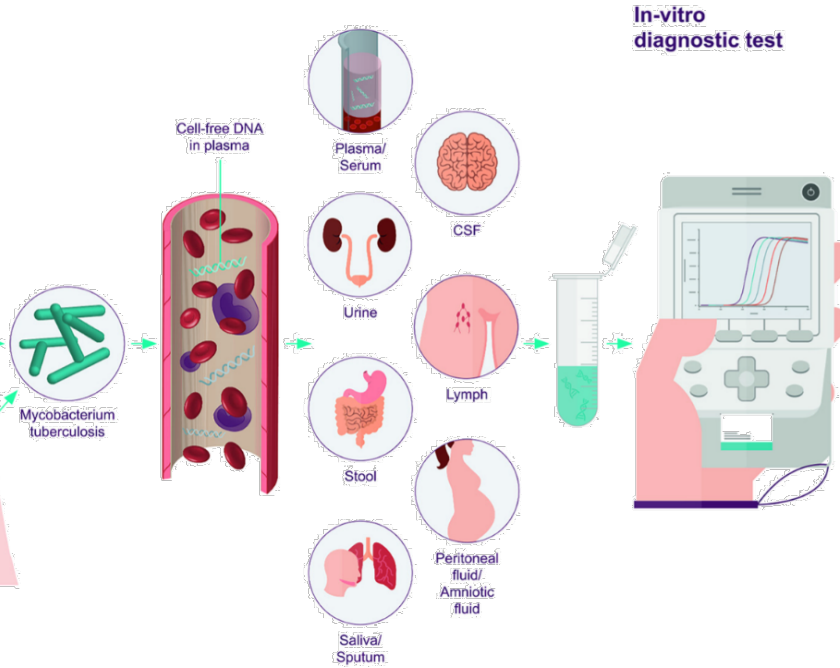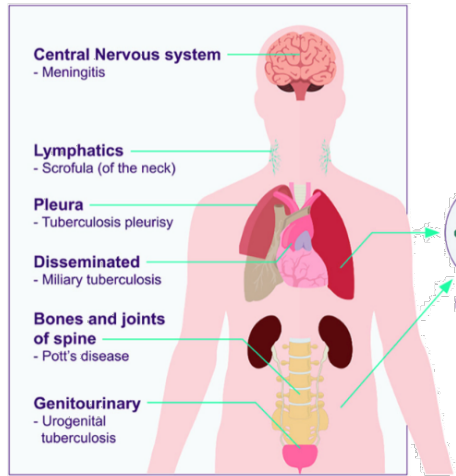
- Ease of collection via non-invasive sampling methods (face mask)
- Link to infectiousness and transmission

45 individuals screened for eligibility

25 did not report tuberculosis symptoms

20 people confirmed eligible

12 participants with Xpert-negative sputum and face-mask samples

1 participant with Xpert-positive sputum sample and Xpert-negative face-mask sample

1 participant with Xpert-positive face-mask and sputum samples

6 participants with Xpert-positive face-mask sample and Xpert-negative sputum sample

1 participant lost to follow-up

12 no incident tuberculosis infections detected at 10 months

2 began tuberculosis treatment and not followed up further

5 followed up at 6 weeks and 20 weeks*

	Initial screening		6-week follow-up						20-week follow-up			
	Sputum sample	Face-mask sample	Sputum sample	Face-mask sample	Chest radiography	Bronchoalveolar lavage result	PET-CT	Outcome	Sputum sample	Face-mask sample*	PET-CT	Outcome
2	–	+	–	+	–	–	–	Treatment withheld and observed	–	–	–	Alternative diagnosis made
3	–	+	+	+	–	+	+	Tuberculosis treatment commenced	–	–	–	Completed treatment
7	–	+	+	+	–	+	+	Tuberculosis treatment commenced	–	–	–	Completed treatment
13	–	+	+	+	–	–	+	Tuberculosis treatment commenced	–	–	–	Completed treatment
17	–	+	+	+	–	–	+	Tuberculosis treatment commenced	–	–	–	Completed treatment

Williams CM et al, Lancet ID 2020

cfDNA (urine/blood) – Can it transform TB like it has cancer?

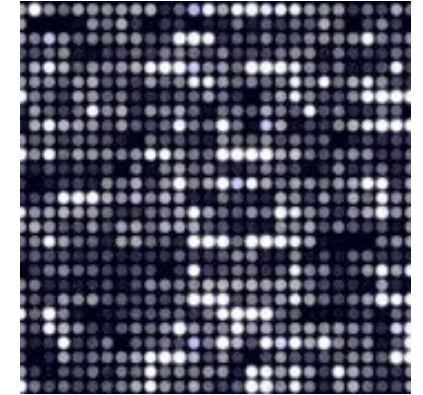
Fernandez Carballo BL et al, J Clin Microbiol 2016

Karius Test: A CLIA-certified cfDNA assay

■ Single blood test that detects cfDNA from >1000 microbial pathogens, including *M. tuberculosis*
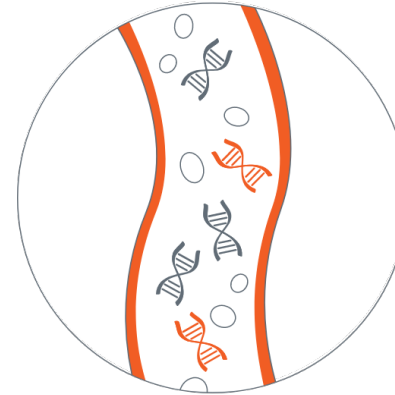
Targeted NGS   **AI technology**

■ Limited use as a routine TB diagnostic

-- Moderate sensitivity in smear-positive TB (40-50%)

-- Poor sensitivity in smear-negative TB (0%)

Age group	TB status	Cell-free DNA detected by commercial-reporting threshold n/N (%) (95% CI)
Children		
	Sm+/Cx+ (*n* = 4)	2/4 (50) (7–93)
	Sm−/Cx+ (*n* = 6)	0/6 (0) (0–46)
	Clinically diagnosed (Sm−/Cx−) (*n* = 10)	0/10 (0) (0–31)
	TB ruled out (Sm−/Cx−) (*n* = 10)	0/10 (0) (0–31)
Adults		
	Sm+/Cx+ (*n* = 5)	3/5 (60) (15–95)
	Sm−/Cx+ (*n* = 5)	0/5 (0) (0–52)

Pollock N et al, IJTLD 2021

Next-generation urine LAM assays: Fujifilm SILVAMP TB LAM

- High affinity monoclonal antibodies against *M. tuberculosis*-specific LAM epitopes

- Silver amplification step that increases the visibility of the test lines

**IPD meta-analysis: banked urine from 5 prospective cohorts of PLHIV**

Test (N=1595)	Sensitivity (N=724)	Specificity (N=871)
SILVAMP-LAM	70.7 (59.0-80.8)	90.8 (86.0-94.4)
LF-LAM	34.9 (19.5-50.9)	95.3 (87.7-98.8)
Difference	35.8	-4.4

CD4 stratum	Sensitivity	Specificity
0-100	84.2 (71.4-91.4)	85 (75.8-91.7)
101-200	60.6 (44.4-72.5)	89.6 (78.5-98.1)
>200	44.0 (29.7-58.5)	97.0 (92.5-99.3)

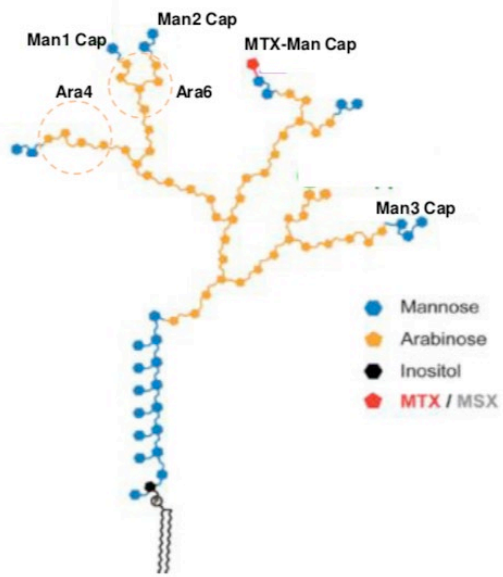# …and people living without HIV?

Diagnostic accuracy of three urine lipoarabinomannan (LAM) tuberculosis assays in HIV-negative outpatients
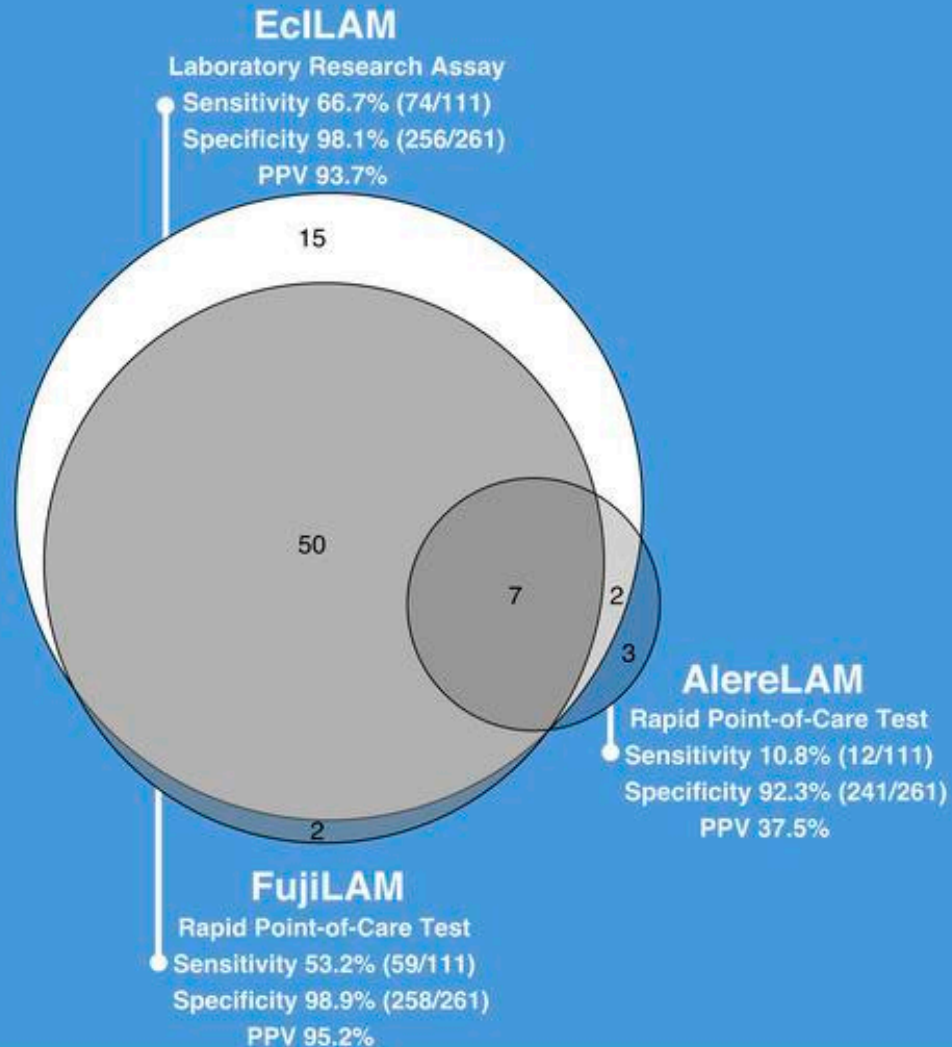
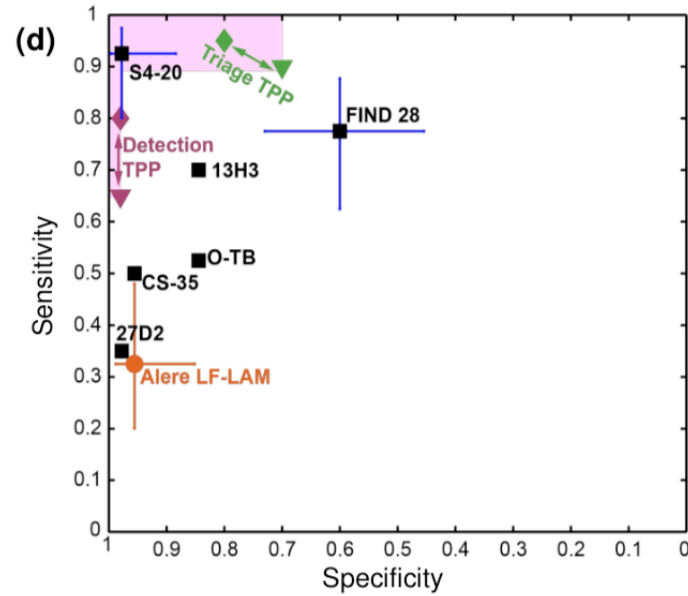| Test	Sensitivity (N=111)	Specificity (N=871)
LF-LAM	10.8	92.3
SILVAMP-LAM	53.2	98.9
EcILAM	66.7	98.1

Broger T et al, J Clin Invest 2021

NEXT STEPS: THIRD GENERATION OF URINE LAM TESTS

Better knowledge

LAM structure epitope mapping

Better antibodies

Generation of new **high affinity antibodies**

Better detection

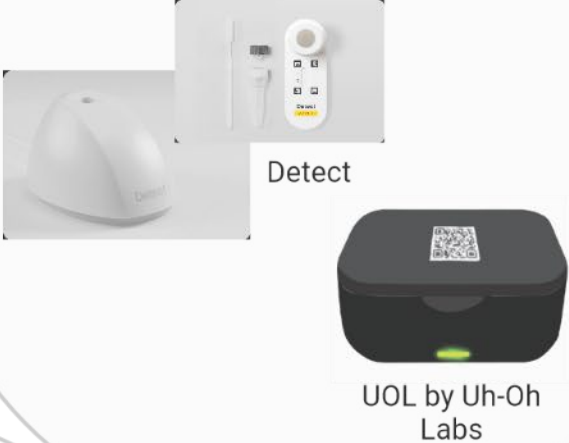Better ability to **capture, concentrate & detect**

3ʳᵈ gen tests

"Best of the best" approach - BMGF

- Salus
- Mologic
- Abbott
- Boditech
- Others

Broger T et al, J Clin Micro 2018

Technological innovation that enables POC testing

LAMP Assays

Detect

UOL by Uh-Oh Labs

Instrument-Free Molecular Dx

Lucira Health

Visby Medical

Veros by Sense Biodetection

Instrument-based Immunoassays

Sofia by Quidel Corporation

Omnia by Qorvo Biotechnologies

LumiraDx

Clip by Luminostics

Summary

- Molecular testing should be current standard for diagnosis of pulmonary TB
  - Low complexity tests: Xpert MTB RIF, Xpert MTB RIF Ultra, Truenat
  - Moderate complexity tests: 4 endorsed by WHO to date

- Innovations in sample type and platforms for molecular testing anticipated over next few years
  - Oral swabs and mask-based aerosol
  - Cell-free DNA
  - POC platforms

- Urine LAM testing showing increasing promise, with third-generation assays expected to undergo accuracy trialing in 2023

Thank you!