MAYO CLINIC

Center for Tuberculosis

TB Diagnostics Demystified: Practical Insights for Clinical Decision-Making

Dr. Madiha Fida, M.D, D(ABMM)
Assistant Professor of Medicine
Infectious diseases
Mayo Clinic Rochester

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Accreditation Statement
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- 1.00 Attendance
- 1.00 IPCE

Disclosures
Madiha Fida

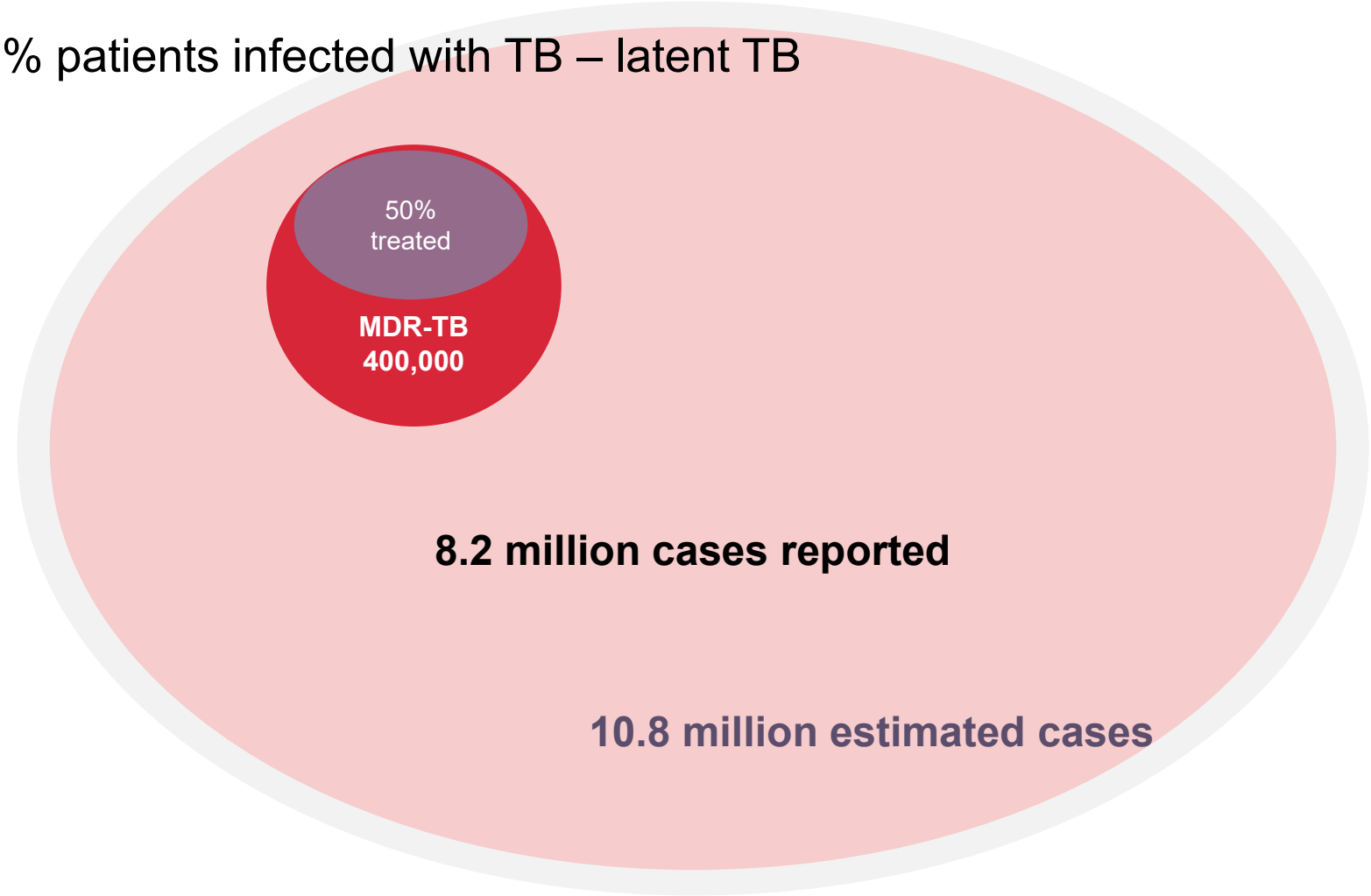
No relevant financial disclosures to report and no mention of off-label use of any medications or products

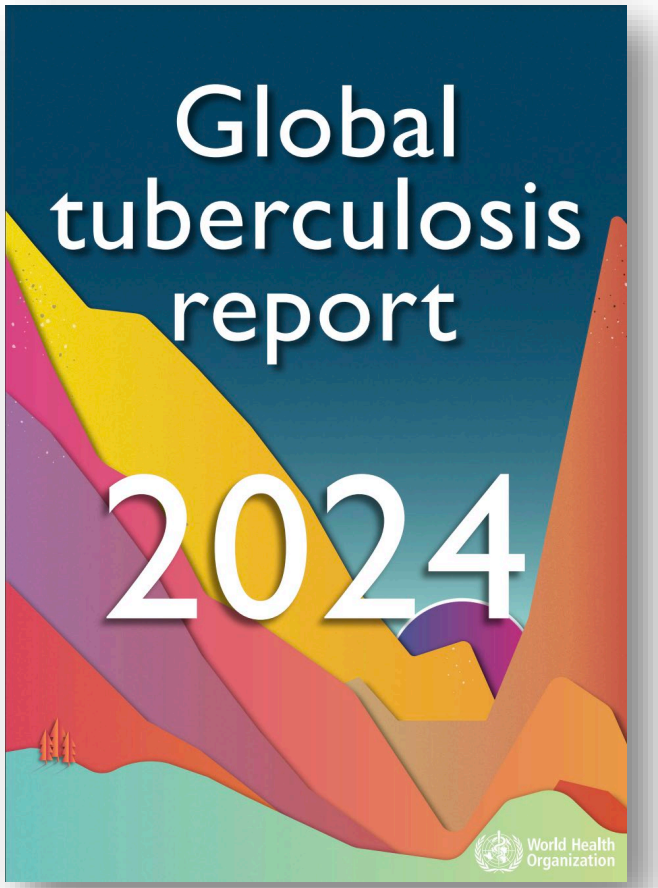
Learning Objectives

- Interpret interferon-gamma release assay (IGRA) results to guide clinical decision making.
- Analyze culture results to accurately diagnose TB disease.
- Evaluate new diagnostic tools and techniques for TB detection.

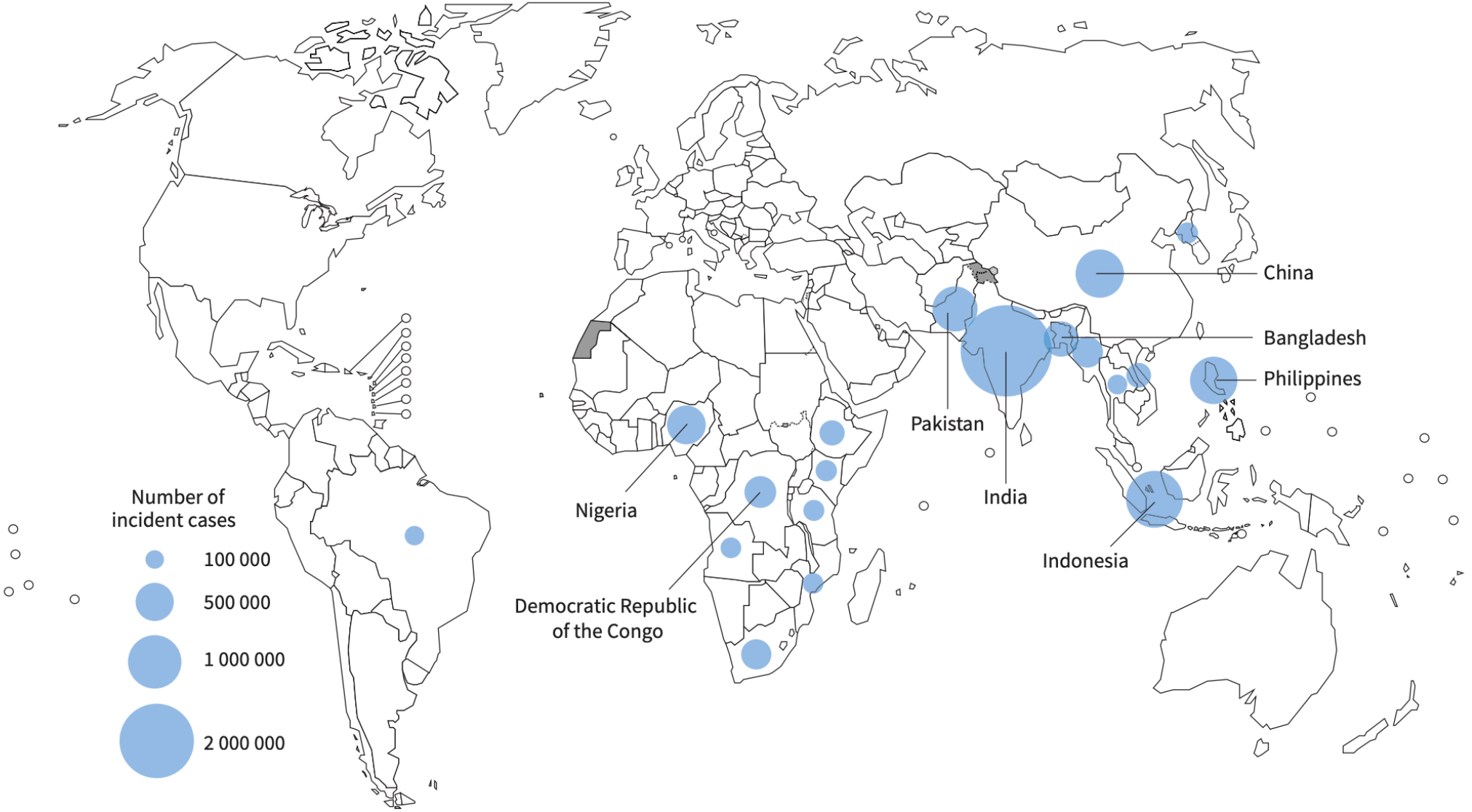
Global Impact of *M. tuberculosis*

- Leading cause of death from a single infectious agent in 2023 (more than COVID in 2023)
- 1.23 million deaths
- 25% patients infected with TB – latent TB





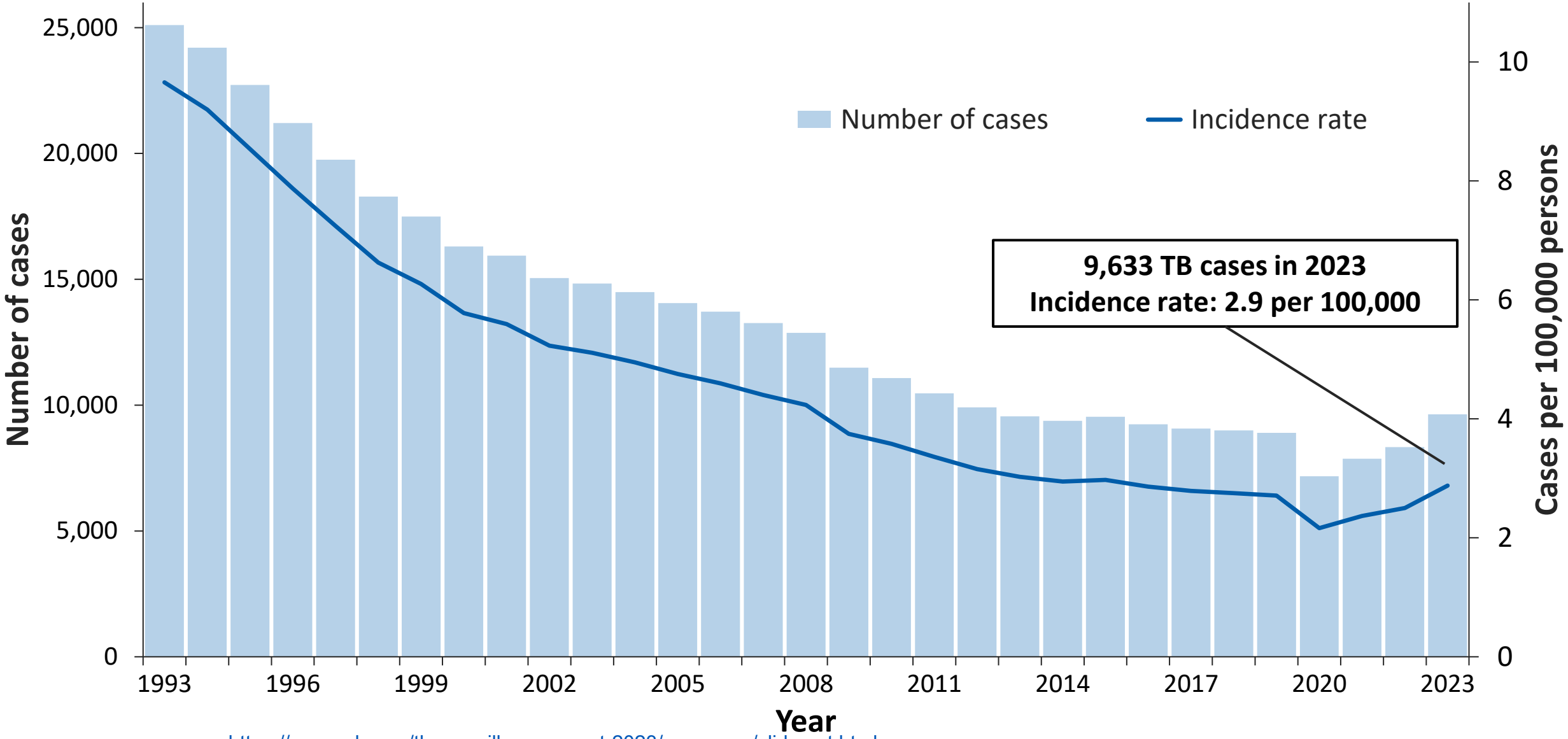
Estimated number of incident TB cases in 2023, for countries with at least 100 000 incident cases[a]



[a] The labels show the eight countries that accounted for about two thirds of the global number of people estimated to have developed TB in 2023.

Source: WHO Global TB Report 2024

TB Cases and Incidence Rates, United States, 1993–2023



https://www.cdc.gov/tb-surveillance-report-2023/resources/slide-set.html

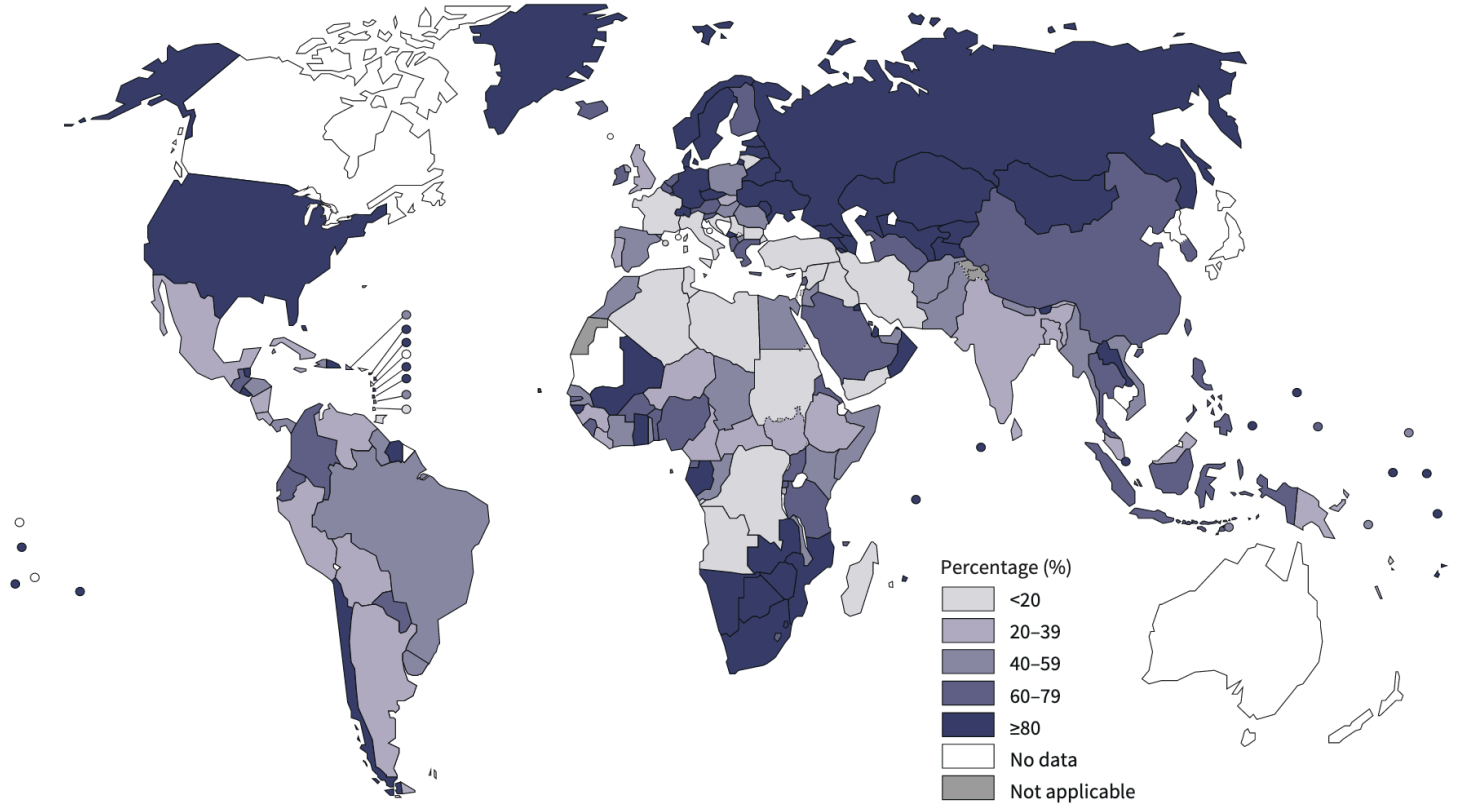
END-TB Targets for Diagnosis

1. Use of WHO-recommended rapid diagnostic tests

Goal: ≥90% of TB cases by **2027**

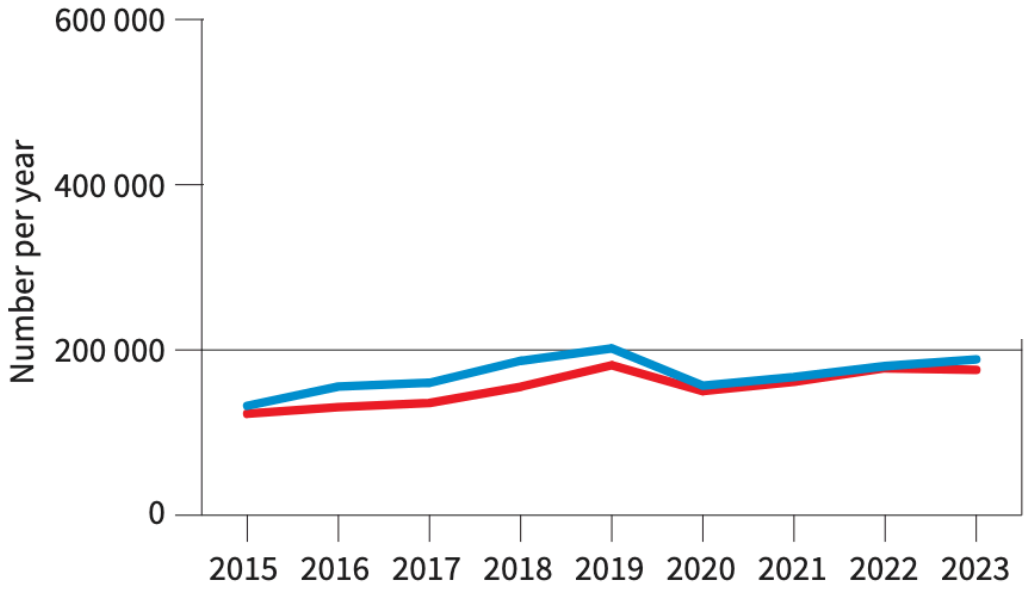
Current (2023): Only **48%**

Percentage of people newly diagnosed with TB who were initially tested with a WHO-recommended rapid diagnostic test (WRD), by country, 2023



Source: WHO Global TB Report 2023

END-TB Targets for Diagnosis



[a] The time period corresponds to the period for which estimates of the incidence of MDR/RR-TB are available.

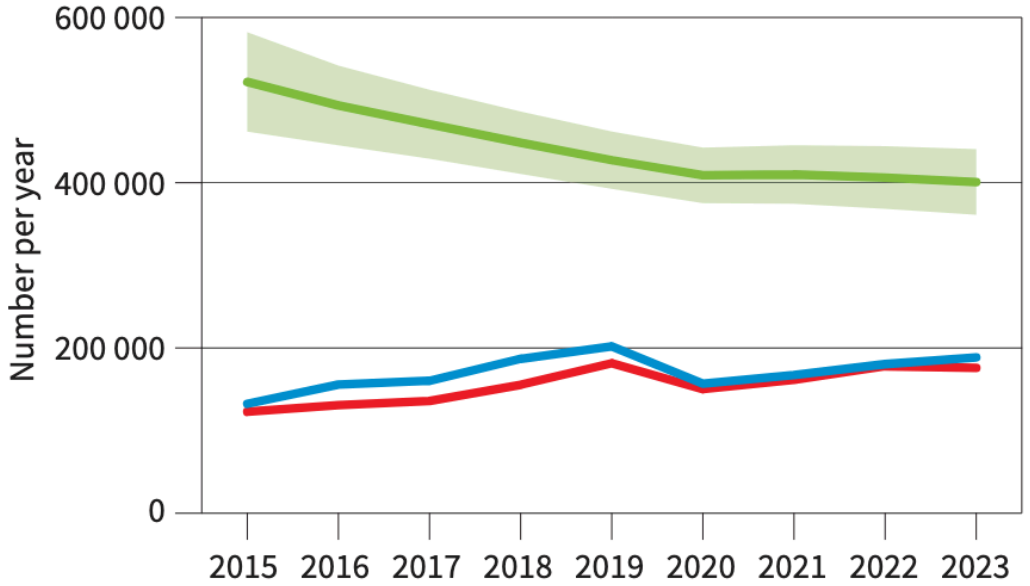
Global number of people diagnosed with MDR/RR-TB (blue)
Number enrolled on an MDR-TB treatment regimen (red)

Source: WHO Global TB Report 2024

END-TB Targets for Diagnosis

Drug Susceptibility testing coverage for tb patients → 100% by 2035

2023: **79%** of bacteriologically confirmed pulmonary TB cases tested for **rifampicin resistance** (↑ from 62% in 2019)



[a] The time period corresponds to the period for which estimates of the incidence of MDR/RR-TB are available.

Estimates of the global number of incident cases of MDR/RR-TB
(95% uncertainty interval shown in green), 2015–2023"

Global number of people diagnosed with MDR/RR-TB (blue)
Number enrolled on an MDR-TB treatment regimen (red)

Source: WHO Global TB Report 2024

Case Study

IGRA

54-year-old man with DM, HTN and CKD5 admitted for worsening hyperkalemia with plans to start HD.

As part of his pre-dialysis evaluation, he undergoes routine screening, including a QuantiFERON-TB Gold test. Results shown below

QuantiFERON-TB Gold Plus Result	Indeterminate
TB1 Ag minus Nil Result	**0.00**
TB2 Ag minus Nil Result	**0.00**
Mitogen minus Nil Result	**0.10**
Nil Result	**0.02**

Which of the following is true regarding this result?

QuantiFERON-TB Gold Plus Result	Indeterminate
TB1 Ag minus Nil Result	**0.00**
TB2 Ag minus Nil Result	**0.00**
Mitogen minus Nil Result	**0.10**
Nil Result	**0.02**

A. Patient is likely immunosuppressed

B. This is due to overstimulated T cells

C. Indeterminate test means weakly positive

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B. This is due to overstimulated T cells

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Interferon gamma release assays

Importance of Identifying Latent TB Infections

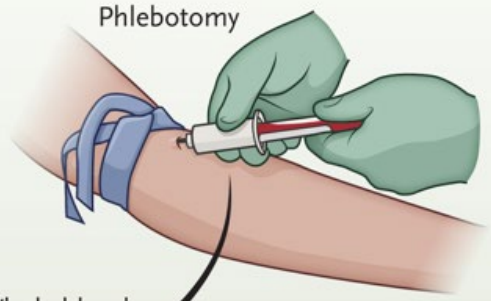
- Identify LTBI in at-risk individuals who would benefit from treatment
  - High risk of acquisition (close contacts)
  - High risk of transmission (crowded living conditions)
  - High risk of reactivation (immunosuppression)

  - Decision to test for LTBI should presuppose decision to treat if positive

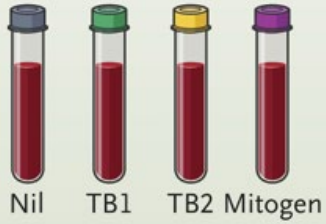
- **~80% of active TB cases in the U.S.** stem from untreated LTBI
- No ‘gold standard’ method for detection of LTBI
  - All methods are *indirect* approaches detecting host immune response to *M. tuberculosis*

Shea KM, Kammerer JS, Winston CA, Navin TR, Horsburgh CR Jr. 2014. Estimated rate of reactivation of latent tuberculosis infection in the United States, overall and by population subgroup. Am J Epidemiol 179: 216–225

QuantiFERON-TB Gold Plus IGRA

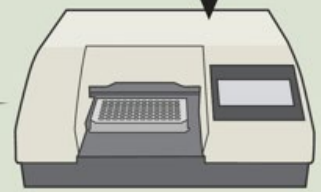


Whole blood

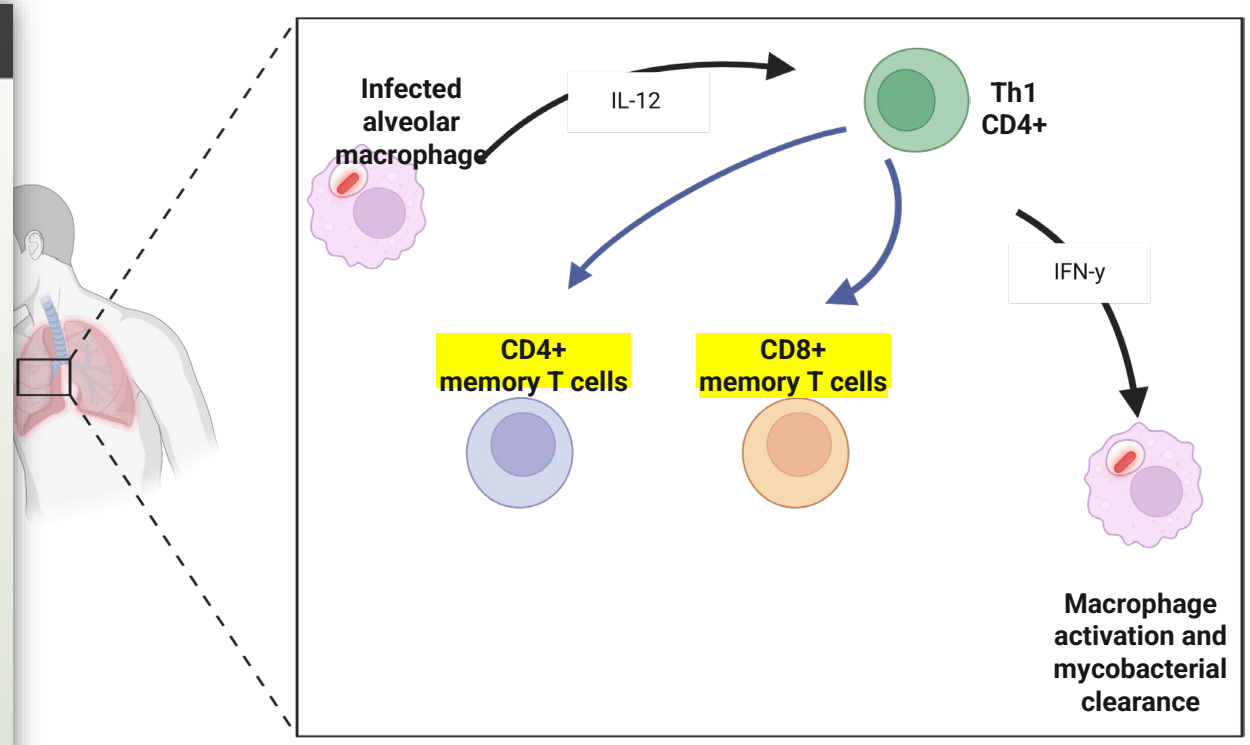


Add blood to tubes precoated with nil control, *M. tuberculosis* antigens (tubes TB1 and TB2), and mitogen positive control.

Incubate 16–24 hr. Add aliquot of stimulated plasma to wells of ELISA plate that contains interferon-γ antibodies.



A secondary enzyme-linked antibody is added and binds to interferon-γ. A detection reagent is added, and absorbance at 450 nm is measured. Concentration of interferon-γ is calculated on the basis of a standard curve.



IGRAs are **indirect** tests for infection with MTB

Patients infected with MTb have primed T-cells

Exposure of primed T-cells to MTb antigens induces IFN-γ production

IFN-γ detected by EIA/CIA or ELISPOT methods

2 FDA-cleared assays :

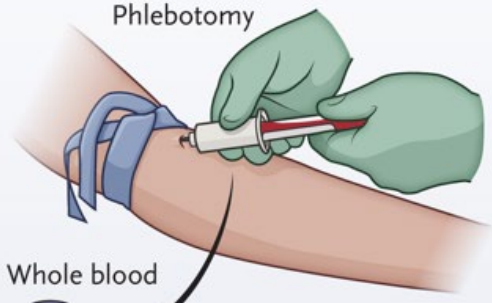
T.SPOT ®-TB (Oxford Immunotec)

QuantiFERON® TB-Gold Plus (QFT-Plus; Qiagen)

Both stimulate $CD4^+$ and $CD8^+$ T-cells

Modified from Maunank Shah et al, 2021 NEJM

T-SPOT.*TB* IGRA

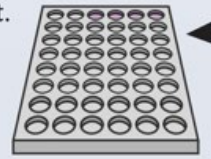


Whole blood

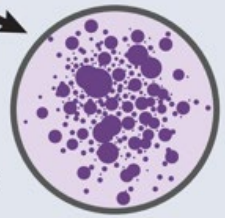
PBMCs

Centrifugation

Plate 250,000 cells into each of four wells per patient.



Add nil control, *M. tuberculosis* antigens (×2), and mitogen positive control. Incubate for 16–20 hr.



Interferon-γ antibodies capture interferon-γ as it is released from cells. A secondary enzyme-labeled antibody is added and binds to captured interferon-γ. A detection reagent is added, resulting in spots that are a footprint of the location where the interferon-γ was released by a cell. Spots are counted.

QuantiFeron-Tb Gold Plus

	Nil Control	Tb 1 Antigens	Tb 2 Antigens	Mitogen Control
				
Antigens	None			PHA phythaemagglutinin-P
T cell stimulated	N/A			Universal T-cell stimulant
Purpose	Adjusts for background IFN-γ levels			Low response may indicate inability to generate IFN-

QuantiFeron-Tb Gold Plus

	Nil Control	Tb 1 Antigens	Tb 2 Antigens	Mitogen Control
				
Antigens	None	**ESAT-6 CFP-10**	**ESAT-6 CFP-10**	PHA phythaemagglutinin-P
T cell stimulated	N/A	**CD4+**	**CD4+/ CD8+**	Universal T-cell stimulant
Purpose	Adjusts for background IFN-γ levels			Low response may indicate inability to generate IFN-

QuantiFeron-Tb Gold Plus

	Nil Control	Tb 1 Antigens	Tb 2 Antigens	Mitogen Control
				
Antigens	None	**ESAT-6 CFP-10**	**ESAT-6 CFP-10**	PHA phythaemagglutinin-P
T cell stimulated	N/A	**CD4+**	**CD4+/ CD8+**	Universal T-cell
Purpose	Adjusts for background IFN-γ levels			Low response may indicate inability to generate IFN-

Negative Result:
IFN-γ levels <0.35 IU/mL in ***both*** Tb antigen tubes and <25% of Nil

Positive Result:
IFN-γ levels ≥ 0.35 IU/mL in ***either one or both*** Tb antigen tubes

QuantiFERON-Tb Gold Plus

	Nil Control	Tb 1 Antigens	Tb 2 Antigens	Mitogen Control
				
Antigens	None	**ESAT-6 CFP-10**	**ESAT-6 CFP-10**	PHA phythaemagglutinin
T cell stimulated	N/A	**CD4+**	**CD4+/ CD8+**	Universal T-cell stimulant
Purpose	Adjusts for background IFN-γ levels			Low response may indicate inability to generate IFN-

Mitogen not reacting

Indeterminate results due to poor mitogen response

Immunosuppressed patients
Technical issues

What to do?

Consider getting T spot test

QuantiFERON-Tb Gold Plus

	Nil Control	Tb 1 Antigens	Tb 2 Antigens	Mitogen Control
				
Antigens	None	**ESAT-6 CFP-10**	**ESAT-6 CFP-10**	PHA phythaemagglutinin-P
T cell stimulated	N/A	**CD4+**	**CD4+/ CD8+**	Universal T-cell stimulant
Purpose	Adjusts for background IFN-γ levels			Low response may indicate inability to generate IFN-

Nil reacting

Indeterminate results due to Nil reacting

Elevated baseline IFN-γ levels, such as certain infections, autoimmune diseases, or other inflammatory conditions

What to do?

- Consider repeating QuantiFERON
- Get T-spot test

2021 Meta-Analysis on Performance of QFT-Plus vs. Alternative LTBI Assays

- 24 studies included
  - High- and low-income countries
  - QFT-Plus vs. QFT-Gold vs. T-Spot

		No. of Studies	Pooled Values
Sensitivity (vs. culture)	QFT-Plus	7	92.6%
	QFT-Gold	7	91.8%
	T.Spot	2	90.2%
Specificity	QFT-Plus	2	97.8%
	QFT-Gold	2	98.7%
	T.Spot	1	98.1%

Oh CE, et al. *Clin Infect Dis*. 2021;73:e116-e1125

Detection of Latent TB Infection via IGRAs

- **False Positives:**
  - *M. marinum, M. kansasii, M. szulgai, M. flavescens*
  - Pre-analytic processing errors
- **False Negatives:**
  - Immunosuppression
  - Pre-analytic processing errors

- **Can IGRAs be used to monitor response to therapy?**
  - "…monitoring IGRA changes over time seems to have a speculative value only." (Chiappini, Clin. Therapy 2012)

- **Do IGRAs have a predictive value for progression to active TB?**
  - Predictive value of IGRAs is low.

Variability for the QFT Assays

With-in subject variability: ± 0.60 IU/mL

Assay cut-off for positive: ≥ 0.35 IU/mL

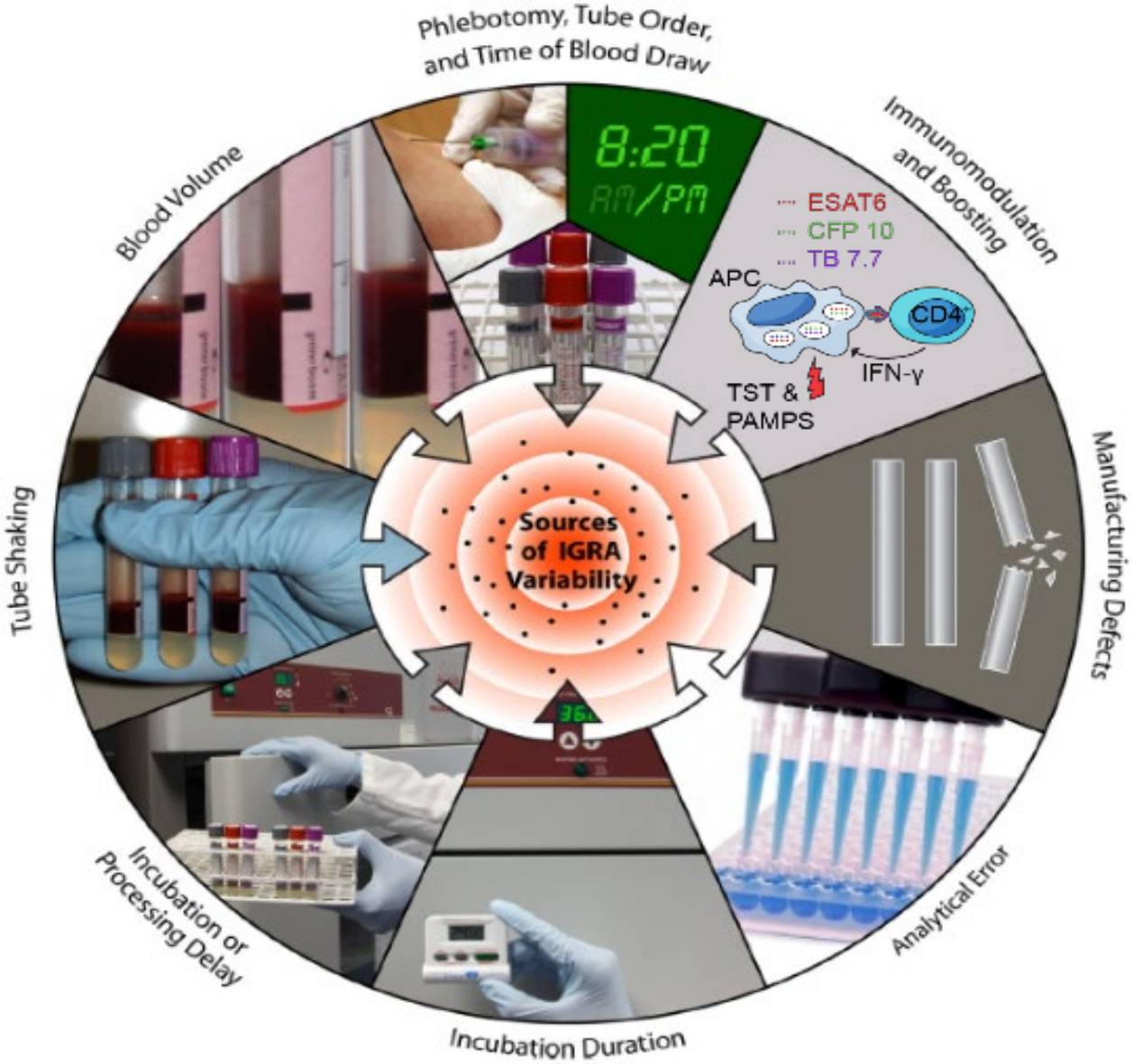
Interpret positive QFT values between

0.35 - 0.95 IU/mL with caution!

High rates of spontaneous conversion/reversion:

QFT-Plus: 22/196 **(11%)**

QFT-Gold: 16/188 **(8.5%)**



Sources of variability for the QFT Assays

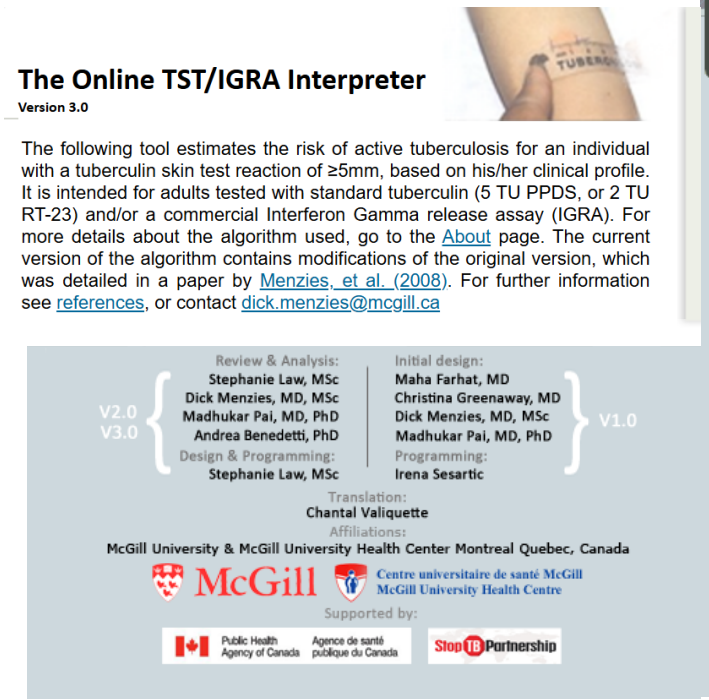
Pai M. et. al. Clin Micro Rev. 2014;27(1):3-39, Oh CE, et al. *Clin Infect Dis*. 2021;73:e116-e1125

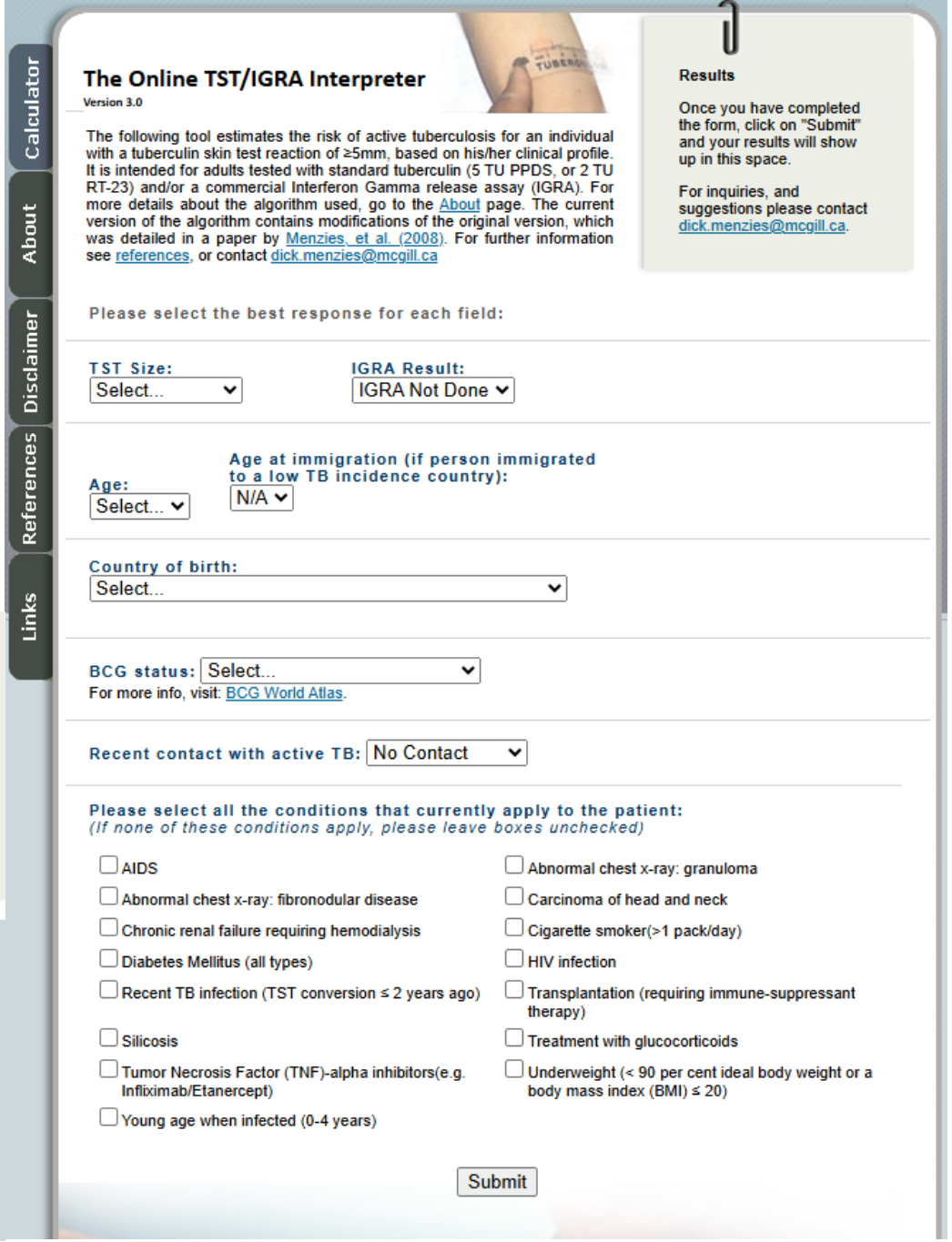
Metcalfe JZ et. al. Am J Respir Crit Care Med. 2013;187(2): 206-211

TST/IGRA Online Interpreter

- Estimates the risk of active TB based on results of TST and/or IGRA and clinical profile

https://www.tstin3d.com/en/calc.html





TST/IGRA Online

- Estimates the risk of active
on results of TST and/or IG
clinical profile

Results

Printable version

Below are the results for a patient with a **Positive** QFT Test, who is **33** years old, born in **Indonesia**, whose BCG status is **Never vaccinated or unknown**, who has had **no contact** with active TB, and who can be characterized by:

- **Transplantation (requiring immune-suppressant therapy)**

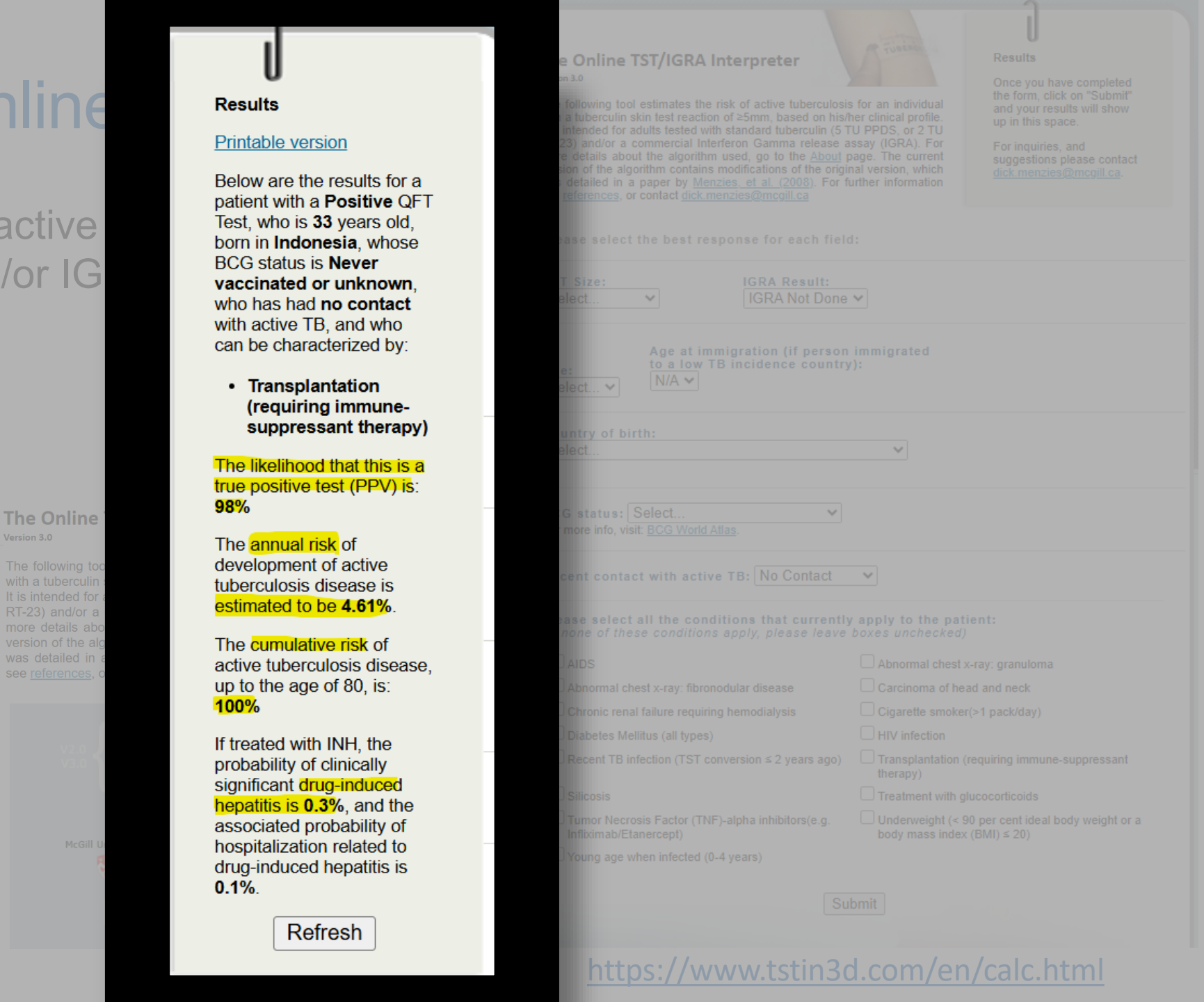
The likelihood that this is a true positive test (PPV) is: **98%**

The annual risk of development of active tuberculosis disease is estimated to be **4.61%**.

The cumulative risk of active tuberculosis disease, up to the age of 80, is: **100%**

If treated with INH, the probability of clinically significant drug-induced hepatitis is **0.3%**, and the associated probability of hospitalization related to drug-induced hepatitis is **0.1%**.

Refresh



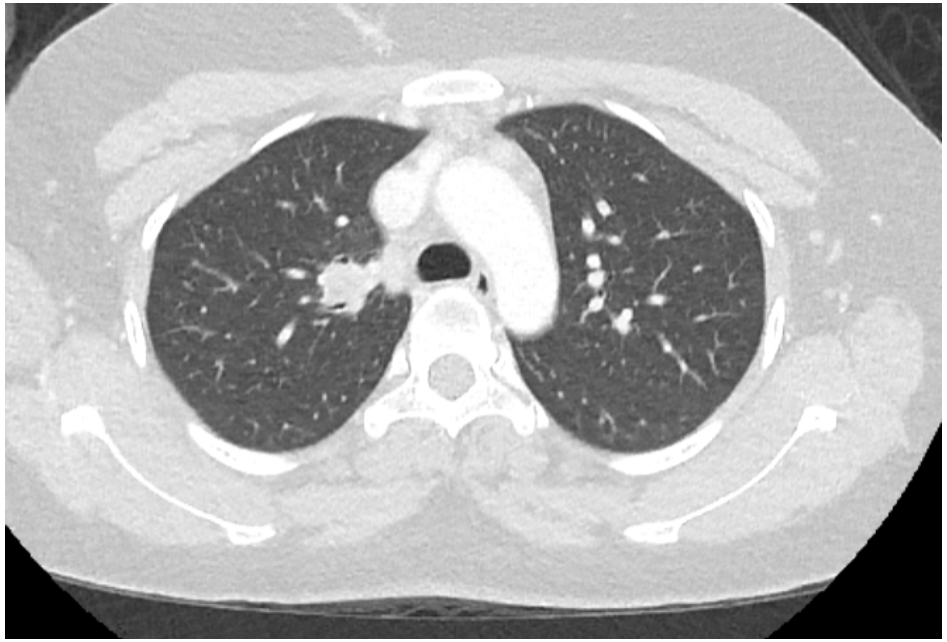
https://www.tstin3d.com/en/calc.html

Patient: 27-year-old woman, born in Somalia, immigrated to U.S. 8 years ago, visited Kenya recently

Current symptoms: Dry cough, shortness of breath, fatigue x 3 months and intermittent night sweats

CT imaging: Clustered nodularity in the medial right apex measuring up to 6 x 4 mm, solid 17 x 14 mm suprahilar right upper lobe nodule/lymphadenopathy, separate right hilar lymphadenopathy, and multistation mediastinal lymphadenopathy.

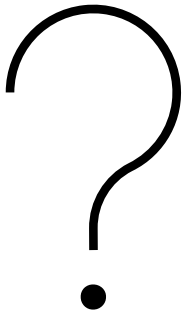
No previous QuantiFERON, Current QuantiFERON is positive



What is the best next step?

Clinical case

Polling Question

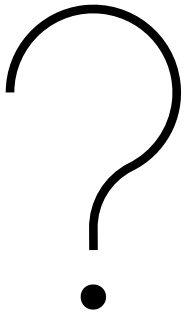


Given the previous history of TB with incomplete therapy, underlying suspicion for TB was high.

Which of the following will be the best next step?

A	3 x sputum samples obtained ≥8 hours apart for AFB smear and mycobacterial cultures
B	3 x sputum samples obtained ≥8 hours apart for AFB smear with mycobacterial cultures and nucleic acid amplification test (NAAT)
C	1 x sputum samples for AFB smear and mycobacterial cultures and MTB PCR

Polling Question



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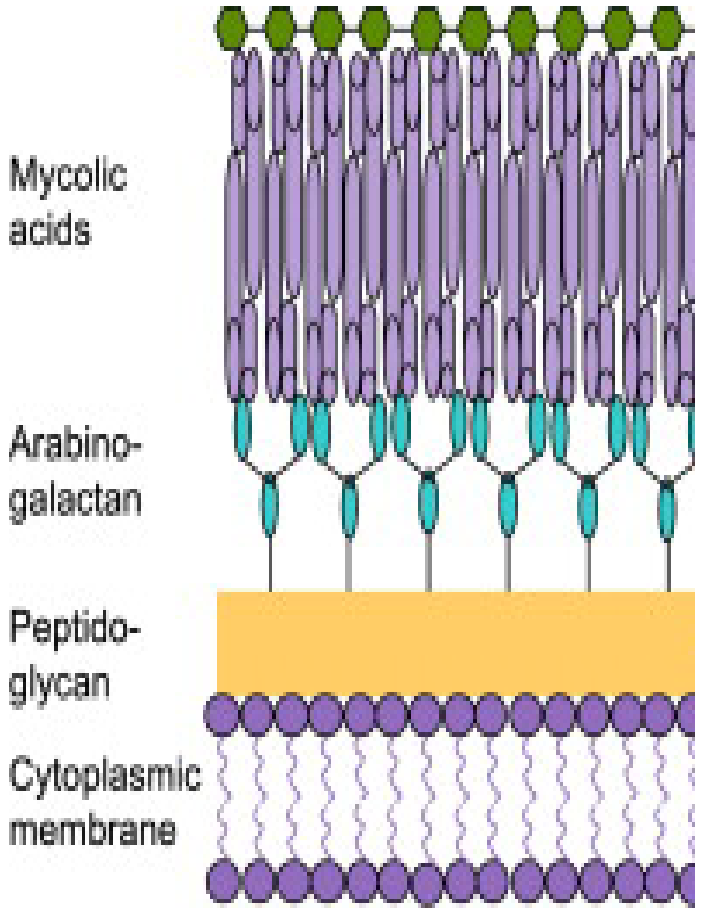
B	3 x sputum samples obtained ≥8 hours apart for AFB smear with mycobacterial cultures and nucleic acid amplification test (NAAT)
C	1 x sputum samples for AFB smear and mycobacterial cultures and MTB PCR

Acid-fast stains for mycobacteria

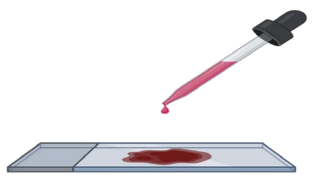
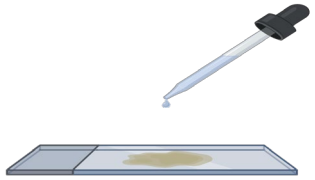
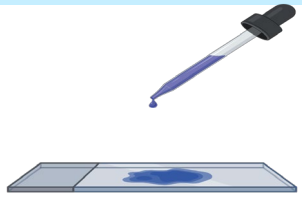
- Inexpensive, minimal infrastructure needed
- Results available within **24 hours**

Mycobacteria = AFB (Acid-Fast Bacilli)

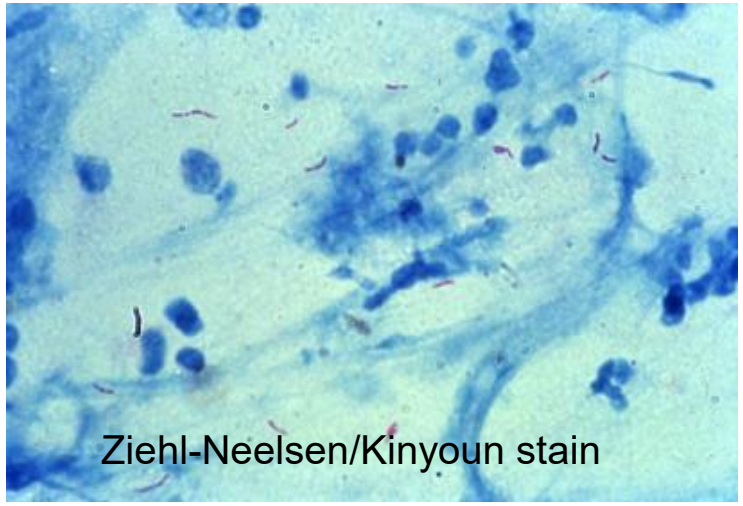
- Cell walls contain **mycolic acid**, which forms a complex with stain (e.g., carbol-fuchsin or auramine O)
- This complex resists decolorization by mineral acid → "acid fast"



AFB Stains for mycobacteria

	Application of primary stain to specimen smear	Decolorization of the sample with acid-alcohol	Application of counterstain to the sample
			
Carbolfuschin based stains	*Carbolfushin + heat fixation (Ziehl-Nielsen stain)* *Carbolfuschin without heat (Kinyoun stain)*	*Acid alcohol decolorizer*	*Methylene blue*

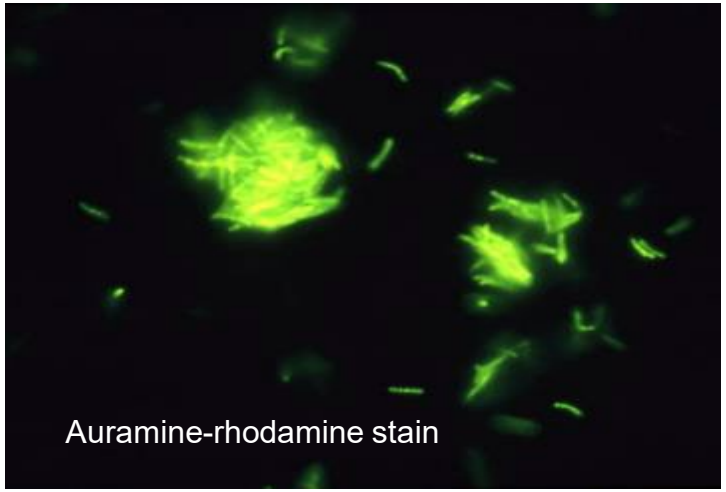
Sensitivity: **20–70% vs culture**



Ziehl-Neelsen/Kinyoun stain

Auramine – Rhodamine stain	*Auromine O stain*	*Auramine O decolorizer*	*Permanganate*

↑ Sensitivity by ~ **5–10%**



Auramine-rhodamine stain

Clinical case

Patient: 42-year-old male, born in Indonesia, immigrated to U.S. 5 years ago

PMH: Diagnosed with TB 10 years ago, stopped treatment at 4 months

Current symptoms

Dry cough, intermittent fevers, hemoptysis x 1 month

Shortness of breath on exertion, night sweats, ↓ appetite

Exam: Cachectic, mild respiratory distress

- T 38.2°C, HR 110, RR 22, SpO_2 94%
- Diffuse crackles, ↓ breath sounds in upper lobes

Imaging: CXR: RLL infiltrate

- CT: RLL consolidation with central cavitation + LUL cavitary lesion

Labs: Normal WBC, normal kidney/liver function

Given the previous history of TB with incomplete therapy, underlying suspicion for TB was high.

Which of the following will be the best next step?

AFB stain

Sensitivity

AFB load matters:

~100% positivity with **10^6 AFB/mL**

Only **60% positivity** with **10^4 AFB/mL**

Specimen volume:

>5 mL of sputum improves sensitivity significantly

Key factors influencing sensitivity:

Specimen type (respiratory specimens best)

Staining technique (fluorochrome ~10% more sensitive than carbolfuchsin)

Reader experience

Direct vs. indirect smear

Patient population

Specificity

Very high specificity due to mycolic acid-rich cell walls → acid-fast staining

Ideally, **<1% of smear-positive cases are culture-negative**

Smear-positive/culture-negative results

Over-decontamination → loss of mycobacterial viability

Inadequate culture duration

Fastidious species (e.g., *M. ulcerans*, *M. marinum*, *M. haemophilum*) requiring special conditions

Patients on TB treatment: may show positive smears with **delayed or negative cultures** (2–10 weeks)SD

How many smears?

Smear Sensitivity vs. Culture

- **1st smear**: ~53.8% sensitivity
- **2nd** adds ~11.1%
- **3rd** adds only 2–5% (ATS Guidelines)

WHO recommends:

- **2 spot samples**, ≥1 hour apart
- Based on minimal added yield from 3rd sample
- Same-day diagnosis

CDC recommends:

- **3 sputum samples** 8-24 hours apart
- At least **1 morning specimen** (higher yield)
- Purpose: Optimal detection & to **discontinue airborne isolation**

Morning sputum

Higher sensitivity than spot specimens
Likely due to **overnight accumulation of mycobacteria** → ↑ **sensitivity by ~12%**

Optimal volume

5–10 mL
<3 mL reduces smear & culture sensitivity

Detection Limits

Smear: Needs ~5,000–10,000 AFB/mL
Culture: Detects as few as **10–100 bacilli/mL**

AFB smear in extrapulmonary samples

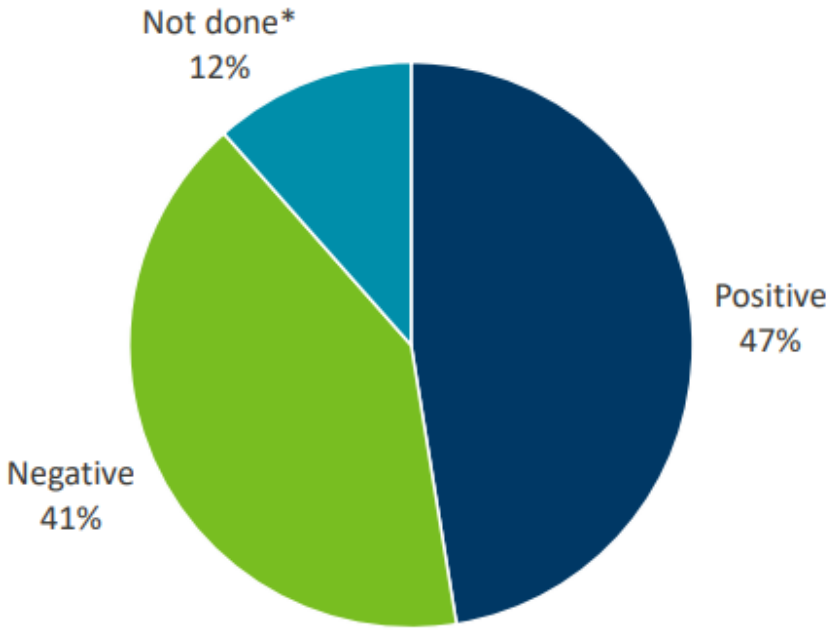
- Overall low sensitivity in extrapulmonary TB→ 22%
- Lymph node aspirates 25%-50% depending on whether direct smear vs concentration method used
- Low sensitivity → ~9% in CSF

Tadesse M et al 2014, Tiwari V et al 2003, Stadelman AM et al 2021

AFB Stains for mycobacteria

TB Cases with Pulmonary Involvement by Initial Sputum AFB Smear Result
Minnesota, 2018-2022

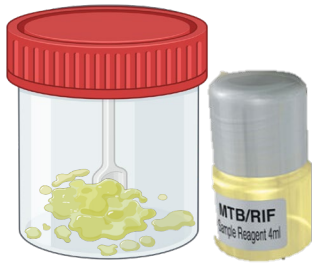
N = 485



**50% of pulmonary cases without sputum smear results were under 15 years of age*

30

https://www.health.state.mn.us/diseases/tb/stats/tbepislidesnotes.pdf



Sputum liquefaction and inactivation with 2:1 sample reagent

Transfer of 2 mL material into test cartridge

Cartridge inserted into test platform

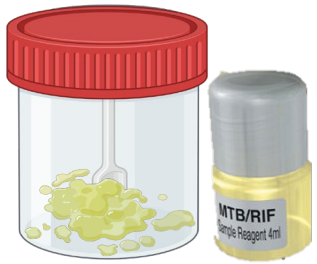
- Sample filtered and washed
- Lysis of organisms to release DNA
- DNA mixed with PCR reagents
- Real time amplification and detection in integrated reaction tube

Printable test results

Results in **~2 hours**, minimal hands-on time

Cepheid Xpert® MTB/RIF Test

Adapted from Boehme et al



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- Lysis of organisms to release DNA
- DNA mixed with PCR reagents
- Real time amplification and detection in integrated reaction tube

Printable test results

Detects MTB complex and rifampin (RIF) resistance
Off-label use: CSF, BAL

✅ WHO-endorsed,
✅ FDA-approved (U.S.: respiratory specimens only)

Results in **~2 hours**, minimal hands-on time

Cepheid Xpert® MTB/RIF Test

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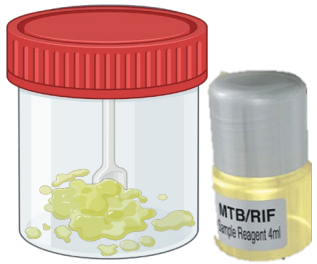

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Transfer of 2 mL material into test cartridge

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- Sample filtered and washed
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- Real time amplification and detection in integrated reaction tube

Printable test results

	Sensitivity (%)	Specificity (%)
Smear-Positive	96.7%	99.2%
Smear-Negative	59.3% – 73.1%	99.1%

Results in **~2 hours**, minimal hands-on time

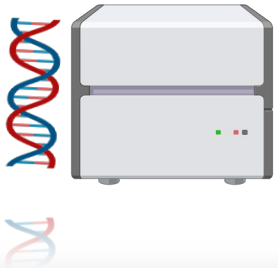
Cepheid Xpert® MTB/RIF Test

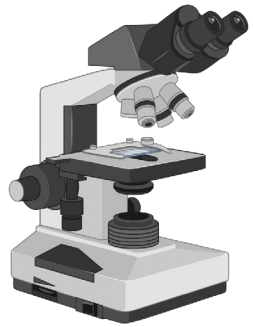
Adapted from Boehme et al

Additional Test Variants (not FDA approved)

Assay	Targets	Sensitivity	Notes
Xpert® MTB/RIF	Dual multicopy targets (IS6110/IS1081) + RIF	63–90% (↑ in smear–/HIV)	Improved LoD (16 vs. 114 cfu/mL); detects silent mutations
Xpert® MTB/XDR	INH (katG, inhA), FQs, injectables, ETH	Similar to MTB/RIF	Used **after** RIF testing; result in 90 mins

NAAT vs AFB Smear Microscopy – Roles in TB Diagnosis





NAAT (e.g., Xpert MTB/RIF)

- **Higher sensitivity & specificity** than AFB smear
  - Especially effective in **HIV+** & **paucibacillary TB**
- **WHO (2020)**: Recommends **NAAT over AFB** for initial diagnosis
- Especially valuable in **high TB burden** areas

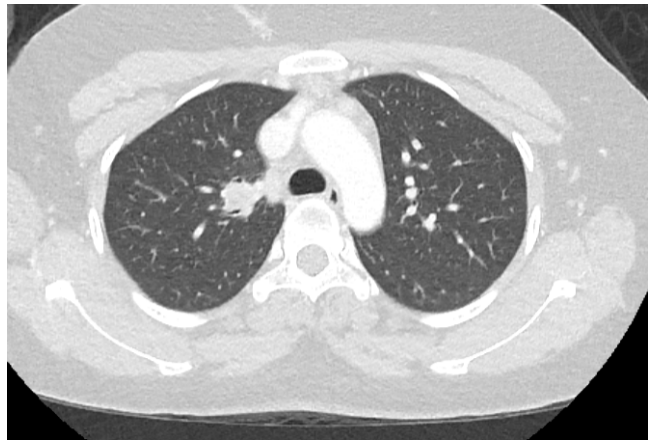
AFB Smear Microscopy

- Still useful in **low TB incidence** settings for:
  - Assessing **infectiousness**
  - **Monitoring treatment response**
  - Deciding on **airborne isolation release (AII)**
  - **Screening for NTM**, which are increasingly common
- Can **support interpretation of NAAT results**

Patient: 27-year-old woman, born in Somalia, immigrated to U.S. 8 years ago, visited Kenya recently

Current symptoms: Dry cough, shortness of breath, fatigue x 3 months and intermittent night sweats

CT imaging: Clustered nodularity in the medial right apex measuring up to 6 x 4 mm, solid 17 x 14 mm suprahilar right upper lobe nodule/lymphadenopathy, separate right hilar lymphadenopathy, and multistation mediastinal lymphadenopathy.



No previous QuantiFERON, Current QuantiFERON is positive

Patient could not give sputum samples. Induced sputum x 3 obtained

Sputum AFB smears x 3 are negative with negative MTB PCR.

Cultures after 5 weeks grew MTB, negative resistance testing and patient was treated for 6 months with **standard therapy** (RIPE x 2 months then INH + RIF x 4 months)

Clinical case

Next steps??

A. **Repeat biopsy and obtain mycobacterial culture**
B. **Repeat biopsy and obtain MTB PCR**
C. **Treat for latent TB with rifampin**
D. **Treat for latent TB with INH**

- 52 yo woman from Sudan undergoing workup for cough. Imaging shows cervical LAD
- Lymph node FNA of her Rt neck, which showed "granulomatous inflammation".
- AFB stains negative on path. Cultures not obtained
- CT chest – Waxing and waning GGOs
- Plans for immunosuppression for diagnosis of ILD
- Testing shows positive QuantiFERON
- Referred for LTBI treatment.
- Born in Sudan and immigrated to US 10 years ago

Next steps??

A. **Repeat biopsy and obtain mycobacterial culture**

B. **Repeat biopsy and obtain MTB PCR**

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Mycobacterial cultures → Most sensitive test

Culture generally uses a higher specimen volume so sensitivity can be better than PCR due to sampling issues

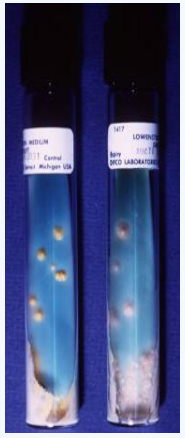
Allows for **phenotypic DST** and often is very helpful for **genotypic DST**

It might not be TB; it may be a **nontuberculous *Mycobacterium*** that will be missed by an Mtb-specific PCR test

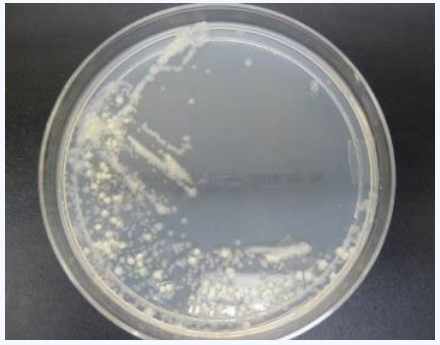
Gold standard
Only need 10-100 AFB/mL of specimen
Incubated for 6 weeks

Mycobacterial culture

Agar/solid medium



Lowenstein-Jensen (LJ) egg-based

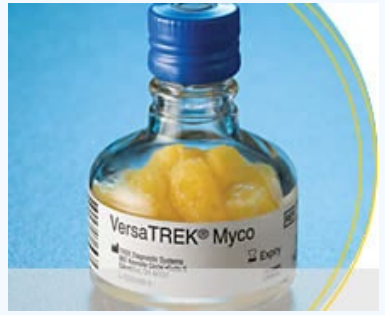


Middlebrook enriched agar

Slower growth
Allows colony observation
Useful for morphology
Time to detection: ~25 days

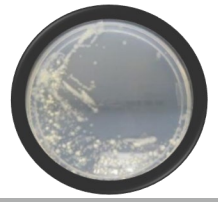
Broth/liquid medium

BACTEC MGIT (BD)



VersaTREK (TF)

Faster growth
Higher yield
Automation possible
Time to detection: ~17 days



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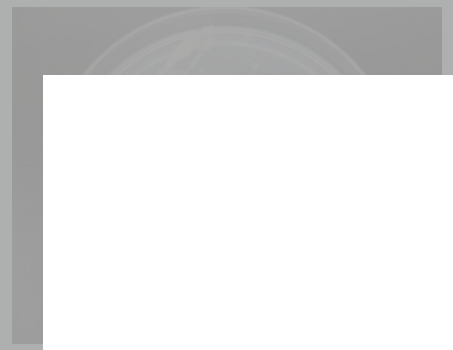

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VersaTREK (TF)

Faster growth
Higher yield
Automation possible
Time to detection: ~25 days

Slower growth
Allows colony observation
Useful for morphology
Time to detection: ~17 days

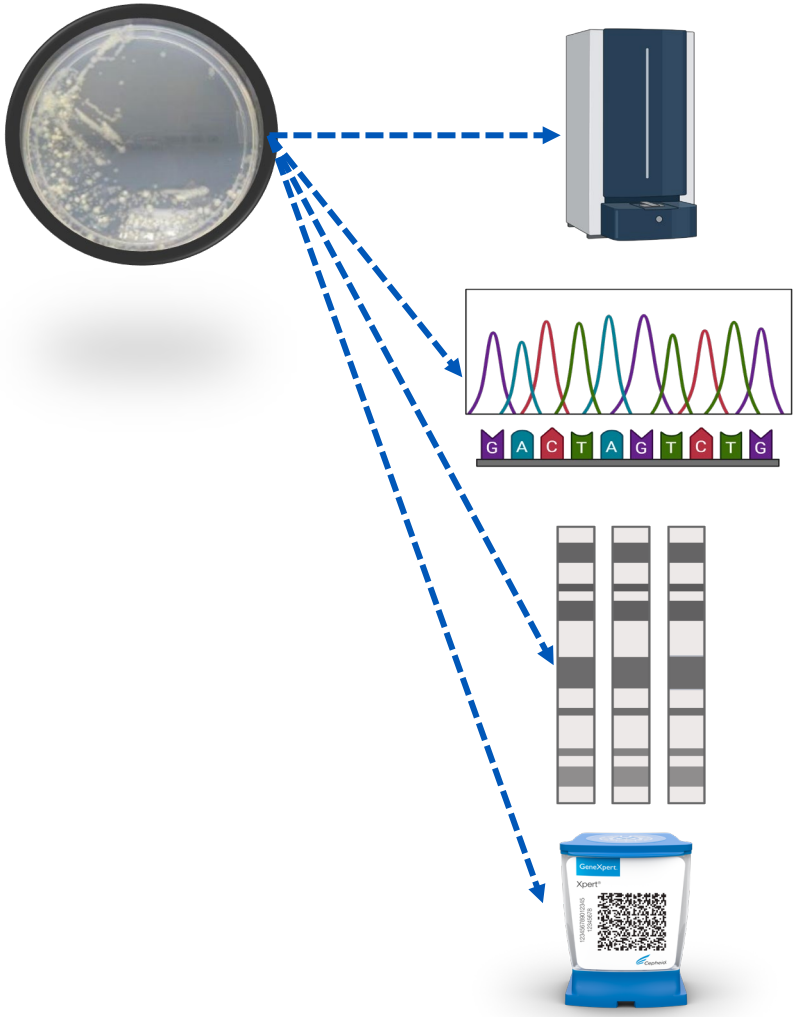
Accuracy & Limitations

Sensitivity: 88–90%

Specificity: >99%

False positives due to contamination, mislabeling, or specimen mix-ups.

Identification of *M. tuberculosis* after growth in culture



✖ Biochemical tests

MALDI-TOF (highly accurate; requires decent amount of growth so it may be a little slower than “probes”)

Sanger DNA sequencing good targets are 16S rRNA gene, *rpoB*, *hsp*65 technically complex; takes about 1 day after culture growth

Line Probe Assays Hain Lifesciences (Bruker) and Inno-LIPA (Fujirebio) not approved for diagnostic use in U.S. (clinical labs often don’t have them; public health labs may)

Targeted PCR assays Cepheid GeneXpert MTB/RIF, LDT PCRs – fast, accurate, only for Mtb (not NTMs yet)

Repeat LN biopsy grew *Mycobacterium tuberculosis* at 42 days

Treated with 6 months of treatment for TB lymphadenitis

- 52 yo woman from Sudan undergoing workup for cough Imaging shows cervical LAD
- Lymph node FNA of her Rt neck, which showed "granulomatous inflammation".
- AFB stains negative on path. Cultures not obtained
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- Plans for immunosuppression for diagnosis of ILD
- Testing shows positive QuantiFERON
- Referred for LTBI treatment.
- Born in Sudan and immigrated to US 10 years ago

Patient: 42-year-old male, born in Mexico, immigrated to U.S. 5 years ago

Current symptoms

Dry cough, intermittent fevers, hemoptysis x 1 month

Shortness of breath on exertion, night sweats, ↓ appetite

PMH: Diagnosed with TB 10 years ago, stopped treatment at 4 months

Exam: Cachectic, mild respiratory distress

- T 38.2°C, HR 110, RR 22, SpO_2 94%
- Diffuse crackles, ↓ breath sounds in upper lobes

Imaging: CXR: RLL infiltrate

- CT: RLL consolidation with central cavitation + LUL cavitary lesion

Labs: Normal WBC, normal kidney/liver function

Sputum smear is positive for AFB**.**

Should rapid molecular drug susceptibility testing be performed in this case?

Clinical case

A. Rapid molecular susceptibility is indicated since the smear is positive

B. Rapid molecular susceptibility testing is indicated due to history of previously treated tuberculosis

C. Rapid molecular susceptibility is **not indicated** since there is no history of contact with MDR tuberculosis

D. Rapid molecular susceptibility is **not indicated** since it is not sensitive for detection of resistance mutations

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Sputum smear is positive for AFB**.**

Should rapid molecular drug susceptibility testing be performed in this case?

Drug susceptibility testing

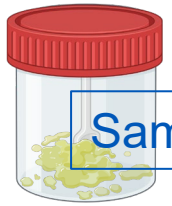
ATS/IDSA Recommendations for rapid molecular DST:

Test if patient has any of the following:

- Prior **TB treatment**
- Birth/residence ≥1 year in country with:
  - TB incidence ≥20 per 100,000, or
  - Drug resistance prevalence ≥2%
- Contact with **MDR-TB**
- **HIV infection**

Predictive Value Depends on Prevalence

- Rifampin resistance assays:
  - Low false-negative & false-positive rates (<3%)
  - **Low PPV** in U.S. due to **low prevalence**
- Isoniazid resistance assays:
  - Up to **10% false-negative rate**
  - **Higher INH resistance prevalence** (~8%) in U.S.

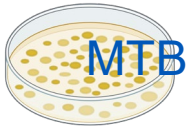


Sample

Smear +/-

Genotypic susceptibility

Mycobacterial cultures

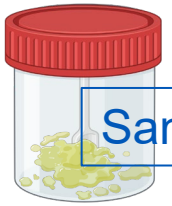


MTB complex

Phenotypic susceptibility

Genotypic susceptibility

MTB susceptibility testing



Sample

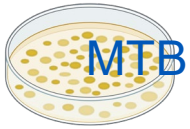
Smear +/-

Genotypic susceptibility

Phenotypic susceptibility

Compare growth of MTB in the presence of drugs vs no drugs.

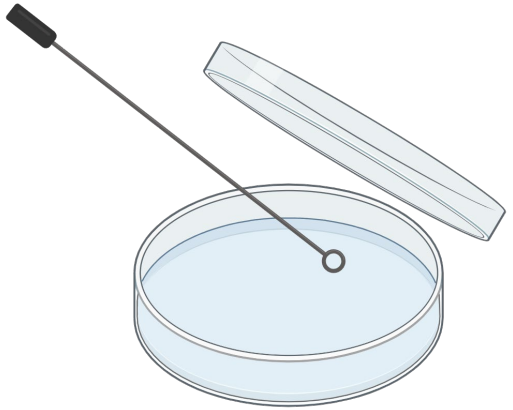
Mycobacterial cultures



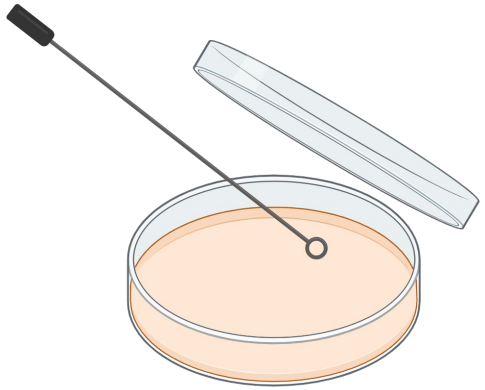
MTB complex

Phenotypic susceptibility

Genotypic susceptibility



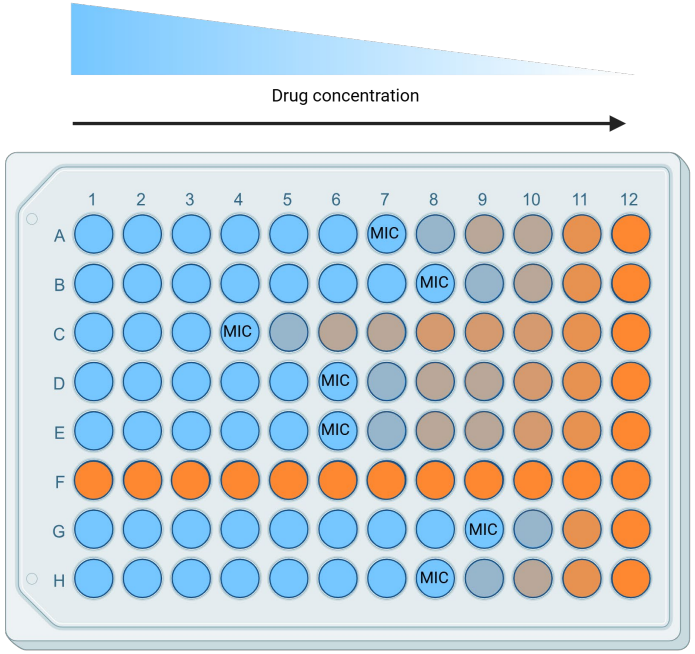
Drug-free medium



Drug-containing medium

Growth or No growth

Concept of "Critical concentrations"



Typical MICs (minimum inhibitory concentration)

+

Pharmacokinetic and pharmacodynamic data

Safely achievable tissue concentrations expected when drug is given at standard dose

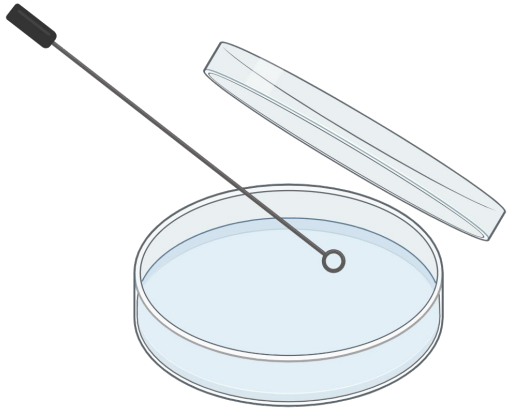
Clinical breakpoints

Critical concentration

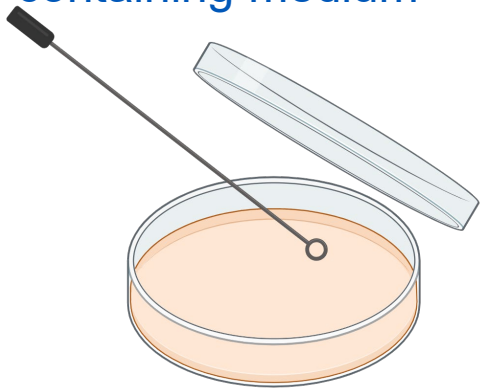
Historically set by WHO in the 1960s
Adapted from **LJ** to **Middlebrook 7H10/7H11/7H9**

Compare growth on drug-containing media to drug-free media

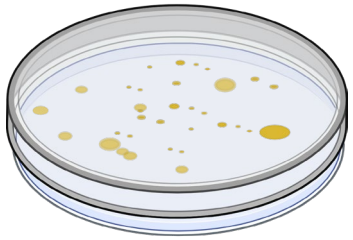
Inoculation in drug-free medium



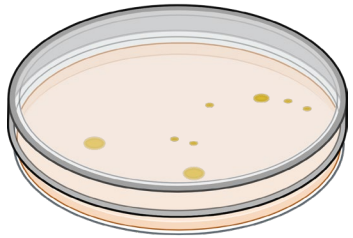
Inoculation in drug-containing medium



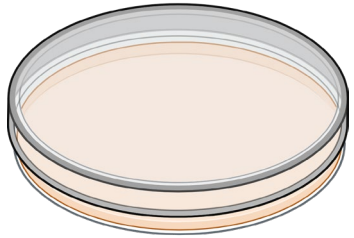
Growth is drug free media



>1% growth
Resistant



≥99% growth inhibition
Susceptible



CC ≠ MIC
(Minimum Inhibitory Concentration)
Lowest concentration that inhibits visible growth (specific to isolate)
Used to assess resistance level

CC ≠ clinical breakpoint
Clinical Breakpoint: Drug concentration that predicts **clinical response**
-Guides treatment decisions

Limitations of CC:
No integration of **PK/PD** data
Cannot distinguish **low/moderate/high-level** resistance
May **misclassify** resistance for drugs like EMB, ethionamide, ofloxacin

Evolution of Standards – Toward MIC & ECOFF

- WHO (2018) attempted to redefine CCs using **ECOFFs***
  - Lacked consistent MIC data & QC targets
- **EUCAST (2016–2019)**:
  - Developed **reference broth microdilution MIC method**
  - Uses 7H9 broth + standardized inoculum
  - Now endorsed as a **reference method**
- **Rifampin CC revised**:
  - WHO (2021) & CLSI (2023): lowered from **1.0 → 0.5 mg/L**
  - Improves detection of **borderline resistance**

***Epidemiological cutoff value (ECOFF)** is a threshold used in microbiology to differentiate between wild-type (WT) microorganisms, which do not have acquired resistance mechanisms, and non-wild-type (NWT) microorganisms,

Phenotypic susceptibility testing

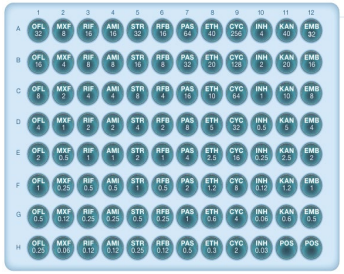
Solid media detect mixed populations better
Requires **~3 weeks** incubation

Medium	Notes
LJ medium	Most widely used; slowest
7H10 / 7H11	Faster than LJ; used with OADC* enrichment

Liquid media
May **miss heteroresistance** and borderline mutations

System	Method	Drugs	TAT	Limitations
MGIT™ 960 (BD BACTEC™)	Oxygen consumption (fluorescence)	1st line (FDA), 2nd line (validated)	**4–13 days**	Low reproducibility for PZA, EMB; may miss low-level RIF resistance
VersaTREK™ (Thermo Scientific™)	Pressure change	1st line (FDA)	**≤3 days**	Similar limitations; contamination risk

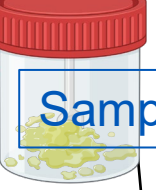
Broth Microdilution
requires ~ 2 weeks, Contains INH, RIF, EMB and 9 other drugs, Provides MIC endpoint



TF Sensititre MYCOTB MIC Plate LDT

Requires skilled staff to setup and read plates

*Oleic Albumin Dextrose Catalase Growth



Sample

Genotypic susceptibility

Smear +/-

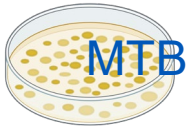
Initial tests

✅ Available in USA

Test	Drugs	TAT
✅**Xpert MTB RIF**	**RIF**	<2 hours
Xpert MTB RIF ULTRA	RIF	<2 hours
Truenat MTB plus and MTB/RIF Dx assays	RIF	~2 hours
✅**Abbott RealTime MTB and RealTime RIF/INH**	**RIF, INH**	~11 hours
✅**BD MAX™ MDR-TB**	**RIF, INH**	<4 hours
FluoroType® MTB and MTBDR	RIF, INH	~2.5 hours
✅**Roche cobas® MTB assay and cobas® MTB RIF/INH assay**	**RIF, INH**	~v4.5 hours

Smear +

Mycobacterial cultures



MTB complex

Follow on tests

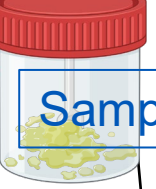
Test	Drugs Detected	Turnaround Time
Xpert MTB/XDR	INH, FQ, ETH, SLID	<2 hours
MTBDRplus assay	**INH, RIF**	~5 hours
Genoscholar™ PZA-TB	**PZA**	~1 day
GenoType® MTBDR v1.0	**FQ, SLID**	~5 hours
GenoType® MTBDRsl, versions 1.0 and 2.0	**FQ, SLID**	~5 hours

Phenotypic susceptibility

Genotypic susceptibility

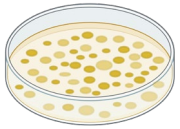
Deeplex® Myc-TB

Rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin.



Sample

Mycobacterial cultures



MTB complex

Phenotypic susceptibility

Genotypic susceptibility

NAAT testing
Line probe assay
Multiplex real-time PCR

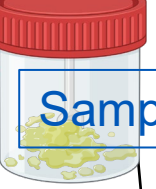
Available in USA
Cepheid Xpert MTB/RIF – for RIF only
LDT PCRs – e.g., MTBRP for INH only
LDT Sanger sequencing – e.g., *pncA* for PZA

Targeted NGS

Deeplex MDDR

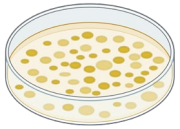
MDDR (CDC, 2023): tNGS available for select M. tuberculosis isolates and NAAT-positive sputum with pre-approval (e.g., history suggestive of MDR TB).

https://www.cdc.gov/tb/php/laboratory-information/mddr-user-guide.html



Sample

Mycobacterial cultures



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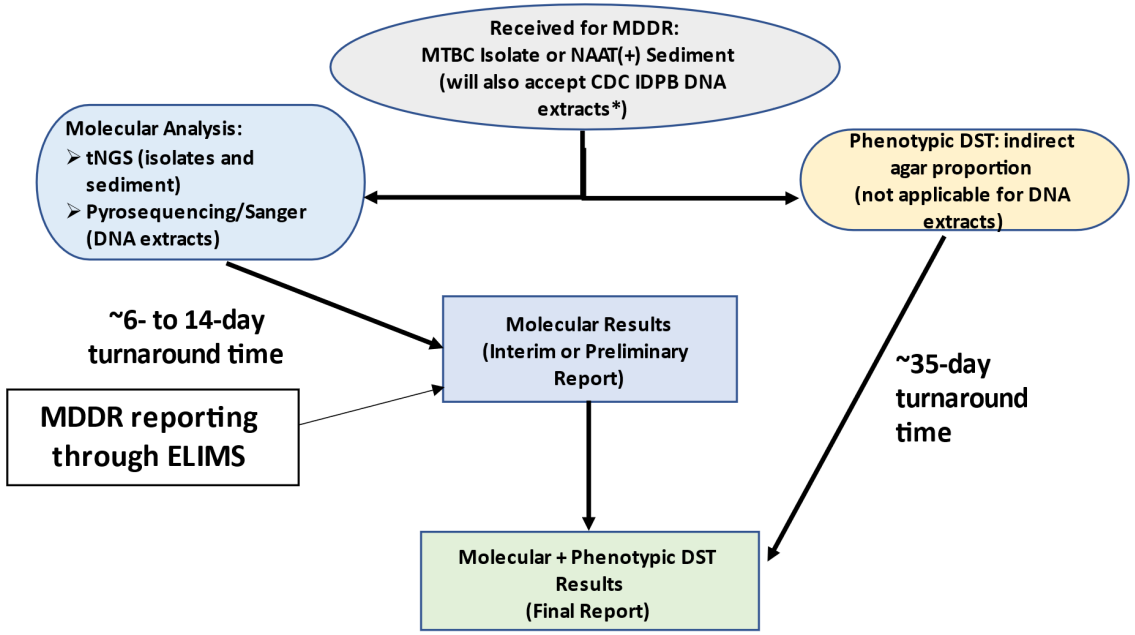
Targeted NGS

Deeplex MDDR

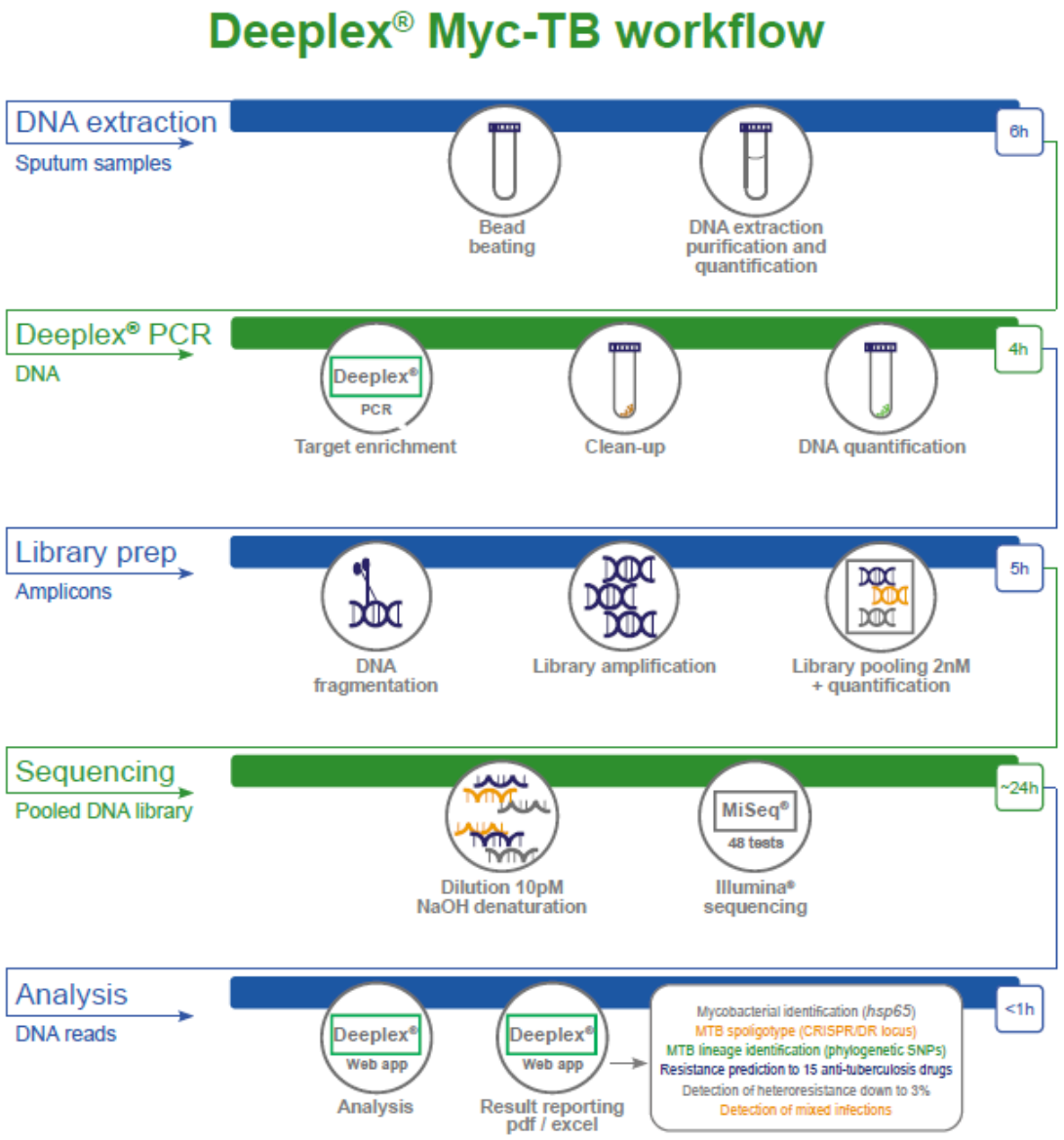
MDDR Algorithm



*DNA extracts only accepted from CDC IDPB and will be tested by conventional sequencing methods (not tNGS)

MTBC *Mycobacterium tuberculosis* complex, IDPB: Infectious Diseases Pathology Branch, ELIMS: enterprise laboratory information management system

https://www.cdc.gov/tb/php/laboratory-information/mddr-user-guide.html



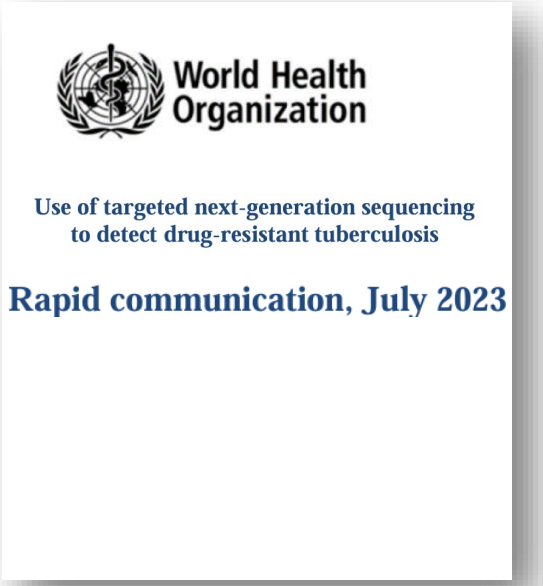
References

1. World Health Organisation, **Global tuberculosis report**. 2021.
2. Rahman, A. *et al.* Comparison of Xpert MTB/RIF assay and genotype MTBDRplus DNA probes for detection of mutations associated with rifampicin resistance in *Mycobacterium tuberculosis*. ***PLoS One* 11**, 1–11 (2016).
3. Rufai, S. B. *et al.* Comparison of xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* *52*, 1846–1852 (2014).
4. Singhal, R. & Myneedu, V. P. Microscopy as a diagnostic tool in pulmonary tuberculosis. *Int. J. Mycobacteriology* 4, 1–6 (2015).
5. Dai J, Chen Y, Lauzardo M. Web-accessible database of hsp65 sequences from Mycobacterium reference strains. J Clin Microbiol 2011; 49: 2296–303.

- **Identification of the Mycobacterial species** by sequence analysis of the *hsp65* gene in combination with select other targets for closely related species
- Smear positive sputum and culture isolates

Genomic target	Drug
rpoB	rifampicin
inhA	isoniazid, ethionamide
fabG1	isoniazid, ethionamide
katG	isoniazid
ahpC	isoniazid
*pncA**	pyrazinamide
embB	ethambutol
*gidB**	streptomycin
*rpsL**	streptomycin
*rrs**	streptomycin, amikacin, kanamycin, capreomycin
eis	kanamycin
*tlyA**	capreomycin
gyrA	fluoroquinolones
gyrB	fluoroquinolones
*ethA**	ethionamide
rrl	linezolid
rplC	linezolid
*rv0678**	bedaquiline, clofazimine

WHO now recommends BPaLM (6-month all-oral regimen) for MDR/RR-TB—including pre-XDR cases.
Emerging resistance to key drugs like bedaquiline and linezolid highlights the need for comprehensive DST.



tNGS → Detects resistance to multiple drugs in a single test, including new/repurposed ones (e.g., BDQ, LZD, DLM, Pa).

Diagnostic accuracy
Pulmonary TB (bacteriologically confirmed):
Sensitivity ≥95% for RIF, INH, MFX, EMB; 94% LFX; 88% PZA
Specificity ≥96% for all

Diagnostic accuracy
RR-TB:
Sensitivity ≥95% for INH, LFX, MFX, PZA, EMB
Lower for BDQ (68%), LZD (69%), CFZ (70%), AMK (87%), STR (90%)
Specificity ≥95% except STR (75%)

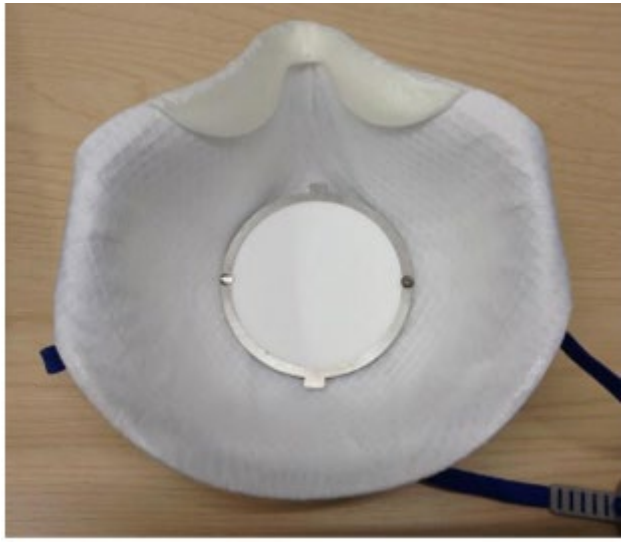
WHO recommendations

- Targeted NGS can guide treatment decisions post-TB diagnosis in prioritized patients.
- Still need phenotypic DST for drugs with suboptimal NGS sensitivity.
- Not yet available for all drugs or settings—only 3 products met inclusion criteria:
- **Deeplex® Myc-TB**: RIF, INH, PZA, EMB, FQs, BDQ, LZD, CFZ, AMK, STR
- **NanoTB®**: RIF, INH, FQs, LZD, AMK, STR
- **TBseq®**: EMB

New sample types

Exhaled Mycobacterium tuberculosis output by face-mask sampling

- Non-invasive, more sensitive than face-mask
  - Mtb was detected in 86% of face-mask samples and 21% of sputum samples over 24 hours.



Tongue swabs → Molecular testing

- Lower sensitivity than sputum but non-invasive, easier to collect

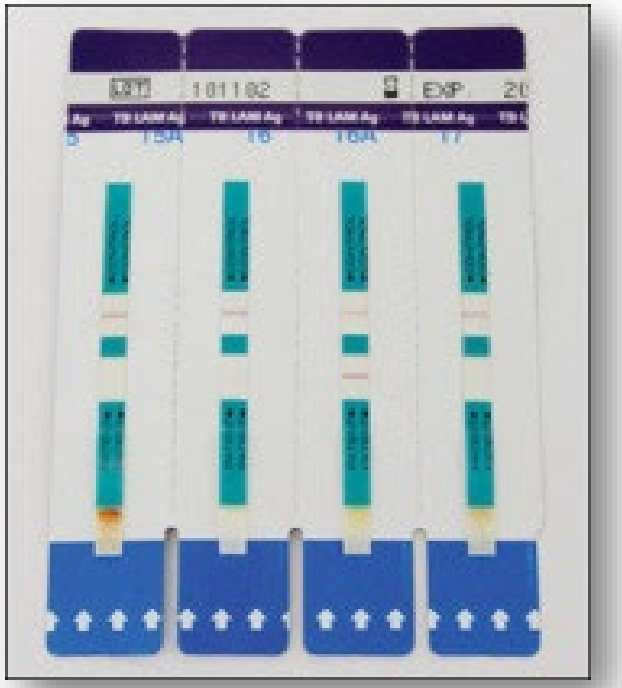
Williams, Caroline M et al, Steadman A et al 2024, Olson AM 2025

LAM

Lipoarabinomannan (LAM) is a glycolipid in the cell wall of mycobacteria

- Detected in urine
- Point of care lateral flow assay
- Sensitivity increases as CD4 count declines

Assay	Setting	Sensitivity
LF-LAM (Alere)	Inpatients	52%
	Outpatients	29%
SILVAMP-LAM (Fujifilm)	Mixed (mostly inpatients) CD4 count ≤ 100 cells/μL	87.1% (95% CI: 79.3–93.6%)



Kerkhoff AD et al 2016

Thank you